A systematic literature review and meta-analysis of the effectiveness of vitamin D supplementation in maintaining or restoring vitamin D levels in Duchenne muscular dystrophy

Jing Guo[1] and Karen Anthony[2]

[1]Nutritional Epidemiology Group, School of Food Science and Nutrition, University of Leeds, Leeds, LS2 9JT, United Kingdom.

[2]Centre for Physical Activity and Life Sciences, University of Northampton, University Drive, Northampton, NN1 5PH, United Kingdom.

Corresponding author: Karen Anthony (karen.anthony@northampton.ac.uk), University of Northampton, University Drive, Northampton, NN1 5PH, United Kingdom. Tel ++44 1604 89 3467; Fax: 01604 720636.

Declarations of interest: none.

Abstract

We conducted a systematic literature review and meta-analysis on the effectiveness of vitamin D supplementation in maintaining or restoring vitamin D levels in Duchenne muscular dystrophy. Due to a lack of randomised controlled trials, cross-sectional and retrospective and prospective cohort studies were taken as the best available evidence. Inclusion criteria included reporting mean serum vitamin D levels in a supplement-taking group. After screening 102 records; 13 were included in a narrative synthesis and eight of these in a meta-analysis. We show that current dosing regimens are preventing severe deficiency but are not effective at maintaining sufficient vitamin D levels within the Duchenne population. Despite high levels of daily vitamin D supplementation (>1000 International Units), at least 20% of people with Duchenne remain vitamin D deficient. No significant association between dose and serum vitamin D levels was found ($r^2 = 0.3$, $p = 0.237$). A meta-analysis of mean serum vitamin D levels across eight studies also revealed substantial variability in response to vitamin D supplementation and high heterogeneity ($I^2 = 99.59$ %). These data could impact on an individual's risk and severity of osteoporosis and vertebral fractures.

Keywords:

- Duchenne muscular dystrophy
- Vitamin D
- 25-Hydroxyvitamin D
- 25(OH)D
- Calcifediol
- Nutrition

1. Introduction

Duchenne muscular dystrophy (DMD) is a life-limiting genetic disease that primarily affects boys, characterized by progressive muscle weakness and wasting. Life expectancy for people with DMD is improving, it is now common for individuals to live into their 30's[1–4]. The disease is caused by mutations in the *DMD* gene, which codes for a protein called dystrophin that is essential for maintaining the structural integrity of muscle fibres [5]. Established treatments focus on managing the symptoms of the disease, such as corticosteroids and physical therapy, and delaying the progression of muscle weakness to improve quality of life. More recently, dystrophin restorative therapies have emerged, however clinical studies are ongoing to determine their long-term effectiveness in improving muscle function [6]. Individuals with DMD are at an increased risk of bone fractures and osteoporosis, likely due to corticosteroid therapy and/or the progressive muscle wasting and decreased physical activity associated with the disease.

Vitamin D is a fat-soluble vitamin that promotes the absorption of calcium from the gastrointestinal system allowing mineralisation of the bone and optimal skeletal muscle function; it is crucial for maintaining a healthy bone density. There are two main dietary forms of vitamin D: vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol). Vitamin D2 is primarily derived from plant sources, such as mushrooms, while vitamin D3 is synthesised by the skin upon exposure to ultraviolet (UV) B radiation from sunlight and can also be obtained from some animal-derived foods, such as fatty fish and egg yolks. Once in the body, both forms of vitamin D undergo metabolic processes in the liver and kidney to produce the biologically active form of vitamin D, 1,25-dihydroxyvitamin D (calcitriol) [7]. However, the most measured marker of vitamin D status is 25-hydroxyvitamin D, 25-(OH)D (calcifediol), which reflects the combined levels of vitamin D2 and D3 in the body. This metabolite is used

as a proxy for vitamin D status because it has a longer half-life in the blood than the active form of vitamin D and is more stable than other metabolites of vitamin D [7].

In 2016, The UK Scientific Advisory Committee on Nutrition (SACN) set a Reference Nutrient Intake of 10 µg (400 International Units, IU) of vitamin D per day for the general population (≥4 years of age) to achieve a serum 25-(OH)D status above 25 nmol/l, however, the vitamin D dietary requirement for vulnerable sub-groups, including Duchenne muscular dystrophy (DMD) are unclear [8]. Insufficiency or deficiency of vitamin D is prevalent in individuals with DMD and is thought to be exacerbated by prolonged glucocorticoid therapy [9,10]. Corticosteroids are a part of the accepted standard of care and early, daily, treatment (5 - 6 years of age) can delay the loss of ambulation to age 15 years and above [11]. However, unwanted effects include altering vitamin D metabolism, a decrease in osteoblast function and an even greater risk of osteoporosis [12]. There is a lack of consensus and evidence on the use of nutritional supplements in DMD despite both nutrition and bone health being key features of management guidelines. The current guidelines surrounding vitamin D monitoring and supplementation in DMD are based on expert opinion rather than scientific evidence and discrepancies exist; they recommend annual monitoring of 25-(OH)D, daily supplementation and treating deficiency by maintaining a serum 25-(OH)D concentration above either 75 nmol/l [13] or 50 nmol/l [14]. There is no consensus on the recommended daily dose, nor on the definition of deficiency which can be ≤25 nmol/l, ≤50 nmol/l or ≤75 nmol/l depending on the location and/or opinion of the clinical care team(s)[15–18]. Despite high compliance of daily supplementation, reports of vitamin D deficiency or insufficiency in DMD cohorts both on corticosteroids and in steroid naive individuals are not unusual [9,10,19,20]. One study on the UK Duchenne population found that most individuals on maintenance doses of 200 or 400 IU/day were still vitamin D deficient, with optimal levels

(serum 25-OHD above 75 nmol/l) achieved by those taking a higher dose of at least 1000 IU/day [10].

Given the lack of evidence and conflicting guidelines surrounding the effectiveness of vitamin D supplementation in the Duchenne population we undertook a comprehensive systematic literature review and meta-analysis of the best available evidence to help inform and direct new research in this area.

2. Materials and methods

This systematic literature review was performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 statement [21], a flow chart illustrating the number of records identified, included and excluded and the reasons for exclusions is provided in figure 1.

2.1. Eligibility criteria

Peer-reviewed randomised controlled trials (RCTs), cross-sectional and retrospective and prospective cohort studies were all screened. Reviews, opinion pieces, commentaries, single case studies, animal studies, posters, abstracts and articles not in English were excluded. Only studies on DMD patients were included. Regardless of study aims and objectives, studies were included if they reported serum/plasma vitamin D levels in supplement versus no supplement-taking groups and/or vitamin D levels in a supplement-taking group only. Studies were also included if they provide data on vitamin D insufficiency amongst a supplement-taking group. Studies were excluded from meta-analysis if they did not report, or provide sufficient data to allow the calculation of, the mean serum vitamin D level (±SD) in a supplement-taking group of DMD patients. We did not exclude studies based on subject age, ambulation, geographic location, steroid use, supplement type, regimen or dose but this information was collected and considered in our narrative synthesis.

2.2. Information sources

The following online libraries were searched: PubMed, Web of Science, Cochrane Central, Medline and CINAHL. Google Scholar, reference lists and relevant review articles were also

examined to identify additional studies. Database searches were initially conducted on the 24th October 2022 and updated before submission to capture more recent articles.

2.3. Search strategy

The search strategy was the same for all databases. Search terms for vitamin D and search terms for Duchenne muscular dystrophy were combined with the Boolean operators 'AND' and 'OR'. No filters or limits were used. Search terms included both medical subject headings (MeSH) and free text terms (keywords), as applicable. Vitamin D search terms were: Vitamin D, Cholecalciferol, 25-Hydroxyvitamin D, 25-Hydroxyvitamin D2 and 25-hydroxyvitamin D3. Duchenne search terms were: Duchenne muscular dystrophy, Duchenne, and muscular dystrophy.

2.4. Study selection

Database search results were exported into Microsoft Excel and duplicates removed. The remaining titles and abstracts were first screened against the inclusion criteria and independently verified by a second reviewer to minimise bias and the risk of missed studies. Second, full-text screening was conducted for articles that passed initial screening to confirm they met the full inclusion criteria required for data extraction. Full-texts were screened in cases where it was unclear from the title and abstract alone if the required data was present. The final articles for inclusion were determined by consensus between the two reviewers; borderline and disputable studies were each discussed before their inclusion or exclusion.

2.5. Risk of bias

A critical appraisal of the research evidence was conducted using the Joanna Briggs Institute (JBI) Critical Appraisal Tools [22,23]. The tools are designed to assess the methodological

quality and the possibility of bias in study design, conduct and analysis. All articles meeting the inclusion criteria were independently assessed by both authors. Depending on the study type, either the checklist for analytical cross-sectional studies, the checklist for cohort studies or the checklist for randomised controlled trials was used and the results collated using Microsoft Excel.

2.6. Data extraction

Data extraction was performed manually using pre-designed electronic tables in Microsoft Excel and was independently verified by a second reviewer. The effect measure used in the synthesis and presentation of results was the mean (±SD) serum 25(OH)D level in nmol/l. Units were converted when there were differences and weighted means and SD used where appropriate. Missing SD's were imputed where possible according to the Cochrane Handbook for Systematic Reviews of Interventions [24]. For the Alshaikh *et al.* paper, which presents data grouped by dosage regimes [10], only data from the daily dose groups was used and a weighted mean ±SD were calculated. Additional data collected from each article, where available, was: study design, geographic location, primary research aims, number of participants, testing method, adherence, age of participants, supplementation regimen and steroid usage.

2.7. Meta-analysis

Due to a lack of randomised control trials meeting the eligibility criteria, we proceeded with a quantitative synthesis (meta-analysis) of the best available evidence which included several study types (table 1). Given the likely heterogeneity of mean vitamin D levels amongst study cohorts with different geographical locations and demographics, a random-effects meta-analysis (DerSimonian-Laird method) was conducted using the OpenMeta software with the

assumption that the effects being estimated in the different studies are not identical but follow some distribution. Studies are weighted more equally in random-effects analysis than in a fixed-effects analysis [25]. Study characteristics suspected to influence the effect size were identified a priori as supplement dose and latitude; random-effects meta-regression was performed using these as covariates in OpenMeta. Statistical heterogeneity among studies was assessed in OpenMeta using the I^2 statistic which describes the percentage of variation across studies due to heterogeneity rather than chance. In line with convention, I^2 values below 25 % were considered low, between 25 % and 75 % moderate and above 75 % high.

3. Results

Data from 13 articles met the inclusion criteria and were included in the narrative synthesis. Of these, eight articles were included in a meta-analysis. Figure 1 illustrates the study selection process. Studies excluded due to insufficient data most commonly did not provide clear confirmation on whether their cohorts were taking supplements, one study [26] was excluded due to uncertainty over the reported units of measurement. Table 1 provides a summary of paper characteristics. Six studies were conducted in the continent of Europe (United Kingdom, Sweden, Italy, Germany, Denmark and Greece [10,27–31]), three in North America (Canada and the United States of America [32–34]), three in Australasia (Australia and New Zealand [35–37]) and one in Asia (India, [38]). Only three studies [10,30,32] had the primary aim of determining the effectiveness of vitamin D supplementation. The mean age (where available) of participants in the included studies varied between eight and 17 years of age and as expected of DMD cohorts, a large percentage of (if not all) individuals from each study were taking corticosteroids (Table 1). Only two of the 13 studies were randomised controlled trials [35,36], but the intervention/study drug was not vitamin D supplementation in either case. The remaining studies were a mix of retrospective case note reviews (n=4) and prospective (n=3) or cross-sectional (n=4) observational studies. A risk of bias analysis (Supplementary Figure 1) highlights an overall low risk of bias for all but one study which was ranked as having a moderate risk of bias (a RCT which returned 5/13 'no' responses for questions on randomisation and blinding [36].

The dosage of vitamin D supplementation, and the quality of reporting it, varied among the 13 studies (Table 1). Interestingly, despite supplementation, nine out of the 13 included studies (69 %, Table 2 and Figure 2) reported vitamin D deficiency within supplement taking

cohorts (Table 2). The definition of deficiency differed (Table 2), though most studies defined vitamin D deficiency as 25(OH)D serum levels <50 nmol/l. Amongst these studies the number of individuals reported as deficient decreased from 65 % (400 IU/day) to 25 % (1627 IU/day) as the reported daily dose increased (Figure 2). The relationship between serum vitamin D levels and daily supplementation dose was therefore explored using data available from six independent studies (Figure 3). Regression analysis revealed a weak non-significant relationship between serum vitamin D levels and daily dose (r^2 = 0.3, p = 0.237). We next assessed the 13 studies for accounts of adherence/compliance. Given the retrospective nature of most of our included studies, adherence was not always captured. Five out of the 13 studies commented on adherence, or compliance, to vitamin D supplementation. The studies of Bianchi *et al*., Davidson *et al*. and Zacharin *et al*. reported high adherence rates to supplementation of approximately 80% [30,35,36]. Vather-Wu *et al*. note that compliance is typically high in their clinic population [32] and Perera *et al*. similarly allude to a high level of compliance in stating that non-adherence to supplementation was identified as a factor in only 15% of cases with declining vitamin D levels [37].

Eight studies reporting mean serum vitamin D levels ±SD in a supplement taking group were included in a random effects meta-analysis ([10,30,31,33–36,38], Figure 4A). The weighted pooling of the reported means was 74.6 nmol/l (95 % CI: 56.0 – 93.12, I^2 = 99.59 %). Only one study reported a mean serum vitamin D level below 50 nmol/l (Suthar *et al*., [38]). A high I^2 value of 99.59 % indicates substantial heterogeneity between included studies. Subgroup analysis of dose and latitude (identified a priori) did not reduce heterogeneity (data not shown). We performed a sensitivity (leave one out) analysis to evaluate the influence of each study and to identify potential influential, or outlier, studies (Figure 4B). The pooled estimate was relatively stable and not substantially influenced by a specific study. Nasomyont *et al*.

showed the largest influence reducing the pooled estimate from 74.6 nmol/l to 66.66 nmol/l when removed [33]. Cumulative meta-analyses over time (date of acceptance) and by study size were also performed to explore trends in the pooled estimate (Figure 5). Other than an initial increase between the Söderpalm *et al*. study in 2007 [31] and the Bianchi *et al*. study in 2011 [30] (52.05 to 74.6 nmol/l, Figure 5A) there is not a large cumulative effect on the estimate over time. However, there is a notable and consistent decrease in the cumulative estimate with increasing study size (131 to 74.6 nmol/l, Figure 5B). To determine whether a linear relationship exists between serum vitamin D levels and co-variates identified a priori, a meta-regression analysis for daily dose ($p = 0.107$) and latitude ($p = 0.66$) was performed, they did not show any statistically significant influences on serum vitamin D levels (Figure 6); this is in agreement with the analysis in Figure 3.

4. Discussion

We conducted a systematic literature review of the best available evidence concerning the effectiveness of vitamin D supplementation in maintaining or restoring vitamin D levels in the DMD population. Very few studies have addressed this directly and there is a lack of randomised controlled trials. A limitation of our review is therefore the use of uncontrolled studies which are deemed low in the hierarchy of evidence. We also observed high heterogeneity and cannot identify whether this results from clinical or methodological diversity or both. Based on the evidence presented, we are therefore unable to make a clear recommendation concerning the most effective vitamin D regimen for the DMD population. It is natural to suggest more studies are required to provide definitive conclusions; however, we acknowledge the difficulties in controlling for diet and the use of other supplements as well as the unethical nature of withholding a preventative treatment for osteoporosis, for which individuals with DMD are already at an increased risk. Future studies might therefore focus on comparing different vitamin D dose regimens.

Whilst it can be argued that a meta-analysis should not have been conducted with such high heterogeneity, tests for heterogeneity are irrelevant to the choice of analysis since clinical and methodological diversity will always occur in a meta-analysis [39]. It was therefore decided a priori to summarise the evidence and measure inconsistency using I^2 making efforts to minimise it by using a random effects model, subgroup analysis and sensitivity analysis. Ultimately, we could not explain the statistical heterogeneity by subgroup or meta-regression analysis suggesting there are unidentified sources of clinical and/or methodological heterogeneity. We kept the meta-analysis to show the estimates and dispersion of mean vitamin D levels for the included studies and present some valuable findings from collating the presented evidence.

In agreement with other chronic diseases where long term corticosteroid treatment is widespread [40,41], our review is suggestive of an increased risk for vitamin D insufficiency amongst DMD patients with at least 20% of individuals with DMD remaining vitamin D insufficient or deficient despite high levels of supplementation. Naume *et al*. found, in a small study of children with neuromuscular disorders (n = 44), high counts of vitamin D deficiency (approximately 30%) amongst their DMD cohort where seven out of 23 DMD individuals were deficient or insufficient versus one each of spinal muscular atrophy type II (n = 9), Bethlam myopathy (n = 1) and Charcot-Marie-Tooth neuropathy (n = 2) [27]. However, whilst this does appear to be a feature of DMD, Alshaikh *et al*. found no significant differences (taking dosage into account) in mean serum vitamin D levels between those taking corticosteroids or not [10]. In 2003 Bianchi *et al*. also reported low serum 25(OH)D levels in ten DMD individuals not taking corticosteroids (with normal diet and sunlight exposure), though notably the levels in a corticosteroid-taking group of boys were significantly lower [19]. The classic function of vitamin D is keeping bone healthy by promoting calcium absorption [8] and there is clear evidence that vitamin D supplementation can mitigate against the known bone-related effects of long-term corticosteroid treatment. For example, bone mass loss and an increased risk of fractures is well documented amongst cohorts taking corticosteroids in the tong-term [42]. The additional muscle impairment and loss of weight-bearing activity amongst individuals with DMD adds additional risk for osteopenia and osteoporosis. Bianchi *et al*. 2011 conducted the first prospective study on the effects of vitamin D and calcium treatment in DMD [30]. In a cohort of 33 DMD individuals, all taking prednisolone, they showed that calcifediol plus adequate dietary calcium was an effective treatment to control bone turnover and increase bone mineral density for two thirds of individuals with DMD; persistently high bone turnover was a feature in the third of non-responding children. They observed a

negative correlation between the cumulative dose of corticosteroids and spine bone mineral density in their DMD cohort. Deficiency of vitamin D is known to stimulate parathyroid hormone (PTH) secretion which in turn stimulates the release of calcium from bone [43]. Twenty percent of the bone mineral content (total body) increase observed with vitamin D and calcium treatment in the Bianchi *et al*. study was explained by a decrease in PTH level and 26% was explained by an increase in 25(OH)D and decrease in N-telopeptide (NTx), a marker of bone turnover [30]. These results support the evaluation of bone and mineral metabolism in clinical practice for DMD.

The largest study captured in our analysis was Alshaikh *et al*., who conducted a retrospective case note review of boys with DMD at a large specialist neuromuscular centre located in London [10]. Amongst a cohort of 197 individuals, they found a high prevalence of vitamin D deficiency and insufficiency and a weak but positive trend between supplement dose and serum 25(OH)D levels. In their analysis, optimal vitamin D levels were achieved only with maintenance doses of at least 1000 IU/day [10]. Here, our analysis of multiple studies shows a non-significant relationship between daily dose and 25(OH)D levels; this is perhaps not surprising given the heterogeneity, global distribution of the studies and known risk factors such as latitude and ethnicity for vitamin D deficiency [44]. Information on the ethnicity of the study cohorts was not available and a meta-regression analysis for study latitude was non-significant. An American study conducted in Cincinnati (Nasomyont *et al*., six participants [33]) had the highest mean serum 25(OH)D levels and the North Indian study (Suthar *et al*., 76 participants [38]) had the lowest. High levels of vitamin D deficiency are well documented within the Indian population highlighting that the global DMD population likely also replicates such trends [44]. Interestingly, Alshaikh *et al.* found no significant differences between 25(OH)D levels in their DMD cohorts when tested in the summer versus winter, there were

also no differences in levels between ambulant and non-ambulant boys [10]. Other factors such as body mass index (BMI) may also be important given increased obesity and growth delay within the DMD population. Indeed, it is established that people with obesity need a higher vitamin D intake to maintain adequate vitamin D levels, as vitamin D accumulates in adipose tissue decreasing its bioavailability [45,46]. Interestingly however, a systematic review of a very heterogenous, and globally distributed, group of studies on various other adult populations found a significant dose-response between vitamin D supplementation and serum vitamin D levels independently of multiple confounders [47]. These authors also highlight a wider spread lack of randomised studies assessing the efficacy of vitamin D supplementation on adult populations. The fact that the dose response analyses from our study and the Alshaikh *et al.* study [10] were weak, variable and non-significant justifies regular monitoring of blood levels and highlights a need to determine whether or not the increased risk of vitamin D deficiency or insufficiency is due to the pathogenesis of DMD and/or long-term corticosteroid treatment.

The current consensus guidelines for the standard of care of children and adults with DMD [13,14] state vitamin D supplements should be given to all patients and serum 25(OH)D levels checked annually to maintain levels above 50 nmol/l (adult) or 75 nmol/l (children). The adult guideline refers to the fact that although most clinics will aim to maintain serum 25(OH)D levels above 75 nmol/l, there is no direct evidence from clinical trials to support this. Both guidelines state that dietary calcium intake should be adequate and monitored since low vitamin D levels are known to reduce intestinal calcium absorption and cause hypocalciuria [13,14]. There is no recommendation on the daily dosage required to maintain the recommended vitamin D levels. A previous version (published in 2010) of the care recommendations [4] did advise that for levels between 20 and 31 nmol/l to give 1000 IU

orally twice daily and for levels <20 nmol/l to give 2000 IU orally twice per day. It was advised to recheck levels after three months and encourage weight-bearing activities. Our pooled mean serum 25(OH)D level across eight studies was 74.6 nmol/l and lower than what one might expect given these guidelines. One study mean fell below 50 nmol/l and five studies fell below 75 nmol/l highlighting that whilst the dosing regimens currently in use may be preventing severe deficiency, they are not effective at maintaining sufficient vitamin D levels within the DMD population. This is in agreement with Alshaikh *et al*. who concluded that children with DMD should not be given small doses of 200 - 800 IU/day but rather a maintenance dose of 1000 – 1500 IU/day following a three-month replenishment of 6000 IU cholecalciferol per day where required [10]. Additional interventions such as bisphosphonates (e.g. zoledronic acid) may also be considered where a poor response to vitamin D supplementation is observed. Indeed, a randomised controlled trial included in our analysis showed that zoledronic acid infusions plus vitamin D and calcium improves bone mineral density in glucocorticoid dependent DMD boys [36].

Our cumulative meta-analysis by study size showed a notable decrease in the pooled estimate with increasing study size; this highlights a potential small study effect and a noteworthy variance of mean vitamin D levels within the DMD population despite high levels of, and adherence to, supplementation. The true pooled estimate may therefore be even lower than that presented here. Advances in therapeutic development and standards of care have already begun to extend survival times for individuals with Duchenne, the effects of vitamin D deficiency and insufficiency may therefore soon become even more relevant to maintaining or improving quality of life.

In summary, although there is a lack of randomised controlled trials, our collated evidence highlights the importance of regular vitamin D monitoring (and replenishment where

required) in people with DMD. Doses should be adjusted depending upon an individual's response; high levels of dosing may be required for some individuals and consensus guidelines in this area should be revisited.

5. Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. For the purpose of open access, the author has applied a Creative Commons Attribution (CC BY) licence to any Author Accepted Manuscript version arising from this submission.

6. Data statement

All data underpinning this publication are openly available from the University of Northampton Research Explorer at DOI: 10.24339/258c4713-fa3f-46f0-9d75-481c9f37b15a

7. References

[1] Broomfield J, Hill M, Guglieri M, Crowther M, Abrams K. Life Expectancy in Duchenne Muscular Dystrophy: Reproduced Individual Patient Data Meta-analysis. Neurology 2021;97:e2304–14. https://doi.org/10.1212/WNL.0000000000012910.

[2] Landfeldt E, Thompson R, Sejersen T, McMillan HJ, Kirschner J, Lochmüller H. Life expectancy at birth in Duchenne muscular dystrophy: a systematic review and meta-analysis. Eur J Epidemiol 2020;35:643–53. https://doi.org/10.1007/s10654-020-00613-8.

[3] Bushby K, Finkel R, Birnkrant DJ, Case LE, Clemens PR, Cripe L, et al. Diagnosis and management of Duchenne muscular dystrophy, part 1: diagnosis, and pharmacological and psychosocial management. Lancet Neurol 2010;9:77–93. https://doi.org/10.1016/S1474-4422(09)70271-6.

[4] Bushby K, Finkel R, Birnkrant DJ, Case LE, Clemens PR, Cripe L, et al. Diagnosis and management of Duchenne muscular dystrophy, part 2: implementation of multidisciplinary care. Lancet Neurol 2010;9:177–89. https://doi.org/10.1016/S1474-4422(09)70272-8.

[5] Muntoni F, Torelli S, Ferlini A. Dystrophin and mutations: One gene, several proteins, multiple phenotypes. Lancet Neurol 2003;2:731–40. https://doi.org/10.1016/S1474-4422(03)00585-4.

[6] Filonova G, Aartsma-Rus A. Next steps for the optimization of exon therapy for Duchenne muscular dystrophy. Expert Opin Biol Ther 2023;23:133–43. https://doi.org/10.1080/14712598.2023.2169070.

[7] Thacher TD, Clarke BL. Vitamin D Insufficiency. Mayo Clin Proc 2011;86:50–60. https://doi.org/10.4065/mcp.2010.0567.

[8] SACN vitamin D and health report. GOVUK n.d. https://www.gov.uk/government/publications/sacn-vitamin-d-and-health-report (accessed July 27, 2023).

[9] Larson CM, Henderson RC. Bone mineral density and fractures in boys with Duchenne muscular dystrophy. J Pediatr Orthop 2000;20:71–4.

[10] Alshaikh N, Brunklaus A, Davis T, Robb SA, Quinlivan R, Munot P, et al. Vitamin D in corticosteroid-naïve and corticosteroid-treated Duchenne muscular dystrophy: what dose achieves optimal 25(OH) vitamin D levels? Arch Dis Child 2016;101:957–61. https://doi.org/10.1136/archdischild-2015-308825.

[11] Guglieri M, Bushby K, McDermott MP, Hart KA, Tawil R, Martens WB, et al. Effect of Different Corticosteroid Dosing Regimens on Clinical Outcomes in Boys With Duchenne Muscular Dystrophy: A Randomized Clinical Trial. JAMA 2022;327:1456–68. https://doi.org/10.1001/jama.2022.4315.

[12] Ward LM, Hadjiyannakis S, McMillan HJ, Noritz G, Weber DR. Bone Health and Osteoporosis Management of the Patient With Duchenne Muscular Dystrophy. Pediatrics 2018;142:S34–42. https://doi.org/10.1542/peds.2018-0333E.

[13] Birnkrant DJ, Bushby K, Bann CM, Apkon SD, Blackwell A, Brumbaugh D, et al. Diagnosis and management of Duchenne muscular dystrophy, part 1: diagnosis, and neuromuscular, rehabilitation, endocrine, and gastrointestinal and nutritional management. Lancet Neurol 2018;17:251–67. https://doi.org/10.1016/S1474-4422(18)30024-3.

[14] Quinlivan R, Messer B, Murphy P, Astin R, Mukherjee R, Khan J, et al. Adult North Star Network (ANSN): Consensus Guideline For The Standard Of Care Of Adults With

Duchenne Muscular Dystrophy. J Neuromuscul Dis 2021;8:899–926. https://doi.org/10.3233/JND-200609.
[15] McCarthy M. Evidence for routine vitamin D screening is insufficient, US panel concludes. BMJ 2014;348:g4277. https://doi.org/10.1136/bmj.g4277.
[16] Binkley N, Ramamurthy R, Krueger D. Low Vitamin D Status: Definition, Prevalence, Consequences and Correction. Endocrinol Metab Clin North Am 2010;39:287-contents. https://doi.org/10.1016/j.ecl.2010.02.008.
[17] Amrein K, Scherkl M, Hoffmann M, Neuwersch-Sommeregger S, Köstenberger M, Tmava Berisha A, et al. Vitamin D deficiency 2.0: an update on the current status worldwide. Eur J Clin Nutr 2020;74:1498–513. https://doi.org/10.1038/s41430-020-0558-y.
[18] Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 2011;96:1911–30. https://doi.org/10.1210/jc.2011-0385.
[19] Bianchi ML, Mazzanti A, Galbiati E, Saraifoger S, Dubini A, Cornelio F, et al. Bone mineral density and bone metabolism in Duchenne muscular dystrophy. Osteoporos Int J Establ Result Coop Eur Found Osteoporos Natl Osteoporos Found USA 2003;14:761–7. https://doi.org/10.1007/s00198-003-1443-y.
[20] Aparicio LF, Jurkovic M, DeLullo J. Decreased bone density in ambulatory patients with duchenne muscular dystrophy. J Pediatr Orthop 2002;22:179–81.
[21] Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. https://doi.org/10.1136/bmj.n71.
[22] Munn Z, Stone JC, Aromataris E, Klugar M, Sears K, Leonardi-Bee J, et al. Assessing the risk of bias of quantitative analytical studies: introducing the vision for critical appraisal within JBI systematic reviews. JBI Evid Synth 2023;21:467–71. https://doi.org/10.11124/JBIES-22-00224.
[23] Barker TH, Stone JC, Sears K, Klugar M, Leonardi-Bee J, Tufanaru C, et al. Revising the JBI quantitative critical appraisal tools to improve their applicability: an overview of methods and the development process. JBI Evid Synth 2023;21:478–93. https://doi.org/10.11124/JBIES-22-00125.
[24] Higgins JPT, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, Welch VA (editors). Cochrane Handbook for Systematic Reviews of Interventions version 6.3 (updated February 2022) 2022.
[25] Dettori JR, Norvell DC, Chapman JR. Fixed-Effect vs Random-Effects Models for Meta-Analysis: 3 Points to Consider. Glob Spine J 2022;12:1624–6. https://doi.org/10.1177/21925682221110527.
[26] Liu C, Yang D-D, Zhang L, Lei X-G, Jia F-L, Liao Y, et al. Bone Mineral Density Assessment by Quantitative Computed Tomography in Glucocorticoid-Treated Boys With Duchenne Muscular Dystrophy: A Linear Mixed-Effects Modeling Approach. Front Endocrinol 2022;13.
[27] Naume MM, Jørgensen MH, Høi-Hansen CE, Born AP, Vissing J, Borgwardt L, et al. Metabolic assessment in children with neuromuscular disorders shows risk of liver enlargement, steatosis and fibrosis. Acta Paediatr Oslo Nor 1992 2023;112:846–53. https://doi.org/10.1111/apa.16649.

[28] Doulgeraki AE, Athanasopoulou HI, Katsalouli MS, Petrocheilou GM, Paspati IN, Monopolis IK. Body composition of patients with Duchenne muscular dystrophy: the Greek experience. Acta Neurol Belg 2016;116:565–72. https://doi.org/10.1007/s13760-015-0582-1.
[29] Razmdjou S, Seufert J, Rensing-Zimmermann C, Kirschner J, Korinthenberg R. Differential Analysis of Bone Density in Children and Adolescents with Neuromuscular Disorders and Cerebral Palsy. Neuropediatrics 2015;46:385–91. https://doi.org/10.1055/s-0035-1563695.
[30] Bianchi ML, Morandi L, Andreucci E, Vai S, Frasunkiewicz J, Cottafava R. Low bone density and bone metabolism alterations in Duchenne muscular dystrophy: response to calcium and vitamin D treatment. Osteoporos Int 2011;22:529–39. https://doi.org/10.1007/s00198-010-1275-5.
[31] Söderpalm A-C, Magnusson P, Ahlander A-C, Karlsson J, Kroksmark A-K, Tulinius M, et al. Low bone mineral density and decreased bone turnover in Duchenne muscular dystrophy. Neuromuscul Disord NMD 2007;17:919–28. https://doi.org/10.1016/j.nmd.2007.05.008.
[32] Vather-Wu N, Krasowski MD, Mathews KD, Shibli-Rahhal A. Vitamin D Level Stability in Dystrophinopathy Patients on Vitamin D Supplementation. J Neuromuscul Dis 2021;8:481–7. https://doi.org/10.3233/JND-200625.
[33] Nasomyont N, Keefe C, Tian C, Hornung L, Khoury J, Tilden JC, et al. Safety and efficacy of teriparatide treatment for severe osteoporosis in patients with Duchenne muscular dystrophy. Osteoporos Int J Establ Result Coop Eur Found Osteoporos Natl Osteoporos Found USA 2020;31:2449–59. https://doi.org/10.1007/s00198-020-05549-z.
[34] Bian Q, McAdam L, Grynpas M, Mitchell J, Harrington J. Increased Rates of Vitamin D Insufficiency in Boys With Duchenne Muscular Dystrophy Despite Higher Vitamin D3 Supplementation. Glob Pediatr Health 2019;6:2333794X19835661. https://doi.org/10.1177/2333794X19835661.
[35] Davidson ZE, Hughes I, Ryan MM, Kornberg AJ, Cairns AG, Jones K, et al. Effect of a multicomponent nutritional supplement on functional outcomes for Duchenne muscular dystrophy: A randomized controlled trial. Clin Nutr Edinb Scotl 2021;40:4702–11. https://doi.org/10.1016/j.clnu.2021.06.008.
[36] Zacharin M, Lim A, Gryllakis J, Siafarikas A, Jefferies C, Briody J, et al. Randomized Controlled Trial Evaluating the Use of Zoledronic Acid in Duchenne Muscular Dystrophy. J Clin Endocrinol Metab 2021;106:2328–42. https://doi.org/10.1210/clinem/dgab302.
[37] Perera N, Sampaio H, Woodhead H, Farrar M. Fracture in Duchenne Muscular Dystrophy: Natural History and Vitamin D Deficiency. J Child Neurol 2016;31:1181–7. https://doi.org/10.1177/0883073816650034.
[38] Suthar R, Reddy BVC, Malviya M, Sirari T, Attri SV, Patial A, et al. Bone density and bone health alteration in boys with Duchenne Muscular Dystrophy: a prospective observational study. J Pediatr Endocrinol Metab 2021;34:573–81. https://doi.org/10.1515/jpem-2020-0680.
[39] Higgins JPT, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ 2003;327:557–60. https://doi.org/10.1136/bmj.327.7414.557.
[40] Bardare M, Bianchi ML, Furia M, Gandolini GG, Cohen E, Montesano A. Bone mineral metabolism in juvenile chronic arthritis: the influence of steroids. Clin Exp Rheumatol 1991;9 Suppl 6:29–31.

[41] Klein RG, Arnaud SB, Gallagher JC, Deluca HF, Riggs BL. Intestinal calcium absorption in exogenous hypercortisonism. Role of 25-hydroxyvitamin D and corticosteroid dose. J Clin Invest 1977;60:253–9. https://doi.org/10.1172/JCI108762.
[42] Van Staa TP, Leufkens HG, Abenhaim L, Zhang B, Cooper C. Use of oral corticosteroids and risk of fractures. J Bone Miner Res Off J Am Soc Bone Miner Res 2000;15:993–1000. https://doi.org/10.1359/jbmr.2000.15.6.993.
[43] Nussey S, Whitehead S. The parathyroid glands and vitamin D. Endocrinol. Integr. Approach, BIOS Scientific Publishers; 2001.
[44] Guo J, Lovegrove JA, Givens DI. A Narrative Review of The Role of Foods as Dietary Sources of Vitamin D of Ethnic Minority Populations with Darker Skin: The Underestimated Challenge. Nutrients 2019;11:81. https://doi.org/10.3390/nu11010081.
[45] Holick MF, Chen TC. Vitamin D deficiency: a worldwide problem with health consequences. Am J Clin Nutr 2008;87:1080S-6S. https://doi.org/10.1093/ajcn/87.4.1080S.
[46] Wortsman J, Matsuoka LY, Chen TC, Lu Z, Holick MF. Decreased bioavailability of vitamin D in obesity. Am J Clin Nutr 2000;72:690–3. https://doi.org/10.1093/ajcn/72.3.690.
[47] Jarrett F. The Effect of Oral Supplementation of Vitamin D3 on Serum Levels of Vitamin D: A Review. Epidemiol Open Access 2014;04. https://doi.org/10.4172/2161-1165.1000148.

8. Figure legends

Figure 1. A PRISMA flow chart of study selection.

Figure 2. Evidence supporting vitamin D deficiency amongst DMD patients despite supplementation. A. The number of included studies reporting vitamin D deficiency despite supplementation is 69%; percentages do not equal 100 due to rounding. B. Most included studies defined vitamin D deficiency as 25(OH)D serum levels <50 nmol/l; where available, for these studies the percentage that was deficient is plotted according to the reported daily dose (IU) of supplementary vitamin D.

Figure 3. Evidence on the relationship between serum vitamin D levels and daily supplementation dose. Data was available from six studies (from low to high dose: Söderpalm *et al*., Alshaikh *et al*., Zacharin *et al*., Bianchi *et al*., Bian *et al*., Davidson *et al*.) and presented

as (A) a bar chart and (B) a scatter plot with linear regression line (equation: Y = 0.01825*X + 52.49; r2 = 0.3; p = 0.237).

Figure 4. (A) Meta-analysis comparing mean serum vitamin D levels of included studies. (B) Leave one out meta-analysis of mean serum vitamin D levels.

Figure 5. (A) Cumulative meta-analysis by date of publication (acceptance, earliest – latest) of mean serum vitamin D levels. (B) Cumulative meta-analysis by study size (smallest – largest) of mean serum vitamin D levels

Figure 6. Random effects meta-regression analysis of mean serum vitamin D levels with (A) daily dose and (B) latitude as co-variates. Larger circles indicate studies with greater weight and more precision of effect (i.e. less variance).

Supplementary figure 1. Risk of bias analysis of included studies performed using JBI Critical Appraisal Tools. Study type is indicated on the right-hand side. Either the checklist for (A) randomised controlled trials (RCT), (B) the checklist for analytical cross-sectional studies or (C) the checklist for cohort studies (retrospective and prospective) was used. Y: yes; N: no; U: unclear and white: non-applicable. Questions that were non-applicable for all studies are removed for clarity.

9. List of Tables

Table 1. Summary table of included study characteristics

Table 2. Evidence supporting vitamin D deficiency amongst DMD patients after supplementation

Figure 1

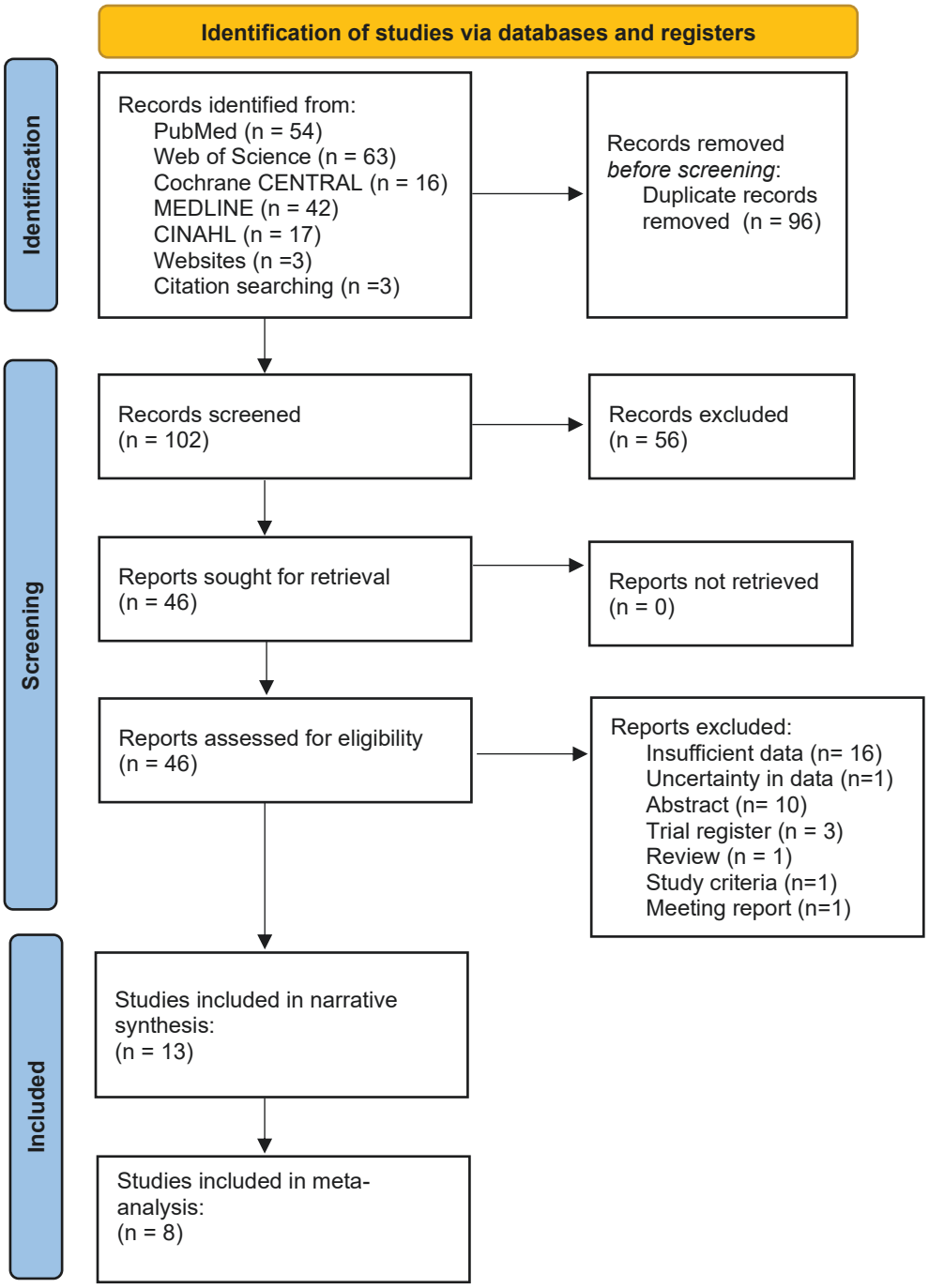

Identification of studies via databases and registers
Identification
Records identified from:
PubMed (n = 54)
Web of Science (n = 63)
Cochrane CENTRAL (n = 16)
MEDLINE (n = 42)
CINAHL (n = 17)
Websites (n =3)
Citation searching (n =3)
Records removed
before screening:
Duplicate records
removed (n = 96)
Screening
Records screened
(n = 102)
Records excluded
(n = 56)
Reports sought for retrieval
(n = 46)
Reports not retrieved
(n = 0)
Reports assessed for eligibility
(n = 46)
Reports excluded:
Insufficient data (n= 16)
Uncertainty in data (n=1)
Abstract (n= 10)
Trial register (n = 3)
Review (n = 1)
Study criteria (n=1)
Meeting report (n=1)
Included
Studies included in narrative
synthesis:
(n = 13)
Studies included in meta-
analysis:
(n = 8)

Figure 2.

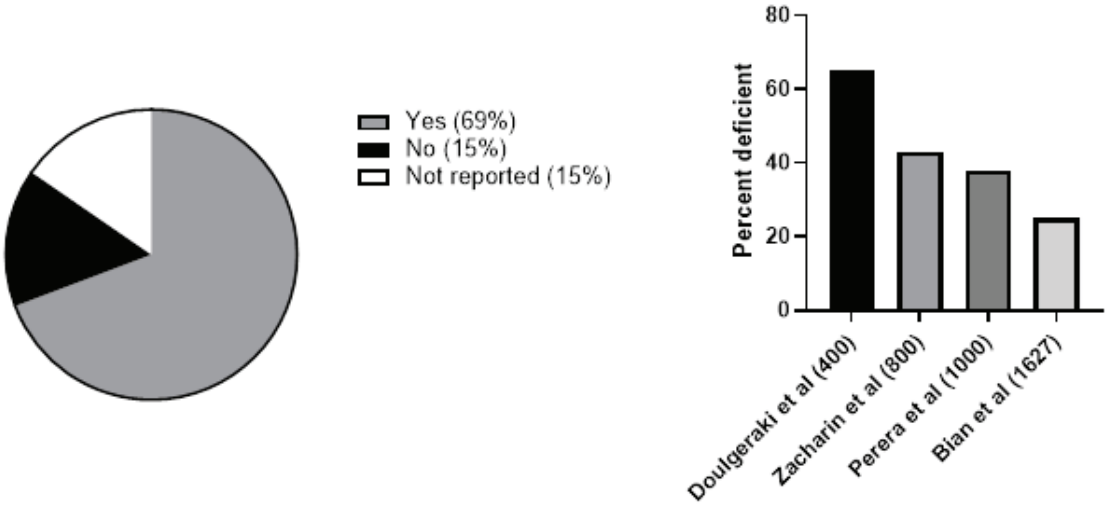

Yes (69%)
No (15%)
Not reported (15%)
Percent deficient
80
60
40
20
0
Doulgeraki et al (400)
Zacharin et al (800)
Perera et al (1000)
Bian et al (1627)

Figure 3.

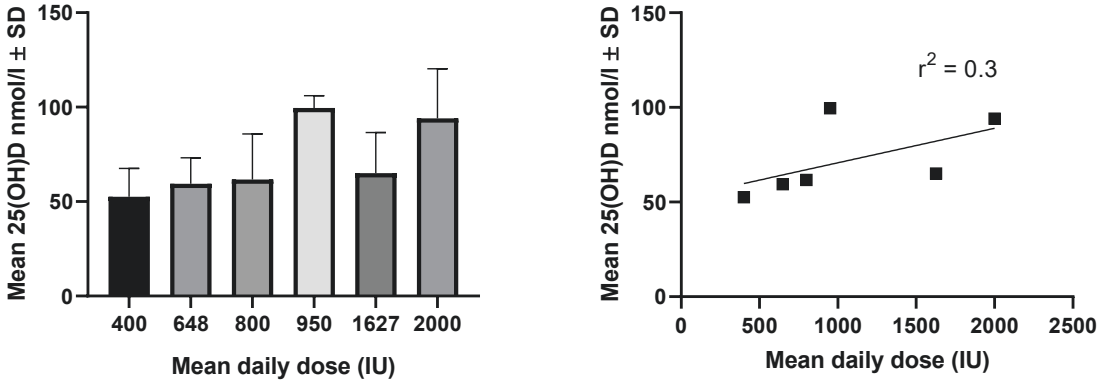
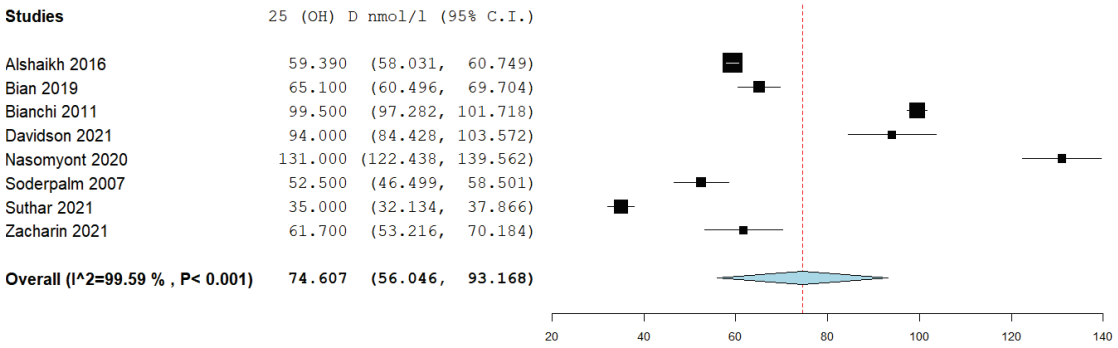

Mean 25(OH)D nmol/l ± SD
150
100
50
0
400
648
800
950
1627
2000
Mean daily dose (IU)
Mean 25(OH)D nmol/l ± SD
150
100
50
0
r2 = 0.3
0
500
1000
1500
2000
2500
Mean daily dose (IU)

Figure 4.

A


Studies
25 (OH) D nmol/l (95% C.I.)
Alshaikh 2016 59.390 (58.031, 60.749)
Bian 2019 65.100 (60.496, 69.704)
Bianchi 2011 99.500 (97.282, 101.718)
Davidson 2021 94.000 (84.428, 103.572)
Nasomyont 2020 131.000 (122.438, 139.562)
Soderpalm 2007 52.500 (46.499, 58.501)
Suthar 2021 35.000 (32.134, 37.866)
Zacharin 2021 61.700 (53.216, 70.184)
Overall (I^2=99.59 % , P< 0.001) 74.607 (56.046, 93.168)
20
40
60
80
100
120
140

B

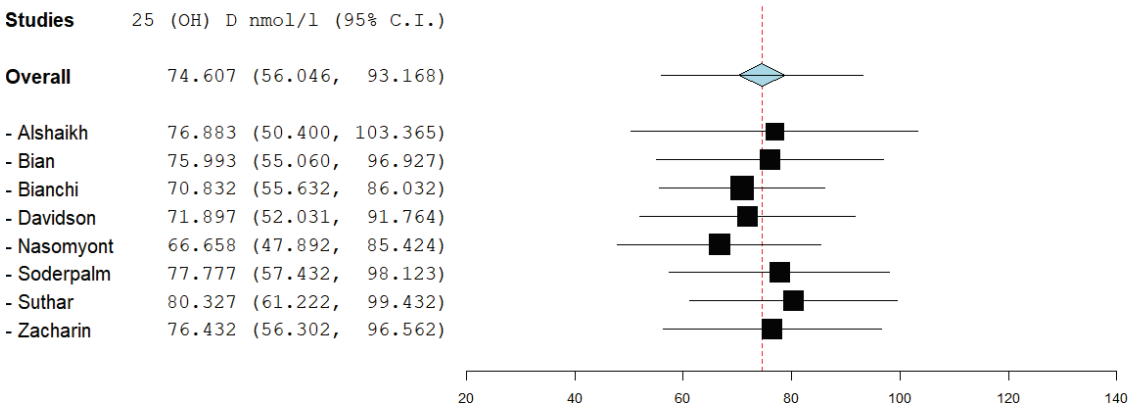
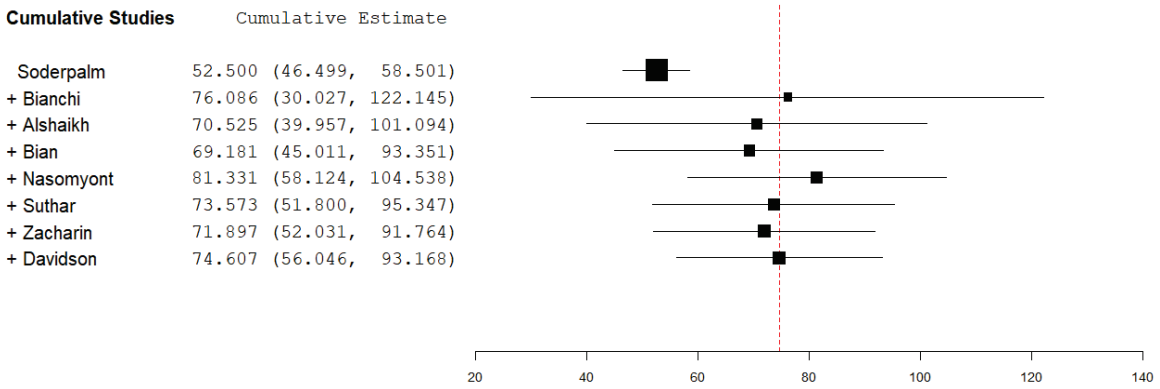

Studies
25 (OH) D nmol/l (95% C.I.)
Overall 74.607 (56.046, 93.168)
- Alshaikh 76.883 (50.400, 103.365)
- Bian 75.993 (55.060, 96.927)
- Bianchi 70.832 (55.632, 86.032)
- Davidson 71.897 (52.031, 91.764)
- Nasomyont 66.658 (47.892, 85.424)
- Soderpalm 77.777 (57.432, 98.123)
- Suthar 80.327 (61.222, 99.432)
- Zacharin 76.432 (56.302, 96.562)
20
40
60
80
100
120
140

Figure 5.

A


Cumulative Studies
Cumulative Estimate
Soderpalm 52.500 (46.499, 58.501)
+ Bianchi 76.086 (30.027, 122.145)
+ Alshaikh 70.525 (39.957, 101.094)
+ Bian 69.181 (45.011, 93.351)
+ Nasomyont 81.331 (58.124, 104.538)
+ Suthar 73.573 (51.800, 95.347)
+ Zacharin 71.897 (52.031, 91.764)
+ Davidson 74.607 (56.046, 93.168)
20
40
60
80
100
120
140

B

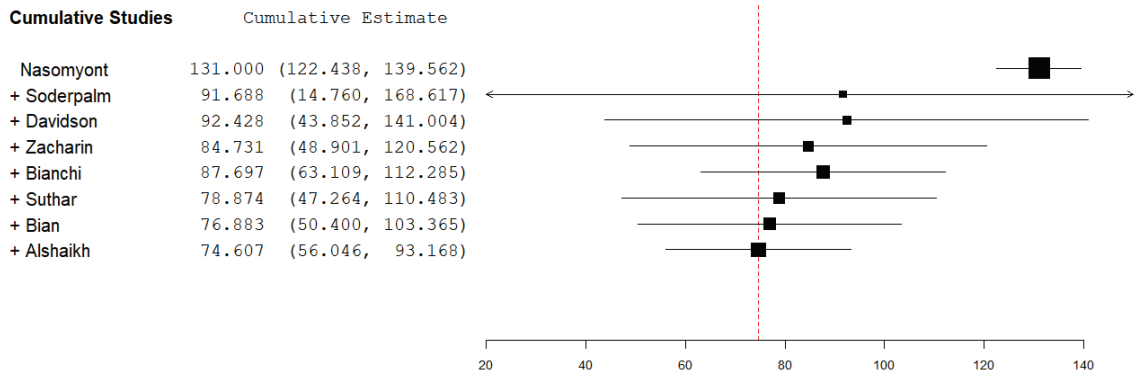
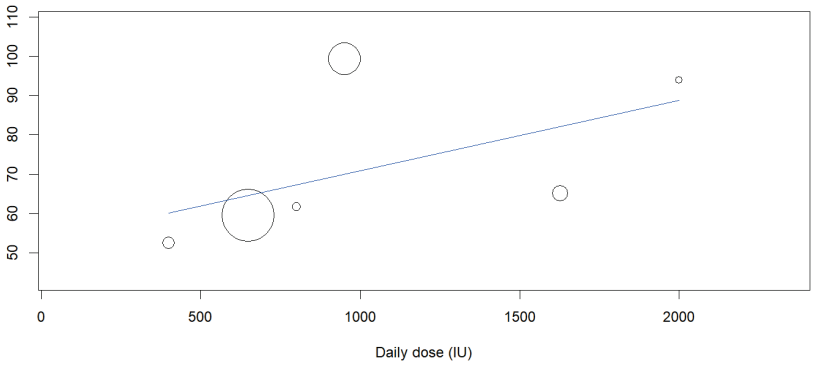
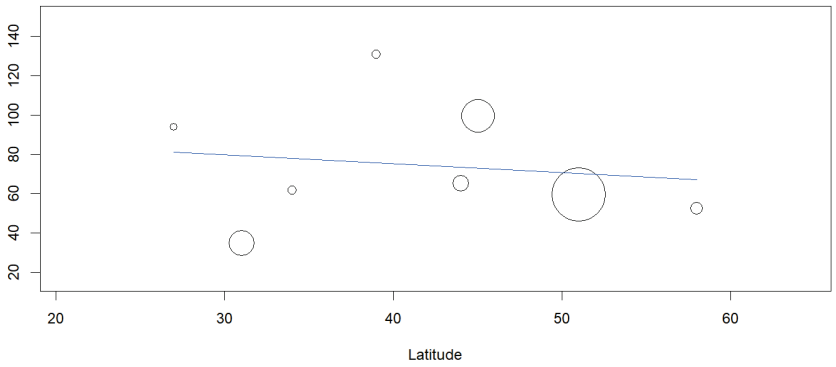

Cumulative Studies
Cumulative Estimate
Nasomyont 131.000 (122.438, 139.562)
+ Soderpalm 91.688 (14.760, 168.617)
+ Davidson 92.428 (43.852, 141.004)
+ Zacharin 84.731 (48.901, 120.562)
+ Bianchi 87.697 (63.109, 112.285)
+ Suthar 78.874 (47.264, 110.483)
+ Bian 76.883 (50.400, 103.365)
+ Alshaikh 74.607 (56.046, 93.168)
20
40
60
80
100
120
140

Figure 6.



P = 0.107



P = 0.66

Supplementary Figure 1.

A

	Was true randomization used?	Was allocation to treatment groups concealed?	Were treatment groups similar at the baseline?	Were participants blind to treatment assignment?	Were those delivering treatment blind to treatment assignment?	Were outcomes assessors blind to treatment assignment?	Were treatment groups treated identically other than the intervention of interest?	Was follow up complete and if not, were group differences adequately described and an	Were participants analyzed in the groups to which they were randomized?	Were outcomes measured in the same way for treatment groups?	Were outcomes measured in a reliable way?	Was appropriate statistical analysis used?	Was the trial design appropriate?	
Zacharin et al.	N	N	Y	N	N	Y	N	Y	Y	Y	Y	Y	Y	RCT
Davidson et al.	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	U	Y	Y	RCT

B

	Were the criteria for inclusion in the sample clearly defined?	Were the study subjects and the setting described in detail?	Were objective, standard criteria used for measurement of the condition?	Were confounding factors identified?	Were strategies to deal with confounding factors stated?	Were the outcomes measured in a valid and reliable way?	Was appropriate statistical analysis used?	Were the outcomes measured in a valid and reliable way?	Was appropriate statistical analysis used?	
Söderpalm et al.	N	Y	Y	Y	Y	Y	Y	Y	Y	Cross-sectional
Razmdjou et al.	Y	Y	Y	Y	Y	Y	Y	Y	Y	Cross-sectional
Doulgeraki et al.	Y	Y	Y	Y	Y	Y	Y	Y	Y	Cross-sectional
Naume et al.	Y	Y	Y	Y	Y	Y	Y	Y	Y	Cross-sectional

Table 1. Summary table of included study characteristics

Study	Year	Study type	Vitamin D supplementation effectiveness as primary aim?	Study location	n	Age in years	Vitamin D testing method	Dose	Steroid use	Included in meta-analysis?
Söderpalm *et al.*	2007	Cross-sectional study on bone health	No	Sweden	24	Mean ± SD: 11.9 ± 5.2	RIA	400 IU recommended dosage	Prednisolone (16 patients)	Yes
Bianchi *et al.*	2011	Prospective study to evaluate vitamin D and calcium treatment	Yes, on BMC and BMD	Italy	33	Mean ± SD: 8.4 ± 2.6	RIA	32 IU/kg	Prednisolone (all)	Yes, mean and SD imputed from GraphReader
Razmdjou *et al.*	2015	Cross-sectional study on bone density	No	Germany	20*	*Mean ± SD: 11.6 ± 3.7	ELISA	Unknown	*Prednisolone (at least 4 patients), Deflazacort (at least 3 patients)	No (insufficient data)
Alshaikh *et al.*	2016	Retrospective case note review to assess efficacy of vitamin D supplementation	Yes, on serum levels	United Kingdom	197	Mean: 9.7 (range 2–18)	LC/MS/MS	Weighted mean: 648 IU 200 IU (22 samples), 400 IU (182 samples), 800 IU (97 samples), 1000 IU (81 samples), 1500 IU (14 samples)	69% of samples were from patients taking corticosteroids	Yes, weighted mean and SD computed from all daily dose groups
Perera *et al.*	2016	Retrospective case note review to assess natural history of fractures and vitamin D deficiency	No	Australia	48	Mean ± SD: 13.6 ± 4.7	Unknown	1000 IU prescribed in most cases	33 patients (69%) received chronic corticosteroid therapy	No (insufficient data)
Doulgeraki *et al.*	2016	Cross-sectional study on body composition	No	Greece	42	Median (range): 9.5 (12.4)	ECLIA	400 IU	Prednisolone or deflazacort (all)	No (insufficient data)
Bian *et al.*	2019	Retrospective case note review on vitamin D status	No	Canada	83*	*Mean (SD): 10.3 (3.8)	Unknown	*Mean dose: 1627 IU ± 1011 SD	*87% taking glucocorticoids	Yes
Nasomyont *et al.*	2020	Prospective study on the safety and efficacy of teriparatide treatment	No	United States of America	6	Mean ± SD [range]: 17.9 ± 3.2 [13.9, 22.1]	Unknown	Unknown maintenance dose	Concurrent glucocorticoid therapy (all)	Yes, SD computed from range
Zacharin *et al.*	2021	RCT on the use of	No	Australia & New	62	Eligible	CMIA	800 IU	All: prednisolone (76%),	Yes

		zoledronic acid		Zealand		range: 6-16			deflazacort (24%)	
Suthar *et al.*	2021	Prospective observational study on bone health	No	North India	76	Median: 8.5, IQR= 7-10.7	LC/MS	Unknown maintenance dose	All: 56 (74%) prednisolone, and 20 (26%) deflazacort	Yes
Vather-Wu *et al.*	2021	Retrospective case note review to determine vitamin D level stability	Yes, on serum stability	United States of America	27 *	Median: 15, IQR= 2	Multiple (majority ECLIA)	*Median dose: 2000 IU	*93% on glucocorticoids	No (incompatible data)
Davidson *et al.*	2021	RCT on effects of a standard versus enhanced nutritional supplement	No	Australia	36	Eligible range: 5-13	Unknown	2000 IU	All taking corticosteroids	Yes, SD computed from 95 % CI
Naume *et al.*	2023	Cross-sectional study on metabolic and nutritional health	No	Denmark	23 *	*Mean (SD): 9.3 (4–16)	Unknown	Mean dose: 680 IU	20/23 taking glucocorticoids	No (insufficient data)

*BMC: bone mineral content; BMD: bone mineral density; RCT: randomised controlled trial; IQR: interquartile range; RIA: radioimmunoassay; ELISA: Enzyme-linked immunosorbent assay; LC/MS/MS: Liquid chromatography coupled to tandem mass spectrometry; ECLIA: electro-chemiluminescence immunoassay; CMIA: chemiluminescence microparticle immunoassay; IU: international unit. *These studies included other patient groups, the data listed refer to DMD patient groups only.*

Table 2. Evidence supporting vitamin D deficiency amongst DMD patients after supplementation

Study	% deficient	Definition of deficiency	Daily dose (IU)
Söderpalm *et al.* (2007)	Not reported	n/a	400
Bianchi *et al.* (2011)	Not reported	n/a	950
Razmdjou *et al.* (2015)	0	<25 nmol/l	Unknown
Naume *et al.* (2023)	30	<25 nmol/l	680
Alshaikh *et al.* (2016) (weighted)	6	<25 nmol/l	648
Alshaikh cohort 1	23	<25 nmol/l	200
Alshaikh cohort 2	10	<25 nmol/l	400
Alshaikh cohort 3	2	<25 nmol/l	800
Alshaikh cohort 4	0	<25 nmol/l	1000
Alshaikh cohort 5	0	<25 nmol/l	1500
Perera *et al.* (2016)	38	<50 nmol/l	1000
Doulgeraki *et al. (*2016)	65	<50 nmol/l	400
Bian *et al.* (2019)	25	<50 nmol/l	1627
Zacharin *et al.* (2021)	43	<50 nmol/l	800
Suthar *et al.* (2021)	89	<50 nmol/l	Unknown
Nasomyont *et al.* (2020)	0	<75 nmol/l	Unknown
Vather-Wu *et al.* (2021)	22	<75 nmol/l	2000
Davidson *et al.* (2021)	19	<75 nmol/l	2000