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## *Letter to the Editor*

# Response to invited commentary: Vitamin $D_3$ supplementation for 8 weeks leads to improved haematological status following the consumption of an iron-fortified breakfast cereal: a double-blind randomised controlled trial in iron-deficient women

We would like to respond to the invited commentary by Blanco-Rojo[1] pertaining to our published findings on the improvement of Fe status indices following an 8-week vitamin D supplementation with Fe-fortified cereals in UK Fe-deficient women[2]. The key findings of our study supported the study hypothesis and suggest a role for vitamin D in Fe regulation. In particular, our study demonstrated a significant effect of vitamin $D_3$ supplementation in improving the participants' Fe status, indicated by the increase in Hb concentrations and haematocrit levels, despite the non-significant changes in hepcidin and ferritin concentrations. The commentary author correctly pointed out that low hepcidin concentration may have hindered the detection of a possible decrease. This is plausible, as the recruited participants for the study were Fe-deficient. Ganz *et al.*[3] who developed and validated the first ELISA specific for hepcidin reported that serum hepcidin concentrations were undetectable in 18–19 Fe-deficient patients. Multiple clinical factors that may be present simultaneously have been shown to influence the concentration of circulating hepcidin[4]. A potential decrease may be insufficient to be observed due to the lack of sensitivity of the immunoassay used in the study which actually recognises all isoforms of hepcidin (–20, –22, –24 and –25). Hepcidin exists in various isoforms[5], and hepcidin-25 has been distinctively identified to play a role in Fe regulation[6] with unclear underlying mechanisms[7,8].

Focusing on the use of breakfast cereals in the study, Blanco-Rojo[1] inferred that the presence of phytic acid, Ca and casein in the milk, with the lack of ascorbic acid, might inhibit Fe absorption, which resulted in no statistically significant increase in ferritin concentrations, and this cannot be ruled out due to the nature of the phytate content of breakfast cereals in particular. However, depending on the conditions and chemical forms of Fe itself (haem or non-haem)[9], the overall percentage of Fe absorption for an individual is reported to be classically low and ranges from as low as 5 % to as high as 35 %[10]. Previous studies that used cereal-based meals also reported a wide range of Fe absorption, of between 0·56 and 18·8 %[11–16]. The amount of phytates in ready-made cereal products varied from 0·05 to 3·29 %[17]. A molar ratio of <1:1 for phytates and Fe in a meal was proposed to counteract the inhibition effect of phytates on the Fe absorption; however, it was shown that phytates can still interfere with Fe absorption at a ratio of as low as 0·2:1[14]. However, no means of measuring phytates was carried out in our study, which should be taken into account in future research in order to make a fair comparison in terms of the effect of phytates in the intervention food product on Fe absorption in general. Cereals and cereal products represent the highest percentage of food (38 %) that contributes to Fe intake among UK women based on the latest National Dietary Nutrition Survey[18], where high-fibre breakfast cereals represented 7 %. This explains why it was selected as means of providing dietary Fe to the participants in our study. The use of semi-skimmed milk in the study provides approximately 250 mg of Ca with no clear underlying mechanism on how Ca affects Fe uptake and bioavailability[19]. A dose-dependent effect of 40–600 mg of Ca in foods on Fe absorption was observed in one of the earliest studies by Hallberg *et al.*[20]. In a recent study, Candia *et al.*[21] investigated the effects of various Ca salts on non-haem Fe bioavailability in fasted women of childbearing age and found 800 mg of supplemental calcium citrate significantly decreased non-haem Fe bioavailability. Additionally, several studies have reported conflicting findings, due to factors such as variations in the forms of Ca; differing administration routes, including within foods in a single meal; complex meals; or as supplements, in addition to varied doses[20,22–25].

Blanco-Rojo[1] suggested that the food fortification vehicles used in previous studies may have contributed to low Fe absorption in the first place, but the limited number of recent Fe fortification studies carried out in various settings have reported discrepancies in the improvement of Fe status biomarkers (Table 1). There was no significant increase in the daily Fe intake in our study; however, the intake was 1·7-fold higher at baseline, relative to typical Fe intake of adult women reported in the National Dietary Nutrition Survey, which accounted to approximately 116 % of the UK reference nutrient intake. We agree that future randomised controlled trials should consider the importance of addressing the use of food products that taken into account the bioavailability of both Fe and vitamin D. Findings from such studies have the potential to provide an alternative or adjunct route to manage Fe deficiency, as opposed to the therapeutic oral Fe therapy that can lead to poor adherence due to the adverse gastrointestinal events.

**Table 1.** Recent iron fortification studies using cereal and cereal-based products

| Study | Population | Age (years) Mean | SD | Range | Intervention/duration | Main findings/comments |
|---|---|---|---|---|---|---|
| Fuzi & Mushtaq[2] (UK) | Fe-deficient women (*n* 50) | 27·4 | 9·4 | | Fe-fortified cereal/8 weeks | Mean Hb concentration was significantly higher at post-intervention in the vitamin D group (8·59 (SD 0·61) mmol/l) compared with the placebo group (7·96 (SD 0·78) mmol/l) ($P < 0·05$) |
| Bal *et al.*[26] (India) | Children (*n* 112) | | | 6–12 | Fe-fortified biscuits (high or low Fe)/4 months | Mean Hb concentration was significantly higher at post-intervention in the high Fe group (6·53 (SD 0·50) mmol/l) compared with the low Fe group (6·22 (SD 0·45) mmol/l) ($P < 0·01$)<br>The study showed that Hb concentration can be increased in 4 months. The present study showed that Hb concentration is increased at a shorter duration of 8 weeks. The high Fe group received 30 mg of Fe/serving of biscuits, whilst Fe-fortified cereal used in the present study consisted of 9 mg Fe/serving |
| Quintero-Gutiérrez *et al*[27] (Mexico) | Children (*n* 47) | | | 3–6 | A: fortified biscuit (FS), B: fortified biscuit (HIC), C: placebo biscuit/10 weeks | Mean Hb concentration was significantly higher at post-intervention in groups A (9·25 (SD 0·12) mmol/l) and B (9·12 (SD 0·19) mmol/l) compared with the placebo group (9·50 (SD 0·19) mmol/l) ($P < 0·05$)<br>Participants in group C were non-anaemic for comparison with anaemic participants in groups A and B, hence the higher Hb concentration at post-intervention. None of the other Fe status biomarkers measured in the study (erythrocytes, MCV, MCH, MCHC and SF) was affected by the intervention, comparable with the present study |
| Powers *et al.*[28] (UK) | Adolescents (*n* 71) | | | 6–19 | Fe-fortified or unfortified breakfast cereals/12 weeks | Mean SF concentration was significantly higher at post-intervention in the intervention group (22·1 (SD 16·7) μg/l) compared with the unfortified group (18·4 (SD 11·6) μg/l) ($P < 0·001$)<br>The study found no effect of intervention on the other Fe biomarkers including Hb, haematocrit and MCV, contrary to the present study. The participants in the study were not Fe-deficient as opposed to the present study but were recruited based on riboflavin status (EGRAC > 1·4 and Hb < 8·5 mmol/l). The significant observation reported in the study may be due to a longer study duration, as opposed to 8 weeks in the present study |

FS, ferrous sulphate; HIC, haem Fe concentrate; MCV, mean corpuscular volume; MCH, mean corpuscular Hb; MCHC, mean corpuscular Hb concentration; SF, serum ferritin; EGRAC, erythrocyte glutathione reductase activation coefficient.

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