Title: Micronutrient deficiencies in children with coeliac disease; a double-edged sword of both untreated disease and treatment with gluten-free diet

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Authors contributions

LM and MA carried the data analysis and drafted the results section of the paper; MM carried out recruitment and collected samples; FS, AC, JW performed the analysis of micronutrients; EB, TC, HD introduced the study to the participants and collected clinical data; RH provided clinical commentary, RKR co-ordinated research activities at the clinical site; CAE & PM designed the study, applied for funding and co-supervised the research student; DT organised and supervised the laboratory analysis of samples; KG designed the study, applied for ethical permission and funding

award, co-supervised the research student; co-ordinated the study; revised statistical analysis and drafted the full manuscript. All authors approved the final version submitted.

Conflicts of interest

RH has received speaker's fees, conference support or consultancy fees from Nutricia and 4D Pharma. RKR has received speaker's fees, travel support, and participated in medical board meetings with Abbvie, Janssen, Takeda, Celltrion, Pharmacosmos and Nestle. KG reports personal fees from Nutricia, research grants and personal fees from Nestle, personal fees from Dr Falk and Baxter; CAE is chair of working group for ILSI Europe. The remaining authors have no conflicts of interest to disclose.

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ABSTRACT

Introduction: In coeliac disease (CD) micronutrient deficiencies may occur due to malabsorption in active disease and diminished intake during treatment with a gluten-free diet (GFD). This study assessed the micronutrient status in children with CD at diagnosis and follow-up.

Methods: Fifteen micronutrients were analysed in 106 blood samples from newly diagnosed CD and from patients on a GFD for <6 months, 6-12 months and with longstanding disease (>12 months). Predictors of micronutrient status included: demographics, disease duration, anthropometry, gastrointestinal symptoms, raised tissue transglutaminase antibodies (TGA), multivitamin use and faecal gluten immunogenic peptide (GIP). Micronutrient levels were compared against laboratory reference values.

Results: At CD diagnosis (n=25), low levels in ≥10% of patients were observed for: vitamins E (88%), B1 (71%), D (24%), K (21%), A (20%) and B6 (12%), ferritin (79%), and zinc (33%). One year post-diagnosis, repletion of vitamins E, K, B6 and B1 was observed (<10% patients). In contrast, deficiencies for vitamins D, A and zinc did not change significantly post-diagnosis. Copper, selenium and magnesium did not differ significantly between diagnosis and follow-up. All samples for B2, folate, vitamin C (except for one sample) and B12 were normal. A raised TGA at follow-up was associated with low vitamins A and B1 (raised vs normal TGA; vitamin A: 40% vs 17%, p=0.044, vitamin B1: 37% vs 13%, p=0.028). Low vitamin A (p=0.009) and vitamin D (p=0.001) were more common in samples collected during winter. There were no associations between micronutrient status with GIP, body mass index, height, socioeconomic status, or gastrointestinal symptom. Multivitamin use was less common in patients with low vitamin D.

Conclusions: Several micronutrient deficiencies in CD respond to a GFD but others need to be monitored long-term and supplemented where indicated.

Keywords

Celiac disease, micronutrient, gluten free diet, vitamin, mineral, trace element, children

INTRODUCTION

Coeliac disease (CD) is a chronic autoimmune enteropathy characterised by an aberrant immune reaction to dietary gluten. It evokes histopathological changes in the small bowel mucosa, typically villous atrophy and crypt hyperplasia. These changes cause nutrient malabsorption leading to the onset of intestinal symptoms, such as diarrhoea, and extra-intestinal manifestations including growth failure, osteopenia and anaemia[1].

Cereals are a major staple in the Western diet and an important source of B complex vitamins and trace elements. It is therefore possible that their dietary exclusion, during treatment with gluten-free diet (GFD), increases the risk of secondary nutrient deficiencies, particularly when diet variety and food options are diminished and manufactured gluten-free products often contain lower amounts of micronutrients than their gluten-containing equivalents[2, 3]. Several studies have shown that patients on GFD have lower mean dietary intakes of micronutrients than either their healthy peers or their recommended daily intakes, inferring risk of deficiency[2, 4-7].

The use of micronutrient biomarkers is the recommended method to assess adequacy of body stores[8, 9]. Most previous research that assessed the micronutrient status of children with CD was based on retrospective review of medical charts which evaluated the blood levels of specific vitamins and trace elements such as vitamins B12 and D, folate, iron and magnesium, as these nutrients are routinely monitored in contemporary clinical practice[10, 11]. Sparse literature has assessed the status of other micronutrients such as vitamins B1, B2, B6, A, E and K and trace elements such as copper, zinc and selenium in children with CD. As the main sources of several of these nutrients are cereals, their exclusion from the diet of those with CD could potentially increase the risk of deficiencies.

In the present study, we hypothesised that children with CD are at risk of micronutrient deficiencies, but the type and prevalence of these deficiencies will vary throughout the natural course of the disease and its management with GFD. Certain deficiencies seen in newly diagnosed patients with CD will diminish following treatment with GFD, and restoration of the absorptive capacity of the small bowel mucosa. For other micronutrients, deficiencies will persist or potentially arise as the result of diminished micronutrient intake resulting from the dietary restrictions imposed during a GFD.

METHODS

Participants

Children with established CD and clinically recommended to follow a GFD, and patients with suspected CD who were still consuming gluten-containing foods were eligible to participate in the study. All

children were recruited and followed up at the Royal Hospital of Sick Children, Glasgow, UK between August 2011 to August 2013. Patients with suspected CD were identified at the point of referral from primary care. Patients who were previously diagnosed with CD were recruited from outpatient review clinics where all patients are seen at least annually. All patients had a confirmed CD diagnosis including positive tissue transglutaminase antibodies (TGA) and small intestinal biopsy according to the British Society of Paediatric Gastroenterology, Hepatology and Nutrition guidelines in place at the time of the study[12]. For patients with established CD, a single blood sample was collected at the end of their routine clinical appointment. For the newly diagnosed patients, a maximum of three samples were collected longitudinally; a first sample at disease diagnosis and while the patients were still consuming gluten in their diet, plus a second and third follow-up sample, 6 and 12 months post-diagnosis.

To gain statistical power in comparative analysis, data from the longitudinal and cross-sectional groups were merged, but subgroup analysis based on the time elapsed between CD diagnosis and assessment (i.e. disease duration) is presented separately. Four subgroups are presented here; a first group of children newly diagnosed with CD who were still consuming gluten, and three other groups of patients who had been on a GFD for <6 months, between 6 to 12 months or for longer than 12 months. We chose these timeframes to match the research visits of the patients with their routine clinical follow-up appointments.

Dietary assessment was initially performed using the Scottish Collaborative FFQ for children[13]. However, as the micronutrient composition of proprietary gluten-free products is unknown and use of such products is common by patients with CD, results were not deemed representative of patients' true intake and inclusion of this analysis was therefore abandoned. Gastrointestinal symptoms were assessed using the PedsQL™ Gastrointestinal Symptom Scale (PedQL-GSS) which is a 9-item scale[14]. Each item takes a score on a Likert scale from 0 (Never) to 4 (Almost always). A higher score represents more gastrointestinal symptoms. Socioeconomic classification was assigned in quintiles using the Scottish Index of Multiple Deprivation https://www.gov.scot/collections/scottish-index-of-multiple-deprivation-2020/.

Micronutrient analysis

Research bloods were collected in lithium heparin tubes in addition to the tubes for the patients' routine clinical blood tests. Routine clinical blood tests were prioritised, particularly when the volume of blood available was small. Except for ferritin, folate, vitamins B12 and D, all other micronutrients were analysed for research purposes only and results were not readily available to the clinical

management team. Haematology, liver function test, serum albumin and parathyroid hormone results were available from patients' routine clinical tests obtained as the same time as micronutrient assessment.

Micronutrients were analysed by the Scottish Trace Element and Micronutrient Diagnostic and Research Laboratory, a national accredited service and as described previously[9, 15]. Quality assurance and quality controls were assessed using Certified Reference Materials and through external Quality Assessment schemes (data available on request). Plasma vitamins A (retinol) and E (α-tocopherol) were determined by high-performance liquid chromatography (HPLC). Plasma vitamin E was corrected for plasma total cholesterol to adjust for variations in plasma lipids. Plasma Vitamin K (phylloquinone) was assessed using liquid chromatography tandem mass spectrometry and corrected for triglyceride levels. Vitamin B1 (thiamine diphosphate) was measured in whole blood using HPLC with post-column ferricyanide derivatisation and fluorometric detection. Vitamin B2 (flavin adenine dinucleotide) was measured in erythrocytes with an isocratic HPLC system with a reversed-phase C18 column and fluorescence detection. Vitamin B6 (pyridoxyl phosphate) concentrations in erythrocytes was measured by HPLC using precolumn semicarbaside derivatisation and fluorescence detection. Vitamin B2 and B6 concentrations in erythrocytes were adjusted to haemoglobin. Vitamin C status was assessed by measuring ascorbic acid in plasma on a C18 reversed-phase analytical column with electrochemical detection. Inductively coupled plasma mass spectrometry (Agilent Technologies, Cheadle, UK) was used to measure plasma zinc, copper, and selenium using germanium and scandium as an internal standard. Serum ferritin, folate, B12 and magnesium were available from the routine clinical tests of the patients. The between batch coefficient of variation (%CV) of all methods described above was <10%. Micronutrient status of biochemical sufficiency and deficiency were evaluated against the laboratory reference intervals which are used for all clinical services across Scotland and were age and/or sex adjusted where this was relevant https://www.trace-elements.co.uk/

Recent ingestion of gluten

Recent ingestion of gluten, as a proxy marker of GFD adherence, was evaluated by measuring the faecal concentration of gluten immunogenic peptide (GIP) levels (iVYLISA, Biomedal, Spain)[16]. Patients and health care providers were blinded to this assessment. A positive test was suggestive of recent intake of gluten in the diet.

Statistical analysis

Statistical analysis was performed using Minitab version 17. Chi-square tests were used to assess differences between groups in qualitative data (e.g. differences in prevalence of micronutrient deficiencies between CD subgroups). Differences in continuous variables between groups were explored using the General Linear Model and following logarithmic transformation to improve data distribution normality. Predictors of micronutrient status included: patient age, gender, the quintile score of the Scottish Index of Multiple Deprivation, season sample was collected, BMI and height z-score and the total score of PedQL-GSS. In the subgroups of patients at follow-up additional predictors studied included raised TGA, use of multivitamin, iron and/or vitamin D supplements and a detectable GIP in faeces.

Ethical considerations

The study was approved by the West of Scotland Research Ethics Committee (Ref:11/WS/0006). All authors had access to the study data and reviewed and approved the final manuscript.

RESULTS

Subject and sample characteristics

In this single centre study, 78 distinct patients provided a total of 106 blood samples in which 15 different micronutrients were analysed (Table 1). Twenty-eight of these 106 samples were repeated samples from the newly diagnosed patients after 6 (n=16) and/or 12 (n=12) months follow up (Table 1 & Supplementary Figure 1). There were no significant differences between the newly diagnosed patients and the other three subgroups of patients at follow up for age, BMI and height z-scores. Five patients were classed as thin (BMI z-score < -2 SD) and three as short (Height z-score < -2 SD) (Table 1). Routine liver function tests (alkaline phosphatase, alanine transaminase, and aspartate transaminase and total bilirubin) and plasma urea were within normal levels for all samples, except for a sample with a marginally elevated alanine transaminase; albumin was low in a single blood sample and no patient had raised plasma CRP or raised thyroid stimulating hormone. Three patients had type 1 diabetes and were diagnosed with CD following screening. Anaemia was more common in the group of newly diagnosed patients and the group of CD diagnosed for more than 12 months (Table 1). All but one patient (96%) had raised TGA at disease diagnosis. This was the case for 47%, and 33% of the children at 6 and 12 months follow up, respectively, and for 16% for children with longstanding disease for more than 12 months (Table 1). A total of 82 faecal samples were provided; approximately 20% of the samples collected from the subgroups of patients at follow-up had detectable GIP suggesting recent consumption of gluten (Table 1). The PedQL-GSS was significantly higher in newly diagnosed patients than in the subgroups of patients at follow up (Table 1). Micronutrient supplements were used by 36 (34%) of patients of which 16 (15%) received medically prescribed supplements (e.g. vitamin D, iron, calcium supplements) and the remaining 20 (19%) reported use of prescribed and over the counter multivitamins.

Micronutrient status at CD diagnosis

At the time of disease diagnosis, and while the patients were still exposed to a diet containing gluten, low levels for ≥10% of patients were observed for: vitamin E (total 88%, deficiency:72% & insufficiency:16%), vitamin B1 (total 71%, deficiency:21% & insufficiency:50%), ferritin (total: 79%), zinc (total 33%, deficiency:0% & insufficiency:33%), vitamin D (total 24%, deficiency:20% & insufficiency:4%), vitamin K (total: 21%), vitamin A (total: 20%) and B6 (total: 12.5%) (Figure 1). Fewer than 10% of the patients had low levels for selenium, magnesium and copper and all had blood levels for B2, folate and B12 within the normal reference intervals (Figure 1).

Micronutrients whose status improved at follow up

Patients who had been on GFD for more than 12 months had the highest concentration of vitamins E, K, B1 and newly-diagnosed patients the lowest (Supplementary Table 1, Figure 2). Ferritin concentration was higher in patients who had been on GFD for 12 months or longer (Supplementary Table 1, Figure 2). Longer disease duration was associated with fewer patients presenting with low micronutrient status for vitamins K, E, B1 and ferritin than at diagnosis (Figure 1). Of note, in the group of patients with longstanding CD for longer than 12 months, no patients had low levels for vitamins E and K compared with 88% and 21% of the newly diagnosed patients, respectively (Figure 1). Likewise, for vitamin B1, the proportion of patients with low levels dropped from 71% at disease diagnosis to 5% in those with a disease duration longer than 12 months, and for ferritin from 79% to 14% at 12 months follow up (Figure 1).

Micronutrients whose status declined at follow up

For all but one sample, B6 levels were within the reference intervals. However, patients with disease duration of less than 12 months, including those who were newly diagnosed, had higher mean concentrations of B6 than those patients with longstanding CD for over 12 months; suggesting a gradual deterioration of their B6 status over time (Supplementary Table 1, Figure 2). Of note, few patients (n=12) had supraphysiological concentrations (> 2,000 pmol/g Hb) greatly exceeding the upper reference interval of B6 in erythrocytes.

In the subgroups of patients at follow up, the proportion of children with low vitamin D levels was higher the longer they were diagnosed, and this relationship reached significance in the subgroup of children with longstanding disease for longer than 12 months and compared with the newly diagnosed patients (p=0.011) (Supplementary Table 1). When comparing patients with low and normal vitamin D levels, parathyroid hormone (PTH) levels were elevated in those with lower vitamin D levels suggesting secondary hyperparathyroidism (26% elevated PTH in low vitamin D vs 9% elevated PTH in normal vitamin D; p=0.039).

Likewise, a higher proportion of patients at follow up had low levels of copper (6 months: 19%, 12 months: 18%, >12 months: 15%) than at diagnosis (4%) although this difference did not reach statistical significance (p=0.117). Due to a batch failure, vitamin C levels were available for only 46 samples from patients with established CD, the majority of which (37/45) had CD for longer than 12 months. In this group, low levels of vitamin C were observed in a single sample (2.2%).

Micronutrients whose status did not change at follow up

Compared with CD diagnosis, there was neither improvement nor deterioration in the micronutrient status of vitamin A, zinc or selenium between the newly diagnosed patients and the other three subgroups of patients at follow-up (Supplementary Table 1). In all four subgroups, the prevalence of low vitamin A levels varied (8% to 28%) whereas biochemical deficiencies for zinc were particularly high in the group of patients with longstanding disease of more than 12 months; in this group, almost half had plasma zinc levels below the reference intervals (Figure 1). The proportion of patients with low levels of selenium remained the same during treatment with GFD, as with the new disease diagnosis group, with a non-significantly higher proportion of patients with low levels of selenium at 12 months post-diagnosis (Figure 1).

Almost all (96%) patients in the newly diagnosed group had magnesium concentrations within the reference intervals, and this was the case for the subgroups of patients at follow up for less than 6 months (100%), between 6 and 12 months (91%), and for longer than 12 months (98%) (Figure 1). Like at disease diagnosis, biochemical deficiencies were not observed for any of the blood samples with available routine clinical results for folate and B12 (Figure 1).

Associates of micronutrient status

Relationships between micronutrient status, patients' demographics, socioeconomic status, micronutrient supplementation, anthropometry, the PedQL-GSS, raised TGA titres and positive GIP results (for the subgroups of patients on GFD) were explored. A raised TGA at follow-up was associated with low levels of vitamins A and B1 (raised vs normal TGA; vitamin A: 40% vs 17%, p=0.044, vitamin B1: 37% vs 13%, p=0.028). Likewise, a higher proportion of samples collected during winter months had low levels of vitamin A (winter: 41%, vs spring: 17%, vs summer: 23%, vs autumn: 0%; p=0.009), and vitamin D (winter: 73%, vs spring: 39%, vs summer: 26%, vs autumn: 28%; p=0.001). Patients with low B1 levels were younger and low levels of ferritin were more common in boys than in girls (boys vs girls; ferritin: 63% vs 9% p<0.001). Use of multivitamins was associated with fewer patients with low vitamin D levels at follow up. There were no associations between micronutrient status and faecal GIP measurements, BMI z-score, height z-score, the Scottish Index of Multiple Deprivation, or PedQL-GSS.

Discussion

This study assessed the micronutrient status of patients with CD using objective biomarkers in blood adding to previous research which assessed risk of micronutrient deficiencies using dietary assessment[2, 4, 13]. We identified several micronutrients with blood levels below the laboratory reference intervals for a substantial number of patients. This was the case for all fat-soluble vitamins (A, D, E and K), for the water-soluble vitamins B1, B6 and for most trace elements studied, in particular copper and zinc. Based on the findings of this single centre, we make suggestions for micronutrient monitoring and intervention which might support improved nutritional status in newly diagnosed CD patients commencing a GFD (Table 2) at least in our local population.

Most importantly, we described two broad patterns of micronutrient status following disease diagnosis and during treatment with GFD. The first represents reversible vitamin and mineral deficiencies which were frequent in more than 20% of patients at disease diagnosis but which resolved in the vast majority following treatment with GFD. This group included vitamin B1, vitamin E, vitamin K and ferritin. For all three of these vitamins, and less so for ferritin, it needed a year after diagnosis to reach values within the reference intervals (Figure 2). As the levels of these micronutrients will normalise following initiation of treatment with a GFD and parallel mucosal healing, we advocate screening selected only groups at diagnosis rather than all patients (Table 2). For these micronutrients, supplementation may receive less priority by the clinical team and may only be reserved for patients who are still deficient one year after disease diagnosis (Table 2). Of interest, the prevalence of anaemia was lower in samples collected 6 and 12 months after disease diagnosis but present in more than 10% of patients with a CD duration longer than 12 months. This may indicate gradual loss of compliance in patients with longstanding disease or merely reflect the occurrence of iron deficiency and anaemia in the general paediatric population.

The second group of micronutrients identified in the current study comprised essential trace elements like zinc, copper and selenium, and fat-soluble vitamins like A and D. For this group, blood levels remained either consistently below the reference intervals, in more than ~10% of samples tested and regardless of disease duration, or micronutrients which appeared to decline over the course of the disease. This represents a group of micronutrients whose levels either failed to improve or got worse on GFD. In the case of vitamin B6, mean concentrations in erythrocytes were highest in patients with newly diagnosed CD, and lowest for patients with established CD for longer than 12 months. This group refers to micronutrients which might be influenced by low intake due to the dietary exclusions imposed during treatment with GFD and may therefore need specific supplementation. For such nutrients, we suggest monitoring of their levels every 6 months for patients with deficient levels and annually in patients with results within normal ranges. At the same time,

these patients should receive dietary advice which extends beyond the principles of adherence to a GFD with guidance on how to choose naturally gluten-free foods which are rich sources of these micronutrients or nutrient supplementation as in the case of vitamin D[17]. Example foods which are rich sources of trace elements include nuts and lean meat and pseudo-cereals like quinoa and buckwheat. Regarding proprietary gluten-free products, the observations of the current study pinpoint nutrients that might need to be fortified in these manufactured products to ensure nutritional adequacy in those patients who are high users.

A very large proportion of children had deficient levels of vitamin E at disease diagnosis which improved following CD diagnosis. As vitamin E is abundant in food and deficiency is rare in the general healthy UK population, it seems unlikely that the low levels observed in the current study are not due to a poor dietary intake but rather to intestinal malabsorption; similar findings were observed for vitamin A and K levels, albeit at a lower extent than vitamin E. This concept of fat malabsorption leading to vitamin E deficiency is supported by our results, with no children who had been recommended to adhere to a GFD for longer than a year presenting low levels of vitamin E. The results of the current study are in close agreement with previous research in Polish children which proposed testing and supplementation of vitamin E following the diagnosis of CD [18]. Vitamin E has antioxidant, immunomodulatory and anti-platelet functions and it is thought that deficiency can contribute to neurological complications, myopathy and impairment of immune response and reproductive disorders. Vitamin E deficiency has been implicated in myelopathy, neuropathy, ophthalmoplegia and cerebellar ataxia in adult CD[19].

Vitamin D deficiency in CD is well documented in the literature alongside the potential implications for bone health[11, 20]. Vitamin D is primarily absorbed from the gastrointestinal tract in the duodenum, an area affected by villous atrophy in CD. In contrast to vitamin E, vitamin D levels did not significantly improve with time spent on a GFD but, on the contrary, more patients with longstanding disease suffered from biochemical deficiencies than newly diagnosed patients. This suggests that biochemical deficiency in this population, supported by the prevalence of secondary hyperparathyroidism in few patients, is the effect of low intake and ethnicity as well as low exposure to ultraviolet radiation during winter, particularly in countries of the North, like Scotland, where this study took place and as showed in the current study. Therefore, we suggest active monitoring of vitamin D status and treatment where necessary at follow-up of all coeliac patients[17]. However, this practice may also vary in different geographic regions with more exposure to sunlight.

In clinical practice folate and B12 status are assessed routinely, perhaps because of their ready availability as important and common haematological markers of clinical deficiencies. However, in the

current study none of the of the samples for which clinical routine measurements of folate and vitamin B12 were available presented low levels. The haematological profile of these patients was within normal values with no patient presenting with raised mean corpuscular volume suggestive of macrocytic anaemia. These findings are in agreement with previous research[10] and along with measurements of magnesium, which are also often measured in routine clinical practice, were within the normal ranges in approximately 96% of the samples tested in this study. We believe that routine screening of these three micronutrients is unnecessary without specific clinical indications (Table 2).

The current study explored disease characteristics as predictors of micronutrient status, including GIP, a novel biomarker of recent gluten ingestion and by extension proxy of compliance with GFD. We did not find any significant relationships between low BMI or short stature and micronutrient deficiencies. This most likely indicates that micronutrient deficiencies are not the result of a diet inadequate in calorific content but rather the result of a diet of poor nutritional quality in patients on GFD. Likewise, we did not find a significant association between biomarkers of recent ingestion of gluten, a proxy of compliance with GFD, and micronutrient deficiencies. This is most likely because patients with CD may deviate from their GFD recommendations, but such dietary transgressions may be too short-term to cause nutritional imbalances. It is also likely that this study was underpowered to address all secondary analyses. A few patients had very high levels of B6, potentially indicating supplementation but this was not confirmed based on the self-reported intake of prescribed and over-the-counter supplementation.

Limitations of the current study are the lack of information on the micronutrient content of gluten-free products; hence unravelling the underlying origins of micronutrient deficiencies and whether these are attributed to low intake or excessive losses from malabsorption and increased epithelial cell turnover. Another limitation is the lack of correlation between any deficiencies identified and their clinical manifestations, and symptoms to corroborate the impact of the biochemical deficiencies observed here. However, for several of these nutrients clinical symptoms are subtle and non-specific and, although subclinical deficiencies lead to metabolic, immunological, cognitive and work capacity impairment, overt clinical symptoms may only appear when body stores are severely depleted, such as in the case of vitamin E deficiency, zinc and the B complex vitamins. In the current study, assessment of body micronutrient status relied on direct measurements of micronutrients in blood and in comparison, with reference intervals. However, there are several caveats with this approach summarised recently in a position paper by the European Society of Paediatric Gastroenterology, Hepatology and Nutrition[8]. For example, biochemical measurements of micronutrients in blood do not always have biological relevance in reflecting true tissue deficiency and ideally should be complemented with functional biomarkers of deficiencies (e.g. erythrocyte

transketolase activity in the case of vitamin B1), dietary and clinical review. Nonetheless, functional biomarkers are rarely measured in routine clinical practice, a least in the UK clinical laboratories, and therefore assessment of micronutrient deficiencies and initiation of supplementation is based solely on biochemically low levels of the biomarkers presented here. Systemic inflammatory response can also influence the concentration of plasma vitamins and minerals independently of body stores, but in the current study no patient had raised CRP, thus offering reassurance that our measured concentrations have not been significantly influenced by such factors[8]. Although the present study is the most comprehensive micronutrient analysis in one of the largest populations reported in the literature, confirmation of the findings of this single centre study is necessary in independent multicentre research and in other settings. Inclusion of age and gender matched controls might be useful to include, particularly when one would like to compare the prevalence of deficiencies in the CD population compared with that in the general population. However, it is unlikely that this would explain the temporal effects we observed in the current study. Future research should aim to relate micronutrient deficiencies with histological damage scoring (e.g. Marsh score) which was not available for the participants of the present study.

The strengths of the current study include the substantial number of samples assayed and repeated cross-sectional analysis to allow time to assess changes relating to treatment.

In conclusion, this study identified certain micronutrients which might need monitoring after the first year following disease diagnosis and others throughout the natural course of the disease as suggested in Table 2. The findings of the current study, especially if independently replicated in larger international studies, may have implications for the dietary management of patients with CD and potentially for micronutrient fortification of proprietary gluten-free products.

Figure Legends

Figure 1: Prevalence of micronutrient deficiencies in paediatric patients with coeliac disease at diagnosis and during follow-up

ND: At diagnosis, 6mo: 6 months on GFD, 12mo: 12 months on GFD, >12mo: >12 months on GFD

Grey line indicates micronutrient which were below reference ranges for more than 10% of samples assayed.

Figure 2: Micronutrient levels in paediatric patients with coeliac disease at diagnosis and during follow-up

ND: At diagnosis, 6mo: 6 months on GFD, 12mo: 12 months on GFD, >12mo: >12 months on GFD

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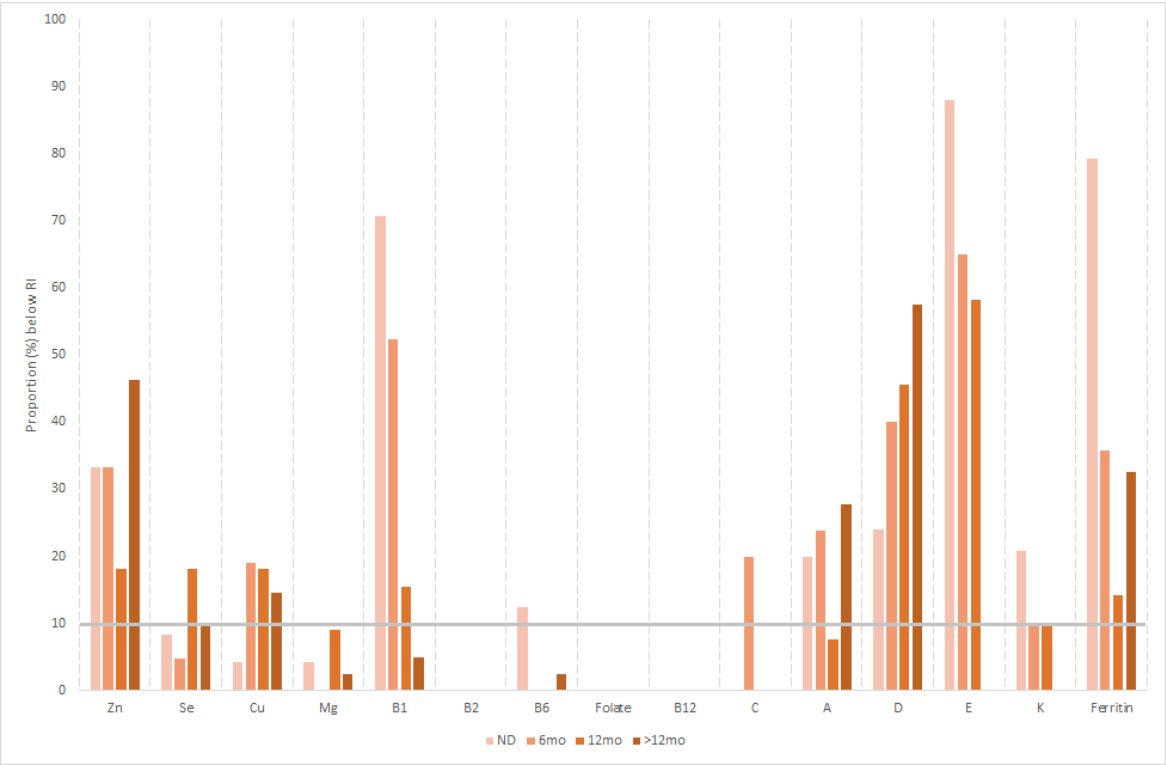

Figure 1

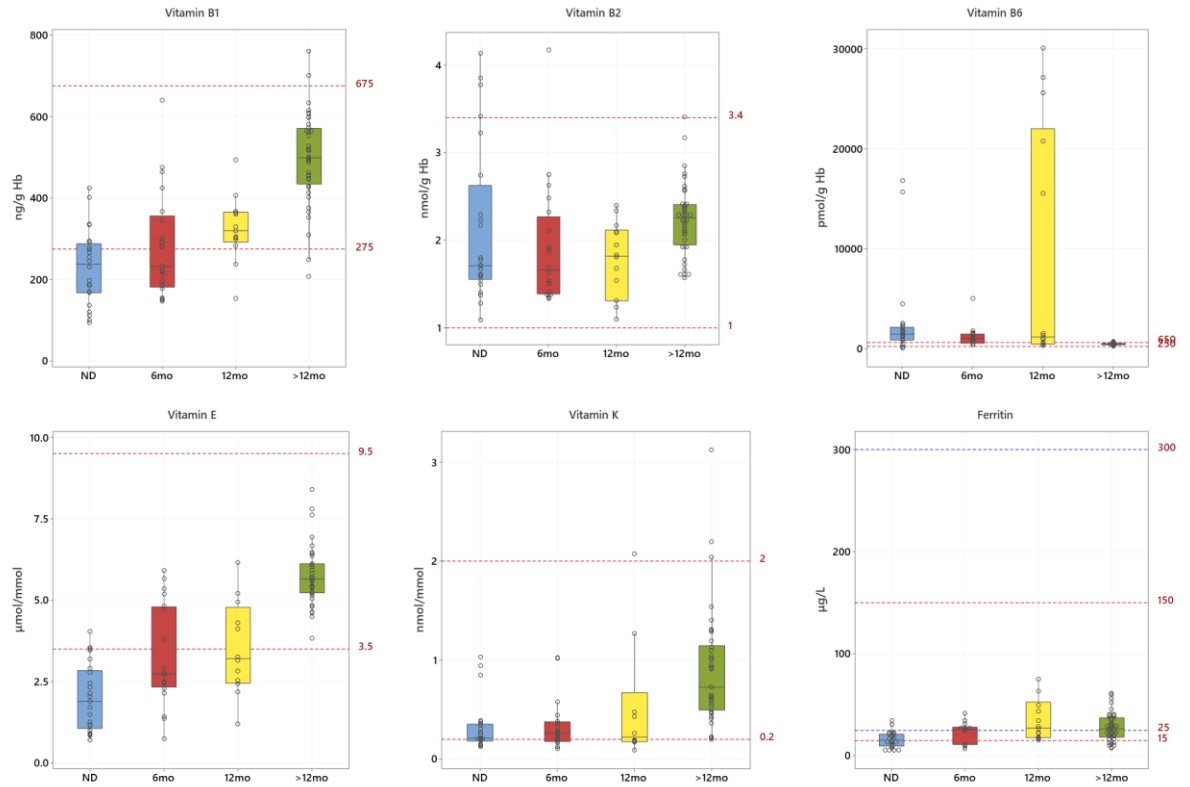

Figure 2

Table 1: Participants characteristics

	ND (n=25)	6mo* (n=21)	12mo* (n=16)	>12mo (n=44)	p-value
Gender, (M)	11/25 (56%)	8/21 (62%)	6/16 (62%)	18/44 (59%)	0.971
Age, y	9.3 (3.5)	9.4 (3.9)	10.7 (3.3)	9.3 (3.2)	0.502
Disease duration, y	0.0 (0.0)	0.6 (0.1)	1.1 (0.1)	5.5 (3.4)	<0.001
BMI z-score, SD	-0.1 (1.1)	-0.1 (1.3)	0.0 (1.1)	0.0 (0.9)	0.964
Thin < -2 SD	2/25 (8%)	2/21 (9.5%)	1/16 (6.2%)	0/43 (0%)	n/a
Height z-score, SD	-0.1 (1.1)	-0.3 (1.0)	0.1 (0.6)	0.0 (1.0)	0.685
Short < -2 SD	2/25 (8%)	1/21 (5%)	0/16 (0%)	0/43 (0%)	n/a
Raised TGA	24/25 (96%)	9/19 (47%)	5/15 (33%)	7/44 (16%)	<0.001
Anaemia	3/25 (12%)	1/15 (7%)	0/12 (0%)	6/43 (14%)	0.525
Low albumin	0/25 (0%)	0/14 (0%)	0/14 (0%)	1/41 (2%%)	n/a
Raised TSH	0/25 (0%)	0/12 (0%)	0/14 (0%)	0/42 (0%)	n/a
Raised aspartate transaminase	0/25 (0%)	0/11 (0%)	0/14 (0%)	0/42 (0%)	n/a
Raised alanine transaminase	0/25 (0%)	0/14 (0%)	0/10 (0%)	0/42 (0%)	n/a
Raised plasma urea	0/25 (0%)	0/13 (0%)	0/14 (0%)	0/42 (0%)	n/a
Positive faecal GIP	17/18 (94%)	3/15 (20%)	4/16 (25%)	6/33 (18%)	
GI symptoms severity	16.2 (7.0)	10.2 (6.6)	9.2 (7.9)	8.0 (6.4)	<0.001

*including samples from the prospective (n=16 at 6mo & n=12 at 12mo) and cross-sectional cohorts (n=5 at 6mo & n=4 at 12mo); BMI: body mass index, TGA: tissue transglutaminase antibodies, TSH: thyroid stimulating hormone, GIP: gluten immunogenic peptide, GI: gastrointestinal; ND: Newly diagnosed patients, 6mo: 6 months post-diagnosis, 12mo: 12 months post-diagnosis, >12mo: 12 months post-diagnosis; n/a: number of patients per group is too low hence statistical analysis is invalid; Data are presented with mean (SD) or frequencies with (%) for continuous and categorical variables, respectively

Table 2: Suggested monitoring of biochemical micronutrient status and suggested interventions in children with coeliac disease at diagnosis and at follow-up in the West of Scotland

	Deficiencies reversible with GFD	Treatment persistent or new onset deficiencies	Low risk of deficiencies

Micronutrients	B1, K, E, Ferritin	Zn, Se, Cu, A, D, B6	C**, B2, Folate, B12, Mg,
Frequency of monitoring	• Not all patients at diagnosis except for ferritin • Selected groups at diagnosis only* • At 12-month post-diagnosis	• At diagnosis • Annually if normal levels • Every 6-months if deficient	• Selected groups only at diagnosis and follow up*
Intervention	• Dietary counselling • Supplementation if still deficient at 12 months	• Dietary counselling • Supplementation	

*E.g. treatment refractory disease, very poor compliance to GFD, abnormal haematological profile, undernutrition, short stature despite good compliance to GFD. ** suggestions for vitamin C data are made considering the caveats of the data as described in the manuscript