Iron Deficiency Uniquely Reprograms the Amino Acid and Lipid Metabolism of Red Pulp Macrophages to Support Enhanced Iron Recycling Functions

ABSTRACT

Iron deficiency is the most common nutritional disorder worldwide, yet the ways in which specialized cell types adapt to limited iron availability remain incompletely understood. Splenic red pulp macrophages (RPMs) play a crucial role in systemic iron homeostasis. They continuously remove aged red blood cells (RBCs) through a process known as erythrophagocytosis (EP) and recycle iron back into the circulation. However, the impact of iron-deficient (ID) conditions on the function and metabolic programs of RPMs has not been clearly established.

This dissertation builds on previous findings to demonstrate that ID conditions do not impair the function of RPMs; rather, they induce a coordinated adaptive state characterized by enhanced EP, increased mitochondrial activity, and significant metabolic remodeling that supports heightened oxidative metabolism. More specifically, this thesis leverages proteomic analyses and functional approaches to demonstrate that this adaptive state relies on the selective activation of oxidative metabolic pathways, particularly branched-chain amino acid (BCAA) catabolism and fatty acid β-oxidation. This coordinated response is highly specific to this cell type and is not observed in other macrophage populations. Notably, BCAA metabolism emerges as a crucial aspect of the metabolic adaptation observed in ID RPMs. The rate-limiting enzyme branched-chain α-ketoacid dehydrogenase subunit B (BCKDHB) is selectively upregulated, and pharmacological inhibition of BCAA catabolism reverses the heightened EP and mitochondrial activity that occur under ID conditions, both *in vitro* and *in vivo*. Meanwhile, lipid metabolism shifts toward increased β-oxidation and decreased lipid storage; inhibiting fatty acid oxidation similarly disrupts EP. Together, these findings identify BCAA- and lipid-derived substrates as key drivers of the increased bioenergetic demands associated with sustained RBC clearance.

Additionally, this metabolic reprogramming is linked to the systemic regulation of iron homeostasis. The low hepcidin-high ferroportin (FPN) axis, along with spleen tyrosine kinase (SYK) signaling, establishes a regulatory framework that connects iron export to these metabolic adaptations and increased mitochondrial respiration in ID RPMs. Within this framework, the data presented here define how these conditions are accompanied by the selective engagement of metabolic pathways, including BCAA catabolism and fatty acid β-oxidation, that support increased mitochondrial activity and sustained EP.

In summary, this thesis defines how the coordinated activation of amino acid and lipid metabolic pathways supports RPM function under ID conditions. Specifically, the data demonstrate that BCAA catabolism and fatty acid β-oxidation contribute to sustaining mitochondrial activity and EP, thereby supporting RBC clearance during iron limitation. These findings establish a functional link between metabolic pathway engagement and the capacity of RPMs to maintain iron-recycling activity under conditions of restricted iron availability.