Author’s response to reviews

Title: The effects of single high-dose or daily low-dosage oral colecalciferol treatment on vitamin D levels and muscle strength in postmenopausal women

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Author’s response to reviews:

We express our special thanks to the reviewer for his/her careful reading of the paper, and for providing valuable comments and suggestions which have helped improved both the content and the presentation. We believe that the revision has been successful and the paper has been improved. The responses to reviewer's comments are available in this document.

Response to the comments of Reviewer

Thank you for your insightful assessment of the manuscript. We have addressed the issues you noted in this revision.

1. 10 nmol/L and mmol/L may be helpful for wider audience when discussion 25-OHD and serum calcium concentrations.

-- 10 nmol/L and mmol/L values of 25-OHD and serum calcium concentrations were added.
2. What method of randomization was used? Were clinicians/study team blinded to this?

--Patients consecutively enrolled to study. The neurologist who performed muscle strength tests was blinded to study patients however, endocrinologists known the patients belonging to which group.

3. A comment on study limitations and effects of multiple comparisons is needed (particularly with regards the different groups and muscle function measures).

--“Being a single center study and a relatively small sample size were the limitations of the study. Being at lower threshold levels of vitamin D3 which known to decrease fracture risk and falls at 1st and 3th of months of study could be another limitation of the study, as it may make difficult to interpret the results of the study. Additionally, none of our patients was evaluated for vitamin D metabolites which has been demonstrated to be related to muscle function in recent studies. This is another limitation of our study.” was added as an explanation for study limitations.

--“It is known that vitamin D has an important role in muscle strength and function. This condition is more prominent in proximal muscles of lower extremity. Because of this, we evaluated knee muscles strength such as quadriceps and hamstrings. It is difficult to evaluate the hip muscles strength with isokinetic device, therefore we selected the knee muscles (Rejnmark L. Effects of vitamin D on muscle function and performance: a review of evidence from randomized controlled trials. Ther Adv Chronic Dis 2011;2:25-37).

4. It appears that there is a trend to increased muscle strength across the different measures, but only 2 groups (daily dosage dominant, knee extension and daily dosage non-dominant, knee flexion) reached statistical significance - as the authors state, it may be that the study is underpowered. How were power calculations made? Alternatively, recent evidence has suggested that there are differential effects of different vitamin D metabolites on muscle (PLoS One. 2017 Feb 15; 12(2): e0170665). In particular, 1,25OHD has direct effects on muscle mitochondrial function and gene expression (Endocrinology 2016 Jan; 157(1):98-111, J Biol Chem 2016 15;29(3):1514-28). This may explain some of the complexity in relationships observed in this study between post-treatment vitamin D status (serum 25OHD) and muscle function. Multi-metabolite analysis in a future study would be interesting.

--Unfortunately, different Vitamin D metabolites were not checked as they are not a component of the routine laboratory evaluation. This was added to discussion part as a limitation of the study.

5. There have been some concerns regarding the safety of single large doses of vitamin D supplementation with regards to falls risk - some acknowledgment of this would be useful.
These findings might support the results of a randomized controlled trial which demonstrated high-dose colecalciferol leads to a higher risk of falls and fractures in older community-dwelling women (Sanders KM, Stuart AL, Williamson EJ, Simpson JA, Kotowicz MA, Young D et al. Annual high-dose oral vitamin D and falls and fractures in older women: a randomized controlled trial. JAMA. 2010 May 12;303(18):1815-22) sentence was added to discussion part.

6. Table 4 is confusing to look at in current format. May be better presented as a graph.

--Table 4 was modified

A couple of minor points on typos and grammar:

Colecalciferol preferred to cholecalciferol.

--Corrected

Unclear meaning of phrase: 'On the other hand, the form of vitamin D therapy, the dose and dosing interval, and route of administration is considered negligible because it does not deserve specific recommendations or guidelines'

--This sentence was modified as “The form of vitamin D therapy, the dose and dosing interval, and route of administration are not given much importance because there are no specific recommendations or guidelines in this regard”

Difficult to follow, would review language and consider fragmenting this sentence: 'In a systematic review of studies using large, single dose, oral vitamin D supplementation in adult populations, the authors mentioned that a single vitamin D3 dose of ≥100,000 IU provided a perdurable effective means of increasing short-term vitamin D concentrations to >20 ng/mL, although vitamin D3 doses of ≥300,000 IU were required to achieve 25(OH)D3 concentrations >30 ng/mL and decreased plasma PTH concentrations [20].'

--This sentence was corrected as “In a systematic review of studies using large, single-dose, oral vitamin D supplementation in adult populations, a single vitamin D3 dose of ≥100,000 IU was shown to provide a perdurable effective means of increasing short-term vitamin D concentrations to >20 ng/mL”

Martin Hewison (Reviewer 2): Please include all comments for the authors in this box rather than uploading your report as an attachment. Please only upload as attachments annotated versions of manuscripts, graphs, supporting materials or other aspects of your report which cannot be included in a text format.

Please overwrite this text when adding your comments to the authors.
The manuscript by Apaydin et al describes a relatively straightforward but nevertheless interesting study in which the authors have compared the impact of two different vitamin D supplementation regimens on serum vitamin D 'status' and muscle function in a cohort of postmenopausal women who were vitamin D-deficient (< 50 nmol/L) at the start. The two types of vitamin D supplementation used in the study were: 800 IU/day or 300,000 IU single dose. Rather predictably the latter regimen was better at increasing serum 25-hydroxyvitamin D (25D) than the former, although both increased 25D relative to baseline. However, muscle function appeared to improve only in the group receiving daily dosing with vitamin D. These observations provide plenty of talking points, but to make these meaningful talking points, the authors need to perhaps do some additional analyses and some data reorganization.

Specific comments:

1. The crucial observation from this study is that simply elevating serum does not guarantee success with vitamin D supplementation. The authors need to provide more information on why this occurs. One possibility is that the different dosing regimens have different effects on vitamin D metabolites other than serum 25D. Are serum 1,25-dihydroxyvitamin D (1,25D) or 24,25-dihydroxyvitamin D (24,25D) different? Measuring these metabolites is more work but it may provide crucial new information. For example, recent studies have shown that muscle function in humans correlates more closely with 1,25D than 25D. Is 1,25D higher with the daily dosing? Or, indeed, is the catabolic metabolite 24,25D higher with the single large dose - this has been suggested previously. The authors should measure other serum vitamin D metabolites

-- Unfortunately, different Vitamin D metabolites were not checked as they are not a component of the routine laboratory evaluation.

2. The authors should re-structure Table 2 to place the Single Dose and Daily Dose data side-by-side. This will allow much easier comparison of these two sets of data.

--Table 2 was revised

3. In Table 4 the authors have stratified data according to serum 25D level (<20, >20, <30, >30). This stratification should be applied to the muscle function data. In other words, at week 4 the single dose group had many more women >30 ng/ml serum 25D but this did not have a dramatic effect on muscle function. Therefore, which group of women from the daily dosing saw the most improved muscle function - those < 20, those >20???

--Daily dose patients was divided into two groups. First group consist of patients achieved vitD level>20 while second group <20. Muscle function tests measured at 1st month were similar between groups (p>0.05). A statement of “Muscle function tests measured at 1st month in daily dose group were similar between patients who achieved 25OHvitD3 level of >20 IU/L and patients with lower than 20 IU/L” was added to results part.