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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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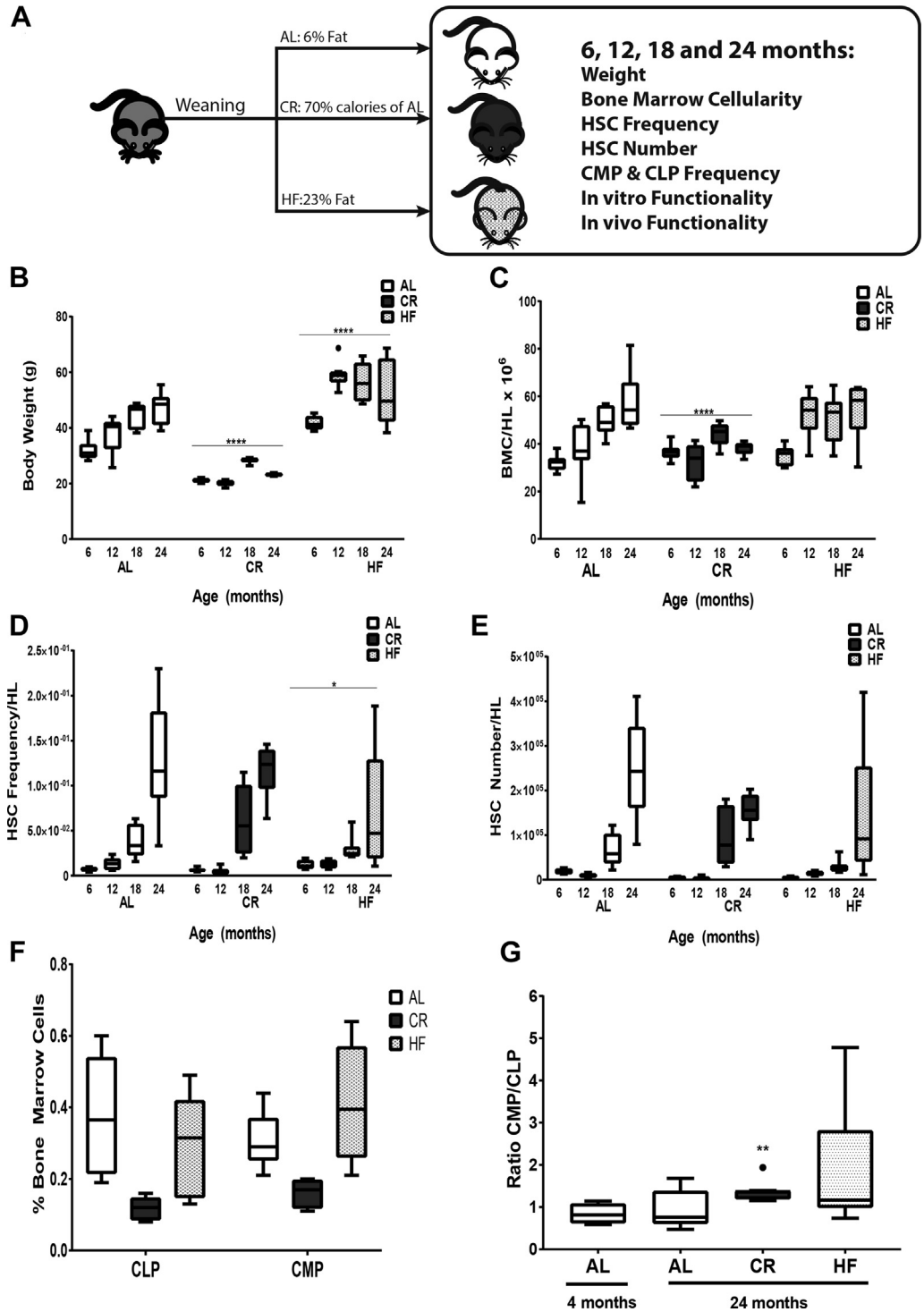

Figure 1. (**A**) Experimental setup. (**B**) Body weights of all cohorts. (**C**) Number of nucleated cells per hind limb. (**D**) Frequency of $Lin^{-}Sca1^{+}c\text{-}kit^{+}Epcr^{+}CD34^{-}CD48^{-}CD150^{+}$ cells per hind limb. (**E**) Absolute number of $Lin^{-}Sca1^{+}c\text{-}kit^{+}Epcr^{+}CD34^{-}CD48^{-}CD150^{+}$ 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

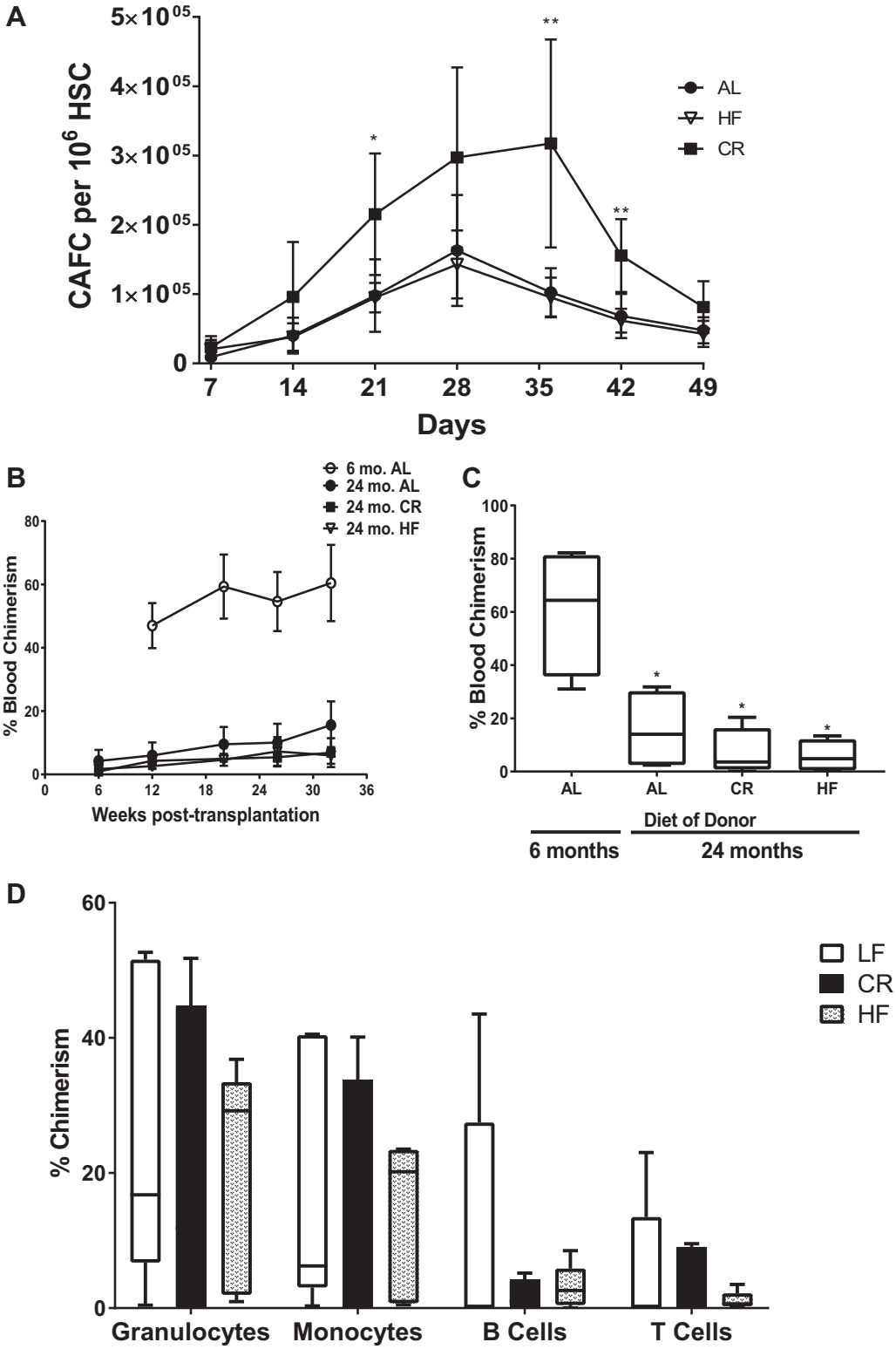

Figure 2. (**A**) Cobblestone-area-forming cell frequencies of highly purified Lin$^-$Sca1$^+$c-kit$^+$Epcr$^+$CD34$^-$CD48$^-$CD150$^+$ cells isolated from aged mice on three different diets. (**B, C**) Percentage of donor-derived peripheral blood cells in sublethally conditioned W41.SJL recipients transplanted with 15 young or 30 aged Lin$^-$Sca1$^+$c-kit$^+$Epcr$^+$CD34$^-$CD48$^-$CD150$^+$ cells. (**D**) Lineage contribution in recipients transplanted with aged Lin$^-$Sca1$^+$c-kit$^+$Epcr$^+$CD34$^-$CD48$^-$CD150$^+$ cells isolated from mice given low-fat (ad libitum), calorie-restricted, or high-fat diets. Asterisks refer to statistical significant differences compared with mice fed ad libitum (**A**) or with young mice (**C**).

variation in the population with age (Fig. 1C, 1D, and 1E; significant *F*-test). Whereas hematopoietic parameters were highly similar in young mice, upon aging, phenotypes in individual mice started to deviate substantially. Because it is now well established that during aging, HSCs become more prone to producing myeloid cells at the expense of lymphoid cell production [6–9], we also assessed whether the frequency and ratio of common myeloid progenitors (CMPs) and common lymphoid progenitors (CLPs) in the bone marrow would be affected by diet. Although the relative frequency of both CMPs and CLPs was somewhat lower in calorie-restricted mice (Fig. 1F), the ratio of these two progenitor subsets in aged mice was not affected (Fig. 1G).

We also investigated whether aging on a calorie-restricted or high-fat diet would affect HSC function. $Lin^{-}Sca1^{+}c\text{-}kit^{+}Epcr^{+}CD34^{-}CD48^{-}CD150^{+}$ HSCs were isolated from bone marrow of mice that were fed ad libitum or kept on a calorie-restricted or a high-fat diet for 24 months. Purified HSCs were seeded on stromal cell layers to assess cobblestone-area-forming cell frequencies or were transplanted in W41.SJL recipient mice that were irradiated with 2,5 Gy. For transplantations, 15 young or 30 aged C57BL/6 HSCs, isolated from all cohorts, were transplanted in conjunction with 1×10^6 W41.SJL competitor cells. In vitro, long-term HSCs (LT-HSCs) isolated from calorie-restricted mice showed a somewhat higher potential to generate cobblestones that appeared at late time points, whereas a high-fat diet did not affect in vitro colony growth (Fig. 2A). In vivo studies did not reveal any significant functional difference between the three cohorts, and caloric restriction failed to improve the functional impairment in engraftment of aged HSCs (Fig. 2B and 2C). Even though twofold more LT-HSCs from aged mice were transplanted, young cells led to threefold to fourfold higher engraftment levels. This decline in functional capacity of aged (24-month-old) HSCs was consistent with previous studies, including our own [6,8,10,11]. Finally, we assessed the lineage contribution of aged LT-HSCs from the three cohorts at 30 weeks after transplantation (Fig. 2D). In all instances, transplanted LT-HSCs resulted in the typical myeloid skewing pattern associated with aging, and dietary intake did not affect this significantly.

In conclusion, although lifelong caloric restriction affected total bone marrow cellularity, we failed to detect any functional improvement of aged primitive HSCs to contribute to hematopoiesis. In addition, a lifelong high-fat diet also did not affect HSC function negatively. Our results are seemingly at odds with a previously published study in which improved functioning of HSCs upon caloric restriction was observed [12]. It is not evident how this discrepancy can be explained, but there are multiple differences in experimental setup. First, we only performed transplantations after lifelong (24 months) caloric restriction, whereas Tang et al. [12] restricted caloric intake for 6 or 12 months, starting at only 3 months of age, and HSCs were analyzed at 12 months of age. It is possible that the duration of caloric restriction affects HSC functionality. In addition, the composition of the standard diet may affect the consequences of caloric restriction. It has been reported recently that dietary depletion of specific amino acids, notably valine, can have severe consequences for HSC functioning [13]. It is therefore conceivable that subtle changes in the amino acid composition of food intake affect the outcome of dietary restriction experiments. In addition, the transplantation model that we used (i.e., transplanting a very low number of HSCs in sublethally irradiated recipients with a compromised hematopoietic system) is different from the model used by Tang et al. [12]. In addition, because the variation in HSC phenotypes between individual mice increases dramatically during aging, irrespective of dietary intervention, it is possible that caloric restriction affects individual mice differently. In contrast to Tang et al. [12], in our experiments, HSCs from donor mice were not pooled, but rather HSCs from individual donors were transplanted separately in cohorts of recipients. Finally, the outcome of caloric restriction studies is dependent on many parameters, and seemingly conflicting results in this field have been reported previously [14]. Carefully designed multicenter experiments are warranted to confirm or refute whether and how caloric intake can affect HSC functioning.

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