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# Comparative Analysis of Hepatic Iron Storage and Stable Isotope Ratios ($\delta^{56}$Fe) Across Vertebrate Species

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The scarcity of bioavailable iron has driven the evolution of regulatory mechanisms in vertebrates that prioritize iron acquisition over excretion. Environmental differences in iron availability have likely resulted in interspecific variation in iron demand and storage strategies. The stable iron isotope ratio ($\delta^{56}$Fe) records biological fractionation during intestinal absorption and intracellular storage and thus provides a powerful tracer of iron metabolic regulation. However, whether $\delta^{56}$Fe systematically reflects interspecific differences in iron storage strategies remains unclear.

Here, we investigated species-specific differences in hepatic iron storage and their isotopic signatures across 14 vertebrate species (8 mammals, 2 birds, 2 reptiles, 2 fish), together with multi-organ analysis in laboratory mice (*Mus musculus*, Slc:ddY, male, n = 5). Storage iron fractions were quantified using Fe K-edge X-ray Absorption Near-Edge Structure (XANES) spectroscopy at KEK (Japan). Stable iron isotope ratios were measured using MC-ICP-MS (Neptune Plus) with the addition of double-spikes ($^{57}$Fe–$^{58}$Fe) to correct measurement errors. Analytical accuracy was validated using DOLT-5 (NRC Canada) and two in-house standards (maximum deviation ±0.2‰).

Among mouse tissues, $\delta^{56}$Fe values were highest in the liver and spleen (−1.52‰ to −0.80‰; −1.58‰ to −1.12‰, respectively). The proportion of storage iron (ferritin-bound iron) per total iron was highest in the spleen (64–89%) and testis (65–79%), followed by liver (18–54%). Across tissues, $\delta^{56}$Fe positively correlated with storage iron fraction ($r = 0.55$, $p = 0.01$), indicating preferential retention of heavier isotopes in storage pools.

Interspecific comparison of liver samples revealed that mammals (−1.27‰ to −1.18‰) exhibited higher $\delta^{56}$Fe values than birds (−2.17‰ to −1.56‰) and reptiles (−3.44‰ to −1.94‰). Hierarchical Bayesian modeling demonstrated a negative correlation between hepatic $\delta^{56}$Fe and storage iron across species ($r = -0.42$, $p = 0.01$), opposite to the intra-tissue trend observed in mice. This pattern suggests that species with high storage iron may exhibit lower intestinal absorption efficiency, favoring uptake of isotopically lighter iron.

These findings demonstrate that tissue $\delta^{56}$Fe captures both intra-organ iron partitioning and interspecific differences in iron acquisition strategies, highlighting its potential as a mechanistic tracer for comparative studies of iron metabolism in both wild and domestic vertebrates.