Effect of a 12-Month Exercise Intervention on the Apoptotic Regulating Proteins Bax and Bcl-2 in Colon Crypts: A Randomized Controlled Trial

Kristin L. Campbell,[1] Anne McTiernan,[1,2,3] Shuying S. Li,[1] Bess E. Sorensen,[1] Yutaka Yasui,[7] Johanna W. Lampe,[1,2] Irena B. King,[1] Cornelia M. Ulrich,[1] Rebecca E. Rudolph,[1,3,5] Melinda L. Irwin,[8] Christina Surawicz,[3] Kamran Ayub,[6] John D. Potter,[1,2] and Paul D. Lampe[1,4]

[1]Cancer Prevention Program, Division of Public Health Sciences, The Fred Hutchinson Cancer Research Center; Departments of [2]Epidemiology, [3]Medicine, and [4]Pathobiology, University of Washington; [5]Health Services Research and Development Program, Veterans Affairs Puget Sound Health Care System; [6]Virginia Mason Medical Center, Seattle, Washington; [7]Department of Public Health Sciences, University of Alberta, Edmonton, Alberta, Canada; and [8]Department of Public Health, Yale University School of Medicine, New Haven, Connecticut

Abstract

Background: **Cellular proliferation and apoptosis (cell death) are highly regulated in the colon as insufficient apoptosis may lead to polyps and cancer. Physical activity decreases risk of colon cancer in observational studies, but the biological basis is not well defined. The objective of this study is to examine the effects of a 12-month aerobic exercise program on expression of proteins that promote (Bax) or inhibit (Bcl-2) apoptosis in colon crypts.**

Methods: **Two hundred two sedentary participants, 40 to 75 years, were randomly assigned to moderate-to-vigorous intensity exercise for 60 min per day, 6 days per week for 12 months, or usual lifestyle. Colon crypt samples were obtained at baseline and 12 months. Bcl-2 and Bax expression was measured by immunohistochemistry.**

Results: **Bax density at the bottom of crypts increased in male exercisers versus controls (+0.87 versus −0.18; $P = 0.05$), whereas the ratio of Bcl-2 to Bax at the bottom and middle of crypts decreased as aerobic fitness (VO_2max) increased (P trend = 0.02 and 0.05, respectively). In female exercisers, Bax density in the middle of crypts decreased (−0.36 versus +0.69; $P = 0.03$) and Bcl-2 to Bax ratio at the top of crypts increased versus controls (+0.46 versus −0.85; $P = 0.03$). Bax density in the middle of crypts also decreased as minutes per week of exercise increased (P trend = 0.03).**

Conclusions: **A 12-month exercise intervention resulted in greater expression of proteins that promote apoptosis at the bottom of colon crypts in men and decreased expression of proteins that promote apoptosis at the middle and top of colon crypts in women. The difference in effect by gender and location of observed changes warrants further study.** (Cancer Epidemiol Biomarkers Prev 2007;16(9):1767–74)

Introduction

There is convincing observational evidence of an inverse association between physical activity and colon cancer risk (1-3). This relationship has been observed in all age groups, in various racial and ethnic groups, and in diverse geographic areas around the world. A dose-response relationship has been noted, with a risk reduction of 70% at the highest levels of physical activity (1). Adjustment for potential confounding factors, such as age, diet, and obesity, does not diminish the observed protective association (1, 3). Current guidelines from the American Cancer Society suggest engaging in at least 30 min of moderate-to-vigorous physical activity on 5 or more days of the week to lower cancer risk, but 45 to 60 min of physical activity at this frequency are probably needed to reduce colon cancer risk (4).

Alterations in control of cellular proliferation and survival may be an important early step in the development of colonic neoplasms (5). New cells are produced rapidly and continuously from stem cells at the base of colonic crypts. Older cells undergo apoptosis (programmed cell death) and are sloughed into the colonic lumen. To maintain crypt organization and structure, cellular proliferation and apoptosis must be tightly controlled. Failure of these controls may lead to the formation of colonic neoplasms (5).

Previous research suggests that Bcl-2 and Bax proteins are key regulators of apoptosis in colonic crypts (6). Bcl-2 has been shown to prolong cell survival and to inhibit apoptosis (7, 8). In the normal colon, Bcl-2 is expressed primarily at the base of crypts and to a much lesser extent by cells closer to the lumen (9, 10). In contrast, in the settings of colonic dysplasia and cancer, Bcl-2 can be expressed at relatively high levels in cells close to the lumen (9, 11, 12). Bax has the opposite effect of Bcl-2. It promotes apoptosis through negative regulation of Bcl-2

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Requests for reprints: Anne McTiernan, Fred Hutchinson Cancer Research Center, 1100 Fairview Avenue North, M4-B402, Seattle, WA 98109-1024. Phone: 206-667-7979; Fax: 206-667-7850. E-mail: amctiern@fhcrc.org

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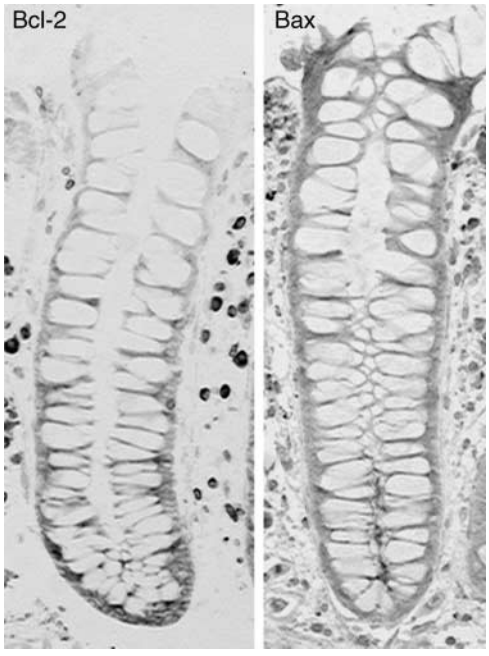

Figure 1. Examples of colon crypt images taken after staining for Bcl-2 and Bax. Black and white images of immunostain without any counterstain are shown. Note that Bcl-2 expression was higher in the lower regions of the crypt, whereas Bax is more evenly distributed.

as well as other biological pathways (13). Bax is normally more highly expressed at the top of crypts (Fig. 1; ref. 13).

In this study, we tested the hypothesis that exercise reduces risk of colon cancer by promoting optimal expression of Bcl-2 and Bax in colonic crypts. To that end, we recruited healthy men and women to participate in a randomized controlled trial in which the intervention was 12 months of moderate-to-vigorous intensity exercise, 60 min per day, 6 days per week. The effects of this intervention on expression of Bcl-2 and Bax were assessed through immunohistochemical analysis of samples from normal-appearing colonic epithelium.

Materials and Methods

As part of a 12-month randomized controlled clinical trial evaluating the effects of an aerobic exercise intervention, we examined changes in Bcl-2 and Bax, markers of colon crypt apoptosis and potential markers of colon cancer risk, in association with the exercise intervention. A detailed description of the clinical trial methods has been reported previously (14, 15).

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. Eligibility criteria were as follows: sedentary [i.e., engaging in <90 min of moderate-to-vigorous exercise per week in the past 3 months, or if reports of exercise were questionable, a low VO_2max indicating a low fitness level (16)], normal response to an exercise tolerance test (17), consumption of less than two alcoholic beverages per day, and no personal history of invasive cancer or other serious medical conditions, such as cardiovascular disease, stroke, diabetes, uncontrolled hypertension, ulcerative colitis, or short bowel disease. To avoid potential confounding by 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 ''excessive'' use of laxatives (three or more per week), enema use, or use of any medication that might interfere with colonic crypt studies, including chronic use of anticoagulants or corticosteroids. Use of nonsteroidal anti-inflammatory medications was allowed up to two times per week if the person was able to stop use safely for 2 weeks before and after obtaining colorectal biopsies.

Participants were recruited between 2001 and 2004 primarily through gastroenterology practices, where potentially eligible persons were identified from medical records and sent an invitation letter by their physicians. Additional recruitment methods included medium placements, flyers, a study Web site, and referrals. After extensive screening, 202 (102 men and 100 women) were enrolled in the trial with consent. Flow of participants through the trial has been reported previously (14, 15). Study procedures, including the informed consent, were approved by the Fred Hutchinson Cancer Research Center Institutional Review Board.

Randomization and Blinding. Participants were randomized in equal numbers to exercise or control group (referred hereafter as ''exercisers'' and ''controls,'' respectively). Randomization was blocked on sex, use of nonsteroidal anti-inflammatory medications [regular use (more than two times per week) versus less], and current smoking status (yes versus no) and, among women, on menopausal status (premenopausal or perimenopausal versus postmenopausal) and current use (yes/no) of hormone replacement therapy to ensure balance in these factors between intervention arms. Staff and scientists involved in end point determinations were blinded to intervention group and to whether samples were obtained at baseline or 12 months.

Baseline and Follow-up Measures

Measurement of Bcl-2 and Bax in Colonic Crypts. Immunohistochemical staining of the colonic crypts has been detailed previously (14). In brief, samples were collected from each participant at a prerandomization and a 12-month poststudy flexible sigmoidoscopy by physicians trained in the procedure using jumbo biopsy forceps (Olympus FB-50U1-1; Olympus America, Inc.). Biopsies from the sigmoid colon (30-35 cm from the level of the external anal aperture) were oriented, processed, and embedded into paraffin blocks. Eight to 12 sections, at least 50 μm apart (to ensure that the same was not evaluated in more than one section), were cut per biopsy, mounted with positive control (human tonsil) and negative control tissues (colonic tissue from the same participant with all reagents except primary antibody), deparaffinized, and antigen retrieved. 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 stained on a Techmate 1000 Staining System automatic immunostainer (Ventana Medical Systems,

Inc.). After blocking with 4% normal horse serum, slides were incubated with a mouse anti-Bcl-2 (Dako Corp.) or anti-Bax (Beckman Coulter) at a 1:500 dilution for 1 h 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 IgG (Vector Laboratories, Inc.) at a dilution of 1:200 for 30 min in PBS with 1% bovine serum albumin with 0.05% Tween 20 and then buffer washed. Endogenous peroxidases were inactivated and slides were washed and immersed in avidin-biotin complex from the Vectastain Elite Standard ABC kit (Vector Laboratories) for 30 min. After washing, slides were developed using 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.) to identify acceptable colonic crypts (mid-axial, U-shaped sections extending from muscularis to lumen with an intact structure). Images of the crypts were captured by using a digital camera (Hamamatsu Photonics KK) set at constant illumination and exposure settings. Macros developed for the NIH Image software program[9] allowed the collection and analysis of images.

To measure expression (i.e., density) of Bcl-2 and Bax, crypt images were measured and divided longitudinally into 10 segments of equal length and then divided into three regions based on deciles: bottom (interval, 0.1-0.3), middle (interval, 0.4-0.6), and top (interval, 0.7-1.0). Then, the number and average intensity of pixels above background were determined. Density of Bcl-2 and Bax within each crypt was calculated as the size of the stained area multiplied by the intensity of the stain. The ratio of Bcl-2 to Bax at each time point was calculated as the mean density of Bcl-2 divided by the mean density of Bax in the specified interval. At least 5 and up to 10 crypts per biopsy were analyzed.

Exercise Data. To determine cardiopulmonary fitness, we assessed maximal oxygen consumption (VO_2max, mL/kg/min) at baseline and 12 months (18). Details of the exercise test are presented elsewhere (14, 15). In brief, participants completed a maximal-graded treadmill test, with heart rate and oxygen uptake monitored by a MedGraphics automated metabolic cart (MedGraphics).

Demographics and Anthropometrics. 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. 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 as kg/m^2.

Intervention

Exercisers. The intervention was a 12-month moderate-to-vigorous intensity aerobic exercise program. The goal was 60 min per day, 6 days per week, to be achieved gradually over 12 weeks and then continued throughout the 12-month intervention. The intervention included both facility- and home-based programs. Participants did aerobic exercise for 60 min per session on machines (primarily treadmills and stationary bikes, supplemented with rowing machines and elliptic machines). During these exercise sessions, each participant's goal heart rate corresponded to 60% to 85% of the maximal heart rate achieved on the baseline VO_2max test. Heart rate was measured with Polar heart rate monitors (Polar Electro, Inc.). Approximately 5 to 10 min of warm-up, cooldown, and stretching exercise were done in addition to the 60-min exercise sessions.

Participants also were asked to do three additional unsupervised exercise sessions per week either at a facility or at home. The primary home exercises were outdoor walking, jogging, or biking. Participants wore heart rate monitors during these unsupervised exercise sessions, with the same instructions about goal ranges and recording, as for their supervised sessions.

Adherence was calculated weekly as number of facility sessions attended, minutes of moderate-to-vigorous exercise per week, and percent of the goal of 360 min of moderate-to-vigorous exercise per week. Good adherence was defined as meeting at least 80% of the goal of 360 min of moderate-to-vigorous exercise per week. Exercisers were asked not to change their dietary habits during the trial.

Controls. Controls were asked not to change their exercise or diet habits during 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.

Statistical Analyses. The crypt height and mean density of Bcl-2, Bax, and Bcl-2 to Bax ratio in colonic crypts was determined for each participant at baseline and 12 months. Analyses of the effects of the exercise intervention on the outcome variables (crypt height, mean density Bcl-2, mean density Bax, and Bcl-2 to Bax ratio) were based on assigned treatment at the time of randomization regardless of adherence status (i.e., intent to treat). All outcomes were log transformed due to the lack of normality; therefore, our results are presented on the log scale. The intervention effects were evaluated by assessing the differences in the log mean changes in the outcome variables from baseline to 12 months between exercisers and controls. The significance of any differences was determined by using the generalized estimating equation modification to linear regression models to account for the longitudinal nature of the data. Primary analyses were unadjusted in line with the randomized design of the study. However, an additional analysis was completed adjusting for blocking factors (history of adenomatous polyps, etc.) and these factors did not influence the results.

Baseline and 12-month apoptosis data were available for 101 of 102 randomized men and for 98 of 100 randomized women. Inadequate amount of sample was available for the three missing individuals. Participants with at least one outcome value were included in the analyses. Before the intervention effect analysis,

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

Table 1. Baseline characteristics for participants by gender and intervention

	Women			Men		
	Exercisers (n = 48), mean (SD)	Controls (n = 50), mean (SD)	P*	Exercisers (n = 50), mean (SD)	Controls (n = 50), mean (SD)	P †
Age (y)	53.7 (5.65)	54.4 (7.13)	0.61	56.6 (7.63)	56.2 (6.66)	0.78
BMI (kg/m^2)	28.6 (4.80)	28.7 (5.41)	0.84	30.1 (4.84)	29.7 (3.73)	0.68
Energy intake (kcal)	1,583 (555)	1,491 (628)	0.44	1,668 (566)	1,692 (639)	0.85
Fiber intake (g)	15.0 (8.85)	15.9 (6.23)	0.58	14.4 (5.92)	15.2 (7.14)	0.55
	n (%)	n (%)	P	n (%)	n (%)	P
NSAID use	15 (31.3)	19 (27.3)	0.53	22 (43.1)	22 (43.1)	>0.99
Recent adenomatous polyps	15 (31.3)	12 (23.5)	0.39	30 (58.8)	31 (60.8)	0.84
First-degree relative with colon cancer	21 (43.8)	19 (37.3)	0.51	15 (29.4)	18 (35.3)	0.53

Abbreviations: BMI, body mass index; NSAID, nonsteroidal anti-inflammatory drug.
*P value comparing female exercisers and controls at baseline.
† P value comparing male exercisers and controls at baseline.

exploratory data analysis examined the association between crypt height and Bcl-2 or Bax density at baseline using Pearson correlations.

As exploratory analysis, we examined whether the extent of change in mean crypt height, Bax density, mean Bcl-2 density, or Bcl-2 to Bax ratio was different among exercisers with stronger adherence to the intervention than among exercisers with lesser adherence or controls to provide additional important mechanistic information on the effect of physical activity on colon crypt biology. For these analyses, exercisers were grouped into tertiles of each of two measures of adherence to the exercise program: mean change in VO_2max from baseline to 12 months and mean number of minutes exercised per week. To test for trends across levels of adherence, four-category adherence variables were created that included controls and the three tertiles of adherence. To do tests for trend, the four-category adherence variable was included in models as a continuous variable.

All analyses were conducted separately for men and women by study design. Differences between women and men were tested by a gender-intervention interaction term into the models. All statistical tests were two sided. Statistical analyses were done using Statistical Analysis System software (version 8.2; SAS Institute, Inc.).

Results

Study Participants. Exercisers and controls were similar with regards to age, body mass index, energy intake, fiber intake, use of nonsteroidal anti-inflammatory medications, history of adenomatous polyps, and family history of colorectal cancer (Table 1). The majority of participants were overweight and non-Hispanic Whites. Overall, both men and women had low levels of aerobic fitness at baseline, consistent with the eligibility criteria targeting a sedentary population (16).

During the trial, 80% of exercisers achieved ≥80% of their 360 min of moderate-to-vigorous intensity exercise a week. Male exercisers completed a mean 370 min per week (102.7% of goal), whereas female exercisers completed a mean 295 min per week (82% of goal). Two of 51 (4%) male exercisers and 4 of 49 (8%) female exercisers dropped out of the intervention. Duration of self-reported moderate-to-vigorous exercise decreased or was unchanged among controls (mean minutes per week of 113 at baseline and 54 min at 12 months), with the exception of one male control who reported a mean of 360 min per week of moderate-to-vigorous exercise at 12 months. Mean aerobic fitness (VO_2max) increased by 2.5 mL/kg/min (10.5%) in female exercisers and 3.3 mg/kg/min (11%) in male exercisers but decreased in both female and male controls ($P < 0.001$ comparing exercisers with controls). Exercisers lost more weight compared with controls in both women (−1.4 kg versus +0.9 kg) and men (−1.8 kg versus −0.1 kg; ref. 15). No significant change in mean total daily caloric intake was observed among exercisers or controls (data not shown).

Pattern of Bcl-2 and Bax Density within Colonic Crypts at Baseline. The pattern of Bax density, Bcl-2 density, and Bcl-2 to Bax ratio within colonic crypts at baseline is shown in Fig. 2. We did not observe any difference between exercisers and controls in Bcl-2 density, Bax density, or Bcl-2 to Bax ratio at baseline (data not shown). Among both men and women, Bax density had a U-shaped distribution with higher density at the bottom and top intervals of the crypts and lower density in the middle of the crypts (Fig. 2A), whereas Bcl-2 density (Fig. 2B) and Bcl-2 to Bax ratio (Fig. 2C) declined steadily from the bottom intervals to the top intervals of the crypts.

Baseline Association between Crypt Height and Bcl-2 Density and Bax Density. At baseline, height of colonic crypts was positively associated with Bcl-2 density and Bcl-2 to Bax density at the bottom of crypts in both men ($r = 0.31$, $P = 0.002$ and $r = 0.30$, $P = 0.003$, respectively) and women ($r = 0.23$, $P = 0.02$ and $r = 0.28$, $P = 0.006$, respectively). Bax density was not associated with crypt height in either men or women (Table 2). In men, Bcl-2 to Bax density ratio over the entire crypt ($r = 0.21$, $P = 0.04$) and at the middle ($r = 0.21$, $P = 0.04$) of crypts was also associated with overall crypt height, with a similar trend in women that did not achieve significance ($r = 0.18$, $P = 0.08$ and $r = 0.79$, $P = 0.08$).

Intervention Effects on Bcl-2 Density, Bax Density, and Bcl-2 to Bax Ratio. During the trial, mean Bax density in the bottom third of crypts increased among male exercisers compared with controls [mean change in

density at 12 months: exercisers (+0.87) versus controls (−0.18); $P = 0.05$; Table 3]. In addition, among male exercisers, there was a trend toward a decrease in mean Bcl-2 to Bax ratio in the bottom third of crypts [mean change in ratio at 12 months: exercisers (−0.16) versus controls (+0.78); $P = 0.06$]. Among females, there was a decrease in mean Bax density across the crypts as a whole [mean change in density at 12-months: exercisers (−0.20) versus controls (+0.36); $P = 0.05$] as well as the middle third of crypts [mean change in density at 12 months: exercisers (−0.36) versus controls (+0.69); $P = 0.03$] in exercisers compared with controls. In addition, higher Bcl-2 to Bax ratio was seen in the top of crypts [mean change in density at 12 months; exercisers (+0.46) versus controls (−0.85); $P = 0.03$] in female exercisers compared with controls (Table 4). Crypt height did not change significantly with exercise in men or women (Tables 3 and 4).

Effect of Adherence on Colon Crypt Cell Apoptosis. To provide additional important mechanistic information on the effect of physical activity on colon crypt biology, we examined the changes in the outcome variables by tertiles of the two measures of adherence: amount of activity (i.e., minutes per week of moderate-to-vigorous activity) and change in fitness (i.e., VO_2max). Compared with controls, no change in Bax, Bcl-2, or Bcl-2 to Bax ratio by adherence (minute per week) was seen in male exercisers, whereas in female exercisers Bax density was lower at the middle of crypts with higher adherence (P trend = 0.03). For male exercisers, Bcl-2 to Bax ratio was lower at the bottom of crypts with improvements in VO_2max of >15% (P trend = 0.02) compared with controls. In the middle of crypts, a lower Bcl-2 to Bax ratio was also seen in male exercisers with greater improvement in VO_2max (P trend = 0.05) compared with controls and when comparing exercisers who increased >15% with those with lesser gains (P trend = 0.02). In male exercisers, an increase in Bax in the middle of the crypts was also seen with improvements in VO_2max of >15% (P trend = 0.03). Among female exercisers, there was no effect of change in VO_2max compared with control. Unadjusted analyses are presented as no difference was noted with adjustment for age, body mass index, fiber, family history of colon cancer, and history of adenomatous polyps.

Table 2. Pearson correlations between crypt height and antigen density

Section	Women (n = 97)		Men (n = 99)	
	ρ	P	ρ	P
Bax density				
Crypt bottom (0.1-0.3)	−0.074	0.47	−0.018	0.86
Crypt middle (0.4-0.6)	−0.137	0.18	−0.102	0.32
Crypt top (0.7-1.0)	−0.146	0.15	−0.026	0.80
Entire crypt (0.1-1.0)	−0.126	0.22	−0.049	0.63
Bcl-2 density				
Crypt bottom (0.1-0.3)	+0.230	0.02	+0.306	0.002
Crypt middle (0.4-0.6)	+0.062	0.55	+0.13	0.19
Crypt top (0.7-1.0)	−0.060	0.56	+0.092	0.36
Entire crypt (0.1-1.0)	+0.052	0.61	+0.182	0.07
Bcl-2 to Bax ratio				
Crypt bottom (0.1-0.3)	+0.275	0.006	+0.303	0.003
Crypt middle (0.4-0.6)	+0.790	0.08	0.208	0.04
Crypt top (0.7-1.0)	+0.071	0.49	+0.09	0.35
Entire crypt (0.1-1.0)	+0.177	0.08	+0.21	0.04

NOTE: n = 194 for Bcl-2 to Bax ratio. n = 97 for Bcl-2 to Bax ratio. n = 97 for Bcl-2 to Bax ratio.

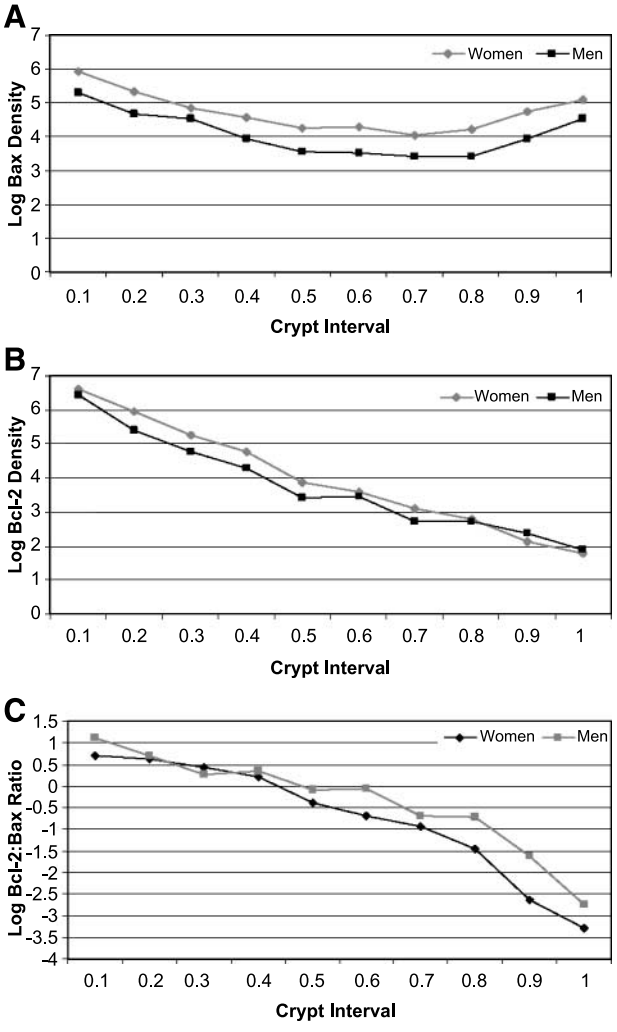

Figure 2. Baseline antigen density pattern across colon crypt length in deciles for men (n = 101) and women (n = 98). **A,** Bax. **B,** Bcl-2. **C,** Bcl-2 to Bax ratio.

Discussion

This randomized controlled clinical trial of a 12-month moderate-to-vigorous intensity exercise intervention with exercise goals of 60 min per day, 6 days per week, resulted in an increased density of the proapoptotic protein Bax at the bottom of crypts in male exercisers compared with controls. In contrast, among women, there was a decrease in the proapoptotic protein Bax in the middle of crypts, with an increase in the ratio of Bcl-2 to Bax density at the top of crypts in exercisers compared with controls, suggesting a decrease in apoptosis for exercisers.

Our exploratory analyses indicate that male exercisers who showed >15% improvement in aerobic fitness

Table 3. Baseline and follow-up means for Bax and Bcl-2 densities for males

	Baseline		Follow-up				
	Exercisers*	Controls †	Exercisers		Controls		*P*
	Mean (SD)	Mean (SD)	Mean (SD)	Δ	Mean (SD)	Δ	
Crypt bottom (intervals, 0.1-0.3)							
Bax density	4.63 (2.32)	5.02 (2.33)	5.52 (2.59)	0.87	4.84 (2.37)	−0.18	0.05
Bcl-2 density	5.60 (1.72)	5.47 (2.07)	6.02 (1.81)	0.42	6.02 (1.33)	0.55	0.51
Bcl-2 to Bax density ratio	0.89 (1.69)	0.51 (2.16)	0.73 (2.68)	−0.16	1.29 (2.17)	0.78	0.06
Crypt middle (intervals, 0.4-0.6)							
Bax density	3.68 (2.23)	3.65 (2.86)	4.66 (2.71)	0.98	3.96 (2.57)	0.31	0.23
Bcl-2 density	3.79 (1.90)	2.67 (2.30)	4.20 (2.22)	0.41	4.25 (1.42)	1.58	0.80
Bcl-2 to Bax density ratio	0.02 (2.01)	0.10 (2.56)	−0.21 (2.70)	−0.23	0.37 (2.31)	0.27	0.40
Crypt top (intervals, 0.7-1.0)							
Bax density	3.83 (2.28)	3.83 (2.88)	4.51 (2.87)	0.68	4.61 (2.05)	0.78	0.76
Bcl-2 density	2.77 (1.65)	2.07 (2.51)	2.60 (2.25)	−0.17	2.54 (1.35)	0.47	0.23
Bcl-2 to Bax density ratio	−1.14 (2.14)	−1.74 (2.68)	−1.66 (2.77)	−0.52	−1.98 (2.35)	−0.24	0.69
Entire crypt (intervals, 1.0-1.0)							
Bax density	4.03 (2.18)	4.13 (2.65)	4.86 (2.70)	0.83	4.49 (2.17)	0.36	0.37
Bcl-2 density	3.92 (1.49)	3.57 (0.16)	4.11 (1.93)	0.19	4.10 (1.04)	0.53	0.40
Bcl-2 to Bax density ratio	−0.18 (1.63)	−0.51 (2.29)	−0.51 (2.52)	−0.33	−0.29 (1.93)	0.22	0.24
Crypt height	423 (61.3)	433 (74.0)	451 (61.1)	28	449 (73.5)	16	0.54

*n = 50 for Bax density and n = 49 for Bcl-2 density and Bcl-2 to Bax density ratio at baseline. n = 48 for Bax density and n = 46 for Bcl-2 density and Bcl-2 to Bax density ratio at follow-up.
† n = 49 for Bax density, n = 50 for Bcl-2 density, and n = 48 for Bcl-2 to Bax density ratio at baseline. n = 46 for Bax density, n = 45 for Bcl-2 density, and n = 44 for Bcl-2 to Bax density ratio at follow-up.

(VO_2max) decreased their Bcl-2 to Bax ratio in the bottom and middle of crypts compared with controls. In the middle of crypts, an increase in Bax density and decreased Bcl-2 to Bax ratio was observed in exercisers who showed >15% improvement in aerobic fitness (VO_2max) compared with those with less improvement or decrease in aerobic fitness. This suggests a shift toward greater apoptosis at the bottom and middle of the colon crypt related to greater physical activity. No change in Bax or Bcl-2 pattern was seen with change in aerobic fitness in female exercisers compared with controls or in exercisers with varied improvements in VO_2max. However, in females who exercised ≥250 min per week, there was a decrease in Bax at the middle of crypts, suggesting a shift toward less apoptosis, compared with the exercisers who exercised <250 min per week and controls. No change in Bax or Bcl-2 pattern was seen in men based on exercise adherence. These exploratory analyses were preplanned and done to provide additional important mechanistic information

Table 4. Baseline and follow-up means for Bax and Bcl-2 densities for females

	Baseline		Follow-up				
	Exercisers*	Controls †	Exercisers		Controls		*P*
	Mean (SD)	Mean (SD)	Mean (SD)	Δ	Mean (SD)	Δ	
Crypt bottom (intervals, 0.1-0.3)							
Bax density	5.41 (2.43)	5.31 (1.98)	5.33 (1.88)	−0.08	5.84 (1.89)	0.53	0.13
Bcl-2 density	5.97 (1.81)	5.92 (1.66)	6.27 (1.37)	0.30	6.18 (1.86)	0.26	0.33
Bcl-2 to Bax density ratio	0.56 (2.29)	0.61 (1.78)	1.03 (1.82)	0.47	0.47 (1.89)	−0.14	0.25
Crypt middle (intervals, 0.4-0.6)							
Bax density	4.50 (2.29)	4.24 (2.11)	4.14 (1.92)	−0.36	4.93 (2.09)	0.69	0.03
Bcl-2 density	4.28 (2.36)	3.87 (2.14)	4.27 (1.81)	−0.01	4.28 (2.53)	0.41	0.41
Bcl-2 to Bax density ratio	−0.22 (2.68)	−0.35 (2.24)	0.26 (2.09)	0.48	−0.53 (2.51)	−0.18	0.36
Crypt top (intervals, 0.7-1.0)							
Bax density	4.54 (2.46)	4.51 (1.83)	4.39 (1.89)	−0.15	5.17 (1.98)	0.66	0.09
Bcl-2 density	2.45 (2.67)	2.44 (2.18)	2.65 (1.71)	0.20	2.17 (2.97)	−0.27	0.49
Bcl-2 to Bax density ratio	−2.09 (2.95)	−2.07 (1.68)	−1.63 (1.98)	0.46	−2.92 (2.65)	−0.85	0.03
Entire crypt (intervals, 1.0-1.0)							
Bax density	4.79 (2.34)	4.67 (1.87)	4.59 (1.82)	−0.20	5.03 (1.88)	0.36	0.05
Bcl-2 density	4.06 (2.19)	3.92 (1.75)	4.22 (1.49)	0.16	4.01 (2.32)	0.09	0.99
Bcl-2 to Bax density ratio	−0.73 (2.52)	−0.75 (1.56)	−0.26 (1.74)	0.51	−1.19 (2.14)	−0.44	0.08
Crypt height	418 (62.3)	426 (86.5)	427 (66.7)	9	410 (82.5)	−16	0.22

*n = 48 for Bax density, Bcl-2 density, and Bcl-2 to Bax density ratio at baseline. n = 40 for Bax density and Bcl-2 to Bax density ratio and n = 43 for Bcl-2 at follow-up.
† n = 50 for Bax density and n = 49 for Bcl-2 density and Bcl-2 to Bax density ratio at baseline. n = 46 for Bax density and Bcl-2 density and n = 44 for Bcl-2 to Bax density ratio at follow-up.

on the effect of physical activity on colon crypt biology. The results should be interpreted conservatively.

A crucial early step in the development of colorectal neoplasia is the accumulation of abnormal cells at the luminal surface (19). Failure of these cells to slough could be a consequence of abnormal apoptosis, potentially reflected by decrease in Bax or increase in Bcl-2 density. A decrease in Bax density at the middle of crypts was seen in women compared with controls and in women with greater exercise adherence. This finding along with the observed increase in Bcl-2 to Bax ratio at the top of crypts in women compared with controls is inconsistent with a hypothesized protective role of physical activity by promoting apoptosis at the top of the crypt. In male exercisers compared with controls and in male exercisers with greater adherence and improvements in fitness, the role of the observed decrease in Bcl-2 and increase in Bax expression at the bottom and middle of crypts is consistent with the hypothesis.

The pattern of Bcl-2 and Bax expression in the colon suggests that Bcl-2 is normally expressed primarily at the base of colon crypts (9, 10), whereas Bax is primarily found at the top of crypts (13, 20). Consistent with these previous observations, at baseline, we noted the highest Bcl-2 density at the bottom of the crypt and a steady decrease in Bcl-2 level in each decile above. However, Bax density was also high at the bottom of the crypt, lower in the middle of the crypt, and higher again at the top of the crypt. The highest Bcl-2 to Bax ratio was at the bottom of the crypt and there was a drop in ratio in each decile above, suggesting greater apoptotic potential at the top of the crypt. Overall, the pattern of Bcl-2 and Bax suggests a balance of differentiation and apoptosis along the crypt, which may be disrupted in the transition toward carcinogenesis (21). The ratio of Bcl-2 to Bax was presented rather than Bax to Bcl-2 as previously reported by other investigators (20) because of the decreasing density of Bcl-2 along the crypt and more stable Bax density. At the top of the crypt using Bcl-2 as the denominator could cause instability of the ratio by creating a large ratio, potentially magnifying the potential effect of outliers.

At baseline, we also observed that colon crypt height was associated with Bcl-2 density and Bcl-2 to Bax ratio at the bottom of the crypt in both men and women. This suggests that greater amounts of Bcl-2 at the bottom of the crypt may dictate the height of the crypt, which has not been reported previously. The implications of this with regards to physical activity are not known. We did not see a significant change in crypt height in exercisers compared with controls. However, a marginally significant lengthening of crypt height in exercisers compared with controls in this sample has been previously reported (14). Whereas the effect of crypt height on colon cancer risk is unknown, in animal models high-fiber diets significantly lengthen crypt height (22, 23).

This is the first trial to examine the effect of a physical activity intervention on Bcl-2 and Bax expression. In animal experiments of colon carcinogenesis, results have ranged from a reduction in tumors with voluntary exercise (24, 25) to an increase in tumor markers with exhaustive forced exercise (26). Colbert et al. (27, 28) 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 in *ad libitum*–fed (27) or in energy intake–controlled mice with negative energy balance due solely to exercise (28). However, male exercising mice had fewer jejunal polyps compared with controls (28).

Epidemiologic evidence suggests that physical activity may play a stronger role in colon cancer risk reduction in men (3) than in women (29); therefore, the effect of exercise on proposed biomarkers of colon cancer risk may be, plausibly, more evident in men. We observed a varied effect of exercise on markers of apoptosis potential in women compared with men, consistent with our recent findings on the colon crypt proliferation marker Ki67. Men who exercised at least 250 min or increased VO_2max >5% had a decrease in mean height of Ki67-positive nuclei, a measure of proliferation, whereas there was no change in women (14). Although further investigation into the possible reasons for these sex differences is needed, suggested explanations include greater intensity of exercise undertaken by men as vigorous exercise may be more potent in reducing colon cancer risk (i.e., 3.5 and 4 h of vigorous activity per week to optimize protection; ref. 30), a differential effect of obesity on cancer risk between men and women (31), and a reduction in the protective effects of estrogen with physical activity in women because with increased exercise estrogen levels decrease in both premenopausal and postmenopausal women (32, 33), thereby potentially reducing colon cancer protection. There is a considerable body of literature from early observations (34, 35) to the recent findings of the Women's Health Initiative Hormone Replacement Therapy Trial (36) that show consistently that estrogen reduces risk of colon cancer; the effects of exercise on apoptosis may differ between the sexes, if estrogen plays a role.

One limitation of our study was that a portion of the exercise intervention was home based. We therefore had to rely on self-reported daily activity logs rather than validation by direct observation during the supervised sessions at fitness facilities. However, observed improvements in VO_2max compared with no change in controls support the self-reported logs. Another limitation to our study was that participants recorded peak heart rate but did not record the duration of exercise at this heart rate. This prevented accurate determination of total caloric expenditure during each exercise session. In addition, the complexity of the data resulted in a large number of comparisons that may have resulted in finding statistically significant associations by chance. However, all comparisons, including the intervention effect assessment by adherence and change in aerobic fitness, were hypothesis-driven and preplanned analyses.

The major strengths of the study include the use of randomized controlled clinical trial design, in which the effects of confounding variables are minimized, participation in exercise can be documented, and significant improvements in aerobic fitness can be achieved. Further strengths of the study were the excellent adherence to the exercise program and low drop-out rate. Finally, the trial was designed to have statistical power to examine the effects separately in men and women.

In conclusion, a year-long randomized controlled trial of moderate-to-vigorous intensity exercise intervention resulted in an increase in the potential for apoptosis at

the bottom of the crypt in male exercisers compared with controls, whereas in contrast there was a decrease in the potential for apoptosis at the top and middle of the crypt in women. In addition, this pattern was also seen with improvements in aerobic fitness in men, whereas for women this pattern occurred only with increased minutes per week of exercise. The difference in effect by gender and in location of observed changes warrants further study. Overall, our findings suggest that Bcl-2 and Bax may be important biomarkers in providing mechanistic data to support the epidemiologic evidence for the role of physical activity in reduction of colon cancer risk.

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