Dear Lian-Sheng Ma,

Thank you for considering our manuscript WJG no. 62755**:**

Hypophosphatemia after high-dose intravenous iron treatment in patients with inflammatory bowel disease – mechanisms and possible clinical impact

for publication in World Journal of Gastroenterology and give us the opportunity to revise it. We appreciate the thorough and good comments given by the reviewers and yourself. We have made several changes and amendments, which we think have improved the manuscript.

Below we have addressed the editor and reviewers´ comments point-by-point. For clarity, we have organised all review comments in black font and our corresponding response in red font.

The revised version has been approved by all co-authors and we hope that this paper will be of interest to the readers of WJG and are looking forward to hear from you.

Yours sincerely,

Trond Espen Detlie

Editor

We thank you and the reviewers for your comprehensive review of the manuscript and appreciate your comments and suggestions for improvement which we have taken into consideration. Please see details below.

1. Comment on scientific quality: Self-cited references: There are 4 self-cited references out of a total of 35 references. The self-referencing rate should be less than 10%.
    a. Response: Thank you for noticing this error. We will remove the reference number 33 in the first submitted version of the article and the self-cited references should then be below 10%.
    b. Action: The following reference has been removed: **Jahnsen J**, Falch JA, Mowinckel P, Aadland E. Vitamin D status, parathyroid hormone and bone mineral density in patients with inflammatory bowel disease. *Scand J Gastroenterol* 2002; **37**: (2): 192-199 [PMID: 11843057 DOI: 10.1080/003655202753416876].

2. Comment on issues raised: (1) The authors did not provide the approved grant application form(s). Please upload the approved grant application form(s) or funding agency copy of any approval document(s)

a. Response: Thank you for raising this question. We did not receive any grants for this study. The study was supported by the involved institutions; Akershus University Hospital Gastroenterology department, Oslo University Hospital Ullevål Gastroenterology department and the University of Oslo. The funding from the institutions was part of everyday practice and research strategy, and hence, no grant number supplied.
b. Action: No changes in the manuscript.

3. Comment on issues raised: (2) The authors did not provide original pictures. Please provide the original figure documents. Please prepare and arrange the figures using PowerPoint to ensure that all graphs or arrows or text portions can be reprocessed by the editor.
    a. Response: Thank you for noticing. We believed that the pictures provided were the originals, but not editable. We will of course provide the same pictures/graphs in an editable version using power point.
    b. Action: Added 2 power point files for figures 1 and 2.

4. Comment on issues raised: (3) The "Article Highlights" section is missing. Please add the "Article Highlights" section at the end of the main text.
    a. Response: Thank you for making us aware that the section "Article Highlights" is missing. We sincerely apologize that we did not notice this section. Please find the implemented section in the new version
    b. Action: The section "Article highlights" is implemented in the new version as follows:

Research background

High-dose intravenous iron is an effective and frequently used treatment option for iron deficiency or iron deficiency anaemia in inflammatory bowel disease. However, treatment with ferric carboxymaltose has been associated with the development of hypophosphatemia.

Research motivation

We aimed to investigate the occurrence of hypophosphatemia after treatment with either ferric carboxymaltose and ferric derisomaltose for iron deficiency or iron deficiency anaemia in patients with inflammatory bowel disease.

Research objectives

In this part of the study, we aimed to disclose underlying mechanism behind the development of hypophosphatemia after treatment with high dose intravenous iron and whether hypophosphatemia had a clinical impact on these patients.

Research methods

A prospective observational study of adult IBD patients with iron deficiency or iron deficiency anaemia was conducted between 1 February 2017 and 1 July 2018 at two separate university hospitals in the southeast region of Norway. Patients were recruited consecutively and received one dose of 1000 mg of either ferric carboxymaltose or ferric derisomaltose, and were followed for an observation period of at least 7 weeks at three timepoints; baseline, week 2 and week 6. Blood and urine

samples were collected for relevant analyses at all three visits in addition to assessment of clinical symptoms using a respiratory function test, a visual analogue scale, and a health-related quality of life questionnaire.

Research results

Our study results demonstrate an association between ferric carboxymaltose treatment and the development of hypophosphatemia by increasing the level of intact Fibroblast Growth Factor 23 (iFGF23) and phosphate wasting in the urine. Moreover, we observed a significant decline in active Vitamin D and ionised calcium. No clinical impact was detected by applying Short Form – 36 questionnaire, VAS score and MicroRPM^{TM} respiratory test in an observation period of six weeks.

Research conclusions

Ferric carboxymaltose treatment is associated with the development of hypophosphatemia in patients with inflammatory bowel disease. This is due to increased formation of iFGF23 which in turn probably results in an increase of urinary phosphate output. No clinical impact was detected nor excluded. Assumably our study is underpowered together with a too short observation period to provide solid information with regard to clinical impact of hypophosphatemia.

Research prospective

Based on our results we encourage clinicians to be aware of the risk of developing hypophosphatemia after treatment with ferric carboxymaltose. Larger studies with a longer observation period to detect possible clinical impact of hypophosphatemia is desirable.

Reviewer #1

1. Comment on introduction: (1) this is too long; much of the text (esp. the 3rd paragraph, beginning “Many organ systems…”) is more of discussion than introduction – this should be addressed.
    a. Response: Thank you for this constructive feedback. The 3rd paragraph aims to create awareness among clinicians of the potential complications that hypophosphatemia may produce. Our experience is that clinicians/gastroenterologists in general possess limited knowledge in this field. The introduction therefore aims to give the reader not only information in regard to this study, but also to give the reader an illustration of the potential problem of hypophosphatemia, underlining its potential complications and possible severeness. However, we completely agree that this makes the introduction somewhat long and will comply with the comment and remove the 3rd paragraph.
    b. Action: The 3rd paragraph in the introduction has been removed from the manuscript.

2. Comment on methods: (1) Although the authors have previously published the methodology in a previous manuscript, this paper should still be able to be read independently. Readers should not have to read 2 papers in order to understand the methods and outcomes. Although a brief outline is fine, the authors still need to clearly provide inclusion and exclusion criteria, definitions of iron-deficiency and iron deficiency anaemia, whether patients were recruited randomly or consecutively and if patients were treated per protocol or by physician choice. Additionally, there is no mention of the study visit times points. I would suggest that possibly a flow diagram of the study recruitment may address many of these issues without needing a significant amount of text.
    a. Response: Thank you for this important comment. We fully agree and to comply we have expanded the section with additional information. Moreover, we suggest that a flow diagram could be attached as supplementary.
    b. Action: According to the comment the following text (in italics) is added: In brief, adult IBD patients *(>18 years*) diagnosed with ID or IDA (*according to ECCO guidelines*[2]) were recruited at two separate study sites in the southeast region of Norway and treated with either FCM or FDI.
    Eligible patients were prescribed 1,000 mg of high-dose intravenous iron, ferric carboxymaltose (50 mg/ml) or iron derisomaltose (100 mg/ml), administered as a single dose. Patients who had received high-dose intravenous iron treatment or a packed red blood cell transfusion within 3 months of study entry, or for whom high-dose intravenous iron treatment was contraindicated, were not included in the study.
    Enrolment continued until at least 50 consecutive patients with complete adherence to the study protocol were recruited at each site (a total of more than 100 patients). The enrolment period was followed by a prospective observation period, which lasted ≤7 weeks for each patient and included three study visits.
    Study inclusion was performed at baseline, at which time intravenous iron treatment was administered. Patients attended the clinic at Week 2 (10–15 days) and at Week 6 (5–7 weeks) following intravenous iron treatment. Each patient could receive only one infusion within an approximate 2-month period after consenting to study participation.

    Also, please find a flow diagram attached as supplementary information.

3. Comment on methods: (2) Does using the FEPO4 formula negate the fact that two different assays were used? I don't think it does. The formula is merely a function of the inputs (which vary by assay) – would a brief analysis of variance of the formulas address this better?
    a. Response: Thank you for bringing this important remark to our attention. The $FEPO_4$ formula do not fully negate the fact that two different assays were used, and unfortunately, we do not possess a variance analysis. According to our two chemical laboratories the difference seems to be that the analysis of urine phosphate applied at Akershus University Hospital results in somewhat higher levels compared to the analysis applied at Oslo University Hospital Ullevål. The different analysis methods have been controlled by an external

quality control analysis 12 times during the study period and resulted in an average difference of 8,2% higher levels at Akershus University Hospital compared to Oslo University Hospital Ullevål. Both analysis methods were found to be precise with a coefficient of variation (CV%) of 3.1 and 4.6 respectively. When applying the $FEPO_4$ formulae the difference between the two methods gets even smaller. Since there are no variance analysis available, we have chosen not to comment this.
Moreover, and most importantly, applying this analysis is first and foremost within the treatment group investigating the change from baseline. We therefore believe that the difference between the two analyses methods has little importance. However, we are happy to comment on this in the discussion section if desired.

b. Action: No changes in the manuscript.

4. Comment on methods: (3) Why was hypophosphataemia defined as <0.8? This seems quite high (i.e. is 0.79 clinical relevant)?
    a. Response: Thank you for bringing this to our attention. Hypophosphatemia is defined at a level below 0,8 mmol/L in Europe. The criteria for hypophosphatemia followed the UK National Health Service (NHS) Guideline for the Treatment of Hypophosphatemia in Adults, published in March 2016. Reference number 30 in the re-submitted article. The NHS guidelines categorise hypophosphatemia as mild (serum phosphate level: 0.65–0.79 mmol/l), moderate (0.32–0.64 mmol/l), and severe (<0.32 mmol/l) (normal range: 0.8–1.45 mmol/l). We believe the same criteria is used in the CTCAE version 5 by the U.S Department of Health and human services.
    b. Action: No changes in the manuscript.

5. Comment on methods: (4) Although the authors have provided a biostatistical letter of approval I am not sure that in this scenario if a pair t-test is appropriate. Would a two-way ANOVA not be the test of choice?
    a. Response: Thank you for this important remark. We believe a paired t-test is the appropriate analysis to answer our research questions. Our main interest lies in change from baseline, at two time points, hence taking paired differences are reasonable. We don't quite follow the reviewers´ suggestion about a two-way-Anova. In addition to the treatment variable, what would be the second factor to add to the analysis?
    b. Action: No changes in the manuscript.

6. Comment on results: (1) In the phosphate results section it is important to know/understand if the 21.6% group is a subset of the 72.5% group; i.e. looking at these groups separately as a whole is not useful – each patient's phosphate level (and change over time) is the important factor. So for both the FCM and FDI groups did 11 of the 37 (FCM) and 2 of the 6 (FDI) groups have low phosphate levels at both time points? This is a vital distinction to make.
    a. Response: Thank you for this important observation and comment. Yes, there were no new incidences of hypophosphatemia at week 6. This means that some of those who developed hypophosphatemia at week 2 still had

hypophosphatemia at week 6 and some did not. So, the 11 of the 37 (FCM) and 2 of the 6 (FDI) had low phosphate levels at both timepoints.

   b. Action: To avoid any misunderstanding we have added the sentence "There were no new incidences of hypophosphatemia at week 6" as a third sentence in that paragraph.

7. Comment on results: (2) Although the distribution of Vit D deficiency was the same in both groups, were those with low Vit D have lower phosphate levels (i.e. was there any correlation?)
   a. Response: Thank you for this interesting observation. At baseline the number of patients with low vitamin D levels was equal in the two treatment groups and there were no association between low vitamin D level and development of hypophosphatemia in either of the two groups.
   b. Action: On page 12, paragraph 1 we have added "Moreover, we found no association between low levels of vitamin D and development of hypophosphatemia."

8. Comment on discussion: This is also very low and could do with being more succinct/focussed
   a. Response: Thank you for your feedback on this issue. We agree that the discussion seems a bit long. However, we felt it necessary to comment on all our important findings in the study, as well as try to explain the relevance of the results. We feel that removing some of the content will leave the reader with unexplained issues and ultimately open for questions in regard to interpretation.
   b. Action: No changes in the manuscript.

Reviewer #2

Comments to address:

1. I would include the normal values in Tables 1 and 2 for reference.
   a. Response: Thank you for this important observation. We fully agree and act accordingly.
   b. Action: Implemented normal reference values in table 1 and 2.

2. Comment references: Does the manuscript cite appropriately the latest, important and authoritative references in the introduction and discussion sections? Does the author self-cite, omit, incorrectly cite and/or over-cite references? Yes
   a. Response: Thank you for these important comments. We believe the references applied in both the introduction and the discussion sections meet the specific requirements. The second comment has been addressed by the Editor as well.
   b. Action: The reference number 33 in the first submitted version of the article has been removed.

3. Comment on further critique: (1) The data presented in Detlie et al APT 2019 show that FCM resulted in a more sustained improvement in serum iron and related

indices. The findings of low phosphorus and other indices described in the current manuscript are of questionable significance, but the more effective iron repletion may actually be the more important clinical outcome here.

a. Response: Thank you for addressing this interesting issue. In our paper Detlie et al APT 2019 we show that patients treated with FDI suffered from a more severe ID/IDA compared to the patients treated with FCM at baseline. Comparing the effectiveness of treatment between the two groups therefore seems inaccurate. Also, patients treated with FDI had a more effective response in regard to haemoglobin correction and there is only minimal difference between the two groups in regard to ReticulocyteHb, Transferrin receptor and Transferrin saturation. Changes from baseline are also more significant in the FDI treated group compared to the FCM treated group. (This is addressed in the discussion of the same article). Moreover, the increase in ferritin levels by FCM is also believed to be a possible part, or result, of the mechanism behind the high risk of developing hypophosphatemia. However, the latter has not yet been proven.
   Importantly, patients were not treated according to need i.e dose adjusted treatment, but rather treated with a fixed dose regime in order to compare the two groups in regard to the risk of developing hypophosphatemia. In fact, based on the results one might argue that FDI is more effective, but this again would be a wrong interpretation due to the difference at baseline between the two groups. Since this study was not designed to compare effectiveness between FCM and FDI, we believe it would be incorrect to speculate on this topic in this paper.
b. Action: No changes in the manuscript.

4. Comment on further critique: (2) The overall mean vitamin D levels at baseline, while reported as deficient in 36% of patients at baseline, are actually substantially higher than most IBD cohorts. In my hospital, vitamin D >30 is listed as sufficient and most IBD patients, especially in the Winter, have vitamin D levels in the 10's and 20's. It is possible that if this population had lower baseline values, the effect of phosphorus depletion might actually correlate with clinically meaningful changes in vitamin D and PTH metabolism. The findings in this manuscript mostly highlight the difference in 1,25 Vitamin D and there was no major impact on 25-OH-vitamin D.
   a. Response: Thank you for this important comment. It is well recognized that low levels of vitamin D might play a negative role in IBD patients. (Fletcher J, et al. The role of Vitamin D in inflammatory bowel disease: Mechanism to management. Nutrients. 2019 May; 11(5): 1019. PMID: 31067701, DOI: 10.3390/nu11051019). In Norway, and in Europe, we follow ECCO guidelines (*Journal of Crohn's and Colitis*, Volume 13, Issue 2, February 2019, Pages 144–164K, https://doi.org/10.1093/ecco-jcc/jjy113, that states that Vitamin D levels should be monitored and treated if low. Other guidelines and studies suggest that levels of 25-OH-vitamin D below 25–50 nmol/L constitute deficiency with levels > 75 nmol/L indicating sufficiency. The threshold between 51–74 nmol/L 25-OH-vitamin D may then be termed insufficiency. Treatment for deficiency has been recommended at a cut off of <50 nmol/L

(Francis R. et al. National Osteoporosis Society practical clinical guideline on vitamin D and bone health. Maturitas. 2015;80:119–121.)
In this paper we have highlight the difference of 1.25 $(OH)_2$ vitamin D which is the active form of vitamin D due to the fact that FGF 23 interfere with the hydroxylation of 25-OH-vitamin D to 1.25 $(OH)_2$ vitamin D in the kidneys.

b. Action: No changes in the manuscript.

5. Comment on further critique: (3) Please reference the normal ranges for serum phosphorus and other lab values in the tables. The units of mmol/L are different than the values commonly used in US clinical practice and should be placed in context.
    a. Response: Thank you for your valued feedback on this issue. We fully agree and will make changes accordingly.
    b. Action: We have now added the references of normal values in the tables and moreover added information in table text comparing mmol/L to mg/dl.

6. Comment on further critique: (4) The impact of hypophosphatemia on serum ionized calcium, respiratory function tests, SF-36, and VAS was minimal between groups suggesting that the differences, although statistically significance, may have limited clinical relevance or only theoretical clinical impact.
    a. Response: Thank you for your comment. This is in line with our interpretation as stated in our discussion. However, we also believe that this question has not been answered in full and that a larger study with a longer observation time might give more information in this regard. Case reports clearly shows that hypophosphatemia might result in severe complications, however, the incidence of severe complications is unknown.
    b. Action: No changes in the manuscript.

7. Comment on further critique: (5) It is hypothesized that FCM may have a direct impact on FGF23 cleavage. Is data presented to justify this conclusion? If purely speculative, this should be clarified in the discussion (Page 14, first paragraph)
    a. Response: Thank you for this important remark. We believe that there is no doubt about the effect of FCM on FGF23 cleavage. The difference between the two treatment groups is highly significant in this regard, and there are no other theories to explain this result. Also, our findings are similar to, and support, other publications on the same issue, e.g. Wolf et al Effects of Iron Isomaltoside vs Ferric Carboxymaltose on Hypophosphatemia in Iron-Deficiency Anemia Two Randomized Clinical Trials, JAMA. 2020;323(5):432-443. doi:10.1001/jama.2019.22450
    Actually, it is remarkable how reproduceable the results seem to be regardless of different patient groups, and these findings are no longer considered speculative as far as we know. We therefore feel that this is adequately addressed in the discussion as it is.
    b. Action: No changes in the manuscript.

Reviewer #3

General comments: (1) The numbers of data points (eg subjects) for the various analyses is difficult to determine in many cases. This needs to be made more explicit. As an example, the number of subjects is not even mentioned in the methods. Similarly, the massive SD's with some of the analyses would suggest either under-powering or technical problems. Figure 2 is a good example. Numbers/group should be stated in the figure legends.

a. Response: Thank you for bringing this to our attention. The number of data points are clearly shown in table two and there are very few missing values indicating no or insignificant influence on the analysis. However, it is a correct observation in regard to the standard deviations. As stated in the method section under the paragraph “data analysis” we inform the reader that this part of the study was not used to justify sample size. Looking at the results this becomes clear that some of the analyses are probably under-powered. We can confirm that we did not experience any technical difficulties.
b. Action: No changes in the manuscript.

General comments: (2) Some attention to grammar is required. For example, the abstract could be made more concise...eg 'has shown to be associated'; could just be 'has been associated'. For some reason a dot-point style has been used for the aims. It should either be 'A total of 106 patients was available' or either '106 patients were available'; the word 'total' is not plural.

a. Response: Thank you for the detection of errors. We agree and will make changes accordingly in the re-submitted manuscript. We are, however, not certain of what you mean by dot-point style for the aims. If you mean that it involves two “statements”, the reason is due to the number of words allowed. Hence, in order to comply it would prove difficult to change this. However, we would be grateful of suggestions in this matter.
b. Action: The text has been changed according to comments. Awaiting feedback on the aims section.