Author's response to reviews

Title: Effect of Physical Activity and Sun Exposure on Vitamin D Status of Saudi Children and Adolescents

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Version: 3 Date: 13 April 2012

Author's response to reviews: see over
The authors have examined the association between 25-OH-VitD level, physical activity and sun exposure in apparently healthy children and adolescents living in a sun soaked country such as Saudi. From the basis of the study there were clear associations between 25-OH-VitD, the level of physical activity and sun exposure whilst BMI or serum calcium did not influence the 3 groups analysed due to changes in sun exposure or physical activity.

Minor essential revisions

1. Abstract: under the results the authors need to add clarity to their statements, what does the 71.6% represent? Also add further data, % or p values around the comparison of changes in Vit D based around physical activity.

- Done

2. Last line of results section which begins BMI was lowest???.group, could the authors rephrase this and indicate the meaning of the sentence more clearly.

- The results section were revised accordingly.

3. The authors should add in intra and inter CV values for 25-OH-VitD levels.

- This comment is noted and was added in the revised methods section.

4. There are several grammatical errors which need to be addressed as detailed below.
   Page 2 line 1: ??suggests ?an? increased prevalence?..
   Page 2 line 13: group remain?ed? deficient??
   Page 2 last line: population, vitamin D supplementation is still required in all groups, including those with the highest sun exposure and physical activity?
   Please amend text as detailed below:
   Page 3: line 4: ..India and Lebanon ?have? 25(OH)D?.
   Page 3 line 5: ??vitamin D deficiency may ?arise? due to low exposure of ?the? skin??
   Page 3 line 14: effects of ?a? season to vitamin D?.
   Page 3 Line 15: as compared ?with the? summer?.
   Page 3 line 16: outdoors during ?the? daytime
   Page 3 Line 19: add in coma as follows: dark veils completely, for cultural and religious reasons
   Page 3 line 21: The aim of the present study ?was? to determine?.
Page 6 Line 8-9: rephrase these lines as follows: These were, no exposure (0 min), daily exposure (10-30 min) and weekly exposure (40-160 min).
Page 6 line 14: differences were noted due to BMI or serum calcium?..
Page 6 line 19: The important ?findings? of this study reveals??.
Page 6 Line 24: vitamin D status besides body mass index (BMI; >30kg/m2) and calcium supplement intake was physical activity. Whilst it is well established that physical ??.
Page 6 last line: ?.absorption efficiency [17], it also increases serum calcium?..
Page 7 Line 1-4: ?. In addition, physical activity which is known to reduce body weight by increasing the rate of lipolysis [18] may enhance mobilization of vitamin D from adipose tissue?..
Page 7 line 6: ?.in the cohort could also be attributed to growth spurts of peak b one mass, requiring higher Vitamin D?..
Page 7 Line 11: ??skin pigmentation, clothing, cultural practices??
Page 7 line 14: Serum Vitamin D levels were highest in the group exposed to sunlight on a daily basis as anticipated, but were nevertheless still vitamin D deficient?..
Page 7 Line 18: ??UV radiation; since the dust is composed of mineral particles that both absorb and scatter sunlight and this may affect vitamin D ??
Page 7 Line 21: Our study acknowledges ?a? few limitations??

- All the suggested changes were incorporated, and the final revised manuscript was proof read before submission.

5. Discretionary revisions

The authors note that one factor which may account for the increased levels of Vitamin D in physically active subjects is the level of lipolysis which helps to release Vit D into the circulation as a result. From the previous literature it appears that at least in adults, women have higher rates of noted induced systemic lipolysis than men. As such in this study did the subjects show a gender divide in the amount of Vit D that was observed in the circulation with the highest level of activity. If so this may add further weight to the stated concept around lipolysis activity and Vitamin D levels. It should be noted however that comparable studies of lipolysis and Vit D do not appear to have been undertaken to date to address this in children, this study may shed some light on this.

Horton TJ, Dow S, Armstrong M, Donahoo WT. Greater systemic lipolysis in women compared with men during moderate-dose infusion of epinephrine

- This comment is noted and the reference was cited in the revised discussion section.

**Referee 2**

**Page 2:**

Background:
Accumulating evidence suggests an increased prevalence of vitamin D deficiency in the Middle East as compared to European countries. In this context, we aimed to determine whether the prevalence of vitamin D deficiency is related to degree of physical activity and sun exposure among healthy Saudi children and adolescents, a little studied population.

Results:
All subjects were vitamin D deficient, the majority being moderately deficient (71.6%). Body Mass Index (BMI) was also lowest in the most physically active group (p < 0.05).

**Page 3:**

Background:
.... The kingdom of Saudi Arabia (KSA) has unique conditions that influence vitamin D status even though Saudi Arabia is sun-drenched throughout the year and temperatures often rise above 50°C (122°F) during summer. In summer months, for example, parents do not allow their children to engage in outdoor activities during daytime. Women in particular receive little or no sun-light since they cover their bodies with dark veils completely, for cultural and religious reasons. The aim of the present study is to determine the prevalence of vitamin D deficiency and its association with behavioral factors like physical activity and exposure to sunlight in an otherwise healthy group of Saudi children, an understudied population in terms of vitamin D deficiency. The results should help in raising awareness as well as suggesting strategies to combat vitamin D deficiency in this part of the world and thus in reducing the incidence of various chronic ...

**Page 4, Methods:**

A pre-designed and approved questionnaire which includes medical history are answered due to all participants.

**Page 6:**

...The BMI was highest in the physically inactive group (P<0.05) (Table 2). Discussion:
Consistent with our findings, Brock et al. reported that the major modifiable predictors of low vitamin D status were body mass index (BMI) > 30 kg/m² and physical inactivity and calcium supplement intake [16].

...Our study acknowledges a few limitations. The cross-sectional nature of the study makes it difficult to determine the true effect of physical activity on the vitamin D status of the cohort.

- All the suggested changes were incorporated, and the final revised manuscript was proof read before submission.

Referee 3

MAJOR COMPULSORY REVISION:

1) Authors cannot conclude that physical activity determines by itself the level of vitamin D in this population. They conclude that lack of physical activity is associated with vitamin D deficiency, but lack of physical activity is associated with lack of sun exposure and that is the mayor condition associated with vitamin D deficiency. So they have to compare time of sun exposure and level of physical activity, if there are difference between subjects with the same frequency of sun exposure but with different levels of physical activity, they would be very interesting and, in those case, the authors could conclude that physical activity is associated with vitamin D deficiency in an independent way this population.

- This comment was well noted and an additional figure was done showing that physical activity is affecting levels of 25 (OH) vitamin D independent of sun exposure.

2) The authors have to redefine the groups based on vitamin D status. They define mild vitamin D deficiency as vitamin D > 25 nmol/l. But it is known that levels of vitamin D > 30 nmol/l are sufficient. So the authors have to define the cut-off for mild vitamin D deficiency as levels of vitamin D between 25-29.9 nmol/l and they have to include a new group of subjects: no vitamin D deficiency for subjects with levels of vitamin D > 30 nmol/l. If there are no subjects with levels of vitamin D > 30 nmol/l in this population, the authors could conclude that all subjects of the study were vitamin D deficient.

- The cut-off for vitamin D deficiency has been revised accordingly.

3) The authors cannot conclude BMI is associated with vitamin deficiency in the study population, because they have found an association between BMI and physical
activity, but not between levels of vitamin D and BMI. They have to compare BMI and levels of vitamin D to be able to find an association between BMI and vitamin D status.

- Our conclusion was vitamin D status was affected by levels of physical activity and sun exposure and not BMI, because this was not proven in the present study.

MINOR ESSENTIAL REVISION:

1) It would be interesting to know how many Primary Health Care Center have participated in the study and how many cities or areas have been involved in the study. It would be interesting too, to know how the authors chose the different Center to make the study (by a random method or not?).

- No randomization was done. Saudi children who were coming from the 4 different PHCCs were enrolled after consent and assent were provided.

2) Because it’s known that vitamin D status varies with seasons, it would be interesting to know when the study was carrying out: in a range of date, in one year, at the same time in all subjects?

- The recruitment phase was done on March-December 2010 and majority were recruited during the summer period, as mentioned in the revised methods section.

3) Authors describe in methods, they ask about parts of body exposed to sun, use of sun protection creams and type of physical activity, but they don’t show the results. They have to show the results or not talk about these concepts in methods.

- This point is noted and was removed from the revised methods section.

4) Authors should define how they divided the population in three groups based on the level of physical activity: based on intensity of the activity, based on the time expended doing physical activity? And they have to explain what the cut-off for the different groups is.

- This point is noted and the cut-offs were explained in the revised results section.

DISCRETIONARY REVISIONS:

1) The authors could analyze different in levels of vitamin D between boys and girl. They could find different in relation to different in parts of body exposed to sun (some girls maybe use veils) and in relation to more boys are physically
active than girls in the study population. It would be interesting to know if girls have more risk to hypovitaminosis D than boys.

- We did regression analysis and for this specific cohort we found no influence of gender over 25 (OH)D levels although this finding does not supersede previous findings.

Referee 4

Vitamin D deficiency in the middle-east seems high, but only few papers have been published on this topic. This is a nice cross-sectional study on a good sample. Unfortunately the analysis is done too simplistically and many details are lacking.

Abstract

Results state that all subjects were vitamin D deficient. This is not stated in the discussion. The conclusion regarding counteracting is unjustified given that even the highest sun and physical activity group was deficient.

- This comment was noted and additional statements were provided in the discussion section.

Background

You state information about the Saudi behaviour in page 3, par 2. Could your study contribute to that? Can you see that the outdoor behaviour is different in summer vs winter?

- The majority of children enrolled in the study were recruited during the summer months and therefore seasonal variation was not apparent.

Methods

You state that 331 boys and girls were selected. There is no information on the participation rate and how many children were actually in the study. How many were excluded?

- In the original proposal that was approved by the grant provider it was decided that 400 children were to be included but only 331 were recruited. Those children who were seen but excluded were not included in the final count.

There is no information over which period the children were measured. All in 1 season or throughout the year?
This comment was noted and the recruitment time was mentioned in the revised subjects section of the methods.

You measured sun exposure but do not indicate over which period you measured sun exposure (last summer, last winter, the last month??). Same with physical activity. You measured Ca but do not provide data in your paper.

This comment was noted and was provided in the revised version.

Your statistical analysis are too simplistic. Multivariable linear regression should be used with 25(OH)D as an outcome (probably transformed to satisfy normality, then back transformed to obtain estimates in units that people can interpret). What do you mean with age-adjusted groups and how did you achieve that?

The comment is noted and additional statistics were provided. We did correlation and stepwise regression analyses and results were given in the revised results section. We changed age-adjusted to age-matched, since there was no significant difference between the ages of subjects across groups, which was the major confounder of the variable of interest.

Results

Prevalence: your categories are <12.5, 12.5-24.9 and >25. Should the last category be 25-50 nmol/L for mild deficiency? It is now unclear how many had sufficient levels. Figure 1 should be dropped. You already said it with three numbers. Please examine the seasonality in vitamin D levels? that is interesting. Also examine seasonality in other variables such as physical activity, sun exposure and amount of skin exposed.

The new cut-offs have been provided and it was a typographical error not to provide the range of the mild deficiency.

Sun exposure and vitamin D: your sun exposure variable does not have mutually exclusive groups. Suggest to express in hrs/wk.

You should use multivariable analysis so you can examine both sun exposure and physical activity at the same time. Examine what happens to the magnitude when you add the other in. Then you can say something about the relative importance of sun versus physical activity. You need to add in age, sex, bmi, clothing, etc. You also need to take seasonality into account. There are different ways for doing that. Was there a difference by age, by sex, etc, after taking the other factors into account. You can express the increases/decreases 25(OH)D per hr/wk increase in sun or hr/wk increase in physical activity.
We added an additional figure revealing the influence of physical activity in children having the same sun exposure.

Discussion
You don't state your prevalence findings clearly in the discussion. I don't see the issue with a cross-sectional study. The period they need to recall back is not long. An experimental design does not make sense here. Instead, analysing it correctly by taking confounders into account would be much better. Can you compare your results to other countries in the middle east.

This was noted and additional references were added.

Referee 5

Major Compulsory Revisions

Abstract Revisions

1. Recommend starting the results section of abstract by describing the study subjects with at least one demographic variable such as age.

   - This comment is noted and the whole abstract has been revised accordingly.

2. The authors report the results in the first sentence of the abstract as ?All subjects were vitamin D deficient, majority of whom were moderately deficient (71.6%)? It is important to define what ?moderately deficient? is using the cut off for vitamin D levels.

   - This comment is noted and the whole abstract has been revised accordingly.

3. Similarly the next two sentences in the abstract ?Age-adjusted comparisons revealed that vitamin D status was highest (report value) among the most physically active group and most frequently sun exposed, though levels in this group remain deficient. BMI was also lowest (report value) in the most physically active group (p < 0.05).? also need to include the absolute values in addition to the interpretation.

   - This comment is noted and the whole abstract has been revised accordingly.

4. Suggest starting the conclusion by a sentence that describes the main finding, such the high prevalence of vitamin D deficiency.

   - Done
Background Revisions

Background, last sentence: It might be helpful for the readers to have a sentence that describes the various chronic diseases associated with vitamin D deficiency.
- Noted and additional diseases related to vitamin D deficiency were mentioned

Methods Revisions

6) Methods, 2nd paragraph: The questionnaire also sought information about sun exposure (frequency of exposure, time of exposure, parts of body mostly exposed to sun, parts of body not exposed to sun and use of sun protection creams) and physical activity, which is self reported (frequency and type of activities performed along with duration ? number of minutes per week). Please clarify whether all the subjects ages 6-18 years read the questionnaire and self report the information? Or was the questionnaire administered by someone else to the subjects. It is also important to state whether any or all of this information was obtained from the parents.
- The methods were revised accordingly.

7) Methods, 4th paragraph: Please state the manufacture?s normal reference range for this assay after the sentence ?Vitamin D was measured using enzyme-linked immunosobent assay (ELISA) (I.D.S., Tyne & Wear, UK)?
- Done

8) It is important to define to the primary outcome measure vitamin D deficiency and also to state clearly what evidence that definition was based on.
- Noted and done

Results Revisions

9) Results, 1st paragraph : The subjects of this study divided into three groups based on their vitamin D deficiency status using different cut-off values: severe (<12.5 nmol/l), moderate (12.5-24.9 nmol/l), and ?mild (>25 nmol/l - ?)[14]. Please fill in the missing information shown in red.
- Noted and done

10) Results , 2nd paragraph: The lowest level of vitamin D in serum (report levels 17.7 ± 1.6 nmol/l vs 22.7 ± 1.5 nmol/l) was found in the physically inactive group (P<0.05). The
BMI was highest (state the BMI values) in the physical inactive group (P<0.05) (Table 2).

- Noted and Done

11) Please make a reference to figure 1 in the appropriate part of the results section.

- Figure 1 was replaced and was cited in the revised results section.

12) It might be helpful to the readers if the values for severe, moderate and mild hypovitaminosis D were in the legend of the figure.

- Figure 1 was replaced and results on deficiency were just mentioned in the text.

13) Table 1: Please put the number of subject in the title of Table 1, and in the column headings for physically inactive, moderate active, and physically active. Moderate active title should be changed to moderate physical activity.

- Noted and Done

14) Table 2: Please put the number of subjects in the column headings for physically inactive, moderate active, and physically active.

- Noted and Done

Discussions Revisions

1) Discussion, 1st paragraph: Consistent with our findings, Brock et al. reported that the major modifiable predictors of low vitamin D status were body mass index (BMI) >30 kg/m2 and physical inactivity. calcium supplement intake [16]. Please revise this sentence and remove the period between inactivity and calcium.

- Noted and Done

Minor Essential Revisions

Background, 2nd sentence: Avoid starting a sentences with a numerals? 30 to 50% of children and adults in the United Arab Emirates, Australia, Turkey, India, and Lebanon had 25(OH)D levels below 20ng/ml [4-7]. Suggest rewriting it as Thirty to Fifty percent ..? This is seen also seen in 1st paragraph of results section ?11.4% of subjects had severe vitamin D deficiency while 71.6% ? and 16.8% of subjects showed moderate and mild deficiencies, respectively.
Methods, 4th paragraph: Spelling corrections for the word immunosorbent? Vitamin D was measured using enzyme-linked immunosorbent assay (ELISA) (I.D.S., Tyne & Wear, UK).? Done

Results, 1st paragraph is missing a verb ?were?: The subjects of this study were divided into three groups based on their vitamin D deficiency status using different cut-off values: severe (<12.5 nmol/l), moderate (12.5-24.9 nmol/l), and >25 nmol/l [14]. 11.4% of subjects had severe vitamin D deficiency while 71.6% and 16.8% of subjects showed moderate and mild deficiencies, respectively.? Done

Results, 2nd paragraph: Consider changing from a passive voice to active voice? Based on the level physical activity, subjects were divided into three groups? to ? The subjects were divided into three groups based on their level of physical activity: inactive, moderately active and active.? Done

Results, 3rd paragraph: These were: no exposure, daily exposure (on average 10 minutes to 30) minutes and weekly exposure (on average 40 minutes to 160 minutes per week).? Suggest a space between average and 40. Done

- All changes were incorporated and a final proof reading was done prior to resubmission.

Discretionary Revisions

1) Discussion, 4th paragraph: The cross-sectional nature of the study makes it difficult to determine the true effect of physical activity to the vitamin D status of the cohort.? Perhaps it would be better to end the sentence as vitamin D status of our subjects instead of cohort, since the words cross-sectional and cohort used in the same sentence are both terms used for study designs. Done

- The limitations were changed accordingly.