LAY ABSTRACT

It is estimated that 3% of infants born in the U.S. have at least one serious congenital malformation. We suggest that suboptimal nutrition during pregnancy is a key modulator of embryonic and fetal development. In all species studied to date, a deficit of copper (Cu) during pregnancy results in cardiovascular and skeletal defects, and neurological and immune system abnormalities that can persist into adulthood. Organ meats, seafood, wheat bran cereals, whole grain and cocoa products, nuts and seeds are good food sources of Cu however the trend in the U.S. is the increased consumption of refined foods and poor food choices leading to lower quality diets. Low diet quality has been associated with an increased risk of congenital malformations in California women. A substantial percentage of adults have dietary Cu intakes less than the recommended dietary allowance (RDA) of 0.9 mg/day, which is further increased in populations with food insecurity. Additionally, high zinc and iron intakes (commonly used mineral supplements) can lead to suboptimal Cu status fueling the concern that marginal Cu deficiency is a public health problem.

One mechanism that may underlie Cu deficiency-induced tissue pathology is an increase in tissue damage by reactive nitrogen molecules. Under conditions of low Cu, the activity of Cu-zinc superoxide dismutase, a protein that helps defend the body against oxygen free radicals, decreases. The result is an increase in oxygen free radicals. These oxygen free radicals can combine further with an important biological signaling molecule, nitric oxide, to produce peroxynitrite which is a very strong initiator of oxidative damage. Peroxynitrite can nitrate proteins which can negatively alter a protein's function. Peroxynitrite-induced damage is thought to be involved in the development and progression of a number of neurodegenerative diseases including Alzheimer's disease, Parkinson's disease, multiple sclerosis, amyotrophic lateral sclerosis (ALS), prion disease, and traumatic brain injury. Several of these neurodegenerative diseases are also linked with abnormal Cu metabolism. Several lines of evidence show that Cu deficiency leads to brain defects and neurodegeneration. First, infants with Menkes disease who have mutations in a Cu-transporting protein that leads to Cu deficiency, are characterized by mental retardation, seizures, neural degeneration and neuronal cell death. Second, offspring of Cu deficient rodent dams have neurodegeneration, and abnormal motor function which persists even after adequate levels of Cu are given back in the diet. We, and others, have shown that the Cu deficient embryos and offspring have increased levels of protein nitration. In this proposal, we will determine if Cu deficiency nitrates proteins in the brains of offspring at three developmentally important timepoints, identify whether there are negative functional consequences of this protein nitration, and determine whether these changes occur early in fetal development, and persist into early adulthood. In addition to dietary Cu deficiency, a model of genetic Cu deficiency will be used since humans have been shown to carry mutations in Cu genes. The uptake of Cu into a cell is modulated by a protein called Cu transporter-1 (Ctr-1). Mice that do not carry the gene for Ctr-1 die in mid-gestation. Mice that have one copy of the Ctr-1 gene (Ctr-$1^{+/-}$) survive and appear to be normal. However, Ctr-$1^{+/-}$ mice have lower Cu concentrations in several parts of the brain compared to Ctr-1 mice who have two copies of the Ctr-1 gene indicating that the lack of one allele of the Ctr-1 gene results in altered Cu homeostasis. In this proposal, we will determine whether dietary or genetic Cu deficiency leads to the nitration of proteins that contribute to Cu deficiency-induced brain pathology. These proteins include tyrosine hydroxylase (an enzyme involved in neurotransmitter synthesis), tubulin and actin (involved in the cytoskeleton and the production of neurons), and heat shock protein 90 (a molecule that is increased under conditions of stress and is involved in cell signaling and survival). We will also examine if protein nitration alters the activity or function of the protein. Combined information from the above work will provide new insights into the mechanisms underlying Cu deficiency-induced pathologies, with ramifications for pregnant women as well as for the general population. The finding that an imbalance of the metal ion, Cu, leads to brain nitration and protein dysfunction will underscore the fact that a nutritional insult can have profound effects on brain development and pathology.