Comparison of Intestinal Iron Uptake From Different Plant and Animal Proteins and Food Matrices: Studies Using an In Vitro Digestion/Caco-2 Cell Culture Model

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Objectives: The aim of this study was to assess the effect of cyanocobalamin on intestinal iron uptake in the presence of different plant and animal proteins and food matrices.

Methods: Different proteins and foods with added iron and cyanocobalamin were digested using Infogest digestion protocol with minor changes. Concentrations of added ferrous sulphate (FeSO<sub>4</sub>) and  $B_{12}$  was 10  $\mu$ mol/L and 50  $\mu$ mol/L in the digesta. Digesta was introduced into Caco-2 cells for 12 hours. Growth medium was removed, and cells were washed twice with ice cold Phosphatebuffered saline (PBS). Cells were harvested by adding an aliquot of deionized water, placing them in a sonicator at 4°C for 15 min, then scraped, collected with 2 mL water in each well, stored at  $-20^{\circ}$ C. The harvested cell suspension was used to analyse ferritin and total protein concentrations using ferritin solid-phase sandwich enzymelinked immunosorbent assay (ELISA) and Pierce Bicinchoninic acid assay (BCA) respectively. The ratio of ferritin/total protein expressed as ng ferritin/mg protein was used as an index of cellular iron uptake.

Results: Bioavailability of FeSO<sub>4</sub> was higher in the presence of animal proteins (22.20  $\pm$  3.35, 19.77  $\pm$  2.90, 41.52  $\pm$  2.74 ng ferritin/mg protein for casein, egg albumin, myoglobin respectively) when compared with plant proteins (16.47  $\pm$  1.63, 15.84  $\pm$  2.40,  $13.37 \pm 3.68$  ng ferritin/mg protein for gluten, rice, pea protein respectively). Vitamin B<sub>12</sub> improved the bioavailability of FeSO<sub>4</sub> in the Caco-2 cell model. With the final concentration of cyanocobalamin at 50 mmol/L, ferritin-protein ratio for casein, egg albumin, myoglobin, gluten, rice, pea protein increased 1.3, 1.3, 1.2, 1.6, 1.5, 1.6 times respectively. Similarly, for milk, yogurt, Indian flat bread, bread, rice pancake and rice-pea pancake, addition of vitamin B12 improved ferritin-protein ratio 1.5, 1.1, 1.4, 1.2, 1.6, 1.5 times respectively compared to only FeSO<sub>4</sub> fortification.

Conclusions: Cyanocobalamin promotes iron uptake from FeSO<sub>4</sub> in presence of different proteins. Whole foods initiate more iron uptake than protein isolates. Among the protein isolates, myoglobin shows highest iron uptake. Yogurt shows highest intestinal iron uptake in the presence of cyanocobalamin and rice-pea mix could be one of the best options for vegan diet in terms of iron bioavailability.

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