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Negative energy balance induced by voluntary wheel running inhibits polyp development in APC^{Min} mice

Lisa H.Colbert*, Volker Mai¹, Janet A.Tooze², Susan N.Perkins³, David Berrigan³ and Stephen D.Hursting⁴

Department of Kinesiology and Comprehensive Cancer Center, University of Wisconsin, Madison, WI, USA,¹Department of Epidemiology and Preventive Medicine, University of Maryland, Baltimore, MD, USA,²Wake Forest University School of Medicine, Winston-Salem, NC, USA,³National Cancer Institute, Bethesda, MD, USA and ⁴Department of Human Ecology, University of Texas, Austin, TX, USA

*To whom correspondence should be addressed.
Email: lhc Colbert@education.wisc.edu

Treadmill running of ~0.9 km/day has had inconsistent effects on spontaneous intestinal polyposis in C57BL/6J-Apc^{Min}/J (Min) mice; the amount of energy expenditure and/or a lack of hormonal changes could account for this variability. The purpose of this study was to examine the effects of a negative energy balance induced by voluntary wheel running on polyps, insulin-like growth factor-1 (IGF-1) and corticosterone in Min mice. Seven-week-old male Min mice were randomly assigned to control (CON, n = 23) or wheel running (EX, n = 24) conditions for a 10-week study period. All mice had water and AIN-76A diet ad libitum for the first ~3 weeks on study, after which the EX group was pair-fed to the CON group to maintain a negative energy balance due to the exercise. EX mice voluntarily ran 3.8 km/day (2.7–6.0 km/day) (median, interquartile range) and weighing less than CON mice throughout the study. More CON mice died before the end of the study versus EX mice (26 versus 0%, P < 0.01). CON mice had significantly more polyps versus EX mice (21.6 ± 1.5 versus 16.9 ± 2.0, P < 0.01; mean ± SE), and daily running distance in EX was inversely correlated with total polyp number (r = −0.70, P < 0.01). Urinary corticosterone output (P < 0.01) and serum IGF-1 were significantly higher in EX than CON (P < 0.001); however, total polyp number was unrelated to corticosterone (r = 0.05, P = 0.84) and IGF-1 (r = −0.01, P = 0.93). In this study, a negative energy balance produced by wheel running exercise and restricted feeding decreased polyp burden in male Min mice and appeared to have a dose–response effect on polyp number. Although EX affected IGF-1 and corticosterone, neither marker was related to total polyp number.

Introduction

Epidemiological evidence is quite consistent in demonstrating that persons who have higher levels of physical activity have a lower risk for colorectal cancer (reviewed in ref. 1). Similarly, exercise training in models of chemically induced carcinogenesis in rats have been consistent in demonstrating that rats who run voluntarily on wheels (2,3) or involuntarily on treadmills (4,5) are protected to various degrees against the development of chemically induced tumors. In an attempt to explore potential mechanisms that might mediate the beneficial effects of exercise on colon cancer, we previously ran C57BL/6J-Apc^{Min}/J (Min) mice on a rodent treadmill. We found no effect of the treadmill running in female mice, and only minimal effects in male mice, on the number of intestinal adenomatous polyps that spontaneously develop in this model (6,7). Calorie restriction (CR) in these same mice, however, dramatically reduced polyp number (8). Given these data, it is possible that the minimal dose of treadmill exercise imposed was simply not enough of a stimulus to reduce polyp number, and we hypothesized that the greater caloric expenditure that can be achieved through voluntary wheel running might be more effective than involuntary treadmill running. As in our prior study (7), we chose to restrict the food consumption of the exercised mice to that of the non-exercised mice in order to produce a negative energy balance due to the exercise.

Mechanisms to explain the effects of exercise on carcinogenesis are largely speculative. More work has been done on changing energy intake through CR than on the energy expenditure side of the energy balance equation. With CR, both insulin-like growth factor-1 (IGF-1) and corticosterone have been found to mediate risk in various cancer types. Higher IGF-1 levels have been associated with the risk of colon cancer in humans (9) and found to be directly related to the CR effects on tumorigenesis of other types (10). Higher corticosterone levels have also been directly implicated in the protective effect of CR on mammary and skin carcinogenesis in rodent models (11,12). Our previous studies in Min mice found that CR, but not treadmill running, decreased IGF-1 levels and increased urinary corticosterone production (8). Additionally, body weight and fatness were affected much more with CR than with treadmill running. We therefore hypothesized that a greater exercise stimulus achieved through 24 h access to running wheels, and thus a larger negative energy balance, might affect body composition and these hormonal markers to a greater extent than scheduled, short-term treadmill running, leading to a reduction in polyp number.

Therefore, the purpose of the current study was to examine the effects of a voluntary wheel running-induced negative energy balance on polyp number, polyp location, body composition and hormonal changes in male Min mice.

Materials and methods

Animals

All animal protocols were approved by the National Cancer Institute’s Animal Care and Use Committee. Male Min mice (~7 weeks of age) were purchased from The Jackson Laboratories (Bar Harbor, ME) and delivered in two separate shipments (referred to in this text as blocks). Mice were randomized and housed individually within 7 days of arrival, given water and AIN-76A diet ad libitum, and kept on a 12 h light/dark cycle. All mice spent the first

Abbreviations: ANCOVA, analysis of covariance; CR, calorie restriction; IGF-1, insulin-like growth factor-1.
~3 weeks in quarantine, during which sentinel mice were screened for a standard battery of pathogens. Once cleared, mice were transferred to the main animal facility.

**Experimental design**

The animals described in the current study were part of a larger experiment designed to examine the role of corticosterone and CR on polyp development in Min mice. For this reason, all mice in the current experiment were subcutaneously implanted with a 0.5 cm placebo pellet (Innovative Research of America, Sarasota, FL) when they were released from quarantine at ~10 weeks of age. To address the hypotheses outlined in this study, we compared non-exercise control (CON) and voluntary wheel running (EX) conditions. Within each block, mice were randomly assigned to either the CON or the EX conditions for a total of 24 mice in each group. Mice in the EX group had access to running wheels in their cages for ~10 weeks, from the time they were singly housed in quarantine at ~7 weeks of age until the experiment was terminated; the mice ~13–16 weeks of age. All mice had nestlets placed in their cages for enrichment. One mouse in the CON group was found dead at 14 weeks of age and was therefore removed from the study. Six mice from the CON group had to be killed at ~15–16 weeks of age owing to morbidity (assessed by appearance and behavior including hunching, ruffled fur, signs of dehydration or signs of anemia such as pale ears or feet). On the basis of high corticosterone and low hematocrit levels as described in the Results, these premature deaths appeared to be due to polyp-related anemia. The first of these CON mice to be killed owing to morbidity was noted to have multiple polyps, but they were not quantified. For this mouse, total polyp number was imputed from the mean of the latter five moribund-kill mice. Removing this mouse from the total polyp count number did not affect the results. No marker markers were measured in these six mice.

Wheel running activity was monitored periodically throughout the study using magnetic switches in conjunction with Vital View software (Mini Mitter, Bend, OR). Daily running activity was recorded for 14 days (study weeks 7 and 8) for the mice in the first block and 21 days (study weeks 6–8) for the mice in the second block. Body weight was measured weekly in all mice, and food consumption was measured weekly in CON mice throughout the study period by institutional animal handling personnel without knowledge of study hypotheses. The difference in weight of the food placed into the cages from that of any food remaining in the cage at the end of the week was considered to be the weight of food consumed. From the time they were released from quarantine, EX mice were given daily aliquots of AIN-76A diet equal to the average daily consumption of CON mice during the previous week in order to maintain a negative energy balance due to the exercise. At ~8 weeks of study, mice were placed in metabolic cages for 48 h, with urine collected after each 24 h period. The mice did not have access to their wheels for these 48 h.

**Necropsy**

At the end of the study, mice were killed by continuous CO₂ inhalation in accordance with current National Institutes of Health (NIH) guidelines. The necropsies took place over several days so that all mice could be killed in the morning, allowing for comparability in serum IGF-1 and hematocrit measures. Serum was collected, immediately frozen in liquid nitrogen and then stored at ~70°C until analyzed. The necropsy procedure involved removal of the entire gastrointestinal tract. The small intestine was divided into three segments of approximately equal length (i.e. duodenum, jejunum and ileum), and the colon was left intact. Neither the stomach nor the cecum was evaluated for polyps. The intestinal segments were washed with phosphate-buffered saline to remove intestinal contents and opened longitudinally. Polyps ≥ 0.5 mm in each of the segments were counted using a dissecting microscope, and the size (<2, 2–4 and >4 mm) and the location of each polyp was recorded.

**Body composition**

Fat and lean mass was measured after necropsy on all mice that survived until the end of the study with dual-energy x-ray absorptiometry (DXA) (GE Lunar PIXIImus II, Madison, WI), with each mouse scanned three times. We have previously validated the use of DXA on necropsied mice through comparison with gravimetric and chemical (Soxhlet) extraction methods (13).

**Hormonal measures**

Urinary corticosterone was measured with a rat corticosterone radioimmunoassay (RIA) (ICN Biomedicals, Costa Mesa, CA) in a random sample (n = 9, CON; n = 10, EX) of mice that survived until the end of the study and were collected during the eighth week of the study. Urine from the second 24 h collection period was used in this analysis, and the urinary excretion of corticosterone in nanograms per day (ng/day) was determined by adjusting for the volume of urine collected in that 24 h period. Serum IGF-1 was measured in all mice that survived until the end of the study (n = 17, CON; n = 24, EX) with a rat/mouse IGF-1 RIA (Diagnostic Systems Laboratories, Webster, TX). Intra-assay coefficients of variation were 5.8 and 6.2% for corticosterone and IGF-1, respectively.

**Statistical analysis**

Wheel running activity is expressed as the median and interquartile range, as the daily running among the mice was not normally distributed. For the IGF-1 analysis, one EX mouse had IGF-1 levels that were >3 interquartile ranges higher than the 75th percentile, and so this outlier was removed. The proportion of mice that survived until the end of the study was compared using Fisher’s exact test. The total number of polyps, number of polyps ≥2 or 4 mm, hematocrit, corticosterone, IGF-1 and body weight were compared between the two groups using analysis of covariance (ANCOVA), adjusting for the study block. Body composition was also analyzed by ANCOVA with adjustment for study block, and for relevant covariates (lean mass, body weight), as noted in Table I. Differences in body weight were assessed using a mixed model that included block, study week and a random subject effect. Linear associations between total polyp number and various markers were evaluated with Spearman correlations.

**Results**

Survival to the end of the study varied by treatment group, with 6 out of 23 (26%) CON mice killed before the end of the study owing to morbidity while 0 out of 24 (0%) of EX mice were killed early (P = 0.009). The CON mice killed early had hematocrit values of 12.7 ± 2.9 versus 33.2 ± 12.7 (mean ± SD) for CON mice that survived until the end of the 9 week study. Total polyp number was higher in those mice killed early compared with mice that survived until the end of the study (26.7 ± 5.8 versus 18.1 ± 8.7, respectively). The hematocrit and polyp numbers suggest that these mice were moribund owing to polyp-related anemia.

Total polyp numbers as well as location and size of polyps by treatment group are presented in Figure 1. EX significantly reduced total polyp number, polyps of greater size (both ≥2 and ≥4 mm) and polyps in both the small intestine and colon, with the small intestinal difference localized to the jejunum. We also analyzed total polyp number with the analysis restricted to those mice that survived to the end of the study and found that EX had lower polyp numbers than CON (16.1 ± 1.3 versus 20.7 ± 1.5, P = 0.015). The median distance run by EX mice during the study was 3.8 km/day (interquartile range: 2.7–6.0 km). Running activity (mean revolutions per day; rev/day) among the EX mice was inversely correlated with

**Table I.** Body composition, hormonal and hematocrit measures by treatment group among mice surviving until the end of the study

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control (n = 17)</th>
<th>Exercise (n = 24)</th>
<th>Adjusted mean difference* (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body composition</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>6.2 ± 0.3</td>
<td>7.5 ± 0.3</td>
<td>-1.2 (0.4); P = 0.008</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>27.2 ± 0.9</td>
<td>30.8 ± 0.7</td>
<td>-3.6 (1.1); P = 0.002</td>
</tr>
<tr>
<td>Lean body mass (g)</td>
<td>17.5 ± 0.4</td>
<td>15.9 ± 0.3</td>
<td>1.6 (0.5); P = 0.003</td>
</tr>
<tr>
<td>BMD (mg/cm²)</td>
<td>53.2 ± 0.8</td>
<td>55.1 ± 0.7</td>
<td>-1.9 (1.0); P = 0.007</td>
</tr>
<tr>
<td><strong>Hormonal measures</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGF-1 (ng/ml)</td>
<td>201 ± 6</td>
<td>230 ± 5</td>
<td>-28.7 (7.3); P = 0.0005</td>
</tr>
<tr>
<td>Corticosterone (ng/day)</td>
<td>21.1 ± 11</td>
<td>77.3 ± 10.7</td>
<td>-56.2 (14.8); P = 0.002</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>22.6 ± 2.2</td>
<td>39.0 ± 2.1</td>
<td>-16.5 (3.0); P &lt; 0.0001</td>
</tr>
</tbody>
</table>

*Adjusted for block.

1Adjusted for block and lean mass.

2BMD adjusted for block and total body weight.

3Control, n = 9; Exercise, n = 10.

4Control, n = 22.
both total polyp number ($r = -0.70, P < 0.001$) (Figure 2) and number of polyps $>2$ mm ($r = -0.74, P < 0.001$).

Weekly body weights are presented in Figure 3. There was a significant interaction between treatment and time on study ($P < 0.001$), with the rate of change in body weight different between CON and EX. There was no difference in body weight between groups at Week 1 on study ($P = 0.46$), but body weight was significantly lower in EX versus CON at Week 9 ($P = 0.03$).

Body composition data for all necropsied mice that survived until the end of the study are presented in Table I. Bone mineral density (BMD) tended to be higher in EX versus CON as anticipated. There was an unexpected difference in body composition that differed from our observations in an earlier study of the effects of treadmill running (7) such that the EX mice had higher fat and lower lean masses than the surviving CON mice at the time of killing. Percent body fat and total polyp number were inversely related ($r = -0.34, P = 0.03$).

Measures of IGF-1, corticosterone and hematocrit are also presented in Table I. IGF-1 and corticosterone were significantly higher in the EX mice compared with those in CON. Neither IGF-1 ($r = -0.01, P = 0.93$) nor corticosterone ($r = 0.05, P = 0.84$) were significantly correlated with total polyp number. The random sample of EX mice chosen for corticosterone measurement did not differ from the other EX mice in their average amount of daily running ($P = 0.30$).

Hematocrit was also significantly higher in EX versus CON, consistent with the lower polyp burden.

Discussion

On the basis of our prior work with treadmill running exercise in male Min mice in which the relatively small dose of exercise had only minimal effects on polyp number (6,7), we had hypothesized that a greater dose of exercise and thus a greater energy expenditure might be more effective in reducing tumor burden. In this study, a negative energy balance induced by voluntary wheel running significantly decreased total polyp number in male Min/+ mice and prolonged their survival. The daily distance voluntarily run by the mice here was four times that imposed in our previous treadmill running study (3.8 versus 0.9 km/day) (7). Consistent with the notion that the total amount of running (and thus energy expenditure) is related to polyp number, we saw moderate inverse correlations between running volume (rev/day) and both total polyp number and the number of polyps of larger size ($>2$ mm).
In contrast to our data suggesting running volume-dependent effects on polyps, a recent study that examined both treadmill and voluntary wheel running in male Min mice found a significant reduction in polyp number in treadmill runners but not in wheel runners compared with sedentary control mice (14). In that study, the running volumes were similar to ours, with their treadmill runners and wheel runners covering an average of 1.1 and 4.7 km/day, respectively. Other differences in the studies may explain the disparate results. Although both studies utilized a 9–10-week training period, the mice in the study by Mehl et al. (14) began the exercise treatment at 3.5 weeks of age, whereas our mice began treatment at 7 weeks of age. More importantly, feeding regimens were also different, with our EX mice pair-fed to the CON mice in order to produce a negative energy balance due to the exercise, while the mice in Mehl et al. (14) were fed ad libitum. As a result, while our EX mice maintained lower body weights throughout the study compared with CON, the wheel runners in Mehl et al. (14) had body weights no different from their non-running controls. Consequently, it could be the negative energy balance rather than the exercise per se that led to the reduction in polyps. Interestingly, however, treadmill running was effective in the Mehl et al. (14) study despite no change in body weight in those mice, which suggests that some non-energy balance-dependent effect of exercise conferred the protection seen in that study. Our study also used a purified AIN-76A diet while the other study used Harlan Teklad Rodent diet no. 8604. While both diets have a similar fat composition (~5%), the vitamin and mineral levels differ. These differences in diet composition may interact with the exercise, resulting in the different effects noted, as dietary composition can clearly affect polyp number in Min mice as we, and others, have reported (8,15). Finally, the fact that all of our mice were surgically implanted with placebo pellets as part of a larger experiment may have influenced our results. While the internal comparison between EX and CON would still be valid, the external validity of our results may be limited.

In our previous study of involuntary treadmill running in Min mice, the treatment had little effect on either polyp number or body fat (7). In contrast, prolonged daily treadmill running decreased both body fat and colon adenomas in a prior study of chemically induced intestinal tumors in rats (4). In the current study with voluntary running wheel exercise, we saw decreased polyp numbers but a modest increase in body fat in the EX mice compared with CON. The percent body fat seen in our CON mice is lower while the polyp numbers were considerably higher than what we saw in our previous study (7), and so it may be possible that our mice were in the early stages of cachexia. Consistent with this idea, a loss of skeletal muscle mass has been documented in Min mice at older ages (16). Additionally, hematocrit values were significantly lower in the CON mice than in the EX mice, probably reflecting anemia made more severe by the increased intestinal polyp burden. Moreover, 26% of CON mice had to be killed early owing to morbidity, while all of the EX mice survived until the end of the study. The higher percent body fat found in the EX mice in this study may thus be indicative of a healthier mouse; by attenuating polyp development, EX also ameliorated the consequent anemia and thereby improved health status and overall survival.

Evidence from both epidemiological and experimental studies suggests that IGF-1 is related to the development of cancer (17). In particular, increased circulating IGF-1 has been directly associated with an increased risk of colon cancer in humans in some studies (18,19). Furthermore, supplementation of tissue culture media with IGF-1 stimulates the in vitro growth of colorectal cells (20) and preneoplastic, but not normal, mouse epithelial cells (21). Liver-specific IGF-1-deficient mice have been used to elegantly demonstrate that circulating IGF-1 plays an important role in the growth and metastasis of transplanted colon adenocarcinomas (22). Given that higher IGF-1 is associated with colorectal cancer and that CR dramatically decreases both polyps and IGF-1 in Min/+ mice (8), it is not unreasonable to hypothesize that physical activity may inhibit colon cancer through a reduction in IGF-1. However, in our study, the voluntary wheel running treatment was associated with an increase in IGF-1 at the time of killing. This may be related to the body composition data such that IGF-1 was upregulated to counteract the decreases in lean mass that we observed in the EX group. Previously, we saw no effect of treadmill exercise on IGF-1 (7), while Mehl et al. (14) saw no effect of either treadmill or wheel running exercise on IGF-1 in Min mice. Other exercise studies in both hamsters and rats have shown no effect of running exercise on IGF-1 levels (23,24). The responses seen in animals are consistent with conclusions drawn in a recent comprehensive review of physical activity and IGF-1 studies in humans (25). Among longitudinal exercise studies in humans, 50% have found no change in IGF-1, 28% an increase in IGF-1 and 23% a decrease in IGF-1 (25). Importantly, there was no association between IGF-1 and the total number of polyps in our study. It is possible that IGF-1 is related to later stages in the carcinogenic process and not to the development of adenomatous polyps; however, this cannot be addressed in Min mice in which more invasive lesions do not develop. It is also possible, however, that without measures of IGF binding proteins, we do not have an accurate picture of the IGF axis. Further studies that measure these binding proteins in addition to IGF-1 may be more informative.

Corticosterone has been implicated in the inhibitory effects of CR on tumorigenesis of various types (12,26–28). Earlier studies suggested that an intact adrenal gland was necessary in order to see the protective effects of CR on skin and lung tumorigenesis (26,27). More recently, adrenalectomy with glucocorticoid supplementation demonstrated that corticosterone was responsible for some, but not all, of the CR effects on skin tumorigenesis (12). In contrast, adrenalectomy did not interfere with the inhibition of chemically induced mammary carcinogenesis by CR in rats (29). Concomitant with a reduction in polyp number due to CR in Min mice, we saw a significant increase in urinary corticosterone output (8). In the current study, urinary corticosterone was increased by the voluntary wheel running-induced negative energy balance but to a lesser extent than that seen with 40% CR (8). This exercise-induced increase could be due to the exercise itself, as has been reported in other mouse models of voluntary wheel running (30,31), or it may be due to the energy deficit created by the pair-feeding to the CON mice in our study. We did not, however, see a significant correlation between levels of corticosterone and polyp number among all mice in this study. This suggests that the higher urinary corticosterone levels associated with the exercise may not have significant direct effects on the inhibition of polyp number.

In summary, 10 weeks of voluntary wheel running exercise increased survival and decreased polyp number by 25% in...
Min mice compared with mice without wheel access. The reduction in polyw npo was correlated with the average amount of running in the EX mice. The exercise treatment with paired feeding to the CON mice was sufficient to induce a negative energy balance as indicated by a lower body weight among the EX mice during the study, although at the time of killing EX mice retained more body fat than CON. Both IGF-1 and corticosterone were increased with voluntary wheel running; however, neither marker was associated with the total polyw npo numbers. These data suggest that voluntary wheel running that induces a negative energy balance can decrease tumorigenesis in Min mice, but that the mechanism is probably unrelated to body composition, IGF-1 or corticosterone.

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Conflict of Interest Statement: None declared.

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