Author’s response to reviews

Title: Dynamics of Vitamin D in Stable Patients with Inflammatory Bowel Disease and their Families

Authors:

Avigyle Grunbaum (avgrunbaum@gmail.com)
Christina A Holcroft (stats.holcroft@gmail.com)
Debra Heilpern (debheilpern@hotmail.com)
Stephanie Gladman (stephanie.gladman@mail.mcgill.ca)
Barry Burstein (barry.burstein@gmail.com)
Maryse Menard (marie.menard@mail.mcgill.ca)
Jasim Al-Abbad (jasim.al-abbad@mail.mcgill.ca)
Jamie Cassoff (jamie.cassoff@mail.mcgill.ca)
Elizabeth MacNamara (elizabeth.macnamara@mail.mcgill.ca)
Philip H Gordon (philip.gordon@mail.mcgill.ca)
Andrew Szilagyi (aszilagy@gas.jgh.mcgill.ca)

Version: 2 Date: 27 September 2013

Author’s response to reviews: see over
Dear Dr Nagaraj Nagathihalli

Editor: Nutrition Journal

Sept 27/2013

We thank you for your consideration of our paper, MS: 177784220100378 for possible publication in Nutrition Journal. We have changed the title to “Dynamics of Vitamin D in Patients with Mild or Inactive Inflammatory Bowel Disease and Their Families”. We offer the following point by point reply to the reviewers. Changes in the manuscript are highlighted in yellow.

**Response to Reviewers**

**Reviewer 1**

Major Concerns;

Demographics: It is mentioned that ‘controls were profiled to resemble patients with respect to age, sex, ethnicity and weight’. Race, which is known to influence vitamin D status, was not included in this list. Study results a significant difference in race between the IBD patients (94% Caucasian) and controls (79%) and higher serum 25(OH)D levels in Caucasians. The authors mention that removal of non-Caucasians did not significantly change the outcome (serum vitamin D status) between the two groups of patients. But, could this explain the apparently higher vitamin D status of the patient family group?

**Response;** The inclusion of more non-Caucasians in the control group was inadvertent. We were certainly aware of different expected vitamin D levels affected by race. We do concede that this tactical error could influence our main outcomes both statistically and a different intrafamilial dynamic could function in non-Caucasians. (These were discussed in the original MS now on pg 17 bottom as a chance occurrence or pg 18 specifically because of the inclusions of more non-Caucasians). The statistical impact of the uneven number of non-Caucasians is described on pgs 13 and 14 in the Result section. However after careful thinking about our results we emphasize that in this study the different aspects noted in the patients and their interactions within the IBD/family unit discloses different dynamics in this unit compared with the control/family unit. This is noted in the conclusions of the Abstract and the Conclusions of the Discussion. Because of the inherent error in the participants’ recruitment we are unable to give clear cause(s) for these observations and suggest however that future studies would need to be carried out
to confirm this potential interaction. However we clearly ruled out our initial hypothesis that IBD family vitamin D dynamics pose a potential risk for IBD precipitation.

Missing data: There appears to be considerable missing data in this study – listed as up to 20% for ‘smoking’ and in the range of 10-40% for other variables known to influence vitamin D status such as tanning salon use and vacation in sunny climates. It is unclear how such missing data was treated in the statistical analysis. Further, the ‘n’ for the various groups changes depending on the comparisons made, making it extremely difficult to get a clear picture of the study results.

**Response:** We agree with the reviewer that missing numbers and information are troubling and confusing. Unfortunately despite attempting to recall patients during the study period for additional information we were not able to fill in all missing values. We therefore handled the data according to available numbers for individual outcomes. The “n” values change because they represent actual numbers used in analyzing a particular outcome. We tried to note missing information with asterisks in the Tables to point out discrepancies in totals or frequencies of participants. Missing data was not imputed because most of our analysis is univariate and exploratory in nature. When conducting multivariable analysis, we did not put many variables together since it will result in a much limited data set. In addition, only five variables had shown statistical significance in univariate analysis. The limitation is discussed on pg 17 and 18.

Disease state: Although the study is reported to be conducted in stable IBD patients, mild disease activity was reported 19% of the Crohn’s patients. It is unclear whether these individuals were examined individually, or if/how the disease status influenced the study outcome.

**Response:** (also includes response to question 6 of reviewer 2): Simple Clinical Indexes were chosen in our study to classify IBD patients because diagnostic tests like endoscopy or radiology were not done immediately prior to entry. The diagnoses were based on the accumulation of previous diagnostic elements and course of disease. The Harvey Bradshaw index is a recognized instrument often used to classify clinical status of patients with Crohn’s disease (ref 34). The five criteria (General well being, Abdominal pain, number of liquid stools per day, presence of abdominal mass and any extra intestinal manifestations) are based on purely clinical grounds and can classify patients on these criteria alone. There is a 0.93 correlation with the more extensive Crohn’s
Disease Activity Index which is based on a week of clinical criteria and some biochemical criteria as well. Clinical indexes are routinely used to include IBD patients in studies. As stated in the methods all patients and controls underwent history and physical exam on the day of blood testing and diet interview.

According to the HBI remission is considered a score of less than 4. Seven (not 6, corrected on bottom of pg 10) patients of 34 CD had borderline score with average 5.7 (eg 1-2 more liquid tools or mild abdominal pain, or patients were tired, etc). None had abdominal mass on examination at entry into the study. These scores are used to define clinical remission or activity although they are not necessarily perfect for accuracy.

As outlined in our methods section (pg 7) we did measure blood tests including CRP (which is a non specific indicator of proinflammatory cytokine synthesis in the liver and is used as an indicator of active IBD). However these were not part of the clinical indexes and were not used.

For the purpose of a response to the reviewers we examined the CRP values and compared serum vitamin 25(OH) D results to see whether there was an effect on serum vitamin D or altered clinical indexes. We expect that there would be a positive correlation between scores and CRP if the latter was an accurate indicator of activity (in the current study).

Of the 206 participants, 190 had CRP measured (no CRP available for 2 patients with Crohn’s disease, 2 Ulcerative colitis, 6 IBD family members, 4 controls and 2 control family members). Of the 190 available, 15 had elevated CRP (4 controls or family members without IBD, 4 with UC and 7 with CD: If we consider these as more reliable for activity than the clinical scores, then 7/32 (22%) of CD patients and 4/19 (21%) of UC patients had some degree of activity. Nevertheless vitamin 25(OH) D levels were nonsignificantly different between the IBD and control groups. Furthermore, for the purpose of responding to this question, we evaluated Pearson’s correlation between vitamin 25(OH) D levels, and C-reactive protein in 10 (1/11 Vitamin
D missing) patients. The correlation between CRP and Vitamin 25(OH) D was \( r = 0.029 \) (n =10). The expectation would be a negative one. Nevertheless, we have modified the title, Background (pg4), Results (as mentioned above pg 10), Discussion (pg 14) and outline the limitations of the use of clinical indexes (pg 17). All reflect that some of the patients had some IBD activity.

Statistics – In general, the statistical analyses are not well described and do not appear to match the study objectives. For example, the authors proposed to describe differences in serum vitamin D and vitamin D and calcium intake across ‘the four study groups’, yet the primary statistical test used was the student T which allows comparison of only 2 groups. Further in some instances it is hinted that frequencies (i.e vitamin D distributions) differed across the four groups, but means of testing was not indicated. Additional detail should also be included with regard to the models used for regression analysis.

**Response:** The section on Statistics in the Methods was rewritten pg 9.

Other comments/suggestions:

Pg 4, 2nd P The meaning of the 1st sentence is unclear. CD and UC are thought to be ‘leading contenders’ of what?

**Response:** the confusion has now been corrected on pg 4.

Pg 5, 1st P The 1st sentence is poorly worded and does not properly represent the results of the cited study which found prolonged breast feeding, less sunshine exposure and lower vitamin D supplementation in the rachitic children.

**Response:** the quotation has been now changed

Pg 5, line 10 ‘levels’ is misspelled

**Response:** Unclear question; spell-check did not single out “levels”

Pg 6, 3rd P BMI is an abbreviation for ‘body mass index’, not basal metabolic Index (and elsewhere)

**Response:** We have now changed BMI to the appropriate wording

Pg 10, 1st P The following abbreviations/acronyms have not? been defined: HBI, SCCAI, 5ASA
Response: HBI and SCCAI are defined on pg 6 of methods and 5ASA was written out on pg 10

Pg 11, line 8 The suggestion that those taking supplemental vitamin D have intakes which ‘approaches levels above daily recommendations’ seems unnecessarily alarming. Intake levels of those taking supplements are in the range of ~1100 – 1350 IU. Although higher than the RDA, these do not approach the tolerable upper limit (UL) for vitamin D of 4000 IU for children > 9 yrs. and adults.

Response: we now clarified the statement with the suggested levels.

Pg 11, line 11 Suggest changing to ‘the effect of vitamin D supplementation and season on serum vitamin D.

Response: The heading was changed but also added intake of calcium which was prominent in the multivariable analysis. However the impact was mainly noted in controls (possibly due to higher intake of dairy foods pg 13). A new reference 46 has been added.

Pg 11, last P The correlation between total intake and serum vitamin D across all groups was not significant. The description of the results is hard to follow and adds little to the paper. Suggest deleting this paragraph.

Response: Deleted as requested

Pg 12, line 15 The p-value for patients indicated here is ‘p<0.03’, but indicated as p=0.003 in the figure legend.

Response: the p values were changed (now on pg 13) p = 0.003

Pg 13, 2nd P It is indicated that ‘there was a higher level of vitamin D’ between Jewish and non-Jewish patients. Based on the values presented, it is assumed that the reference is to serum 25(OH) vitamin D levels. Here and elsewhere in the manuscript this terminology should be used where appropriate in lieu of the less specific ‘vitamin D levels’. Further, while it is mentioned that the ‘vitamin D’ levels are higher in Jewish vs. non-Jewish patients, no indication of significance of the finding is indicated. If not statistically significant, suggest deleting the paragraph.

Response: As suggested we reexamined serum vitamin D levels between Jewish and non Jewish patients. We found that in fact there was no statistically significant difference
between the patients \((p = 0.1)\). However there is a difference in serum vitamin D levels when using log values between Jewish/non Jewish controls. This observation appears to drive the significant findings in the regression analysis. As suggested we therefore deleted this paragraph.

Table 1a/b Although some of the parameters/data reported in Table 1a would be pertinent to only the patients (disease activity, disease site, medical, surgical history), I nonetheless suggest combining Tables 1a and 1b. This would allow ease of comparison between patients vs. family members and patients vs. healthy controls.

**Response:** The tables were combined

• The percentage of UC patients sampled in less sunny months appears to be incorrect.

**Response:** The percentage is now corrected

Table 2 Some of the information accompanying the table (table heading) seems more appropriated for the results section. The units for serum Vit D (actually 25(OH)D) are not given. Suggest spelling out ‘Mean’ in column 2, rather than using ‘\(\mu\)’ which is confusing. • Also suggest including % vitamin D supplement use in the table.

**Response:** The heading of Table 2 was changed including the units of serum vitamin 25(OH) D and the word “means” was spelled out. We have reworked the Table to reflect vitamin D supplement intake in each group and category of serum vitamin D levels (and noted in the legend the total number of analyzed serum vitamin D samples). We also compared statistical significance (using Chi2) between the number taking supplements in replete with deficient categories. None were statistically significant.

Table 3 The set-up of the table is a bit confusing. It would seem more logical to list dietary vitamin D intake first, followed by total vitamin D intake, and then the two subgroups of total intake +/- supplemental vitamin D. As in, it is not at first obvious that the third and fourth sets of data represent subgroups (particularly since the ‘n’ of the two groups does not always match up with the ‘n’ for total vitamin D intake. Further there is no indication of significance in the table, although the text mentions that the total intake was doubled with supplementation. Was that result significant?
Response: The sequence of headings has been changed. As well the means ± SD were compared and statistical significance is marked in the appropriate values. In all groups supplemental vitamin D significantly increased total intake. This significant change has been noted in the results section on pg 11 and with asterix and a footnote for Table 3.

Table 4 The title and table heading does not fully or accurately represent the data presented. The table appears to present the effect of supplement use or season on serum 25(OH)D – not the effects of vitamin D intake. Further, significant differences, if any, are not indicated. Also, suggest deleting data on vacation.

Response: The headings for vitamin D intake have been changed to indicate intake with supplements or without. Serum vitamin D level in patients who consumed vitamin D supplements was significantly higher than in those not taking supplements using means ± SD. Using log vitamin D there was only borderline significance. All others were not significant. The patients serum vitamin D levels taken during sunny months was significantly higher than levels in patients blood tests done during less sunny months. Others were not significant. Missing numbers have been suggested by rewriting in the main group headings in the Table the original number of participants in addition to the n values shown for actual analysis. As suggested vacation results were removed as well.

Reviewer 2

1. A number of areas of the manuscript are unclear and require revision (and shortening) to enhance readability and clarity.

Response: We have rewritten many parts of the manuscript and these have been highlighted in yellow.

2. The Introduction suggests and CD and UC are leading contenders - but it does not indicate what they are contenders for.

Response: The confusion has now been corrected in the background (on pg 4).

3. The Aims at the end of the Introduction should be rewritten to the past tense Only
Response: The wording was changed to past tense.

4. The selection of first degree family members (by the patient or control subject) introduces bias that is not considered.

Response: The choice of first degree family members raises the possibility that if we do find a relationship within family units the explanation may introduce genetic causes as previously suggested by a few papers which evaluated vitamin D binding proteins. As noted however previous quoted papers from Canada and Jordan offered different environmental explanations for any similarities of vitamin D levels within families. As far as we are aware there has been no previous study which evaluated within family relationships in IBD. One of our stated purposes was to evaluate such a relationship. Unfortunately only a modest correlation was found and there is no strong implication that genetic factors are at play.

It is not clear what bias would be introduced by our method. Ideally both parents of younger patients would increase the likelihood of finding genetic influences. Using a single first degree relative at random (random was determined by the participants agreeing to join the study) could reduce the likelihood of finding genetic effects. In our study however Dr CH was concerned that correlation (genetic or environmental) within family units would bias the results and p-values. Even though these correlations turned out to be weak in our study, from a statistical perspective, these correlations should always be accounted for and we did so by applying paired tests as appropriate. From a clinical perspective, the correlations were not as high as expected and became statistically significant in the patient /IBD family unit in summer only. Therefore if there was a genetic influence it may have been diluted by the method chosen to include family members. This fact may also have contributed to the modest correlations found. This comment is made in the discussion on limitations, pg 18.

5. The Methods indicates that patients who are "stable" or with mild activity were included. Is the word stable to indicate those in remission? or those with stable disease regardless of the degree of activity? Further those with mild disease should be excluded in the concept is to examine patients who are in remission.

Response: We agree with the reviewer that the word stable is unclear. We therefore clarified it to mean 1) patients in remission and 2) no recent change in medications (for 3
months). We changed the title, abstract, background, methods (pg 6), results (pg 10), discussion (pg 15, 18) and alluded to this fact in the Conclusions (pg 19).

6. In addition, remission is documented only with clinical indices (subjective). The authors should include an impression of biological remission (e.g., even CRP level) to assess remission. Clearly clinical symptoms are not good indicators of remission.

**Response:** We agree with the reviewer that clinical symptoms are relatively poor indicators of true (endoscopic or histological remission). See above 3rd question reviewer

1. Clinical Indexes for IBD are recognized instruments to gauge disease activity. The limitations of such instruments are discussed on pg 18. As stated in the methods we did take blood samples for multiple targets, including CRP. However CRP is not a feature of the clinical indexes and so they were not used. Moreover as stated above we found no correlation between serum vitamin D levels and CRP (perhaps because CRP is non-specific and was raised in some controls as well {Vermeire et al Gut 55; 2006). However patients with increased CRP likely had some activity. As stated above we have now modified the MS to reflect some cases with more active disease.

7. Under clinical assessment BMI is incorrectly defined. Please correct this

**Response:** We have now changed BMI to the appropriate wording

8. In regards the number of patients included. It is unclear why the authors included 13 patients with no vitamin measurements, given that this was the crux of their assessment. This should be reconsidered.

**Response:** Upon recruitment of participants all expected data were collected. Those that were missing generally were due to inadvertent mistakes at the level of interviewers or as in the case of vitamin D levels at the level of the blood takers or at the laboratory. It is true that our primary outcomes were serum 25(OH) vitamin D levels, however we also sought modifiers of those levels. As such we were able to use data from participants where belatedly we found vitamin D levels missing to assess intakes of vitamin D and their sources within the different groups. When data modifying vitamin D levels were assessed the participants whose levels were lost were excluded from particular analyses. These missing data are clearly outlined in the Tables.
9. Evidently none of the patients with CD had upper gut involvement - had all patients undergone upper endoscopy to confirm this?

**Response:** All patients underwent either endoscopy or radiological methods but not immediately prior to study entry. In the methods, the diagnosis is explained to have been based on several accepted criteria and on previous records. In addition none of the patients reported upper gastrointestinal symptoms when evaluated for the study.

10. It is unclear why the control subjects should be having a different rate of supplementation, given that all are in effect control subjects.

**Response:** I am not certain what question is asked here. On pg 11 and 12 we state that patients and controls had similar “rate” meaning “frequency” of intake. Of the patients 53% consumed supplements, while 48% of the controls consumed supplements and this difference was not significant. The requirement for vitamin D supplementation was not a criteria for entry into the study. In fact it was one of the outcomes we sought that may be associated with serum levels.

11. In the correlations section, the authors suggest that a p value is nearly significant. A relationship is either significant (i.e. p <0.05) or not, there should not be any in between.

**Response:** This question is tricky and with all due respect disagree with the reviewer. Current statistical practice no longer follows the strict (but arbitrary) α level which states that all values below 0.05 % are significant and all above are non significant. The p value which gives an exact number for α was difficult to calculate before the advent of rapid computerized calculations. As a result a p value is now standard calculation and estimates need not be followed. The consequence is that exact numbers can be used and the readers form an opinion about the significance of a comparison. For example it makes no logical sense to call a p value of 0.04999 significant to reject the null hypothesis and a p value of 0.0501 as non significant. A value approaching from 0.1 toward the conventional 0.05 has less likelihood of predicting future outcomes but the paradigm is similar to that with a p value less than 0.05 (1/20). For example in betting for a win at a horse race 10 to 1 odds are still pretty good so the probability between 0.05 – 0.1 remains a grey area on decision.
whether an event is trending toward a true effect. However we have changed the wording and kept the data largely as statistically significant or insignificant.

12. The Discussion does not have any heading (page 15)

**Response:** The Discussion Heading has been added

We hope these responses are adequate

Sincerely

Andrew Szilagyi