Vitamin D Supplementation in Children. Oral versus Parenteral! D2 versus D3!

BY:

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Abstract:

Background: The global prevalence of vitamin D deficiency has been studied thoroughly. Either ergocalciferol (vitamin D2) or cholecalciferol (vitamin D3) supplements treat such deficiency. However, their relative efficacy was explored in many researches.

Aim: To compare the effects of vitamin D supplementation with either the enteral or parenteral formulations of cholecalciferol and ergocalciferol on raising serum 25-hydroxyvitamin D levels in children.

Methods: This is a randomised controlled clinical trial that included 120 Egyptian school-aged children (5-10 years) randomly selected from the Pediatric outpatient clinic at Ain Shams University Hospitals from January 2021 to December 2022. Sequential randomization allocated the participants into 4 equal groups. Group A: received 10,000 IU of oral ergocalciferol every 4 days for 3 months, Group B: received 2400 IU of oral cholecalciferol daily for 3 months, Group C: received 200,000 IU of intramuscular ergocalciferol once, and Group D: received 200,000 IU of intramuscular cholecalciferol once. serum 25(OH)D was measured at baseline, 1,2, and 3 months after supplementation.

Results: The mean ages of the recruited children were 7.40±1.33, 7.40±1.45, 8.28±2.02, and 7.23±1.65 years for groups A, B, C, and D respectively. Injectable vitamin D3 achieved the highest increments in serum 25 (OH) D after 3 months of supplementation followed by injectable vitamin D2, oral vitamin D3 and oral vitamin D2 respectively. 100% of injectable vitamin D3 recipients, 76.7 % of injectable vitamin D2 recipients, 23.3% of oral vitamin D3 recipients and 20 % of oral vitamin D2 recipients achieved sufficient vitamin D levels after 1 month of supplementation. Compliance with oral therapy was assured by asking the patients to return empty bottles.

Conclusion: A loading dose of intramuscular vitamin D3 200.000 IU is the most potent, cost-effective and rapid regimen in correcting vitamin D deficiency/insufficiency in children and sustains sufficient 25 (OH) D levels up to 3 months after injection, followed by injectable vitamin D2, oral vitamin D3 and oral vitamin D2 respectively.

Keywords: vitamin D deficiency, ergocalciferol, cholecalciferol, pediatrics, parenteral vitamin D2, oral D2, parenteral vitamin D3, oral vitamin D3.

Introduction

Vitamin D exists in two forms: ergocalciferol (D2) and cholecalciferol (D3). Cholecalciferol is available in animal-source foods or formed in the skin on exposure to ultraviolet-B radiation . Ergocalciferol is found in plant sources, including mushrooms and yeast. Vitamin D2 and vitamin D3 are subjected to two different hydroxylation reactions to achieve biological activity. The first enzymatic hydroxylation occurs in the hepatocytes, converting D2 and D3 to 25(OH)D2 and 25(OH)D3, respectively. This is followed by renal hydroxylation, converting 25(OH)D2 and 25(OH)D3 to the active forms (1,25 dihydroxy vitamin D2) and (1,25 dihydroxy vitamin D3) respectively (Institute of Medicine Committee to Review Dietary Reference Intakes for Vitamin and Calcium, 2011). Serum total 25(OH)D is considered a reflection of the vitamin D status (DeLuca, 1979). Both ergocalciferol and cholecalciferol were believed to have similar potency in curing vitamin D deficiency rickets (Institute of Medicine Committee to Review Dietary Reference Intakes for Vitamin and Calcium, 2011).

Vitamin D deficiency represents a universal health problem. In a study conducted on healthy Egyptian adolescents, inadequate vitamin D levels were found in 99% of the studied adolescents, 94.8% were vitamin D deficient and 4.2% were insufficient with a higher prevalence of vitamin D deficiency in girls than boys (Sherief et al., 2021). The predisposing factors could be multi-factorial including sociocultural practices of avoiding sun exposure, restricted diet, environmental pollution, obesity, and genetic causes (Gil et al., 2018).

Even tropical countries with abundant sunlight have a high prevalence of vitamin D deficiency in both adults and children (G and Gupta, 2014), (Health et al., 2019).

Eradicating vitamin D deficiency has great benefits to public health. To improve vitamin D status, multiple regimens of therapeutic supplementation and/or food fortification are usually tried. Both vitamers (D2 and D3) have proven efficacy in raising serum levels of 25 (OH) D when used as a single large bolus or given in divided doses by oral and parenteral routes (Garg et al., 2013, Khadgawat et al., 2013). However, the relative efficacy of both vitamers remains unclear. Many national guidelines regarding food fortification recommend an equal dose of D2 and D3 assuming similar biological activities and potency of both vitamers (Ghoul, 2013). The equivalent potency of the two vitamers is based on various studies regarding the prevention and cure of rickets with either of the two vitamers in either experimental animals or humans (Shohl et al., 1928). Evaluation of the relative efficacy of D2 and D3 in raising serum 25 (OH)D is essential to improve the quality of food fortification programs, this needs further studies comparing increments in vitamin D levels from baseline with different doses, routes, frequency of dosing and duration of supplementation of the 2 forms of vitamin D.

Ethical considerations:

- **Ethics approval:** The research was reviewed and approved by the local ethics committee of the Faculty of Medicine, Ain Shams University, approval no. FMASU MS 693/2021 following the Declaration of Helsinki.
- **Consent to participate**: informed consent was obtained from the parents or guardians of the patients
- **Availability of data and materials:** The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.
- **Competing interests:** The authors declare that they have no competing interests.
- **Funding:** No external funding sources received
- **The patient has the right to withdraw from the study at any time.**
- **Cnfidentialy of the data and results of the study.**

Sample size calculation:

The estimated sample size was statistically calculated, and the expected effect size of difference is between medium to large. A sample size of at least 26 cases per group totalling 104 achieves a power of at least 80 % to detect an effect size of 0.8 (a large effect size) comparing the differences by independent t-test with a level of significance of 0.05. To consider the dropouts the sample should increase to include 30 cases per group totaling 120 cases in the 4 groups (Tripkovic et al., 2012). Accordingly, a total of 120 children were recruited. Sequential randomisation was performed to divide them into 4 groups with 30 participants in each group.

- ***The inclusion criteria*** were laboratory
- evidence of vitamin D deficiency or insufficiency without clinical signs of rickets in children aged 5 – 10 years after obtaining informed consent from the parents or guardians of the patients.
- ***The exclusion criteria*** were vitamin D or calcium supplementation within the last 6 months before inclusion, patients with chronic diseases such as diabetes mellitus, chronic liver disease and kidney disease, congenital or rheumatic heart disease, malabsorption syndromes, thyroid disease and juvenile rheumatic diseases or other bone disorders.
- **Study Procedure:** all the studied children were subjected to the following

a) **Full history taking** to exclude chronic illnesses, skeletal dysplasias, symptoms or signs of rickets or inherited forms of rickets, developmental history.

b) **A thorough clinical examination** was performed, and various anthropometric measurements including weight, height, and body mass index (BMI) were measured with

the calculation of weight SDS, height SDS and BMI SDS (Cole et al., 2007).

- Total serum calcium, serum PO4, ALP and PTH were measured at baseline in serum
- 25 (OH) D levels were measured in serum at baseline and after 1, 2 and 3 months of supplementation using enzyme

c) **Laboratory evaluation including:**

using a (Cobas 5000 analyser, Roch Dagnostics)

immunoassay test kit 96-well catalogue number 10501 by (PerkinElmer (USA)).

Intervention:

The commercially available intramuscular (IM) cholecalciferol formulation (200,000IU) taken once every 3 months was used as the standard regimen in our trial, a similar dose of IM ergocalciferol was used, oral ergocalciferol and cholecalciferol doses were chosen to be 2400-2500 IU daily to reach a cumulative dose of nearly 200,000 units after 3 months in each. Group A: oral vitamin D2 group, received 10,000 IU of ergocalciferol every 4 days for 3 months, Group B: oral vitamin D3 group received 2400 IU of cholecalciferol daily for 3 months, Group C: parenteral vitamin D2, group received IM 200,000 IU of ergocalciferol once, and Group D: parenteral vitamin D3 group received IM 200,000 IU of cholecalciferol once.

Outcomes: The primary outcome was comparing the increments in serum 25(OH)D after 1,2 and 3 months of supplementation with either forms of vitamin D2 or D3. The secondary outcomes were comparing the percentage of patients reaching sufficiency at different time points after supplementation between the different routes and forms of vitamin D. According to the Endocrine Society's definition, vitamin D deficiency was defined as serum 25(OH)D below 20 ng/mL, and vitamin D insufficiency was defined 25(OH)D of 20 ng/mL to less than 30 ng/mL (Holick et al., 2011).

Statistical analysis:

The statistical package for social sciences, version 23.0 (SPSS Inc., Chicago, Illinois, USA), was used to analyze the recorded data. Mean ± standard deviation and ranges were used to present quantitative data when normally distributed, while non-parametric data were presented as median with inter-quartile range (IQR). For qualitative variables, numbers and percentages were used. Kolmogorov-Smirnov and Shapiro-Wilk Tests were used to explore the data for normality. One-way analysis of variance (ANOVA) was used to compare more than two means, and Post HOC: Tukey's test was used for multiple comparisons between different variables. Kruskall Wallis test was used for multiple-group comparisons in non-parametric data, and Mann Whitney U test was used for two-group comparisons in non-parametric data. An independent-sample t-test was used to compare between two means. The comparison between groups with qualitative data was done using the Chi-square test. Fisher's exact test was used instead of the Chi-square test only when the expected count in any cell was less than 5. The margin of error accepted was set to 5%, and the confidence interval was set to 95%.

Results

Our result will be demonstrated in the following tables and figure:

The mean ages of the studied children were 7.40±1.33, 7.40±1.45, 8.28±2.02, and 7.23±1.65 for groups A, B, C and D respectively, with insignificant differences in gender distribution among the studied groups.

Table (1): Comparison between the 4 studied groups regarding baseline anthropometric measurements

Baseline anthropometric measurements	**Group A: Oral vitamin D2 (n=30)**	**Group B: Oral vitamin D3 (n=30)**	**Group C: Injectable vitamin D2 (n=30)**	**Group D: Injectable vitamin D3 (n=30)**	**Test value**	**p-value**
Weight/kg						
Mean ± SD	26.63±5.77	26.67±5.46	26.88±6.19	24.00±6.47	1.5	0.202
Range	16-38	16-37	16.5-36	15.8-39		
Weight SDS						
Median (IQR)	**0.54 (0.15 to 1.12)**	**0.61 (0.21 to 1.15)**	**0.00 (-0.29 to 0.34)**	**-0.17 (-0.62 to 0.34)**	8.9	0.000
Range	-1.03-2.850	-1.85-2.250	-0.81-0.950	-1.28-1.380		
Height/cm						
Mean±SD	122.17 ± 9.24	119.27±10.60	126.18±11.50	121.88 ± 11.46	2.6	0.052
Range	105-140	103-140	104-139.7	105-143		
Height SDS						
Mean±SD	0.04±0.83	-0.58±1.23	-0.30±0.44	-0.27±0.52	2.8	0.042
Range	-1.28-2.7	-2.48-2.87	-1.24-0.88	-1.26-0.67		
Tanner staging						
I	29 (96.7%)	28 (93.3%)	29 (96.7%)	29 (96.7%)	0.6	0.890
II	1 (3.3%)	2 (6.7%)	1 (3.3%)	1 (3.3%)		

This table shows the baseline anthropometric measurements with insignificant differences between the studied groups.

Table (2): Comparison between the 4 studied groups according to baseline laboratory parameters.

Laboratory data	**Group A: Oral vitamin D2 (n=30)**	**Group B: Oral vitamin D3 (n=30)**	**Group C: Injectable vitamin D2 (n=30)**	**Group D: Injectable vitamin D3 (n=30)**	**Test value**	**p-value**
Serum calcium (mg/dl)						
Mean±SD	8.60±0.36	8.45±0.32	8.54±0.49	8.44±0.36	1.154	0.330
Range	8.1-9.7	8.1-9.5	7.9-10	7.9-9.5		
Serum Phosphorus (mg/dl)						
Mean±SD	3.91±0.65	3.67±0.62	3.81±0.64	3.74±0.54	0.853	0.468
Range	2.9-5.4	2.5-5.1	2.6-5.6	2.5-4.9		
Serum Alkaline phosphatase (U/L)						
Mean±SD	301.40±95.82	251.63±83.78	248.10±113.95	239.97±69.76	2.733	0.047
Range	113-471	125-420	111-505	122-350		
Serum Parathyroid Hormone (pg/ml)						
Mean±SD	37.23±8.95	38.83±11.56	51.40±15.96	54.47±14.28	13.561	0.000
Range	22-66	19-66	20-82	33-79		

This table shows the baseline laboratory parameters with insignificant differences between the studied groups except for serum Parathyroid hormone, a significant difference was found but all within normal range.

Table (3): Comparison between the studied groups according to the degree of deficiency at the baseline levels of 25 (OH)D.

Baseline 25 OHD	**Group A: Oral vitamin D2 (n=30)**	**Group B: Oral vitamin D3 (n=30)**	**Group C: Injectable vitamin D2 (n=30)**	**Group D: Injectable vitamin D3 (n=30)**	**Test value**	**p-value**
Severe deficiency (<10 ng/ml)	6 (20.0%)	8 (26.7%)	8 (26.7%)	16 (53.3%)	32.2	0.000
Deficiency (10 - <20 ng/ml)	14 (46.7%)	8 (26.7%)	21 (70.0%)	13 (43.3%)		
Insufficiency (≥20-30 ng/ml)	10 (33.3%)	14 (46.7%)	1 (3.3%)	1 (3.3%)		

Groups C and D had a significantly higher percentage of patients with severe vitamin D deficiency in comparison to groups A and B.

Table (4): Comparison between the 4 groups regarding 25 (OH)D levels before and after vitamin D supplementations and increments in 25(OH)D at different time points from supplementation (ng/ml)

25(OH)D (ng/ml)	**Group A: Oral vitamin D2 (n=30)**	**Group B: Oral vitamin D3 (n=30)**	**Group C: Injectable vitamin D2 (n=30)**	**Group D: Injectable vitamin D3 (n=30)**	**Test value**	**p-value**
Baseline 25(OH)D (ng/ml)						
Mean ± SD	15.17±5.93	15.07±3.53	11.50±3.15	9.67±2.04	8.5	0.000
Range	5-28	5-28	7-20	4-20		
25(OH)D level (ng/ml) at 1 month						
Mean ± SD	24.27±4.92	24.30±6.76	35.57±8.27	51.90±8.13	100.0	0.000
Range	15-38	13-42	25-62	42-71		
25(OH)D level (ng/ml) at 2 months						
Mean ± SD	33.00±6.60	40.87±8.56	45.70±8.75	63.83±8.36	77.9	0.000
Range	20-45	25-58	32-69	50-84		
25(OH)D level (ng/ml) at 3 months						
Mean ± SD	43.07±5.67	60.20±6.28	53.67±7.32	72.60±9.49	85.1	0.000
Range	30-50	45-70	40-73	55-93		

25(OH)D increment after 1 month						
Mean ± SD	9.50±2.31	9.27±2.00	24.17±9.66	42.37±10.75	98.8	0.000
Range	-2_25	-2_22	11-52	22-65		
25(OH)D increment after 2 months						
Mean ± SD	8.80±2.78	17.23±4.35	34.30±8.81	54.30±11.06	179.8	0.000
Range	0-20	8-28	18-59	35-78		
25(OH)D increment after 3 months						
Mean ± SD	10.23±2.15	19.80±5.06	42.33±8.47	63.07±12.06	224.5	0.000
Range	2-20	7-40	26-63	41-86		
25(OH)D increment from one month to two months						
Mean ± SD	8.83±2.05	16.57±4.14	10.27±2.72	12.07±2.63	20.4	0.000
Range	0-20	8-28	5-15	7-17		
25(OH)D increment from one month to three months						
Mean ± SD	18.80±4.89	35.90±6.19	18.30±3.15	20.87±4.23	93.1	0.000
Range	8-28	23-50	11-25	13-29		
25(OH)D increment from two months to three months						
Mean ± SD	10.07±2.13	19.33±5.58	8.07±2.60	8.87±2.84	34.3	0.000
Range	0-20	7-40	4-14	5-16		

Injectable D3 (group D) group had the highest 25(OH) D level and increment from baseline after 1 month from supplementation followed by the injectable D2 group (group C) (Table 3), Daily oral supplementation with 2400 IU of vitamin D3 for 3 months achieved a higher serum 25(OH) D level at 3 months of supplementation with a (mean +/- SD) of 60.20 ± 6.28 ng/ml in comparison to 43.07±5.67 ng/ml after 3 months of oral vitamin D2 supplementation at a daily dose of 2400 IU. Injectable D2 was superior to oral D2, also injectable D3 was superior to oral D3 supplementation when comparing serum 25(OH) D levels and increments after 1, 2 and 3 months of supplementation.

Table (5): Comparison between groups regarding the percentage of patients who reached sufficiency after 1, 2 and 3 months.

Time to reach sufficiency in 25-OHD level	**Group A: Oral vitamin D2 *(n=30)***		**Group B: Oral vitamin D3 *(n=30)***		**Group C: Injectable vitamin D2 *(n=30)***		**Group D: Injectable vitamin D3 *(n=30)***		**Test value**	**p-value**
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**		
One Month	6	20.0%	7	23.3%	23	76.7%	30	100.0%	24.5	<0.001**
Two Months	16	53.3%	22	73.3%	7	23.3%	0	0.0%		
Three Months	8	26.7%	1	3.3%	0	0.0%	0	0.0%		

100% of group D patients, 76.7 % of group C, 23.3% of group B and 20 % of group A patients reached sufficiency (25(OH) D) level >30 ng/ml) after 1 month of supplementation despite the fact that group D had the highest percentage of patients with severe vitamin D deficiency (53.3%)

Table (6): Comparison between groups regarding the time in months to reach sufficiency in 25 (OH) D Level.

Time in months to reach sufficiency in 25- OHD Level	**Group A: Oral vitamin D2 (n=30)**	**Group B: Oral vitamin D3 (n=30)**	**Group C: Injectable vitamin D2 (n=30)**	**Group D: Injectable vitamin D3 (n=30)**	**Test value**	**p-value**
Median (IQR)	**2 (2-3) A**	**2 (2-2) A**	**1 (1-1) B**	**1 (1-1) B**	61.0	0.000
Range	1-3	1-3	1-2	1-1		

IQR: Interquartile range

The median time in months to reach vitamin D sufficiency in each group is explained.

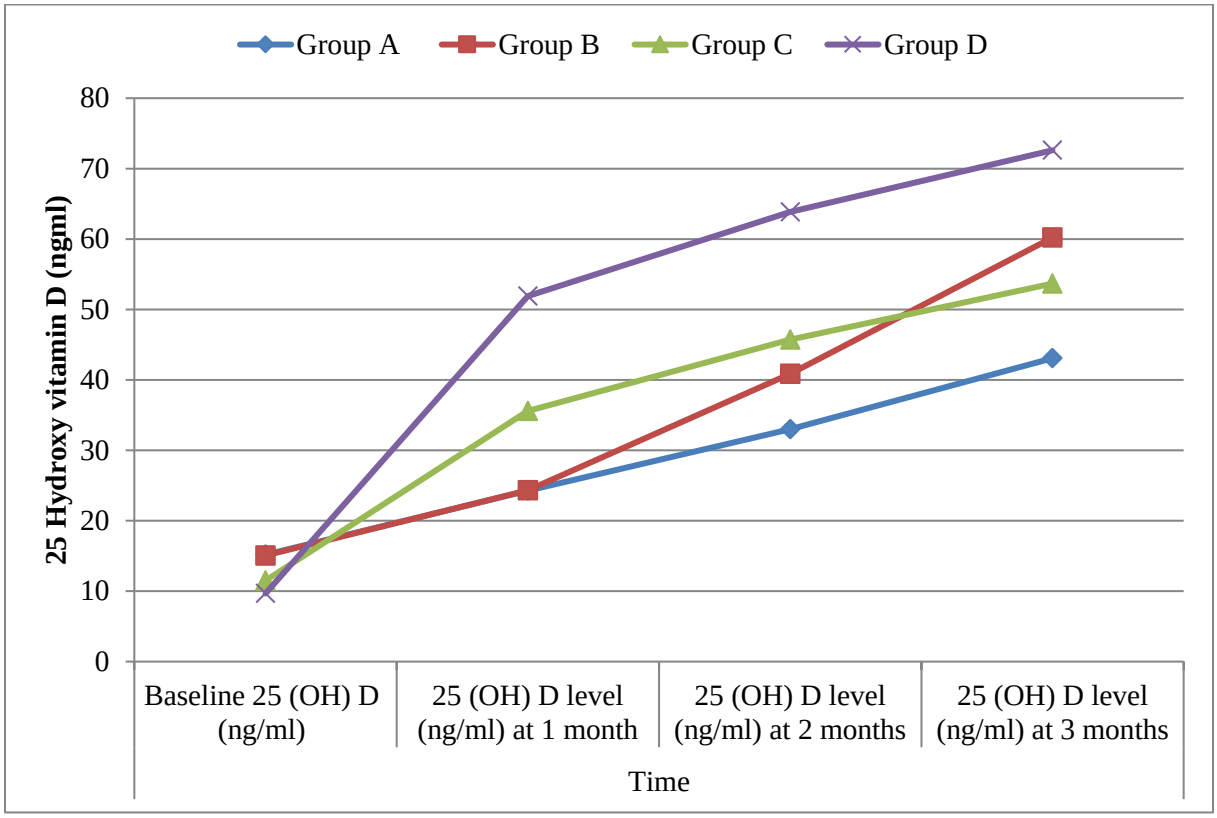

Figure. (1): Comparison between groups according to 25 hydroxy vitamin D (ng/ml).

No significant difference was noticed between oral vitamin D2 and oral vitamin D3 in the ability to raise serum 25(OH) D level after 1 month of supplementation, while oral vitamin D3 supplementation was superior to oral vitamin D2 in raising serum 25(OH)D level from one to two months, two to three months and from one to three months after supplementation, (Figure.1).

Discussion:

In our study, we compared 25 (OH) D levels in four groups of vitamin D-deficient children after receiving different modalities of vitamin D replacement over 3 months period. The degree of deficiency in vitamin D was classified according to baseline 25(OH) D serum levels into severe deficiency for serum 25(OH) D levels <10 ng/ml, deficiency for serum 25(OH) D levels ranging from (10 - <20) ng/ml and insufficiency for serum 25 (OH) D levels (≥20-30) ng/ml. Because some studies have shown rickets can manifest in patients with 25(OH)D concentration up to 20 ng/ml (Spence and Serwint, 2004), as well as the higher values that are needed for non-bony outcomes (Abrams, 2011). Also, a serum 25 (OH) D level of 30–32 ng/ml results in maximal suppression of PTH, optimal intestinal calcium absorption and prevention of fractures (Boonen et al., 2007), (Khazai et al., 2008), (Heaney et al., 2003). Accordingly, we used 30 ng/ml as a cut-off value for vitamin D sufficiency. We chose the dose of vitamin D supplementation near the

upper limit allowed for the pediatric age group due to various factors related to family habits hindering proper sun exposure (Tsiaras and Weinstock, 2011). A daily dose of 2400 IU was used for the oral D3 daily regimen, 10,000 IU were used for oral D2 every 4 days and a single IM dose of 200000 IU was used for both D2 and D3, the daily dose is close to the dose recommended by the global consensus for prevention and management of nutritional rickets (Munns et al., 2016).

When comparing the effect of oral D3 to oral D2, a nearly equal rise in serum 25(OH) D levels was observed after one month of supplementation demonstrating comparable oral absorption and hepatic hydroxylation of both vitamins to 25- hydroxy metabolites in our studied patients. Still, in the subsequent 2 months, oral D3 achieved higher increments in serum 25(OH) D levels than oral D2 which could be explained by the more rapid clearance of ergocalciferol (Romagnoli et al., 2008b).

A single dose of IM vitamin D3 at a dose of 200,000 IU succeeded in achieving sufficiency in 100% of group D patients after 1 month despite having lower baseline serum 25(OH)D levels in that group. Single-dose injectable Vitamin D can be better tolerated and increase patients' compliance, in addition to its cost-effectiveness in developing countries. The pharmacokinetics of IM D3 administration and lack of serum 25(OH) D level fluctuations after IM administration suggest its advantage, especially in obesity, malabsorption, or poor compliance (Vieth, 2011). However, judicial use of the parenteral route must be assured to avoid hypercalcemia, hypercalciuria, and Vitamin D toxicity (Kaur et al., 2015).

Both oral and injectable forms of vitamin D3 were superior to oral and injectable forms of D2 in raising and maintaining serum 25(OH) D levels after 1, 2 and 3 months of vitamin D supplementation, Although both vitamins D2 and D3 were believed to undergo identical hydroxylation processes that result in the same active metabolite (calcitriol), recent data suggests that there may be a difference in their efficacy in raising serum 25(OH)D, the established marker of vitamin D status (Trang et al., 1998), (Romagnoli et al., 2008a),. The proposed differences between the two calciferols are attributed to their differing affinities for the vitamin D receptor (VDR), as well as an additional step of 24-hydroxylation that inactivates calcitriol (Houghton and Vieth, 2006). Additionally, vitamin D3 is possibly the preferred substrate for hepatic 25-hydroxylase (Holmberg et al., 1986), which, combined with the potential difference in the 24-hydroxylation rate, may explain the superiority of vitamin D3 to vitamin D2 in supplementation. In a study by (Balachandar et al., 2021) vitamin D3 has proven superiority in raising serum 25 (OH) D regardless of the route of administration either oral, parenteral or supplementation of various foods as orange juice, biscuits or bread. However, other clinical trials couldn't prove a difference in the efficacy of D2 compared with D3 which indicates that other underlying factors need to be studied as genetic differences in the manipulation of drugs (Holick et al., 2008), (Biancuzzo et al., 2010).

A systematic review and meta-analysis comparing various regimens of vitamin D supplementation in adults showed the superiority of vitamin D3 over vitamin D2 regimens in raising serum 25(OH) D levels (Tripkovic et al., 2012).

However, further studies are needed in pediatrics in various populations to reach the best cost-effective regimen of supplementation.

Conclusion and Recommendations: Vitamin D3 is superior to vitamin D2 in correcting vitamin D deficiency/ insufficiency**,** A loading dose of intramuscular vitamin D3 around 200.000 IU is preferable in correcting vitamin D deficiency/insufficiency in pediatrics as it is more potent and cost-effective than oral D3. Oral D3 is superior to oral D2 in correcting vitamin D deficiency. Parenteral D3 is more superior and cost-effective than parentral D2 in correcting vitamin D deficiency.

Limitations: Some patients refused to participate as multiple follow-up visits with follow-up blood samples were an essential part of the study, follow-up of urinary calcium excretion especially after parenteral doses of D2 and D3 would have been of benefit to exclude Calciurea but couldn't be done due to lack of fund.

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References:

ABRAMS, S. A. 2011. Vitamin D requirements in adolescents: what is the target? *Am J Clin Nutr,* 93**,** 483-4.

BALACHANDAR, R., PULLAKHANDAM, R., KULKARNI, B. & SACHDEV, H. S. 2021. Relative Efficacy of Vitamin D2 and Vitamin D3 in improving Vitamin D status: Systematic review and meta-Analysis. *Nutrients,* 13**,** 3328.

BIANCUZZO, R. M., YOUNG, A., BIBULD, D., CAI, M. H., WINTER, M. R., KLEIN, E. K., AMERI, A., REITZ, R., SALAMEH, W., CHEN, T. C. & HOLICK, M. F. 2010. Fortification of orange juice with vitamin D(2) or vitamin D(3) is as effective as an oral supplement in maintaining vitamin D status in adults. *Am J Clin Nutr,* 91**,** 1621-6.

BOONEN, S., LIPS, P., BOUILLON, R., BISCHOFF-FERRARI, H. A., VANDERSCHUEREN, D. & HAENTJENS, P. 2007. Need for additional calcium to reduce the risk of hip fracture with vitamin d supplementation: evidence from a comparative metaanalysis of randomized controlled trials. *J Clin Endocrinol Metab,* 92**,** 1415-23.

COLE, T. J., FLEGAL, K. M., NICHOLLS, D. & JACKSON, A. A. 2007. Body mass index cut offs to define thinness in children and adolescents: international survey. *Bmj,* 335**,** 194.

DELUCA, H. F. 1979. The transformation of a vitamin into a hormone: the vitamin D story. *Harvey Lect,* 75**,** 333-79.

G, R. & GUPTA, A. 2014. Vitamin D deficiency in India: prevalence, causalities and interventions. *Nutrients,* 6**,** 729-75.

GARG, M. K., MARWAHA, R. K., KHADGAWAT, R., RAMOT, R., OBROI, A. K., MEHAN, N., GUPTA, N. & MADAN, R. 2013. Efficacy of vitamin D loading doses on serum 25-hydroxy vitamin D levels in school going adolescents: an open label non-randomized prospective trial. *J Pediatr Endocrinol Metab,* 26**,** 515-23.

GHOUL, I. 2013. Food safety and standards authority of India (FSSAI). *Training Manual for Food Safety Regulators. New Delhi: Food Safety and Standards Authority of India,* 1.

GIL, Á., PLAZA-DIAZ, J. & MESA, M. D. 2018. Vitamin D: Classic and Novel Actions. *Ann Nutr Metab,* 72**,** 87-95.

HEALTH, M. O., FAMILY WELFARE , G. O. I., UNICEF & COUNCIL, P. 2019. Comprehensive national nutrition survey (CNNS) national report. Ministry of Health and Family Welfare New Delhi (India).

HEANEY, R. P., DOWELL, M. S., HALE, C. A. & BENDICH, A. 2003. Calcium absorption varies within the reference range for serum 25-hydroxyvitamin D. *J Am Coll Nutr,* 22**,** 142-6.

HOLICK, M. F., BIANCUZZO, R. M., CHEN, T. C., KLEIN, E. K., YOUNG, A., BIBULD, D., REITZ, R., SALAMEH, W., AMERI, A. & TANNENBAUM, A. D. 2008. Vitamin D2 is as effective as vitamin D3 in maintaining circulating concentrations of 25-hydroxyvitamin D. *J Clin Endocrinol Metab,* 93**,** 677-81.

HOLICK, M. F., BINKLEY, N. C., BISCHOFF-FERRARI, H. A., GORDON, C. M., HANLEY, D. A., HEANEY, R. P., MURAD, M. H. & WEAVER, C. M. 2011. Evaluation, Treatment, and Prevention of Vitamin D Deficiency: an Endocrine Society Clinical Practice Guideline. *The Journal of Clinical Endocrinology & Metabolism,* 96**,** 1911-1930.

HOLMBERG, I., BERLIN, T., EWERTH, S. & BJÖRKHEM, I. 1986. 25-Hydroxylase activity in subcellular fractions from human liver. Evidence for different rates of mitochondrial hydroxylation of vitamin D2 and D3. *Scand J Clin Lab Invest,* 46**,** 785-90.

HOUGHTON, L. A. & VIETH, R. 2006. The case against ergocalciferol (vitamin D2) as a vitamin supplement. *Am J Clin Nutr,* 84**,** 694-7.

INSTITUTE OF MEDICINE COMMITTEE TO REVIEW DIETARY REFERENCE INTAKES FOR VITAMIN, D. & CALCIUM 2011. The National Academies Collection: Reports funded by National

Institutes of Health. ***In:*** **ROSS, A. C., TAYLOR, C. L.,** YAKTINE, A. L. & DEL VALLE, H. B. (eds.) *Dietary Reference Intakes for Calcium and Vitamin D.* Washington (DC): National Academies Press (US)

KAUR, P., MISHRA, S. K. & MITHAL, A. 2015. Vitamin D toxicity resulting from overzealous correction of vitamin D deficiency. *Clin Endocrinol (Oxf),* 83**,** 327-31.

KHADGAWAT, R., MARWAHA, R. K., GARG, M. K., RAMOT, R., OBEROI, A. K., SREENIVAS, V., **GAHLOT, M., MEHAN, N., MATHUR, P. & GUPTA, N. 2013**. Impact of vitamin D fortified milk supplementation on vitamin D status of healthy school children aged 10-14 years. *Osteoporos Int,* 24**,** 2335-43.

KHAZAI, N., JUDD, S. E. & TANGPRICHA, V. 2008. Calcium and vitamin D: skeletal and extraskeletal health. *Curr Rheumatol Rep,* 10**,** 110-7.

MUNNS, C. F., SHAW, N., KIELY, M., SPECKER, B. L., THACHER, T. D., OZONO, K., MICHIGAMI, T., TIOSANO, D., MUGHAL, M. Z. & MÄKITIE, O. 2016. Global consensus recommendations on prevention and management of nutritional rickets. *The Journal of Clinical Endocrinology & Metabolism,* 101**,** 394-415.

ROMAGNOLI, E., MASCIA, M. L., CIPRIANI, C., FASSINO, V., MAZZEI, F., D'ERASMO, E., CARNEVALE, V., SCILLITANI, A. & MINISOLA, S. 2008a. Short and long-term variations in serum calciotropic hormones after a single very large dose of ergocalciferol (vitamin D2) or cholecalciferol (vitamin D3) in the elderly. *J Clin Endocrinol Metab,* 93**,** 3015-20.

ROMAGNOLI, E., MASCIA, M. L., CIPRIANI, C., FASSINO, V., MAZZEI, F., D'ERASMO, E., CARNEVALE, V., SCILLITANI, A. & MINISOLA, S. 2008b. Short and long-term variations in serum calciotropic hormones after a single very large dose of ergocalciferol (vitamin D2) or cholecalciferol (vitamin D3) in the elderly. *The Journal of Clinical Endocrinology & Metabolism,* 93**,** 3015-3020.

SHERIEF, L. M., ALI, A., GABALLA, A., ABDELLATIF, G. M., KAMAL, N. M., AFIFY, M. R., ABDELMALEK, D. H., EL-EMARI, S. A., SOLIMAN, A. S. A. & MOKHTAR, W. A. 2021. Vitamin D status and healthy Egyptian adolescents Where do we stand? *Medicine,* 100**,** 6.

SHOHL, A. T., BENNETT, H. B. & WEED, K. L. 1928. Rickets in Rats. V. Comparison of Effects of Irradiated Ergosterol and Cod Liver Oil. *Proceedings of the Society for Experimental Biology and Medicine,* 25**,** 551-554.

SPENCE, J. T. & SERWINT, J. R. 2004. Secondary prevention of vitamin D-deficiency rickets. *Pediatrics,* 113**,** e70-2.

TRANG, H. M., COLE, D. E., RUBIN, L. A., PIERRATOS, A., SIU, S. & VIETH, R. 1998. Evidence that vitamin D3 increases serum 25-hydroxyvitamin D more efficiently than does vitamin D2. *Am J Clin Nutr,* 68**,** 854-8.

TRIPKOVIC, L., LAMBERT, H., HART, K., SMITH, C. P., BUCCA, G., PENSON, S., CHOPE, G., HYPPÖNEN, E., BERRY, J. & VIETH, R. 2012. Comparison of vitamin D2 and vitamin D3 supplementation in raising serum 25-hydroxyvitamin D status: a systematic review and meta-analysis. *The American journal of clinical nutrition,* 95**,** 1357-1364.

TSIARAS, W. G. & WEINSTOCK, M. A. 2011. Factors influencing vitamin D status. *Acta Dermato Venereologica,* 91**,** 115.

VIETH, R. 2011. Why the minimum desirable serum 25-hydroxyvitamin D level should be 75 nmol/L (30 ng/ml). *Best Practice & Research Clinical Endocrinology & Metabolism,* 25**,** 681-691.