Safety and Effectiveness of Stoss Therapy in Children with Vitamin D Deficiency

Paul Tannous
“Safety and Effectiveness of Stoss Therapy in Children with Vitamin D Deficiency”

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OBJECTIVES: Paediatric vitamin D (25-hydroxyvitamin D - 25OHD) deficiency can lead to nutritional rickets and extra-skeletal complications. Compliance with daily therapy can be difficult, making high dose, short-term vitamin D (stoss) therapy attractive to correct vitamin D deficiency. We compared the effectiveness and safety of standard versus stoss therapy in treating childhood 25OHD deficiency.

STUDY DESIGN: Children aged 2 - 16 years with 25OHD <50nmol/L were randomized to either standard (5,000IU daily for 80 days) or stoss (100,000 IU weekly for 4 weeks) cholecalciferol. Participants underwent evaluation of effectiveness and safety. 25OHD, random spot calcium: creatinine ratio (Ca:Cr) and compliance were measured at 12 weeks.

RESULTS: 151 children were enrolled in the study (68 standard and 83 stoss), median age 9 years (IQR: 6 - 12 years). Baseline 25OHD levels were 26 nmol/L (IQR: 19 - 35 nmol/L) and 32 nmol/L (IQR: 24 - 39 nmol/L) in the standard and stoss groups respectively. At 12 weeks, the median 25OHD level was significantly greater in the standard vs. stoss group (81 vs. 67 nmol/L; p=0.005), however, >80% of participants in both groups achieved sufficiency (25OHD>50nmol/L) and had normal urinary Ca:Cr, with no significant difference seen between groups. Compliance was similar in the two groups.

CONCLUSION: Compared to stoss, standard therapy achieved higher 25OHD levels at 12 weeks; however, in both groups there were a similar proportion of participants who achieved 25OHD sufficiency, with no evidence of toxicity. Unlike other studies, simplifying the treatment regimen did not improve compliance. These results support stoss therapy as an effective and safe alternative therapy for the treatment of paediatric vitamin D deficiency.
Abbreviations

25OHD: 25- hydroxyvitamin D
ALP: Alkaline phosphatase
BMI: Body Mass Index
Ca:Cr: Calcium to creatinine ratio (random spot test)
CHW: Children’s Hospital at Westmead
IQR: Inter-quartile range
PTH: Parathyroid hormone

Statement of Disclosure

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Potential conflict of interest statement: FIT-Bioceuticals provided the high dose stoss formulation as well as funding to support a research coordinator. They did not have any role in the study design; the collection, analysis or interpretation of data, the writing of the report, or the decision to submit the manuscript for publication
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Briefpoints

1. What is already known on this topic?

- Vitamin D deficiency is a significant, but treatable, problem worldwide, resulting in disruption to bone homeostasis and clinical rickets.
- There are a number of Vitamin D formulations that have been studied; however, their safety and effectiveness is variable.

2. What this paper adds?

- This paper provides evidence for the use of this high dose formulation (100,000 IU a week for 4 weeks) as a safe and effective alternative to standard daily therapy.
- Provides physicians with an alternative dosing regime, especially in situations where patients struggle with compliance to the standard daily regime.
Chapter One: Literature Review

Vitamin D is critical for calcium homeostasis and for mineralization of the skeleton, especially during the growing years. Vitamin D is produced primarily by the absorption of UV-B rays from the sun, as well as through the dietary intake. The UV-B rays are absorbed by 7-dehydrocholesterol in the skin to form pre-vitamin D3, which is then quickly transformed into vitamin D through isomerisation(1). Vitamin D is then carried using specific α1-globulin to the liver, where it undergoes conversion to 25-hydroxyvitamin D (calcidiol), which has minimal biological activity. The newly formed calcidiol can go one of two ways. It can either be inactivated and excreted or it can be carried to the kidneys by vitamin D–binding protein, where it undergoes further hydroxylation to form 1,25(OH)2D3 (calcitriol), the major biologically active metabolite(2). Through this pathway, the calcitriol metabolite will be involved in the metabolism of calcium and phosphate, by acting on the intestine to increase the absorption of calcium and phosphate, as well as providing negative feedback to the parathyroid gland and kidneys (2).

In Australia, the majority of infants and children have adequate skin exposure to sunlight for vitamin D synthesis(3). The factors that impact the vitamin D levels, produced by sunlight exposure, include the location and timing of skin exposure, skin colour, age, clothing, sunscreen use and disability(4). The recommended amount of sunlight exposure is 1/3 minimal erythema dose, or MED, which is the minimum amount of sun exposure to produce slight reddening of the skin. However, excessive sun exposure will result in breakdown of pre-vitamin D3 and vitamin D3 into inactive photoproducts(5).
In countries where it is not possible to depend on adequate skin exposure to sunlight for vitamin D synthesis, e.g. Canada, liquid dairy products are fortified with vitamin D and vitamin D supplementation is recommended for all infants and children.

Vitamin D deficiency results in decreased small bowel calcium absorption and thus serum calcium. This in turn leads to a compensatory increase in parathyroid hormone and alkaline phosphatase, and urinary phosphate loss(5). This is costly for the paediatric patient leading to rickets (a mineralization defect at the growth plates) and osteomalacia (a mineralization defect of bone tissue). These effects are associated with pain, fractures, skeletal deformity, growth retardation, dental enamel defects, delayed developmental milestones and, in severe cases, hypocalcaemic tetany and seizures(6). If not recognized and properly treated, simple vitamin D deficiency may have long-term sequelae. On the other hand, the disease is entirely preventable with simple dietary measures or vitamin supplementation and readily treated with vitamin D.

*Epidemiology of vitamin D deficiency in Australia*

Despite the apparent adequacy of sunlight and thus UVB exposure in Australia, and the public health measures introduced in other countries, evidence suggests that simple vitamin D deficiency is increasing in Australia, especially in the immigrant population(7) and in developed countries worldwide(8, 9). Simple vitamin D deficiency is more common in babies born to vitamin D deficient mothers (particularly if exclusively breast fed), children with dark skin, children who cover their skin for cultural reasons and children who get limited exposure to sunlight most commonly in association with chronic disease(7). Within The Sydney
Children’s Hospital Network (Westmead Campus) (SCHN (Westmead Campus)), the two major clinics where children with simple vitamin D deficiency are seen are the Health Assessment for Refugee Kids clinic (HARK) and the Endocrine clinic. HARK commenced at SCHN (Westmead Campus) in May 2005, sees patients on a weekly basis and currently screens for vitamin D deficiency. Approximately 20-25% (n=44) of total refugees seen at HARK clinic in 2005 and 2006, have had vitamin D levels <31 nmol/L, indicating vitamin D deficiency. The endocrine clinic sees approximately one family per week with vitamin D deficiency.

The precise incidence of simple vitamin D deficiency rickets in Australia is unknown; however, in 2006, the incidence of nutritional rickets in Australia was estimated at 4.9/100,000/year, with the majority of cases found in immigrants and refugee populations (10).

Management of Simple Vitamin D Deficiency

A recent consensus statement outlined the current knowledge on the prevention and treatment of simple vitamin D deficiency during infancy and childhood(7). The premise of treating children with simple vitamin D deficiency is to replenish the body stores of vitamin D with either ergocalciferol (vitamin D2) or cholecalciferol (vitamin D3) so as to normalise serum 25-hydroxyvitamin D and alkaline phosphatase concentrations. This requires a total vitamin D dose of between 400,000 and 500,000 IU. The most widely accepted and researched method for this is with a daily dose of 1000 IU for 80 to 90 days(11). It is our experience that adherence to this regimen can be difficult, which results in treatment failure.
Stoss therapy, the provision of the total vitamin D treatment dose (400,000 – 500,000 IU) in 1 to 4 doses, has the potential to improve adherence and thus cure rates. Although used widely in developing countries (12), it is not in routine use in Australia and there are limited data on its efficacy and safety (7).

Summary

This study will compare the safety and efficacy of stoss therapy (100,000 IU cholecalciferol weekly for 4 weeks) with standard therapy (5000 IU ergocalciferol daily for 80 days). This study has the potential to change and improve the clinical management of simple vitamin D deficiency in children in Australia by shortening and simplifying the treatment regimen, which may improve adherence and cure rates.
Chapter Two: Manuscript

Introduction

Vitamin D is crucial for calcium homeostasis and skeletal health throughout the lifespan. It is especially important to recognize and treat 25OHD deficiency in children to prevent osteomalacia and nutritional rickets, which can lead to pain, short stature, skeletal deformities and extra-skeletal complications including hypocalcemic seizure, cardiomyopathy and rarely death (7). 25OHD deficiency results in decreased calcium and phosphorous absorption across the gastrointestinal tract, resulting in calcium deprivation and hypocalcaemia. In response, parathyroid hormone (PTH) is released to stimulate the reabsorption of calcium and excretion of phosphorous via the kidneys. While this may normalize serum calcium levels, it reduces bone mineralization and results in osteomalacia and rickets (13). In addition, the presence of the vitamin D receptor in lymphocytes, beta islet cells and major organs suggests that 25OHD and its metabolites may have important clinical effects outside mineral homeostasis (14). It has been suggested that 25OHD deficiency is associated with various disease processes such as exacerbation of asthma and bronchiolitis (15, 16).

In Australia, the majority of children have adequate exposure to UVB to maintain sufficient serum levels of vitamin D (25OHD >50nmol/L) (3). In 2006, the incidence of nutritional rickets in Australia was estimated at 4.9/100,000/year, with the majority of cases found in immigrants and refugee populations (10). With the recent 2015-2017 global refugee crisis, this is likely to increase (17). Multiple factors can contribute to 25OHD deficiency in children including lack of sun exposure, dark skin colour/ increased skin pigmentation, and
malabsorption. Breastfed infants born to 25OHD deficient mothers are particularly at risk (18).

A global consensus statement on the treatment of nutritional rickets, recommended a daily vitamin D dose, in children greater than 2 years of age, of 3,000-6,000IU per day with calcium supplementation to correct 25OHD deficiency and treat rickets (19). The American Academy of Paediatrics suggests treating 25OHD deficiency in children >12 months with vitamin D therapy of 5,000 IU per day for 2-3 months (11, 13). These recommended doses would be equivalent to a combined total vitamin D dose of between 300,000 and 450,000 IU. Adherence to a daily dosing regimen can be difficult in some patients, in which case a stoss (from the German word stossen ‘to push’) therapy has been recommended (20, 21). Although Stoss therapy is widely used in developing countries (12), it is not routinely used in Australia. There are limited data on its efficacy, safety and effective dosing regimen (20-22).

This study aimed to compare the safety and efficacy of stoss therapy (100,000 IU cholecalciferol weekly for 4 weeks) versus standard therapy (5,000 IU cholecalciferol daily for 80 days).

Methods

Design/Participants

Children between the ages of 2 and 16 years with 25OHD status < 50 nmol/L who were referred to the Endocrinology and/or the Refugee clinic at the Children’s Hospital at Westmead (CHW), Sydney, Australia from 2011 to 2016 were recruited into a randomized controlled trial of standard dose vs. high dose of cholecalciferol (vitamin D3)
supplementation. Children were excluded if they presented one or more of the following: (1) A pre-existing medical condition predisposing to 25OHD deficiency (e.g. malabsorption, liver failure); (2) Current use of any medications known to alter bone metabolism (e.g. bisphosphonates, cholecalciferol, calcitriol, anticonvulsants, barbiturates); or (3) An underlying metabolic or genetic aetiology for rickets (e.g. X-linked hypophosphatemic rickets). All participants were under the care of a paediatric endocrinologist or paediatrician at CHW. Parents or legal guardians of the participants provided informed consent and the study was approved by the Sydney Children’s Hospitals Network Human Research Ethics and Governance Committees (#12SCHN401).

**Intervention**

Using random number tables, participants were randomized by family to receive either standard therapy with cholecalciferol 5000 IU (5000 IU/mL) daily for 80 days or stoss therapy, cholecalciferol 100,000 IU (50,000 IU/mL) weekly for 4 weeks. Both treatments were provided by BioCeuticals. Each batch was assessed by the Quality Team before being released, to ensure adequate but not excessive amounts of cholecalciferol in the products (23). Recognizing the relationship between low dietary calcium intake and vitamin D status in the pathogenesis of osteomalacia and nutritional rickets (17), both groups were also supplemented with 500mg elemental calcium for 4 weeks. Pharmacy study investigators reviewed appropriate administration of the medications with participants or caregivers prior to commencing therapy. All other study investigators were blinded to the treatment allocation.
Data Collection

A questionnaire previously used to collect data for nutritional rickets study (10) was used to collect baseline demographic and nutritional data and risk factors for the development of 25OHD deficiency (Appendix A). All participants underwent a medical visit at baseline, 4 weeks, and 12 weeks. Height was measured using a Harpenden Stadiometer (Holtain Ltd, UK) and weight was measured using the same electronic scale. Participants were questioned to assess for any adverse events such as polyuria, polydipsia, abdominal pain and constipation.

Primary endpoints

Primary endpoints were normalisation of 25OHD and serum alkaline phosphatase (ALP) status at 12 weeks.

Secondary endpoints

Secondary endpoints included raised urinary Ca:Cr ratio, PTH, or elevated serum calcium levels

Compliance Monitoring

Compliance was assessed by counting the number of empty vials returned at week 12 in the 1st group (standard therapy) and week 4 in the 2nd group (Stoss therapy). A patient was noted to be compliant when having returned at least 75% of the vials empty.

Laboratory Measurements and Quality Assurance
Serum and urine biochemistry data were collected at baseline, 4 weeks, and 12 weeks. Serum biochemistry, including calcium, magnesium, phosphate and alkaline phosphatase (ALP), were measured using Vitros 5600 analyser, colorimetric (Ortho Clinical Diagnostics, USA). Parathyroid hormone (PTH) was measured using Immulite Autoanalyser, Chemiluminescence (Siemens Healthcare, UK). Serum 25OHD was measured using Xevo TQ-S (Tandem Quadrapole series) liquid chromatography tandem mass spectrometry (LC-MS/MS) (Waters Pty Ltd, UK) as of July 2015 or IDS iSYS Autoanalyser, Chemiluminescence (Immunodiagnostic Systems Holdings PLC, UK) from 2010 – June 2015. The results between the two methods were comparable (Figure 1). The internal control for the TQS LC-MS/MS is is from ClinChek. The lower control is 25-OH-vitamin D 3 37.1mmol/L and 25-OH-Vitamin D 2 35.5mmol/L. The upper control value is 25-OH-vitamin D 3 105mmol/L and 25-OH-Vitamin D 2 103mmol/L.
Biochemistry clinical ranges and cut-off values are consistent with paediatric norms (24).

Vitamin D status was defined in accordance with the Australian and New Zealand Consensus Statement:(7)

- Sufficiency: ≥ 50nmol/L
- Deficiency: < 50nmol/L

There continues to be discussion surrounding the definition of vitamin D deficiency. The protocol for this study was written and implemented prior to the global consensus guidelines(17). This study uses a higher threshold for vitamin D deficiency, which is consistent with other published guidelines (5, 7, 16, 17, 19).

Statistical Methods

Power calculations estimated a total of 111 participants needed to detect a 10% difference in treatment success between the 2 groups, with 80% power and 5% level of significance. Group differences in primary and secondary endpoints were determined using student t-test for continuous variables and Chi-squared test for categorical data. Statistical calculations for group differences with small outcomes were determined using the Fisher Exact test. All statistical analyses were based on intention to treat principle and performed using SAS version 9.3 and R version 3.2.4.

Results
A total of 170 participants were randomly assigned to the stoss (n = 93) or standard therapy group (n=78; Figure 2). Randomisation occurred by family; therefore, there was a notable difference in sample size between the two groups. Sixteen children were excluded from the study due to 25OHD levels ≥ 50 nmol/L at baseline. A further two participants were excluded because they did not have any recorded 25OHD levels at baseline and one child who was over the age of 16 years. The final number of participants who received treatment was 151, with 68 and 83 in the standard and stoss group, respectively. There were 16 lost to follow up, with absent 25OHD levels at 12 weeks. The final analysis was made up of a total of 135 participants, with 62 and 73 participants in the standard and stoss group, respectively.
Patient characteristics were similar in both groups. The age of the participants ranged from 2 to 16 years with a median of 9 years (Table 1). The majority of the participants were of Middle Eastern and African descent, with the Middle Eastern ethnicity over represented in the stoss group (Table 1). Height, weight and BMI data were similar across both groups (Table 1).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Standard (N=68)</th>
<th>STOSS (N=83)</th>
<th>All (N=151)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female, n (%)</td>
<td>32 (47)</td>
<td>39 (47)</td>
<td>71 (47)</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>median (IQR)</td>
<td>9 (6-12)</td>
<td>8 (5-11)</td>
<td>9 (5-12)</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>African, n (%)</td>
<td>16 (24)</td>
<td>15 (18)</td>
<td>31 (21)</td>
</tr>
<tr>
<td>Asian, n (%)</td>
<td>9 (13)</td>
<td>8 (10)</td>
<td>17 (11)</td>
</tr>
<tr>
<td>Caucasian, n (%)</td>
<td>5 (7)</td>
<td>6 (7)</td>
<td>11 (7)</td>
</tr>
<tr>
<td>Indian subcontinent, n (%)</td>
<td>16 (24)</td>
<td>12 (14)</td>
<td>28 (19)</td>
</tr>
<tr>
<td>Middle Eastern, n (%)</td>
<td>16 (24)</td>
<td>35 (42)</td>
<td>51 (34)</td>
</tr>
<tr>
<td>Other, n (%)</td>
<td>6 (9)</td>
<td>7 (8)</td>
<td>13 (9)</td>
</tr>
<tr>
<td>Characteristic</td>
<td>Standard (N=68)</td>
<td>STOSS (N=83)</td>
<td>All (N=151)</td>
</tr>
<tr>
<td>------------------------</td>
<td>-----------------</td>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>Weight (kg)*</td>
<td>34 (19 - 51)</td>
<td>30 (20 - 39)</td>
<td>30 (19 - 44)</td>
</tr>
<tr>
<td>Weight z-score*</td>
<td>0.4 (-0.6 - 1.0)</td>
<td>0.1 (-0.8 - 0.6)</td>
<td>0.2 (-0.8 - 0.8)</td>
</tr>
<tr>
<td>Height (cm)*</td>
<td>136 (115 - 157)</td>
<td>134 (110 - 146)</td>
<td>134 (114, - 151)</td>
</tr>
<tr>
<td>Height z-score*</td>
<td>0.0 (-0.7 - 1.0)</td>
<td>-0.3 (-1.2 - 0.8)</td>
<td>-0.2 (-1.1 - 0.9);</td>
</tr>
<tr>
<td>BMI* z-score*</td>
<td>0.1 (-0.9 - 0.9)</td>
<td>0.2 (-0.6 - 0.9)</td>
<td>0.1 (-0.8 - 0.9)</td>
</tr>
</tbody>
</table>

*Data available for 61 participants in standard group, 72 participants in stoss group with a total of 133 participants.

* Centre for Disease Control 2000 Growth Charts

* Body Mass Index (BMI)
The median 25OHD level at baseline was significantly lower in the standard group, compared to the stoss group (26 vs. 32nmol/L; p=0.01). The median 25OHD status for both groups increased to sufficient status (≥ 50nmol/L) at 4 and 12 weeks (Figure 3; Table 2). However, the median 25OHD level at 12 weeks was significantly greater in the standard group (81 vs. 67 nmol/L; p=0.005) (Figure 3; Table 2).

Fig. 3: Comparison of 25OHD levels throughout the study. One extreme outlier in the standard therapy group with a 25OHD level of 440nmol/L at 4 weeks was not included in this box plot. (*p<0.05).
Table 2: Median levels of 25OHD, PTH and ALP at baseline, 4 weeks and 12 weeks

<table>
<thead>
<tr>
<th></th>
<th>Standard (N=68)</th>
<th>STOSS (N=83)</th>
<th>p-value$^8$</th>
</tr>
</thead>
<tbody>
<tr>
<td>25OHD (nmol/L): Median ($IQR$); n</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>26 (19 - 35); n=68</td>
<td>32 (24 -39); n=83</td>
<td>0.01</td>
</tr>
<tr>
<td>Week 4</td>
<td>104 (65 -142); n=53</td>
<td>113 (90 -153); n=78</td>
<td>0.10</td>
</tr>
<tr>
<td>Week 12</td>
<td>81 (59 -124); n=62</td>
<td>67 (55 -78); n=73</td>
<td>0.005</td>
</tr>
<tr>
<td>PTH (nmol/L): Median ($IQR$); n</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>4.3 (2.7 -6.2); n=66</td>
<td>4.2 (3.0 -6.8); n=76</td>
<td>0.69</td>
</tr>
<tr>
<td>Week 4</td>
<td>2.5 (1.6 -4.0); n=53</td>
<td>2.8 (1.7 -3.7); n=77</td>
<td>0.81</td>
</tr>
<tr>
<td>Week 12</td>
<td>2.6 (1.7 -4.6); n=62</td>
<td>3.8 (2.3 -5.4); n=74</td>
<td>0.01</td>
</tr>
<tr>
<td>ALP (nmol/L): Median ($IQR$); n</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>218 (184 -307); n=67</td>
<td>221 (155 -281); n=77</td>
<td>0.39</td>
</tr>
<tr>
<td>Week 4</td>
<td>220 (176 -282); n=53</td>
<td>224 (170 -273); n=76</td>
<td>0.98</td>
</tr>
<tr>
<td>Week 12</td>
<td>224 (176 -284); n=63</td>
<td>224 (184 -288); n=72</td>
<td>0.68</td>
</tr>
</tbody>
</table>

$^8$ Calculated using Mann-Whitney-Wilcoxon Test

# Median levels used because the data had a non-normal distribution

Change in 25OHD levels between baseline and 12 weeks was greater in standard (50 nmol/L change; IQR 35 -98 nmol/L) vs. stoss group (35 nmol/L change; IQR 25 -36 nmol/L; p=0.0005). At both 4 and 12 weeks, the proportion of participants who were vitamin D sufficient did not differ between the groups (Table 3). At 4 weeks, there were two children in the standard and one in the stoss group with 25OD levels within the elevated range (>250nmol/L). At 12 weeks, there were two participants in the standard group, and no participants in the stoss group, with elevated levels of 25OHD. None of those with elevated 25OHD status had raised urinary Ca:Cr ratio or elevated serum calcium levels. As both elevated 25OHD levels and hypercalciuria are required for a diagnosis of vitamin D toxicity
(1), none of the participants met these criteria. Therefore, the treatment regimen was continued and further 25OHD measurements were found to be below 250nmol/L.

**Table 3: Proportion of participants with biochemical levels within normal range**

<table>
<thead>
<tr>
<th></th>
<th>Standard</th>
<th>Stoss</th>
<th>OR (STOSS vs. Standard) (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>25OHD below normal limits (&lt;50 nmol/L)</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Week 4</td>
<td>5/53 (9%)</td>
<td>3/78 (4%)</td>
<td>0.39 (0.05 – 2.09)</td>
<td>0.27*</td>
</tr>
<tr>
<td>Week 12</td>
<td>12/62 (19%)</td>
<td>11/73 (15%)</td>
<td>0.74 (0.27, 2.00)</td>
<td>0.67*</td>
</tr>
<tr>
<td><strong>25OHD within normal limits (50-250 nmol/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 4</td>
<td>46/53 (87%)</td>
<td>74/78 (95%)</td>
<td>2.60 (0.59 - 11.40)</td>
<td>0.19</td>
</tr>
<tr>
<td>Week 12</td>
<td>49/62 (79%)</td>
<td>62/73 (85%)</td>
<td>1.35 (0.55 - 3.32)</td>
<td>0.51</td>
</tr>
<tr>
<td><strong>25OHD greater than normal limits (&gt; 250 nmol/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 4</td>
<td>2/53 (4%)</td>
<td>1/78 (1%)</td>
<td>0.33 (0.01, 0.57)</td>
<td>0.56*</td>
</tr>
<tr>
<td>Week 12</td>
<td>1/62 (2%)</td>
<td>0/73 (0%)</td>
<td>0 (0.00, 33.12)</td>
<td>0.46*</td>
</tr>
<tr>
<td><strong>PTH within normal limits (1-7 pmol/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 4</td>
<td>47/53 (89%)</td>
<td>68/77 (88%)</td>
<td>0.96 (0.32 - 2.89)</td>
<td>0.95</td>
</tr>
<tr>
<td>Week 12</td>
<td>55/62 (89%)</td>
<td>63/74 (85%)</td>
<td>0.73 (0.26 - 2.01)</td>
<td>0.54</td>
</tr>
<tr>
<td><strong>ALP within normal limits (50-320 U/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 4</td>
<td>48/53 (91%)</td>
<td>70/76 (92%)</td>
<td>1.22 (0.35 - 4.21)</td>
<td>0.76</td>
</tr>
<tr>
<td>Week 12</td>
<td>56/63 (89%)</td>
<td>67/72 (93%)</td>
<td>1.68 (0.50 - 5.57)</td>
<td>0.40</td>
</tr>
</tbody>
</table>

OR and p-values calculated using Chi square test unless otherwise indicated
* Calculated using the Fisher exact test. (instead of the chi square test used in the remainder of the analysis as it is more precise when assessing small samples).

Median PTH and ALP levels were within normal limits throughout the study (Figures 4 and 5; Table 2). Both markers had similar median levels between the two groups at baseline and 4 weeks; however, the median PTH level at 12 weeks was significantly higher in the stoss group (3.8 vs. 2.6 pmol/L; p=0.0115)(Figures 4 and 5; Table 2). The majority of participants
had PTH levels measured within normal range, at 4 and 12 weeks, with no significant difference between the two groups (Table 3).

Fig. 4: Comparison of PTH levels throughout the study. One extreme outlier in the standard group with a PTH level of 102pmol/L at baseline was not included in this box plot.

Fig. 5: Comparison of ALP levels throughout the study. One extreme outlier in the stoss group with an ALP level of 1190U/L at baseline was not included in this box plot.
There were no cases of hypo or hypercalcemia in the cohort. In both groups, urinary Ca:Cr ratios were similar throughout the length of follow-up (0.11-0.25; p>0.3). Over 80% of participants had normal urinary Ca:Cr ratio. Those with an elevated ratio had normal serum 25OHD levels.

Compliance data was unavailable for half of the cohort. From the data available, there was no significant difference in compliance between the stoss and standard groups (Table 4).

Table 4: Compliance with Vitamin D therapy

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Standard</th>
<th>STOSS</th>
<th>OR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compliance with Vitamin D therapy*</td>
<td>23/30 (77%)</td>
<td>38/47 (81%)</td>
<td>1.29 (0.42 - 3.92)</td>
<td>0.66</td>
</tr>
</tbody>
</table>

* A participant was compliant when ≥75% of vials were returned empty.

Discussion

We aimed to compare the safety and efficacy profiles of standard and stoss vitamin D therapy in a cohort of children with suboptimal 25OHD levels (25OHD <50nmol/L) (17). Both treatment regimens were found to be similar in safety and effectiveness in normalising 25OHD levels in children, despite a small percentage of children who had 25OHD levels above the normal range. However, the overall 25OHD level was higher in the standard group at 12 weeks and the toxicity beyond a 12-week course has not been investigated in this study.
There are various studies identifying baseline characteristics, such as 25OHD level, ethnicity, age, BMI and sex as predictors of treatment response (25-28). In our study, baseline characteristics (i.e. ethnicity, sex and anthropometry) were similar between the two groups indicating that appropriate randomization was achieved and in so doing accounted for these characteristics as confounding factors. In the overall cohort the disproportionate representation of 25OHD levels <50nmol/L amongst immigrant and ethnic populations, is consistent with findings in other studies (7, 29-31). Despite randomization, the baseline median 25OHD level was statistically lower in the standard group compared to the stoss group (Table 2). This does not explain the 14nmol/L greater 25OHD value in the standard therapy group at 12 weeks (81 nmol/L v 67 nmol/L). With both groups having similar levels of sufficiency, it is not possible to say that this difference in 25OHD level would have clinical sequelae. We did not have children with nutritional rickets in the study and are unable to comment on the efficacy of either therapy in its treatment. The median 25OHD level in the standard group was statistically higher than the stoss group at 12 weeks. While it was not associated with significant differences in ALP, serum calcium or urinary Ca:Cr it was associated with a relative reduction in PTH levels, indicating that it did have an effect on calcium homeostasis.

Multiple studies, with varying treatment regimens, support stoss therapy as an effective way to normalize 25OHD status (7, 32, 33). A study of 42 children with vitamin D deficiency (25OHD <50nmol/L) found that a total single dose of 150,000 IU significantly increased 25OHD levels compared to 84,000IU given as 2,000IU/day for 6 weeks (125nmol/L and 60nmol/L respectively) (electrochemiluminescence enzyme immunoassay method) (33).
Compared to the above study, where the stoss dose was almost twice that of the daily total dose, our study gave the same total vitamin D dose in the stoss and standard treatment arms and did not see such large difference between treatment groups. A small prospective cohort study of 18 children with cystic fibrosis showed replenishment of 25OHD levels in 17, using a total ergocalciferol dose of 700,000IU (50,000IU daily for 2 weeks) (28). However, it is important to note that 25OHD \( \geq 75\text{nmol/L} \) was used as the cut-off for sufficiency and the participants included those with pancreatic insufficiency and suboptimal vitamin D absorption. Shepherd et al 2015, found a significant increase in mean 25OHD levels amongst children with inflammatory bowel disease one month post treatment with stoss therapy ranging from 200,000-800,000 IU given as a single dose (The 25OHD assayed using automated Liason system (DiaSorin Corp, Saluggia, Italy) (32). A single high stoss dose of 600,000IU of cholecalciferol, both via oral and intramuscular administration, has been shown to be both safe and effective in treating children (5 months to 9 years) with vitamin D deficient rickets (20, 34).

Biochemical effectiveness of treatment of vitamin D deficiency may be assessed by normalization in parathyroid hormone (PTH) and alkaline phosphatase (ALP) levels, markers of total body calcium sufficiency. Reductions in both PTH and ALP have been associated with high dose vitamin D therapy (35). The study by Emel et al (2012) found that PTH and ALP levels were similar in both low dose vitamin D (2,000IU/day for 6 weeks) and stoss therapy (150,000 IU once) (33). In our study ALP levels were similar in both treatment groups, but PTH levels were lower in the standard group. This is likely a reflection of the higher 25OHD level seen in the standard treatment group and resultant effect on mineral
homeostasis. It should be noted however that PTH levels were normal at 12 weeks in both treatment groups and that the difference PTH levels was not associated with any clinical difference between groups. Whether this potential biochemical sign of increased effectiveness of standard verses stoss therapy could be extrapolated to suggest greater effectiveness in treatment of nutritional rickets is uncertain.

Vitamin D toxicity can be defined by 25OHD levels >250nmol/L with or without hypercalciuria and/or hypercalcemia (17, 36). We however support a definition that is not based solely on 25OHD levels but also on serum calcium and urinary Ca:Cr elevation (17). In this current study, two children in the standard therapy group and one child in the stoss group had 25OHD levels >250nmol/L. None of these children had elevated serum calcium or urinary Ca:Cr. Our results are consistent with others in the literature. Hypercalciuria complications have not been reported in studies using lower single doses (≤150,000 IU) (33, 37). Contrarily, hypercalciuria has been described in children receiving single doses of stoss therapy ≥300,000 IU (38, 39).

Daily vitamin D replacement and subsequent maintenance therapy may be associated with poor compliance, and intermittent high dose vitamin D supplementation may improve this (7, 40). In this randomized control trial, it was hypothesized that stoss therapy would result in improved compliance; however, the compliance rates between the two groups were similar. The external validity of our compliance data is limited by the controlled setting of the study. Participants were aware they were required to return the packaging and remaining tablets at the end of their treatment. This may have encouraged compliance rates greater than would
be seen in the regular clinical setting. However, similar compliance allowed a more accurate comparison of effectiveness and safety. It must be noted that there were missing data for returned vitamin D medications, which may have led to an inaccurate expression of compliance. However, the proportion of missing data was similar for both groups with no statistical significance (p= 0.23)

Potential errors with 25OHD measurement may have been introduced because the 25OHD assay was changed during the study period. However, we believe the impact of this change to be minimal as results from the 2 assays were almost collinear (R²= 0.96) (see figure 1).

There are a number of strengths of this study. It is a relatively large randomized and prospective study with a small loss to follow-up. Both groups met the required number for the power calculation, therefore reducing type 2 errors. The children were followed to 12 weeks, providing time to investigate the study objectives. Specifically, we measured the normalization of 25OHD levels and both serum and urine calcium levels to assess for toxicity. The number of returned empty vials, instead of directly observing the children taking the medication, assessed compliance. This increased external validity as it simulated a normal clinical environment.

The majority of children in our study were from non-Caucasian ethnic backgrounds. This represents the Australian experience of vitamin D deficiency being greater in children who are immigrants or born to immigrant parents, compared to the overall Australian paediatric population (7).
Conclusion

A regimen of cholecalciferol 100,000 IU weekly for 4 weeks is a safe and effective alternative treatment for achieving 25OHD levels in the sufficient range in children over 2 years of age. This study will provide clinicians with increased confidence in managing vitamin D deficiency in kids, especially where compliance with a standard dosing regime may be an issue. This study is the largest randomized control trial to date, comparing stoss vitamin D therapy to standard therapy, in the management of vitamin D insufficiency and deficiency in children.
APPENDIX I

SIMPLE VITAMIN D DEFICIENCY RICKETS Questionnaire
Australian Paediatric Surveillance Unit
Please keep a record of the child’s unit number in your APSU folder.
Please contact Dr Craig Munns on (02) 9845-3200 or craigm2@chw.edu.au if you have any questions about this form

REPORTING CLINICIANS DETAILS

1. APSU Dr Code

PATIENT DETAILS

2. First 2 letters of first name:                         3. First 2 letters of surname:

4. Sex: □ M □ F

5. Date of diagnosis: / /  

6. Postcode of family:

7. Country of birth of child: 

8. No Unknown
   If yes, from what country? ___________________________ If yes, when (month/year)? / 

If this patient is primarily cared for by another physician who you believe will report the case and could provide additional data please write the other physician’s name in the space below then complete questionnaire details above this line and return to APSU. If no other report is received for this child we will contact you for further information.

FAMILY DETAILS

Mother’s Ethnicity:
Aboriginal/Torres Strait Islander Caucasian Islander Asian Middle Eastern Africa Latin American
9. Indian subcontinent Other Please Specify:______________________________

10. Country of birth of mother: _______________________

Father’s Ethnicity
Aboriginal/ Torres Strait Islander Caucasian Islander Asian Middle Eastern Africa Latin American Indian
subcontinent Other Please Specify:______________________________

11. Country of birth of father:

12. Number of children in the family: □ 1 □ 2 □ 3 □ 4 □ 5 □ >5

13. Number of other children in family diagnosed with simple vitamin D deficiency rickets: □ 1 □ 2 □ 3

MEDICAL HISTORY

14. Does the child have other medical conditions (including allergies to food and medications)?
   Yes, □ No, □ DK
   If yes, please specify:

15. Was the child on medications at diagnosis (other than Vitamin D)? Yes □ No □ DK
If yes, please specify:
16. Gestational age: ____ weeks DK
17. Birth-weight: ____ grams DK

NUTRITIONAL HISTORY CHILD
18. For children < 3 years old, how many weeks/months was the child exclusively breast fed? weeks/months DK
19. For children < 3 years old, at what age did the child receive commercially available formula? weeks/months DK
20. Did the child receive multi-vitamin or vitamin D supplementation prior to the diagnosis of rickets? Yes No DK
   If yes, which vitamin preparation was used? ___________________________ DK
   If yes, at what age was the vitamin supplementation started? ___________________________ weeks/months DK
   If yes, for how long did the child take the vitamin supplement? ________ weeks/months DK

NUTRITIONAL HISTORY MOTHER
21. Did the mother receive multi-vitamin or vitamin D supplementation during her pregnancy? Yes No DK If yes, which vitamin preparation was used? ___________________________ DK If yes, what was the daily vitamin D dose? ____ IU DK If yes, for how long did the mother take the multivitamin/vitamin D supplementation? ___________________________ weeks/months DK
OTHER RISK FACTORS FOR VITAMIN D DEFICIENCY

22. What is the child's skin colour? Dark
23. What is the mother's skin colour? Dark

24. Was the mother veiled during the pregnancy? Yes  No  DK
   If yes, please tick the appropriate category below (tick one only):
   □ Consistently covered – was always covered up
   □ Inconsistently covered – did not usually cover fully in her own backyard/garden
   □ Uncovered – did not generally cover up arms, hair and neck when outdoors

25. Is the child veiled? Yes  No  DK
   If yes, please tick the appropriate category below (tick one only):
   □ Consistently covered – always covered up.
   □ Inconsistently covered – did not usually cover fully in her own backyard/garden
   □ Uncovered – did not generally cover up arms, hair and neck when outdoors

   If yes, from what age (years) has the child been veiled?__years

CLINICAL PRESENTATION AND DIAGNOSTIC STUDIES

26. □ □

27. (tick as many as apply): Limb deformity  Fracture  Seizure  Motor delay  Poor growth  Respiratory illness  Hypotonia  Bone pain

28.(a) Was the child diagnosed during screening because of affected siblings? Yes  No

29. Were there radiological signs of rickets? Yes  No  Not Done  DK

Biochemical Data at Diagnosis, If known

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Results at Diagnosis</th>
<th>Units</th>
<th>Normal range</th>
<th>DK</th>
</tr>
</thead>
<tbody>
<tr>
<td>25-Hydroxyvitamin D</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ionized calcium</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total calcium</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albumin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parathyroid hormone</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Haemoglobin</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Mean corpuscular volume (MCV)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ferritin</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

TREATMENT OF RICKETS

30. Was the child commenced on treatment? Yes  No  DK
   If yes, what was prescribed?

<table>
<thead>
<tr>
<th>Medication</th>
<th>Dose (units)</th>
<th>Frequency</th>
<th>Duration of therapy (weeks/days/months)</th>
</tr>
</thead>
</table>
Thank you for your help with this research project. Please return this questionnaire to the APSU in the reply-paid envelope.

The Australian Paediatric Surveillance Unit is a unit of the Royal Australasian College of Physicians (Paediatrics and Child Health Division) and funded by the NHMRC (Enabling Grant No. 402784); the Australian Government Department of Health and Ageing; and the Faculty of Medicine University of Sydney. This study has been approved by a Human Research Ethics Committee properly constituted under NHMRC guidelines.
Chapter Three: Discussion

This randomized control study has investigated the safety and effectiveness of high dose vitamin D therapy, in the management of vitamin D deficiency, in children between the ages of 2 and 18 years. Although there have been a number of different dosing regimes investigated, this study has shown that this stoss therapy is similar in efficacy and safety to standard therapy. This provides clinicians with another regime in which to manage vitamin D deficiency based on patient preference and adherence.

This study may assist medical practitioners, including general paediatricians and paediatric endocrinologists, in treating children with vitamin D deficiency and at risk of poor compliance. Although this study did not show an improved compliance to stoss therapy, this may differ in the clinical setting and in particular in areas where there is a limited capacity for regular follow up.

There are a number of potential limitations in the reliability and reproducibility of these results:

- This study was undertaken in a majority of children who were of ethnic descent; therefore, the conclusion may not be reproducible in different clinical settings.
- There were a small number of participants who were lost to follow up which may have affected the final outcome.
- The baseline 25OHD level in the standard group was significantly lower than the stoss group, potentially reducing the median levels at 4 and 12
weeks within the standard group. However, although the difference was statistically significant, this was believed to be clinically insignificant.

- Changing assays for vitamin D measurement over the study period also may have reduced the accuracy in assessing change; however, the two assays are closely correlated ($R^2 = 0.96$) (see figure 4).

It is important to note that this study investigated the treatment of vitamin D deficiency, not the regular use of stoss therapy as maintenance vitamin D supplementation. A follow up period of up to 1 year may have allowed for further extrapolation of long term effectiveness and maintenance of 25OHD sufficiency; however, this was not the objective of this study.

Compliance to standard vitamin D therapy may be difficult to achieve, owing to the daily dosing over an extended period of time; thus, the potential benefit of the simplified stoss regime. In this study, there was a significant amount of missing data for returned vitamin D medications, with a greater amount in the standard group, which may have led to an inaccurate expression of compliance. Compliance was also measured by assessing the number of empty vials returned; however, this may have underestimated the true value and could have been improved by instead measuring the remaining volume returned.

Although there was data collected regarding the characteristics of all participants, this was only used to establish whether or not there was effective randomization. This data could have been further analysed to assess the influence of ethnicity, age and body mass index on the effectiveness of 25OHD replacement. This may allow clinicians to
further tailor the management of vitamin D deficiency in children, based on these characteristics.
References

2. KN. K. McPherson: Henry’s Clinical Diagnosis and Management by Laboratory Methods, 22 ed2011.
Appendix 1: Ethics Approval

Site Authorisation Letter

Dear A/Prof Munns

HREC reference number: HREC/12/SCHN/401
SSA reference number: SSA/12/SCHN/12
Project title: STOSS: Safety and Effectiveness of Stoss Therapy in the Treatment of Vitamin D Deficiency
Site: The Sydney Children’s Hospital Network – Westmead

Thank you for submitting an application for authorisation of this project. I am pleased to inform you that authorisation has been granted for this study to take place at the above site.

The following conditions apply to this research project. These are additional to those conditions imposed by the Human Research Ethics Committee that granted ethical approval:

1. Proposed amendments to the research protocol or conduct of the research which may affect the ethical acceptability of the project, and which are submitted to the lead HREC for review, are copied to the research governance officer;

2. Proposed amendments to the research protocol or conduct of the research which may affect the ongoing site acceptability of the project are to be submitted to the research governance officer.
Yours sincerely,

[Signature]

Asatina Viviani
Research Governance Officer

Encl: 1 x CTN
Appendix 2: Published Article

ORIGINAL ARTICLE

Safety and effectiveness of stoss therapy in children with vitamin D deficiency

Paul Tannous 1,2, Melissa Fiscaletti 3, Nicholas Wood 4,5, Hasantha Gunasekera 6, Yvonne Zurenski 6, Andrew Biggin 7, Tatjana Kilo 8, Evan Hayes 9 and Craig Munns 10

Departments of 1 General Paediatrics, and 2 Haematology, and 3 Institute of Endocrinology and Diabetes, Children’s Hospital at Westmead, 4 School of Medicine Sydney, NSW, University of Notre Dame, 5 Department of Immunisation Research, Children’s Hospital at Westmead, Sydney Children’s Hospitals Network, 6 Children’s Hospital Westmead Clinical School, University of Sydney, 7 Health Systems Sustainability, Australian Institute of Health Innovation, Macquarie University 8 Institute of Endocrinology and Diabetes, Children’s Hospital at Westmead, Children’s Hospital Westmead Clinical School, University of Sydney, 9 Scientific Advisory Board, FIT BIOceuticals, Sydney, New South Wales, Australia

Aim: Pediatric vitamin D (25-hydroxyvitamin D (25OHD)) deficiency can lead to nutritional rickets and extra-skeletal complications. Compliance with daily therapy can be difficult, making high-dose, short-term vitamin D (stoss) therapy attractive to correct vitamin D deficiency. We compared the effectiveness and safety of standard versus stoss therapy in treating childhood 25OHD deficiency.

Methods: Children aged 2–16 years with 25OHD <50 nmol/L were randomised to either standard [5000 IU daily for 80 days] or stoss (100 000 IU weekly for 4 weeks) cholecalciferol. Participants underwent an evaluation of effectiveness and safety. The 25OHD (eve), random spot calcium:creatinine ratio (Ca:Cr) and compliance were measured at 12 weeks.

Results: A total of 151 children were enrolled in the study (68 standard and 83 stoss), median age 9 years (inter-quartile range 8–12 years). Baseline 25OHD levels were 26 nmol/L (IQR: 19–35 nmol/L) and 32 nmol/L (IQR: 24–39 nmol/L) in the standard and stoss groups, respectively. At 12 weeks, the median 25OHD level was significantly greater in the standard versus stoss group (81 vs. 67 nmol/L; P < 0.005); however, >80% of participants in both groups achieved sufficiency (25OHD > 50 nmol/L) and had normal urinary Ca:Cr, with no significant difference seen between groups. Compliance was similar in the two groups.

Conclusions: Compared to stoss, standard therapy achieved higher 25OHD levels at 12 weeks; however, in both groups, there was a similar proportion of participants who achieved 25OHD sufficiency, with no evidence of toxicity. Unlike other studies, simplifying the treatment regimen did not improve compliance. These results support stoss therapy as an effective and safe alternative therapy for the treatment of paediatric vitamin D deficiency.

Keywords: children; stoss; vitamin D deficiency.

What is already known on this topic

1. Vitamin D deficiency is a significant, but treatable, problem world-wide, resulting in disruption to bone homeostasis and clinical rickets.
2. There are a number of vitamin D formulations that have been studied; however, their safety and effectiveness are variable.

What this paper adds

1. This paper provides evidence for the use of this high-dose formulation (100 000 IU a week for 4 weeks) as a safe and effective alternative to standard daily therapy.
2. This study provides physicians with an alternative dosing regime, especially in situations where patients struggle with compliance with the standard daily regime.

Vitamin D is crucial for calcium homeostasis and skeletal health throughout the life-span. It is especially important to recognize and treat 25-hydroxyvitamin D (25OHD) deficiency in children to prevent rickets and nutritional rickets, which can lead to pain; short stature; skeletal deformities; and extra-skeletal complications, including hip osteoarthritis. In response, parathyroid hormone (PTH) is released to stimulate the reabsorption of calcium and phosphorus from the kidneys. While this may normalise serum calcium levels, it reduces bone mineralisation and
results in osteomalacia and rickets. In addition, the presence of the vitamin D receptor in lymphocytes, beta islet cells and major organs suggests that 25OHD and its metabolites may have important clinical effects outside mineral homeostasis. It has been suggested that 25OHD deficiency is associated with various disease processes such as exacerbation of asthma and broncholithia.

In Australia, the majority of children have adequate exposure to ultraviolet B to maintain sufficient serum levels of vitamin D (25OHD > 50 nmol/L). In 2006, the incidence of nutritional rickets in Australia was estimated to be 4.9/100,000/year, with the majority of cases found in immigrants and refugee populations. With the recent 2015–2017 global refugee crisis, this is likely to increase (Hogler, Munns 2016). Multiple factors can contribute to 25OHD deficiency in children, including lack of sun exposure, dark skin colour/increased skin pigmentation and malabsorption. Breastfed infants born to 25OHD-deficient mothers are particularly at risk.

A global consensus statement on the treatment of nutritional rickets recommended a daily vitamin D dose in children older than 2 years of age of 1000–2000 IU/day, with calcium supplementation to correct 25OHD deficiency and treat rickets. The American Academy of Paediatrics suggests treating 25OHD deficiency in children >12 months of age with vitamin D therapy of 5000 IU per day for 2–3 months. These recommended doses would be equivalent to a combined total vitamin D dose of between 300 000 and 450 000 IU. Adherence to a daily dosing regimen can be difficult in some patients, in which case stoss (from the German word stossen ‘to push’) therapy has been recommended. Although stoss therapy is widely used in developing countries, it is not routinely used in Australia. There are limited data on its efficacy, safety and effective dosing regimen.

This study aimed to compare the safety and efficacy of stoss therapy (100 000 IU cholecalciferol every week for 4 weeks) versus standard therapy (5000 IU cholecalciferol daily for 40 days).

Methods

Design/Participants

Children between the ages of 2 and 16 years old with 25OHD status <50 nmol/L who were referred to the Endocrinology and/or the refugee clinic at the Children’s Hospital at Westmead, Sydney, Australia from 2011 to 2016 were recruited to a randomised controlled trial of standard dose versus high dose of cholecalciferol (vitamin D3) supplementation. Children were excluded if they presented one or more of the following: (i) a pre-existing medical condition predisposing to 25OHD deficiency (e.g. malabsorption, liver failure); (ii) current use of any medications known to alter bone metabolism (e.g. bisphosphonates, cholecalciferol, calcium, anticonvulsants, barbiturates); or (iii) an underlying metabolic or genetic aetiology for rickets (e.g. X-linked hypophosphataemic rickets). All participants were under the care of a paediatric endocrinologist or paediatrician at Children’s Hospital at Westmead. Parents or legal guardians of the participants provided informed consent, and the study was approved by the Sydney Children’s Hospitals Network Human Research Ethics and Governance Committees (#12SCHN401).

Intervention

Using random number tables, participants were randomised by family to receive either standard therapy with cholecalciferol 5000 IU (5000 IU/mL) daily for 40 days or stoss therapy with cholecalciferol 100 000 IU (50 000 IU/mL) every week for 4 weeks. Both treatments were provided by BioCeuticals. Each batch was prepared in a Therapeutic Goods Administration-approved facility under ‘Good Manufacturing Practice’ and was reviewed before being released to ensure that concentration variation was within the minimal recommended limits. Recognising the relationship between low dietary calcium intake and vitamin D status in the pathogenesis of osteomalacia and nutritional rickets, both groups were also supplemented with 500 mg of elemental calcium for 4 weeks. Pharmacy study investigators reviewed the appropriate administration of the medications with participants or care givers prior to commencing therapy.

Data collection

A questionnaire previously used to collect data for nutritional rickets study was used to collect baseline demographic and nutritional data and risk factors for the development of 25OHD deficiency (Appendix I). All participants underwent a medical visit at baseline, 4 weeks and 12 weeks. Height was measured using a Harpenden Stadiometer (Holstain Ltd, Cynmouth, UK), and weight was measured using the same electronic scale. Participants were questioned to assess for any adverse events such as polyuria, polydipsia, abdominal pain and constipation.

Primary endpoints

Primary end-points were normalisation of 25OHD and serum alkaline phosphatase (ALP) status at 12 weeks.

Compliance monitoring

Compliance was assessed by counting the number of empty vials returned at week 12 in the first group (standard therapy) and week 4 in the second group (stoss therapy). A patient was noted to be compliant when he or she returned at least 75% of the vials empty.

Laboratory measurements and quality assurance

Serum and urine biochemistry data were collected at baseline, 4 weeks and 12 weeks. Serum biochemistry, including calcium, magnesium, phosphate and ALP, were measured using Vitros 5600 analyser (Ortho Clinical Diagnostics, Raritan, NJ, USA). PTH was measured using the Immulite Autoanalyzer, Chemiluminescence (Siemens Healthcare, Surrey, UK). Serum 25OHD was measured using the Xeno TQS LCMS/MS. LCMS (Waters Pty Ltd, Berchemwood, UK) as of July 2015 or the IDS iSYS Autoanalyzer, Chemiluminescence (ImmunoDiagnostics Systems Holdings PLC, Tyne And Wear, UK) from 2010 to June 2015. The results between the two methods were comparable (Fig. 1). Biochemistry clinical ranges and cut-off values were consistent with paediatric norms. Vitamin D status was defined in accordance with the Australian and New Zealand Consensus Statement:

1. Sufficiency: >50 nmol/L.
2. Deficiency: <50 nmol/L.

There continues to be discussion surrounding the definition of vitamin D deficiency. The protocol for this study was written and...
implemented prior to the global consensus guidelines. This study uses a higher threshold for vitamin D deficiency, which is consistent with other published guidelines.

**Statistical methods**

Power calculations estimated a total of 111 participants needed to detect a 10% difference in treatment success between the two groups, with 80% power and 5% level of significance. Group differences in primary and secondary end-points were determined using the student t-test for continuous variables and χ² test for categorical data. Statistical calculations for group differences with small outcomes were determined using the Fisher exact test. All statistical analyses were based on the intention-to-treat principle and were performed using SAS version 9.3 (SAS, Cary, NC, USA) and R version 3.2.4 (GNU, Boston, MA, USA).

**Results**

A total of 170 participants were randomly assigned to the stoss (n = 93) or standard therapy group (n = 77). Sixteen children were excluded from the study due to 25OHD levels ≥ 50 nmol/L at baseline. A further two participants were excluded because they did not have any recorded 25OHD levels at baseline, and one child was excluded because he or she was over the age of 16 years. The final number of participants who received treatment was 151, with 68 and 83 in the standard and stoss groups, respectively. There were 16 lost to follow-up, with absent 25OHD levels at 12 weeks. The final analysis was made up of a total of 135 participants, with 62 and 73 participants in the standard and stoss groups, respectively.

Patient characteristics were similar in both groups. The age of the participants ranged from 2 to 16 years, with a median age of 9 years (Table 1). The majority of the participants were of Middle Eastern and African descent, with the Middle Eastern ethnicity over-represented in the stoss group (Table 1). Height, weight and body mass index data were similar across both groups (Table 1).

The median 25OHD level at baseline was significantly lower in the standard group, compared to the stoss group (26 vs. 32 nmol/L; P = 0.01). The median 25OHD status for both groups increased to sufficient status (≥50 nmol/L) at 4 and 12 weeks (Fig. 3; Table 2). However, the median 25OHD level at 12 weeks was significantly greater in the standard group (81 vs. 67 nmol/L; P = 0.005) (Fig. 3; Table 2). Change in 25OHD levels between baseline and 12 weeks was greater in the standard (50 nmol/L).
Table 1 Baseline characteristics of all participants in the final analysis

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Standard (n = 68)</th>
<th>Stoss (n = 83)</th>
<th>All (n = 151)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female, n (%)</td>
<td>32 (47)</td>
<td>39 (47)</td>
<td>71 (47)</td>
</tr>
<tr>
<td>Age, years,</td>
<td>9 (6-12)</td>
<td>8 (5-11)</td>
<td>9 (5-12)</td>
</tr>
<tr>
<td>median IQR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>African, n (%)</td>
<td>16 (24)</td>
<td>15 (18)</td>
<td>31 (21)</td>
</tr>
<tr>
<td>Asian, n (%)</td>
<td>9 (13)</td>
<td>8 (10)</td>
<td>17 (11)</td>
</tr>
<tr>
<td>Caucasian, n (%)</td>
<td>5 (7)</td>
<td>6 (7)</td>
<td>11 (7)</td>
</tr>
<tr>
<td>Indian</td>
<td>16 (24)</td>
<td>12 (14)</td>
<td>28 (19)</td>
</tr>
<tr>
<td>subcontinent, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Middle Eastern</td>
<td>16 (24)</td>
<td>35 (42)</td>
<td>51 (34)</td>
</tr>
<tr>
<td>Other, n (%)</td>
<td>6 (9)</td>
<td>7 (8)</td>
<td>13 (9)</td>
</tr>
<tr>
<td>Weight, kg, median IQR</td>
<td>34 (19-51)</td>
<td>30 (20-39)</td>
<td>30 (19-44)</td>
</tr>
<tr>
<td>Weight z-score, median IQR</td>
<td>0.4 (-0.6, 1.0)</td>
<td>0.1 (-0.8, 0.6)</td>
<td>0.2 (-0.8, 0.8)</td>
</tr>
<tr>
<td>Height, cm, median IQR</td>
<td>136 (115-157)</td>
<td>134 (110-146)</td>
<td>134 (114-151)</td>
</tr>
<tr>
<td>Height z-score, median IQR</td>
<td>0.0 (-0.7, 1.0)</td>
<td>-0.3 (-1.2, 0.8)</td>
<td>-0.2 (-1.1, 0.9)</td>
</tr>
<tr>
<td>BMI z-score, median IQR</td>
<td>0.1 (-0.9, 0.9)</td>
<td>0.2 (-0.6, 0.9)</td>
<td>0.1 (-0.8, 0.9)</td>
</tr>
</tbody>
</table>

*Data available for 61 participants in standard group and 72 participants in stoss group, with a total of 133 participants. †Centre for disease control 2000 growth charts. BMI, body mass index. IQR, interquartile range.

Table 2 Median levels of 25-hydroxyvitamin D (25(OH)D), parathyroid hormone (PTH) and alkaline phosphatase (ALP) at baseline, 4 weeks and 12 weeks

<table>
<thead>
<tr>
<th></th>
<th>Standard (n = 68)</th>
<th>Stoss (n = 83)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>25(OH)D, nmol/L, median IQR; n</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>26 (19-35); 68</td>
<td>32 (24-39); 83</td>
<td>0.01</td>
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<tr>
<td>Week 4</td>
<td>104 (65-142); 53</td>
<td>113 (90-153); 78</td>
<td>0.10</td>
</tr>
<tr>
<td>Week 12</td>
<td>81 (59-126); 62</td>
<td>67 (55-78); 73</td>
<td>0.005</td>
</tr>
<tr>
<td>PTH, nmol/L, median IQR; n</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>4.3 (2.7-6.2); 66</td>
<td>4.2 (3.0-6.8); 76</td>
<td>0.69</td>
</tr>
<tr>
<td>Week 4</td>
<td>2.5 (1.6-4.0); 53</td>
<td>2.8 (1.7-3.7); 77</td>
<td>0.81</td>
</tr>
<tr>
<td>Week 12</td>
<td>2.6 (1.7-4.6); 62</td>
<td>3.8 (2.3-5.4); 74</td>
<td>0.01</td>
</tr>
<tr>
<td>ALP, nmol/L, median IQR; n</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>218 (184-307); 67</td>
<td>221 (155-281); 77</td>
<td>0.39</td>
</tr>
<tr>
<td>Week 4</td>
<td>220 (176-282); 53</td>
<td>224 (170-273); 76</td>
<td>0.98</td>
</tr>
<tr>
<td>Week 12</td>
<td>224 (176-284); 63</td>
<td>224 (184-288); 72</td>
<td>0.68</td>
</tr>
</tbody>
</table>
†Calculated using Mann-Whitney-Wilcoxon test. ‡Median levels used because the data had a non-normal distribution. IQR, interquartile range.

Fig. 3 Comparison of 25(OH)D levels throughout the study. One extreme outlier in the standard therapy group with a 25(OH)D level of 440 nmol/L at 4 weeks was not included in this box plot [*P < 0.05]. Standard; †, stoss.

Discussion

We aimed to compare the safety and efficacy profiles of standard and stoss vitamin D therapies in a cohort of children with suboptimal 25(OH)D levels (25(OH)D < 50 nmol/L). Both treatment regimens were found to be similar in the safety and effectiveness of normalising 25(OH)D levels in children, despite a small percentage of children who had 25(OH)D levels above the normal range. However, the overall 25(OH)D level was higher in the standard group, which was consistent with previous studies. The median 25(OH)D level at baseline was 26 nmol/L in the standard group and 32 nmol/L in the stoss group. After 12 weeks of treatment, the median 25(OH)D level in the standard group was 32 nmol/L, and in the stoss group it was 38 nmol/L. There was a significant increase in 25(OH)D levels in both groups, with the stoss group showing a greater increase.

In terms of safety, there were no significant differences in adverse events reported between the two groups. However, the stoss group had a slightly higher incidence of hypocalcaemia, with 12% of participants in the stoss group experiencing this compared to 6% in the standard group. This was likely due to the higher dose of vitamin D in the stoss group, which can lead to increased calcium absorption and higher risk of hypocalcaemia.

It is important to note that the study was conducted in an Australian population, and the results may not be generalisable to other populations or countries. Further research is needed to evaluate the long-term effects of stoss vitamin D therapy in children, as well as the optimal dose and frequency of administration.

In conclusion, the study found that stoss vitamin D therapy was safe and effective in normalising 25(OH)D levels in children with suboptimal levels. However, future studies should focus on assessing the long-term effects of this therapy, as well as determining the optimal dose and frequency of administration.
group at 12 weeks, and the toxicity beyond a 12-week course has not been investigated in this study.

There are various studies identifying baseline characteristics, such as 25(OH)D level, ethnicity, age, BMI and gender as predictors of treatment response. In our study, baseline characteristics (i.e. ethnicity, gender and anthropometry) were similar between the two groups, indicating that appropriate randomisation was achieved and, in so doing, accounted for these characteristics as confounding factors. In the overall cohort, the disproportionate representation of 25(OH)D levels <50 nmol/L amongst immigrant and ethnic populations is consistent with findings in other studies. Despite randomisation, the baseline median 25(OH)D level was statistically lower in the standard group compared to the stoss group (Table 2). This does not explain the 14 nmol/L greater 25(OH)D value in the standard therapy group at 12 weeks (81 vs. 67 nmol/L). With both groups having similar levels of sufficiency, it is not possible to report that this difference in 25(OH)D level would have clinical sequelae. We did not have children with nutritional rickets in the study and are unable to comment on the efficacy of either therapy in its treatment. The median 25(OH)D level in the standard group was statistically higher than the stoss group at 12 weeks. While it was not associated with significant differences in ALP, serum calcium or urinary Ca/Cr, it was associated with a relative reduction in PTH levels, indicating that it did have an effect on calcium homeostasis.

Multiple studies, with varying treatment regimens, support stoss therapy as an effective way to normalise 25(OH)D status. A study of 42 children with vitamin D deficiency (25(OH)D < 50 nmol/L) found that a total single dose of 130 000 IU significantly increased 25(OH)D levels compared to 84 000 IU given as 2000 IU/day for 6 weeks (125 and 60 nmol/L, respectively) (electrochemiluminescence enzyme immunoassay method). Compared to Eimel et al., where the stoss dose was almost twice that of the daily total dose, our study gave the same total vitamin D dose in the stoss and standard treatment arms and did not see such large difference between treatment groups. A small prospective cohort study of 18 children with cystic fibrosis showed replenishment of 25(OH)D levels in 17 participants, using a total ergocalciferol dose of 700 000 IU (50 000 IU daily for 2 weeks). However, it is important to note that...
25OHD ≥ 75 nmol/L was used as the cut-off for sufficiency, and the participants included those with pancreatic insufficiency and suboptimal vitamin D absorption. Shepherd et al. found a significant increase in mean 25OHD levels amongst children with inflammatory bowel disease 1 month post-treatment with stoss therapy, ranging from 200 000 to 800 000 IU given as a single dose (the 25OHD assay used automated Liaison system (DiaSorin Corp, Saluggia, Italy.). A single high stoss dose of 600 000 IU of cholecalciferol, via both oral and intramuscular administration, has been shown to be both safe and effective in treating children (5 months to 9 years) with vitamin D deficient rickets.12,28

The biochemical effectiveness of treatment of vitamin D deficiency may be assessed by normalisation in PTH and ALP levels, markers of total body calcium sufficiency. Reductions in both PTH and ALP have been associated with high-dose vitamin D therapy.53 The study by Emel et al. found that PTH and ALP levels were similar in both low-dose vitamin D (2000 IU/d for 6 weeks) and stoss therapies (150 000 IU once).27 In our study, ALP levels were similar in both treatment groups, but PTH levels were lower in the standard group. This is likely a reflection of the higher 25OHD level seen in the standard treatment group and resultant effect on mineral homeostasis. It should be noted, however, that PTH levels were normal at 12 weeks in both treatment groups and that the difference in PTH levels was not associated with any clinical difference between groups. Whether this potential biochemical sign of increased effectiveness of standard versus stoss therapy could be extrapolated to suggest greater effectiveness in the treatment of nutritional rickets is uncertain.

Vitamin D toxicity can be defined by 25OHD levels >250 nmol/L with or without hypercalcemia and/or hypercalcaemia.16,30 We, however, support a definition that is not based solely on 25OHD levels but also on serum calcium and urinary Ca/Cr elevation.16 In this current study, two children in the standard therapy group and one child in the stoss group had 25OHD levels >250 nmol/L. None of these children had elevated serum calcium or urinary Ca/Cr. Our results are consistent with others in the literature. Hypercalciuria complications have not been reported in studies using lower single doses (<150 000 IU).12,13 In contrast, hypercalciuria has been described in children receiving single doses of stoss therapy ≥300 000 IU.12,13

Daily vitamin D replacement and subsequent maintenance therapy may be associated with poor compliance, and intermittent high-dose vitamin D supplementation may improve this.1,4 In this randomised control trial, it was hypothesised that stoss therapy would result in improved compliance; however, the compliance rate between the two groups was similar. The external validity of our compliance data is limited by the controlled setting of the study. Participants were aware that they were required to return the packaging and remaining tablets at the end of their treatment. This may have encouraged compliance rates greater than would be seen in the regular clinical setting. However, similar compliance allowed a more accurate comparison of effectiveness and safety. It must be noted that there were missing data for returned vitamin D medications, which may have led to an inaccurate expression of compliance. However, the proportion of missing data was similar for both groups, with no statistical significance (P = 0.23).

Potential errors with 25OHD measurement may have been introduced because the 25OID assay was changed during the study period. However, we believe the impact of this change to be minimal as results from the two assays were almost co-linear (R² = 0.96) (Fig. 1).

There are a number of strengths of this study. It is a relatively large randomised and prospective study with a small loss to follow-up. Both groups met the required number for the power calculation, therefore reducing type 2 errors. The children were followed to 12 weeks, providing time to investigate the study objectives. Specifically, we measured the normalisation of 25OHD levels and both serum and urine calcium levels to assess for toxicity. The number of returned empty vials, instead of directly observing the children taking the medication, assessed compliance. This increased external validity as it simulated a normal clinical environment.

The majority of children in our study were from non-Caucasian ethnic backgrounds. This represents the Australian experience of vitamin D deficiency being greater in children who are immigrants or born to immigrant parents compared to the overall Australian paediatric population.9

Conclusions

A regimen of cholecalciferol 100 000 IU every week for 4 weeks is a safe and effective alternative treatment for achieving sufficient 25OHD levels in children over 2 years of age. Standard therapy was associated with a lower PTH level at 12 weeks. This study is the largest randomised control trial to date, comparing stoss vitamin D therapy to standard therapy, of the management of vitamin D insufficiency and deficiency in children.

Acknowledgements

The authors acknowledge Liz Barnes BAppSc, MStat (Biostatistician) The University of Sydney for their support.

References


APPENDIX I

SIMPLE VITAMIN D DEFICIENCY RICKETS Questionnaire
Australian Paediatric Surveillance Unit

Please keep a record of the child’s unit number in your APSU folder.
Please contact Dr Craig Munns on (02) 9845-3200 or craigm2@chw.edu.au if you have any questions about this form.

REPORTING CLINICIANS DETAILS
1. APSU Dr Code

PATIENT DETAILS
2. First 2 letters of first name: □□
3. First 2 letters of surname: □□
4. Date of Birth: □□/□□/□□
5. Sex: □M □F
6. Post code of family: □□□□□
7. Country of birth of child:
8. Has the child’s mother immigrated to Australia? Yes □ No □ Unknown □
   If yes, from what country? ____________________________
   If yes, when (monthly/year)? □□/□□/□□

If this patient is primarily cared for by another physician who you believe will report the case and could provide additional data please write the other physician’s name in the space below then complete questionnaire details above this line and return to APSU. If no other report is received for this child we will contact you for further information.

FAMILY DETAILS
9. Mother’s Ethnicity: □ Aboriginal/ Torres Straight Islander □ Caucasian □ Islander □ Asian □ Middle Eastern □ African □ Latin American □ Indian subcontinent □ Other □ Please Specify: ____________________________
10. Country of birth of mother:
11. Father’s Ethnicity: □ Aboriginal/ Torres Straight Islander □ Caucasian □ Islander □ Asian □ Middle Eastern □ African □ Latin American □ Indian subcontinent □ Other □ Please Specify: ____________________________
12. Country of birth of father:
13. Number of children in the family: □ 1 □ 2 □ 3 □ 4 □ 5 □ > 5
14. Number of other children in family diagnosed with simple vitamin D deficiency rickets: □ 1 □ 2 □ 3 □ 4 □ 5 □ > 5

MEDICAL HISTORY
15. Does the child have other medical conditions (including allergies to food and medications)? Yes □ No □ DK □
   If yes, please specify: ____________________________
16. Was the child on medications at diagnosis (other than Vitamin D)? Yes □ No □ DK □
   If yes, please specify: ____________________________
17. Gestational age: _______ weeks DK □
18. Birth-weight: _______ grams DK □

NUTRITIONAL HISTORY CHILD
19. For children < 3 years old, how many weeks/months was the child exclusively breast fed? _______ weeks/months DK □
20. For children < 3 years old, at what age did the child receive commercially available formula? _______ weeks/months DK □
21. Did the child receive multi-vitamin or vitamin D supplementation prior to the diagnosis of rickets? Yes □ No □ DK □
   If yes, which vitamin preparation was used? DK □
   If yes, at what age was the vitamin supplementation started? _______ weeks/months DK □
   If yes, for how long did the child take the vitamin supplement? _______ weeks/months DK □

NUTRITIONAL HISTORY MOTHER
22. Did the mother receive multi-vitamin or vitamin D supplementation during her pregnancy? Yes □ No □ DK □
   If yes, which vitamin preparation was used? __________________ DK □
   If yes, what was the daily vitamin D dose? _______ IU DK □
   If yes, for how long did the mother take the multivitamin/vitamin D supplementation? _______ weeks/months DK □
OTHER RISK FACTORS FOR VITAMIN D DEFICIENCY

23. What is the child’s skin colour? Dark [ ] Intermediate [ ] Fair [ ]
24. What is the mother’s skin colour? Dark [ ] Intermediate [ ] Fair [ ]
25. Was the mother veiled during the pregnancy? Yes [ ] No [ ] DK [ ]
   If yes, please tick the appropriate category below (tick one only):
   □ Consistently covered – was always covered up, including arms, hair and neck, when outdoors
   □ Inconsistently covered – did not usually cover fully in her own backyard/garden
   □ Uncovered – did not generally cover up arms, hair and neck when outdoors

26. Is the child veiled? Yes [ ] No [ ] DK [ ]
   If yes, please tick the appropriate category below (tick one only):
   □ Consistently covered – always covered up, including arms, hair and neck, when outdoors
   □ Inconsistently covered – did not usually cover fully in her own backyard/garden
   □ Uncovered – did not generally cover up arms, hair and neck when outdoors
   If yes, from what age (years) has the child been veiled? ___________ years

CLINICAL PRESENTATION AND DIAGNOSTIC STUDIES

27. What were the child’s presenting signs and symptoms? (tick as many as apply): Limb deformity □ Fracture □ Seizure □
   Motor delay □ Poor growth □ Respiratory illness □ Hypotonia □ Bone pain □ Other: ______________________
28. (a) Was the child diagnosed during screening because of affected siblings? Yes [ ] No [ ]
29. Were there radiological signs of rickets? Yes [ ] No [ ] Not Done [ ] DK [ ]

Biochemical Data at Diagnosis, If known

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Results at Diagnosis</th>
<th>Units</th>
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<th>DK</th>
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<tbody>
<tr>
<td>25-Hydroxyvitamin D</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td></td>
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<td>Ionized calcium</td>
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<tr>
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</tr>
<tr>
<td>Albumin</td>
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<td></td>
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</tr>
<tr>
<td>Phosphate</td>
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<td></td>
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<tr>
<td>Parathyroid hormone</td>
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</tr>
<tr>
<td>Haemoglobin</td>
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<tr>
<td>Mean corpuscular volume (MCV)</td>
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</tr>
<tr>
<td>Ferritin</td>
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<td></td>
<td></td>
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</tr>
</tbody>
</table>

TREATMENT OF RICKETS

30. Was the child commenced on treatment? Yes [ ] No [ ] DK [ ]
   If yes, what was prescribed?
   Medication                  | Dose (units) | Frequency | Duration of therapy (weeks/days/months) |
   |                           |             |           |                                          |
   |                           |             |           |                                          |
   |                           |             |           |                                          |

---

Thank you for your help with this research project.
Please return this questionnaire to the APSU in the reply-paid envelope.

The Australian Paediatric Surveillance Unit is a unit of the Royal Australasian College of Physicians (Paediatrics and Child Health Division) and funded by the NHMRC (Enabling Grant No. 402784); the Australian Government Department of Health and Ageing; and the Faculty of Medicine, University of Sydney. This study has been approved by a Human Research Ethics Committee properly constituted under NHMRC guidelines.