



A pilot interventional study to evaluate the impact of cholecalciferol treatment on HbA1c in type 1 diabetes (T1D)

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Abstract

Background: Higher 25(OH)D3 levels are associated with lower HbA1c, but there are limited UK interventional trials assessing the effect of cholecalciferol on HbA1c.

Aims: (1) To assess the baseline 25(OH)D3 status in a Manchester cohort of children with type 1 diabetes (T1D). (2) To determine the effect of cholecalciferol administration on HbA1c.

Methods: Children with T1D attending routine clinic appointments over three months in late winter/early spring had blood samples taken with consent. Participants with a 25(OH)D3 level <50 nmol/L were treated with a one-off cholecalciferol dose of 100,000 (2–10 years) or 160,000 (>10 years) units. HbA1c levels before and after treatment were recorded.

Results: Vitamin D levels were obtained from 51 children. 35 were Caucasian, 11 South Asian and 5 from other ethnic groups. 42 were vitamin D deficient, but 2 were excluded from the analysis. All South Asian children were vitamin D deficient, with mean 25(OH)D3 of 28 nmol/L. In Caucasians, there was a negative relationship between baseline 25(OH)D3 level and HbA1c ($r = -0.484$, $P < 0.01$). In treated participants, there was no significant difference in mean HbA1c at 3 months ($t = 1.010$, $P = 0.328$) or at 1 year ($t = -1.173$, $P = 0.248$) before and after treatment. One-way ANCOVA, controlling for age, gender, ethnicity, BMI and diabetes duration showed no difference in Δ HbA1c level.

Conclusion: We report important findings at baseline, but in children treated with a stat dose of cholecalciferol, there was no effect on HbA1c. Further studies with larger sample sizes and using maintenance therapy are required.

Key Words

- ▶ diabetes
- ▶ bone
- ▶ paediatric endocrinology
- ▶ vitamin D
- ▶ HbA1c

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Introduction

Type 1 diabetes (T1D) affects 25,000 children and young adults in the United Kingdom (1). Intensive glycaemic control is crucial to avoid long-term complications (2) but only 18.4% are meeting the National Institute for Health and Care Excellence (NICE) HbA1c target of <6.5%. The incidence of vitamin D deficiency, as reliably defined by serum 25-hydroxyvitamin D [25(OH)D] <50 nmol/L (3, 4, 5, 6), has been estimated at 8–24% in UK children (7), with particularly high figures of 38% (8) to 43% (9) reported in T1D.

Vitamin D has been implicated in the pathogenesis of diabetes; inverse relationships have been described between serum 25(OH)D level and impaired glucose tolerance (10) as well as impaired fasting glucose (11). An association between vitamin D deficiency and diabetes development has been shown in diabetic mice displaying beta cell destruction (12, 13), and in these mice, administering 1,25-dihydroxy vitamin D, the biologically active metabolite of vitamin D, prevented diabetes development (12). Further evidence can be drawn from cohort studies. In a Finnish cohort study (14), infants who received the recommended cholecalciferol dose of 2000 units/day had a lower risk of developing T1D. A Danish cohort study (15) found that males were at higher risk of developing T1D if they were born in the spring compared with the autumn, demonstrating an important role for maternal ultraviolet B (UVB) exposure during late gestation. This seasonality was eliminated with a vitamin D-fortified diet (16). In a systematic review and meta-analysis conducted by Zipitis and coworkers (17), the risk of T1D was significantly reduced in individuals who had received cholecalciferol in early infancy compared with those who had not (pooled odds ratio 0.7, 95% CI, 0.6–0.8).

Despite promising evidence from these studies, data from interventional studies are limited and contradictory. One study (18) showed that in 44 vitamin D-deficient children with T1D, one-off treatment improved HbA1c levels (mean HbA1c $9.7 \pm 1.9\%$ before compared to $8.6 \pm 1.9\%$ three months later). Another study found that in eighty patients with T1D who had baseline 25(OH)D levels <50 nmol/L, vitamin D repletion to >75 nmol/L (following 12 weeks' daily supplementation) was associated with lower HbA1c levels compared with those whose 25(OH)D levels remained <75 nmol/L (mean HbA1c $7.3 \pm 1.5\%$ vs $9.1 \pm 2.4\%$ respectively) (19). In contrast, a retrospective study of 131 people with T1D did not find an effect of cholecalciferol supplementation

on HbA1c after three-month treatment (8.5 ± 1.2 – $8.5 \pm 1.1\%$) (20). In addition, a recent study in the United States found no significant relationship between 25(OH)D and HbA1c in 7- to 18-year-old children with T1D (21). To date, there have been no prospective UK studies assessing the effect of cholecalciferol on HbA1c in T1D.

Aims

To determine the prevalence of vitamin D deficiency among children with T1D, attending our Paediatric Diabetes clinic over three consecutive months in late winter/early spring (Feb–April).

To determine the impact of a single dose of cholecalciferol supplement on subsequent HbA1c levels in children with both T1D and low serum 25(OH)D levels.

Methods

Participants

All children aged <19 years with a diagnosis of T1D, who attended the diabetes clinic at Royal Manchester Children's Hospital (Latitude 53° 28 North), over three consecutive months in late winter/early spring were invited to take part in the study. A diagnosis of T1D was assigned after careful consideration of the clinical presentation, biochemical abnormalities, the need for insulin and presence of islet autoantibodies in line with American Diabetes Association (22) and International Society for Paediatric and Adolescent Diabetes (23) guidelines. Ethical approval was obtained from the regional Research Ethics Committee (REC reference 10/H1008/133, IRAS project ID 64935). Informed consent was obtained from children and parents/guardians prior to recruitment.

Measurements

Height was measured using a wall mounted Harpenden stadiometer to the nearest 0.1 cm, and weight was measured using a digital weighing scale to the nearest 0.1 kg. BMI was calculated using the formula weight [kg]/height [m]². HbA1c was routinely measured using the Siemens DCA Vantage or the BioRad Variant (intra- and

inter-assay CV <6%), and total daily insulin dose was collected where possible.

At the time of clinic attendance, serum samples were taken for estimation of HbA1c and 25(OH)D level, as well as any other clinically indicated biochemical and haematological parameters. All data were anonymised and stored on a secure server.

Participants with a serum 25(OH)D level of <50 nmol/L (5) were treated with a single oral cholecalciferol dose of 100,000 (2–10 years) or 160,000 (>10 years) units according to local guidelines. Serum samples of 25(OH)D were measured in a sample of participants at follow-up, to assess the adequacy of treatment.

Vitamin D (serum 25(OH)D) assay

Blood samples were centrifuged at 3000g for 10 min at 17°C, and aliquots of sera were stored at –80°C. 25(OH)D was assayed using a 25-hydroxy vitamin D mass spectrometry method (Chromsystems) on an AB Sciex 5500 instrument. This assay is traceable to NIST standard reference material 972a, and repeatability (within run) imprecision is usually <6%, with intermediate precision (between runs) being typically 5–7% at 20 and 80 nmol/L.

Statistical analysis

Diabetes duration, body mass index (BMI), change in mean insulin dose (Δ insulin dose) and change in mean HbA1c (Δ HbA1c) were calculated. Standard deviation scores (SDS) for height, weight and BMI were derived using an online SDS calculator. Statistical analyses of baseline characteristics were performed in IBM SPSS (version 22).

Paired *t* tests were performed to compare the mean HbA1c in the year before and after treatment and 3 months before and after treatment. Analysis of covariance (ANCOVA) was used to determine any effect of treatment with cholecalciferol on Δ HbA1c, controlling for the confounding factors of age, gender, BMI, ethnicity, diabetes duration, month of treatment, daylight hours and Δ insulin dose. Random forest analysis using R version 3.2.2 was also performed to determine the influence of treatment on Δ HbA1c. Random forest is a useful tool for making predictions based on multiple ‘decision trees’, eliminating the high variance and high level of bias posed by a single decision tree.

Results

Fifty-three children, representing 80% of those who were approached, agreed to take part in the study. Blood samples were obtained from 51 (Fig. 1). Of these, 42 had a serum 25(OH)D level <50 nmol/L and were prescribed cholecalciferol (Dekristol 20,000 IU capsules, MIBE GMBH, Germany); 9 were not deficient. Thirty-two participants were seen at follow-up and confirmed that they had taken the treatment. One patient returned the cholecalciferol in the post, and HbA1c results after cholecalciferol treatment were not available for one participant who moved away. To assess the effect of cholecalciferol treatment, 40 participants were included on an intention-to-treat basis. To check that vitamin D levels had improved after treatment, we performed serum measurements of 25(OH)D on a subset of 13 patients at follow-up, and all were in the non-deficient range (52.1–117.2 nmol/L).

At baseline

The baseline characteristics of the participants are shown in Table 1. 84% of those recruited were vitamin D deficient (11 South Asian, 27 White Caucasian and 4 other). For deficient and non-deficient groups, the median (range) serum 25(OH)D levels were 35.4 (30.0) nmol/L and 53.7 (16.2) nmol/L (very close to the threshold of deficiency) respectively. Multiple regression analysis identified ethnicity ($t=2.46$, $P=0.02$) and daylight hours ($t=2.081$, $P=0.05$) as key parameters affecting baseline vitamin D status.

At baseline, there was no significant correlation between serum 25(OH)D and HbA1c level in the whole cohort. However, in White Caucasian children, there was an inverse correlation between HbA1c and serum 25(OH)D levels (correlation coefficient, -0.5 ; $P=0.03$, $n=34$). Regression analysis found 23% of the HbA1c value was predicted by 25(OH)D level in White Caucasian children.

All South Asian children were vitamin D deficient, and the 25(OH)D level of South Asian children (median 26 nmol/L, range 19.3 nmol/L) was significantly lower than that of White Caucasians (median 40.5 nmol/L range 43 nmol/L, $z=-3.5$, $P<0.0001$).

When participants were categorised according to age, gender and diabetes duration, no significant differences were found in 25(OH)D levels.

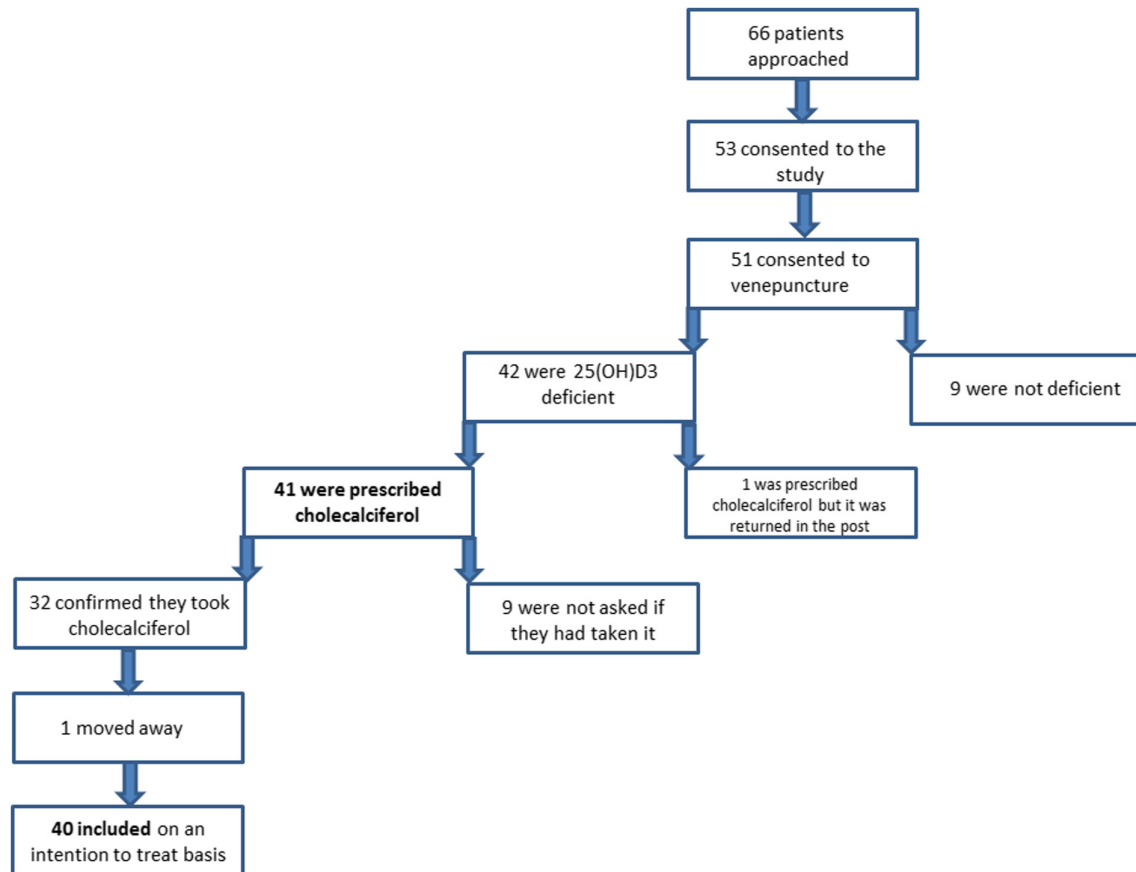


Figure 1

Flow diagram to show the recruitment of patients with T1D from the clinic.

Following treatment

A paired *t* test assessing Δ HbA1c in the treated group alone showed no significant difference in mean HbA1c levels for one year before ($8.9 \pm 1.2\%$) and one year after ($9.1 \pm 1.4\%$) treatment ($t = -1.2$, $P = 0.2$, $n = 39$) or for 3 months before ($8.6 \pm 1.3\%$) and after ($8.4 \pm 1.1\%$) treatment ($t = 1.0$, $P = 0.3$, $n = 16$). 16 children had a mean HbA1c level that was *higher* in the year after treatment than in the year

before. Figure 2 shows the trends in HbA1c levels for individual participants.

A one-way ANCOVA was performed to determine whether there was a difference between being treated or not treated with cholecalciferol on Δ HbA1c, at one year before and after treatment, controlling for age, gender, ethnicity, BMI, diabetes duration, Δ total insulin dose,

Table 1 Baseline characteristics of subjects who had vitamin D levels checked.

	All subjects			Vit D-deficient subjects			Vit D-sufficient subjects		
Number	51			42			9		
Age (years)	12.5 (3.5)			12.7 (3.1)			11.2 (5.4)		
Gender	Boys	Girls		Boys	Girls		Boys	Girls	
	28	23		24	18		4	5	
Ethnicity	Caucasian	South Asian	Other	Caucasian	South Asian	Other	Caucasian	South Asian	Other
	35	11	5	27	11	4	8	0	1
Duration of diabetes (years)	4.8 (3.3)			5.0 (3.2)			3.4 (2.9)		
Vitamin D level mean (range) (nmol/L)	37.8 (11)			34 (9)			55 (5.5)		

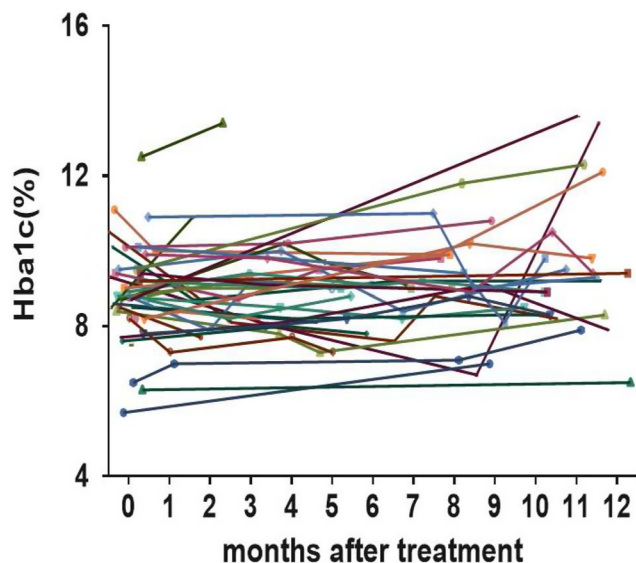


Figure 2
Changes in HbA1c levels over time after treatment with cholecalciferol.

month and daylight hours. No difference was found ($F=1.3$, $P=0.3$). To evaluate the effect of missing data, the ANCOVA was repeated with Δ total insulin dose excluded, and this confirmed no significant difference ($F=1.1$, $P=0.4$). The analyses were repeated at three months before and after treatment, and no significant differences were found ($F=4.8$, $P=0.3$, $F=2.0$, $P=0.1$ with insulin excluded).

Random forest analysis, based on division of the data by median values, was used to determine the influence of age, gender, ethnicity, baseline vitamin D status, treated or not, month of recruitment, number of daylight hours at recruitment, diabetes duration, BMI SDS and Δ total insulin dose on Δ HbA1c level. This analysis revealed an AUC of 0.43 (95% CI, 0.37–0.49) at 3 months and AUC of 0.58 (95% CI 0.44–0.72) at 1 year, illustrating that Δ HbA1c cannot be predicted from these factors. When ‘treated or not’ was removed, random forest analysis showed an AUC of 0.59 (95% CI 0.46–0.73), with baseline insulin dose as the strongest predictor of baseline HbA1c, demonstrating the validity of this analysis.

Discussion

We confirmed a high prevalence of vitamin D deficiency in this cohort of children with T1D. At baseline, we observed an inverse relationship between HbA1c and serum 25(OH) D level in White Caucasians, but not in South Asians. In deficient participants, treatment with cholecalciferol did not influence HbA1c.

Our findings of high prevalence of vitamin D deficiency are in accordance with previous studies in children with T1D (21, 24, 25). It has been suggested that vitamin D deficiency disturbs glucose homeostasis (26) by exacerbated cytokine damage of beta-cells (27), 70–80% of which has already occurred by diagnosis (28).

South Asian children are prone to vitamin D deficiency due to their increased skin pigmentation, with melanin affecting cutaneous pre-cholecalciferol synthesis, and all South Asian patients in our cohort were vitamin D deficient. In addition, dress practices may result in poor skin exposure to UVB radiation, which is already limited in Manchester (<http://www.manchester.climateps.com/sunlight.php>).

We found no significant correlation between serum 25(OH)D level and HbA1c in South Asian children. We postulate that this is due to this group having a reduced range of 25(OH)D values, as all children were deficient. This is a known phenomenon in correlational research (29). Nevertheless, a negative trend was observed. South Asian children had a similar mean HbA1c level to White Caucasian children, despite having much lower 25(OH) D values. This could be explained by the South Asian group having a higher mean age (13.3 vs 12.0 years of age) and duration of diabetes (5.3 vs 4.9 years) or by the fact that the 25(OH)D levels were too low to detect any significant trends.

Tests to assess the effect of treatment with cholecalciferol, including control for confounding variables, showed no difference in mean HbA1c before and after treatment. Therefore, our study does not support the previous studies that were conducted in Iran and Saudi Arabia, where average daylight hours are longer. Instead, our findings are in line with a more recent study in the United States (21), which found no relationship between 25(OH)D and HbA1c in T1D.

Other large epidemiological studies (24, 25) provide evidence of an association between higher baseline serum 25(OH)D and lower HbA1c, but data from interventional trials are limited. To our knowledge, this is the first prospective UK-based study examining whether treatment with cholecalciferol has an effect on HbA1c levels in T1D.

The main limitation of our pilot study is the small sample size, which has insufficient power to detect a small effect size. Our findings must therefore be interpreted with caution, and a larger, adequately powered study is required. Another limitation is that there are many confounding variables that affect HbA1c in patients with diabetes. For example, an individual with a short duration of diabetes may have residual insulin secretion

i.e. a ‘honeymoon effect’ resulting in lower HbA1c. Measurements of C peptide might have been valuable to detect these situations. Whilst we have attempted to control for these variables, one major factor influencing HbA1C, adherence to treatment is challenging to accurately measure and control. Total daily insulin dose acted as a surrogate for the impact of puberty. However, total daily insulin dose was not available for 25 participants where diabetes control was centred on carbohydrate counting. This limited our analysis and the ability of our models to control for this. A further study limitation is that data on daylight hours were used, rather than measuring exposure to ambient sunlight with polysulphone badges. All blood samples were taken between February and April, so prevalence of vitamin D deficiency may not be representative.

Participants were treated with a single dose of cholecalciferol, according to hospital guidelines. Studies comparing single one-off with maintenance vitamin D therapy have shown it to be safe and effective (30, 31), even up to 12 months after administration (32). However, randomised controlled trials provide some evidence to support the value of vitamin D maintenance therapy, rather than single doses, in paediatric asthma patients to prevent exacerbations (33). There is also some evidence of benefit in paediatric patients with inflammatory bowel disease (34). It has been proposed that the effects on immune action are through the production of cathelicidins, a family of polypeptides found in lysosomes of macrophages and polymorphonuclear leukocytes (35). Furthermore, single-dose vitamin D treatment in critically ill patients has been shown to have no effect on length of stay in hospital (36).

In conclusion, this is the first prospective UK-based study examining the effect of vitamin D treatment on HbA1c levels in T1D. We report important findings at baseline, but found no improvement in HbA1c levels following treatment with a single dose of cholecalciferol. We suggest randomised controlled trials in larger cohorts comparing single versus maintenance doses of cholecalciferol, to further examine these findings.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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