Reviewer’s report

Title: Vitamin-D2 treatment-associated decrease in 25(OH)D3 level is a reciprocal phenomenon: a randomized controlled trial

Version: 0 Date: 27 Jul 2018

Reviewer: Michael Holick

Reviewer's report:

1. The authors have misspelled 1,25-dihydroxyvitamin D this should be corrected.

2. The comment about vitamin D2 supplementation associated with decreased 25-hydroxyvitamin D3 and 1,25-dihydroxyvitamin D3 is misleading. The statement should be modified to make it clear that the total levels were not changed.

3. There is a simple explanation for why blood levels of 25-hydroxyvitamin D3 decrease when 50,000 international units of vitamin D2 was given. There is much more substrate of vitamin D2 for the hepatic 25 hydroxylase and therefore it is preferentially converted to 25-hydroxyvitamin D2. This results in an increase in the blood levels of 25-hydroxyvitamin D2. As a result there is a decrease in 25-hydroxyvitamin D3 synthesis since very little vitamin D3 is being converted to 25-hydroxyvitamin D3 at the same time that vitamin D2 is converted to 25-hydroxyvitamin D2. See reference 19

4. Their hypothesis that vitamin D2 treatment is associated decreased 25-hydroxyvitamin D3 may be related to an increase in total 25-hydroxyvitamin D levels has are ready been proven several times including refs 2, 19. What is novel about their observation is that they saw the same phenomenon when they gave 50,000 international units of vitamin D3 and showed to decrease in 25-hydroxyvitamin D2. Therefore the authors have confirmed previous observations about the reciprocal effect of vitamin D2 on serum 25-hydroxyvitamin D3 levels. They have now added a new observation regarding the reciprocal effect of vitamin D3 on 25-hydroxyvitamin D2. This should be made clear that conclusion.

5. In the discussion it is misleading to suggest a significant decrease in 1,25-dihydroxyvitamin D3 levels in response to 1000 international units of vitamin D2 daily when the total blood levels remained the same demonstrating that there is very tight control on the total 1,25-dihydroxyvitamin D. Furthermore this suggests that since the levels remained the same that the 1,25-dihydroxyvitamin D2 was having the same biologic effect on calcium metabolism as 1,25-dihydroxyvitamin D3.

6. The relative potency and discussion about it is reasonable but another possibility is the design of the previous studies. What is not taken into account is that it's likely that body fat contained vitamin D3 that was released and metabolized to 25-hydroxyvitamin D3 whereas there is no fat
stores of vitamin D2.

7. Figure 1 needs error bars and statistics.

**Are the methods appropriate and well described?**
If not, please specify what is required in your comments to the authors.

Yes

**Does the work include the necessary controls?**
If not, please specify which controls are required in your comments to the authors.

Yes

**Are the conclusions drawn adequately supported by the data shown?**
If not, please explain in your comments to the authors.

Yes

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