The Effects of Nutrients on Stem Cell Function and Regeneration in Bone in Response to Ectopic Adipogenesis

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Abstract

During aging, adipocytes accumulate in the cavities of long bones by slowly replacing the red, hematopoietic bone marrow. Obesity and caloric restriction (CR) accelerate the expansion of bone marrow adipose tissue (BMAT). BMAT secretes adipokines and provides lipids, thereby exerting paracrine and endocrine effects on local stem cells. Excessive BMAT accumulation seems to impair regenerative processes, including hematopoiesis and osteogenesis. In mice, a similar pattern of BMAT accumulation occurs. We find BMAT accumulation changes in response to aging and dietary challenges, such as high sucrose and/or high fat intake, as well as caloric restriction. Additionally, we find alterations in gene expression and the fatty acid composition in defined bone compartments. In summary, our results demonstrate critical changes in the bone marrow niche as a response to dietary exposure. They highlight nutrient-dependent mechanisms that modulate bone regeneration and BMAT formation as a function of age and diet.

Strategy of nutritional challenges

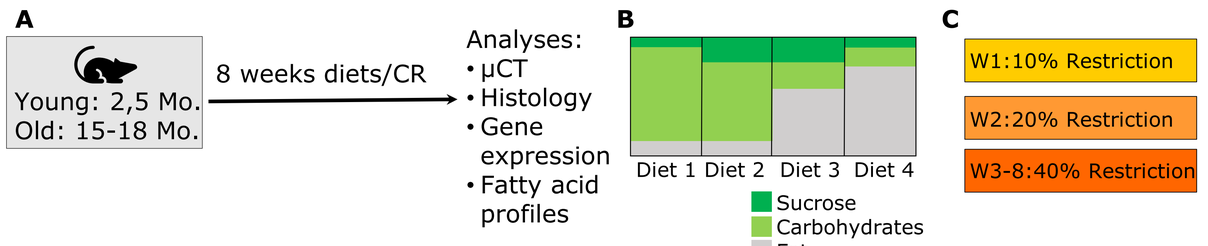

Figure 1: Experimental set up to investigate dietary effects on different parameters of bone health. (A) Young (2,5 Months old) or old (15-18 Months old) mice received CR or HFD for 8 weeks. Upon sacrifice, different analysis were carried out. **(B)** Nutrient composition of 4 high-sucrose/high-fat diet interventions. Diet 1: 7 kcal% sucrose, 10 kcal% fat; Diet 2: 17 kcal% sucrose, 10 kcal% fat; Diet 3: 17 kcal% sucrose, 45 %kcal fat, Diet 4: 7 kcal% sucrose, 60 kcal% fat. Diet 1 serves as a control-diet, Diet 2 is a high-sucrose diet, diet 3+4 serve as HFDs. All diets contain 20 kcal% of protein. **(C)** Caloric restriction with micronutrient-enriched diet: CR mice received a stepwise reduction of food intake, a fortified diet was used to prevent any micronutrient deficiencies.

Nutritional disruptions decreased bone quality in aged mice

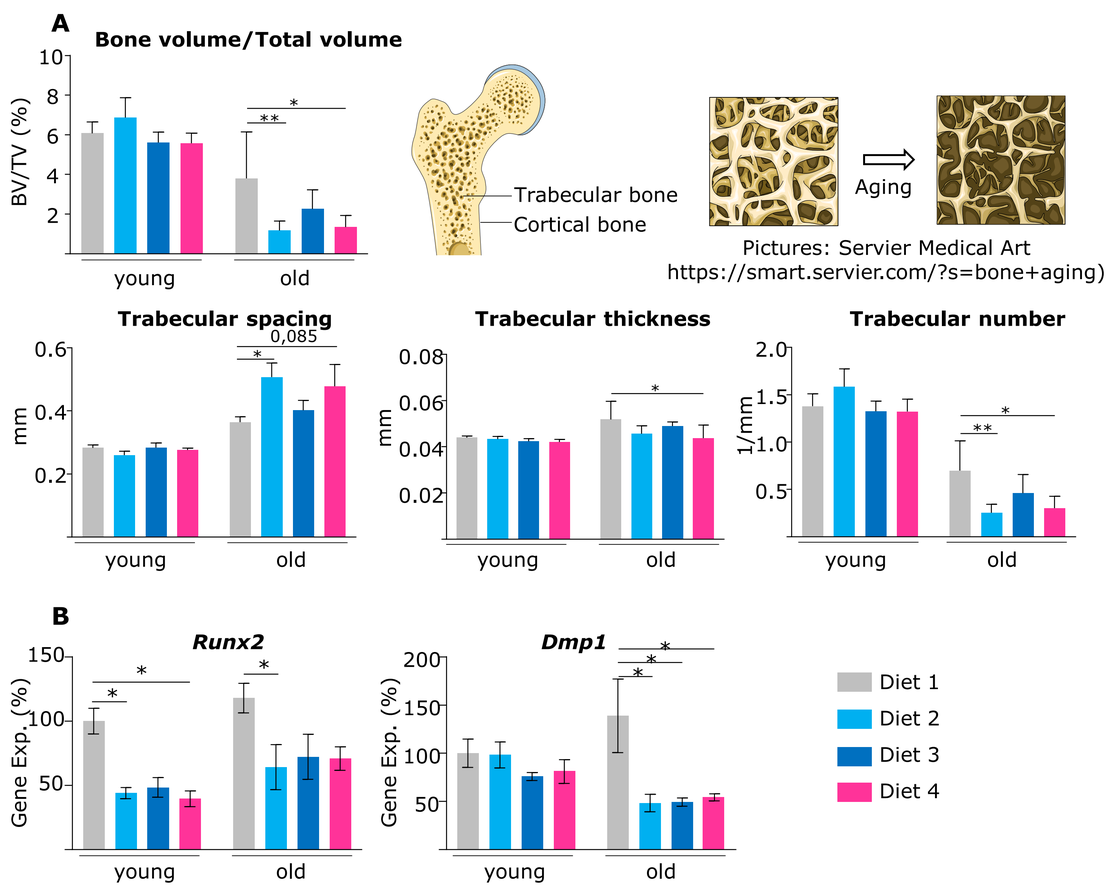

Figure 2: Nutritional challenges decreased bone quality in old mice. (A) µCT Analysis of tibia from young and old mice on diets 1-4 for 8 weeks. Old mice on high fat and/or sucrose diets displayed a decrease in bone volume/total volume and an increase in trabecular spacing through a reduction in trabecular thickness and number. **(B)** Gene expression of osteogenic markers genes: Runt-related transcription factor 2 (*Runx2*), an early osteogenesis marker decreased in diets high in fat and/or sucrose. Dentin matrix acidic phosphoprotein (*Dmp1*), functionally involved in mineralization, showed reduced expression only in old mice following nutritional challenges. (n=3-4, SEM. Statistical analysis: 2-way ANOVA; *=p<0,05; **=p<0,005)

Caloric restriction ameliorated aged bone-defects

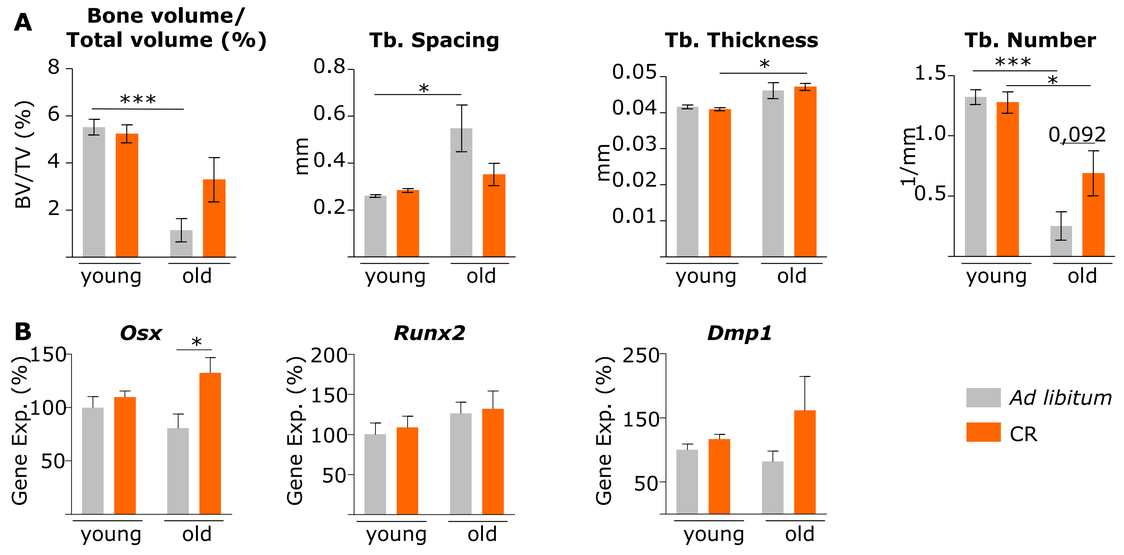

Figure 3: Caloric restriction ameliorated age-related bone loss. (A) Aged mice showed a decrease in bone volume/total volume ratio, which was ameliorated by CR. Old, *ad libitum*-fed mice showed an increase in trabecular spacing and decreased trabecular numbers. This effect was less pronounced in aged animals on caloric restriction, suggesting that this dietary intervention improves age-related bone dysfunction. **(B)** Aged mice on caloric restriction displayed an increased expression of early osteogenic marker Osterix (*Osx*) in tibia bone, and a trend to an increase in *DMP1* mRNA. (n=3-4, SEM. Statistical analysis: 2-way ANOVA; *=p<0,05; **=p<0,005 ***=p<0,0005

Caloric restriction increased BMAT accumulation

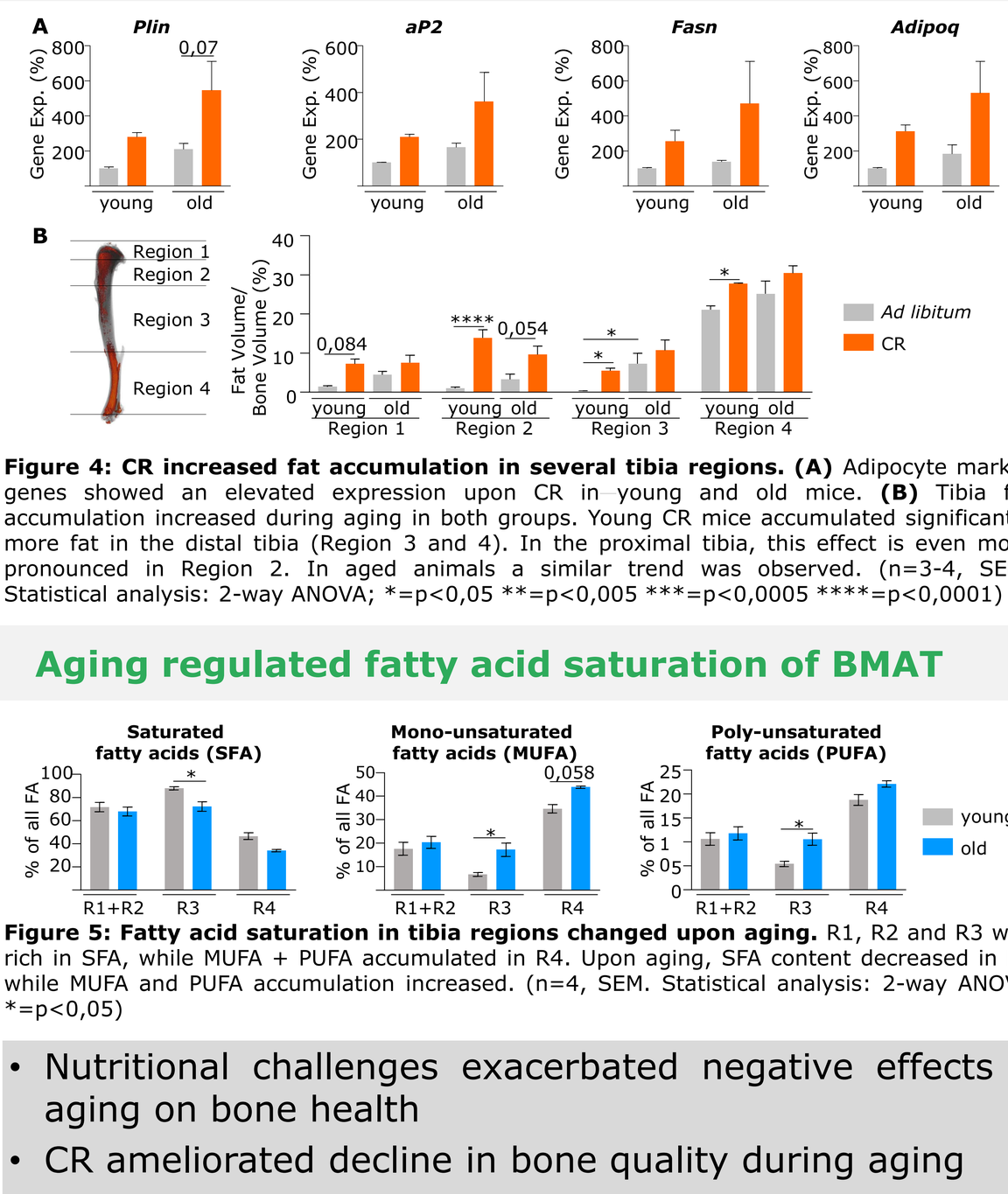

Figure 4: CR increased fat accumulation in several tibia regions. (A) Adipocyte marker genes showed an elevated expression upon CR in young and old mice. **(B)** Tibia fat accumulation increased during aging in both groups. Young CR mice accumulated significantly more fat in the distal tibia (Region 3 and 4). In the proximal tibia, this effect is even more pronounced in Region 2. In aged animals a similar trend was observed. (n=3-4, SEM, Statistical analysis: 2-way ANOVA; *=p<0,05 **=p<0,005 ***=p<0,0005 ****=p<0,0001)

Aging regulated fatty acid saturation of BMAT

Figure 5: Fatty acid saturation in tibia regions changed upon aging. R1, R2 and R3 were rich in SFA, while MUFA + PUFA accumulated in R4. Upon aging, SFA content decreased in R3, while MUFA and PUFA accumulation increased. (n=4, SEM. Statistical analysis: 2-way ANOVA: *=p<0,05)

- Nutritional challenges exacerbated negative effects of aging on bone health
- CR ameliorated decline in bone quality during aging
- CR increased bone fat accumulation and adipocyte marker gene expression
- FA composition was specific to certain bone regions and changed upon aging

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