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Dual iron and zinc deficiency alters cellular metabolism compared with single deficiencies: an in vitro Caco-2 cell study

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Iron and zinc deficiencies are prevalent globally and have been found to co-exist within populations(1). Previous work from our laboratory found dual iron and zinc deficiency alters iron absorption compared with iron deficiency alone (unpublished). Iron deficient (FeD), zinc deficient (ZnD), dual iron and zinc deficient (FeZnD) and healthy Caco-2 cells demonstrated different rates of cell media acidification, indicative of altered cell metabolism. The aim of this study was to understand changes to energy substrate consumption and intracellular concentrations under individual and dual deficient conditions. Iron, zinc and dual iron and zinc deficiencies were induced in Caco-2 cells by Chelex-100 removal of minerals from FBS in the growth media and repleting all but the target mineral. Cell media was changed every second day for the first 9 days post seeding, then every day until day 14 due to increased rate of media acidification particularly in iron deficient cells. Glucose (*Megazyme, GOPOD*), lactate (*Megazyme, K-LATE*) and protein (*Thermo Scientific, SKU# 23225*) contents were analysed using commercial kits. Creatine and phosphocreatine were analysed by HPLC. ANOVA was used for statistical analyses using Tukey's test for post-hoc analyses with a significance of $p < 0.05$. FeZnD cells had significantly higher intracellular glucose ($p = 0.000$), and lower lactate ($p = 0.000$) concentrations compared to healthy control cells. Intracellular glucose and lactate concentrations in cells were also significantly different to that in FeD and ZnD ($p < 0.05$) cells. Iron deficient and zinc deficient cells were not different to healthy cells (control) for either intracellular glucose ($p(FeD) = 0.354$, $p(ZnD) = 0.996$) or lactate ($p(FeD) = 0.251$, $p(ZnD) = 1.000$) concentrations. Iron deficient cells did not show a difference in glucose media loss compared to healthy ($p = 0.715$) cells, whereas zinc deficient ($p = 0.000$) and dual iron and zinc deficient ($p = 0.000$) cells had significantly lower glucose loss than healthy cells. Zinc deficiency and dual iron and zinc deficiency resulted in a significant reduction in total intracellular creatine (phosphocreatine + creatine) compared to control cells ($p = 0.000$). In contrast, zinc deficiency and dual iron and zinc deficiency resulted in a significant increase in phosphocreatine compared to control or iron deficient cells ($p < 0.05$). Changes to glucose, lactate and phosphocreatine found in this study indicate that dual iron and zinc deficient cells do not mirror changes in individual deficiency. How changes in energy substrates affect nutrient absorption is uncertain from this present work. Further research is required to improve understanding with the view to translate findings into effective treatment and prevention of micronutrient deficiencies.

References

1. Ergul AB, Turanoglu C, Karakukcu C *et al.* (2018) *Eurasian J Med* **50**(1), 34–37.