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Authors: Yamauchi, Kazumi, Ono, Tetsuya, Ayabe, Yoshiko, Hisamatsu, Shun'ichi, Yoneya, Manabu, et al.

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SHORT COMMUNICATION

Life-Shortening Effect of Chronic Low-Dose-Rate Irradiation in Calorie-Restricted Mice

Kazumi Yamauchi,[a,1] Tetsuya Ono,[a] Yoshiko Ayabe,[b] Shun'ichi Hisamatsu,[b] Manabu Yoneya,[a] Yuki Tsutsumi[c] and Jun-ichiro Komura[a]

Departments of [a] Radiobiology and [b] Radioecology, Institute for Environmental Sciences, Rokkasho, Kamikita, Aomori, Japan; and [c] Tohoku Environmental Science Service Corporation, 330-2, Noduki, Obuchi, Rokkasho, Kamikita, Aomori, Japan

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Calorie restriction is known to influence several physiological processes and to alleviate the late effects of radiation exposure such as neoplasm induction and life shortening. However, earlier related studies were limited to acute radiation exposure. Therefore, in this study we examined the influence of chronic low-dose-rate irradiation on lifespan. Young male B6C3F1/Jcl mice were divided randomly into two groups, which were fed either a low-calorie (65 kcal/week) or high-calorie (95 kcal/week) diet. The latter is comparable to *ad libitum* feeding. The animals in the irradiated group were continuously exposed to gamma rays for 400 days at 20 mGy/day, resulting in a total dose of 8 Gy. Exposure and calorie restriction were initiated at 8 weeks of age and the diets were maintained for life. The life-shortening effects from chronic whole-body irradiation were compared between the groups. Body weights were reduced in calorie-restricted mice irrespective of radiation treatment. Radiation induced a shortened median lifespan in both groups, but to a greater extent in the calorie-restricted mice. These results suggest that calorie restriction may sensitize mice to chronic low-dose-rate radiation exposure to produce a life-shortening effect rather than alleviating the effects of radiation.

INTRODUCTION

The accidents at nuclear power plants in Chernobyl and Fukushima resulted in chronic low-dose-rate radiation exposure for a large number of people, although the exposures in Fukushima were much lower than those in Chernobyl (*1, 2*). These incidents motivated essential research to better understand the health effects of such exposure and to develop strategies for alleviating the possible long-term radiation effects. The major expected health effects of low-dose-rate radiation exposure are late effects such as neoplasm induction and life shortening (*3*). These effects are well known to be induced with high acute doses of radiation; however, the influence of low or low-dose-rate radiation exposure remains controversial (*4*). The late effects of radiation are known to be influenced by many factors such as dose, dose rate and biological parameters, including sex, genetic background and age at exposure (*3*). Calorie or food restriction has also been proposed to mediate the late effects of radiation, but in contrast to the other factors mentioned above, calorie restriction actually appears to reduce rather than exacerbate the radiation effects. Gross and Dreyfuss (*5, 6*) found that food restriction could reduce the radiation-induced neoplasm incidence in rats and radiation-induced lymphatic leukemia in mice. Subsequently, Yoshida *et al*. (*7*) reported a similar effect on radiation-induced myeloid leukemia in mice using an elaborate calorie restriction experimental design rather than simple food restriction. Furthermore, the lifespans of irradiated rats and mice were also shown to be improved by food or calorie restriction (*7, 8*). More recently, Shang *et al*. (*9*) demonstrated that calorie restriction in male B6C3F1 mice at an adult age (7 weeks old) was effective in reducing the neoplasm induction and life shortening induced by irradiation of 1-week-old infants, suggesting that the late effects of long-term radiation can be modified by calorie restriction.

Despite the clear influence of calorie restriction on late radiation effects, the underlying mechanism remains unclear. However, these effects are likely mediated through the diverse well-known physiological changes induced by calorie or food restriction, including reduction of body weight, suppression of inflammation, increase in lifespan, induction of stress resistance, activation of genome maintenance and elevation of mitochondrial biogenesis, among others (*10*). In fact, Yoshida *et al*. (*7*) reported a reduced life-shortening effect in calorie-restricted mice after 3 Gy irradiation compared to that of mice maintained on a high-calorie diet that was comparable to *ad libitum* feeding.

[1] Address for correspondence: Institute for Environmental Sciences, 1-7 Ienomae, Obuchi, Rokkasho-mura, Kamikita-gun, Aomori 039-3212, Japan; e-mail: yamauchi@ies.or.jp.

However, all of these previously reported studies were limited to examining the late effects induced by acute irradiation; thus, we sought to determine whether similar effects would be induced with low-dose-rate irradiation in calorie-restricted mice. When the dose rate is reduced, many biomarkers are known to lead to smaller radiation effects (i.e., dose-rate effects). Moreover, recently published studies indicate that the molecular responses to low-dose-rate radiation exposure differ from those induced by acute exposure both quantitatively and qualitatively (*11, 12*). In this work, we examined the effect of chronic low-dose-rate gamma-ray irradiation on the lifespan of calorie-restricted mice.

MATERIALS AND METHODS

Animals and Irradiation

Male B6C3F1/Jcl mice were purchased from CLEA Japan Inc. (Tokyo, Japan) at 6 weeks of age, acclimated to our laboratory environment for 2 weeks, then divided randomly among four groups: 65 kcal/week, nonirradiated (n = 62); 65 kcal/week, irradiated (n = 63); 95 kcal/week nonirradiated (n = 62); and 95 kcal/week, irradiated (n = 63). Irradiation and maintenance of mice were performed under specific pathogen-free (SPF) conditions. Exposures using a Cs-137 source were initiated at 56 ± 3 days of age and continued for 400 days at 20 mGy/day, resulting in a total dose of 8 Gy. The dose rate was examined once a year using an ionization chamber, and the dose rate of 20 mGy/day was achieved by adjusting the distance between the radiation source and the mouse cage. Over a 2-h period each day, mice were not exposed (from 10:00 to 12:00), while undergoing a health check and maintenance; thus, the actual dose rate was 20 mGy/22 h.

The details of the experimental conditions are described elsewhere (*13*). However, in the current study, each mouse was kept separately in a plastic box, 9.3 × 20.5 × 12.7 cm (width × depth × height), to reduce variation of body weight among individuals, in contrast to the 4–5 mice housed together in a larger box in the previously reported study. After irradiation for 400 days, the mice were maintained under the SPF condition until their death. Irradiation and calorie restriction time periods are detailed in Fig. 1.

Calorie Restriction

Mice were maintained with regular food (FR-2; Funabashi Farm Co., Chiba, Japan) until reaching 56 ± 3 days of age, at which point they were administered one of the two calorie-controlled diets. Mice receiving the high-calorie diet were provided with 95 kcal/week, which is considered comparable to the calories taken under *ad libitum* feeding (*7*), and mice receiving the calorie-restricted diet were provided with 65 kcal/week. To prepare the diets, we adopted the ingredients developed by Yoshida *et al*. (*7*). The amounts of nutrients such as proteins, minerals and vitamins, were equalized between the two diets. The difference in calories was adjusted only by the amounts of carbohydrate and dextrose. The diet was supplied on Monday, Wednesday and Friday, at 5.7 g, 5.7 g, and 8.5 g for the 65 kcal/week group, and at 8.0 g, 8.0 g, and 12.0 g for the 95 kcal/week group, respectively. Mice provided with 65 kcal/week consumed all foods supplied. However, mice in the 95 kcal/week group left some food. The amount left was weighed every Monday before new food was provided, and the amount consumed per week was estimated by subtracting the amount of food left from the amount provided in the preceding one week. The average amount of consumed food was approximately 25 g/week at 1 week after the beginning of the experiment and gradually increased to 27.5–28 g/week at 400 days of age. The consumed food amount of 28 g/week corresponds to 95 kcal/week. This level was maintained for 300 days followed by a slight decrease. These age-dependent changes showed no difference between nonirradiated and irradiated groups. The body weights of individual mice were monitored weekly.

Most mice died of natural causes, while some were euthanized at moribund states. The numbers of euthanized mice, out of the total number (for each group), were: 8/62 (95 kcal/week, nonirradiated), 10/63 (95 kcal/week, irradiated; 8/62 (65 kcal/week, nonirradiated); and 12/63 (65 kcal/week, irradiated groups). The ratios were not statistically different among the groups.

All experiments were performed according to legal regulations in Japan and followed the Guidelines for Animal Experiments of the Institute for Environmental Sciences.

Statistical Analysis

All statistical analyses were performed using R version 3.4.1 with a GUI front end of EZR version 1.37 (*14*). Since the lifespan data did not follow a normal distribution in the 65 kcal/week group based on the Shapiro-Wilk test and Kolmogorov-Smirnov test, all differences of lifespans were examined using the non-parametric Mann-Whitney U test. The hazard ratios of the risk of radiation and the effects of calorie restriction on lifespan and their interaction were analyzed using a Cox proportional hazard regression model in which radiation exposure and calorie restriction were transformed to nominal values of 0 and 1, with 0 indicating no exposure and 95 kcal/week, respectively. A P value of less than 0.05 was considered to represent a statistically significant difference.

RESULTS

Age-dependent changes of average body weight are shown in Fig. 2A. In the 95 kcal/week group, the body weight of nonirradiated mice increased rapidly between days 56–200, then showed a slow increase until 600 days followed by a mild decline. This change was not affected by radiation. However, in the 65 kcal/week groups, only slight increases in body weight were observed between days 56–200, followed by a steady state until approximately 1,000 days, with slight decreases observed thereafter. The difference between nonirradiated and irradiated groups was not notable.

Figure 2B shows the survival curves of the four groups, and their median lifespans are summarized in Table 1. In the 65 kcal/week group, the median lifespan of the exposed mice was significantly shortened by 143 days. Although exposure also reduced the lifespan in the 95 kcal/week group by 79 days, the difference was not statistically significant. Moreover, comparisons of the different calorie groups showed a highly significant effect of calorie restriction on lifespan extension in both the nonirradiated and irradiated groups (Table 1). Results of the Cox proportional hazard regression analysis are summarized in Table 2. The hazard ratio of radiation exposure was not statistically significant ($P = 0.16$), because we took 0 as the nominal value of 95 kcal/week, and the lifespan shortening due to exposure was not large. However, the hazard ratio for the interaction term of exposure and 65 kcal/week was significant ($P = 0.039$), further confirming that the effect of radiation differed according to diet.

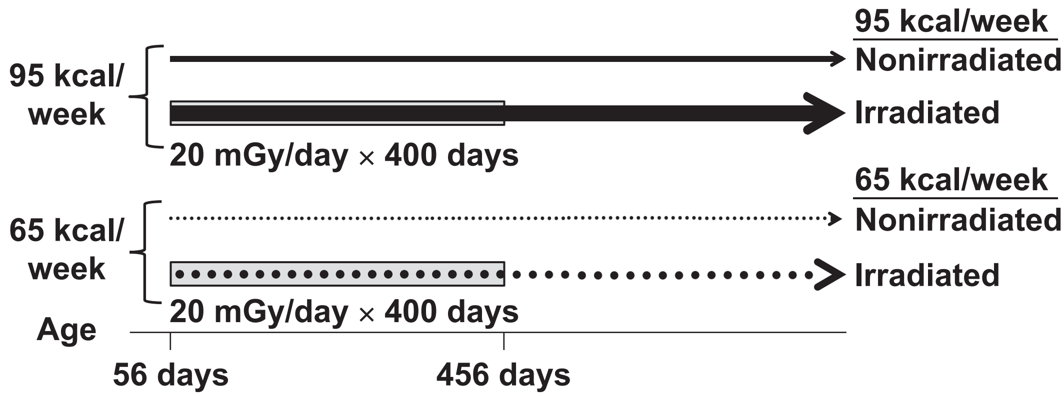

FIG. 1. Schematic of experimental design. Mice fed 95 kcal/week or 65 kcal/week were assigned to either the nonirradiated or irradiated group. The period of chronic γ-ray irradiation (dose rate of 20 mGy/day) is indicated by thick gray lines. Irradiation and calorie-controlled feeding were initiated when animals were 56 days old. Irradiation was discontinued after 400 days (at 456 days of age), and the feeding continued throughout the lifetime of the mice.

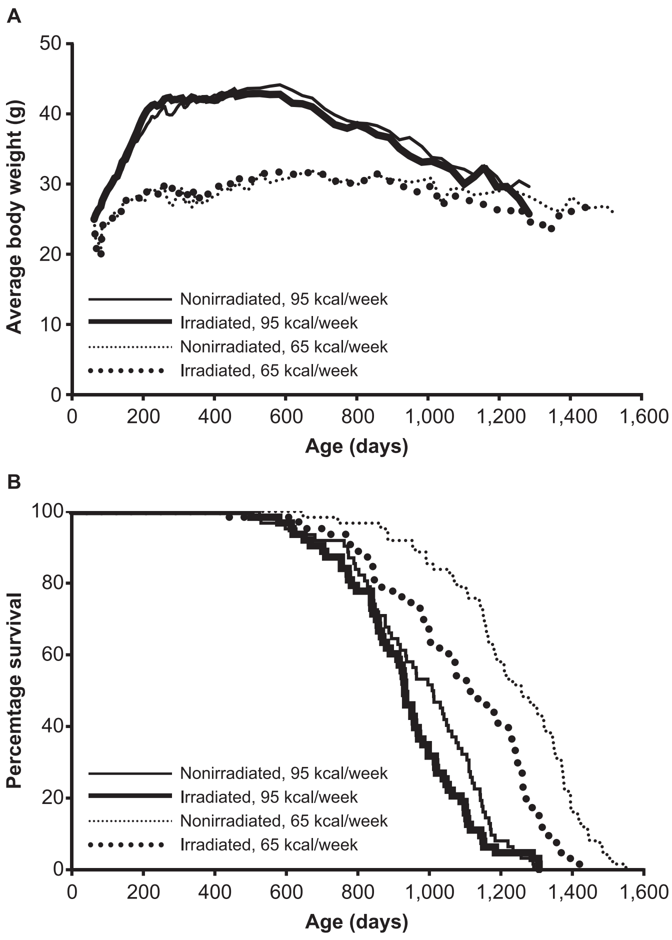

FIG. 2. Time-dependent changes of body weights (panel A) and survival curves (panel B) of the four experimental groups with different calorie (65 kcal/week or 95 kcal/week) and γ-ray irradiation conditions. The body weight of each mouse was measured weekly, and average values of 62–63 mice were plotted.

DISCUSSION

Although previously published studies have demonstrated that calorie restriction can improve the lifespan of mice exposed to acute radiation, the current study showed that chronic low-dose-rate irradiation at 20 mGy/day for 400 days actually shortens the lifespan of calorie-restricted mice. When the degree of shortening was compared to that observed in non-calorie-restricted mice, the effect appeared to be more efficient in calorie-restricted mice: the lifespan was shortened by 143 days (11.4% of nonirradiated lifespan) in the calorie-restricted group and by 79 days (7.9%) in the non-restricted group. By contrast, Yoshida *et al*. (*7*) observed a life-shortening of 59 days (7.1%) in calorie-restricted mice after 3 Gy acute irradiation, but of 113 days (14.4%) in non-restricted mice. Shang *et al*. (*9*) found a reduced lifespan of 393 days (37.5%) in the calorie-restricted group after 3.8 Gy acute irradiation and of 331 days (37.7%) in the non-restricted group. Thus, these studies showed that the life-shortening effect of acute radiation exposure is reduced or similar in calorie-restricted mice compared to that in non-calorie-restricted mice. Our current results showed that the effects of calorie restriction clearly differ between acute and chronic irradiation scenarios. Calorie restriction might make mice more resistant to acute radiation exposure but more sensitive to chronic radiation exposure. Alternatively, these discrepant results could be related to the difference in experimental conditions among studies. In the work of Yoshida *et al*. (*7*) male C3H/HeNirMs mice were irradiated at 10 weeks of age and calorie restriction started right after irradiation. In the work of Shang *et al*. (*9*) male B6C3F1/Crlj mice were irradiated at 1 week of age and calorie restriction started at 7 weeks of age. A definitive conclusion requires simultaneous comparison of acute and chronic effects under the same experimental conditions.

Moreover, it is still unclear whether the processes underlying late-effect induction are the same under acute

TABLE 1
Effects from Chronic Low-Dose-Rate Irradiation on the Lifespans of Mice Fed a Low- or High-Calorie Diet

Irradiation	Median lifespan (days) 65 kcal/week	95 kcal/week	Life extension by calorie restriction (days)	*P* value
–	1,258	1,010	248	1.7×10^{-10}***
+	1,115	931	185	2.0×10^{-5}***
Life shortening by radiation (days)	143	79		
P value	2.7×10^{-4}***	0.10		

*** $P < 0.001$, Mann-Whitney U test.

and low-dose-rate irradiation. Recent comparative studies on the molecular alterations induced by acute and chronic radiation exposure revealed certain quantitative and qualitative differences (*11, 12, 15–17*). For example, Nakajima *et al*. (*17*) compared the mouse liver proteins affected by acute 4 Gy and chronic 8 Gy (20 mGy/day × 400 days) radiation using an antibody microarray assay. They examined the alterations three months postirradiation and found that the affected proteins were completely different between the two groups. This finding suggests that the molecular processes of radiation-induced late-effect could be different, at least in part, between acute and chronic exposure.

Tanaka *et al*. (*13*) reported statistically significant life shortening by 100 days after similar chronic exposures compared to the current study (20 mGy/day × 400 days) using a similar strain of mice (B6C3F1/Nrs) fed *ad libitum*. Although the life shortening of mice under a similar feeding condition was 79 days, this was not a statistically significant change. Since most of the experimental conditions were similar between the two studies, including the genetic background of the mice, sex, radiation exposure facility and maintenance of mice, possible reasons for the different results could be the difference in the number of mice examined (500 in Tanaka's study versus 62 or 63 in the current study), food constituent [FR-2 vs. Yoshida's (*7*) diets] and/or the population density of mice (4–5 mice per cage in Tanaka's study versus one mouse per cage in the current experiment). Indeed, population density was reported to influence many physiological parameters in mice (*18*).

In summary, this study showed that calorie restriction can make mice more sensitive to the life shortening effect induced by chronic low-dose-rate radiation exposure. Since this is in contrast to the reported effects of acute radiation exposure, the complexity of chronic exposure and interactions of physiological mechanisms warrant further investigation. It should be noted that the current study was performed using only male mice, and female mice may respond to the same conditions differently.

TABLE 2
Hazard Ratios of the Effects of Radiation and Calorie Restriction on Lifespan Using the Cox Proportional Hazard Regression Model

Factor	Hazard ratio	95% Confidence limits	*P* value
Irradiation	1.29	0.90–1.83	0.16
65 kcal/week	0.21	0.14–0.32	9.0×10^{-14}***
Interaction	1.72	1.03–2.89	0.039*

Note. Factors of radiation and calorie restriction were addressed as nominal variables of 0 and 1, with 0 indicated for nonirradiation and 95 kcal/week, respectively.

* $P < 0.05$; ***$P < 0.001$.

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