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Lifelong dietary intervention does not affect hematopoietic stem cell function

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Hematopoietic stem cells (HSCs) undergo a profound functional decline during normal aging. Because caloric or dietary restriction has been shown to delay multiple aspects of the aging process in many species, we explored the consequences of lifelong caloric restriction, or conversely, lifelong excess caloric intake, on HSC numbers and function. Although caloric restriction prevented age-dependent increases in bone marrow cellularity, caloric restriction was not able to prevent functional decline of aged, long-term HSC functioning. A lifelong high-fat diet also did not affect HSC function. We conclude that lifelong caloric interventions fail to prevent or induce loss of age-associated HSC functioning.

Hematopoietic stem cells (HSCs) undergo a profound functional decline during normal aging, but their numbers increase [1]. Because caloric or dietary restriction has been shown to delay multiple aspects of the aging process in many species [2], we explored the consequences of lifelong caloric restriction or, conversely, lifelong excess caloric intake, on HSC numbers and function. To this end, mice were aged on an ad libitum diet (2141 AM II diet, AB Diets), a calorie-restricted diet (30% fewer calories than the 2141 AM diet II of ad libitum controls), or a high-fat diet (4031.09 high-fat diet, Lard, AB Diets). Compared with mice fed ad libitum, calorie-restricted mice received 30% fewer calories, and high-fat mice received 23% fat instead of 6% (Fig. 1A). To determine the effect of these lifelong diets on HSC pool size and function, we recorded bone marrow cellularity, HSC frequency, lineage bias, and number at 6-month intervals from 6 to 24 months. We subjected 24-month HSCs from all diets using in vitro and in vivo functional assays.

As expected, diet affected weight fluctuations with age in our aging populations. Although ad libitum and high-fat mice steadily gained weight with age, calorie-restricted mice at 6 months weighed less than other groups, maintained a steady weight throughout their recorded lifespan, and had less variation in this weight in aged populations compared with the other two diets (Fig. 1B).

As has been reported previously [3], ad libitum mice showed consistent increases in bone marrow cellularity with age, displaying significant increases in cellularity between 6 months and 18 to 24 months (Fig. 1C). This steady increase in bone marrow cellularity was not observed in calorie-restricted mice. In fact, calorie-restricted mice had bone marrow cell numbers similar to those of young ad libitum mice, and cellularity did not increase with age (Fig. 1C). In contrast, bone marrow cellularity in mice aged on a high-fat diet increased rapidly between 6 and 12 months, after which it stabilized.

The proportion of bone marrow cells that were phenotypic HSCs (here defined as $Lin^{-}Sca1^{+}c\text{-}kit^{+}Epcr^{+}CD34^{-}CD48^{-}CD150^{+}$) increased significantly with age between 12 and 24 months (compared with 6 months) in all experimental groups equally. This increase was as much as 30-fold in 24-month-old mice (Fig. 1D). This increase in HSC frequency is not different from what has been reported previously by us and by others [4–6].

The total pool size of HSCs per hind leg was calculated by factoring in bone marrow cellularity and HSC frequency.

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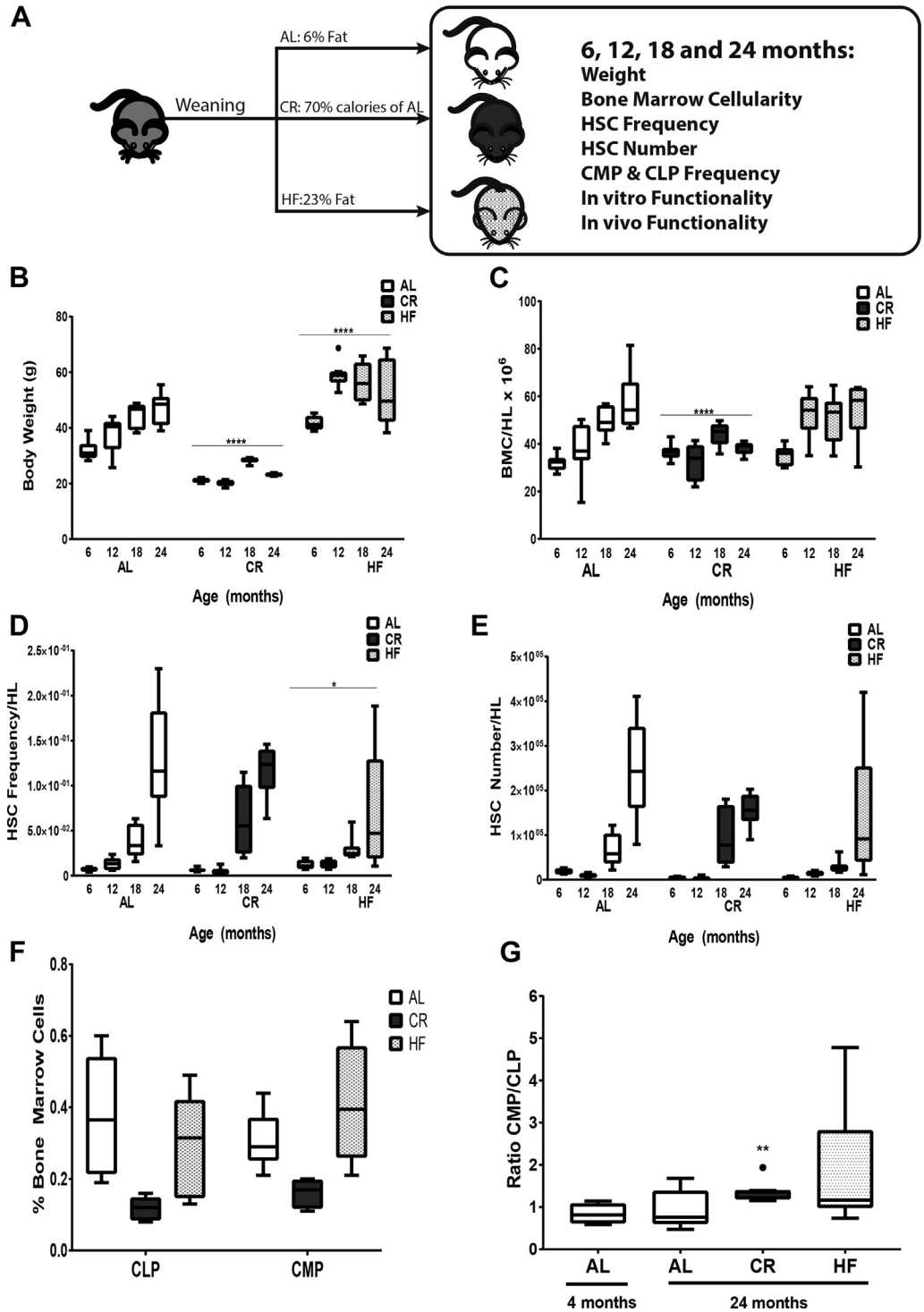

Figure 1. (**A**) Experimental setup. (**B**) Body weights of all cohorts. (**C**) Number of nucleated cells per hind limb. (**D**) Frequency of $Lin^{-}Sca1^{+}c\text{-}kit^{+}Epcr^{+}CD34^{-}CD48^{-}CD150^{+}$ cells per hind limb. (**E**) Absolute number of $Lin^{-}Sca1^{+}c\text{-}kit^{+}Epcr^{+}CD34^{-}CD48^{-}CD150^{+}$ cells per hind limb. (**F**) Frequency of CMPs and CLPs in bone marrow. **(G)** Ratio of CMPs/CLPs in bone marrow of young and aged mice as a function of diet.

Unlike bone marrow cellularity, caloric restriction did not prevent the increase in HSC pool. Mice aged on a high-fat diet did not display accelerated or enhanced increase in HSC frequency or pool. Irrespective of diet, in all experimental groups, total HSC pool size increased during aging (Fig. 1E). There were no statistically significant differences between the three cohorts. Interestingly, all hematopoietic phenotypes showed significantly increased