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Comparison of intestinal iron uptake in presence of cobalamins from different plant and animal proteins: Studies using an in vitro digestion/ caco-2 cell culture model

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Iron deficiency affects one-third of the world's population. In Australia, one in four females and one in 30 males do not meet their daily iron intake according to the Australian Bureau of Statistics,(1) putting them at risk of iron deficiency. Research shows that a patient diagnosed with haemolytic anaemia caused by a lack of vitamin B12 also suffered from iron deficiency anaemia.(2) The aim of this study was to assess the effect of the combination of different cobalamins on intestinal iron uptake in presence of different plant and animal proteins using *in vitro* Caco-2 cells. Several purified proteins with added ferrous sulphate and cyanocobalamin were digested using the INFOGEST digestion protocol(3) with minor changes. The final concentration of FeSO4 was 10 μmol/L and the final molar ratio of vitamin B12 (cyano, aqua, methyl, and adenosyl cobalamin) and FeSO4 was 5:1. Digesta was introduced 14 days post-seeding of Caco-2 cells and incubated for 12 hours. After the incubation, the growth medium was removed, and cells were washed twice with ice-cold PBS. Cells were harvested by adding an aliquot of deionized water, then sonicating at 4°C for 15 min. The cells were then stored at −20°C. The ferritin and total protein concentrations in the harvested cell suspension were analysed with ferritin solid-phase sandwich ELISA and a Pierce BCA Protein assay respectively. The ratio of ferritin/total protein expressed as ng ferritin/mg protein was used as an index of cellular iron uptake. Bioavailability of ferrous sulphate was higher in the presence of animal proteins (22.20 ± 3.35, 19.77 ± 2.90, 41.52 ± 2.74 ng ferritin/mg protein for casein, egg albumin, and myoglobin respectively) when compared with plant proteins (16.47 ± 1.63, 15.84 ± 2.40, 13.37 ± 3.68 ng ferritin/mg protein for gluten, rice, and pea protein respectively). Results show that Vitamin B12 improves the bioavailability of ferrous sulphate in a Caco-2 cell model. With the final concentration of cobalamin at 50 mmol/L, the ferritin-protein ratio increased for casein, egg albumin, myoglobin, gluten, pea, and rice protein (1.7, 1.9, 1.6, 1.8, 2.4, 2.2 times respectively). The presence of all four forms of cobalamins significantly ($p < 0.05$) increased iron uptake when compared with only cyano or aqua cobalamin. The combination of all four different cobalamins promotes higher iron uptake in the presence of different proteins when compared with the individual form. Among the animal protein isolates, myoglobin shows the highest iron uptake and rice protein could be one of the best options for vegans in terms of iron bioavailability.

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