Short-term calorie restriction enhances DNA repair by non-homologous end joining in mice

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Calorie restriction (CR) improves health, reduces cancer incidence and extends lifespan in multiple organisms including mice. CR was shown to enhance base excision repair and nucleotide excision repair pathways of DNA repair, however, whether CR improves repair of DNA double-strand breaks has not been examined in vivo system. Here we utilize non-homologous end joining (NHEJ) reporter mice to show that short-term CR strongly enhances DNA repair by NHEJ, which is associated with elevated levels of DNA-PK and SIRT6.

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Calorie restriction (CR) without malnutrition slows the biological aging process and results in lifespan extension in a number of species. In mice, CR by reduction in calorie intake by 30–40% extends both mean and maximum lifespan by 30–40%2,3. CR provides many beneficial health effects, including: reduced incidence of tumors, obesity, diabetes, autoimmune diseases, sarcopenia, and cardiovascular diseases4,5. CR has also been shown to ameliorate oxidative damage to DNA, protein, and lipids6–7. This reduction in oxidative damage has been attributed to a decline in the generation of reactive oxygen species (ROS)8, an enhancement of protective mechanisms9–12, or an increase in DNA repair capacity13,14. Several studies reported that CR prevents age-related decline in base excision and nucleotide excision repair pathways that repair ROS-induced damage to individual bases and UV-induced DNA adducts, respectively14 (reviewed in ref. 15). The effect of CR on DNA double-strand break (DSB) repair, the pathway that repairs the most severe type of lesion leading to genomic rearrangements, is less studied. DSB repair plays an essential role in the aging process as mutations in multiple genes involved in DSB repair lead to premature aging syndromes and aged tissues accumulate genomic rearrangements consistent with faulty DSB repair (reviewed in ref. 15). Long-term CR prevented age-related decline in the main DSB repair pathway, non-homologous end joining (NHEJ), measured using a linearized DNA ligation in vitro assay16. CR was also found to prevent age-related decline in the levels of Ku protein, a critical component of NHEJ machinery17.

We generated a knock-in reporter mouse that allows to quantitively measure NHEJ efficiency in vivo using I-SceIendonuclease to induce site-specific DNA double-strand breaks within NHEJ reporter cassette integrated in ROSA26 locus18 (Fig. 1a). NHEJ reporter cassette19 consists of the GFP gene interrupted by the rat Pem1 intron, which does not have homology to the mouse genome, and a "killer" exon flanked by I-SceI sites. GFP is inactivated by the presence of the killer exon in unarranged construct. I-SceI cuts lead to the release of the killer exon, while successful NHEJ repair re-ligates the intron leading to reactivation of the GFP gene. NHEJ repair events occurring within the intron tolerate deletions (up to 1 kb on each side of the cut) and insertions without interfering with GFP activity.

The NHEJ reporter mouse model provides a new tool to study the effect of CR on NHEJ efficiency in vivo. Earlier reports did not examine the effect of short-term CR on NHEJ. Notably, it has been reported that 4 weeks of CR is sufficient to ameliorate age-related alterations in DNA methylome20, suggesting that short-term CR may be sufficient to enhance DNA repair efficiency. In this report, we used NHEJ reporter mice to examine the effect of short-term 40% CR on NHEJ efficiency. We found that NHEJ efficiency was strongly improved by short-term CR.

To calculate the daily calorie consumption in NHEJ reporter mice, we fed mice ad libitum with dustless precision pellet diet and counted the number of consumed pellets. Mice consumed an average of 14.3 ± 1.3 kcal per day under ad libitum feeding and maintained their body weight (Fig. 1b). In the CR group, mice were fed with 8.4 kcal/day, which was equal to 60% of the average daily calorie consumption. The CR mice lost body weight in the first two weeks of feeding and then maintained their body weight (Fig. 1c). All mice were single housed to ensure equal food consumption. Mice were sacrificed at 4 weeks from the start of the diet. NHEJ was measured in primary cells isolated from fresh skin, lung, kidneys, and brain of the mice. NHEJ assay was conducted as previously described18. Briefly, cells were co-transfected with a plasmid encoding I-SceI enzyme to induce DSBs and a DsRed plasmid to normalize for transfection efficiency, and analyzed by flow cytometry. NHEJ efficiency was calculated as a ratio of GFP+ to DsRed+ cells. I-SceI, NHEJ-GFP, and DsRed are expressed from a CMV promoter. Importantly, CR did not alter the intensity of DsRed fluorescence (Supplementary Fig. 1c), suggesting that I-SceI expression was not affected by CR.

We found that short-term CR significantly increased NHEJ efficiency in skin, lung, kidney, and brain tissues, compared to ad libitum-fed mice in skin, lung, kidney, and brain (Fig. 1d). This increase was associated with elevated levels of DNA PK (Fig. 1e, f) and SIRT6 (Fig. 1e, g). These results indicate that short-term CR is sufficient to enhance NHEJ.

Very large deletions may extend into GFP-coding region and result in a loss of signal. Although, theoretically a CR treatment can alter the fidelity of repair, from our previous sequencing studies in AL animals of the same strain, the deletions very rarely extend into the coding region of the GFP gene17. Therefore, the observed change in NHEJ efficiency cannot be explained by altered fidelity of NHEJ.

Previous studies showed that CR prevented age-related decline in repair capacity12,18, while here we show that short-term CR enhances NHEJ in the young animals. This short-term CR, or perhaps intermittent fasting, may provide added benefit. Environmental stress
conferred by CR may trigger hormetic response by activation stress-response pathways, such as SIRT6, and upregulating DNA repair machinery. Improved NHEJ efficiency will result in lower levels of persistent DNA damage, reduced cell death, improved genome stability, and reduced mutation load. This improved genome stability is likely to contribute to lower cancer incidence and longer lifespan conferred by CR. Our finding that even a short-term CR is sufficient to trigger these beneficial effects is important for the practical applications of CR in humans where long-term CR is difficult to achieve, while short-term CR or intermittent fasting is more feasible.

**METHODS**

**Animals**

All mouse experiments were performed in accordance with guidelines established by University of Rochester Committee on Animal Resources.

Male mice were used in this study and were all single-housed to perform calorie-controlled feeding experiments. The experiments were performed on 3–5 months old C57BL/6 mice harboring NHEJ reporter cassette in ROSA26 locus generated by Vaidya et al.19.

**CR administration**

Mice were fed with Dustless Precision Pellet diet (BioServ, Cat# F0074). Calculated amount of precision pellet food was placed on the cage floor every morning, leftover food pellets were counted and removed daily before new food was provided. One mouse became moribund and was discontinued from the CR experiments and excluded for NHEJ analysis.

**NHEJ assay**

Primary cell cultures were isolated from skin, lung, kidney, and brain of mice as previously described19. One million primary cultured cells were transfected with 5 µg pCMV-I-SceI plasmid to induce DNA DSBs and 0.1 µg
were derived from the same experiment and were processed in parallel. Over- night at 4 °C with rabbit monoclonal antibodies anti-DNA PKcs (Abcam, ab1791, 1:10,000) in 5% BSA-TBST. After three washes for 10 min with TBST, membranes were incubated for 1 h at room temperature with goat anti-rabbit IgG H&L (HRP) (Abcam, ab6721, 1:5000). After three washes with membranes were incubated for 2 h at room temperature. Membranes were then incubated overnight at 4 °C with rabbit monoclonal antibodies anti-DNA PKcs (Abcam, ab32566, 1:1000); rabbit monoclonal antibodies anti-Sirt6 (CST, #12486, 1:1000) or rabbit polyclonal antibodies anti-Histone H3 (Abcam, ab#12486, 1:1000) in 5% BSA-TBST. After three washes for 10 min with TBST, membranes were incubated for 1 h at room temperature with goat anti-rabbit IgG H&L (HRP) (Abcam, ab6721, 1:5000). After three washes with TBST signal was developed with Clarity Western ECL substrate (Bio-Rad).

**Western blotting**

Exponentially growing cells were harvested with trypsin, counted and 10⁶ cells were resuspended in 100 µL of PBS containing protease inhibitors. 100 µL of 2×Laemmli buffer (Bio-Rad) was added and samples were boiled at 95 °C for 10 min. Samples were separated with 4–20% gradient SDS–PAGE, transferred to the PVDF membrane, and blocked in 5% milk-TBST for 2 h at room temperature. Membranes were then incubated overnight at 4 °C with rabbit monoclonal antibodies anti-DNA PKcs (Abcam, ab32566, 1:1000), rabbit monoclonal antibodies anti-Sirt6 (CST, #12486, 1:1000) or rabbit polyclonal antibodies anti-Histone H3 (Abcam, ab#12486, 1:1000) in 5% BSA-TBST. After three washes for 10 min with TBST, membranes were incubated for 1 h at room temperature with goat anti-rabbit IgG H&L (HRP) (Abcam, ab6721, 1:5000). After three washes with TBST signal was developed with Clarity Western ECL substrate (Bio-Rad). The images were quantified with Image Lab (Bio-Rad). All blots shown were derived from the same experiment and were processed in parallel.

**Reporting summary**

Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**DATA AVAILABILITY**

All data generated or analyzed during this study are included in this published article (and its supplementary information files) or are available from the authors upon a reasonable request.

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**REFERENCES**

1. Fontana, L., Partridge, L. & Longo, V. D. Extending healthy life span—from yeast to humans. Science 328, 321–326 (2010).
2. Mattson, M. P. Energy intake, meal frequency, and health: a neurobiological perspective. Annu. Rev. Nutr. 25, 237–260 (2005).
3. Weindruch, R. & Walford, R. L. Dietary restriction in mice beginning at 1 year of age: effect on life-span and spontaneous cancer incidence. Science 215, 1415–1418 (1982).
4. Anderson, R. M., Shannuganayagam, D. & Weindruch, R. Caloric restriction and aging: studies in mice and monkeys. Toxicol. Pathol. 37, 47–51 (2009).
5. Yu, B. P. Aging and oxidative stress: modulation by dietary restriction. Free Radic. Biol. Med. 21, 651–668 (1996).
6. Zainal, T. A., Oberley, T. D., Allison, D. B., Szveda, L. I. & Weindruch, R. Caloric restriction of rhesus monkeys lowers oxidative damage in skeletal muscle. FASEB J. 14, 1825–1836 (2000).
7. Sohal, R. S., Aparasu, S., Candas, M., Forster, M. J. & Lal, H. Effect of age and caloric restriction on DNA oxidative damage in different tissues of C57BL/6 mice. Mech. Ageing Dev. 76, 215–224 (1994).
8. Feuers, R. J., Weindruch, R. & Hart, R. W. Caloric restriction, aging, and antioxidant enzymes. Mutat. Res. 295, 191–200 (1993).
9. Hyun, D. H., Emerson, S. S., Jo, D. G., Mattson, M. P. & de Cabo, R. Calorie restriction up-regulates the plasma membrane redox system in brain cells and suppresses oxidative stress during aging. Proc. Natl Acad. Sci. USA 103, 19908–19912 (2006).
10. De Cabo, R. et al. Calorie restriction attenuates age-related alterations in the plasma membrane antioxidant system in rat liver. Exp. Gerontol. 39, 297–304 (2004).

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**AUTHOR CONTRIBUTIONS**

Z.K., A.S., and V.G. analyzed data and wrote the paper with input from all authors.

**COMPETING INTERESTS**

The authors declare no competing interests.

**ADDITIONAL INFORMATION**

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