

**Dietary Fat Enhances Intestinal Copper Absorption by
Modulating Copper Transporter 1 (CTR1/SLC31A1)**

Yingze Martin Ma¹, Yu Wang¹, Svetlana Lutsenko¹

¹ Department of Physiology, Pharmacology and Therapeutics, Johns Hopkins University School of Medicine, Baltimore, MD

Copper (Cu) is an essential micronutrient required for numerous biological processes. As the only known high-affinity Cu importer, Cu Transporter 1 (CTR1/SLC31A1) is required for dietary Cu absorption, yet its localization and function in enterocytes remain controversial. Studies of CTR1 mechanisms in the intestine have been complicated by its low abundance and the lack of reliable tools to monitor CTR1 localization. Consequently, to better understand CTR1 localization and function in polarized cells of intestinal origin (Caco-2), we fused a bright fluorescent protein, mStaygold, via a flexible linker to the C-terminus of endogenous CTR1 using CRISPR/Cas9-based genomic knock-in. Successfully edited cell clones were differentiated on transwell inserts and imaged with super-resolution microscopy to reveal CTR1 localization under various nutritional conditions. We found that basolaterally localized CTR1 responds to dietary fat treatment and relocates to the sub-apical compartment of polarized Caco-2 cells. To understand the functional relevance of this phenomenon, we co-treated differentiated Caco-2 cells with Cu and dietary fat. We observed that dietary fat-induced CTR1 relocation enhances Cu absorption in these cells. Immunostaining for the Cu exporter ATP7A confirmed increased Cu entry under this condition. These observations were further validated in human duodenal enteroid monolayers and mouse intestines. In conclusion, we developed an innovative tool to study CTR1 dynamics and identified dietary fat as a novel modulator of intestinal Cu absorption. Further work is needed to understand the mechanism of CTR1 endocytosis. This work was supported by NIH UC2HD113036.