Intestinal tumours, colonic butyrate and sleep in exercised Min mice

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There is strong epidemiological evidence that more physical activity is associated with reduced risk of colon cancer, but the amount or type of activity necessary to invoke this protection is disputed, and the mechanism that is responsible has not been elucidated. The present study compared the effects of two contrasting exercise regimens on intestinal tumourigenesis in Min mice, and investigated two novel mechanistic factors: colonic butyrate and sleep. From 5 weeks of age, Min mice were exercised by running on a treadmill (TR; ≤ 21 m/min, 30–60 min/d, 5 d/week, ≤ 12 weeks). Additional groups of mice were provided with an exercise wheel (WH) or no exercise (CON). Mice had free access to a Western-style, high-fat diet. WH mice ran 3.97 km (females) and 1.92 km (males) daily (P=0.002). There were no differences in body weight gain or body composition between treatment groups. Treadmill running reduced the numbers of larger (≥ 2 mm diameter) tumours (P=0.042), and tended to reduce tumour multiplicity in the colon (P=0.049). TR mice had a higher molar proportion of butyrate in colonic digesta than CON mice (P=0.030), and when treatment groups were combined, there was a weak negative correlation (r=-0.174, P=0.061) between butyrate molar proportion and total tumour number. In a subset of animals in which non-exercise physical activity was monitored, there were strong positive correlations between sleep duration and both tumour multiplicity (P<0.001) and tumour burden (P=0.001). More studies of the effects of sleep and of colonic butyrate in mediating the effects of physical activity on intestinal tumourigenesis are warranted.

Min mice: Exercise: Butyrate: Sleep: Tumours

There is substantial epidemiological evidence (at least fifty studies(1)) to suggest that being physically active can reduce the risk of developing cancer, particularly of the breast and colon(2). Indeed, it has been estimated that high levels of physical activity may reduce the risk of colon cancer by up to 50 % (3). However, physical activity may not help those with a family history of colonic adenomatous polyps(4) or those who have had at least one polyp removed, as physical activity had no effect on polyp recurrence in the Polyp Prevention Trial(5). There may be sex differences in response to physical activity since there was an inverse association between physical activity and polyp recurrence in men only in the Calcium Polyp Prevention Study(6).

A limitation of such observational studies is that they demonstrate associations, but cannot prove causality, between physical activity and colon cancer, and there may be other aspects of lifestyle associated with physical activity (such as diet(7)) that are responsible for the protection against colon cancer. In addition, because physical activity is a complex and heterogeneous behaviour, it is difficult to characterise and quantify, and this imprecision in measurement of exposure exacerbates the difficulty in assessing observational data.

The amount or types of physical activity which may reduce risk of bowel cancer are poorly understood. Human epidemiological studies have used relatively crude instruments to quantify physical activity, including duration and intensity of leisure activity, occupational activity, activity in the past year and lifetime activity. Evidence of the benefit (or otherwise) of specific forms of exercise is obtained more readily from experiments in animal models (as reviewed by Basterfield et al.(8)). Studies using carcinogen-treated rats have been unanimous in finding reduced colon cancer with both voluntary (wheel running) and forced (treadmill running) exercise(9–13). In contrast, the outcomes of studies of exercise in Min mice, which carry a truncating mutation in the Apc gene and develop multiple intestinal tumours spontaneously(14), have been mixed with only a few studies showing protection against tumourigenesis in some circumstances(15–20). The reasons for this heterogeneity in response are not known, but may be related to the use of different sexes, exercise protocols and/or diets. The type of physical activity (treadmill or wheel running), and how much, that protects against intestinal tumourigenesis in Min mice remains unclear, and the mechanisms responsible for protection remain to be discovered.

Poor sleep quality is associated with the metabolic syndrome(21) and obesity(22), and obesity is known to be a risk factor for cancer(7). Interest in the association between

Abbreviations: CON, control treatment; TR, treadmill treatment; WH, wheel treatment.

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sleep and cancer is developing\(^{23}\), although results so far are conflicting\(^{24–28}\). To date, the relationship between sleep and intestinal cancer risk has not been investigated using an animal model of tumourigenesis. The SCFA butyrate is another potentially protective factor which is under-investigated in exercise studies. SCFA are produced by the bacterial fermentation of carbohydrates in the large intestine, and butyrate is used as fuel by colonocytes\(^ {29}\). The anti-bacterial fermentation of carbohydrates in the large intestine, is another potentially protective factor which is under-investigated using an animal model of tumourigenesis. The SCFA butyrate is another potentially protective factor which is under-investigated using an animal model of tumourigenesis. The SCFA butyrate is another potentially protective factor which is under-investigated using an animal model of tumourigenesis. The SCFA butyrate is another potentially protective factor which is under-investigated using an animal model of tumourigenesis. The SCFA butyrate is another potentially protective factor which is under-investigated using an animal model of tumourigenesis. The SCFA butyrate is another potentially protective factor which is under-investigated using an animal model of tumourigenesis. The SCFA butyrate is another potentially protective factor which is under-investigated using an animal model of tumourigenesis. The SCFA butyrate is another potentially protective factor which is under-investigated using an animal model of tumourigenesis. The SCFA butyrate is another potentially protective factor which is under-investigated using an animal model of tumourigenesis. The SCFA butyrate is another potentially protective factor which is under-investigated using an animal model of tumourigenesis. The SCFA butyrate is another potentially protective factor which is under-investigated using an animal model of tumourigenesis. The SCFA butyrate is another potentially protective factor which is under-investigated using an animal model of tumourigenesis. The SCFA butyrate is another potentially protective factor which is under-investigated using an animal model of tumourigenesis. The SCFA butyrate is another potentially protective factor which is under-investigated using an animal model of tumourigenesis. The SCFA butyrate is another potentially protective factor which is under-investigated using an animal model of tumourigenesis. The SCFA butyrate is another potentially protective factor which is under-investigated using an animal model of tumourigenesis. The SCFA butyrate is another potentially protective factor which is under-investigated using an animal model of tumourigenesis. The SCFA butyrate is another potentially protective factor which is under-investigated using an animal model of tumourigenesis. The SCFA butyrate is another potentially protective factor which is under-investigated using an animal model of tumourigenesis. The SCFA butyrate is another potentially protective factor which is under-investigated using an animal model of tumourigenesis. The SCFA butyrate is another potentially protective factor which is under-investigated using an animal model of tumourigenesis. The SCFA butyrate is another potentially protective factor which is under-investigated using an animal model of tumourigenesis. The SCFA butyrate is another potentially protective factor which is under-investigated using an animal model of tumourigenesis. The SCFA butyrate is another potentially protective factor which is under-investigated using an animal model of tumourigenesis. The SCFA butyrate is another potentially protective factor which is under-investigated using an animal model of tumourigenesis. The SCFA butyrate is another potentially protective factor which is under-investigated using an animal model of tumourigenesis. The SCFA butyrate is another potentially protective factor which is under-investigated using an animal model of tumourigenesis. The SCFA butyrate is another potentially protective factor which is under-investigated using an animal model of tumourigenesis. The SCFA butyrate is another potentially protective factor which is under-investigated using an animal model of tumourigenesis. The SCFA butyrate is another potentially protective factor which is under-investigated using an animal model of tumourigenesis. The SCFA butyrate is another potentially protective factor which is under-investigated using an animal model of tumourigenesis. The SCFA butyrate is another potentially protective factor which is under-investigated using an animal model of tumourigenesis. The SCFA butyrate is another potentially protective factor which is under-investigated using an animal model of tumourigenesis.
Exercise and tumourigenesis in Min mice

Table 1. Food intake, weight gain and percentage body fat of mice by exercise group
(Mean values with their standard errors and 95 % confidence intervals)

<table>
<thead>
<tr>
<th>Variable</th>
<th>TR (n 41)</th>
<th>WH (n 43)</th>
<th>CON (n 52)</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food intake (g/d)</td>
<td>2.08 ± 0.03</td>
<td>2.26 ± 0.03</td>
<td>2.14 ± 0.03</td>
<td>0.364</td>
</tr>
<tr>
<td>Weight gain over study (g)</td>
<td>7.3 ± 3-8.4</td>
<td>7.2 ± 6-8.3</td>
<td>8.1 ± 7.1, 9.1</td>
<td>0.459</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>30.9 ± 2.09</td>
<td>23.5 ± 1.82</td>
<td>23.9 ± 2.05</td>
<td>0.037</td>
</tr>
</tbody>
</table>

TR, treadmill running; WH, wheel running; CON, control (not exercised).

Results

Seven female mice and twelve male mice did not complete the initial 2-week training period on the treadmill satisfactorily, and were removed from the study, as per previous investigations (16,17), leaving twenty-four female and seventeen male mice in the TR treatment group. A higher proportion of female (18/24) than of male (8/17) mice ran on every possible day (P = 0.048). There was no difference between the sexes in the median time spent on the treadmill in each session. As they completed the majority of the exercise protocol (twenty-six mice ran on all days, nine ran between 81 and 99 % of days, and six ran between 68 and 80 % of days), results from all mice are included in this analysis (intention to treat analysis). During the 10-week study, female WH mice ran approximately twice as far (P = 0.002) in their wheels as did males, and the mean distances run were 276 (SEM 26.0) v. 136 (SEM 32.1) km, which equate to 3.97 and 1.92 km/d for female and male mice, respectively.

WH mice consumed significantly (P = 0.015) more food than CON mice (Table 1) whose intake was similar to that of TR mice. There was no difference between treatment groups for either carcass weight, weight gained over the study or body composition (Table 1). Weekly weight gain was similar for each treatment group, except at week 6 when CON mice gained 0.5 g more than both TR and WH mice (P = 0.017 for TR v. CON, P = 0.021 WH v. CON).

Total numbers of intestinal tumours were very similar for WH, CON and TR mice (Table 2), but TR mice had significantly (P = 0.042) fewer larger (≥ 2 mm) tumours than CON mice. TR mice showed a trend for fewer tumours in all sections of the intestine, but, with the exception of the colon (P = 0.049), the differences between TR and CON mice were NS (P > 0.05) when analysed by anatomical site. For the WH mice, there was no correlation between distance run in wheels in 10 weeks and the total number of tumours (r = -0.096, P = 0.539), or the tumour burden (the sum of tumour diameters, r = -0.120, P = 0.444). Combined data showed a negative correlation between weight gained over the study and total tumour number (r = -0.372, P < 0.001).

The haematocrits of TR (29.6 ± 1.3 %) and WH (28.4 ± 1.4 %) mice were very similar, and both were significantly higher than that of CON (24.5 ± 1.2 %) mice (P = 0.009 and 0.040, respectively). The spleens of the two exercising groups were significantly lighter (TR 197 mg, WH 247 mg) than those of the CON mice (334 mg; P = 0.007 and 0.055 for comparisons of TR and WH with CON, respectively). There was a strong inverse correlation (r = -0.580, P < 0.001) between spleen weight and haematocrit (Fig. 1), and spleen weight was positively correlated with tumour number (r = 0.373, P = 0.002).

The type of exercise had a significant effect on the pattern of colonic SCFA (Table 3). For TR mice, the reduction in acetate molar proportion approached significance compared with the CON mice (P = 0.067), whereas butyrate molar proportion was increased by 50 % compared with the CON mice (P = 0.030). For WH mice, propionate was increased significantly (P = 0.019) compared with the CON mice. This observation of differential effects of exercise regimen on colonic SCFA pattern highlights an area of ignorance about the impact of physical activity on intestinal function. It may be relevant to note that treadmill running was restricted to

![Image](https://i.imgur.com/5EY.png)
the light phase of the day, whereas the majority of the wheel running occurred in the dark phase. Given the diurnal patterns of food consumption by mice that were fed ad libitum (most food is eaten in the dark phase of the day), there may be some interaction between timing of food ingestion, exercise and large bowel bacterial activity. When the treatment groups were combined, there was a negative correlation (\( r = -0.174 \), \( P = 0.061 \)) between butyrate molar proportion and total tumour number.

Non-exercise physical activity was measured in a subsample of twelve mice (seven TR and five CON), with a mean of five separate 23 h continuous recordings per mouse. With this subsample of mice, there was no significant (\( P>0.05 \)) difference in tumour number or tumour burden between the groups, but the TR mice tended to have fewer tumours (12.1 (SEM 4.3) for TR and 24.1 (SEM 5.0) for CON, \( P=0.104 \)) in parallel with the findings of the main study. During the 23 h per day when they were in their home cage, there was a trend for TR mice to be more active than CON mice (1377 ± 240 vs. 1132 ± 192 counts, \( P = 0.084 \)), with this difference in activity being confined to the dark period. There was no difference in total sleep time between the groups (\( P = 0.125 \)), or in sleep during the dark (\( P = 0.111 \)) or light (\( P = 0.793 \)) phase. However, there were negative correlations between total activity and tumour burden (Pearson’s \( r = -0.559 \), \( P = 0.059 \), Fig. 2(a)), total activity and tumour number (\( r = -0.558 \), \( P = 0.059 \), Fig. 2(b)), and dark activity and tumour burden (\( r = -0.559 \), \( P = 0.040 \)). There were significant positive correlations between dark sleep and tumour burden (\( r = 0.835 \), \( P = 0.001 \), Fig. 2(c)), total sleep time and tumour number (\( r = 0.872 \), \( P = 0.001 \), Fig. 2(d)), and total sleep and tumour burden (\( r = 0.841 \), \( P = 0.001 \)). There was no correlation between activity and final body mass.

### Discussion

The present study adds to the body of work on the effects of exercise on tumourigenesis in Min mice by demonstrating a small reduction in larger tumours (\( P = 0.042 \)) and in colon tumours (\( P = 0.049 \)) following treadmill running. We report the novel observation of altered SCFA proportions in the colon, in particular, an increase in the anti-neoplastic butyrate, in response to treadmill running. Furthermore, we provide preliminary evidence that time spent sleeping is correlated strongly with both tumour number and tumour burden.

The aim of the present study was to help resolve controversies arising from the six previously published studies of the effect of exercise on tumourigenesis in Min mice, which have reported conflicting results (see Table 4 for summary). In contrast with the heterogeneity of outcomes from Min mouse studies, there were consistently protective effects of both voluntary and involuntary forms of exercise in previous experiments with carcinogen-treated rats (9–13). There is no readily apparent reason for the inconsistent effects of exercise in the Min mouse studies, but differences in experimental protocols between the studies, e.g. the ages at the start of the procedures, the length of the study and the number of tumours generated per mouse, may help explain the differing results (Table 4). In the rat studies where a protective effect of exercise was apparent, only males were used, whereas both male and female Min mice have been used in most studies. There may be interactions between sex and environmental factors on tumourigenesis in Min mice since McKay et al. (33) observed more tumours in females than in males, and this difference and total tumour numbers were reduced by feeding a low-folate diet.

The optimum mode of exercise (forced treadmill running or voluntary wheel running) to reduce tumourigenesis in Min mice has not been established yet. Although it seems counter-intuitive, treadmill running (which results in shorter running distances than wheel running) reduced tumour

### Table 3. Effect of treadmill (TR) and wheel (WH) running on molar proportions (mmol/mol) of SCFA in the colon of Min mice compared with the control (CON) animals

<table>
<thead>
<tr>
<th>SCFA</th>
<th>TR (n 30) Mean (95% CI)</th>
<th>WH (n 47) Mean (95% CI)</th>
<th>CON (n 41) Mean (95% CI)</th>
<th>Probability of effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetate</td>
<td>748 (723, 773)</td>
<td>757 (737, 777)</td>
<td>782 (758, 807)</td>
<td>0.067</td>
</tr>
<tr>
<td>Propionate</td>
<td>107 (95, 120)</td>
<td>123 (112, 135)</td>
<td>103 (92, 115)</td>
<td>0.842</td>
</tr>
<tr>
<td>Isobutyrate</td>
<td>14 (12, 16)</td>
<td>16 (14, 17)</td>
<td>14 (12, 16)</td>
<td>0.942</td>
</tr>
<tr>
<td>Butyrate</td>
<td>75 (58, 95)</td>
<td>50 (39, 62)</td>
<td>50 (38, 62)</td>
<td>0.030</td>
</tr>
<tr>
<td>Isovalerate</td>
<td>20 (17, 22)</td>
<td>21 (19, 23)</td>
<td>19 (17, 21)</td>
<td>0.717</td>
</tr>
<tr>
<td>Valerate</td>
<td>13 (9, 16)</td>
<td>12 (10, 14)</td>
<td>10 (7, 13)</td>
<td>0.327</td>
</tr>
</tbody>
</table>

TR, treadmill running; WH, wheel running; CON, control (not exercised).
number in male Min mice, whereas wheel running had no effect in the study done by Mehl et al. (17). This mode of exercise difference was confirmed in the present study where tumours were smaller and numbers were lower in all sections of the intestine of TR mice but not of WH mice (Table 2).

Increased adiposity is a risk factor for cancer (7), and energetic restriction studies (which reduce body mass and adiposity) decrease tumour risk in human subjects (36) and Min mice (37). Therefore, if exercise lowers body mass it may also lead to a reduction in tumour load. Four of five carcinogen-treated rat studies (9–12) reported that exercise decreased body weight, and a similar effect was observed in two Min mice studies (16,18), one of which found an inverse relationship between polypl number and percentage body fat (18), but not in the other two studies, neither of which reports any associations between body weight and tumour multiplicity (15,17). Ju et al. (19) reported a reduction in body fat but not in body mass with wheel running exercise in female Min mice, and Baltgalvis et al. (20) did not observe a reduction in either body mass or fat pad mass with exercise. In the present study, we did not observe an effect of exercise on either final weight or body composition, in agreement with recent studies reporting no effect of wheel running on body mass or fat mass in female C57BL/6 mice (38). However, where adiposity is measured at the end of the studies of tumourigenesis in Min mice (i.e., all studies to date), outcomes should be interpreted with caution since cachexia accompanying intestinal tumourigenesis may have profound effects on food intake and body composition. Colbert et al. (18) observed that male wheel-running mice had higher levels of body fat, and suggested that this may indicate that WH mice were healthier than the CON mice which showed signs of cachexia (loss of muscle and fat tissue) (18,39).

A recent study (40) has suggested that the severest cachectic symptoms are observed in Min mice with the highest levels of circulating IL-6 and greatest tumour multiplicity. Although we did not find a significant correlation between tumour number and body fat ($r^2 = 0.214, P = 0.098$), an observation also noted by Ju et al. (19) and Baltgalvis et al. (20), there was a significant negative correlation between total tumour number and weight gained over the study ($P < 0.001$).

Inter-study differences in tumour outcome with different modes of exercise may be explained by differential effects on the energy balance of the experimental mice. Most previous studies of effects of exercise in Min mice have used low-fat diets, but tumour number can be increased by feeding a higher fat diet (41). Our mice were given a high-fat diet that reflected human Western diets, as high-fat diets are associated with increased risk of colon cancer in human subjects (42). WH mice, but not TR mice, ate significantly more than CON mice, presumably to compensate for their extra energy expenditure. This greater energy intake by the WH mice may explain the lack of difference in body weights compared with CON mice. The provision of energy-dense food ad libitum in the
| Types of exercise | Exercise protocol | Study duration | Males | | | Females | | | | | Tumour multiplicity (polyps/mouse) |
| Treadmill | 60 min/d at 18–21 m/min, 5% gradient, 5 d/week | 7 weeks (4 weeks old at the start) | Control | 36.8 | 4.9 | | | | | | Control | 38.0 | 6.3 | | | | | | | | No significant effect of exercise for either sex |
| Treadmill | 45 min at 20 m/min, 5% gradient, 5 d/week | 8 weeks (5–6 weeks old at the start, 0–3 weeks in wheel, treadmill thereafter) | Control | 8.8 | 1.2 | | | | | | Control | 6.5 | 0.9 | | | | | | | | No significant effect of exercise for either sex |
| Treadmill | 60 min/d at 18 m/min, 5% gradient, 6 d/week or free access to wheel | 9 weeks (3–5 weeks old at the start) | Control | 93.0 | 7.0 | | | | | | Control | 103.0 | 8.0 | | | | | | | | No effect of exercise Studies in males only |
| Wheel | Free access | 10 weeks (7 weeks old at the start) | Control | 20.7 | 1.5 | | | | | | Control | 20.2 | 0.6 | | | | | | | | Studies in females only P=0.015 NS (colon) |
| Wheel | Free access (shared housing) | 6 weeks (5 weeks old at the start) | Control | 29.2 (SI) | 4.4 (SI) | | | | | | Control | 20.4 (SI) | 0.6 (colon) | | | | | | | | Studies in females only P=0.015 NS (colon) |
| Wheel (high-fat diet) | Free access (shared housing) | 9 weeks (5 weeks old at the start) | Control | 30.8 (SI) | 3.4 (SI) | | | | | | Control | 22.0 (SI) | 0.4 (colon) | | | | | | | | Studies in females only P=0.05 NS (colon) |
| Treadmill | 60 min/d at 18 m/min, 5% gradient, 6 d/week | 6 weeks (4 weeks old at the start) | Control | ~20† | | | | | | Control | ~10† | | | | | | | | Studies in males only P=0.05 |
| Treadmill (high-fat diet) | 60 min/d at 18 m/min, 5% gradient, 6 d/week | 6 weeks (4 weeks old at the start) | Control | ~35† | | | | | | Control | ~43† | | | | | | | | Studies in males only P=0.05 |

SI, small intestine.
* Adapted from Basterfield et al. (8).
† Exact data not published.
present study may have enabled our exercising mice to match their greater energy outputs with higher energy intakes, so preventing any change in energy balance, and this may explain the more modest effects of exercise on tumourigenesis seen in the present study. In contrast, some previous studies have ‘pair-matched’ food intake of exercising mice with that of non-exercising controls to induce negative energy balance.16,18) Mehli et al.17 who fed their mice ad libitum also found reduced tumour numbers with only treadmill running, and reported increased food intake by WH-running mice. Baltgalvis et al.20 showed that male TR mice on a low-fat diet had fewer tumours than controls, an effect that was negated by feeding a high-fat diet.20 Thus, changes in energy balance may be more important than energy intake per se in determining effects on intestinal tumourigenesis, and this area deserves further exploration.

Insulin-like growth factor 1 (IGF-1)16,18, IL-617, corticosterone16,18, inflammation17,20 and leptin16,20 have been investigated as possible mechanisms or mediators for the protective effect of exercise, but with no clear results. In the present study, we investigated the possibility that exercise alters the exposure of intestinal epithelial cells (the cells from which tumours develop) to pro- or anti-neoplastic substrates. We focused on butyrate which is one of the most potent anti-neoplastic components of the intestinal milieu and which suppresses tumour cell proliferation, increases apoptosis, induces differentiation and enhances immunosurveillance.30 Although several factors in food are known to alter SCFA concentrations34,43 and changes in the microbiota in the large intestine in response to diet are associated with reduced tumour number in Min mice44, the present study is the first to report effects of exercise on colonic SCFA in Min mice. We observed that the colon contents of TR mice contained a significantly (P=0.030) greater proportion of butyrate than those of CON mice, and this greater exposure to butyrate may help to explain the reduced colonic tumour number and smaller tumours in TR mice. There was a negative correlation (r = -0.174, P=0.061) between butyrate molar proportion and total tumour number in the present study. The mechanism through which exercise alters intestinal production of butyrate (and other SCFA) is not known, but evidence from rats indicates that changes in the caecal microbiota may be critical in mediating increases in caecal butyrate.45 There are several potential means by which exercise could affect intestinal microbiota, including changes in intestinal transit time,46 intestinal immune system or endogenous substances such as mucin.45 In the present study, we found no effect of exercise on caecal transit time (data not shown). However, these observations suggest that future intervention studies should also investigate butyrate production.

There is increasing interest in the role of sleep in modulating cancer risk with evidence that increased sleep may reduce the risk of both breast24,25,27 and prostate cancers.26 In contrast, other studies have reported no convincing evidence for a relationship between sleep duration and breast cancer risk28 or a modest increased risk with greater sleep duration.47. Relationships between sleep and bowel cancer risk are not known. We observed strong positive correlations between sleep duration and tumour multiplicity (P<0.0001) and sleep during the dark phase and tumour burden (P=0.001; Fig. 2). It has been hypothesised that there is mutual reinforcement between circadian rhythms of melatonin production, the sleep/wake cycle and immune function23 and evidence that biological clock proteins may interact with the cell cycle/DNA damage response pathway to alter cancer risk. Others28 have observed an increase in sleep with increased body weight in mice fed a high-fat diet, and the complex interrelationships between physical activity, sleep, energy balance and cancer risk are a potentially important new research focus. The availability of robust techniques for quantifying sleep duration in animal models such as Min mice provides an exciting opportunity to investigate the putative relationship between sleep patterns and development of cancer.

Stress is another potentially important mediator of the effects of exercise regimens on intestinal tumourigenesis. TR mice were potentially exposed to extra stress during the current regimen, as the treadmill protocol occurred during the light phase of the day (when mice are often asleep), whereas most WH activity took place during the dark phase. Our investigation of non-exercise physical activity, however, suggests that TR mice did not behave differently from CON mice after they were returned to their home cages, as the amount of activity and sleep during the light period (when the intervention occurred) was not different between the two groups. This suggests that treadmill running is no more stressful than moving the CON mice to the individual control lanes, but the effect of exercise in the light phase v. dark phase of the day on intestinal tumourigenesis remains to be investigated. Our observation that greater non-exercise physical activity was associated with reduced tumourigenesis is difficult to explain in light of the lack of effect of wheel running, and warrants further investigation.

Chronic inflammation increases the risk of colorectal cancer possibly through oxidative damage from reactive oxygen and nitrogen species generated by neutrophils and macrophages in the inflamed tissue.50 An enlarged spleen is symptomatic of chronic inflammation, as demonstrated by the development of colitis in IL-2-deficient mice.51 Mehli et al.17 observed lower spleen size with exercise in male, but not in female, Min mice, and the authors suggested that reduced systemic inflammation in male mice may have been partially responsible for the reduction in tumour number. Our pooled data showing a positive correlation between spleen weight and tumour number support this hypothesis. In female C57BL/6 mice, Rogers et al.38 observed that wheel running reduced spleen weight and increased mucosal cytokine production and T-cell proliferation in Peyer’s patch cells.

In summary, treadmill running, but not wheel running, reduced slightly the number of larger (≥2 mm) intestinal tumours, and tended to reduce tumour multiplicity in the colon (P=0.049) of Min mice. We have observed for the first time that treadmill exercise increases colonic butyrate in Min mice, and that there was an inverse correlation between butyrate and tumour multiplicity. Finally, we present novel observations of relationships between sleep duration and both tumour multiplicity and tumour burden. These findings offer novel avenues for exploration of mechanisms through which exercise (and other lifestyle interventions) may prevent intestinal tumourigenesis.
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References


