Decreased intestinal polyp multiplicity is related to exercise mode and gender in Apc<sup>Min/+</sup> mice

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Mehl, Kristen A., J. Mark Davis, Julie M. Clements, Franklin G. Berger, Maria M. Pena, and James A. Carson. Decreased intestinal polyp multiplicity is related to exercise mode and gender in Apc<sup>Min/+</sup> mice. J Appl Physiol 98: 2219–2225, 2005; doi:10.1152/japplphysiol.00975.2004.-Moderate-intensity treadmill running can alter male Apc<sup>Min/+</sup> mouse polyp formation. This purpose of this study was to examine whether exercise mode differentially affects Apc<sup>Min/+</sup> mouse intestinal polyp development in male and female mice. Male and female Apc<sup>Min/+</sup> mice were randomly assigned to control, treadmill (18 m/min; 60 min/day; 6 days/wk), or voluntary wheel running (24-h access) groups. Nine weeks of training decreased total intestinal polyps by 29% in male treadmill runners (66 ± 9; P = 0.038) compared with male controls (93 ± 7). The number of large polyps (≥1-mm diameter) were also reduced by 38% in male treadmill runners (49 ± 6; P = 0.005) compared with male controls (79 ± 6). Treadmill running in female Apc<sup>Min/+</sup> mice and wheel running in both genders did not affect polyp number or size. Spleen weight decreased in male treadmill runners (91 ± 9 mg; P = 0.011) and wheel runners (75 ± 6 mg; P = 0.004) compared with controls (141 ± 13 mg). Plasma IL-6 was reduced by 96% in male treadmill runners (1.2 ± 0.6 pg/ml) and 78% in male wheel runners (6.6 ± 3.3 pg/ml) compared with control mice (27.9 ± 2.8 pg/ml; P < 0.05). Female mice responded similarly with an 86% decrease in plasma IL-6 with treadmill running (3.2 ± 1.2 pg/ml) and 90% decrease with wheel running (2.9 ± 2.0 pg/ml) compared with control mice (21.1 ± 5.3 pg/ml; P < 0.05). The crypt depth-to-villus height ratio in the intestine, an indirect marker of intestinal inflammation, decreased by 21% (P = 0.024) and 24% (P = 0.029), respectively, in male and female treadmill runners but not wheel runners. Physical activity-induced attenuation of intestinal polyp number and size is dependent on exercise mode and differs between genders. The modulation of systemic and intestinal inflammation may also depend on exercise mode.

colon cancer; treadmill running; activity wheel; interleukin-6; adenomatous polyposis coli

Colon cancer is the second leading cause of cancer death in the United States for both men and women with an estimated 148,300 new cases and 56,600 deaths annually (1). Although increased physical activity in men is associated with decreased colon cancer risk, there have been conflicting reports in women (13, 23, 32, 34). Epidemiological evidence clearly demonstrates an inverse relationship between physical activity and colon cancer risk in men, with a higher accumulation of physical activity being more protective (28).

Animal models of cancer have been and remain important for elucidating physical activity-induced mechanisms related to colon cancer prevention. One such model, the Apc<sup>Min/+</sup> mouse, has a nonsense mutation at codon 850 in the adenomatous polyposis coli (Apc) gene, which results in a truncated protein product that predisposes these mice to both small and large intestine adenomas (21). Germline mutations in the APC gene in humans are responsible for the initiation and progression of familial adenomatous polyposis, a type of colon cancer that is inherited as an autosomal-dominant disorder (15). Two previous studies have investigated the potential preventative effect of physical activity on adenoma development in the Apc<sup>Min/+</sup> mouse. Colbert et al. (8) found a trend toward a decrease in total polyp number in male Apc<sup>Min/+</sup> mice after 7 wk of treadmill training, but the statistical power of the study was weakened by unexpected gender differences. A second study examining physical activity on Apc<sup>Min/+</sup> mouse polyp formation employed an unconventional exercise design that initially used voluntary wheel running for 3 wk, followed by treadmill running for 5 wk. The study reported no physical activity-induced decrease in total intestinal polyp number but a regional polyp number decrease in the jejunum of male mice (8). Both of these studies using Apc<sup>Min/+</sup> mice point to gender as an important variable in modulating intestinal polyp development. However, a major caveat related to the second study was the extremely low number of intestinal polyps in control Apc<sup>Min/+</sup> mice compared with other published studies (8, 16, 33).

The exercise paradigm is also an important limitation of these Apc<sup>Min/+</sup> physical activity studies, since the type of physical activity can influence the subsequent physiological adaptations. Both treadmill- and wheel-running models demonstrate cardiac and skeletal muscle adaptations similar to human endurance exercise (2, 17). Treadmill exercise can induce additional physiological changes in rats, including adrenal hypertrophy and thymic involution, which can be associated with chronic stress (20). With wheel running, mice typically run much farther than during treadmill running and cardiac and skeletal muscle adaptations can be detected with a shorter duration of training (2). Therefore, physical activity...
paradigms that elicit different physiological changes related to stress hormone release could have altered inflammatory effects.

Systemic and intestinal inflammation also contributes to cancer onset and severity, including colon cancer. During intestinal inflammation, the villi shorten while the crypt region thickens due to the infiltration of immune cells (18). Macrophage and neutrophil secretion of interleukin (IL)-6 and TNF-α contribute to elevated cyclooxygenase-2 expression and activity and subsequent colon polyp growth (22). Nonsteroidal anti-inflammatory drugs, such as sulindac and celecoxib, inhibit cyclooxygenase-2 activity, as well as IL-6 and TNF-α production, and have been shown to be extremely effective for prevention of tumor development and colon cancer progression (30). Moderate physical activity induces stress hormone release and can decrease monocyte and macrophage IL-6, TNF-α, and IL-1β production (12). Additionally, women tend to have a heightened inflammatory response to certain stimuli, such as infection (3). Therefore, inflammatory pathway inhibition is a potential mechanism for effect of physical activity on colon cancer development but may be different between men and women.

There are many potential mechanisms associated with increased physical activity that could affect cancer incidence, but inflammatory processes appear to be extremely important. The purpose of the present study was to examine the impact of exercise mode on intestinal polyp number and size in the ApcMin/+ mouse and whether the exercise effect differs between genders. Based on previous studies that have suggested a gender difference (7, 8), our primary hypothesis was that both treadmill running and voluntary wheel running would cause a reduction in polyp number and size in male but not female ApcMin/+ mice. Our secondary hypothesis was that the physical activity-induced attenuation of polyp number would be associated with decreased systemic and intestinal mucosa inflammation.

MATERIALS AND METHODS

Animals. ApcMin/+ male mice on a C57BL/6 background (Jackson Laboratories) were purchased and bred with female C57BL/6 mice in the University of South Carolina’s animal resource facility. Offspring were genotyped as heterozygotes by RT-PCR for the Apc gene by taking tail snips at weaning. The primer sequences were sense: 5'-TGAGAAAGACAGAAGTTA-3' and antisense: 5'-TTCCACCTTGCGATAAGGC-3'. The room was maintained on a 12:12-h light-dark cycle with the light period starting at 0700. Mice were provided standard rodent chow (HarlanTeklad Rodent Diet, no. 8604) and water ad libitum. All animal experimentation was approved by the University of South Carolina’s Institutional Animal Care and Use Committee.

Treadmill protocol. At 3.5 wk of age, mice were randomly divided into four groups: male control (n = 14), female control (n = 7), male activity wheel (n = 11), and female activity wheel (n = 8). Mice were housed individually, and body weights and food intake were measured weekly. All activity wheel runners were placed in cages housed with 9.5-in.-diameter stainless steel activity wheels (MiniMitter), and running activity was monitored over 9 wk. Bicycle computers (Specialized) using magnetic sensors measured average speed, distance, time, and maximum speed, and the data were recorded daily. Controls were housed with immovable wheels in their cages. Activity wheel runners were left in their cages for the duration of the study, since placing them in another cage induces a stress response. All mice were killed ~18 h after their last bout of exercise. One male activity wheel runner failed to complete the 9-wk protocol and was removed from the study.

Activity wheel protocol. At 4 wk of age, mice were randomly divided into four groups: male control (n = 14), female control (n = 7), male activity wheel (n = 11), and female activity wheel (n = 8). Mice were housed individually, and body weights and food intake were measured weekly. All activity wheel runners were placed in cages housed with 9.5-in.-diameter stainless steel activity wheels (MiniMitter), and running activity was monitored over 9 wk. Bicycle computers (Specialized) using magnetic sensors measured average speed, distance, time, and maximum speed, and the data were recorded daily. Controls were housed with immovable wheels in their cages. Activity wheel runners were left in their cages for the duration of the study, since placing them in another cage induces a stress response. All mice were killed ~18 h after their last bout of exercise. One male activity wheel runner failed to complete the 9-wk protocol and was removed from the study.

Tissue collection. Mice were given a subcutaneous injection of ketamine-xylazine-acepromazine cocktail (1.4 ml/kg body wt). The small intestine was carefully dissected distally to the stomach and proximal to the cecum. The large intestine was removed from the distal end of the cecum to the anus. Mesentery tissue was removed with tweezers, and the small intestine was cut into four equal sections. All intestinal sections were flushed with PBS, opened longitudinally, and flattened with a cotton swab. All sections were fixed in 10% buffered formalin (Fisher) for 24 h. Spleens, hearts, and gastrocnemius muscles were also removed, rinsed in PBS, snap frozen in liquid nitrogen, and stored at −80°C until further analysis. Tissues were also removed as an indicator of body size.

Polyp counts. Formalin-fixed intestinal sections from all animals were rinsed in deionized water, briefly stained in 0.1% methylene blue, and counted by the same investigator who was blinded to the treatments. Polyps were counted under a dissecting microscope, using tweezers to pick through the intestinal villi and identify polyps. Polyps were categorized as ≥1 mm or <1 mm in the small intestine and >2 mm, 1–2 mm, and <1 mm in the large intestine. After polyps were...
counted, intestinal sections were placed in 70% ethanol for further analysis. Pilot data yielded an inter assay coefficient of variation of 4%.

*Citr a synthase assay.* Citrate synthase (CS) activity was determined in snap-frozen gastrocnemius muscles based on a method modified by Srere (31). Samples from seven to eight animals from each group were randomly selected for this assay. The tissue (0.025 g) was homogenized at a 1:21 dilution in homogenizing buffer (0.175 M KCl, 0.002 M EDTA, pH 7.4). The CS reaction was carried out in a 1 ml reaction cocktail containing (in mM) 100 Tris–HCl buffer, pH 8.3 (0.700 ml), 1 5,5′-dithiobis-2-nitrobenzoate (0.100 ml), 10 oxaloacetate (0.050 ml), and 3 acetyl-CoA (0.150 ml). Tissue homogenate was added (5–10 μl) to the cocktail, and the absorbance was recorded every 15 s for 3 min at 412 nm. CS activity (mmol min⁻¹ g⁻¹) was calculated based on the extinction coefficient for 5,5′-dithiobis-2-nitrobenzoate at 412 nm (13,6000) using the following equation: (change in absorbance·min⁻¹)(1/13.6 mmol·ml⁻¹) (dilution of sample in reaction cocktail)(dilution of muscle homogenate).

*Plasma IGF-I and IL-6.* Blood samples were collected during death under anesthesia via the inferior vena cava. Samples were kept on ice and centrifuged at 1,000 g for 10 min at 4°C. Plasma was aliquotted and stored at −80°C until analysis. Plasma IGF-I from five to eight randomly selected mice were measured in a rat IGF-I EIA (Diagnostic Labs, Webster, TX) that was cross-reactive with mouse IGF-I. Samples were measured in duplicate, and the inter-assay coefficient of variation was 1.4%. Plasma from all exercised mice and one-half of the control mice was measured with a mouse IL-6 ELISA (Biosource, Camarillo, CA) and performed according to manufacturer’s instructions.

*Crypt depth to villus height ratio.* Methylen blue-stained intestinal sections of the distal ileum (n = 7–8 per group) were Swiss rolled, paraffin embedded, sectioned on a microtome cut in 10-μm sections, and stained with hematoxylin and eosin. Images were digitized (×4) and evaluated by a technician blinded to the treatments. Digital imaging software (Scion Image, Frederick, MD) was used to measure crypt depth and villus height (mm/mm). Preliminary data determined that >50 crypt depth-to-villus height ratio (CVR) measurements did not induce a change in the standard deviation of the mean CVR per animal. Fifty measurements of the distal ileum were performed on each animal, and the mean CVR for each animal was averaged for each treatment group.

*Statistical analysis.* Results are reported as means ± SE. Independent t-tests were performed between treadmill control mice and wheel-running control mice for poly number, body weight, gastrocnemius weight, spleen weight, and CS activity. No differences existed between these groups, so the control groups were combined for all analyses. Food intake and body weight were analyzed with a repeated-measures two-way ANOVA to assess differences between treatment groups within each gender over time. All other variables were analyzed with a one-way ANOVA within each gender. Post hoc analyses within each gender were performed with Student-Newman-Keuls multiple comparison procedures. The accepted level of statistical significance was P ≤ 0.05.

## RESULTS

*Body and tissue weight.* There were no differences in body weight between controls, treadmill runners, or wheel runners within each gender (Table 1). There was a significant interaction between treatment and time (P < 0.05) on food intake within the male animals. There were no differences in food consumption between male controls and treadmill runners, except at week 2 (Fig. 1A). However, male wheel runners ate more than male controls at all time points (P < 0.05). Within the female groups, there was a main effect of treatment group (P < 0.05) and a main effect of time (P < 0.05) on food intake. Female wheel runners ate more than female controls at all time points, but female treadmill runners ate the same as controls (Fig. 1B). Wheel running increased female heart weight by 13% and heart weight-to-tibia length by 11%, but these increases did not occur with treadmill running or among the males. Gastrocnemius weight was smaller in male wheel runners (P < 0.05) and female wheel runners (P < 0.05) compared with the other groups. Wheel running decreased male gastrocnemius weight and gastrocnemius weight-to-tibia length ratio by 15 and 12%, respectively (P < 0.05). The female animals responded similarly with 15 and 17% decreases in gastrocnemius weight and gastrocnemius weight-to-tibia length ratios, respectively. Treadmill running did not induce a change in the standard deviation of the mean CVR per animal. Fifty measurements of the distal ileum were performed on each animal, and the mean CVR for each animal was averaged for each treatment group.

*Training adaptations.* Male activity wheel runners ran an average of 4.7 ± 0.4 km/day and 167 ± 14 min/day. Female activity wheel runners ran an average of 3.5 ± 0.2 km/day and 150 ± 6 min/day. However, male and female treadmill runners ran 11.1 km/day and 60 min/day, which is ~25% the total distance covered by male and female wheel runners. Despite the differences in total level of physical activity, both treadmill running (47 ± 4 mmol·g⁻¹·min⁻¹) and activity wheel running (75 ± 5 mmol·g⁻¹·min⁻¹) in male mice induced 42 and 127% increases in CS activity (P < 0.05), respectively, compared with control mice (33 ± 3 mmol·g⁻¹·min⁻¹) in the gastrocnemius muscle, indicating that these animals were aerobically trained (Fig. 2). However, although both treadmill (46 ± 3 mmol·g⁻¹·min⁻¹) and wheel running

| Table 1. Effects of exercise in ApcMin/+ mice on body, gastrocnemius, and heart weight in 13-wk-old controls, treadmill runners, and activity wheel runners |
|---|---|---|---|---|---|---|
| Treatment | n | BW, g | Tibia, mm | Gastroc, mg | Gastroc/Tibia, mg/mm | Heart, mg | Heart/Tibia, mg/mm |
| **Males** | | | | | | | |
| Control | 27 | 23.3±0.3 | 15.7±0.1 | 115±4 | 7.4±0.2 | 113±3 | 7.2±0.2 |
| Treadmill | 12 | 24.3±0.5 | 15.8±0.0 | 123±3 | 7.9±0.2 | 107±3 | 6.7±0.2 |
| Wheel | 7 | 22.7±0.6 | 15.6±0.1 | 102±4† | 6.5±0.3† | 116±4 | 7.5±0.3 |
| **Females** | | | | | | | |
| Control | 23 | 19.8±0.4 | 15.6±0.1 | 97±4 | 6.3±0.3 | 99±4 | 6.2±0.2 |
| Treadmill | 16 | 20.0±0.4 | 15.4±0.1 | 94±3 | 6.1±0.1 | 93±3 | 6.0±0.2 |
| Wheel | 11 | 20.9±0.5 | 15.6±0.1 | 82±3† | 5.2±0.2† | 109±1† | 7.0±0.1† |

Values are means ± SE. n, No. of mice; BW, body weight; Gastroc, gastrocnemius. Data were analyzed with a one-way ANOVA within each gender. Significance was set at P < 0.05. *Significant difference from control (P < 0.05). †Significant difference from treadmill (P < 0.05).
Spleen weight in age-matched C57BL/6 mice is directed toward specific tissues needing repair (4). The average other inflammatory challenges, initiating an immune response immunological tissue that increases in size during infection and However, there were no differences in colon polyp number

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\text{polyp size was quantified as } \text{H}11022
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occurring in the proximal end. Colon polyps represented

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\text{polyps throughout the gastrointestinal tract in the male or}
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polyps. Exercise training also did not affect the distribution of

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\text{polyp number in male treadmill runners (66 } \pm 9\text{)}
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male or female treatment groups. Unlike the male groups, exercise training, regardless of

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\text{large polyps (}\geq 1\text{-mm diameter) were reduced in male treadmill runners (49 } \pm 6\text{) by 38\% compared with}
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controls (79 } \pm 6\text{; } P = 0.005) (Fig. 3B). The number of large polyps did not differ between male wheel runners (87 } \pm 7\text{) and controls. The number of small polyps (<1-mm diameter) (Fig. 3C) was not different between any of the male treatment groups. Unlike the male groups, exercise training, regardless of mode, did not affect female total polyps, large polyps, or small polyps. Exercise training also did not affect the distribution of polyps throughout the gastrointestinal tract in the male or female mice. The majority of polyps appeared in the small intestine (~95\%). Within the small intestine, the majority of polyps appeared in the distal end (~75\%) with only ~25\% occurring in the proximal end. Colon polyps represented ~5\% of the total polyp burden. There was a trend for male treadmill runners to decrease the number of colon polyps compared with controls (\(P = 0.09\), but not in females (data not shown). Colon polyp size was quantified as >2 mm, 1–2 mm, or <1 mm. However, there were no differences in colon polyp number when categorized by size.

Systemic and intestinal inflammation. The spleen is an immuno-ligical tissue that increases in size during infection and other inflammatory challenges, initiating an immune response directed toward specific tissues needing repair (4). The average spleen weight in age-matched C57BL/6 mice is ~50\% less

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\text{than } Apc^{Min/+}\text{ mice at 13 wk of age, indicating that } Apc^{Min/+}\text{ mice undergo splenomegaly (data not shown). This spleen weight was reduced by 35\% in male treadmill runners (91 } \pm 9
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mg; \(P = 0.011\)) and 47\% in male wheel runners (75 } \pm 6\text{ mg; } P = 0.004) compared with male Apc\textit{Min/+} controls (141 } \pm 13\text{ mg) (Fig. 4A). Spleen weight changes with exercise were not detected in the females. There was a positive correlation between spleen weight and polyp number (Fig. 4B) in male controls (\(r = 0.458\); \(P = 0.0281\)) and female controls (\(r = 0.772\); \(P < 0.001\)). This correlation was also present in male treadmill runners (\(r = 0.821\); \(P = 0.001\)) but not female treadmill runners, male wheel runners, or female wheel runners.

Plasma IL-6 was reduced by 96\% in male treadmill runners (1.2 } \pm 0.6\text{ pg/ml) and 78\% in male wheel runners (6.6 } \pm 3.3\text{ pg/ml) compared with controls (27.9 } \pm 2.8\text{; } P < 0.05\) (Fig. 4C). Females responded similarly with an 86\% decrease in plasma IL-6 with treadmill running (3.2 } \pm 1.2\text{ pg/ml) and 90\%

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\text{Fig. 2. Gastrocnemius citrate synthase activity after 9 wk of exercise training in male and female } Apc^{Min/+}\text{ mice. Solid bars, control; open bars, treadmill;}
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hatched bars, wheel. Values are means } \pm \text{SE. } *\text{Significant difference from control (} P < 0.05\text{). } +\text{Significant difference from treadmill (} P < 0.05\text{).}

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\text{Fig. 3. Effect of exercise on intestinal polyp number and size in } Apc^{Min/+}\text{ mice. Male and female controls, treadmill runners, and wheel runners were}
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killed after 9 wk of exercise training. Both small and large intestinal polyps

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\text{were counted. } \text{A: total intestinal polyps. B: intestinal polyps of } \geq 1\text{-mm diameter. C: intestinal polyps of } <1\text{-mm diameter. Values are means } \pm \text{SE. Solid bars, control; open bars, treadmill; hatched bars, wheel. } *\text{Significant difference from control (} P < 0.05\text{).}
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\text{Fig. 4. Relationship between spleen weight and polyp number in } Apc^{Min/+}\text{ mice. A: total spleen weight. B: spleen weight in male and female mice. C: spleen weight in male and female mice after 9 wk of exercise training. Solid bars,}
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control; open bars, treadmill. *\text{Significant difference from control (} P < 0.05\text{)}.
decrease with wheel running (2.9 ± 0.2 pg/ml) compared with controls (21.1 ± 5.3 pg/ml; \(P < 0.05\)). We did not detect any circulating IL-6 in age-matched C57BL/6 mice, indicating that ApcMin/− mice have elevated levels of circulating IL-6 (data not shown).

CVR is an indirect measurement of intestinal inflammation, with an increased number indicating an inflamed intestinal tract (18). Hematoxylin and eosin (\(\times 4\))-stained sections (Fig. 5A) were used to quantify CVR. Fifty measurements were performed on each sample, and a mean CVR for each animal was determined for the distal ileum. CVR decreased by 21% with treadmill training (\(P = 0.024\)) but not wheel running in male ApcMin/− mice (Fig. 5B). Among the female ApcMin/− mice, CVR also decreased by 24% with treadmill running (\(P = 0.029\)) but not wheel running. The ApcMin/− mouse response to exercise training is summarized in Table 2.

**Fig. 4. Systemic inflammatory markers in male and female ApcMin/− mice after 9 wk of exercise training. A: mean spleen weight. B: spleen weight vs. polyp number correlation. ●, Male controls (\(r = 0.458; P = 0.0281\)); ◆, female controls (\(r = 0.772; P < 0.001\)). C: plasma IL-6. Plasma IL-6 was measured via ELISA. IL-6 was measured on all exercised animals and one-half of the control animals. Values are means ± SE. Solid bars, control; open bars, treadmill; hatched bars, wheel. *Significant difference from control (\(P < 0.05\)).**

**Fig. 5. Crypt depth:villus height ratio (CVR) in ApcMin/− mice after 9 wk of exercise training. Formalin-fixed sections of the distal ileum were stained with hematoxylin and eosin, and \(\times 4\) digital images were analyzed by a blinded investigator. Fifty measurements were made per animal, and a mean CVR was designated to each animal. A: representative \(\times 4\) image of a hematoxylin and eosin-stained ileum. B: mean CVR. Values are means ± SE. Closed bars, control; open bars, treadmill; hatched bars, wheel. *Significant difference from control (\(P < 0.05\)).**

**DISCUSSION**

Epidemiological evidence suggests that colon cancer risk is affected by both genes and environmental factors, such as physical activity. Although two previous studies using ApcMin/− mice have attempted to assess this interaction, the results are equivocal. To our knowledge, this is the first study designed to directly compare distinct exercise paradigms (treadmill running and voluntary wheel running) and whether these responses differ between genders. Additionally, inflammation’s role in altering cancer progression in ApcMin/− mice by physical activity was investigated. The present study demonstrates

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<th>Variable</th>
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<td></td>
<td>Treadmill</td>
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<td>BW</td>
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<td>Gastroc weight</td>
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Increased or decreased is relative to ApcMin/− controls. NC, no change.
that exercise can decrease polyp formation and size, but the exercise effect is related to both gender and the type of physical activity. Physical activity also altered both the systemic and intestinal mucosa inflammatory state. The relationship between inflammation and \( Apc^{Min/+} \) polyp formation appears to be complex and possibly modulated by several factors, including stress hormones and sex steroids.

Improved immune system function is a potential exercise-induced mechanism for colon cancer prevention (28). The immune system is composed of many cell types that detect and destroy foreign pathogens, such as cancerous cells. It is well documented that moderate exercise enhances, and strenuous exercises represses, immune cell number and function in both human and animal models (25). Lymphocyte production occurs in the white pulp of the spleen and various inflammatory processes, such as acute infection or lipopolysaccharide stimulation, can stimulate spleen enlargement (4). The reduction in spleen size in male exercisers could be a marker of reduced systemic inflammation.

\( Apc^{Min/+} \) mice suffer from lymphocyte and natural killer cell depletion of the thymus and spleen between 83 and 120 days of age (9), and the present study extended into this time period. Lymphodepletion is also seen with human colorectal cancer and occurs as a result of immune system dysfunction (24). Exercise-induced decreases in splenomegaly were gender dependent, being present only in male \( Apc^{Min/+} \) mice. The reduction in spleen weight among all male exercisers suggests that exercise may be attenuating \( Apc^{Min/+} \) mouse lymphodepletion. \( Apc^{Min/+} \) mouse lymphodepletion is also associated with increased plasma IL-6 (24). IL-6 is normally not present or expressed at low levels, except during infection, trauma, or other stress (11). Serum IL-6 levels are higher in colon cancer patients and correlated with larger tumor size (5). Inhibition of inflammatory pathways by pharmacological and dietary substances have shown promise in the treatment and prevention of colon cancer (10). Epidemiological evidence suggests that physical activity is inversely correlated with serum IL-6 (26). In the present study, plasma IL-6 was decreased with both forms of exercise in both genders of \( Apc^{Min/+} \) mice. Exercise-induced decreases in polyp number and plasma IL-6 were present only in male treadmill runners. Inhibition of systemic IL-6 production with treadmill running in male \( Apc^{Min/+} \) mice could be partially responsible for attenuating polyp size. Although spleen size and plasma IL-6 may reflect important exercise-induced systemic changes, the data show that decreased spleen size and changes in plasma IL-6 alone are not sufficient for altered polyp number.

Intestinal inflammation, as measured by cyclooxygenase-2 gene expression and activity, also contributes to the onset of \( Apc^{Min/+} \) mouse polyp formation and cancer progression (29). Inflammatory signaling can recruit immune cells to the site of neoplasia, which are used to fight the disease, but they can also become transformed cells and compose part of the tumor (14). CVR is increased during intestinal inflammation due to immune cell infiltration to the crypt area and villus shortening (18). Although CVR is not different between wild-type and \( Apc^{Min/+} \) mice (18), dietary treatments can alter intestinal macrophage and lymphocyte number in \( Apc^{Min/+} \) mice, which correspond to a reduction in adenoma number and size (6). Treadmill running in both male and female \( Apc^{Min/+} \) mice decreased CVR. The exercise effect on intestinal mucosa inflammation appears to be mode dependent, because wheel running had no effect on CVR. Male wheel runners demonstrated a trend for a decrease in intestinal inflammation. However, there was extensive variability in this group, possibly due to the distance and speed differences run between mice. Because systemic inflammation and intestinal inflammation decreases were apparent only in male treadmill runners, the combinatorial effect of systemic and intestinal inflammation may be critical for altered \( Apc^{Min/+} \) mouse polyp number.

Although inflammatory markers did not appear different between male treadmill runners and wheel runners, differences in energy balance may have contributed to this disparity. It is counterintuitive that more physical activity, as demonstrated by a higher amount of accumulated distance by the wheel runners, was not protective against intestinal cancer in the \( Apc^{Min/+} \) mouse. Activity wheel runners ran approximately three times more than treadmill runners, but activity wheel runners also consumed 25% more calories than controls. However, treadmill runners are similarly to controls. An increase in physical activity without a change in food intake should have placed the treadmill runners in a state of negative caloric balance. Caloric restriction studies in \( Apc^{Min/+} \) mice have demonstrated a decrease on tumor formation and size, which is associated with a decrease in body weight (16, 19). The decrease in body weight comes from decreased skeletal muscle and fat. In the present study, a decrease in body weight or muscle weight with treadmill running was not detected and fat mass was not measured. Circulating IGF-I increases in obese individuals and has been shown to decrease (19) or not change (16) after decreased tumor burden with caloric restriction in \( Apc^{Min/+} \) mice. In the present study, a moderate increase in energy expenditure induced by treadmill running did not change circulating IGF-I, which agrees with previous \( Apc^{Min/+} \) mouse exercise studies (7, 8). However, acute changes in circulating IGF-I after exercise were not measured. Although the treadmill runners were in a small state of negative caloric balance, this stimulus does not appear to induce major alterations in body composition, indicative of caloric restriction.

Acute exercise-induced hormonal changes may be an important factor for the regulation of polyp growth. Glycogen synthase kinase (GSK)-3 is a cellular energy-sensing enzyme that is stimulated by insulin receptor signaling through the phosphatidylinositol 3-kinase pathway. An increase in insulin or IGF-I stimulates phosphatidylinositol 3-kinase, which in turn inhibits GSK-3 function. GSK-3 is responsible for the phosphorylation and subsequent degradation of β-catenin. APC protein loss decreases GSK-3 activity, leading to nuclear β-catenin accumulation. Metabolic changes that increase GSK-3 activity may provide the link between an exercise-induced effect on polyp number and dysfunction of the APC β-catenin pathway during colon cancer. Although exercise has been shown to alter GSK-3 activity, β-catenin, and APC protein levels in human skeletal muscle (35), the effect of exercise on the intestinal mucosa is not known. Further work is needed to assess the cellular signaling related to altered energy balance, body composition, adipose tissue hormone release, and their relationship to intestinal polyp development.

In conclusion, treadmill exercise reduced polyp number and size in male but not female mice. Male exercisers had a differential response to systemic inflammation compared with females. Reductions in intestinal inflammation were related to
exercise mode. The synergistic intestinal and systemic anti-inflammatory effect with treadmill running in male Apc<sup>Min/+</sup> mice was associated with a decrease in polyp number. Although activity wheel running initiated a greater training response, it did not elicit a change in polyp number or size. Overall, these data suggest that a greater increase in total energy expenditure is not more protective against intestinal cancer in the Apc<sup>Min/+</sup> mouse. Data generated with this study opens up possible future investigations of an interaction between sex steroids and/or anti-inflammatory agents with physical activity on intestinal polyp formation.

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