Long-term aerobic swimming training by rats reduces the number of aberrant crypt foci in 1,2-dimethylhydrazine-induced colon cancer

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We determined the effect of long-term aerobic swimming training regimens of different intensities on colonic carcinogenesis in rats. Male Wistar rats (11 weeks old) were given 4 subcutaneous injections (40 mg/kg body weight each) of 1,2-dimethyl-hydrazine (DMH, dissolved in 0.9% NaCl containing 1.5% EDTA, pH 6.5), at 3-day intervals and divided into three exercise groups that swam with 0% body weight (EG1, N = 11), 2% body weight (EG2, N = 11), and 4% body weight of load (EG3, N = 10), 20 min/day, 5 days/week for 35 weeks, and one sedentary control group (CG, N = 10). At sacrifice, the colon was removed and counted for tumors and aberrant crypt foci. Tumor size was measured and intra-abdominal fat was weighed. The mean number of aberrant crypt foci was reduced only for EG2 compared to CG (26.21 ± 2.99 vs 36.40 ± 1.53 crypts; P < 0.05). Tumor incidence was not significantly different among groups (CG: 90%; EG1: 72.7%; EG2: 90%; EG3: 80%). Swimming training did not affect either tumor multiplicity (CG: 2.30 ± 0.58; EG1: 2.09 ± 0.44; EG2: 1.27 ± 0.19; EG3: 1.50 ± 0.48 tumors) or size (CG: 1.78 ± 0.24; EG1: 1.81 ± 0.14; EG2: 1.55 ± 0.21; EG3: 2.17 ± 0.22 cm³). Intra-abdominal fat was not significantly different among groups (CG: 10.54 ± 2.73; EG1: 6.12 ± 1.15; EG2: 7.85 ± 1.24; EG3: 5.11 ± 0.74 g). Aerobic swimming training with 2% body weight of load protected against the DMH-induced preneoplastic colon lesions, but not against tumor development in the rat.

Key words: Physical exercise; Exercise intensity; 1,2-Dimethylhydrazine; Colorectal carcinoma; Aberrant crypt foci

Introduction

The process of carcinogenesis can be divided into three major stages: initiation, promotion and progression. Aberrant crypt foci (ACF) are considered to be putative preneoplastic colon lesions that may be early indicators of colon carcinogenesis (1).

Descriptive studies have suggested that an inverse association exists between regular physical activity and the risk of developing colon cancer (2). However, such studies suffer from some methodological limitations such as lack or absence of standardization to assess the frequency, duration and intensity of the activity. Experimental studies on the effects of exercise training on colon cancer, however, have reported controversial results. For example, animal studies have shown that both long-term voluntary

(3) and low-intensity treadmill (4) running trainings reduced tumor incidence in rats injected with 1,2-dimethylhydrazine (DMH), although, other interventional studies have failed to show the protective effects of short-term moderate-intensity treadmill running training against tumor development in mice with genetic predisposition (APCmin mouse) (5,6). Recently, Fuku et al. (7) found that short-term low-intensity treadmill running training reduced the number of DMH-induced ACF in rats.

The positive association of the intensity of physical activity and protection against carcinogenesis has also been suggested (8). However, depending on its intensity exercise may have either harmful or beneficial effects concerning colon carcinogenesis (9). For example, exercise may cause immune function depression or improvement (10), increase in free radical production (11) or improvement in the antioxidant enzymatic defense system (12). Thus, exercise may protect against or promote colorectal carcinogenesis depending on its intensity. For instance, Demarzo and Garcia (13) showed that a single session of exhaustive swimming increased the number of DMH-induced ACF in rat colons.

To date, no experimental study has assessed the effects of long-term aerobic exercise training of different intensities on colon cancer and which step of the carcinogenic process physical exercise training exerts its preventive effects in rodents injected with carcinogens remains unknown. Our aim was therefore to verify the effects of long-term aerobic swimming training regimens of different intensities on the colonic carcinogenic process in rats treated with DMH.

Material and Methods

Eleven-week-old male Wistar rats acquired from the Central Animal House at the Federal University of Viçosa, MG, were maintained in individual cages in a temperature-controlled room at $22 \pm 2°C$ with lights on from 7:00 am to 7:00 pm. Animals received standard chow and water *ad libitum*. All experimentation was conducted in accordance with internationally accepted ethical principles concerning the care and use of laboratory animals and was approved by the animal care and use committee of the Federal University of Viçosa, MG, Brazil.

The animals were randomly divided into 3 exercise groups of 12 animals each (EG1, EG2, EG3) and a control group (CG, N = 10). During the first 2 weeks of the experiment, all rats were given 4 subcutaneous injections of 40 mg/kg body weight DMH (Sigma, USA), two injections in the 1st week, and two in the 2nd week, on nonconsecutive days. DMH was prepared immediately before use, dissolved in 0.9% NaCl containing 1.5% EDTA as vehicle at a final concentration of 10 mg/mL, and final pH was adjusted to 6.5.

The exercise program was carried out in a tank that was 100 cm high, filled to a depth of 45 cm with water, maintained at 28-30°C. All animals were prevented from floating and made to swim continuously. The rats in the CG were kept in a tank with water (10 cm deep) for the same amount of time as that of the exercised groups. One animal from EG1, 1 from EG2, and 2 from EG3 drowned while exercising.

Twenty-four hours after the first injection of DMH, animals of the 3 exercise groups started exercising. During the first 2 weeks they exercised without additional load, 5 to 15 min/day, 5 days/week. In order to maintain the animals swimming aerobically at different intensities below their anaerobic threshold (4.95% body weight, 7.17 mmol/L) (14), different loads attached to the animal body were used as follows: EG1, 0% body weight (intensity 1), EG2, 2% body weight (intensity 2), and EG3, 4% body weight (intensity 3). From weeks 3 to 5, the animals swam with their respective loads, 5 to 20 min/day, 5 days/week. From week 6 to 35, the animals swam with their respective loads, 20 min/day, 5 days/week.

To avoid any interference of the acute effects of exercise, 72 h after the last exercise session rats were sacrificed and the large intestine was removed, opened longitudinally, washed gently, and fixed flat on a Styrofoam board in 10% buffered formaldehyde for 48 h. Intra-abdominal fat was excised and weighed.

The intestine was then divided into three fragments of equal length: proximal, mid- and distal regions, which were stained with 0.1% methylene blue for approximately 2 min and washed in phosphate buffer. The ACF were identified and counted under a light microscope (100X) by two pathologists unaware of the treatment (15). The ACF were grouped as a function of the size of the foci: small ACF (aberrant crypts per focus ≤ 3, ACF ≤ 3), and large ACF (aberrant crypts per focus ≥ 4, ACF ≥ 4) (15). The tumors were classified visually and counted. The heights, as well as the smallest and largest diameters of the tumors, were measured to estimate the tumor size.

Data for body weight gain, mean number of ACF, ACF ≤ 3, tumor multiplicity and size were compared by one-way ANOVA followed by the Tukey test when main effects or interaction were observed. Data for intra-abdominal fat and ACF ≥ 4 were compared by the Kruskal-Wallis test because data were not normally distributed. Data for tumor incidence were analyzed by the chi-square test. All data were analyzed with Sigma Stat for Windows (SPSS Inc., USA). Differences were considered to be significant at $P \leq 0.05$.

Results

The body weight gain was not significantly different among groups (CG: 162.1 ± 19.23; EG1: 169.0 ± 13.72; EG2: 162.45 ± 8.09; EG3: 141.7 ± 12.56 g; means ± SEM). Similarly, intra-abdominal fat did not differ among groups (CG: 10.54 ± 2.73; EG1: 6.12 ± 1.15; EG2: 7.85 ± 1.24; EG3: 5.11 ± 0.74 g). The total number of ACF of EG2 rats was significantly lower than that of CG rats for the proximal and distal regions (Table 1). A higher number ($p < 0.05$) of ACF was observed in all exercised groups for the distal region compared with the proximal region, and for the mid-region when compared to the proximal region (Table 1). The mean ACF was also significantly lower in EG2 (26.21 ± 2.99 crypts) than that in CG (36.40 ± 1.53 crypts), regardless of the intestinal regions. Independent of the experimental group, the distal (37.73 ± 1.85 crypts) and mid-regions (35.86 ± 2.38 crypts) presented significantly higher numbers of ACF than the proximal region (17.34 ± 1.39 crypts). The number of small ACF (ACF ≤3) in EG2 was also significantly reduced compared to CG (61.00 ± 5.98 vs 85.00 ± 4.73 crypts, respectively). However, no significant differences among groups were observed for the number of large ACF (ACF ≥4; CG: 24.15 ± 2.26 crypts). Furthermore, the best indices for potency, relative risk, ACF reduction percentage and reduction efficiency in the ACF size were also shown by EG2 rats (Table 2). No statistical differences in tumor incidence (animals with tumor number ≥1) among groups were observed (CG: 90.0%). Similarly, swimming training did not significantly affect either tumor multiplicity (tumor per animal, CG: 2.30 ± 0.58 tumors) or size (CG: 1.78 ± 0.24 cm^3).

Discussion

These are the first observations regarding the effects of different aerobic swimming training intensities on distinct steps of colon carcinogenesis. Our data showed lower values of total ACF number, mean ACF number and number of ACF ≤3 only in animals of EG2 that exercised at intensity 2 for 35 weeks when compared with non-exercised animals (CG). These effects were not observed in rats swimming with either 0% body weight (EG1) or 4% body weight of load (EG3). The EG2 rats also presented better indices for potency, relative risk, ACF reduction percentage, and reduction efficiency in ACF size, compared with EG1 and EG3 animals. These findings suggest that long-term aerobic swimming training protected against the initiation of ACF development in the rat colon and emphasize that there is an optimal exercise intensity to reach this protection, even if exercising below the anaerobic threshold.

Recently, Fuku et al. (7) demonstrated a reduced number of DMH-induced ACF in rats submitted to a daily two-hour low-intensity treadmill running for 4 weeks (short-term). The present study differs from theirs in terms of exercise model and duration (35 weeks), although similar effects of exercise on ACF reduction were observed in both studies. Thus, it seems that exercise intensity is important regarding ACF development. In fact, it was demonstrated that long-term moderate-to-vigorous intensity daily exercise of 60 min by men resulted in an increased expression of Bax at the bottom of colon crypts (16). This

Table 1. Effect of swimming training on aberrant crypt foci (ACF) in regions of the large intestine of rats treated with 1,2-dimethylhydrazine.

Group	Number of ACF		
	Proximal region	Mid-region	Distal region
CG (N = 10)	26.15 ± 2.62	36.55 ± 4.36	46.50 ± 2.56[+]
EG1 (N = 11)	18.23 ± 3.81	28.36 ± 2.92[+]	39.50 ± 2.74[+]
EG2 (N = 11)	13.32 ± 2.37*	33.05 ± 4.89[+]	32.27 ± 3.49*[+]
EG3 (N = 10)	17.40 ± 1.90	41.35 ± 4.35[+]	40.95 ± 3.42[+]

Data are reported as means ± SEM. Rats received four subcutaneous injections of 1,2-dimethylhydrazine (40 mg/kg body weight each) twice a week, on nonconsecutive days, for 2 weeks and were submitted to exercise intensity 1 (EG1), intensity 2 (EG2), and intensity 3 (EG3) for 35 weeks or were sedentary controls (CG). N = number of animals. *$p < 0.05$ compared to CG; [+]$p < 0.05$ compared to proximal region (ANOVA followed by the Tukey test).

Table 2. Effect of swimming training on the potency, relative risk, aberrant crypt foci reduction percentage, and reduction efficiency in the aberrant crypt foci size in the large intestine of rats treated with 1,2-dimethylhydrazine.

	EG1	EG2	EG3
P	1.27	1.39	1.10
RR	0.79	0.72	0.91
%R	21.15	27.99	8.71
PRT	1.36	1.37	1.07

Rats received four subcutaneous injections of 1,2-dimethylhydrazine (40 mg/kg body weight each) twice a week, on nonconsecutive days, for 2 weeks and were submitted to exercise intensity 1 (EG1), intensity 2 (EG2), and intensity 3 (EG3) for 35 weeks or were sedentary controls (CG). P = potency (mean ACF of the CG/mean ACF of the respective exercise group); RR = relative risk (1/P); %R = % aberrant crypt foci reduction (100 - 100 / P); PRT = reduction efficiency in the aberrant crypt foci size (mean ACF >3 of the CG/mean ACF >3 of the respective exercise group).

protein promotes apoptosis and insufficient apoptosis may lead to ACF development, polyps and cancer (16). However, high-intensity exercise leads to immune function depression, oxidative DNA damage and free radical generation (11,17,18), which could lead to colonic ACF formation. In fact, a single session of exhaustive swimming increased the number of DMH-induced ACF in rat colons (13). While the so-called aerobic exercise has many well-established health benefits that could protect against colorectal carcinogenesis (9-12), we have shown here that neither 0% body weight nor 4% body weight-loaded swimming training reduced ACF development in the rat colon. Our results suggest that the intensity of the exercise plays a key role in this process, even if exercising at intensities below the anaerobic threshold.

However, the mechanisms underlying the exercise-induced protection against preneoplastic lesions are still not clear. It has been suggested that caloric restriction-induced weight loss and exercise-induced negative energy balance inhibit the initiation or proliferation of ACF on the colonic mucosa (19). In the present study, however, the body weight gain and intra-abdominal fat were not significantly reduced by long-term aerobic swimming training of any intensity. Our results are consistent with the suggestion that exercise-training protection against colorectal cancer may occur through mechanisms independent of body weight loss (9).

We also analyzed the effect of long-term aerobic swimming training on a further step of colon cancer. Our results demonstrated that tumor incidence, multiplicity and size were not affected by any intensity of swimming training employed here. In our model, a group of animals was not included without DMH injection followed for 35 weeks because colon cancer in rats does not develop spontaneously with age (20). Experimental studies on colon tumors have presented inconsistent results. For example, colon tumor incidence and multiplicity was inhibited in rats with either DMH- or azoxymethane-induced carcinogenesis submitted to long-term voluntary exercise (3). In addition, low-intensity treadmill running for 38 weeks reduced significantly the number of azoxymethane-induced colon adenocarcinomas in male Fisher rats (4). However, in APC[min] mice submitted to moderate-intensity exercise on a treadmill for 7 weeks (5) and to voluntary exercise for 3 weeks followed by moderate treadmill running for 5 weeks (6), no significant effect of exercise on the number of intestinal polyps was detected. The lack of effect of long-term exercise on tumor size observed in the present study is consistent with other reports (3) in rats treated with DMH and submitted to moderate-intensity exercise. Thus, because these studies differ in exercise model, intensity and duration, and cancer induction process, we cannot compare the results directly. From the results of the present study, it is likely that long-term exercise below the aerobic threshold may not affect colon cancer in the advanced stages of the process. This is reinforced by the fact that the numbers of ACF ≥ 4, which indicates advanced preneoplastic lesions (15), were not significantly different between exercised and control animals in the present study. Moreover, short-term low-intensity running training did not affect the overall mean AC/ACF ratios of rat colon induced by DMH, suggesting that such exercise training was not effective for preventing the proliferation of ACF (7).

In conclusion, aerobic swimming training with 2% body weight of load protected against the DMH-induced preneoplastic colon lesions, but not against tumor development in the rat.

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