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Vitamin D for Growth and Rickets in Stunted Children: A Randomized Trial

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Short running head: Vitamin D for Growth and Rickets in Stunted Children

Abbreviations used: 25-hydroxyvitamin D (25(OH)D), confidence intervals (CIs), low and middle income countries (LMIC), odds ratio (OR), parathyroid hormone (PTH)

Table of Contents: We explore the effect of vitamin D supplementation on growth and rickets in highly stunted, vitamin D and calcium insufficient inner-city Afghan children.

Trial Registration: This trial was registered at clinicaltrials.gov NCT00548379.

Data Sharing Statement: A de-identified individual participant dataset (including data dictionaries) upon which the conclusions of the paper rely will be made available after publication to researchers who provide a methodologically sound proposal for use. Proposals should be submitted to Dr Crowe (F.Crowe@bham.ac.uk)

What's Known on This Subject:
Few trials have examined whether vitamin D supplementation can prevent rickets or improve growth. Daily vitamin D supplementation potentially prevents rickets in vitamin D deficient or insufficient children and may increase height in sub-populations of young teenagers and premature infants.

What This Study Adds:
In a highly stunted, vitamin D and calcium insufficient (not deficient) population using bolus 3-monthly vitamin D supplementation, there was no statistically significant reduction in rickets or improved growth. In those with a high-intake of calcium, height improved.

Contributors Statement:

Dr Crowe designed the analysis plan, analyzed the data, and drafted the manuscript. Professor Mughal made substantial contributions to the conceptualisation and design of the study and drafted the manuscript. Dr Maroof was the trial manager, managed the implementation of the study, and revised the manuscript critically for important intellectual content. Dr Berry led the analysis of blood 25-hydroxyvitamin D and parathyroid hormone, made a substantial contribution to the interpretation of the data, and revised the manuscript critically for important intellectual content. Dr Kaleem led the analysis of the wrist and knee x-rays, made a substantial contribution to the interpretation of the data, and revised the manuscript critically for important intellectual content. Dr Abburu led the analysis of the dietary data, made a substantial contribution to the interpretation of the data, and revised the manuscript critically for important intellectual content. Drs Walraven, Masher and Chandramohan made substantial contributions to the conceptualisation and design of the study, and revised the manuscript critically for important intellectual content. Dr Manaseki-Holland made substantial contributions to the conceptualisation and design of the study, directed the study execution, and drafted the manuscript. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Background and Objectives: Vitamin D is essential for healthy development of bones but little is known about the effects of supplementation on rickets and linear growth in young stunted children in Afghanistan. The objective was to assess the effect of vitamin D supplementation on risk of rickets and linear growth among Afghan children.
Methods: In this double-blind placebo-controlled trial 3,046 children age 1-11 months from inner-city Kabul were randomised to receive oral vitamin D3 (100,000 IU) or placebo every three months for 18 months. Rickets Severity Score was calculated using wrist and knee radiographs for 631 randomly selected infants at 18 months and rickets was defined as a score > 1.5. Weight and length were measured at baseline and 18 months using standard techniques and z-scores calculated.
Results: Mean (95% CI) serum 25(OH)D (seasonally-corrected) and dietary calcium intake were insufficient; 37 (35-39) nmol/L and 372 (327-418) mg/d, respectively. Prevalence of rickets was 5.5% (placebo) and 5.3% (vitamin D); OR: 0.96; 95% CI: 0.48-1.92, p=0.9. Mean difference in height-for-age z-score was 0.05, 95% CI: -0.05-0.15, p=0.3, although the effect of vitamin D was greater for those consuming >300mg/day of dietary calcium (0.14; 95% CI: 0-0.29, p=0.05). There was no between-group differences in weight-for-age or weight-for-height z-scores.
Conclusions: Except in those with higher calcium intake, vitamin D supplementation had no effect on rickets or growth. This may be because these children were insufficient in both vitamin D and calcium (rather than deficient) and vitamin D was given as a bolus dose.

Background

Afghanistan, a low-income country, has one of the highest rates of child mortality among children under age 5 in the region. [1] While improvements in nutrition and the alleviation of poverty have led to a reduction in mortality in recent years,[2] undernutrition still poses a big threat to the health of Afghan children with one of the highest prevalence of childhood stunting ranging from 40%[3] to over 80% in some districts.[4] Factors associated with stunting include sanitation, food insecurity, and low dietary diversity,[2,4] and children in Afghanistan under 5 years have high rates of anaemia, and deficiencies in vitamins A and D.[3]

Vitamin D is essential for children achieving optimal linear growth and development of bones.[5] Nutritional rickets is a condition where there is impaired mineralisation of the growth plate and osteoid in a growing child. It can lead to deformities of the lower limbs and pelvis, and in severe cases is associated with fragility fractures.[6,7] Deficiency in vitamin D or dietary calcium intake, or combination of both these factors can cause nutritional rickets.[6] Vitamin D deficiency arises when there is inadequate exposure of the skin to sunlight or insufficient dietary intake.[8,9] Besides being a major cause of rickets and osteomalacia, vitamin D deficiency might also lead to impaired growth. Randomised control trials of vitamin D supplementation showed improvements in growth in premature low-birth weight neonates in India[10] and in school-aged children (11-13year olds) in Mongolia.[11] The duration of vitamin D supplementation and follow-up in these two trials was only six months and it is not clear whether longer period of vitamin D supplementation as bolus doses in young children undergoing rapid periods of growth (6-24 months) leads to greater improvements in growth or prevents cases of rickets. Additionally, daily and weekly home supplementation regimens often have poor adherence[12,13] and bolus doses implemented during regular child health

contacts (e.g. vaccination) are important supplementation methods for programmatic purposes.

Vitamin D deficiency and malnutrition are common problems in Afghanistan,[3,14] making it an ideal study population in which to examine the effects of vitamin D on bones and growth, which may be relevant to other low and middle-income countries (LMIC). Here we assess the effect of vitamin D supplementation on the risk of rickets as measured by Thacher radiological score, hereafter known as the Rickets Severity Score (RSS) and growth parameters of children who participated in a randomised controlled trial in Afghanistan. Chronically low dietary calcium intake, with or without vitamin D deficiency/insufficiency also leads to rickets in the growing child.[6] Therefore, we have also examine the role that dietary calcium intake may have in the causation of rickets.

Methods

Study design

A more detailed description of the trial methods including the trial profile has been published elsewhere.[15] Briefly, this trial was a community-based double-blind randomised placebo-controlled parallel trial that was conducted between November 2007 and June 2009 in five inner-city districts of Kabul, Afghanistan. The primary objective of the trial was to evaluate the effect of quarterly supplementation of 100,000 IU (2.5 mg) of Cholecalciferol (vitamin D3) on the incidence and/or severity of childhood pneumonia. *A priori* secondary objectives of the study was to assess the effect of vitamin D3 supplementation on the risk of rickets and linear growth among children.

Approvals, consent and registration

The study protocol was approved by the Ethics and Review Board of the Ministry of Public Health of Afghanistan (Reference: 422328) and the Ethics Committee of the London School of Hygiene and Tropical Medicine (Application no. 5117). Thumbprint or signature consent from the mother, father or other head of the household was obtained after either parent read the consent form or it was explained to him/her by the fieldworker. This study was registered at clinicaltrials.gov as NCT00548379.

Participants

The children enrolled in the study mainly were from socioeconomically deprived inner-city districts in Kabul and aged 1 to 11 months. A total of 3,060 children were assessed for eligibility and 3,046 children were randomised to the vitamin D (n=1,524) or placebo group (n=1,522).

Randomisation

An independent statistician individually randomised unique identification numbers in blocks of 20 to vitamin D or placebo group by use of a random number generator with the SAS routine.

Intervention

Supplements and blinding

2.5 mg of vitamin D3 (Cholecalciferol) dissolved in 1 mL of olive oil (vitamin D) or 1 mL of olive oil alone (placebo) were pre-prepared in sealed plastic syringes. The vitamin D in olive oil and placebo syringes were identical in appearance and all families and study personnel including the clinicians were blinded to the group allocation of the child. Vitamin D or placebo was given by the fieldworker to the child on a quarterly basis; doses were given in November 2007, February 2008, May 2008, August 2008, December 2008, and the final (6^{th}) dose given in March 2009. The fieldworkers were unaware of whether the upcoming syringe was vitamin D or placebo and therefore allocation was concealed.

Measurements

At recruitment, data on demography, socio-economic characteristics of the household, and medical history of the child were collected. Principal component analysis of household characteristics and assets were used to measure a wealth index and this was divided into quintiles as a measure of the socioeconomic status of a household.

Dietary intake of energy, protein and calcium

Twice throughout the study (once in the winter and then in the summer months), mothers (or the main caregiver) completed a semi-quantitative food frequency questionnaire (FFQ) that

estimated their child's intake of a list of 56 commonly consumed foods over the past week (**Supplementary methods 1**). Dietary calcium less than 300 mg/d was used as a cut-point to define deficiency.[6]

Adverse events

There were no adverse event reported in the study. Of the children who had serum concentration of 25(OH)D measured, only five in the vitamin D and one in the placebo had 25(OH)D greater than 250 nmol/L (indicative of being at risk of hypercalcemia). None of these children had serum calcium measured.

Anthropometry

Measurement of length/height and weight were performed while the children were wearing light/no clothing and were collected from the children at recruitment and 18 months later (supplementary methods 1).

Radiographs

There were 641 sets of radiographs of the wrist and knee obtained from a subset of children by use of a simple random sample. The radiographs were evaluated by a Consultant Paediatric Radiologist (MK) who was blinded to the group allocation using the RSS[16] for radiological grading of active nutritional rickets (Supplementary methods 1). Of the 641 sets of radiographs, 10 could not be evaluated due to reasons such as poor image quality and so the RSS was available for 631 children (321 in vitamin D and 310 in placebo group).

Measurement of serum 25-hydroxyvitamin D and parathyroid hormone

Five times over the 18 months of the study, approximately 120-140 blood samples (60-70 from the vitamin D and 60-70 from the placebo group) were randomly collected each time from blocks of children. The timing of the blood samples were to allow for measurement of both the pharmacodynamic and seasonal changes in 25(OH)D concentrations.

Serum 25(OH)D was measure by the IDS-iSYS Multi-Discipline Automated Chemiluminescent assay (Immunodiagnostic Systems Ltd, Boldon, Tyne and Wear UK) and Serum intact PTH was measured using the IDS intact PTH ELISA kit (Immunodiagnostic Systems Ltd., Boldon, Tyne and Wear, UK) at Manchester Royal Infirmary (supplementary methods 1).

Power calculation

The trial sample size was based on the outcome measure of first episode of pneumonia.[15]

Statistical analyses

Of the 3,046 children allocated in the trial, anthropometric measurements were available for 2,103 and RSS score was available for 631 children (**Supplementary Figure 1**).

Serum concentrations of 25(OH)D and PTH were log transformed to normalise distributions and difference in the geometric mean concentrations between vitamin D and placebo group were assessed using linear regression analysis.

Logistic regression was used to assess the risk of rickets (RSS > 1.5) in the vitamin D compared to the placebo group. The difference in mean RSS and the mean *z*-score of height-for-age, weight-for-age, and weight-for-height was assessed by using multiple linear

regression. Heterogeneity in the effect of vitamin D on RSS and anthropometric measurements by the subgroup of calcium intake (< 300, ≥ 300 mg/d)[6] was assessed using a standard χ^2 test. This was also done for subgroups of calcium expressed as mg per 1000 kcal (<459, ≥459 mg/1000 kcal). In a sensitivity analysis, the between group differences in the anthropometric *z*-scores were repeated controlling for the respective baseline values.

All statistical analyses were performed using Stata statistical software, V.15 (StataCorp, College Station, Texas, USA). Two-sided *p* values < 0.05 were considered statistically significant.

Results

Characteristics of children for the placebo and vitamin D groups are shown in **Table 1**. The average age at enrolment was 6 months and there were slightly more boys than girls in the trial. More than half of the infants were being breastfed at recruitment and 10% of infants in both the vitamin D and placebo groups were from the poorest households. There was a similar degree of underweight (12%), stunting (13%), and wasting (7%) in the vitamin D and placebo groups. The dietary intake (including breast milk) of energy, protein and calcium were similar between the groups, and the percent of children with intakes of calcium considered deficient (< 300 mg/day) was 50.5% and 50.6% in the vitamin D and placebo group, respectively.

Figure 1 shows the geometric mean (95% CI) serum concentrations of 25(OH)D in a random sample of about 120 infants from the placebo and vitamin D group throughout five time points in the study. Mean serum 25(OH)D was higher in the vitamin D compared to the placebo group after the first dose of vitamin D supplement (115 vs 39 nmol/L, $p < 0.001$), second (49 vs 28 nmol/L; $p < 0.001$) and the third (94 vs 47 nmol/L; $p < 0.001$) dose of vitamin D supplement. There was no difference in mean 25(OH)D concentration between the vitamin D and placebo group at last time point (more than four months after the sixth and final dose of vitamin D). The mean serum 25(OH)D in the subgroup of the placebo group who had 25(OH)D measured was 28 nmol/L in winter months and 47 nmol/L in summer months, giving a seasonally-corrected mean 25(OH)D of 37 nmol/L (95% CI: 35, 39) throughout the trial period. In the placebo group, 26%, 55%, 55%, 21% and 7% were below 30 nmol/L in rounds 1, 2, 3, 4, and 5 respectively

Among the placebo group, PTH increased during the winter months (December 2007 to January 2008 and January to February 2008) and were significantly higher than the vitamin D group at these two time points; 2.6 vs 1.8 pmol/L, $p = 0.004$ and 3.8 vs 2.3 pmol/L, $p < 0.001$ respectively (**Figure 2**). There was no difference in PTH between the placebo and vitamin D group at the other time points.

The proportion of children with a RSS of zero did not differ significantly between the vitamin D and placebo groups (67% vs 69%) and only one child in the placebo group had a RSS of 10 (**Table 2**). The mean RSS, although lower in the vitamin D group, did not differ significantly between the vitamin D and placebo group (0.46 vs 0.35; $p = 0.086$). The proportion of children with a RSS indicative of rickets also did not differ between the two groups (5.5% vs 5.3%; $p = 0.9$). The mean RSS was lower in the vitamin D group but this was not statistically significant by intake of dietary calcium.

The mean height-for-age, weight-for-age and weight-for-height z-scores did not differ significantly between the vitamin D and placebo groups for all children and for those with <300 mg/day dietary calcium (**Table 3**). However, mean height-for-age for children with a calcium intake ≥300 mg/day showed a slight improvement (mean difference 0.14, 95% CI 0, 0.29, $p = 0.05$); the test for interaction by calcium intake was not statistically significant ($p = 0.355$). Mean height-for-age, weight-for-age and weight-for-height z-scores did not differ significantly between the vitamin D and placebo groups for children with calcium intake <459 mg/1000 kcal or those with calcium intake ≥ 459 mg/1000 kcal (supplementary Table 1).

It made little difference to the results if the analysis for the anthropometric z-scores included an adjustment for baseline values or if growth velocity (cm change in height per year) was used (results not shown).

Discussion

Six bolus doses of 2.5 mg of vitamin D every three months given to young highly stunted children with insufficient vitamin D status and calcium intake did not have a significant effect on the prevalence of rickets over a period of 18 months. There was also no significant effect on growth except in a subgroup with high intake of calcium.

The lack of an effect of vitamin D supplementation on the risk of rickets in this trial may have been due to several factors. The overall prevalence of rickets at the end of the trial was lower than anticipated (5%) meaning this study may have been insufficiently powered to detect a statistically significant difference between the groups. Nutritional rickets arises when there is inadequate absorption of dietary calcium due to vitamin D deficiency and/or dietary calcium deficiency or when there is vitamin D insufficiency and dietary calcium deficiency.[6] These factors result in inadequate dietary calcium absorption leading to a fall in serum calcium, which in turn elevates PTH. This secondary hyperparathyroidism increases renal phosphate wastage, resulting in chronic hypophosphatemia. In a growing child, chronic hypophosphatemia causes rickets through failure of maturation and mineralization the growth plate, as well as impaired mineralization of osteoid matrix.[6,17] High rates (73%) of vitamin D deficiency have previously been reported in young Afghan children[3,14] but in this study the seasonally-corrected mean serum 25(OH)D in the subgroup of the placebo group who had 25(OH)D measured was 37 nmol/L (95% CI: 35, 39) throughout the trial period, thus they would be considered vitamin D insufficient rather than deficient. The estimated mean dietary calcium intake in this population was 372 (SD 276) mg/day meaning that this population would be considered insufficient not deficient. While the mean winter PTH concentrations were not above the assay reference range, some of the children may have developed

secondary hyperparathyroidism in the winter months. These findings emphasise the importance of calcium deficiency in combination with vitamin D deficiency or insufficiency in the aetiology of rickets.[6]

There may be other reasons why differences in rickets between groups may not have been detected. A one-year follow-up of Kenyan children who received inpatient treatment for severe acute malnutrition showed that the presence of rickets (13% of the cohort) was associated with increased linear growth compared to children without rickets.[18] Therefore, the impaired growth in this study population, as demonstrated by the high prevalence of stunting, may have contributed to the low rates of rickets irrespective of their level of vitamin D. It is also possible that some features of rickets may be masked in undernourished children. These findings are important for interventions aimed to prevent stunting in LMIC and indicate that for the purpose of growth and rickets prevention, community level vitamin D supplementation programs alone may not be cost-effective for such populations.

In the few existing trials of vitamin D supplementation for improving growth, results are inconsistent and are not easily comparable to our population. Some reporting a lack of effect of vitamin D supplementation on growth of children may be criticised as being underpowered[19] or of insufficient duration.[20] Ganmaa *et al*[11] reported an increase in height of almost 1 cm in older school-aged (11-13 years) children in Mongolia supplemented with 800 IU (20 µg) of vitamin D plus milk daily for 6 months compared to milk alone, and no effect on 9-11 year olds. Kumar *et al*[10] also found a greater length-for-age z-score in premature low-birth-weight infants after 6 months of supplementation with 1400 IU (35 µg) per week of vitamin D with breastmilk compared to breastmilk alone. The trials in Mongolia[11] and India[10] used daily and weekly supplementation, respectively, whereas in our trial young children

were administered bolus doses of vitamin D every 3 months. Differences in the effectiveness of vitamin D for the daily and weekly versus bolus doses have been described for other outcomes such as acute respiratory tract infections[21] and bone outcomes.[22] This has been attributed to the finding that high circulating concentrations of 25(OH)D after bolus dosing may down-regulate activity of enzymes that synthesise and degrade the active form of vitamin D,1,25-dihydroxyvitamin D, leading to lower concentrations of this metabolite in extra-renal tissues.[23] This in turn may have attenuated the effect of vitamin D on growth of these young children by reducing intestinal calcium absorption. Other differences in the trial population and design may have contributed to the contrasting findings. Our population are more typical of the highly malnourished populations in LMIC even though the proportion of children who were vitamin D or calcium deficient was quite low.

Strengths of this trial include the largest sample size than other studies in children, with a long follow-up time, vitamin D and placebo supplements being administered to the children directly, radiographic screening of rickets and use of a validated RSS. The findings are generalizable to Kabul and most of Afghanistan's urban population. Serum 25(OH)D and PTH were not measured in all children who had radiographic screening for rickets making it difficult to know whether the cases of rickets were caused by vitamin D deficiency. Serum concentrations of alkaline phosphatase (that are raised above the reference range in rickets) are used as a screening marker for diagnosis of vitamin D deficiency[24] but were not measured. Lastly, the relative validity of the FFQ used in this study has not been assessed and there was no information on the volume and nutritional composition of breast milk from women in Afghanistan so estimates from Gambian women were used,[25] which may have underestimated the contribution of calcium from breastmilk thus reducing the negative effects of vitamin D deficiency.

In conclusion, among these young Afghan children who were mostly vitamin D and calcium insufficient, there was no effect of 3-monthly bolus vitamin D supplementation for 18 months on the risk of rickets and growth except for those who had high intake of calcium who had marginally better growth. Future research studies of rickets and growth in undernourished children need to consider both the vitamin D dosing regimens as well as calcium supplementation.

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FIGURE 1

Geometric mean (95% CI) serum concentrations of 25-hydroxyvitamin D (nmol/L) in the placebo and vitamin D group over the duration of the study. *$P < 0.001$ for difference between placebo and vitamin D. Arrows indicate when the bolus dose of vitamin D or placebo was administered.

FIGURE 2

Geometric mean (95% CI) serum concentrations of parathyroid hormone (pmol/L) in the placebo and vitamin D group over the duration of the study. *$P < 0.01$ for difference between placebo and vitamin D; **$P < 0.001$ for difference between placebo and vitamin D. Arrows indicate when the bolus dose of vitamin D or placebo was administered.