Effect of a 12-Month Exercise Intervention on Patterns of Cellular Proliferation in Colonic Crypts: A Randomized Controlled Trial

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Abstract

Background: **Colon crypt architecture and proliferation may be appropriate biomarkers for testing prevention interventions. A hypothesized mechanism for exercise-induced colon cancer risk reduction might be through alterations in colon crypt cell architecture and proliferation.**

Methods: **Healthy, sedentary participants with a colonoscopy within the previous 3 years were recruited through gastroenterology practices and media. We randomly assigned 100 women and 102 men, ages 40 to 75 years, to a control group or a 12-month exercise intervention of moderate-to-vigorous aerobic exercise, 60 minutes per day, 6 days per week, and assessed change in number and relative position of Ki67-stained cells in colon mucosal crypts.**

Results: **Exercisers did a mean 370 min/wk (men) and 295 min/wk (women) of exercise (seven dropped the intervention). In men, the mean height of Ki67-positive nuclei relative to total crypt height was related to amount of exercise, with changes from baseline of 0.0% (controls), +0.3% (exercisers <250 min/wk), −1.7% (exercisers 250-300 min/wk), and −2.4% (exercisers >300 min/wk; P_{trend} = 0.03). In male exercisers whose cardiopulmonary fitness (V_{O_2}max) increased >5%, the mean height of Ki67-positive nuclei decreased by 2% versus 0.9% in other exercisers, and versus no change in controls (P_{trend} = 0.05). Similar trends were observed in other proliferation markers. In women, increased amount of exercise or V_{O_2}max did not result in notable changes in proliferation markers.**

Conclusions: **A 12-month moderate-to-vigorous intensity aerobic exercise intervention resulted in significant decreases in colon crypt cell proliferation indices in men who exercised a mean of ≥250 min/wk or whose V_{O_2}max increased by ≥5%.** (Cancer Epidemiol Biomarkers Prev 2006;15(9):1588–97)

Introduction

Colorectal cancer is the second most commonly occurring cancer in the United States and is estimated to affect almost 145,000 men and women in 2005 (1). It is the second leading cause of cancer death and is expected to lead to 56,000 deaths in 2005 (1). Numerous case-control and cohort studies, representing >13,000 colon cancer cases, have found an inverse association between physical activity and risk of colon cancer (2). This relationship has been observed in all age groups, in various racial and ethnic groups, and in diverse geographic areas around the globe. An attenuated effect of exercise on colon cancer risk has been noted in some studies of women (2). This could be due to women being less likely to engage in vigorous activity compared with men, or there could be some additional exercise effects (e.g., lowering endogenous hormones) that interfere with protection of the colorectal epithelium (3, 4). In all of nine cohort studies, the relative risk for colon cancer was decreased in persons in the highest category of either recreational or occupational physical activity compared with persons in the lowest category, with relative risk estimates ranging from 0.40 to 0.90. Case-control studies have supported the cohort study findings. In several cohort and case-control studies, an effect of decreasing incidence of colon cancer with increasing levels of recreational physical activity has been observed (5). Adjustment for potential confounding factors such as age, diet, and obesity has not diminished the observed associations between physical activity and colon cancer occurrence.

We hypothesized that if exercise can change the rate or direction of neoplastic growth, it should be possible to see direct effects on normal colonic mucosa. The cells within a human colon turn over rapidly and continuously (6). Given the complex cellular architecture of the colon, limited and highly regulated proliferation is necessary at all times to maintain proper crypt height and structure. Compared with individuals at low risk for colon cancer, patients with colon cancer and persons at elevated risk of colon cancer (because of a history of sporadic adenoma, familial polyposis, ulcerative colitis, a family history of colon cancer, or age) exhibit in macroscopically normal colorectal mucosa both an increased cell proliferation rate and an extension of the normal proliferative zone from the basal 60% of the crypt toward the luminal 40% (see Fig. 1; ref. 7). In patients with previous colon cancer or sporadic adenomas, these changes also predict adenoma recurrence (8, 9). A luminal shift in the proliferative zone is

Received 3/22/06; revised 6/14/06; accepted 7/6/06.

Grant support: National Cancer Institute grant R01 CA77572-01, National Cancer Institute Cancer Prevention Training grant T32 CA09661 (M.L. Irwin), and NIH grants M01-RR-00037 and AG1094.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Note: A portion of this work was conducted at the Clinical Research Center Facility at the University of Washington.

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doi:10.1158/1055-9965.EPI-06-0223

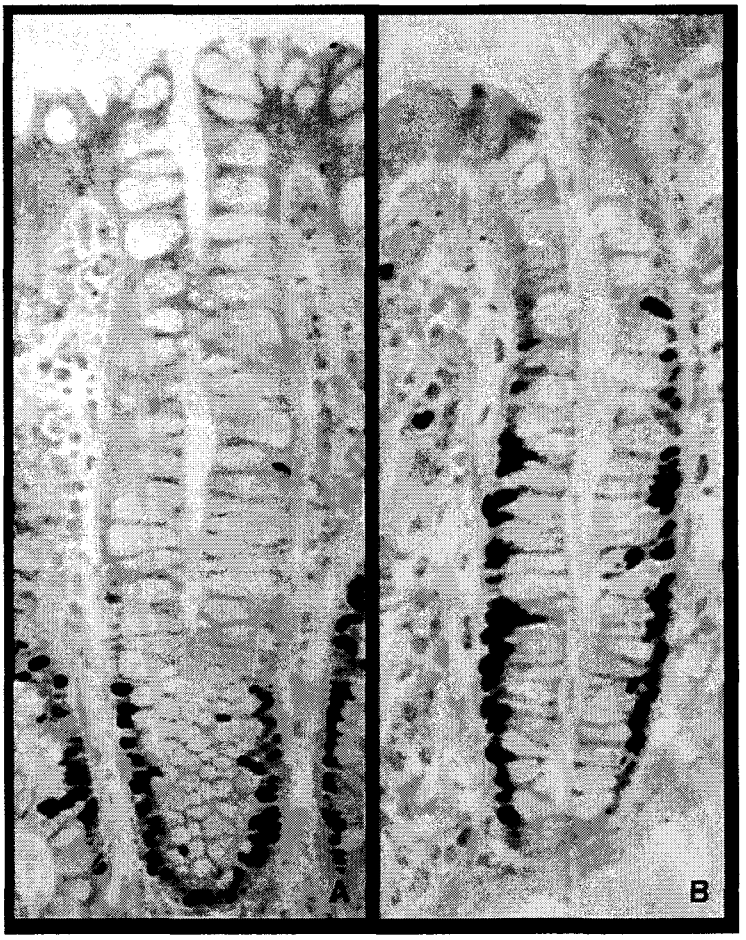

Figure 1. Colon crypt stained with Ki67 proliferation marker. **A.** Normal crypt. **B.** Crypt from normal mucosa of colon cancer patient. Note higher Ki67 staining at luminal edge (*top*) of crypt.

found in cancers and adenomas, but not in hyperplastic polyps (10). Animal experimental evidence and preliminary evidence in humans strongly suggest that these two proliferation abnormalities (overall hyperproliferation and luminal shift of the proliferation zone) are reversible precursors of colon neoplasia (7). Recently, detection of Ki67 antigen, which is expressed in all phases of the cell cycle except G_0 and early G_1, has become widely used as a measure of proliferation (11, 12). Using a commercially available monoclonal Ki67 antibody, it is possible to obtain, on a routine basis, very distinct and clear-cut identification of proliferating cells. Ki67 labeling is considered to have several advantages over other commonly used staining procedures (13).

Colon crypt architecture, in particular crypt height (i.e., the number of cells lining the vertical slopes of the crypt), may also be relevant to carcinogenesis. In animal models, oxidative DNA damage to the large intestine results in significant loss of crypt height (14). Aspirin, an agent that reduces colon cancer risk, was found to reduce crypt height in rats treated with a carcinogen (15). In animal models, fiber of vegetable source lengthens crypt height (16, 17) whereas fiber of animal source or dietary fat of any source reduces crypt height (18, 19). It is not known if crypt height is related to carcinogenesis in humans, although one group reported that colonic crypts were longer in Seventh Day Adventists and health-conscious populations than in groups at high risk of cancer (20).

The purpose of this study was to assess, in a randomized controlled clinical trial, the effect of a 12-month moderate-to-vigorous intensity exercise program (60 minutes per day, 6 days per week,) on colon crypt cell proliferation amount and pattern in women and men.

Materials and Methods

This was a 12-month randomized, controlled clinical trial evaluating the effects of an aerobic exercise intervention on colonic epithelial cell proliferation and other potential markers of colon cancer risk (21).

Participants. Participants were men and women, ages 40 to 75 years, who had a colonoscopy within the previous 3 years so that their recent polyp status was known. Participants must also have been insufficiently active (i.e., engaging in <90 min/wk of moderate-to-vigorous intensity sports, recreational, or walking exercise), drinking less than two alcohol drinks per day, with no personal history of invasive cancer or other serious medical conditions such as cardiovascular disease, stroke, diabetes, or uncontrolled hypertension, and with a normal response to a maximal exercise tolerance test. Furthermore, they must have had normal complete blood count and blood chemistries. To avoid the confounding effects of highly penetrant germ line mutations, we also excluded persons with familial polyposis, Gardner's syndrome, or other known familial colorectal cancer syndromes. Also excluded were persons with ulcerative colitis or short bowel, "excessive" use of laxatives (>3 per week), enema use, or use of any medication that might interfere with colorectal crypt studies including chronic use of anticoagulants or corticosteroids. Use of nonsteroidal anti-inflammatory medications was allowed up to twice a week if the person was able to stop use safely for 2 weeks before and after obtaining colorectal biopsies.

Several methods were used to recruit participants between 2001 and 2004. The primary method was through gastroenterology practices, where potentially eligible persons were

identified from medical records and sent an invitation letter by their physician with an enclosed "Interest Survey" to complete and return to the study center. Additional recruitment methods included media placements, flyers, a study web site, and referrals.

From the 9,828 letters that were mailed to gastroenterology practice patients, 2,033 (21%) responded expressing interest in joining the study, and of these, 956 (47%) were potentially eligible according to age and health status (Fig. 2). These 956 persons were interviewed by phone about eligibility requirements (including current exercise habits, plans for extensive dieting, ability to attend clinic and exercise facility appointments, willingness to be randomly assigned to intervention or control). In addition, there were 1,328 calls to the study line in response to media placements, of which 1,092 interviews were completed. The major reasons for ineligibility were being unwilling to be randomly assigned to exercise or control groups (N = 297), being too physically active (e.g., participating in >90 min/wk of moderate-to-vigorous recreational or occupational activity; N = 339), and not having time available to attend exercise sessions due to work hours (N = 48). A total of 395 attended an information session; 311 were screened in clinic; and 202 (102 men, 100 women) were enrolled in the trial and randomized to either exercise or control group. Informed consent was obtained following the requirements of the Fred Hutchinson Cancer Research Center Institutional Review Board. Participants were paid $50 and $75 after completion of baseline and 12-month data collection, respectively.

Randomization and Blinding. Participants were randomized in equal numbers to exercise or control group (referred to respectively in this article as "exercisers" and "controls"). Randomization was blocked on sex, use of nonsteroidal anti-inflammatory medications [regular use (more than twice a week) versus less], current smoking status (yes versus no), and, among women, on menopausal status (premenopausal or perimenopausal versus postmenopausal) and current use (yes versus no) of hormone replacement therapy. Staff and scientists involved in end point determinations were blinded to participant randomization status and to prerandomization vs. postrandomization status of samples. Randomization was done by the study coordinator (A.M.) via a computerized program developed by the study biostatistician (Y.Y.) that incorporated the blocking factors.

Baseline and Follow-up Measures

Ki67 Labeling of Colonic Epithelial Cell Proliferation. Colonic epithelial cell biopsies were collected at prerandomization and at 12 months poststudy flexible sigmoidoscopy. Before flexible sigmoidoscopy, participants self-administered up to five saline enemas, starting 1.5 hours before the procedure. If the preparation was insufficient, an additional saline enema was administered at the time of sigmoidoscopy.

During the flexible sigmoidoscopy, 1-mm-thick biopsies were collected in a standard manner by physicians trained in the procedure (Drs. Rudolph, Kayub, and Surawicz) using jumbo biopsy forceps (Olympus FB-50U1-1; Olympus America, Inc., Melville, NY). Fourteen biopsies were collected for each participant, of which one biopsy collected from the sigmoid colon (30-35 cm from the level of the external anal aperture) was processed for analysis of proliferation. The remaining biopsies, collected from the sigmoid colon and the rectum, were processed and stored for other colorectal cancer biomarkers.

Biopsies were oriented and placed in 10% neutral buffered formalin for 2 to 3 hours followed by transfer to 70% ethanol. Eight to twelve sections, at least 50 μm apart (to ensure that each section evaluated included sections of different crypts), were cut per biopsy and mounted with positive control

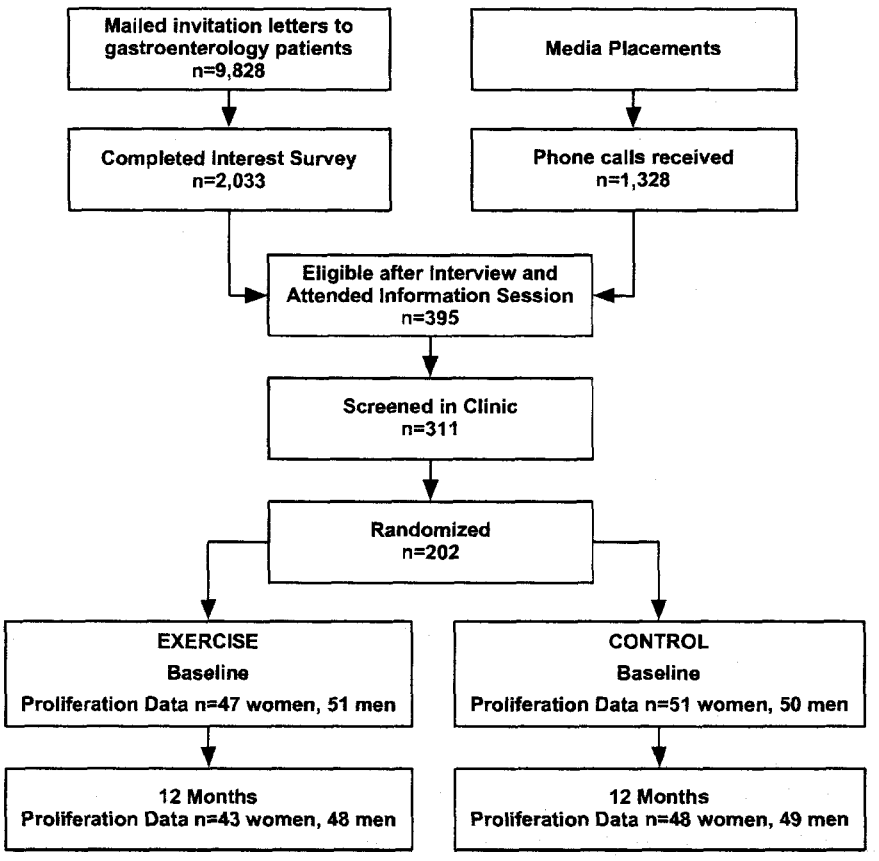

Figure 2. Recruitment and flow of participants in the study.

(human tonsil) and negative control tissues (colon biopsy tissue from the same participant with all reagents except primary antibody). Sections were deparaffinized through xylene/ethanol/water. Slides from a single participant (baseline and follow-up) were stained in the same run to minimize the effect of run-to-run variability in staining intensity inherent in the procedure. Slides were steamed in boiling DAKO Antigen Retrieval Buffer (DAKO Corp., Carpenteria, CA) for 30 minutes. After cooling for 20 minutes, slides were stained on a Techmate 1000 Staining System automatic immunostainer (Ventana Medical Systems, Inc., Tuscon, AZ) using detection reagents from the Vectastain Elite Standard ABC Kit (Vector Laboratories, Inc., Burlingame, CA). Slides were blocked for 5 minutes with 4% normal horse serum in PBS with 0.05% Tween 20, and then buffer washed. Biopsies were stained with the Ki67 (MIB-1) monoclonal antibody (Immunotech, Inc., Miami, FL) at 1:500 dilution for 1 hour in PBS plus 1% bovine serum albumin and 0.05% Tween 20 and washed with buffer. They were immersed in the secondary antibody, biotinylated horse anti-mouse immunoglobulin G (Vector Laboratories), at a dilution of 1:200 for 30 minutes in PBS with 1% bovine serum albumin with 0.05% Tween 20, and then buffer washed. Endogenous peroxidases were inactivated by treatment with 3% hydrogen peroxide for 5 minutes. Slides were then washed and immersed in Avidin-Biotin Complex reagent for 30 minutes. After washing, slides were developed with diaminobenzidine tetrahydrochloride solution at a concentration of 0.5 mg/mL in PBS with 0.1% hydrogen peroxide. The stained slides were then dehydrated and coverslips were applied over mounting medium.

Biopsies were scanned under a microscope (E400, Nikon, Inc., Melville, NY) to identify acceptable colon crypts. Acceptable crypts were defined as those being mid-axial, U-shaped sections extending from muscularis to lumen with an intact structure. Images of the crypts were captured using a digital camera (Hamamatsu Photonics KK, Hamamatsu City, Japan) attached to the microscope that was set at constant illumination and exposure settings. Nuclei were counted if they were positioned on the edge of the crypt cell columns and were fully darkened. Partially darkened nuclei were not counted to exclude nuclei lying below the plane of the cell column. Macros developed for the NIH Image software program[9] allowed the collection and analysis of images. At least 5 and up to 10 crypts per biopsy were scored.

Several outcome variables were defined that could reflect capture crypt cell proliferation. Crypt height was measured as the distance in micrometers from base to lumen of the crypt. Number of Ki67-positive (Ki67+) cells was an absolute count of Ki67+ cells of the crypt. Mean, median, and 75th percentile of Ki67+ cell height as percent of crypt height were calculated by dividing the height of each Ki67+ cell in the crypt by the total crypt height, and then calculating mean, median, and 75th percentile values of the positions of all Ki67+ cells in the crypt. In addition, a weighted mean Ki67+ cell height was calculated, where the weights were proportional to the decile position of the stained cell in the crypt. Thus, Ki67+ cells in the bottom tenth of the crypt received a weight of 0.1, cells in the second lowest decile received a weight of 0.2, cells in the top decile received a weight of 1.0, etc. For each of these measures, the mean value among all biopsied crypts of a subject at a given time (baseline or 12-month follow-up) was calculated and used as the subject's value of the measurement at the time.

Exercise Data. We assessed the previous 3- and 12-month physical activity type, frequency, and duration at baseline, and the previous 3-month physical activity at 3, 6, 9, and 12 months with a physical activity interview adapted from Minnesota Leisure Time Physical Activity Questionnaire (22). The baseline previous 3-month data were used to screen out individuals who were not currently sedentary. Participants reported whether they did any of the 38 recreational or household activities listed. For each activity done, the participants were asked about the number of days per week and minutes per session over the relevant time interval. For eligibility, adherence, and control contamination estimation, we included only sports, recreational, and walking activities with a Metabolic Equivalent Test (MET) level >4.0 (23). Participants were also asked to wear Accusplit pedometers (Accusplit, Inc., San Jose, CA) during waking hours for 1 week at baseline and 3, 6, 9, and 12 months to record their total daily steps in a log, and to turn in the log at their clinic visits.

To determine cardiopulmonary fitness, we assessed maximal oxygen consumption (V_{O_2}max, mL/kg/min) at baseline and 12 months (24). Participants completed a maximal-graded treadmill test, with heart rate and oxygen uptake monitored by a Medgraphics automated metabolic cart (Medgraphics, St. Paul, MN). The test began at 3.0 mph and 0% grade. The speed or grade (2% increments) of the treadmill increased every 2 minutes (i.e., stage II: 3.5 mph, 0% grade; stage II: 3.5 mph, 2% grade; stage IV: 3.5 mph, 4% grade, etc.) until the participant reached volitional fatigue.

Covariates. We collected demographic and medical history information at baseline and 12 months on health habits, smoking status, use of prescription and over-the-counter medications, history of chronic diseases, and reproductive and body-weight history. We estimated total caloric intake, as well as other macronutrients that could be related to colon cancer risk (dietary fat, fiber, and alcohol intake), at baseline and 12 months via a 120-item self-administered food frequency questionnaire (25). With participants wearing light clothing, we measured baseline and 12-month weight and height to the nearest 0.1 kg and 0.1 cm, respectively, with a balance beam scale and stadiometer. Both measurements were taken in duplicate and then averaged. We calculated body mass index (BMI) as kg/m^2.

Exercise Intervention. The intervention was a 12-month moderate-to-vigorous intensity aerobic exercise program. The goal was 60 minutes per day, 6 days per week, to be gradually achieved over 12 weeks and then continued throughout the 12 months of intervention. The intervention included both facility and home-based programs. Three days per week, participants exercised with an exercise physiologist overseeing their sessions, either with a group (~5-10 participants per group) or alone. There were four facilities available to participants: one located at the Fred Hutchinson Cancer Research Center Prevention Center (A. McTiernan, Director) and three private health clubs (participant membership paid by the study, with study exercise physiologist present). At the facilities, participants did aerobic exercise for 60 minutes per session on machines (primarily treadmills and stationary bikes, supplemented with rowing machines and elliptic machines). At the facility sessions, participants wore Polar (Polar Electro, Inc., Lake Success, NY) heart rate monitors. Participants were given an exercise prescription, with a heart rate goal corresponding to 60% to 85% of their maximal heart rate on their baseline V_{O_2}max test, and were instructed to record the maximal heart rate achieved during each exercise session in their daily activity logs (see below). Thus, the actual exercise prescription was 6 days per week of 60 minutes per session of aerobic exercise done at 60% to 85% of maximal heart rate. Approximately 5 to 10 minutes of warm-up, cool-down, and stretching exercise were done in addition to the 60-minute exercise sessions.

Participants also were asked to exercise at home 3 days per week. Some of the study facilities provided the opportunity for participants to work out during any open hours, so some participants did their home exercise as unsupervised gym

[9]Available at http://rsb.nih.gov/nih-image/.

workouts using various aerobic machines (e.g., treadmill, elliptic, stationary bicycle, stairstepper). Other participants chose outdoor walking, jogging, or biking as their primary home exercise. Participants were given heart rate monitors to wear during their home exercise sessions, with the same instructions about goal ranges and recording as for their supervised sessions.

A variety of additional strategies were used to achieve and maintain adherence, including monthly progress review/behavior change meetings individually or in groups, regular monitoring and feedback, newsletters, appropriate incentives as gifts (e.g., pedometers, water bottles), and group social events. Several methods were used to measure and monitor adherence, including required facility class attendance (with data on type, length, and maximal heart rate of exercise session entered in facility logs, verified by the exercise physiologist and data entered for the entire intervention), home exercise logs (with data on type, length, and maximal heart rate of exercise session entered in home logs, submitted weekly, reviewed by the exercise physiologist, and data-entered for the 12 months), quarterly total physical activity questionnaire interviews, V_{O_2}max treadmill tests (baseline and 12 months), and pedometers with recording of daily steps (done quarterly for 1 week). Adherence was calculated weekly as facility sessions attended (minutes per week), metabolic equivalent tests (MET)-min/wk, and percent of goal 360 min/wk, with MET determined by the Exercise Compendium (23). Good adherence was defined as meeting at least 80% of the overall minutes per week goal of moderate-to-vigorous exercise.

Exercisers were asked not to change their dietary habits during the 12-month duration of the trial.

Controls. Controls were asked not to change their exercise or diet habits for the 12-month duration of the trial. They were given the opportunity to participate in exercise classes for 2 months (with the same progression as offered to the exercise-arm participants during months 1-2 of intervention) at the end of the 1-year period, after completion of all end-of-study measures. They completed some of the same exercise variable data collection as exercisers, specifically the quarterly pedometer use with daily logging of steps for 1 week, and quarterly physical activity interviews.

Statistical Analyses. To assess and compare exercisers' and controls' exercise behaviors during the 12 months of study, we calculated mean total number of minutes of moderate-to-vigorous activity per week over the 12-month period using data from the physical activity interviews. We also compared mean number of steps per day at 3, 6, 9, and 12 months of follow-up in exercisers and controls.

Primary outcome analyses were based on assigned treatment at the time of randomization regardless of adherence status (i.e., intent-to-treat). For each of the exercise and control groups, we computed the outcome measures at baseline and 12 months postrandomization. The intervention effects were evaluated by the differences in the mean changes at 12 months between exercisers and controls using the generalized estimating equation modification to linear regression models to account for the longitudinal nature of the data. Primary

Table 1. Baseline characteristics for study participants by sex and intervention versus control group

	P*	Women		P†	Men		P‡
		Exercisers (N = 47)	Controls (N = 51)		Exercisers (N = 51)	Controls (N = 50)	
		Mean (SD) [range]	Mean (SD) [range]		Mean (SD) [range]	Mean (SD) [range]	
Age (y)	0.01	54.3 (7.21) [43.0-73.1]	53.7 (5.65) [42.5-65.2]	0.62	56.2 (6.66) [40.3-69.7]	56.5 (7.63) [40.3-74.7]	0.86
BMI (kg/m^2)	0.08	28.9 (5.36) [21.2-42.9]	28.5 (4.80) [20.3-39.9]	0.73	29.7 (3.73) [23.3-41.7]	30.1 (4.88) [21.3-44.9]	0.70
V_{O_2}max (mL/kg/min)	<0.001	23.9 (5.15) [14.9-37.1]	24.8 (4.34) [17.5-36.9]	0.36	30.1 (5.92) [20.7-44.3]	30.3 (6.77) [15-49.3]	0.87
Energy intake (kcal)*	0.10	1,481 (630.7) [552-3,330]	1,583 (554.6) [610-3,326]	0.40	1,692 (639) [528-3,875]	1,656 (565.8) [662-3,526]	0.77
Fiber intake (g)	0.61	14.7 (8.68) [3.43-39.9]	15.9 (6.33) [6.36-32.3]	0.45	15.2 (7.14) [5.79-43.0]	14.4 (5.98) [5.12-34.0]	0.56
Any Ki67+ cell in the top 40% of crypt	0.43	14 (29.8%)	13 (25.5%)	0.63	15 (29.4%)	18 (36.0%)	0.48
Mean Ki67+ cell height (% of crypt height)	0.57	18.8 (4.93) [0-28.1]	17.6 (5.76) [1.50-29.2]	0.24	18.8 (4.22) [9.0-27.9]	18.4 (5.74) [2.0-29.8]	0.69
Median Ki67+ cell height (% of crypt height)	0.19	16.8 (5.53) [0-26.8]	16.1 (5.21) [1.5-26.9]	0.53	17.7 (4.61) [8.0-29.0]	17.2 (5.89) [2.0-28.2]	0.66
75th percentile for Ki67+ cell height (% of crypt height)	0.92	27.9 (7.93) [0-40.7]	25.9 (8.79) [1.5-42.0]	0.23	26.9 (6.18) [13.0-41.5]	26.6 (8.35) [2.0-44.0]	0.82
Weighted mean Ki67+ cell height	0.45	2.34 (0.53) [0-3.25]	2.23 (0.59) [0.5-3.41]	0.38	2.37 (0.44) [1.33-3.30]	2.32 (0.54) [1-3.42]	0.63
No. Ki67+ cells per crypt	0.76	9.45 (6.21) [0-37]	8.66 (4.32) [1-20]	0.46	9.35 (3.71) [3-20]	9.13 (4.07) [1-19.2]	0.78
Crypt height (μm)	0.82	539.6 (103.8) [256-761]	563.8 (95.7) [372-771]	0.23	536.8 (86.8) [356-697]	574.1 (79.9) [407-788]	0.03
No. crypts	0.12	5.55 (3.06) [1-11]	5.29 (3.23) [1-10]	0.69	6.27 (3.35) [1-11]	6.02 (3.42) [1-13]	0.71
		n (%)	n (%)		n (%)	n (%)	
Race							
American Indian/ Alaskan native	0.20	1 (2.13)	1 (1.96)	0.47	0 (0.00)	0 (0.00)	0.55
Asian or Pacific Islander		4 (8.51)	1 (1.96)		2 (3.92)	3 (6.12)	
African American		1 (2.13)	2 (3.92)		0 (0.00)	0 (0.00)	
Hispanic		1 (2.13)	0 (0.00)		0 (0.00)	0 (0.00)	
White, non-Hispanic		40 (85.1)	47 (92.2)		48 (94.1)	46 (93.9)	
Other		0 (0.00)	0 (0.00)		1 (1.96)	0 (0.00)	
History of adenomatous polyps	<0.001	14 (29.8)	12 (23.5)	0.48	30 (58.8)	30 (61.2)	0.90
First-degree relative with colon cancer	0.23	20 (42.6)	19 (37.3)	0.59	15 (29.4)	17 (34.0)	0.62

*P value comparing males and females at baseline.
†P value comparing female exercisers and controls at baseline.
‡P value comparing male exercisers and controls at baseline.

Table 2. Baseline and follow-up proliferation outcome measures

(A) Women

	Exercisers			Controls			β_{int}	P
	Baseline, $N = 47$	Follow-up, $N = 42$	Δ_E	Baseline, $N = 51$	Follow-up, $N = 47$	Δ_C		
	Mean (SD)	Mean (SD)		Mean (SD)	Mean (SD)			
Mean Ki67+ cell height (% of crypt height)	18.8 (4.93)	19.2 (4.46)	+0.4	17.5 (5.76)	17.2 (6.74)	−0.3	0.002	0.83
Median Ki67+ cell height (% of crypt height)	16.8 (5.53)	17.7 (4.87)	+0.9	16.1 (5.21)	16.0 (6.21)	−0.1	0.001	0.96
75th percentile for Ki67+ cell height (% of crypt height)	27.9 (7.93)	27.9 (5.75)	0.0	25.9 (8.79)	25.3 (11.0)	−0.6	0.004	0.77
Weighted mean Ki67+ cell height	2.34 (0.53)	2.43 (0.44)	+0.9	2.24 (0.59)	2.21 (0.68)	−0.3	0.052	0.63
No. Ki67+ cells per crypt	9.45 (6.21)	10.2 (5.12)	+0.8	8.66 (4.32)	7.86 (4.99)	−0.8	1.153	0.30
Crypt height (μm)	539.6 (103.8)	560.9 (57.6)	+21.3	563.8 (95.7)	532.7 (108.5)	−31.1	47.1	0.06

(B) Men

	Exercisers			Controls			β_{int}	P
	Baseline, $N = 51$	Follow-up, $N = 48$	Δ_E	Baseline, $N = 50$	Follow-up, $N = 45$	Δ_C		
	Mean (SD)	Mean (SD)		Mean (SD)	Mean (SD)			
Mean Ki67+ cell height (% of crypt height)	18.8 (4.22)	17.3 (5.40)	−1.5	18.4 (5.74)	18.4 (4.71)	0.0	−0.008	0.45
Median Ki67+ cell height (% of crypt height)	17.7 (4.61)	16.1 (5.26)	−1.6	17.3 (5.89)	16.7 (4.77)	−0.6	−0.005	0.64
75th percentile for Ki67+ cell height (% of crypt height)	27.0 (6.18)	24.9 (7.91)	−2.1	26.6 (8.38)	26.3 (6.94)	−0.3	−0.011	0.49
Weighted mean Ki67+ cell height	2.37 (0.44)	2.23 (0.51)	−0.14	2.32 (0.54)	2.33 (0.45)	+0.01	−0.090	0.40
No. Ki67+ cells per crypt	9.35 (3.71)	9.23 (4.19)	−0.12	9.13 (4.07)	9.86 (4.68)	+0.73	−0.692	0.46
Crypt height (μm)	536.8 (86.8)	580.2 (87.7)	+43.4	574.1 (79.9)	575.4 (87.5)	+1.3	37.1	0.07

NOTE: Δ_E, change in exercisers; Δ_C, change in controls.

analyses were unadjusted in line with the randomized design of the study.

Baseline and 12-month proliferation data were available for 101 and 97 of 102 randomized men and for 98 and 91 of 100 randomized women, respectively. Participants with missing outcome data were not included in the analyses.

We also conducted stratified analyses to explore differential intervention effects by baseline age (<55 and >55 years), BMI (<25, 25.0-29.9, and >30.0 kg/m^2), history of adenomatous polyps (yes/no), first-degree family history of colon cancer (yes/no), presence of Ki67+ stained cells in the top 40th percent of the crypts versus none, and by median height of Ki67+ stained cells. As a preplanned secondary analysis, we grouped the mean changes of adherence measures (V_{O_2}max and mean number of minutes exercised per week) from baseline to 12 months into tertiles within the exercisers. We also calculated the mean energy expenditure for the year as follows: METs × body weight [kg]/60 [kg] × 1 [kcal/kg/min] × minutes exercised/wk × 4.18 [kJ/kcal] = mean energy expenditure [kJ/wk]. We then divided exercisers into tertiles of this variable and assessed the change in end-point variables over 12 months within each stratum. Tests for trend across the control and the three strata of the adherence in the intervention group were done by placing the four-category adherence variable into models as a continuous covariate. Adjusting for age made no difference in these analyses of adherence subgroups.

All analyses were conducted separately for men and women by study design. Differences between women and men were tested by the gender-intervention interaction term into the models.

All statistical tests were two sided. $P \leq 0.05$ was considered to be statistically significant. Statistical analyses were done using SAS software (version 8.2; SAS Institute, Inc., Cary, NC).

Results

Study Participants. Table 1 presents baseline information on study participants. None of the baseline characteristics was statistically significantly different between exercisers and controls in either men or women, with the exception of a higher crypt height in male controls versus exercisers. Men and women were of similar age (mean ages, 56.4 and 54.0 years, respectively), and most were non-Hispanic White, reflecting the Western Washington population. Participants on average were overweight: the mean baseline BMI for women was 28.7 kg/m^2, and for men it was 29.9 kg/m^2. Men had higher levels of cardiopulmonary fitness (V_{O_2}max) than women as expected from population norms, but both were low, indicating a sedentary population (24). Men were more likely than women to have a history of adenomatous polyps ($P < 0.001$).

Adherence to the program was excellent overall as measured by daily facility and home exercise logs, with 80% of exercisers meeting >80% of their 360 min/wk goal. From the log data over the 12 months of intervention, male exercisers completed a mean 370 min/wk (102.7% of goal) and female exercisers did a mean 295 min/wk (82% of goal). Adherence to facility exercise goals was lower than adherence to home exercise goals for both women and men. Female exercisers completed a mean 89.6 min/wk of facility exercise (56.3% of facility goal) and a mean 145.4 min/wk of home exercise (94.5% of home exercise goal). Male exercisers completed a mean 114.1 min/wk of facility exercise (70.2% of facility goal) and a mean 166.5 min/wk of home exercise (107.4% of home exercise goal). It should be noted that participants were encouraged to do more home exercise when on vacation,

traveling, or otherwise unable to attend the facility. These extra minutes were entered into the Daily Activity Logs and counted with the home exercise minutes. Only 2 of 51 male exercisers and 5 of 49 female exercisers dropped the intervention, and all drops were after 3 months. As reported in the physical activity interview, female and male exercisers significantly increased their mean amount of moderate-to-vigorous recreational physical activity at each time period (baseline, 3-, 6-, 9-, and 12-month means of 24, 309, 364, 306, and 298 min/wk, respectively), whereas controls increased moderate or vigorous recreational physical activity level to a smaller degree (baseline, 3-, 6-, 9-, and 12-month means of 25, 90, 95, 81, and 61 min/wk, respectively; $P < 0.001$). One male control indicated exercising at moderate-to-vigorous levels more than a mean 360 min/wk (i.e., he was a "drop in" to the intervention).

At baseline (prerandomization), exercisers recorded a mean of 5,963 steps per day from their pedometers whereas controls recorded a mean of 6,398 steps per day ($P > 0.05$). Exercisers increased their steps per day by 3,300 to 4,000 at the follow-up points, whereas controls' steps per day remained constant or decreased ($P < 0.001$, comparing change in exercisers versus controls). Because exercisers used a variety of machines, the number of steps per day does not reflect all of their exercise activities. V_{O_2}max increased a mean 2.5 mL/kg/min (10.5%) in female exercisers and 3.3 mL/kg/min (11%) in male exercisers, and decreased in controls ($P < 0.001$, comparing exercisers to controls). Neither exercisers nor controls significantly changed mean total daily caloric intake, intake of fat, fiber, or alcohol, and there were no statistically significant differences for changes in these variables between exercisers and controls in either women or men (data not shown).

Intervention Effects on Colon Crypt Cell Proliferation. Table 2 shows evaluations of intervention effects on the colon crypt cell proliferation measures. Female exercisers experienced an increase in crypt height by 21.3 μm versus a decrease of 31.1 μm in controls ($P = 0.06$; Table 2A). No other proliferation variables changed to a statistically significant degree in female exercisers versus female controls. Similar results were observed among men, among whom exercisers experienced a 43.4-μm increase in crypt height compared with only a 1.3-μm increase in crypt height in controls ($P = 0.07$). Similar to the results for women, no other proliferation variables changed in male exercisers versus male controls overall.

Subgroup Analyses. The effect of exercise on outcome variables did not differ consistently by age (<55 versus >55 years), by baseline BMI, or, among women, by menopausal status. The effect of exercise on outcome variables also did not vary by history of adenomatous colorectal polyps or by first-degree family history of colon cancer.

When we divided the participants into those who had Ki67+ stained cells in the top 40th percentile of the crypts at baseline (approximately one third of the participants) versus those who did not, we did not observe a consistent effect of exercise on any of the proliferation variables. Similarly, when we divided participants by median baseline Ki67+ stained cell height, we saw no consistent effect of exercise on any of the proliferation variables.

Exercise Effect by Intervention Adherence and Change in Cardiopulmonary Fitness. We examined the change in outcome variables among exercisers by mean number of minutes of moderate-to-vigorous exercise per week over the 12-month intervention (Table 3). In female exercisers, there

Table 3. Baseline and follow-up proliferation outcome measures stratified by exercise adherence

	Women				Men			
	Baseline, mean (SD)	Follow-up, mean (SD)	Δ_E	P_{trend}	Baseline, mean (SD)	Follow-up, mean (SD)	Δ_E	P_{trend}
Mean Ki67+ cell height (% of crypt height)								
Controls	17.5 (5.76)	17.2 (6.74)	−0.3	0.69	18.4 (5.74)	18.4 (4.74)	0	0.03
<250 min/wk*	19.6 (4.65)	19.6 (4.68)	0.0		18.1 (3.45)	18.4 (7.67)	+0.3	
250-300 min/wk†	19.3 (4.24)	20.1 (4.44)	+0.8		19.4 (4.28)	17.7 (4.92)	−1.7	
300+ min/wk‡	16.8 (6.15)	17.2 (3.93)	+0.4		18.6 (4.69)	16.2 (4.48)	−2.4	
Median Ki67+ cell height (% of crypt height)								
Controls	16.1 (5.21)	16.0 (6.21)	+0.1	0.43	17.2 (5.89)	16.7 (4.77)	−0.5	0.02
<250 min/wk	17.5 (6.11)	17.9 (4.34)	+0.4		17.1 (4.04)	17.2 (7.51)	+0.1	
250-300 min/wk	17.0 (4.69)	18.9 (5.22)	+1.9		18.3 (4.65)	16.5 (5.08)	−1.8	
300+ min/wk	15.4 (6.03)	15.6 (4.62)	+0.2		17.4 (5.06)	14.9 (3.94)	−2.5	
75th percentile for Ki67+ cell height (% of crypt height)								
Controls	25.9 (8.79)	25.3 (11.0)	−0.6	0.80	26.6 (8.35)	26.3 (6.95)	−0.3	0.05
<250 min/wk	29.7 (7.02)	29.3 (7.73)	−0.4		25.9 (5.50)	26.5 (0.12)	+0.6	
250-300 min/wk	28.8 (7.40)	28.5 (4.33)	−0.3		27.9 (6.43)	25.5 (7.11)	−2.4	
300+ min/wk	24.1 (9.20)	25.4 (4.22)	+1.3		26.5 (6.48)	23.3 (6.24)	−3.2	
Weighted mean Ki67+ cell height								
Controls	2.24 (0.59)	2.21 (0.68)	−0.03	0.34	2.32 (0.54)	2.33 (0.45)	+0.01	0.04
<250 min/wk	2.42 (0.46)	2.47 (0.45)	+0.05		2.29 (0.37)	2.35 (0.71)	+0.06	
250-300 min/wk	2.39 (0.43)	2.52 (0.46)	+0.13		2.43 (0.46)	2.26 (0.49)	−0.17	
300+ min/wk	2.11 (0.74)	2.24 (0.34)	+0.13		2.35 (0.47)	2.12 (0.41)	−0.23	
No. Ki67+ cells per crypt								
Controls	8.66 (4.32)	7.86 (4.99)	−0.8	0.66	9.13 (4.07)	9.86 (4.68)	+0.73	0.89
<250 min/wk	8.37 (3.70)	11.0 (5.37)	+2.62		9.40 (3.68)	8.92 (3.74)	−0.48	
250-300 min/wk	11.1 (8.69)	9.56 (5.02)	−1.54		10.1 (4.91)	10.1 (4.55)	0.0	
300+ min/wk	8.75 (4.93)	10.4 (5.31)	+1.65		8.48 (1.71)	8.49 (4.07)	+0.01	
Crypt height (μm)								
Controls	563.8 (95.7)	532.7 (108.5)	−31.1	0.49	574.1 (79.9)	575.4 (87.5)	+1.1	0.002
<250 min/wk	493.5 (119.0)	515.7 (91.9)	+22.2		521.0 (93.4)	560.9 (81.3)	+39.9	
250-300 min/wk	552.5 (83.5)	589.1 (75.3)	+36.6		553.8 (91.3)	603.8 (100.1)	+50.0	
300+ min/wk	599.6 (67.9)	574.9 (83.5)	−24.7		528.8 (79.0)	564.8 (73.7)	+36.0	

*N women = 19 and 14 at baseline and follow-up; N men = 12 and 10 at baseline and follow-up.
†N women = 17 at baseline and follow-up; N men = 20 at baseline and follow-up.
‡N women = 11 at baseline and follow-up; N men = 19 and 18 at baseline and follow-up.

Table 4. Baseline and follow-up proliferation outcome measures stratified by change in V_{O_2}max

	Women				Men			
	Baseline, mean (SD)	Follow-up, mean (SD)	Δ_E	P_{trend}	Baseline, mean (SD)	Follow-up, mean (SD)	Δ_E	P_{trend}
Mean Ki67+ cell height (% of crypt height)								
Controls*	17.5 (5.76)	17.2 (6.74)	−0.3	0.95	18.4 (5.74)	18.4 (4.74)	0	0.05
Decrease or <5% increase†	18.7 (3.91)	19.3 (2.85)	+0.6		19.3 (3.91)	18.4 (5.13)	−0.9	
>5% increase‡	19.4 (5.44)	19.2 (5.13)	−0.2		18.8 (4.43)	16.8 (5.51)	−2.0	
Median Ki67+ cell height (% of crypt height)								
Controls[1]	16.1 (5.21)	16.0 (6.21)	−0.1	0.68	17.2 (5.89)	16.7 (4.77)	−0.5	0.04
Decrease or <5% increase[2]	17.7 (4.49)	17.6 (2.47)	−0.1		18.0 (4.40)	17.6 (5.10)	−0.4	
>5% increase[3]	17.2 (5.61)	17.8 (5.75)	+0.6		17.8 (4.85)	15.4 (5.27)	−2.4	
75th percentile for Ki67+ cell height (% of crypt height)								
Controls*	25.9 (8.79)	25.3 (11.0)	−0.6	0.99	26.6 (8.35)	26.3 (6.95)	−0.3	0.07
Decrease or <5% increase†	26.4 (6.16)	27.8 (5.37)	+1.4		27.3 (5.67)	26.8 (8.10)	−0.5	
>5% increase‡	28.3 (8.71)	28.0 (6.03)	−0.3		27.0 (6.59)	24.1 (7.23)	−2.9	
Weighted mean Ki67+ cell height								
Controls*	2.24 (0.59)	2.21 (0.68)	−0.03	0.61	2.32 (0.54)	2.63 (0.45)	+0.31	0.07
Decrease or <5% increase†	2.32 (0.39)	2.78 (5.37)	+0.46		2.43 (0.40)	2.33 (0.50)	−0.10	
>5% increase‡	2.39 (0.61)	2.43 (0.51)	+0.04		2.36 (0.46)	2.18 (0.52)	−0.18	
No. Ki67+ cells per crypt								
Controls*	8.66 (4.32)	7.86 (4.99)	+0.8	0.99	9.13 (4.07)	9.86 (4.68)	+0.73	0.50
Decrease or <5% increase†	9.39 (3.90)	11.5 (4.93)	+2.1		8.87 (2.69)	10.1 (3.14)	+1.2	
>5% increase‡	10.2 (7.35)	9.61 (5.18)	−0.6		9.74 (4.15)	8.85 (4.54)	−0.89	
Crypt height (μm)								
Controls*	563.8 (95.7)	532.7 (108.5)	−31.1	0.70	574.1 (79.9)	575.4 (87.5)	+1.1	0.02
Decrease or <5% increase†	544.0 (102.3)	559.1 (57.1)	+15.1		514.3 (74.0)	562.9 (88.5)	+48.6	
>5% increase‡	557.5 (98.1)	561.9 (89.4)	+4.4		553.0 (88.4)	587.4 (87.7)	+34.4	

*N women = 47; N men = 45.
†N women = 14; N men = 14.
‡N women = 28; N men = 28.

was no association between increased exercise duration and changes in proliferation markers. Among male exercisers, in contrast, increasing exercise was associated with decreases in several markers of proliferation. Male exercisers who exercised for a mean 250 to 300 and >300 min/wk experienced mean decreases of 1.7% and 2.4% of mean Ki67+ percent of crypt height, respectively (P_{trend} = 0.03, across controls and all exerciser levels). Similar results were observed for median Ki67+ percent of crypt height (P_{trend} = 0.02), the 75th percentile for Ki67+ crypt height (P_{trend} = 0.05), and the weighted mean Ki67+ relative cell height (P_{trend} = 0.04). In all of these indices, men who exercised for at least 250 minutes experienced decreases in height of the Ki67-positive nuclei in the crypt, with the largest decrease observed in those who exercised ≥300 min/wk.

We then examined the change in proliferation among exercisers by change in V_{O_2}max (Table 4). Among women, change in V_{O_2}max did not alter the lack of exercise effect on any of the proliferation variables. Among men, in contrast, those exercisers whose V_{O_2}max increased by ≥5% (approximately two-thirds of the exercisers) experienced decreases in several proliferation markers. Among this group, the mean Ki67+ percent of crypt height decreased by 2% versus a decrease of 0.9% in those whose V_{O_2}max increased less, and versus no change in controls. The test for trend across the three groups was statistically significant (P = 0.05). A similar result was observed for median Ki67+ percent of crypt height (change of −0.5%, −0.4%, and −2.4% in the three groups, respectively; P_{trend} = 0.04). Similar results were observed for the 75th percentile of Ki67+ cell height, although the test for trend was of only marginal statistical significance (P = 0.07). The decrease in weighted mean Ki67+ cell height was also greatest in male exercisers in the highest category of V_{O_2}max increase (−0.18%), but the test for trend was of marginal statistical significance (P = 0.07).

When we classified exercisers into tertiles of mean weekly energy expenditure, the results largely paralleled the results classified by tertiles of mean minutes of moderate-to-vigorous intensity exercise per week (data not shown).

Discussion

This randomized controlled clinical trial showed that a 12-month moderate-to-vigorous intensity exercise intervention with exercise goals of 60 minutes per day, 6 days per week, such as that recommended by the U.S. Department of Agriculture and Institute of Medicine for weight control (26, 27), results in increased colon crypt height but few other changes overall. However, it also showed that, among men who exercised for at least 250 min/wk on average, several measures of colon crypt proliferation were significantly decreased, and there was a significant trend toward decreasing proliferation with increasing minutes of exercise per week. Proliferation in the upper section of the colon crypt decreased among those exercising for a mean 250 min/wk or greater, which is important because this pattern of proliferation is most associated with risk for colon cancer (13). This amount of exercise—250 min/wk of moderate-to-vigorous intensity physical activity—is consistent with Center for Disease Control guidelines of 30 minutes per day of physical activity on most days for overall health benefits, and is therefore likely to be achievable by motivated adults (24). The greater benefit observed in those exercising for ≥300 min/wk is consistent with epidemiologic evidence that suggests that higher benefit is observed with greater amount of exercise (5). The fact that we did not observe an effect modification with baseline BMI suggests that men of any level of adiposity are likely to derive benefit against colon cancer from exercise, again consistent with existing observational data (5).

It is not clear why a beneficial effect of exercise on colon crypt cell proliferation was not observed in women. However, in epidemiologic studies, women experience less reduction in colon cancer risk with increased exercise (2). One possible explanation may be that with increased exercise, in both premenopausal and postmenopausal women, estrogen levels decrease (28, 29), thereby reducing colon cancer protection. There is a considerable body of literature from early observations (30, 31) to the recent findings of the Women's

Health Initiative Hormone Replacement Therapy Trial (32) that consistently show that higher estrogen levels reduce risk of colon cancer.

Another possible explanation is that the male exercisers may have consistently exercised at more vigorous levels compared with women. The mean percent of maximum heart rate attained over the 12 months was 86.7% in male exercisers and 86.0% in female exercisers, even after adjusting for age, baseline fitness, and use of β-blockers, indicating similar maximal effort levels in male and female exercisers. However, from the exercisers' log data, men spent more mean time jogging or running (19.5 min/wk) compared with women (4.6 min/wk; $P = 0.02$). In contrast, mean minutes of walking per week was similar in male versus female exercisers ($P = 0.70$). Therefore, the male exercisers may have put more sustained intensive effort into their exercise sessions, which could explain the greater exercise effect on outcome in men versus women. Another explanation could be that men changed dietary habits to a greater degree than did women, or that men exercising at higher levels changed their diets differently than did less adherent men. However, we found that exercisers versus controls did not change daily intake of calories, fat, fiber, or alcohol, and that among male exercisers adherence was not related to change in these macronutrients.

In both sexes, we observed a lengthening of crypt height with exercise compared with controls, although the results were of marginal statistical significance. The association between crypt length and colon cancer risk is unknown, although in animal models high-fiber diets significantly lengthen crypt height (16, 17).

No previous study has reported on exercise intervention effects on colon crypt cell proliferation in a human experiment. In animal experiments, diverse results on colon carcinogenesis have been observed, ranging from a reduction in tumors with voluntary exercise (33, 34) to an increase in tumor markers with exhaustive forced exercise (35). Colbert et al. assessed colon polyp development following treadmill exercise in the Min mouse, an induced mutant mouse in which an APC tumor suppressor gene mutation results in multiple intestinal polyps. Polyp development was not significantly affected, neither in *ad libitum* fed (36) nor in energy intake–controlled mice with negative energy balance, due solely to exercise (37).

A limitation of our study was that participants did not record the duration they exercised at peak heart rate. Thus, we were unable to accurately determine total caloric expenditure during the exercise sessions. However, when we estimated the effect of caloric expenditure per week on proliferation markers, trends and effect sizes similar to duration of exercise were observed. Another limitation was that exercise done at home was self-reported in the daily activity log compared with exercise done at the facility, which was also self-reported in the log but validated with direct observation by the exercise physiologist. Nonetheless, a significant increase in the exercisers' overall activity versus no substantial change in controls is supported by participants' reports on the physical activity interview, data from their pedometer logs, and the increase in V_{O_2}max in exercisers versus no change in controls. The effect of exercise on colon crypt cell proliferation pattern may reflect only part of the mechanism linking exercise to cancer risk; in particular, the effect of exercise on apoptosis may be as important as that on proliferation. Future reports from this study will include such markers.

A major strength of our study was the randomized controlled clinical trial design, in which effects of confounding variables are minimized and in which substantial increases in exercise can be achieved and documented. This study was the first randomized clinical trial testing the effect of exercise on colon cancer biomarkers in colon mucosal tissue. Another strength was the excellent adherence to the exercise program and the low drop-out rate. Finally, we designed the trial to be able to test the intervention effects separately in men and women.

In conclusion, this year-long randomized controlled trial testing a moderate-to-vigorous intensity exercise intervention produced statistically significant baseline-to-12-month changes in colon crypt proliferation patterns in men who exercised at least 250 min/wk. This suggests that the epidemiologic observations of reduction in colon cancer risk with regular aerobic exercise are likely to be real and that exercise may reduce colon cancer risk through an alteration in proliferative indices.

Acknowledgments

We thank the study participants for their time and dedication to the project.

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