DEPTOR promotes bone marrow adipose tissue expansion in calorie restricted mice

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Elevated levels of fat in bones is a characteristic feature of osteoporosis and other skeletal disorders. Understanding the preference for adipose differentiation over osteogenesis is key to addressing the rising prevalence of bone fragility in our aging population. While the molecular mechanism behind this shift between bone and fat formation remains incompletely understood, it is evident that the alteration of skeletal stem cell (SSC) differentiation likely plays a pivotal role. In particular, cellular metabolism is critical for SSC maintenance and lineage allocation. Interestingly, both osteoporosis and dietary restrictions lead to similar increased fat/bone ratio. Recently, we showed high levels of DEPTOR, an mTOR inhibitor and therefore a metabolic regulator, in several skeletal dysplasias with altered differentiation of skeletal progenitors. Here, we elucidate the role of DEPTOR in the increase in bone marrow adipose tissue (BMAT) under restricted nutrition. WT mice subjected to a 30% caloric restriction showed decreased trabecular bone and increased BMAT whereas *DEPTOR* KO mice exhibited little to none BMAT increase and even higher trabecular bone parameters compared to WT in response to caloric restriction. Proteomic analysis from bone marrow revealed 1110 upregulated and 478 downregulated proteins under caloric restriction in WT mice. Interestingly, in calorie restricted *DEPTOR* KO mice, more than half of these proteins reverted to levels similar to those observed in WT controls. Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis showed that these proteins contribute to several processes related to lipids such as fatty acid biosynthesis, elongation, and metabolism. These results point to DEPTOR as a key regulator in the metabolic control of skeletal progenitors' differentiation under specific nutritional status. They also provide the rationale for its study as a pathological marker of abnormal bone/fat balance and as a potential target for the treatment of different low bone mass pathologies.

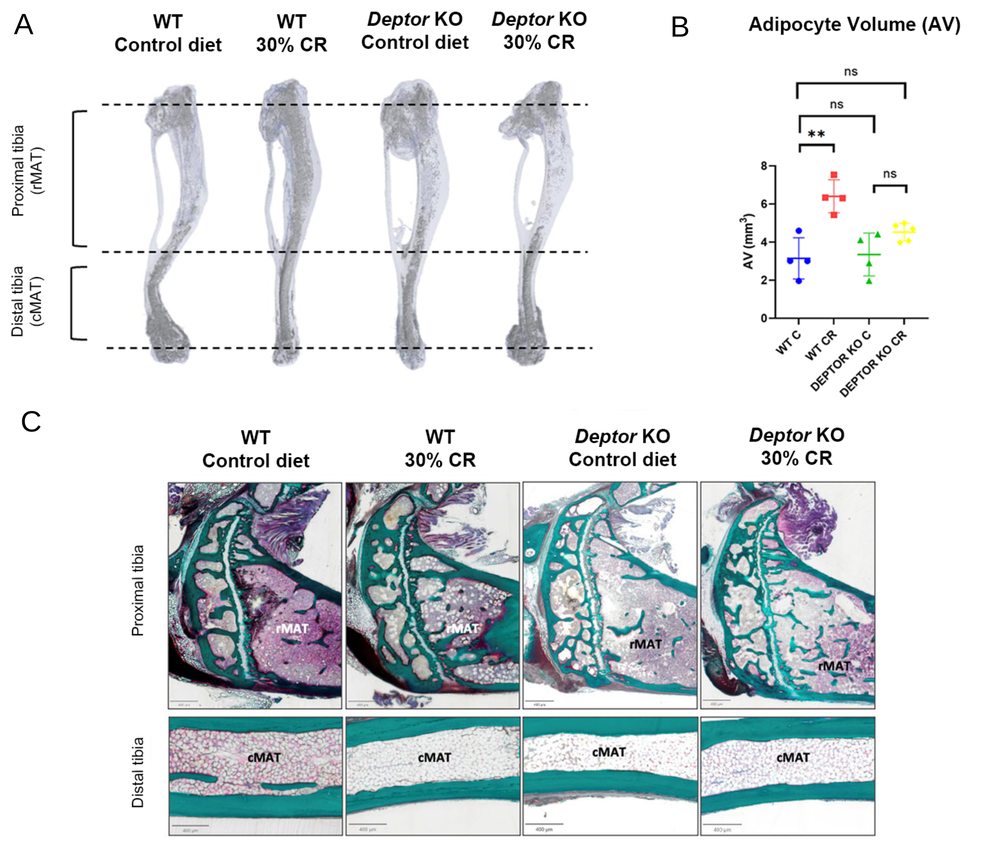

Figure 1. *Deptor* KO mice do not accumulate BMAT under caloric restriction. (A-B) Osmium tetroxide staining (A) and adipocyte volume quantification (B) of wild-type and *Deptor* KO mice fed with control (C) or 30% caloric restriction (CR) diets for 12 weeks. WT mice show increased BMAT due to restricted nutrition whilst *Deptor* KO mice show no fat accumulation. (C) Masson's trichrome staining of methacrylate-embedded tibias showing the same phenotype.

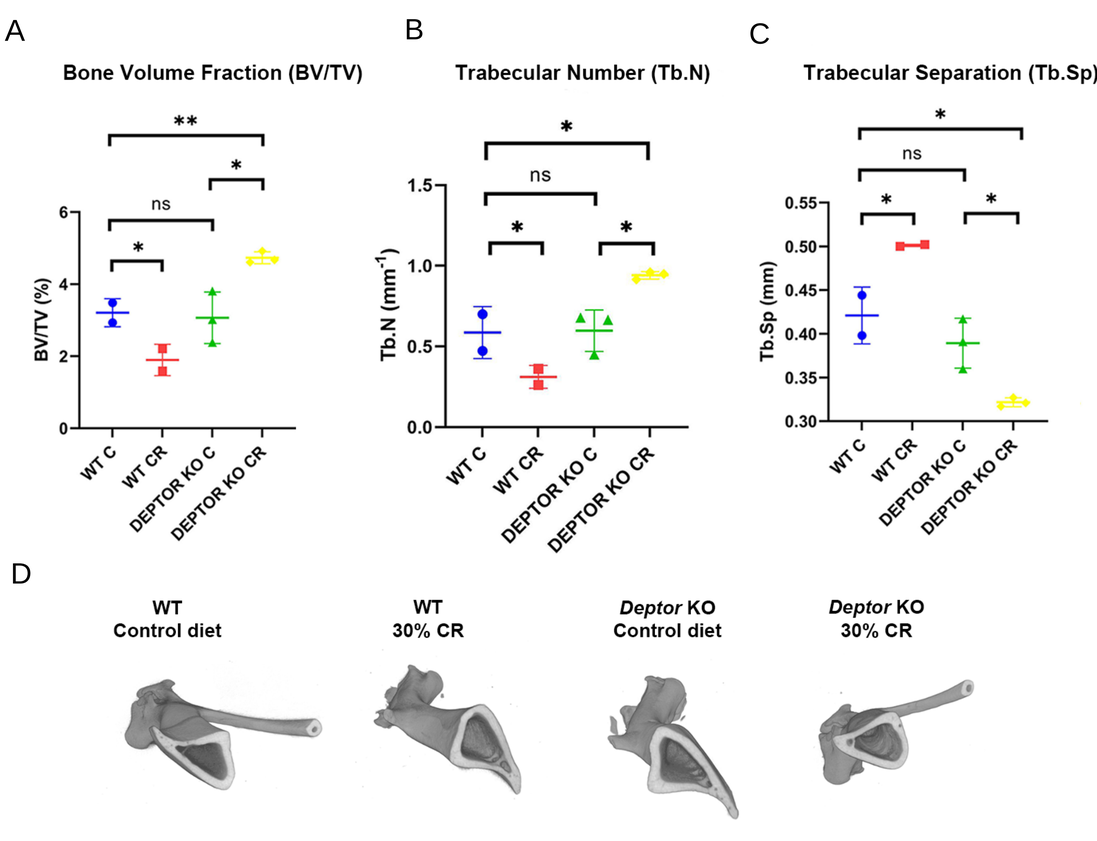

Figure 2. Caloric restriction increases trabecular bone in *Deptor* KO mice. (A-D) Trabecular bone parameters measured by microcomputed tomography (µCT). Bone volumen fraction (A), trabecular number (B) and trabecular separation (C) were analyzed. µCT analysis shows decreased trabecular bone in WT mice after CR, consistent with the observed fat accumulation, but increased trabecular bone in calorie restricted *Deptor* KO mice, being even higher tan controls.

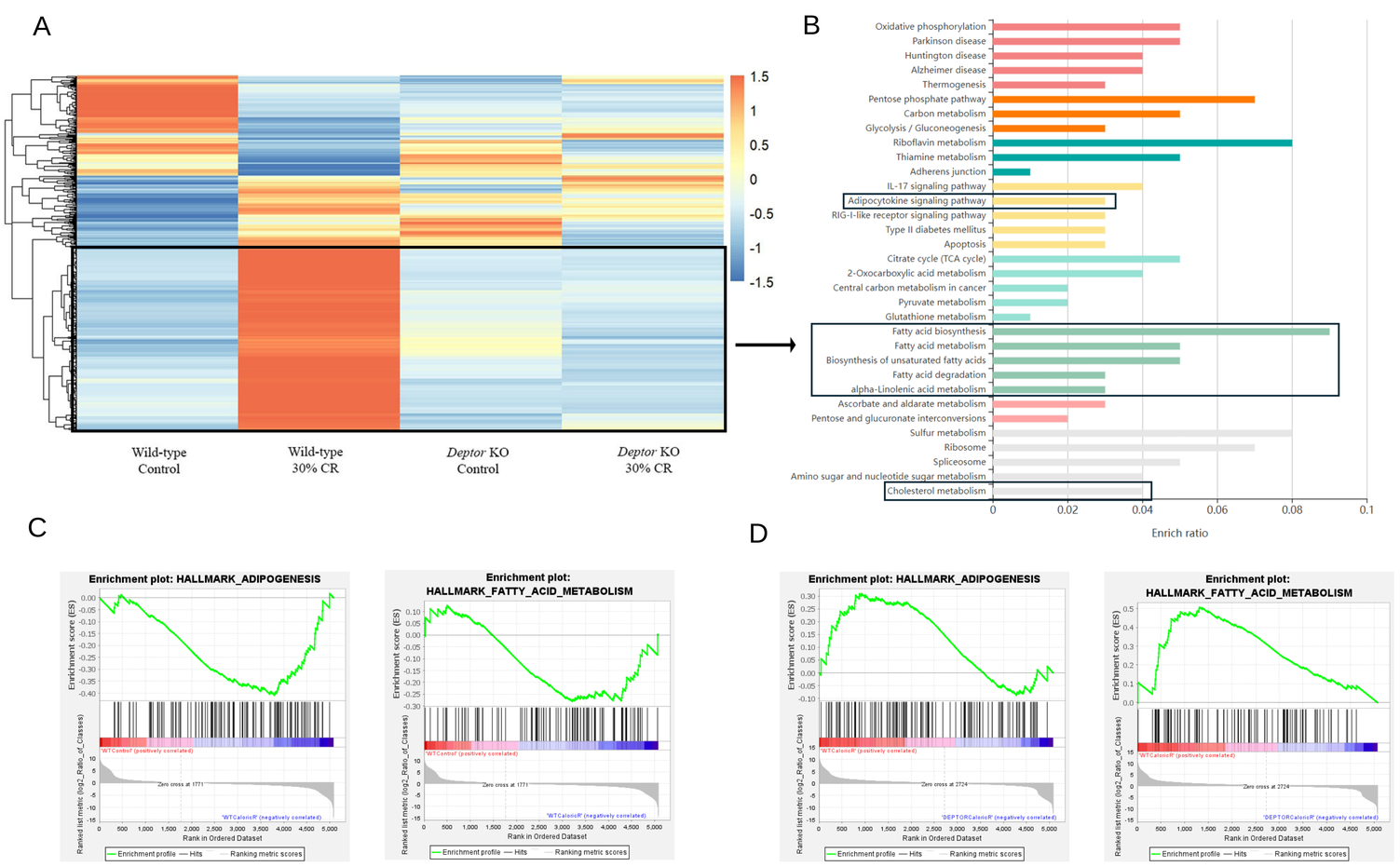

Figure 3. Proteomic analysis of *Deptor* KO bone marrow confirms an aberrant response to CR. Label-free MS-based proteomics from bone marrow extracts was performed. (A) Heatmap showing changes in protein levels in the different experimental groups. In *Deptor* KO mice under CR most proteins that respond to CR have levels similar to WT mice subjected to C diet (marked in rectangle). (B) KEGG enrichment analysis of the proteins pointed in A indicating that these proteins are related to fatty acids, cholesterol and adipocytokine pathways. (C-D) Gene set enrichment analysis of WT C vs WT CR mice (C) and WT CR vs *Deptor* KO CR (D). Mouse hallmark gene set from MSigDB was used. WT mice under dietary restriction show enhanced adipogenesis and fatty acid metabolism protein expresion compared to WT mice in C diet (C) and *Deptor* KO mice in CR (D).

Conclusions:

1. DEPTOR has a key role in the increase of bone marrow adipose tissue observed under caloric restriction.
2. Loss of DEPTOR under restricted nutrition not only impairs adipocyte accumulation in bone marrow but also promotes trabecular bone formation.
3. Bone marrow response to caloric restriction is dysregulated in *Deptor* KO mice in pathways related to adipogenesis and lipid metabolism.