Author's response to reviews

Title: Outdoor exposure and vitamin D levels in urban children with asthma

Authors:

Sonali Bose (sonasona99@gmail.com)
Patrick Breysse (pbreysse@jhsph.edu)
Meredith McCormack (mmccorma@jhsph.edu)
Nadia N Hansel (nhansel1@jhmi.edu)
Robert R Rusher (Robert.Rusher@uscmed.sc.edu)
Elizabeth Matsui (ematsui@jhmi.edu)
Roger Peng (rpeng@jhsph.edu)
Jean Curtin-Brosnan (brosnan@jhmi.edu)
Gregory B Diette (gdiette@jhmi.edu)

Version: 2 Date: 7 May 2013

Author's response to reviews: see over
Re-submission date: May 7, 2013
Nehme Gabriel
Editor-in-Chief
Nutrition Journal

Dear Dr. Gabriel,

We are pleased to re-submit our manuscript entitled, “Outdoor Exposure and Vitamin D Levels in Urban Children with Asthma,” reflecting the comments and suggestions offered by your reviewers. Below is a detailed response to each referee. Suggesting formatting changes to the manuscript have also been made. Please let us know if any additional edits are warranted.

We appreciate your consideration and look forward to your reply.

Sincerely,

Sonali Bose MD MPH
Division of Pulmonary and Critical Care Medicine
Johns Hopkins, Baltimore, MD

REFEREE 1:
Reviewer's report
Title: Outdoor exposure and vitamin D levels in urban children with asthma
Version: 1 Date: 8 January 2013
Reviewer: Lena Lämmle
Reviewer's report:
Review NJ – Outdoor exposure and vitamin D levels in urban children with asthma
Even though the manuscript has its strengths (e.g., the underlying idea of the study, longitudinal data), numerous weaknesses and limitations were evident that dampened my enthusiasm. A primary weakness of the manuscript is that a control group without asthma is lacking. Then, in the result part, a great deal of relevant information is not provided, so that I am not able to follow all of this and must consequently trust the interpretation of the authors. Furthermore, in my opinion, the wrong statistical analyses are used.
P = page L = line

Abstract
There is a lot of information missing in the method part: how many measurement points, duration of the study, gender information, evaluation tools, etc.
Thank you for suggesting that we add additional material to our abstract. We have added additional information to the methods section of the abstract, including the duration of the study, frequency of measurements, and instruments used in our
assessment. Since the study included both girls and boys, we report on this breakdown in the body of the paper and in table 1.

Results: how do you define a modest increase? I don’t think that there are no seasonal variations: please also provide effect size. However, you should discuss this result. How strong is the correlation? And you did not introduce IgE levels.

We agree with you that using the term “modest” is unintentionally vague. We have deleted the words “modest increase” and changed to the more literal word “greater.”

With regard to the seasonal variations in vitamin D levels, we used the Kruskal-Wallis test to determine the relationship of vitamin D and season, and found that there was no significant difference ($p = 0.66$) between levels across four seasons. Below is a representation of this output:

```
.kwallis VitaminD if visit ==1, by( seas1 )

Kruskal-Wallis equality-of-populations rank test
+---------------------------------+-------------------------+-------------------------+
|       seas1 | Obs | Rank Sum |
|-------------------------+-------------------------+-------------------------+
| winter |  25 |  1314.00 |
| spring |  35 |  2157.00 |
| summer |  26 |  1569.00 |
| fall  |  33 |  2100.00 |
+---------------------------------+

chi-squared =     1.610 with 3 d.f.
probability =     0.6571

chi-squared with ties =     1.610 with 3 d.f.
probability =     0.6571
```

To respond to your inquiry regarding seasonal variation, we performed an additional regression analysis of season upon vitamin D levels, using winter season as a reference:

```
*** SEASON CODING ***
tabulation: Freq. Numeric Label
25   1 winter
35   2 spring
26   3 summer
33   4 fall
2   .

*** REGRESS SEASON ON VITAMIN D LEVEL ***
.regress VitaminD i.seas1 if visit ==1

Source |       SS       df       MS              Number of obs =     119
-------------+---------------------------------+-------------------------+
            F(  3,   115) =    0.42
This analysis reveals that variations in vitamin D levels across seasons are minimal, and not statistically significant. We also repeated this regression looking at winter (when levels are expected to be the lowest) vs. all other seasons:

*** Newseason ***

.tab newseason if visit ==1 & VitaminD ~=.

newseason | Freq. Percent Cum.  
-------------+---------------------
          winter | 25  21.01  21.01
          spring/summer/fall | 94  78.99  100.00

          Total | 119 100.00

*** Regress newseason on VitaminD ***

.regress VitaminD i.newseason if visit ==1

Source | SS df MS Number of obs = 119  
-------------+----------------------------------------
Model | 155.033591 1 155.033591 Prob > F = 0.2876
Residual | 15893.0526 117 135.838056 R-squared = 0.0097
-------------+----------------------------------------
Adj R-squared = 0.0012
which yielded a similar minimal effect size and revealed no significant difference. In contrast, seasonal variations in Caucasian populations are known to be much higher. A recent Danish study of healthy Caucasian adolescent girls (11-13 years) reported 25-OH D medians (25, 75 percentiles) between consecutive winter-summer-winter to change from 23.4(16.5, 36.4), to 60.3(42.7, 67.7), and back to 29.5(22.2, 40.4), much higher than the variation noted in our cohort of predominantly-black children. [Andersen R et al., Eur J Clin Nutr. 2013 Mar;67(3):270-4.] Reduced variation in 25-OH D concentrations between seasons has previously been shown in healthy adult black women compared to white women [Harris S et al. Am J Clin Nutr. 1998 Jan;67:1232-6.], rendering our results less surprising.

To respond to your remaining question, we added the outcome of “allergic markers” earlier in the methods of the abstract in order to allow for introduction of the IgE levels prior to reporting its association with 25-OH vitamin D levels in the results. The correlation was of an inverse nature and significant, with a Spearman’s rho of -0.24 and p of 0.03, as noted in the results section.

Conclusion: I think that the conclusion of dietary supplementation is very strong based on your data and the results. Thank you for this comment. We have modified the wording in our conclusion in the abstract section in order to better reflect the extent of our findings.

See also the following.
Introduction
In general: I think the introduction part is really poorly structured (see in the following). Then you also present information which has nothing to do with your research questions (e.g., you do not analyze the effect of vitamin D on cardiovascular processes). Then you switch between the ages, which is confusing. What is the current state in childhood? In summary, I think you have three research questions: 1 prevalence of Vit D, 2 relation to time spent outdoors, 3 relation of asthma and Vit D. So why don’t you structure the introduction part in that way?

Thank you for your very helpful suggestions to revise this section. We have made numerous changes to the organization of these paragraphs to make our points more clearly and follow your suggested order of presentation of our research questions. More
specifically, we changed the structure of the paragraphs to first discuss the prevalence of vitamin D in US minority children, and the factors that may contribute to their low levels, including lack of time spent outdoors, followed by the evidence linking low levels to asthma-specific outcomes, as you suggested.

- P3 L2 Reasons for what? Getting asthma?
We agree with the confusion generated by this sentence and have clarified this statement by adding the phrase “for this disparity.”

- P3 L5 I do not understand the “for example…” sentence in this context. You should first write that there is a correlation between asthma and Vit D and then write that it is necessary to analyze if such populations have deficiencies in Vit D and if there is a moderation effect of Vitamin D. I think that this is your research question.
Thank you for noting this confusing wording. We have changed the structure of this section to better lead into our research question, as you have suggested.

- P3 L15 You cannot conclude this. What you can say is that there is evidence that it should be analyzed if there is a relation.
That is a great point. We have amended this sentence to be more hypothetical in nature. In order to further support our hypothesis, we have also preceded this statement to show that supplementation helps in some populations by referencing the evidence reported by a recent clinical trial published in JACI, which demonstrated an improvement in asthma control (e.g. reduced risk of exacerbation) in children observed after vitamin D supplementation. [Majak P et al. J Allergy Clin Immunol. 2011 May;127(5):1294-6.]

- It is not clear if you would like to analyze a black population or if this is just a characteristic of your sample. This is my impression after reading the abstract. Sample effects should be discussed in the end.
We apologize for the confusion with regards to our study aims. We were interested in examining a predominantly black, inner-city population, due to the fact that this population has a tremendously high and disproportionate burden of asthma in the United States, as referenced. At the same time, blacks in the United States have the highest rates of vitamin D deficiency, and we have added this information with supporting references to highlight this phenomenon in response to your comment. Given that this population is at particular risk of vitamin D deficiency, we aimed to explore if vitamin D deficiency may be playing a role in asthma morbidity, specifically in this population.

- Be careful: for me it sounds like a prejudice that African Americans have an indoor lifestyle.
Thank you for pointing out this unintentional meaning in our text. There are data that demonstrate that people spend a majority of their time indoors overall, and our study results do support that urban children spend a predominant portion of their time indoors; however, it is not clear that this is specific to African-Americans. We have added this information and amended the wording of this sentence to avoid this misperception. Our intention was to indicate that in addition to this widespread indoor time, African-
Americans have the additional risk factors including a darker skin tone and diets low in vitamin D.

Subjects and Methods
In general: You should have at least one control group: one without asthma. Also a white population control group seems to be necessary to me, after reading the introduction part (you don’t treat it as a sample effect…). And I am confused as to which measure you need for which research question. Also it is not clear to me which parameters are used in the regression analysis. And why didn’t you analyze moderation effects?

Thank you for these insightful questions. Our primary interest was to characterize the role of vitamin D status on asthma health in people who already have the disease (respiratory symptoms, in particular), and therefore we did not include children without asthma in our analysis. A non-asthma population would inform the question about whether or not vitamin D impacts the risk of developing asthma which is important, but a different question. Health disparities research can use either comparison between populations or study the target population of interest. In focusing on African-Americans, we have chosen the latter strategy.

The co-variates used in the regression analysis included age, gender, and socioeconomic status, listed in our methods section. We did not explore effect modification due to the small sample sizes in this study, but this would be an interesting question for future studies.

- You only measured vit D at baseline?
  Yes, we only had blood drawn at baseline, and therefore could only measure it at baseline. We have added this detail to the abstract as well for further clarification.

- What is your argument that you use guidelines for bone health as guidelines for asthma?

We agree that this may appear to be inappropriate, however, currently, there are no existing recommendations or guidelines for vitamin D levels in asthma. Traditional cut-offs for sufficiency are based upon PTH suppression, and though the IOM has recently suggested that these thresholds be lowered, this is still under debate. With regards to asthma health, there is not enough evidence at this point to formulate guidelines for 25OH D sufficiency, and therefore the guidelines for bone health are potentially helpful to provide some context for the concepts of sufficiency or insufficiency. This point was further elaborated upon in the discussion section of the paper, and we have modified it to read:

“It is still uncertain what levels constitute sufficiency in asthma and other conditions apart from bone health; nevertheless, regardless of which guidelines are used to determine the optimal 25-OH vitamin D level, our study demonstrates that not only is Baltimore among the cities most affected by asthma, but is also representative of a greater national epidemic of 25-OH D insufficiency in black children.”
- Is the information on allergies relevant for your research questions?
We investigated allergic markers, for example presence/absence of atopy, degree of atopic sensitization, and serum total IgE levels, as they may contribute to the development and control of allergic asthma.

- Reliability information is needed. Is there any information available on quality criteria for the questionnaire? Any publication? Otherwise you should present it.
Thank you for inquiring about our instrument. We have added the source for the asthma questions included in our questionnaire. Asthma symptom questions were derived from standard questions used in several prior studies of inner-city children with asthma, including those used by a multicenter asthma study published by the Inner-City Asthma Study Group (Morgan W et al. NEJM 2004).

- Statistical analysis: where is the gain of your longitudinal data? Is the vitamin D status predictive for 12 months? Provide empirical evidence! You should rather analyze path analysis along with latent growth curve analysis. Or only use baseline information….
- Effect sizes are needed.
The advantage of having longitudinal data with repeated data collection at 3 and 6 months is that it allowed us to gather more data regarding outdoor activities and asthma symptoms from repeated questionnaires and daily diaries, accounting for any variability that occurs in this population over time. You are absolutely correct in pointing out that we did not leverage the full benefit of a longitudinal design, in that we did not have repeated vitamin D measurements over this period. However, given our existing baseline information, we gained by having additional outcome data collected over the 6-month study period.

With regards to our method of analysis, path/latent growth curve models would be ideal methods to determine if changes in 25-OH vitamin D levels affect the trajectory of respiratory outcomes over the 6-month period. This would be an interesting question for future studies. In our study, since we only had baseline vitamin D values, we chose to determine if differences in vitamin D status between subjects were related to differences in their asthma symptoms, rather than are predictive of future paths.

Below are representative coefficients for each of our covariates in our adjusted regression analysis of overall symptoms over the past 2 weeks. Effect sizes, along with p-values, are shown, and demonstrate no significant association between 25-OH vitamin D levels and symptoms in the last 2 weeks in our adjusted model.

```
. regress sx_2wks VitaminD age male neweduca i.seas1 if visit ==1

Source |       SS       df       MS              Number of obs =     119
-------------+---------------------------------------------+---------------------
Model |   121.670621     7   17.3815173           Prob > F      =  0.1383
Residual | 1194.29577   111  10.7594213           R-squared     =  0.0925
```
Result
In general: again, I’m confused. A lot of information is missing (like which parameters are used within multivariate regression models? Which methods are used: enter? Did you observe multicolinearity? How high is the R2?
We made changes to the text to elaborate on our statistical methods, including the clarification that generalizing estimation equations were used to account for the repeated measures of outcomes in our study, as well as that Kruskal-Wallis testing was used to compare vitamin D levels across seasons.

We did not observe multicolinearity. We calculated the variance inflation factor (VIF) test, and found them to range from 1.03-1.71, (mean of 1.32), which is well below a cut-off value of 5 where multicolinearity would be high.

We do not have a calculation of R2, as our variables were categorical, rather than continuous, in nature, and therefore logistic regression was performed.

- “There was no association between the average daily hours spent outdoors and baseline 25-OH D levels (p = 0.49).” ???. This information is also different to the information provided in the abstract. And I do not understand the sense and use of the two following sentences.
That is correct--there was no association between the daily hours spent outdoors and baseline 25-OH D levels. To clarify, in the abstract, we made the more significant point that increased outdoor time was not associated with higher D levels in this predominantly black population, contrary to what may occur in Caucasian skin tones. We have altered
our abstract per our recommendations to make this clearer. We have also deleted the two following sentences that were confusing and did not add anything to the paper, as you rightfully pointed out.

- Effect of season: you should give information why there is no effect of time spent outdoors: this is really surprising…
There indeed was no effect of time spent outdoors upon vitamin D levels, and agree that this finding is novel. We believe this is illustrative of the difference that relates to studying a predominantly African-American cohort of young children, in comparison to previously studied Caucasian groups, whose lighter skin color requires less UV exposure to synthesize 25OH vitamin D from precursors in the skin. In darker skin shades, the melanin in the skin is a competitive inhibitor of UV light, and therefore greater amounts of irradiation is required for vitamin D production (this has been shown in prior *in vitro* and *ex-vivo* experiments). We cite studies such as that of Scragg and Camargo in our discussion to illustrate that such dampened effects in response to sunlight have also been described previously in darker healthy populations, and that this phenomenon may also be true for children with asthma, putting them at risk for the deficiency we observe.

- Provide all the information needed for multiple regression.
Thank you again for pointing out the need for additional information. We have added information to our methods section to clarify our statistical methods.

Discussion
In general: Again it is not structured with regard to the research questions. Then again I’m not sure if you talk about adults or children. Then you report on information which has nothing to do with your research question.
Thank you for your very helpful suggestions with regard to the structure of our paper. Your comments were a helpful guide in the further editing of our manuscript. We have re-structured the discussion section to follow with our three main research questions—that of the prevalence of vitamin D insufficiency in our cohort and within a national context, followed by a discussion of its relationship with outdoor time and seasonal variation, and finally its association with markers of asthma disease. We hope that this helps to clarify our main points.

*Quality of written English:* Acceptable
*Statistical review:* Yes, and I have assessed the statistics in my report.
*Declaration of competing interests:* I declare that I have no competing interests.
REFEREE 2

Reviewer's report

Title: Outdoor exposure and vitamin D levels in urban children with asthma Version: 1
Date: 14 January 2013 Reviewer: Augusto Litonjua

The authors present an analysis of vitamin D levels and outdoor exposure assessment and asthma and allergy phenotypes in a cohort of inner city children. The manuscript is well-written and the authors discuss the limitations.

Minor Essential Revision

I think Figure 1 should be redone to make it clearer. I think a distribution plot would be better - the N on the y axis and vitamin D levels on the x axis. The graph can be a bar graph or a smoothed plot and they can put a line through the mean.

Thank you for your kind and constructive comments. We have revised Figure 1 as follows per your suggestion:

Quality of written English: Acceptable
Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I declare that I have no competing interests.
This is a well written interesting study. Minor essential revisions:

1) The cut-offs chosen for insufficiency and deficiency in this paper are not in keeping with the IOM recommendations from their 2011 report which found that based on the available literature the cut-off for 25(OH)D should be <20ng/ml and not <30ng/ml. The true cut-offs for extraskeletal outcomes has yet to be determined, however using these cutoffs might be overestimating the degree of vit D insufficiency/deficiency. The authors should compare the results using the IOM cutoffs as well and comment.

   Thank you for your discussion on this important point. The IOM did indeed suggest that the cut-off for 25OH D deficiency should be <20ng/mL. Though this is still under debate, if we consider this alternative cutoff, then 23% of subjects would be in the deficient range (vs. 54% if 30ng/ml is used). To your point, we have added this important perspective in our edited manuscript. The remainder of our analyses was performed with the values of 25-OH vitamin D, without any categorization for deficiency.

2) Is there any explanation for no seasonal differences? Comment

   The lack of seasonal differences in Vitamin D in our cohort was one of the striking findings of our study. Prior epidemiologic studies that have demonstrated seasonal differences are mainly reflective of lighter-skinned Caucasian populations. For example, a recent study of healthy adolescent Danish girls reported 25-OH D medians (25, 75 percentiles) between consecutive winter-summer-winter to change from 23.4(16.5, 36.4), to 60.3(42.7, 67.7), and back to 29.5(22.2, 40.4) [Andersen R et al., Eur J Clin Nutr. 2013 Mar;67(3):270-4]. However, in darker-skinned populations such as that of this study, dermal production of vitamin D to UV light is likely diminished (since melanin skin pigmentation is a competitive inhibitor to UV light), rendering blacks less responsive to seasonal variations in sunlight availability. In vitro and ex-vivo studies have long since demonstrated that greater amounts of UV light are needed to result in the same degree of dermal 25-OH vitamin D production and attain the same serum levels [Armas LA et al., J Am Acad Dermatol. 2007; Chen TC et al, Arch Biochem Biophys. 2007] in darker skin compared to lighter skin types. In addition to this biological insensitivity to sunlight, we hypothesize that the minimal amount of outdoor time spent
by children in our cohort of asthmatic children may not afford them the opportunity to be exposed to seasonal differences in UV exposure. Therefore, these children are blunted to this seasonal response. We are currently conducting a study of predominantly African-American children with asthma where we collect more specific information regarding skin pigmentation in order to relate this to serum 25-OH vitamin D levels.

3) Do we know the rates of vitamin supplementation and milk consumption (is milk fortified) in this population? provide if available

The rates of vitamin supplementation and milk consumption are important to fully understand the causes of vitamin D deficiency. Unfortunately, we do not have information in this specific cohort regarding milk consumption and vitamin use. However, this study provided preliminary data for a larger study that is currently underway which addresses this limitation and collects detailed dietary information in order to quantitate vitamin D intake. We have added this limitation to our manuscript to address your point.

4) There does not appear to be an association between degree of illness (ER visits and hospitalizations) and vit D concentration. Please comment.

Thank you for noting this. There was no association between acute care visits (ER visits or hospitalizations) and 25-OH vitamin D levels. We have added this information to the manuscript.

5) There is no control group, a study limitation, therefore causation statements related to vit D insufficiency and asthma cannot be made. Please comment.

Thank you for your comment. We agree that causation conclusions cannot be made with regards to vitamin D insufficiency and asthma from our results. This has been a limitation of observational and longitudinal studies such as ours within this emerging field. In addition to commenting on this point in our limitation paragraphs of the manuscript, we have changed the wording in our introduction and discussion sections to highlight this difference, as well as added an additional reference of a supplementation study in children with asthma that may help to bridge this gap in the literature.

Quality of written English: Acceptable
Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests: 'I declare that I have no competing interests'