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COMMENTARY

A pooled analysis of Vitamin D dose requirements for fracture prevention

Bo Abrahamsen[1,2], Alison Avenell[3], Mark Bolland[4], Lars Rejnmark[5], Graeme S MacLennan[3], Helen M Macdonald[6] and Andrew Grey[4]

[1]Odense Patient Data Exploratory Network (OPEN), Institute of Clinical Research, University of Southern Denmark, Odense, Denmark. [2]Department of Medicine F, Gentofte Hospital, Copenhagen, Denmark. [3]Health Services Research Unit, University of Aberdeen, Aberdeen, UK. [4]Bone and Joint Research Group, Department of Medicine, University of Auckland, Auckland, New Zealand. [5]Aarhus University, Department of Endocrinology and Internal Medicine, Aarhus University Hospital THG, Aarhus, Denmark. [6]Musculoskeletal Research, Health Sciences Building, University of Aberdeen, Aberdeen, UK.

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Uncertainty exists as to whether vitamin D given alone reduces fracture. Certainly, considerable controversy surrounds the optimum vitamin D serum level, dose for fracture prevention, and requirement for supplemental calcium,[1] with the number of meta-analyses exceeding the number of primary trials. Although studies of vitamin D given with calcium have sometimes shown a modestly reduced fracture risk, studies using vitamin D alone have generally failed.[2–4] However, a recent report by Bischoff-Ferrari *et al.*[5] in the *New England Journal of Medicine* using pooled individual patient data from 11 prior clinical trials concludes that vitamin D alone at a dose of 800 IU or more daily may reduce the risk of hip and non-vertebral fractures in persons aged $\geqslant$65. Unfortunately, this conclusion is too optimistic for the data, because of important deficiencies in trial selection and analysis.

Trial Selection

Eligible studies were randomized trials of oral vitamin D $\pm$ calcium in participants $>$65 years, with a control group that received placebo $\pm$ calcium, and reported data on low-trauma fractures. A *PRISMA* (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) statement was not published. Although 14 studies were identified, an eligible study was not included.[6] Others that did not publish fracture data in the primary manuscript[7,8] were not included even though data were available.[2] Unpublished data were not sought from another study,[9] possibly because participants were allowed prescription of HRT or bisphosphonates by their family physicians—however, use of HRT was reported by the study authors to be balanced across the treatment groups.

Studies of vitamin D were excluded because of intramuscular administration,[10] pragmatic study design without placebo medication[11–16] and because participants were $<$65 years of age.[17] As the intention of the analysis was to estimate the effects of vitamin D according to actual intake rather than assigned dose, the rationale for these exclusion criteria is unclear. Among the included trials, the arbitrary age criterion only affected the WHI (Women's Health Initiative) trial,[18] which is very influential because of its large size. Applying it not only violates randomization in WHI, but is likely to have biased the overall findings in favour of vitamin D, because participants in WHI $<$60 years at baseline randomized to active treatment had an elevated hip fracture risk (HR 2.17; 95% CI 1.13–4.18).[18] Lastly, a study of intermittent (annual) high-dose oral vitamin D[19] was excluded from primary analyses because 'it used a different treatment regimen', whereas studies of intermittent (four-monthly) high-dose oral vitamin D were included.[20,21] This bias results in favour of vitamin D, as shown by sensitivity analyses that were included as supplementary material.

Thus, the included studies represent a selective subset of available data, the final pooled dataset is biased in favour of vitamin D, and the balancing effects of randomization were compromised.

Calcium Coadministration

It is surprising that, in an analysis aimed at investigating the effects of vitamin D on fractures, a majority of participants were randomized to vitamin D plus calcium (CaD). This is important, because previous meta-analyses of the same trials reported that CaD marginally reduces fracture risk, vitamin D alone is

without effect, and the efficacy of CaD is similar to that of calcium alone,[2–4] suggesting that the active component of CaD in fracture prevention is calcium.

In the Bischoff-Ferrari analysis,[5] the two trials with most impact in the highest quartile of the actual intake of vitamin D supplement assessed CaD.[18,22] It is therefore inappropriate to attribute treatment effects to the received vitamin D dose. The most influential of all the trials is that by Chapuy *et al.*,[22] which studied very elderly, frail, institutionalized women, with marked vitamin D deficiency and a very high mortality rate (16% by 18 months). The prevalence of osteomalacia in this study population will have been very high as the serum 25OHD levels reported were found to be too high.[23] This study reported the largest fracture risk reduction among CaD trials. Its results largely account for the modest reduction in fracture risk in previous CaD meta-analyses,[2,24] but they have not been replicated in CaD trials in community-dwelling populations.[14,16,18,25] It is therefore unwise to generalize its findings to other populations.

Thus, because of the characteristics of the included trials, the actual-intake results are neither attributable to vitamin D nor applicable to community-dwelling populations.

Approach to Analysis

An intention-to-treat (ITT) analysis includes all participants analysed in the groups they were randomised to, regardless of whether they adhered to trial treatment or not. An ITT analysis answers an effectiveness question, 'Does this treatment work in routine practice?' The ITT analyses conducted by Bischoff-Ferrari *et al.*[5] demonstrated non-significant 10% and small but significant 7% reductions in risk of hip and non-vertebral fractures, respectively. Failure to adhere to treatment within a randomized controlled trial (RCT) can lead researchers to ask the question, 'What was the effect of the treatment in those that took it?' Mistakenly, Bischoff-Ferrari *et al.*[5] believe the most appropriate method to use to answer this question was to conduct a per-protocol analysis, which reclassified participants into vitamin D dose quartiles according to treatment adherence, a post-randomization variable. The immediate problem with per-protocol analyses is that the comparison of treatments is no longer one of randomised groups with baseline comparability but rather one of self-selecting groups, which introduces selection bias and therefore biased estimates of treatment effects. Furthermore, participants that stop taking trial treatments are often those that are of poorest health and are most likely to experience the trial outcome. Results from a per-protocol analysis, even in the unlikely event of being unbiased, are not generalizable to routine practice. An additional 'internal validation' analysis conducted by Bischoff-Ferrari *et al.*[5] is nothing more than an 'as-treated' analysis. In an as-treated analysis, all participants within a RCT are reclassified into treatments groups based on the treatment received, regardless of treatment allocation. Here, the comparison of treatments is no longer protected by randomization. Comparing treatments this way introduces further bias.

For these reasons, the recommended approach for analysing treatment benefits is by ITT. Unsurprisingly, there is evidence of selection bias amongst the treatment-group quartiles constructed by Bischoff-Ferrari *et al.*,[5] who themselves acknowledge that the likelihood of being in a particular quartile group depends on age group, gender and residential institution status at baseline. There is a wide variation in supplemental calcium between quartiles of vitamin D treatment (**Table 1**). The average calcium supplement intake for lowest quartile of vitamin D dose is 396 mg versus 830 mg in the highest vitamin D dose quartile. Similar differences between quartiles exist for institutional status and age (**Table 1**). A comparison between any quartile group and the complete control group is therefore, we believe, an unfair one, adjusted for covariates or not. This cannot be assessed in any meaningful way, as the full results of the models used were not presented, nor is it clear if there was a prespecified analysis plan.

In conclusion, the analysis methods used were suboptimal and in effect reduced the evidence obtained from RCTs to that of observational research. Such analyses needed to be handled with care and appropriate methods are outlined by Emsley *et al.*[26]

Adherence to Trial Supplements

There are difficulties reconciling the adherence data presented in appendix 1 of the published paper with those published originally by the trial authors. Meyer *et al.*[27] reported that 203/569 (36%) of the surviving vitamin D group ceased treatment, but figures in the present Appendix 1 indicate that compliance was 95% for all people randomised.[5] Further, in the 2010 Bischoff-Ferrari trial,[28] 13/86 dropped out (15%), yet compliance was reported as 97%. In the Chapuy study,[22] 17% of subjects had $<70\%$ compliance in the original report, but the present analysis used a figure of 100% being compliant. As patient level data was accessed, it is possible that the analysis corrected errors made in the original papers, but the apparent discrepancies are not explained in the report. Finally, for each of

Table 1 Calcium and vitamin D dose, age, residential status and risk of fractures

Daily supplemental Vitamin D dose	*Daily supplemental calcium dose (mg)*	*Age ⩾85 years (%)*	*Institutionalised (%)*	*Hip fracture RR and 95% CI from Figure 2*	*All nonvertebral fractures RR and 95% CI*
Top quartile (792–1000 IU)	830 ± 460	28% (8.7%)[a]	47	0.72 (0.59–0.89)	0.88 (0.74–1.04)
Third quartile (638–791 IU)	403 ± 436	27.1	52	1.01 (0.82–1.23)	0.89 (0.80–1.01)
Second quartile (361–637 IU)	697 ± 282	5.8	9.9	1.16 (0.76–1.77)	1.12 (0.90–1.40)
Lowest quartile (0–360 IU)	396 ± 393	9.0	14.6	1.13 (0.77–1.67)	0.99 (0.85–1.11)
Controls	84 ± 258	18% (12.8%)[a]	30.7	Reference	Reference

Data extracted from Table 1 and Figure 2, Bischoff-Ferrari *et al*.[5]

[a]The proportions reported (shown in brackets) by Bischoff-Ferrari *et al*.[5] incorrectly classified participants from Chapuy *et al*.[22] All participants in this study were ascribed the mean age of the participants (84 years), therefore grossly underestimating the proportions ⩾85 years. For this table, we assumed that 800 participants in both treatment groups were ⩾85 years.

the four studies with only trial level data, the analysis assumed the same average adherence to trial supplements—the validity of this assumption cannot be tested but would seem unlikely to be correct. Furthermore, the authors added the intake of off-study supplements to that of trial supplements. It is also not clear how compliance to off-protocol supplements was addressed. It may be that compliance was assumed to be the same as for trial supplements. It is not made explicit if the analysis took into account dietary calcium or exposure to vitamin D from other sources, which would have been influenced by diet, food fortification and, in the case of vitamin D, sunlight exposure. In dealing with the off protocol supplements, it is important that there is consistency in the approach; however, it is not clear from the paper whether the control group was treated the same way as the treated group, and whether calcium-containing supplements were included in addition to vitamin D-containing supplements

Other Concerns

The analyses by Bischoff-Ferrari *et al.*[5] have the potential to harm, by diverting resources and inflating the benefits of vitamin D supplementation. Physicians and their patients may assume that simply taking a vitamin D supplement will reduce fracture risk, and may therefore be less inclined to embark on interventions that have proven efficacy. It should be emphasized that vitamin D with or without calcium supplements failed to significantly prevent fractures in many well-designed pragmatic RCTs that provide a 'real world' assessment of their efficacy in the community[10,14–16,18,19,21,25,27,29] Another undesirable consequence of an undue emphasis on vitamin D for fracture prevention lies in the potential overuse of serum 25OH-vitamin D measurements in the general population, as this is costly and there is continued concern regarding the wide variation in measurements between different laboratories.[30] Vitamin D assays have changed substantially over time and this is a major limitation to pooling serum values over decades as is done in their analysis.[31] The problem with assays does not appear to be abating, in spite of the recommendation that the NIST (National Institute of Standards and Technology) standard be used; and the issue of interpreting the results in the light of seasonal variation.[32] It is clear that those who are below appropriate cutoffs levels only in winter will be at less risk of true deficiency compared with those whose measurements are low in summer and autumn.

This recent analysis used suboptimal methods that violate the principle of ITT, compromise randomization and reduce the level of evidence from RCTs to that of observational cohorts by attempting to adjust for post-randomization non-random characteristics such as adherence and own-choice use of off-protocol supplements. Further, the handling of adherence data is not transparent and the included studies represent only a selected subset of those available. Taken together, the methods used have a high likelihood of having misrepresented the benefits of vitamin D, which does not help clinicians or patients to make informed choices when considering treatment options. We suggest that unless robust evidence becomes available that indicates antifracture efficacy of vitamin D in community-dwelling populations,patients at high risk of fracture should be advised instead to consider interventions with proven efficacy. More research is needed on what threshold defines vitamin D deficiency, being the 25-hydroxy vitamin D level below which meaningful clinical benefits arise from vitamin D supplementation.

Conflict of Interest

BA, AA and LR were authors of the DIPART vitamin D analysis. Other disclosures: BA serves on advisory boards for Nycomed and Amgen, has received grant or research support from Novartis, Nycomed, Amgen, and Merck and speaker fees from Nycomed, Merck, Eli Lilly, and Amgen. LR: speakers' fees from Bristol-Myers Squibb and Eli Lilly. All other authors declare no conflict of interest.

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