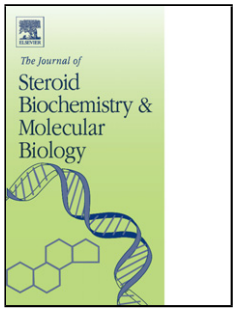

Title: Why vitamin D clinical trials should be based on 25-hydroxyvitamin D concentrations

Authors: William B. Grant, Barbara J. Boucher, Harjit P. Bhattoa, Henry Lahore

PII: S0960-0760(17)30223-6
DOI: http://dx.doi.org/10.1016/j.jsbmb.2017.08.009
Reference: SBMB 5009

To appear in: *Journal of Steroid Biochemistry & Molecular Biology*

Received date: 5-5-2017
Revised date: 10-7-2017
Accepted date: 17-8-2017

Please cite this article as: William B.Grant, Barbara J.Boucher, Harjit P.Bhattoa, Henry Lahore, Why vitamin D clinical trials should be based on 25-hydroxyvitamin D concentrations, Journal of Steroid Biochemistry and Molecular Biologyhttp://dx.doi.org/10.1016/j.jsbmb.2017.08.009

77d-JSBMB July 10, 2017

SBMB-D-17-00161

Why vitamin D clinical trials should be based on 25-hydroxyvitamin D concentrations

William B. Grant,[1] Barbara J. Boucher,[2] Harjit P. Bhattoa,[3] Henry Lahore[4]
[1]Sunlight, Nutrition, and Health Research Center
P.O. Box 641603
San Francisco, CA 94164-1603, USA
wbgrant@infionline.net
www.sunarc.org,

[2]Honorary Professor, Blizard Institute, Barts & The London School of Medicine & Dentistry, Queen Mary University of London, London UK. bboucher@doctors.org.uk,

[3]Department of Laboratory Medicine, Faculty of Medicine, University of Debrecen, Nagyerdei Blvd 98, Debrecen H-4032, Hungary. harjit@med.unideb.hu.

[4]2289 Highland Loop, Port Townsend, WA 98368. VitaminDWiki.com
hlahore@VitaminDWiki.com

Highlights

A revised approach for conducting vitamin D randomized controlled trials is presented.

The trails should be based on 25-hydroxyvitamin D concentrations.

Vitamin D doses should be adjusted for various 25-hydroxyvitamin D concentrations.

Abstract

Many health benefits are attributed to vitamin D, with those findings supported mostly by observational outcome studies of relationships to serum 25-hydroxyvitamin D [25(OH)D]. However, many randomized controlled trials (RCTs) aiming to confirm those findings have failed, perhaps because serum 25(OH)D is an index of UVB exposure and non–vitamin D mechanisms or because disease reduces serum 25(OH)D content. But the most likely reason for that failure is inappropriate design, conduct, analysis, and interpretation of RCTs. Most RCTs used principles designed to test pharmaceutical drugs; that design incorporates the assumptions that the RCT is the sole source of the agent and that dose–response relationships are linear. However, neither assumption is true for vitamin D, since neither vitamin D dose–responses or health outcome–serum 25(OH)D concentration relationships are linear—larger changes being induced with low rather than high baseline 25(OH)D values. Here, we propose a hybrid observational approach to vitamin D RCT design, based primarily on serum 25(OH)D concentration, requiring an understanding of serum 25(OH)D concentration–health outcome relationships, measuring baseline 25(OH)D values, recruiting non-replete subjects, measuring serum 25(OH)D during the trial for adjustment of supplemental doses for achievement of pretrial selection of target 25(OH)D values, where possible, and analyzing health outcomes in relation to those data rather than solely to vitamin D dosages.

Keywords: vitamin D; randomized controlled trial; clinical trial; 25-hydroxyvitamin D; observational study

Introduction

Many health benefits are attributed to higher 25-hydroxyvitamin D [25(OH)D] concentrations. Examples include reduced risk of musculoskeletal disorders, infectious diseases, autoimmune diseases, cardiovascular disease (CVD), diabetes mellitus, several types of cancer, neurocognitive dysfunction, adverse pregnancy and birth outcomes, and all-cause mortality rates [1], [2], [3]. Most such attributions are based on observational studies of health outcomes with respect to 25(OH)D concentrations, with others are based on geographical ecological studies with respect to solar UVB doses [4] or temporal geographical ecological studies [5]. Unfortunately, randomized controlled trials (RCTs) of vitamin D supplementation often fail to support those beneficial findings [6],[7],[8],[9],[10]. One reason for those discrepancies may be that UVB dosage in ecological studies and 25(OH)D concentrations in observational studies act as indexes for non–vitamin D-related mechanisms of solar UVB exposure, as seems to be the case for cardiovascular disease [11],[12]

Another reason for those discrepancies may be inappropriate RCT design, conduct, analyses and interpretation. Vitamin D RCTs are usually based on guidelines designed for pharmaceutical drugs—the underlying assumptions being that the RCT is the only source of the agent and that dose–response relationships are linear. But neither assumption is true for vitamin D RCTs since 25(OH)D concentrations have nonlinear relationships to both baseline 25(OH)D concentration and vitamin D dosages [13], whereas 25(OH)D concentration–health outcome relationships are also nonlinear (e.g., for breast cancer incidence) [14]. The conversion from

intact vitamin D (provided by oral intake or by UVB exposure), to 25(OH)D also varies [13],[15] (e.g., with genetic polymorphisms, intestinal absorption, and body mass). Genetic polymorphisms of CYP24A1 [16]. CYP2R1 and GC genes [17] play important roles, since vitamin D dose-25(OH)D concentration relationships vary from individual to individual [13],[18]. Thus, intact vitamin D having no direct health effects, vitamin D supplemental intake alone is an inadequate index for how vitamin D affects health outcomes.

Robert Heaney proposed guidelines for trials of nutrient effects [19]. Those guidelines include basing RCT design on 25(OH)D concentrations, not vitamin D dosing, which makes baseline and achieved 25(OH)D concentrations important in examining vitamin D–health outcomes, as discussed recently (e.g., for cancer incidence, where results from three vitamin D RCTs were modeled using baseline and achieved [in relation to baseline] 25(OH)D concentrations, vitamin D dosing, expected cancer incidence rates in the population, and number of participant years) [20].

The primary goal of vitamin D RCTs should be to determine relationships between 25(OH)D concentrations achieved with appropriate adjustment of supplementation over time and health outcomes. A secondary goal is to determine whether any adverse effects are associated with increased vitamin D status upon supplementation.

In this paper, therefore, we propose that vitamin D RCTs should be designed, conducted, analyzed, and interpreted based on changes in 25(OH)D concentrations, the role of vitamin D dosing being solely to achieve the targets set for achieved 25(OH)D concentrations.

The approach we propose for vitamin D RCTs is based on 25(OH)D concentrations, so that health outcome relationships to baseline and achieved 25(OH)D concentrations can be determined. In general, expected relationships have been identified by referring to results from

observational studies, including available meta-analyses. As an example, data from a meta-analysis of breast cancer incidence risk in relation to 25(OH)D concentration at diagnosis [14] was used to predict all-cancer incidence, using a geographical ecological study showing that breast cancer and all-cancer mortality rates had similar relations to solar UVB dose [21],[20]. Then, from that relationship, and the average rate of the chosen health outcome in the population, power calculations were made to determine the number of participant-years required to be likely to detect significant relationships between 25(OH)D concentration and health outcomes, an approach incorporating the assumption that vitamin D status will be always be raised to target 25(OH)D concentrations, and using traditional power calculations (e.g., [22]). However, in [20], we developed a different approach to power calculation, based on population distributions of 25(OH)D concentrations, where numbers of cancer cases predicted were used to calculate participant-years necessary for the likely achievement of significant risk reduction through supplementation to target 'achieved' 25(OH)D values; which were noted to be higher for higher baseline 25(OH)D concentrations. Thus, there would be a tradeoff between the effort required to enroll people with low 25(OH)D concentrations and that required to manage larger numbers of participants for longer periods.

In support of this approach to RCT design, previous vitamin D RCTs have clearly been shown to be more likely to find significant benefits of vitamin D supplementation when baseline 25(OH)D concentrations were relatively low, as for cancer incidence [23], respiratory tract infections [24], and blood pressure [25].

A key element of the proposed approach to conducting vitamin D supplementation trials is checking 25(OH)D concentrations periodically during the trial, as well as at baseline, since prospective observational studies with long follow-up periods have shown reduced benefits of

supplementation with higher basal 25(OH)D concentrations, in comparison with case–control studies where 25(OH)D concentration was measured near the time of diagnosis, as is predictable since 25(OH)D concentration changes over time as a result of seasonal and lifestyle variation, and with public and health care views on vitamin D [26]—as has been shown for cancer [14] and all-cause mortality rate [27]. Such variations would be expected in vitamin D RCTs, though their magnitude in the treatment arms should be attenuated. Furthermore, trial compliance is never 100%, and periodically checking serum 25(OH)D concentrations through such RCTs would allow adjustment of supplemental dosages in order to achieve desired target values, as well as yielding data on compliance.

Periodic measurement of 25(OH)D concentration during vitamin D RCTs can be arranged in several ways. Immunoassays are inexpensive and can be run in the laboratories of the researchers conducting the trials. For large-scale trials, where it is difficult to see all participants, blood spot assays are inexpensive and convenient because they can be organized by mail; e.g. Heartland Assays LLC (Ames, IA) offers blood spot 25(OH)D assays using liquid chromatography–tandem mass spectrometry methodology – (interassay coefficient of variation 4.0%; intra-assay coefficient of variation <2.5%; A.J. Makowski, Heartland Assays, LLC, personal communication, Jan. 30, 2017) [20]. Thus, 25(OH)D assessments could be included in RCT planning, as budgets permit, across seasons, and near times of diagnosis of any adverse health outcome. Analyses of health outcome risks with 25(OH)D concentrations (basal, achieved, and over various intervals of time before diagnosis) could then be computed, and the question as to whether disease development reduces 25(OH)D concentrations could also be clarified. As we suggested earlier, “Using basal and achieved vitamin D status [25(OH)D data] in RCT design might appear to make such studies ‘observational’, but the proposed design strategy

simply targets supplementation to chosen baseline status, whilst ensuring achievement of the desired 'target' vitamin D status; thus, such RCTs would clearly continue to be interventional, but with an increased potential for detecting causality." [28]

Proposed approach

1. Obtain data on achieved 25(OH)D concentration for vitamin D supplementation by baseline 25(OH)D for the population of interest [13].
2. Obtain data on known population distributions of serum 25(OH)D concentrations, e.g., for Canadians aged 50–79 yrs [29].
3. Calculate expected achieved 25(OH)D concentration as a function of estimated baseline 25(OH)D concentrations of the population studied, for the planned vitamin D starting doses.
4. Obtain data for relevant observational 25(OH)D concentration–health outcome relationships for the outcomes of interest or related outcomes, e.g., from earlier meta-analyses.
5. Obtain the expected incidence of the disease of interest from the average incidence rates for the age distribution of the population to be studied, data for their baseline 25(OH)D concentration distribution, and the predicted increases in serum 25(OH)D from different amounts of vitamin D supplementation reported in the literature.
6. Calculate the power of the planned trial by using two approaches:(a) using the number of participants and incident cases required to achieve significant reduction of relative risk (RR) (p<0.05) using, e.g., http://www.vassarstats.net/odds2x2.html, adding suitable numbers of participants to cover poor compliance and dropouts; and estimate trial

duration from expected lag times for achieving repletion, and for the time taken for disease development; in addition, (b) calculate the "power" (total numbers needed to treat) by using the expression for standardized difference (St Diff) for binary variables: St Diff = $(P_1–P_2)/ \sqrt{[P_0(1 – P_0)]}$,where P_0 is the estimate of prevalence (proportion)in the total study population [= $(P_1+P_2)/2$], and P_1 and P_2 are the proportional incidence, seen or predicted, (i.e., the fraction of participants expected to develop the health outcome of interest) for the control [P_1] and treatment arms [P_2] of the study. Then, use the calculated St Diff to estimate the number of participants (or the person-years) required (total) for desired "power" and *p*-value (e.g., power 80% [0.8]; p=0.05, e.g. by using the Altman nomogram [22]), noting that numbers per arm = Total numbers/2.

7. Where possible, compare the estimates with those already reported in risk reducing vitamin D RCTs, e.g., for cancer [20].

Examples of analyzing trial results based on 25(OH)D concentrations from the literature

Two vitamin D supplementation RCTs were conducted with pregnant women in South Carolina (ClinicalTrials.gov #NCT00292591 and #NCT00412087).When those results were reanalyzed to look at maternal 25(OH)D concentrations within 6 weeks of birth, rather than by vitamin D supplementation, preterm births decreased steadily as 25(OH)D concentration increased [30]. The gestational week at birth varied from 37.3 weeks for 25(OH)D concentration of 8 ng/mL to 38.9 weeks at 40 ng/mL, with no significant change above 40 ng/mL, whereas raising the 25(OH)D concentration from 20 to 40 ng/mL with supplementation reduced preterm birth risk by 59%. However, the original statistical analysis of those data sets, based solely on

"intention to treat" for vitamin D supplementation, showed no significant reductions in risk [31],[32].

A vitamin D RCT of 4400 versus 400 IU/d of vitamin D_3 was conducted on pregnant women with a high risk of atopic disease in their family, at three centers in the U.S. (ClinicalTrials.gov NCT00920621). The women were supplemented from gestational weeks 10–18, under the hypothesis that a higher dose of supplemental vitamin D_3 would reduce the risk of developing preeclampsia. However, no difference in preeclampsia risk was found by "intention-to-treat"; but the risk of preeclampsia was strongly predicted by baseline serum 25(OH)D concentration, dropping from 11% for values of ~10 ng/mL to <2% for values of ~70 ng/mL [33],[34] another good example of how traditional vitamin D RCT approaches, looking only at vitamin D dosing, cannot be considered reliable in the provision of data suitable for making public health policy decisions.

A recent report of results from a vitamin D plus calcium supplementation RCT reported near-significant reductions in cancer risks based on intention to treat (p = 0.06); [35]. However, the authors noted that "the achieved 25(OH)D level was significantly associated, inversely, with cancer incidence (p=0.03, coeff=–0.02). Compared with 25(OH)D concentrations of 30 ng/mL at baseline, the estimated HR for cancer incidence for basal 25(OH)D concentrations between 30 ng/mL and 55 ng/mL was 0.65 (95% CI, 0.44 to 0.97) (eFigure 2 in Supplement 2." [35].

Furthermore, with these points in mind, if vitamin D dose were titrated so as to achieve certain specified target 25OHD concentrations after determination of baseline concentrations and rechecking concentrations after a few months of dosing, then the study would be a clinical trial of health outcomes with respect to achieved 25OHD concentration. For example, in reporting the recent Lappe clinical trial, the JAMA editors disallowed reporting of

findings in relation to 25OHD concentrations because they had not been specified in the trial description submitted to the registry of clinical trials. Thus, when the RCT design is specified as being based on outcomes related to 25OHD concentrations achieved using oral supplementation (rather than by UV exposure), it can be used to study the effects of vitamin D supplementation.

Additional points to consider in designing, conducting, analyzing, and interpreting RCTs of vitamin D

1. Seek participants with low baseline serum 25(OH)D values.
2. Use vitamin D_3, not vitamin D_2 [36] and at sufficiently high doses, 1000–4000 IU/d.
3. Consider giving a modest loading dose of vitamin D_3 to reach target 25(OH)D concentrations rapidly [37]; [38].
4. Measure baseline serum 25(OH)D concentrations and repeat at suitable intervals to assess compliance and the achievement of target 25(OH)D values (e.g., using blood spot assays, where acceptable, affordable, convenient, and sufficiently accurate).
5. If calcium and magnesium are given, give them in both RCT arms [20].
6. Monitor participants' UVB exposure [39], as well as dietary and supplemental intakes of vitamin D_3 and potential confounders, including obesity and genetic variants.
7. Allow for the natural history of disease development in planning RCT duration and dosing, and for subject age.
8. Analyze results in terms of 25(OH)D values at baseline, at completion, and at intervals before disease diagnosis, rather than solely with vitamin D_3 dose.

9. Carefully consider vitamin D_3 dosing interval with respect to compliance and physiological effect [40],[41],[42].
10. If, for ethical reasons, participants in the control are given 400 IU/d vitamin D_3, the resulting increase in 25(OH)D concentration should be factored into the selection of participants and into outcome analyses.

Conclusion

Hopefully, the hybrid approach for vitamin D clinical trials outlined here, involving vitamin D supplementation but based primarily on 25(OH)D concentrations, will improve the ability of such trials to assess more accurately how long term 25(OH)D concentrations affect health outcomes,

The 20th Workshop on Vitamin D (Orlando March 28-31, 2017), held a constructive debate on observational studies [43] vs. RCTs [44] as the most appropriate way to demonstrate roles of vitamin D in health, concluding that RCTs are, in principle, more reliable indicators of the effectiveness of vitamin D, but are more difficult to conduct properly. We hope, therefore, that the design modifications we suggest can contribute to improving the value of vitamin D RCTs to the point where they can provide definitive answers.

Disclosure

WBG receives funding from Bio-Tech Pharmacal, Inc. (Fayetteville, AR), and has received funding recently from the Vitamin D Society (Woodstock, ON, Canada) and the Vitamin D Council (San Luis Obispo, CA). The other authors have no conflicts of interest to report.

References (PLOS format)

1. Hossein-nezhad A, Holick MF (2013) Vitamin D for health: a global perspective. Mayo Clin Proc 88: 720-755.
2. Pludowski P, Holick MF, Pilz S, Wagner CL, Hollis BW, et al. (2013) Vitamin D effects on musculoskeletal health, immunity, autoimmunity, cardiovascular disease, cancer, fertility, pregnancy, dementia and mortality-a review of recent evidence. Autoimmun Rev 12: 976-989.
3. Garland CF, Kim JJ, Mohr SB, Gorham ED, Grant WB, et al. (2014) Meta-analysis of All-Cause Mortality According to Serum 25-Hydroxyvitamin D. Am J Public Health 104: e43-50.
4. Grant WB (2016) The role of geographical ecological studies in identifying diseases linked to UVB exposure and/or vitamin D. Dermatoendocrinol 8: e1137400.
5. Cannell JJ, Vieth R, Umhau JC, Holick MF, Grant WB, et al. (2006) Epidemic influenza and vitamin D. Epidemiol Infect 134: 1129-1140.
6. Autier P, Boniol M, Pizot C, Mullie P (2014) Vitamin D status and ill health: a systematic review. Lancet Diabetes Endocrinol 2: 76-89.
7. Bolland MJ, Grey A, Gamble GD, Reid IR (2014) The effect of vitamin D supplementation on skeletal, vascular, or cancer outcomes: a trial sequential meta-analysis. Lancet Diabetes Endocrinol 2: 307-320.
8. Cianferotti L, Bertoldo F, Bischoff-Ferrari HA, Bruyere O, Cooper C, et al. (2017) Vitamin D supplementation in the prevention and management of major chronic diseases not related to mineral homeostasis in adults: research for evidence and a scientific statement from the European society for clinical and economic aspects of osteoporosis and osteoarthritis (ESCEO). Endocrine 56: 245-261.
9. Veloudi P, Jones G, Sharman JE (2017) Effectiveness of Vitamin D Supplementation for Cardiovascular Health Outcomes. Pulse (Basel) 4: 193-207.
10. Rejnmark L, Bislev LS, Cashman KD, Eiriksdottir G, Gaksch M, et al. (2017) Non-skeletal health effects of vitamin D supplementation: A systematic review on findings from meta-analyses summarizing trial data. PLoS One 12: e0180512.
11. Brondum-Jacobsen P, Benn M, Afzal S, Nordestgaard BG (2015) No evidence that genetically reduced 25-hydroxyvitamin D is associated with increased risk of ischaemic heart disease or myocardial infarction: a Mendelian randomization study. Int J Epidemiol 44: 651-661.
12. Al Mheid I, Quyyumi AA (2017) Vitamin D and Cardiovascular Disease: Controversy Unresolved. J Am Coll Cardiol 70: 89-100.
13. Garland CF, French CB, Baggerly LL, Heaney RP (2011) Vitamin D supplement doses and serum 25-hydroxyvitamin D in the range associated with cancer prevention. Anticancer Res 31: 607-611.
14. Grant WB (2015) 25-Hydroxyvitamin D and Breast Cancer, Colorectal Cancer, and Colorectal Adenomas: Case-Control versus Nested Case-Control Studies. Anticancer Res 35: 1153-1160.
15. Datta P, Philipsen PA, Olsen P, Petersen B, Johansen P, et al. (2016) Major inter-personal variation in the increase and maximal level of 25-hydroxy vitamin D induced by UVB. Photochem Photobiol Sci 15: 536-545.

16. Hibler EA, Klimentidis YC, Jurutka PW, Kohler LN, Lance P, et al. (2015) CYP24A1 and CYP27B1 Polymorphisms, Concentrations of Vitamin D Metabolites, and Odds of Colorectal Adenoma Recurrence. Nutr Cancer 67: 1131-1141.
17. Zhang M, Zhao LJ, Zhou Y, Badr R, Watson P, et al. (2017) SNP rs11185644 of RXRA gene is identified for dose-response variability to vitamin D3 supplementation: a randomized clinical trial. Sci Rep 7: 40593.
18. Carlberg C, Haq A (2016) The concept of the personal vitamin D response index. J Steroid Biochem Mol Biol.
19. Heaney RP (2014) Guidelines for optimizing design and analysis of clinical studies of nutrient effects. Nutr Rev 72: 48-54.
20. Grant WB, Boucher BJ (2017) Randomized controlled trials of vitamin D and cancer incidence: A modeling study. . PLoS One.
21. Grant WB, Garland CF (2006) The association of solar ultraviolet B (UVB) with reducing risk of cancer: multifactorial ecologic analysis of geographic variation in age-adjusted cancer mortality rates. Anticancer Res 26: 2687-2699.
22. Jones SR, Carley S, Harrison M (2003) An introduction to power and sample size estimation. Emerg Med J 20: 453-458.
23. Bolland MJ, Grey A, Gamble GD, Reid IR (2011) Calcium and vitamin D supplements and health outcomes: a reanalysis of the Women's Health Initiative (WHI) limited-access data set. Am J Clin Nutr 94: 1144-1149.
24. Martineau AR, Jolliffe DA, Hooper RL, Greenberg L, Aloia JF, et al. (2017) Vitamin D supplementation to prevent acute respiratory tract infections: systematic review and meta-analysis of individual participant data. BMJ 356: i6583.
25. Mozaffari-Khosravi H, Loloei S, Mirjalili MR, Barzegar K (2015) The effect of vitamin D supplementation on blood pressure in patients with elevated blood pressure and vitamin D deficiency: a randomized, double-blind, placebo-controlled trial. Blood Press Monit 20: 83-91.
26. Kroll MH, Bi C, Garber CC, Kaufman HW, Liu D, et al. (2015) Temporal relationship between vitamin D status and parathyroid hormone in the United States. PLoS One 10: e0118108.
27. Grant WB (2012) Effect of follow-up time on the relation between prediagnostic serum 25-hydroxyvitamin D and all-cause mortality rate. Dermatoendocrinol 4: 198-202.
28. Grant WB, Boucher BJ (2017) Randomized controlled trials of vitamin D and cancer incidence: A modeling study. PLoS One 12: e0176448.
29. Grant WB, Whiting SJ, Schwalfenberg GK, Genuis SJ, Kimball SM (2016) Estimated economic benefit of increasing 25-hydroxyvitamin D concentrations of Canadians to or above 100 nmol/L. Dermatoendocrinol 8: e1248324.
30. Wagner CL, Baggerly C, McDonnell S, Baggerly KA, French CB, et al. (2016) Post-hoc analysis of vitamin D status and reduced risk of preterm birth in two vitamin D pregnancy cohorts compared with South Carolina March of Dimes 2009-2011 rates. J Steroid Biochem Mol Biol 155: 245-251.
31. Wagner CL, McNeil R, Hamilton SA, Winkler J, Rodriguez Cook C, et al. (2013) A randomized trial of vitamin D supplementation in 2 community health center networks in South Carolina. Am J Obstet Gynecol 208: 137 e131-113.

32. Wagner CL, McNeil RB, Johnson DD, Hulsey TC, Ebeling M, et al. (2013) Health characteristics and outcomes of two randomized vitamin D supplementation trials during pregnancy: a combined analysis. J Steroid Biochem Mol Biol 136: 313-320.
33. Wolsk HM, Harshfield BJ, Laranjo N, Carey VJ, O'Connor G, et al. (2017) Vitamin D supplementation in pregnancy, prenatal 25(OH)D levels, race, and subsequent asthma or recurrent wheeze in offspring: Secondary analyses from the Vitamin D Antenatal Asthma Reduction Trial. J Allergy Clin Immunol.
34. Mirzakhani H, Litonjua AA, McElrath TF, O'Connor G, Lee-Parritz A, et al. (2016) Early pregnancy vitamin D status and risk of preeclampsia. J Clin Invest 126: 4702-4715.
35. Lappe J, Watson P, Travers-Gustafson D, Recker R, Garland C, et al. (2017) Effect of Vitamin D and Calcium Supplementation on Cancer Incidence in Older Women: A Randomized Clinical Trial. JAMA 317: 1234-1243.
36. Tripkovic L, Wilson LR, Hart K, Johnsen S, de Lusignan S, et al. (2017) Daily supplementation with 15 mug vitamin D2 compared with vitamin D3 to increase wintertime 25-hydroxyvitamin D status in healthy South Asian and white European women: a 12-wk randomized, placebo-controlled food-fortification trial. Am J Clin Nutr.
37. Heaney RP, Davies KM, Chen TC, Holick MF, Barger-Lux MJ (2003) Human serum 25-hydroxycholecalciferol response to extended oral dosing with cholecalciferol. Am J Clin Nutr 77: 204-210.
38. McNally JD, Iliriani K, Pojsupap S, Sampson M, O'Hearn K, et al. (2015) Rapid normalization of vitamin D levels: a meta-analysis. Pediatrics 135: e152-166.
39. King L, Xiang F, Swaminathan A, Dear K, Harrison SL, et al. (2017) Validation of Sun Exposure Reported Annually Against Interim Self-report and Daily Sun Diaries. Photochem Photobiol.
40. Hollis BW, Wagner CL (2013) Clinical review: The role of the parent compound vitamin D with respect to metabolism and function: Why clinical dose intervals can affect clinical outcomes. J Clin Endocrinol Metab 98: 4619-4628.
41. Bouillon R (2017) Optimal vitamin D supplementation strategies. Endocrine 56: 225-226.
42. Dalle Carbonare L, Valenti MT, Del Forno F, Caneva E, Pietrobelli A (2017) Vitamin D: Daily vs. Monthly Use in Children and Elderly-What Is Going On? Nutrients 9.
43. Scragg R (2017) Limitations of vitamin D supplementation trials: Why observational studies will continue to help determine the role of vitamin D in health. J Steroid Biochem Mol Biol.
44. Jorde R (2017) RCTS are the only appropriate way to demonstrate the role of vitamin D in health. J Steroid Biochem Mol Biol.