Exercise Improves Cancer-free Survival and Health Span in a Model of Radiation-induced Cancer

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ABSTRACT

FARBER, E., J. M. KWIECIEN, D. BOJIC, M. NGU, P. AKOHENE-MENSAH, J. J. VANHIE, J. LLOYD, J. LARKIN, and M. DE LISIO. Exercise Improves Cancer-free Survival and Health Span in a Model of Radiation-induced Cancer. *Med. Sci. Sports Exerc.*, Vol. 53, No. 11, pp. 2254–2263, 2021. **Introduction:** Radiation therapy increases the risk of secondary malignancy and morbidity in cancer survivors. The role of obesity and exercise training in modulating this risk is not well understood. As such, we used a preclinical model of radiation-induced malignancy to investigate whether diet-induced obesity and/or endurance exercise training altered lifelong survival, cancer incidence, and morbidity. **Methods:** Male CBA mice were randomly divided into control diet/sedentary group (CTRL/SED), high-fat diet (45% fat)/sedentary group (HFD/SED), control diet/exercise group (2–3 $d{\cdot}wk^{-1}$; CTRL/EX), or high-fat diet/exercise group (HFD/EX) groups then exposed to whole-body radiation (3 Gy). End point monitoring and pathology determined mortality and cancer incidence, respectively. Health span index, a measure of morbidity, was determined by a composite measure of 10 anthropometric, metabolic, performance, and behavioral measures. **Results:** Overall survival was higher in HFD/SED compared with CTRL/SED ($P < 0.05$). The risk of cancer-related mortality by 18 months postradiation was 1.99 and 1.63 in HFD/SED compared with CTRL/EX (RR = 1.99, 95% confidence interval = 1.20–3.31, $P = 0.0081$) and CTRL/SED (RR = 1.63, 95% confidence interval = 1.06–2.49, $P = 0.0250$), respectively. The number of mice at end point with cancer was higher in HFD/SED compared with CTRL/EX and CTRL/SED ($P < 0.05$). Health span index was highest in CTRL/EX (score = +2.5), followed by HFD/EX (score = +1), and HFD/SED (score = −1) relative to CTRL/SED. **Conclusion:** This work provides the basis for future preclinical studies investigating the dose–response relationship between exercise training and late effects of radiation therapy as well as the mechanisms responsible for these effects. **Key Words:** PHYSICAL ACTIVITY, HIGH-FAT DIET, OBESITY, CANCER THERAPY, CANCER RECURRENCE, CANCER-RELATED MORTALITY

Cancer survival is currently reported to be 63% and 67% in Canada and the United States, respectively, with the number of cancer survivors expected to increase in the coming years (1,2). Radiation therapy, used to treat at least half of patients with cancer, has numerous negative late effects, including increased risk of developing secondary cancers and morbidity. Adult cancer survivors have a 1.5- to 30-fold higher risk of developing radiation-induced cancers (3), with radiation-induced cancers accounting for 4%–24% of all secondary malignant neoplasms (4). The risk of radiation-induced malignancy is higher in childhood cancer survivors (5), and the prevalence of radiation-induced cancer has not significantly improved despite improvements in radiation delivery (5,6). Incidence of secondary cancers varies based on radiation dose, amount of healthy tissue exposed, age at time of radiation exposure, and length of time since treatment (7). Further, radiation therapy is most often delivered in conjunction with some other treatment modality (i.e., chemotherapy and/or surgery), which can also further increase the risk and prevalence of

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long-term treatment effects. (8). In addition to increased risk of secondary cancers, up to 80% of some cancer survivor populations experience long-term radiation-induced morbidity (9). These long-term consequences related to morbidity include reduced quality of life, decreased physical function, depression, and anxiety (10). Although the biological and treatment-related factors have been the focus of the cancer survivorship literature, comparatively less attention has been paid to the role of lifestyle factors in long-term, late effects of therapy in cancer survivors (3).

A cancer diagnosis is associated with weight gain and reduced physical activity (11). Conversely, maintaining or increasing physical activity through exercise training is associated with a reduced cancer risk in general (12) and improved physical and psychological health, as well as increased overall and cancer-specific survival in cancer survivors (10,13). Obesity and physical inactivity are two such risk factors that have been epidemiologically linked to cancer incidence (14) and morbidity (15) independent of a cancer diagnosis. Further, increased participation in exercise has been linked to decreased cancer recurrence in some studies (16), whereas obesity is associated with reduced survival overall, but data related to recurrence are currently insufficient (17). However, there is limited direct evidence that exercise improves survival in cancer survivors with only a few exploratory studies indicating that exercise may improve overall and progression-free survival in patients with breast, colorectal, and prostate cancer with little consideration on the role of therapy (18,19). Thus, developing a better understanding of the role of exercise training, particularly in the context of obesity, in mitigating the late effects of radiation therapy could have significant clinical relevance.

Given the challenges associated with conducting long-term exercise training studies with sufficient length of patient follow-up to determine the direct effects of exercise training on radiation-induced cancers, translational preclinical studies are warranted. Therefore, we used an established mouse model of radiation-induced cancer (20–22) to evaluate the effects of lifelong endurance exercise training with or without high-fat diet (HFD)-induced obesity on survival, cancer development, and health span after radiation exposure. We hypothesized that sedentary mice with HFD-induced obesity would have lower overall and cancer-related survival, higher incidence of cancer development, and lower health span compared with lean, exercise-trained mice after radiation exposure. Furthermore, we predicted that exercise-trained mice with diet-induced obesity will have longer survival, decreased cancer development, and improved health span compared with sedentary mice with diet-induced obesity, but similar to lean, sedentary mice after radiation exposure.

METHODS

Study Design

Ethical approval for this project was obtained from the University of Ottawa Animal Care and Veterinary Service Committee. Male CBA mice age 4 wk (n = 80; The Jackson Laboratory, Bar Harbor, ME) were provided food and water *ad libitum,* maintained on a 12-h light–dark cycle, and housed under specific pathogen-free conditions for the duration of the study. Male CBA mice are the currently favored model of radiation-induced neoplasms, particularly acute myeloid leukemia (AML) (22). Males from the CBA strain are particularly sensitive to radiation, the radiation-induced pathology closely resembles that in humans (23), spontaneous AML is infrequent (22), and AML is a common secondary neoplasm among patients receiving radiation therapy (24). At 5 wk of age, mice were randomly divided into either a control diet (CTRL, n = 40) or an HFD group (n = 40) and housed at no more than four mice per cage. At 9 wk of age, each dietary group was randomly divided to either an endurance exercise training (EX) or a sedentary (SED) group, making the following four groups: CTRL/SED, CTRL/EX, HFD/SED, and HFD/EX (n = 20 per group). Mice were placed in their respective groups before radiation exposure so they were exposed while obese or exercise trained and continued in with their interventions until end point (Fig. 1). The timing of the interventions relative to radiation exposure allowed physiological adaptations so we could investigate the effects of radiation exposure under these physiological conditions followed by lifelong continuation of this lifestyle. This approach aligns with recommendations for human cancer survivorship studies (25) and builds on our previous preclinical work (26). In addition, a fifth group of male CBA mice was purchased from The Jackson Laboratory at 9 months of age and were not exposed to radiation, fed the CTRL diet, and remained sedentary (NON-IR). One mouse was removed from this group upon arrival because of an injury in shipping (n = 9). NON-IR mice were sacrificed at end point or 23 months of age.

Dietary Intervention

The HFD consisted of 45% kcal from fat (D12451, Research Diet Inc., New Brunswick, NJ) to induce obesity, and the CTRL and NON-IR diet consisted of 9.4% kcal from fat with matched micronutrient content to the HFD (D10012M, Research Diet Inc.). Body weight and food intake (in grams and kilocalories) were measured weekly and averaged across mice in each cage, whereas body composition was measured monthly using an EchoMRI-900 (EchoMRI LLC, Houston, TX).

Exercise Intervention

The endurance exercise intervention was delivered using a motorized treadmill (Exer6; Columbus Instruments, Columbus, OH) as previously described (26–30). Exercise sessions were conducted during the light cycle at approximately the same time each day. Sessions were conducted on three alternating days per week until 14 months of age after which their exercise frequency was reduced to 2 $d \cdot wk^{-1}$. Each session began with a 10-min warm-up at 10 $m \cdot min^{-1}$, followed by a 45-min training period starting at 10 $m \cdot min^{-1}$ with weekly increases of 1 $m \cdot min^{-1}$ up to 24 $m \cdot min^{-1}$. Electric shock was not used, and mice were

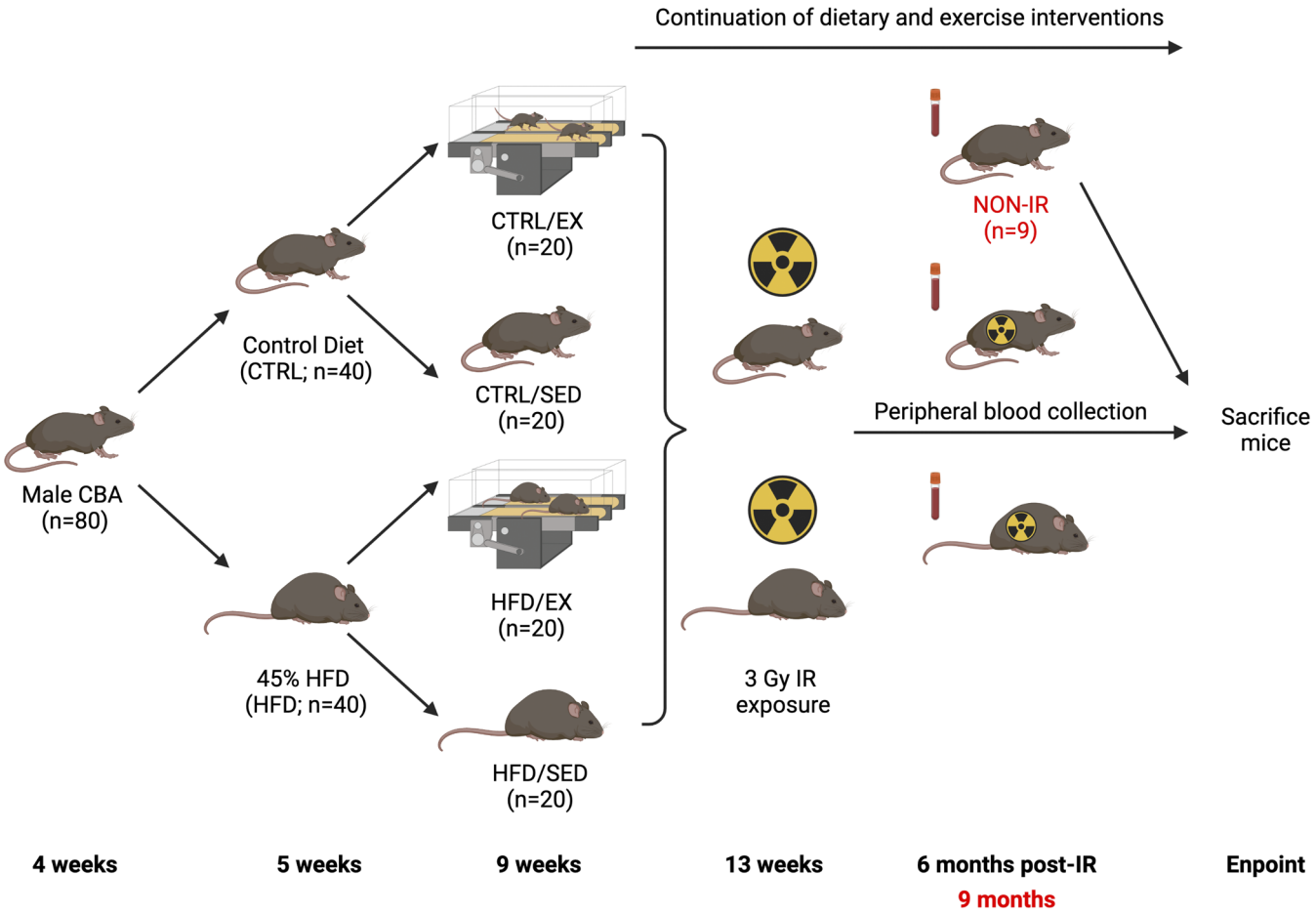


FIGURE 1—Study design. A schematic of the experimental timeline. Male CBA mice were randomized to HFD or control diet group at 5 wk of age. At 9 wk of age, mice in each diet condition were further randomized into sedentary and exercise-trained groups. Mice received a 3-Gy dose of whole body ionizing radiation at 13 wk of age and continued in their intervention group until end point. Peripheral blood was collected bimonthly starting at 6 months postradiation. A group of nonirradiated mice were purchased at 10 months of age and maintained in our facility on the control diet under sedentary conditions until they reached end point or 23 months of age.

encouraged to run by stimulation with the bristles of a paintbrush. Sedentary mice were placed in a sham treadmill for the duration of the exercise training session. This exercise program of 2–3 d·wk^{-1} of moderate-intensity exercise is translatable to current exercise guidelines for cancer survivors recommended by the American College of Sports Medicine (10).

Radiation Challenge

At age 13 wk, all mice were exposed to a single 3 Gy dose of total body gamma radiation over 4.32 min using an XRAD 320 radiation unit (Precision X-Ray Inc., East Haven, CT). This radiation dose administered at this age is sublethal and has been shown to induce AML in 25% of male CBA mice by 18 months after radiation exposure (20). Nonanesthetized mice were confined in a small container during radiation to ensure delivery of a uniform radiation dose with the field of the beam being larger than the container. After radiation exposure, mice were provided Baytril water (50 mg·mL^{-1}) *ad libitum* for 3 wk and were withheld from the subsequent exercise training session.

Cancer Development

At end point, livers and spleens were excised, weighed, visually inspected for macroscopic tumors, and stored in 10% neutral buffered formalin. Both femurs were fixed in 10% neutral buffered formalin for 48 h, placed in a 14% EDTA decalcification solution for 14 d then, stored in 70% ethanol at 4°C (31). Sections at 4 µm thickness were stained with hematoxylin and eosin then evaluated for microscopic tumors by a veterinary research pathologist. From 6 months postradiation until end point, blood was collected bimonthly from the facial vein into EDTA-coated microvette capillary tube (Thermo Fisher Scientific, Waltham, MA, NC9141704). Blood was used for complete blood counts (CBC; Centre for Phenogenomics, Toronto, ON, Canada) or stained with Wright-Giemsa for circulating blast cell quantification.

End Point Monitoring

Health checks were performed several times per week. Mice were considered to be at end point if they displayed

any of the following conditions that did not improve after 3 d: a body condition less than three according to the mouse body condition scoring system, hunched, ruffled coat, skin lesions, dehydration, lethargy, pale mucous membranes, or masses (28,32). Body condition less than three refers to a mouse who is emaciated or underconditioned so that the segmentation of their vertebral column is visible and their dorsal pelvic bones are palpable (28,32). Mice that were moribund or seizing (either lasting longer than 30 s or more than three seizures in less than 1 min) were considered to be immediately at end point (28,32). Mice in the NON-IR group that did not reach end point were sacrificed at 23 months of age. Time to end point was considered mortality for life span analysis.

Behavior Testing

All behavior tests were conducted when mice reached age 17 months and were preceded by a 30-min acclimatization period in the testing room. Cancer survivors face a broad range of negative health consequences, including negative changes to body composition, reduced physical functioning, anxiety, and depression (10). As such, we undertook a comprehensive analysis of these outcomes using well-established preclinical tests. Performance on these tests was combined to calculate an overall health span index as previously described (33).

Exercise endurance test. Exercise endurance tests were conducted as previously described (30,34). Briefly, the test began at a speed of 11 $m \cdot min^{-1}$ and increased by 1 $m \cdot min^{-1}$ every 2 min until a mouse was either resistant to motivation, remained on the treadmill platform for longer than five continuous seconds, or ran approximately one body length away from the platform for longer than 2 min. The speed and time at which each mouse finished the test was recorded. Electric shock was not used during the test.

Metabolic activity. Oxygen consumption ($\dot{V}O_2$ and $\dot{V}CO_2$), heat production, and RER were measured using the OXYMAX Comprehensive Lab Animal Monitoring System (CLAMS; Columbus Instruments). Mice were tested at thermal neutral conditions in individual CLAMS-specific housing chambers for 72 h. The first 48 h was used to allow the mice to habituate to the new environment, and only data collected in the final 24 h were analyzed. Mice were provided water and their specialized diet in powdered form *ad libitum* and maintained on their regular 12-h light–12-h dark cycle during the test.

Beam break (BBK). The BBK test was used to assess anxiety-like behavior (35). Mice were individually housed in new cages placed between photocell emitters, which sent horizontal beams of infrared light through the cage then monitored for 24 h. Ambulatory movement per hour, for each mouse (i.e., number of infrared beams broken during ambulation), was analyzed using the Fusion 5.3 software for Micromax (Omnitech Electronics, Inc., Columbus, OH).

Forelimb grip strength. Forelimb grip strength was evaluated using the Chantillion DFE II (Columbus Instruments) as previously described (36). Mice grabbed the force gauge grid with their front paws and were gently pulled horizontally off the grid with a constant and consistent speed. Eight trials were completed per mouse by the same investigator. Grip strength was averaged after removing the highest and lowest measurement and normalized to body weight.

Tail suspension. The tail suspension test was used test to assess depression-like behavior (37). Mice were taped upside down by their tail to a bar connected to a strain gauge for 6 min. Immobility time was measured as the total amount of time that a mouse spent below the preset lower strain threshold.

Data Analysis

Sample size was calculated using previous data from our laboratory (28) and others (38) that evaluated mortality in mice. Sample size was estimated for a two-sided test of survival probability using the following parameters: accrual time (18 months), follow-up time (24 months), null media survival (18 months), alt median survival (20 months), null survival probability (0.70), and alt survival probability (0.95) at 18 months with $\beta = 0.80$ and $\alpha = 0.05$ (Survival Tool; Cancer Research and Biostatistics, Seattle, WA). Based on the above calculation, a sample size of 11 mice per group would have provided sufficient power. This sample size is more than what has previously been used in the literature to detect significant effects of exercise on mortality (33). We included an $n = 20$ per group to account for potential mouse loss because of unforeseen circumstances. Mice were randomly assigned to each experimental group using simple randomization. Experimenters were blind to group assignment during outcome assessment. Data are presented as mean ± SEM with $P < 0.05$ considered significant.

Kaplan–Meier survival curves were created for each group to assess overall survival. The log-rank test was conducted to determine significant differences between curves. Cumulative incidence and incidence rate of cancer-related mortality curves were created for each group at different points up to 18 months after radiation. Cumulative incidence of cancer-related mortality was calculated as the number of mice that reached end point with cancer over the number of mice at risk of dying from cancer at a given time point. The incidence rate of cancer-related mortality was calculated as the number of mice that reached end point with cancer divided by follow time broken up into 3-month intervals, measured in mouse-months. The average incidence rate was also calculated for each group up to 18 months after radiation and then used to determine the incidence rate ratios for each group relative to the CTRL/SED group. One mouse was removed from the HFD/EX group from all these analyses except overall survival as it died before being exposed to radiation. Relative risk was calculated by dividing the proportion of cancer development of each group by that of all the other groups. Analysis of survival and cumulative incidence of cancer-related mortality was conducted using GraphPad Prism 8.0 software (GraphPad Software, San

Diego, CA). Incidence rates at 3-month intervals were calculated using Microsoft Excel (Microsoft Corporation, Redmond, WA). Lastly, incidence rate ratios, relative risks, and their respective 95% confidence interval (CI) were determined using the epi.2by2 package in RStudio (RStudio Computer Program, Boston, MA).

Cancer development was determined as the number of mice that reached end point with cancer over the total number of mice per group that was at risk of developing radiation-induced cancer. One mouse from the HFD/SED group and three mice from the HFD/EX group were excluded from this analysis because tissue autolysis prevented histological analysis. An additional mouse from the HFD/EX group was excluded because it died before being exposed to radiation. Seizure burden was determined as the number of mice that reached end point because of seizures over the entire sample of their respective group. Statistical analyses were conducted using GraphPad Prism 8.0 software (GraphPad Software).

The health span index table was adapted from Schafer and colleagues (33). It is composed of 10 different parameters, including four anthropometric/metabolic (i.e., body fat [%], lean mass [g], metabolic activity from CLAMS, and HDL/LDL ratio), two performance (i.e., endurance [$m \cdot min^{-1}$] and grip strength/body weight [$F \cdot g^{-1}$]), two behavioral (i.e., anxiety [beams broken] and depression [s]), and two morbidity (cancer development and seizure burden) outcomes. A score ranging from −1 to +1 was assigned to each group based on the P value as determined by a two-factor ANOVA for the anthropometric/metabolic, performance, and behavioral outcomes, or Fisher's exact test for the morbidity outcomes. For outcomes analyzed by a two-factor ANOVA, Dunnett's *post hoc* test was used to determine significant differences between means relative to the CTRL/SED group. Positive outcomes were assigned positive values, and negative outcomes were assigned negative values. Specifically, when compared with the CTRL/SED group, $P > 0.10$ was given a score of 0, $P > 0.05$ and <0.10 was given a score of ±0.5, and $P < 0.05$ was given a score of ±1. The cumulative health span index was then determined by adding the scores for each outcome for each group.

Body weight, body fat percentage, lean mass, food consumption, and endurance exercise capacity were assessed using a three-factor (exercise–diet–time) repeated-measures ANOVA followed by Tukey's *post hoc* test to determine significant differences between means for significant main effects and interactions. Metabolic activity, beam break, grip strength, and tail suspension were assessed using a two-factor (exercise–diet) ANOVA followed by Sidak's *post hoc* test to determine significant differences between means for significant main effects and interactions.

RESULTS

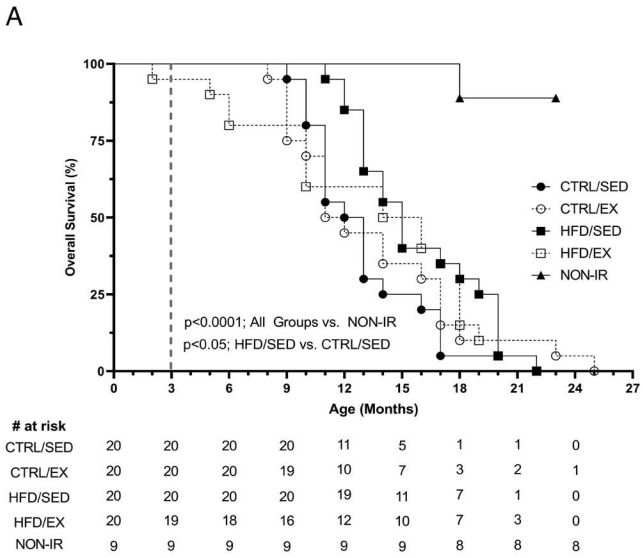
Lifelong exercise reduced cancer-related mortality risk relative to mice with HFD-induced obesity after radiation exposure. Overall survival until end point was significantly shorter in all irradiated groups compared with NON-IR (log-rank, $P < 0.0001$; Fig. 2A). All-cause mortality was significantly lower in HFD/SED compared with CTRL/SED with no effect of exercise (log-rank, $P < 0.05$; Fig. 2A). By 18 months after radiation exposure, there was no significant difference in the cumulative incidence (Fig. 2B) or average incidence rate (Fig. 2C) of cancer-related mortality for each group ($P > 0.05$); however, these outcomes in the irradiated groups were significantly higher versus the NON-IR group. The risk of cancer-related mortality by 18 months after radiation exposure, however, was significantly higher in HFD/SED compared with CTRL/EX (RR = 1.99; 95% CI, 1.20–3.31; $P = 0.0081$) and CTRL/SED (RR = 1.63; 95% CI, 1.06–2.49; $P = 0.0250$) (Table 1). There was a trend for higher risk of cancer-related mortality in HFD/SED compared with HFD/EX (RR = 1.43; 95% CI, 0.95–2.16, $P = 0.086$) (Table 1).

Cancer development was higher in HFD-induced obesity and sedentary mice but attenuated by lifelong exercise after radiation exposure. The most common tumors were AML and lymphocytic lymphoma in the spleen and bone marrow and hepatocellular adenoma and hepatocellular adenocarcinoma in the liver (Fig. 3A–D). Cancer development (Fig. 3E) was significantly higher in HFD/SED (17/19, 89%) compared with CTRL/EX and CTRL/SED (both 11/20, 55%) ($P < 0.05$), and there was a trend for an increase in cancer development in HFD/SED versus HFD/EX (10/16, 63%; $P = 0.1054$).

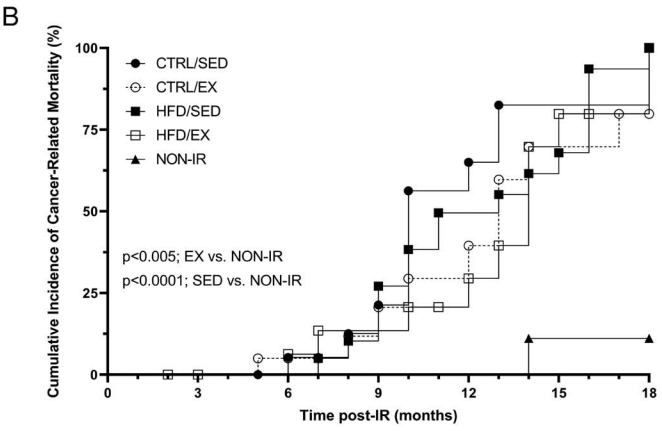
HFD-induced obesity decreased overall health span index, which was higher in lifelong exercise mice after radiation exposure. A health span index was used to quantify overall health span benefits or detriments in each group relative to CTRL/SED (Table 2) (33). The health span index included anthropometric, metabolic, performance, behavior, and morbidity outcomes (see Appendix, Supplemental Digital Content, supplemental information, http://links.lww.com/MSS/C341). The greatest benefit in anthropometric/metabolic outcomes was seen in CTRL/EX (score = 1), with no differences detected in HFD/EX and HFD/SED (both scores = 0). CTRL/EX also had the highest score for performance outcomes (score = 1), with no differences detected in HFD/EX and HFD/SED (both scores = 0). For the behavior outcomes, HFD/EX had the highest score (score = 1) followed by CTRL/EX (score = 0.5) and then HFD/SED (score = 0). Lastly, HFD/SED had the highest morbidity (score = −1), but there were no differences detected in CTRL/EX and HFD/EX (both scores = 0). Cumulatively, CTRL/EX had the highest overall health span index (score = 2.5), followed by HFD/EX (score = 1), and then HFD/SED (score = −1). Results for each component of the health span index are provided in the supplement (see Appendix, Supplemental Digital Content, supplemental information, http://links.lww.com/MSS/C341).

DISCUSSION

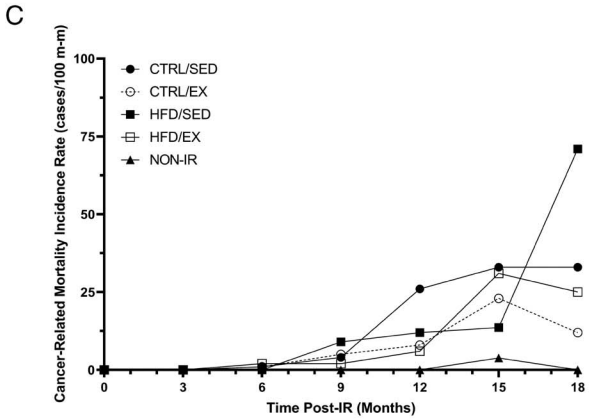
Our overall objective was to evaluate the effects of lifelong endurance exercise and HFD-induced obesity on survival, cancer development, and health span in a mouse model of radiation-induced cancer. Our results demonstrate that lifelong



# at risk									
CTRL/SED	20	20	20	20	11	5	1	1	0
CTRL/EX	20	20	20	19	10	7	3	2	1
HFD/SED	20	20	20	20	19	11	7	1	0
HFD/EX	20	19	18	16	12	10	7	3	0
NON-IR	9	9	9	9	9	9	8	8	8



# at risk							
CTRL/SED	20	20	19	10	5	1	1
CTRL/EX	20	20	17	10	7	3	2
HFD/SED	20	20	20	16	9	6	1
HFD/EX	19	18	16	12	9	6	1
NON-IR	9	9	9	9	9	8	8



Follow-time (Incidence Rate)							Total
CTRL/SED	60 (0)	59 (1.7)	41 (4.9)	19 (26.3)	6 (33.3)	3 (0)	188 (5.9)
CTRL/EX	60 (0)	57 (1.8)	40 (5.0)	23 (8.7)	13 (23.1)	8 (12.5)	201 (5.5)
HFD/SED	60 (0)	60 (0)	54 (9.3)	33 (12.1)	22 (13.6)	7 (71.4)	236 (7.2)
HFD/EX	56 (0)	48 (2.1)	37 (2.7)	31 (6.5)	15 (31.3)	4 (25.0)	192 (5.2)
NON-IR	27 (0)	27 (0)	27 (0)	27 (0)	26 (3.8)	24 (0)	158 (0.6)

FIGURE 2—Lifelong exercise did not reduce all-cause mortality but reduced cancer-related mortality risk relative to mice with HFD-induced obesity after radiation exposure. A, Overall survival across the entire study (*n* = 9–20 mice per group). The *dotted gray line* represents the age at which mice were irradiated. B, The cumulative incidence of cancer-related mortality by 18 months after radiation exposure for each group (*n* = 9–20 mice per group). C, The cancer-related mortality incidence rate per 100 mouse-months divided into 3-month intervals up until 18 months after radiation exposure (*n* = 9–20 mice per group). In panels B and C, one mouse from the HFD/EX group was excluded as it died before being exposed to radiation. Survival was examined by Kaplan–Meier analysis. **P* < 0.05 CTRL/EX vs HFD/SED group.

endurance exercise resulted in lower relative risk of cancer-related mortality, less cancer development, and improved overall health span and that these outcomes were worse in diet-induced obesity. These data extend our previous findings indicating that endurance exercise training attenuated and HFD-induced obesity exacerbated the short-term deleterious effects of radiation (26). Our findings provide direct preclinical evidence that exercise and dietary interventions modulate secondary cancer risk after radiation exposure and provide rationale to evaluate the mechanisms responsible and dose–response relationships for these modifiable lifestyle factors and radiation-induced cancer occurrence.

There is limited direct evidence on the effects of exercise training on overall survival after cancer diagnosis. In the present study, overall survival was not improved by exercise training. Our finding is consistent with a previous clinical study evaluating the effects of exercise on survival (overall and disease-free) in breast cancer survivors (18). However, our findings contrast with another recent clinical trial that evaluated survival in patients undergoing resistance training during radiation therapy compared with those undergoing passive physical therapy (39). The effects of obesity on overall survival after a cancer diagnosis appear to follow a J- or U-shaped curve with some weight gain or elevated body mass index potentially providing some survival benefit (17); however, other studies have not observed the opposite relationship (40). A slight protective effect aligns with our finding that all-cause mortality was lower

TABLE 1. After radiation exposure, HFD-induced obesity increased the relative risk of cancer-related mortality compared with mice without HFD-induced obesity and those who engaged in lifelong exercise training.

	CTRL/SED (n = 20)	CTRL/EX (n = 20)	HFD/SED (n = 19)	HFD/EX (n = 16)
Absolute risk	0.55	0.45	0.89	0.63
Relative risk (95% CI)				
CTRL/SED	1	0.82 (0.44–1.53)	**1.63 (1.06–2.49)**	1.14 (0.66–1.97)
CTRL/EX	1.22 (0.65–2.29)	1	**1.99 (1.20–3.31)**	1.39 (0.75–2.57)
HFD/SED	**0.61 (0.40–0.94)**	**0.50 (0.30–0.84)**	1	0.70 (0.46–1.05)
HFD/EX	0.88 (0.50–1.52)	0.72 (0.39–1.33)	1.43 (0.95–2.16)	1

Statistically significant results are indicated in bold.

in HFD/SED. An important caveat to our data is that body weight and adiposity in HFD groups were no longer significantly different from CTRL-diet groups beyond approximately 12 months of age. This may be explained by reduced sample size because of mortality at that time point, or perhaps it may indicate a survival benefit associated with slight weight gain. Future dose–response studies for both exercise and weight gain will clarify these relationships.

With respect to cancer-specific mortality, a recent systematic review indicated a 28%–44% reduction in risk of cancer-related mortality after cancer diagnosis with no reported adverse effects among those who exercised compared with those who did not (16). Increased participation in exercise was also associated with a reduced incidence of cancer recurrence and all-cause mortality (16). However, these findings were dominated by studies in breast, colorectal, or prostate cancer survivors; did not specifically account for treatment regimen in their analysis; and were not able to identify exercise dose–response or cancer type–, grade-, or stage-specific effects (16). In addition, most of the studies included in the systematic review evaluated exercise participation via questionnaire at variable time points since diagnosis (16). The systematic review identified only four randomized control trials that evaluated cancer survival and recurrence, and these were conducted as exploratory analyses (16). As such, it is not surprising that significant improvements in cancer survival and recurrence have not been detected in the randomized control trials to date (16). We detected a 5.7-fold greater than the cancer incidence rate between 15 and 18 months after radiation in HFD/SED compared with CTRL/EX and 2.9-fold greater than HFD/EX. Therefore, our results provide novel, direct evidence suggesting that endurance exercise training reduces hematopoietic and hepatic cancer development and related mortality after radiation exposure particularly when compared with sedentary, obese conditions.

The model used in this study is the currently preferred model to investigate radiation-induced AML (22). This is because it has a low spontaneous tumor rate (<1%), and a 3-Gy dose of ionizing radiation has been shown to induce AML in 25% of mice by 18 months after radiation that mimics the genetic and

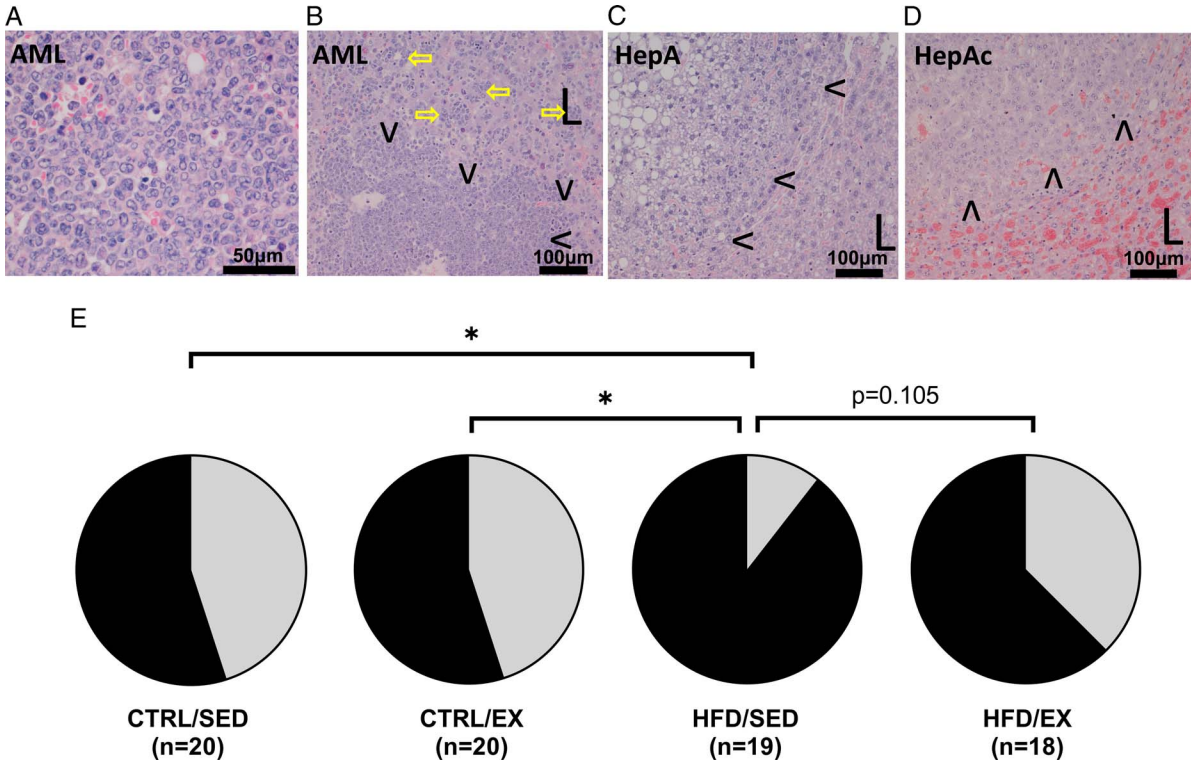


FIGURE 3—HFD-induced obesity and a sedentary lifestyle increased cancer development, which was attenuated by lifelong exercise after radiation exposure. Representative images of various tumors identified. A, Sheets of AML cells in the bone marrow (60×). B, A mass of AML cells (AML) is delineated with black arrowheads from the liver (L) that is infiltrated by small clusters of AML cells in the sinusoids (some indicated by full black arrows) (20×). C, Hepatocellular adenoma (HepA) is delineated by arrowheads from liver (L) (20×). D, Hepatic adenocarcinoma (HepAc) is delineated by arrowheads from the liver (L) (20×). Hematoxylin and eosin stain. E, Pie charts highlighting the distribution of mice who reached end point with cancer and those who did not in each group as identified through histological analysis (n = 16–19 per group). *P < 0.05 compared with the HFD/SED group.

TABLE 2. HFD-induced obesity decreased overall health span index, which was reversed by lifelong exercise after radiation exposure.

		Summary Data, Mean ± SEM or *n* (%)				*P* Value			Score (–1 to + 1)[a]		
Outcomes	**Parameters**	**CTRL/SED**	**CTRL/EX**	**HFD/SED**	**HFD/EX**	**CTRL/EX**	**HFD/SED**	**HFD/EX**	**CTRL/EX**	**HFD/SED**	**HFD/EX**
Anthropometric/metabolic	Body fat (%)[b]	23.9 ± 0.5	20.39 ± 1.0	27.90 ± 0.5	28.13 ± 0.5	**0.0007**	**<0.0001**	**<0.0001**	1	–1	–1
	Lean mass (g)[b]	24.9 ± 0.5	24.9 ± 0.3	28.8 ± 0.4	27.6 ± 0.5	>0.9999	**<0.0001**	**0.0004**	0	1	1
	Metabolism	$\dot{V}O_2$: 94390 ± 10637.6 mL	$\dot{V}O_2$: 106618 ± 2311.9 mL	$\dot{V}O_2$: 108770 ± 6149.1 mL	$\dot{V}O_2$: 105271 ± 3638.6 mL	0.4306	0.2499	0.4667	0	0	0
		$\dot{V}CO_2$: 92015 ± 12584.6 mL	$\dot{V}CO_2$: 103249 ± 3368.8 mL	$\dot{V}CO_2$: 95368 ± 3894.0 mL	$\dot{V}CO_2$: 88416 ± 3392.9 mL	0.4268	0.9441	0.9366	0	0	0
		Heat: 0.47 ± 0.06 kcal	Heat: 0.53 ± 0.01 kcal	Heat: 0.55 ± 0.02 kcal	Heat: 0.52 ± 0.02 kcal	0.3421	0.1339	0.4966	0	0	0
	HDL:LDL ratio (A.U.)	16.17 ± 4.4	19.98 ± 4.9	12.6 ± 3.2	18.22 ± 5.2	0.9156	0.9283	0.9816	0	0	0
Performance	Endurance ($m \cdot min^{-1}$)[c]	26.3 ± 1.6	37.33 ± 1.6	24.43 ± 1.4	33.0 ± 1.4	**<0.0001**	>0.9999	**0.0064**	1	0	1
	Grip strength/BW ($F \cdot g^{-1}$)	4.1 ± 0.3	4.4 ± 0.2	3.7 ± 0.1	3.3 ± 0.2	0.6722	0.3599	**0.0320**	0	0	–1
Behavior	Anxiety (beams broken)	380.8 ± 91.8	552.6 ± 57.7	482.9 ± 26.3	469.1 ± 21.8	**0.0612**	0.3035	0.4259	0.5	0	0
	Depression (s)	118.9 ± 21.7	101.5 ± 17.7	100.4 ± 15.2	42.94 ± 10.4	0.8185	0.7711	0.0228	0	0	1
Morbidity	Cancer development	11/20 (55%)	11/20 (55%)	17/19 (89%)	10/16 (63%)	>0.9999	**0.0310**	0.7412	0	–1	0
	Seizure burden	2/20 (10%)	7/20 (35%)	1/20 (5%)	2/20 (10%)	0.1274	>0.9999	>0.9999	0	0	0
Total									**2.5**	**–1**	**1**

Data for the metabolic, performance, and behavior outcomes are presented as mean ± SEM (n = 4–20/group/outcome).
Body fat and lean mass were measured from the time of radiation exposure (13 wk) until end point.
[a]*P* value: **0** = $P > 0.1$ = 0; **±0.5** = $0.05 < P < 0.01$; **±1** = $P < 0.05$.
[b]Two-factor ANOVA of average per group from first EchoMRI after radiation to end point for each mouse; CTRL/EX, HFD/SED, and HFD/EX with CTRL/SED as control.
[c]Two-factor ANOVA of average per group after radiation; CTRL/EX, HFD/SED, and HFD/EX with CTRL/SED as control.

phenotypic characteristics of human AML (21,22). The rate of AML in mice surviving to 18 months in the present study is consistent with previous work (41). Interestingly, all mice diagnosed with AML were in the HFD/SED group. The most prevalent neoplasm detected in all four experimental groups was hepatic neoplasm, specifically hepatocellular adenoma, which is a benign tumor with the potential for malignant transformation that has been well documented in older mice belonging to the CBA strain (42). Pathological analysis revealed that these tumors lead to gross hepatic damage and were the probable cause of end point. As a result, these tumors were still considered in the cancer development and cancer-related mortality analyses. Radiation-induced hepatic cancers are not well documented, but radiation therapy has been shown to increase liver disease, which in turn can increase liver cancer risk (43). However, previous work (42) and findings from our nonirradiated cohort indicated that radiation did not increase hepatic neoplasms in CBA mice. Interestingly, among irradiated mice, the number of hepatic neoplasms was largely reduced in exercise-trained mice and increased in those with diet-induced obesity. There is strong evidence that obesity increases liver cancer risk; however, there is only limited evidence that exercise can reduce liver cancer risk (12). Our findings provide further support to this previous work and indicate that directed studies evaluating exercise and prevention of hepatic neoplasms are warranted. In addition, seizures are known to occur in the CBA strain and were exacerbated by irradiation in the present study. There have been rare reports of long-term seizure disorders in the clinical literature in patients receiving localized radiation for brain cancer (44); however, this is the first report indicating that whole-body irradiation may increase seizure risk. Future work should examine this outcome directly.

To obtain a comprehensive, integrated assessment of how exercise and diet-induced obesity influenced overall health span in irradiate mice, we combined our metabolic, performance, and mental health (i.e., depression and anxiety) outcomes into a single health span index as previously described (33). We focused on these outcomes as they are among the most common quality of life-related decrements reported in cancer survivors (10,45). Similar to work in nonirradiated mice (33,46), the highest health span index was detected in exercise-trained, lean mice with exercise training partially restoring health span in obese mice. The largest effects of exercise were in endurance performance, anxiety, and depression. This aligns with clinical research indicating that exercise training can improve these outcomes as determined by self-report in cancer survivors (10), and extends this literature by providing objective measures in a preclinical model. Lean mass and muscle strength were not improved in the exercise-trained group. This is likely because of our endurance exercise training model and suggests that future work should include resistance training to preserve muscle mass and strength. Combined, these findings suggest that a combined dietary and exercise intervention should be considered to maximize health span in cancer survivors. The majority of cancer survivors will be over the age of 60 within the next 20 yr (47). As such, our health span results are translationally relevant as they were collected when mice were roughly the equivalent age based on overall life span. Thus, these findings offer valuable insight into the use of lifelong exercise interventions to improve physical and psychological well-being among cancer survivors.

These findings provide the basis for future investigations to identify the mechanisms responsible for improved cancer-related survival, decreased cancer incidence, and improved health span in this model of radiation-induced cancer. Indeed, exercise training in cancer survivors may reduce inflammation,

improve cellular metabolism, induce epigenetic adaptations, and reduce oxidative stress (16). These adaptations would combat several of the cellular mechanisms responsible for the negative effects of radiation on healthy tissue (48). Our previous work has shown that in the short-term after radiation exposure, exercise training reduced radiation-induced cytotoxicity and genotoxicity (27,28), accelerated immune recovery (27,28), and improved muscle architecture and metabolism (49,50). It is likely that the combination of these mechanisms contributed to the improved health span and cancer incidence observed in late time points evaluated in the present study.

A limitation of the study is that we examined radiation in isolation in tumor-free mice. Radiation is often provided in combination with chemotherapy and/or surgery; thus, the combined effects of such treatments could differ from the single treatment used in the present study. Further, irradiating cancer-free mice would be relevant to cases where radiation is used as adjuvant therapy; however, the effects of radiation may differ when an active cancer is present. Lastly, we provided a whole-body, nonlethal dose of radiation according to an established radiation-induced cancer model (22). This protocol differs from localized and fractionated therapy that is often applied in several common cancers that would deliver a higher radiation dose over a series of weeks to a specific body region. No radiation-induced cancer models exist that mimic the human therapy model and reliably induce cancer in mice. Regardless, our model would be relevant to cancers where large volumes of healthy tissue are exposed to radiation. These include common cancers, particularly in young adults, such as lymphoma, breast cancer, sarcoma, and testicular cancer.

In conclusion, our results provide direct evidence that lifelong endurance exercise training reduces cancer development, reduces risk of cancer-related mortality, and improves health span in a mouse model of radiation exposure and that these outcomes are worsened in diet-induced obese, sedentary mice. These findings extend our previous work showing that at acute time points after radiation exposure, exercise training mitigated some of the deleterious effects of radiation (26). Further, our present study extends previous work in age mice not exposed to radiation (33) and supports the limited clinical evidence indicating that exercise may reduce cancer risk and recurrence (18,19). Given that the number of cancer survivors who have been exposed to radiation is increasing, and that there is substantial evidence highlighting the increased cancer risk associated with radiation exposure, developing a better understanding of the optimal exercise prescription and the mechanisms responsible for its beneficial effects would have important clinical implications for cancer survivors.

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The results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation. The results of the present study do not constitute endorsement by the American College of Sports Medicine.

The authors declare no conflict of interest.

E. F. and M. D. contributed to study design and direction. E. F., J. K., D. B., M. N., P. M., J. V., J. Ll., and J. La. completed all formal analyses, investigation, methodology, and validation. E. F. and M. D. contributed to conceptualization and project administration and wrote the original draft. M. D. supervised the project, secured funding, participated in analyses, and is responsible for data curation. All authors reviewed and edited the manuscript and approved final submission.

All data will be made available upon reasonable request.

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