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Et al.

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ABSTRACT

Background: The impact of the anti-inflammatory and immunomodulatory actions of Vitamin D on the duration of partial clinical remission (PR) in youth with type 1 diabetes (T1D) is unclear.

Objective: To determine the effect of adjunctive ergocalciferol on residual β-cell function (RBCF) and PR in youth with newly-diagnosed T1D who were maintained on a standardized insulin treatment protocol.

Hypothesis: Ergocalciferol supplementation increases RBCF and prolongs PR.

Methods: A 12-month randomized, double-blind, placebo-controlled trial of 50,000 IU of ergocalciferol per week for 2 months, and then once every 2 weeks for 10 months, versus placebo in 36 subjects of ages 10-21years(y), with T1D of <3 months, and a stimulated C-peptide (SCP) level of ≥0.2nmol/L (≥0.6ng/mL). The ergocalciferol group had 18 randomized subjects (10m/ 8f), mean age 13.3±2.8y; while the control group had 18 subjects (14m/4f), age 14.3±2.9y.

Results: The ergocalciferol treatment group had significantly higher serum 25-hydroxyvitamin D at 6 months (p=0.01) and 9 months (p=0.02) than the placebo group. At 12 months, the ergocalciferol group had a significantly lower serum TNF-α concentration (p=0.03). There were no significant differences between the groups at each timepoint from baseline to 12 months for SCP concentration (p=0.08), HbA1c (p=0.09), insulin-dose-adjusted A1c (IDAA1c), or total daily dose of insulin. Temporal trends for rising HbA1c (p=0.044) and IDAA1c (p=0.015) were significantly blunted in the ergocalciferol group.

Conclusions: Ergocalciferol significantly reduced serum TNF-α concentration and the rates of increase in both A1c and IDAA1c suggesting a protection of RBCF and PR in youth with newly-diagnosed T1D.

Keywords: type 1 diabetes; ergocalciferol; partial clinical remission; pediatrics; C-peptide

INTRODUCTION

Type 1 diabetes (T1D) is a syndrome of persistent hyperglycemia resulting from autoimmune destruction of pancreatic β-cells causing insulinopenia(1). Fifty-percent of β-cell function may remain at T1D diagnosis and this RBCF may persist for months or years(2-4). Longer duration of the partial clinical remission (PR), or 'honeymoon' phase, of T1D improves glycemic control and reduces long-term complications(5, 6). Efforts to block immune-mediated destruction of β-cells with immunomodulatory and immunosuppressive agents have yielded promising trends but insufficient protection(7-10)**.** Vitamin D is safe and has immunomodulatory functions that could protect RBCF(11). Studies suggest the possibility that vitamin D supplementation may lengthen PR and increase RBCF(6, 11). The rationale for this randomized control trial (RCT) was to establish the effect of an adequate dose of ergocalciferol on PR and RBCF.

We enrolled 48 subjects of 10-21 years with newly-diagnosed T1D in a 12-month RCT of ergocalciferol vs. placebo to determine the impact of vitamin D on RBCF and PR in youth with newly-diagnosed T1D. The hypothesis was that ergocalciferol would increase RBCF and prolong PR. The primary aim was to determine the effect of adjunctive ergocalciferol on RBCF and PR in youth with T1D. The primary outcome was the longitudinal change in peak stimulated C-peptide concentrations (a measure of RBCF).

SUBJECTS AND METHODS

The study protocol(12) was approved by the University of Massachusetts Medical School (UMMS) Institutional Review Board (IRB) on May 27, 2016. The Federal Award Date was July 21, 2017. Study registration at Clinical Trials.gov was completed on February 8, 2017, with a clinical trial identification number of NCT03046927. The FDA Regulatory Document Registration and Investigational New Drug approval were finalized on June 20, 2017. The first study subject was enrolled on October 19, 2017. The last subject completed the study on April 12, 2021 and the study was closed on April 20, 2021. FDA review on Jan 3-8, 2020 found the RCT to be in full compliance with Federal regulations.

Study Design and Setting

This was an investigator-initiated, single-center, randomized, double-blind, parallel trial of ergocalciferol versus placebo treatments in youth with newly diagnosed T1D at a university teaching hospital.

Subjects

Written informed consent was obtained from each subject's parent(s) and assent was obtained from minors. Subjects of ≥18 years signed the consent form. Inclusion criteria were male and female subjects of ages 10-21 years with new-onset T1D of <3mo duration. All subjects had a fasting C-peptide level of >0.1 nmol/L (0.3 ng/mL) or stimulated C-peptide level of ≥0.2 nmol/L (≥0.6 ng/mL). The diagnosis of T1D was established by the presence of autoantibodies against islet antigens(13). Subjects were excluded if they had eating disorders; active neoplasms; 25-hydroxyvitamin D [25(OH)D] level of >70 ng/mL; had received any investigational drug in the prior 6 months; on medications other than insulin

that could affect glycemia; pregnancy, breastfeeding, treatment with weight-altering therapies, systemic illnesses, recurrent hypoglycemia, or a history of ≥2 episodes of diabetic ketoacidosis (DKA) in the preceding 3 months (**Figure 1**). At enrollment, all participants were receiving once-daily subcutaneous basal insulin injection, and pre-meal bolus insulin injections using insulin analogs. All participants employed a self-directed treat-to-target insulin regimen (TTIR) **(Table 1)**. **Figure 1** summarizes the study scheme from screening to study conclusion.

Methods

Participants were evaluated between 8:00 AM - 10:30 AM following an overnight fast.

Anthropometry:

Anthropometric data were collected at each study visit. Height was measured to the nearest 0.1 cm using a wall-mounted stadiometer (Holtain Ltd, Crymych, Dyfed, UK). Weight was measured to the nearest 0.1 kg using an upright scale. BMI was calculated using the formula weight/height^2 (kg/m^2) and expressed as z-scores. Waist circumference was measured to the nearest 0.1 cm at the superior border of the iliac crests.

Biochemical Studies:

Mixed meal tolerance test (MMTT) for Stimulated C-peptide: MMTT was performed between 8:30AM and 10:30 AM following an overnight fast, with no injection of bolus insulin in the preceding 6 hours. Boost (formerly Sustacal, Mead Johnson, Evansville, IN, USA), at a dose of 6 mL/kg (maximum 360 mL), was ingested in <10 minutes. Blood draws were obtained for baseline glucose and C-peptide, and at 30-min, and 90-min for post mixed-meal C-peptide and glucose estimations(14). Glucose and C-peptide were analyzed by the Umass Memorial

Medical Center (UMMC) Biochemistry laboratory. Serum C-peptide (SCP) concentrations were analyzed by ELISA using Quest Immunoassays on an Atellica IM Analyzer (Siemens, Tarrytown, NY, USA) with <12% intra- and inter-assay coefficients of variability.

Hemoglobin A1c: Blood samples for HbA1c were obtained at each visit and were analyzed by the UMMC Biochemistry laboratory. HbA1c was measured by high pressure liquid chromatography which has an inter-assay variability of <1.5%, and intra-assay variability of <2.5%, and a normal range of 4.4.-6.0% (15, 16).

Cytokine assay methodology: Plasma pro- and anti-inflammatory cytokine levels (IFN**γ**, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13 and TNFα) were quantified using the Meso Scale Discovery (MSD) V-PLEX plus kit (Meso Scale Diagnostics, Cat# K15049G). The assay was performed according to the manufacturer's protocol. The intra- and inter-assay coefficients of variability were <10%.

Study Supplies:

Ergocalciferol and placebo were prepared as identical capsules by Boulevard Pharmaceutical Compounding Center, Worcester, Massachusetts, USA(17) and shipped directly to the Investigational Drug Services (IDS) of the University of Massachusetts Medical School (UMMS), which maintained accountability logs for receipt and dispensing of study drugs.

Procedure:

Following enrollment, all subjects entered a run-in phase of 1-2-month duration and started on TTIR.

- *Insulin Adjustment*

At enrollment, all subjects were on multiple daily injections. Long-acting insulin was self-adjusted by subjects using TTIR based on a titration algorithm of (-1)-0-(+1) units to adjust basal insulin dose every 3rd day to maintain fasting plasma glucose (FPG) between 90-120 mg/dL (5.0-6.7 mmol/L) (**Table 1)**. Insulin-to-carbohydrate ratio, correction factor, ideal blood glucose, and insulin sensitivity factor were adjusted as needed for normoglycemia **(Table 2)**.

- *Glucose Data Collection:*

Fingerstick glucose monitoring: Fasting and non-fasting capillary glucose data were uploaded to the UMass MyCareTeam (18) website every 4 weeks.

Continuous glucose monitoring (CGM) using subjects shared their glucose data with the study team via cloud-based data repositories.

Hypoglycemia was classified as nocturnal [plasma glucose of ≤60 mg/dL (3.3 mmol/L) between 11 PM and 6 AM]; symptoms only (plasma glucose of > 60 mg/dL (3.3 mmol/L) or no measurement); minor (plasma glucose < 60 mg/dL (3.3 mmol/L); and major (hypoglycemia requiring third party assistance)(19). To prevent nocturnal hypoglycemia, subjects targeted pre-bedtime and nocturnal glucose level >100 mg/dL (5.6 mmol/L) **(Table 2).**

- *Nutrition and Exercise:*

A registered dietician instructed all subjects on medical nutrition therapy at study entry and at 6 months. No specific exercise regimen was prescribed.

- *Randomization protocol:*

Subjects were randomized to either ergocalciferol or placebo at the conclusion of the run-in phase. The random treatment assignments were generated using a permuted block design with random block sizes of 2, 4, or 6 by the Quantitative Methods Core and administered by the IDS, both at UMMS.

Ergocalciferol treatment

The study group received ergocalciferol 50,000 international units (IU) orally once weekly for 2 months, and then once every other week for 10 months to maintain serum 25(OH)D concentration between 20-100 ng/dL. This dose was determined to be below the standard tolerable upper intake level for ergocalciferol for people >9 yr (20) and thus unlikely to lead to ergocalciferol toxicity. This dosing regimen was chosen to ensure an early rise in the serum 25(OH)D concentration.

Forty-eight participants were enrolled, and 12 subjects were excluded before randomization **(Figure 1)**. Thirty-six subjects were randomized to either ergocalciferol (n=18) or placebo (n=18) after stratification by BMI into normal-weight (BMI<85th percentile) and overweight/obese subjects (BMI ≥85th percentile). Nine subjects from the normal-weight group received ergocalciferol, and 9 subjects from the overweight/obese group received ergocalciferol. Subjects received similar-appearing pills and pill-counting dossettes to monitor compliance.

Randomization-Allocation concealment

Double-blinded treatments were allocated using sequentially numbered drug containers. Concealed treatment allocation was made by the IDS, which also secured blinding codes during the trial. IDS maintained a sealed copy of the randomization sequence at the investigation site in case of the need for emergency unblinding.

Randomization-Implementation:

The IDS established the randomization sequence. Trial endocrinologists enrolled patients to the study. A pharmacist, unconnected with the study, assigned participants to the groups.

Blinding

Subjects and investigators were blinded to the identity of the study products. IDS maintained blinding information throughout the study duration. At study conclusion, the randomization code was decrypted in a two-step procedure: first step, treatment A or B; second step, A = ergocalciferol and B = placebo. All statistical analyses were performed after the second step of unblinding.

Objectives

The primary objective was to determine the impact of adjunctive ergocalciferol on RBCF and PR in youth with newly diagnosed T1D.

The secondary objectives were to determine the longitudinal changes in insulin-dose-adjusted A1c (IDAA1c), HbA1c, fasting blood glucose (FBS), total daily dose of insulin (TDDI), and inflammatory cytokines between the groups.

Outcomes

Primary outcome: Comparison of the changes in baseline and stimulated C-peptide levels between the ergocalciferol and placebo groups over 12 months.

Secondary outcomes: Comparison of the changes in glycemia, IDAA1c, and inflammatory markers.

Safety parameters included monitoring of 25(OH)D, calcium, phosphorus, urinary Ca/Cr ratio in all participants, and pregnancy tests in female subjects.

Protocol Deviations

COVID-19 Pandemic lockdown:

Subject study visits were suspended from March 16th through June 19th, 2020 because of a temporary closure of the Clinical Research Center (CRC) for precautionary measures. Following the lifting of the lockdown, 2 subjects left the study due to fear of contracting the virus at the CRC. One of these 2 subjects was later determined to be in the placebo group, and the other was in the ergocalciferol group.

Compliance monitoring measures

Compliance was monitored by frequent review of MyCareTeam software downloads, along with CGM downloads, counting of pills in the dosettes during clinic visits, review of study drug administration diaries, and the review of subjects' home documentation of glucose data. Analysis of compliance parameters and pharmacy records showed no difference in compliance between the two groups. Data on CGM metrics were not shown as <8 participants used the CGM in each group.

Statistical Analyses

Sample size and power calculation: The planned sample size was based on establishing a stable estimate (with a two-sided 95% confidence interval) for the difference in C-peptide between the two treatment groups. Based on the assumption of a meaningful difference of 20%(21) from a baseline C-peptide level of 0.80 ng/mL(6) with a standard deviation at 12 months of follow-up of 0.38 ng/mL(6), a sample size of 24 per group would produce a distance from the mean difference to the limit of the two-sided 95% confidence interval of ±0.22 ng/mL. This sample size was also sufficient to produce a Cohen's effect size (f^2)of almost 0.30, a moderate-large effect size, for the least-squares adjusted treatment differences, which is expected for this size study. In contrast, we were able to randomize 36 participants, rather than the planned 48. This impacted the size of the 95% confidence interval, increasing it from ±0.22 ng/ml for the planned sample size to ±0.26 ng/mL, but had little impact on the effect size.

Statistical analysis was based on the intent-to-treat principle. Subject characteristics were summarized using means and standard deviations (SD). Group-specific comparisons of anthropometric and biochemical parameters were performed at baseline using two-sample Student's *t* test (or Satterthwaite test for unequal variances) for continuous variables and

Chi-square test for dichotomized variables. Differences in treatment effects between the 2 groups were evaluated by comparison of the 5 study time points for the outcomes of interest, using generalized linear regression model on outcome variables, with regressors being treatment group, time, and their interaction term. A significant interaction term signifies different patterns of trend over time between the 2 groups. Generalized estimating equations method was used to account for correlations between repeated measures. All analyses were performed using SAS9.4.

RESULTS

Baseline Characteristics

Table 3 shows a comparison of the baseline characteristics of the placebo and the ergocalciferol groups. There were no significant differences between the two groups at study entry.

Achieved serum 25-hydroxyvitamin D [25(OH)D] concentration

Serum 25(OH)D concentration (**Figure 2**) was significantly higher in the ergocalciferol group compared to the placebo group at 6 months (p=0.01) and at 9 months (p=0.02).

Anthropometric parameters and C-peptide levels

There were no significant differences in change from baseline to 12 months in systolic and diastolic blood pressure, BMI z-score, and waist circumference between the two groups (**Tables 3 and 4**). There was no significant difference in the overall mean of fasting C-peptide concentration between the 2 groups during the trial (p=0.54) **(Table 5, Figure 3a)**, nor in fasting C-peptide trend over time (p=0.72). Similarly, for stimulated C-peptide neither

the overall mean (p=0.08) **(Table 5, Figure 3b),** nor temporal trend (p=0.31) was different between the groups.

HbA1c, fasting blood glucose (FBG), and total daily dose of insulin (TDDI)

Trend analysis showed a rise in HbA1c for the combined groups (p<0.0001; **Figure 3c**). Although there was no significant difference in the overall mean of HbA1c between the groups (p=0.09) **(Table 5),** there was a faster rate of increase in HbA1c in the placebo group, mean rate of change of 0.46% every 3 months, compared to the ergocalciferol group, mean rate of change of 0.14% every 3 months, (p=0.044) **(Table 5, Figure 3c)**. There were no significant differences in trend between the groups for FBG (p=0.10) and TDDI (p=0.10) **(Table 5).**

Insulin dose adjusted A1c (IDAA1c)

IDAA1c increased over time in the combined placebo and ergocalciferol groups (p<0.0001; **Figure 3d**). IDAA1c was lower in the placebo group at 3 months (p=0.05), but subsequently rose sharply in the placebo group, at a mean rate of change of 0.77 every 3 months, whereas the rise was significantly blunted in the ergocalciferol group, at a mean rate of change of 0.30 every 3 months (p=0.015) **(Figure 3d)**.

Longitudinal changes in cytokines

Figure 4 shows the longitudinal changes in serum TNF-α concentration. The mean rate of change in TNF-α was 0.03 per 3 months for the placebo group, and -0.01 per 3 months for the ergocalciferol group, p=0.20. At 12 months, the ergocalciferol group

had a significantly lower serum TNF-α concentration than the placebo group, 1.12 ± 0.1 vs 1.32 ± 0.3 pg/mL, p=0.03 **(Table 6).**

Occurrence of dysglycemia and other adverse events

Adverse events are summarized in **Table 7.** One subject in the placebo group had an episode of DKA, while another subject in the placebo group had a confirmed COVID-19 infection. Biochemical monitoring showed no evidence for hypercalcemia, hypercalciuria, vitamin D toxicity or pregnancy (data not shown).

DISCUSSION

Despite a statistically significant increase in serum 25(OH)D in the ergocalciferol group compared to the placebo group at 6 and 9 months, this 12-month RCT found no significant differences between the groups for the duration of PR, magnitude of RBCF, IDAA1c, and glycemia. However, a significantly faster rate of rise of HbA1c and IDAA1c values in the placebo group suggested a faster rate of loss of RBCF in that group, which indicates protection of RBCF by high dose ergocalciferol supplementation in the experimental group. These results are supported by a recent report on the limitations of stimulated C-peptide (SCP) concentration to denote PR(22). In that study, 55% of children and adolescents with SCP of >300 pmol/L (0.9 ng/mL) at 14.5 months after T1D diagnosis, had low insulin sensitivity (IS) and thus failed to demonstrate classic PR phenotype when assessed by IDAA1c(22). The authors noted that differences in IS exist in PR and suggested that patients in PR with low IS could benefit from interventions to improve IS, and thus blunt increases in IDAA1c values(22). Thus, IDAA1c is a superior marker for PR than SCP(22); and our RCT showed that vitamin D's actions are better demonstrated by a functional dynamic marker, IDAA1c, than an absolute, static test, SCP.

The significantly lower serum TNF-α concentration, a pro-inflammatory agent(23), in the ergocalciferol group is mechanistically significant, as it suggests that vitamin D could lower inflammation in early T1D by decreasing serum TNF-α concentrations. IL-2 promotes regulatory T-cells(24); however, the apparently higher IL-2 levels in the ergocalciferol group were due to one individual with extremely high levels throughout the study and was not a treatment effect.

Glycemic control was optimized in both groups by the application of TTIR(17, 19) such that the overall average HbA1c levels for the ergocalciferol and placebo groups were 7.51% versus 7.61%, p=0.79. This robust degree of glycemic control from TTIR could have prevented the detection of small differences in glycemia arising from ergocalciferol supplementation**.** Also, despite aggressive ergocalciferol dosing, mean achieved serum 25(OH)D in the treatment group peaked at 30.6 ng/mL, possibly too low for maximal benefit.

It is unclear why the subjects did not attain much higher serum 25(OH)D concentrations than 30.6 ng/mL while receiving 50,000 IU of ergocalciferol weekly for 2 months, and then 25,000 IU weekly for 10 months. However, a similar peak 25(OH)D concentration was reported from India by Khadilkar et al(25), in an RCT of 50 girls of ages 14-15 years who received 300,000 IU of ergocalciferol or placebo 4 times in one year while on 250 mg elemental calcium daily. They reported a peak serum 25(OH)D concentration of 30.2 ng/mL in the experimental arm, and 11.2 ng/mL in the placebo arm. The similarity of these peak 25(OH)D concentrations from studies conducted in both temperate and tropical climates argues against the impact of seasonality on the peak 25(OH)D concentrations.

A number of reasons have been advanced to explain failures of RCTs to demonstrate the expected health benefits of vitamin D supplementation(26). These include study design effects that could impact the attainment and maintenance of elevated 25(OH)D concentrations; variations in 25(OH)D effect thresholds; non-supplemental vitamin D intakes during RCTs and variations in the intestinal absorption of vitamin D; the impact of dietary factors that modulate vitamin D efficiency; and the variation of serum 25OHD values with genetic polymorphisms(11, 26, 27). A possible study design effect on this study is the preponderance of male subjects, who usually have robust PR(28), in the placebo arm compared to the ergocalciferol arm. This could explain the initial robust PR in the placebo arm that was then followed by an accelerated loss of RBCF; whereas subjects in the ergocalciferol arm, though made up of mostly female patients, who experience less robust PR, interestingly had a slower rate of decline in RBCF, suggesting that this mismatch of subjects for sex could have skewed our results, and reduced the observed impact of ergocalciferol on the study outcomes.

Our results are similar to reports in youth that found no significant impact of ergocalciferol supplementation on PR(29) or glycemic control(6, 29) but differ from the study that reported a slower rate of decline of residual β-cell function in a combined population of youth and adults with T1D(6). Mishra et al(29) conducted an RCT of vitamin D and calcium using cholecalciferol 2000 IU per day and placebo in 30 children of 6-12 years old and found no difference in glycemic control or PR at the end of the study. Gabbay et al(6) conducted an 18-month RCT of vitamin D and placebo in a mixed population of 38 children, adolescents, and adults of ages 7-30 years. Using cholecalciferol 2000 IU per day or placebo and an inclusion criterion of a higher serum C-peptide level of 0.6 ng/mL, they found no difference in markers of glycemic control and cytokine levels, but a significant increase in C-peptide in the

first year and a slower C-peptide decay in the second year. Though these 2 studies used a similar dose of cholecalciferol, 2000 IU/day, Gabbay et al(6) reported that 25(OH)D rose from a baseline level of 26.3 ng/mL to a peak level of 60.88 ng/mL at 6 months, whereas Mishra et al(29) reported a baseline level of 27.6 ng/mL that peaked in 6 months at 32.8 ng/mL. The intervention group in our study has a baseline 25(OH)D level of 22 ng/mL, which rose to 30.6 ng/mL at 3 months, and then to 29.2 ng/mL at 6 months and 26.5 ng/mL at 9 months. Thus, it is unclear whether the slower decline in C-peptide in Gabbay's study was due to the robust rise in serum 25(OH)D or due to some other factors such as patient selection or geographical location. Our study, however, is the first to demonstrate significant functional and dynamic differences between ergocalciferol and placebo as depicted by significant reductions in the rates of change of both HbA1c and IDAA1c in the ergocalciferol arm. Our study is also distinct as it is the longest of such studies in an exclusive pediatric T1D population using standardized insulin regimen and high-dose ergocalciferol.

The study's limitation is the single-center setting. Its strengths include a RCT design, long duration of follow-up, the use of a high dose of ergocalciferol, the standardization of insulin therapy in both groups, and adequate sample size for statistical power. The RCT design and subject characteristics make the findings generalizable to youth with new-onset T1D.

CONCLUSIONS

Adjunctive ergocalciferol supplementation significantly reduced serum TNF-α concentration and significantly blunted the rates of increase in both A1c and IDAA1c suggesting a protection of RBCF and PR in youth with newly-diagnosed T1D. This suggests that ergocalciferol slowed the rise in insulin requirements by improving insulin sensitivity in youth with newly-diagnosed T1D. Larger studies are needed to quantify the impact of vitamin D on insulin senstivity in youth with T1D.

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AUTHOR CONTRIBUTIONS

All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission**.** BUN conceived the study and contributed to study design, data acquisition, and interpretation. LCA and BAB contributed to study conception and design. SP, GJ, JF, BUN, and RBS contributed to study design and data acquisition. AFL, BAB, and BUN interpreted and analyzed the data. BUN generated the initial draft of the manuscript.

DISCLOSURE

The authors declare that there is no duality of interest associated with this manuscript.

REGISTRATION

Clinical Trials.gov. Registration Number: NCT03046927

DATA AVAILABILITY STATEMENT

Some or all data generated or analyzed during this study are included in this published article or in the data repositories listed in References.

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Table Headings

Table 1: Titration Algorithm for Long-acting Insulin

Titration Algorithm for Long-acting Insulin	
Average value of fasting plasma glucose for 3 consecutive days	Recommended long-acting insulin dose adjustments
<5.0 mmol/L (90 mg/dL)	subtract 1 unit from the total dose of detemir
5.0-6.7 mmol/L (90 – 120 mg/dL)	no adjustments
>6.7 mmol/L (120 mg/dL)	add 1 unit to the total dose of detemir

Table 2: Summary of Daily Capillary Glucose Goals

Time	Before breakfast	Before lunch or dinner	Before bedtime	2 hours after a meal	At 3AM
Glucose level (mmol/L)	5.0-6.7	4.44-7.22	> 5.56	<12.22	>5.56
Glucose level (mg/dL)	90-120	80-130	> 100	< 220	> 100

Table 3: Baseline anthropometric and biochemical characteristics of subjects

Parameters	Placebo (n=18)		Ergocalciferol (n=18)		p value
	Mean	SD	Mean	SD	
Age (years)	14.28	2.86	13.25	2.76	0.28
Height (cm)	158.65	11.30	156.12	12.77	0.53
Height z-score	0.48	1.18	0.50	0.73	0.93
Weight (kg)	56.16	14.66	53.33	15.19	0.58
Weight z-score	0.67	0.68	0.86	0.81	0.45
Body mass index (kg/m^2)	22.01	4.15	22.03	5.41	0.99
Body mass index z-score	0.74	0.68	0.89	0.94	0.59
Waist circumference (cm)	76.22	11.56	76.16	14.83	0.99
Systolic Blood pressure (mmHg)	104.94	9.06	106.44	10.60	0.65
Diastolic Blood pressure (mmHg)	64.67	6.80	64.72	9.14	0.98
Fasting Plasma Glucose (mg/dL)	111.13	35.78	125.83	25.00	0.18
HbA1c (%)	7.47	1.69	7.62	1.35	0.77
TDD insulin (units/day)	27.17	14.41	37.00	29.61	0.23
TDD insulin (units/kg/day)	0.48	0.23	0.51	0.23	0.72
TDD long-acting insulin only (units)	14.14	7.30	18.50	14.81	0.28
	n	%	n	%	
Gender (male)	14	77.9	10	55.6	0.16
Ethnicity (White)	15	88.2	12	85.7	1.00
Pubertal (Tanner II-V)	10	71.4	12	85.7	0.65

Note: p values for continuous variables were obtained by two sample t test, or Satterthwaite test in case variances were not equal, and for dichotomized variables, either Chi-square or Fisher's exact test whichever was appropriate. SDS=standard deviation score; SD=standard deviation; TDDI=total daily dose of insulin; HbA1c=hemoglobin A1c; %=percentage; n=number

Table 4: Longitudinal changes in clinical parameters during the trial

Parameter	Time	Placebo (n= 18)		Ergocalciferol (n=18)		p value (from LSE estimates)
		mean	SD	Mean	SD	
Systolic Blood pressure (mmHg)	Baseline	104.9	9.1	106.4	10.6	0.64
	Month 3	105.0	10.5	105.0	9.1	0.61
	Month 6	108.8	9.9	107.4	13.8	0.91
	Month 9	110.1	11.5	109.3	13.9	0.88
	Month 12	109.1	10.4	107.3	12.6	0.84
Diastolic Blood pressure (mmHg)	Baseline	64.7	6.8	64.7	9.1	0.98
	Month 3	67.2	7.4	65.6	8.5	0.84
	Month 6	66.5	7.0	67.2	9.2	0.62
	Month 9	69.8	8.1	67.8	9.0	0.71
	Month 12	69.6	10.3	66.3	11.3	0.49
Body mass index (kg/m^2)	Baseline	22.0	4.2	22.0	5.4	0.99
	Month 3	22.2	4.7	22.1	6.1	0.84
	Month 6	22.3	4.4	22.1	6.1	0.59
	Month 9	21.9	4.5	22.1	6.2	0.63
	Month 12	22.0	4.8	22.6	6.0	0.48
Body mass index (z-score)	Baseline	0.74	0.68	0.89	0.94	0.58
	Month 3	0.74	0.82	0.74	0.99	0.68
	Month 6	0.66	0.80	0.65	1.11	0.71
	Month 9	0.51	0.80	0.60	1.13	0.69
	Month 12	0.44	0.89	0.65	1.08	0.32
Waist circumference (cm)	Baseline	76.2	11.6	76.2	14.8	0.96
	Month 3	73.2	14.4	74.1	13.2	0.54
	Month 6	76.2	13.6	73.9	12.0	0.88
	Month 9	72.9	14.4	70.7	15.0	0.85
	Month 12	75.2	11.6	75.3	14.3	0.80

Note: LSE=least square estimates, based on generalized linear model with repeated measures; SD=standard deviation

TABLE 5: Longitudinal changes in therapeutic and biochemical parameters during the trial

Parameter	Placebo (n=18)		Ergocalciferol (n=18)		Placebo (n=18)		Ergocalciferol (n=18)		p value			
	Mean	SD	Mean	SD	LSE Mean	SE	LSE mean	SE	Difference in overall mean	Overall trend	Difference in trend	Difference at specific time (adjusted for multiple comparisons)
TDDI (units/kg/day)									0.046	0.0002	0.0973	
Baseline	0.48	0.23	0.51	0.23	0.48	0.05	0.55	0.06				0.41
Month 3	0.46	0.18	0.62	0.36	0.43	0.05	0.62	0.08				0.0488
Month 6	0.49	0.27	0.62	0.30	0.48	0.06	0.67	0.08				0.06
Month 9	0.62	0.28	0.69	0.26	0.63	0.07	0.73	0.07				0.31
Month 12	0.67	0.30	0.65	0.24	0.67	0.07	0.72	0.07				0.65
FBG (mg/dL)									0.13	0.031	0.10	
Baseline	111	36	126	25	115	9	126	6				0.31
Month 3	131	53	157	61	130	12	157	15				0.17
Month 6	146	72	146	54	146	17	147	14				0.97
Month 9	140	61	141	43	137	16	143	12				0.75
Month 12	158	61	145	49	158	15	145	14				0.52
Fasting C-peptide (ng/mL)									0.54	0.012	0.72	
Baseline	0.68	0.40	0.80	0.82	0.71	0.10	0.80	0.19				0.67
Month 3	0.65	0.38	0.59	0.40	0.69	0.09	0.71	0.12				0.90
Month 6	0.71	0.55	0.46	0.29	0.71	0.13	0.51	0.08				0.19
Month 9	0.44	0.24	0.42	0.24	0.49	0.07	0.46	0.06				0.80
Month 12	0.50	0.47	0.35	0.21	0.50	0.11	0.37	0.06				0.29
Stimulated C-peptide (ng/mL)									0.08	<.0001	0.31	

Baseline	2.22	1.31	1.78	1.18	2.27	0.31	1.78	0.28				0.24
Month 3	1.74	0.94	1.28	0.78	1.76	0.21	1.56	0.25				0.55
Month 6	1.51	1.08	1.17	0.92	1.53	0.25	1.40	0.26				0.73
Month 9	1.35	1.34	1.05	0.84	1.36	0.31	1.25	0.24				0.79
Month 12	1.14	1.27	0.82	0.77	1.15	0.29	1.05	0.22				0.80
HbA1c (%)									0.09	<.0001	0.044	
Baseline	7.47	1.69	7.62	1.35	7.47	0.39	7.62	0.32				0.76
Month 3	6.67	1.23	7.20	1.54	6.67	0.28	7.25	0.37				0.21
Month 6	7.12	1.51	7.26	1.18	7.15	0.34	7.28	0.27				0.77
Month 9	7.59	1.37	7.50	1.42	7.61	0.32	7.48	0.35				0.77
Month 12	8.01	1.70	7.64	2.14	8.03	0.41	7.65	0.57				0.59
IDAA1c									0.025	<.0001	0.0151	
Baseline	9.4	2.4	9.7	1.8	9.4	0.6	9.9	0.4				0.51
Month 3	8.5	1.8	9.7	2.3	8.4	0.4	9.7	0.6				0.05
Month 6	9.0	2.4	9.8	1.9	9.0	0.5	10.0	0.5				0.17
Month 9	10.1	2.2	10.2	2.1	10.1	0.5	10.4	0.5				0.62
Month 12	10.7	2.5	10.3	2.3	10.7	0.6	10.6	0.6				0.89

Note: SD=standard deviation; LSE=least square estimate; SE=standard error; TDDI=total daily dose of insulin; FBG=fasting blood glucose; HbA1c=hemoglobin A1c; IDAA1c=insulin dose adjusted A1c. Note: p values were obtained from repeated measure trend analysis using generalized linear model with dependent variable=clinical parameter, and independent variables=group, time, and their interactions. GEE was used for repeated measures

Table 6: Longitudinal changes in inflammatory cytokines during the trial

Parameters	Time	Placebo (n= 18)		Ergocalciferol (n=18)		p value (from LSE estimates)
		mean	SD	mean	SD	
IL-2 (pg/mL)	Baseline	0.57	0.28	1.74	5.34	0.34
	Month 3	0.48	0.25	1.55	4.12	0.28
	Month 6	0.51	0.23	1.68	4.10	0.26
	Month 9	0.50	0.24	1.67	4.31	0.26
	Month 12	0.47	0.24	1.84	4.55	0.23
IL-4 (pg/mL)	Baseline	0.08	0.06	0.06	0.04	0.18
	Month 3	0.08	0.07	0.07	0.07	0.84
	Month 6	0.10	0.13	0.05	0.03	0.41
	Month 9	0.16	0.41	0.06	0.03	0.39
	Month 12	0.14	0.34	0.06	0.04	0.39
IL-6 (pg/mL)	Baseline	0.67	0.65	0.62	0.33	0.75
	Month 3	0.51	0.25	0.61	0.41	0.35
	Month 6	0.71	0.51	0.80	0.38	0.56
	Month 9	1.12	1.54	0.61	0.33	0.19
	Month 12	0.73	0.60	0.62	0.36	0.57
IL-8 (pg/mL)	Baseline	2.61	0.76	2.79	0.97	0.54
	Month 3	2.74	0.86	3.33	2.12	0.26
	Month 6	2.61	0.58	2.98	1.07	0.12
	Month 9	2.83	1.08	2.88	1.00	0.85
	Month 12	3.20	1.49	2.99	1.27	0.73
IL-10 (pg/mL)	Baseline	0.53	0.30	0.56	0.30	0.75
	Month 3	0.51	0.20	0.54	0.28	0.78
	Month 6	0.64	0.68	0.79	0.94	0.61
	Month 9	0.66	0.61	0.55	0.39	0.53
	Month 12	0.68	0.85	0.48	0.08	0.36
IL-12p70 (pg/mL)	Baseline	0.37	0.15	0.30	0.18	0.20
	Month 3	0.34	0.14	0.35	0.18	0.89

	Month 6	0.35	0.17	0.51	0.78	0.43
	Month 9	0.41	0.42	0.37	0.16	0.70
	Month 12	0.32	0.14	0.32	0.17	0.98
IL-13 (pg/mL)	Baseline	3.24	0.71	2.86	0.58	0.08
	Month 3	3.01	0.58	3.13	0.64	0.72
	Month 6	3.12	0.51	3.20	0.71	0.83
	Month 9	3.05	0.60	3.00	0.70	0.81
	Month 12	2.79	0.49	3.25	0.84	0.09
IL-1β (pg/mL)	Baseline	0.14	0.11	0.13	0.10	0.84
	Month 3	0.11	0.08	0.16	0.15	0.18
	Month 6	0.13	0.08	0.18	0.15	0.12
	Month 9	0.12	0.08	0.16	0.11	0.18
	Month 12	0.11	0.10	0.18	0.11	0.06
IFN-γ (pg/mL)	Baseline	5.87	7.32	5.73	5.68	0.95
	Month 3	3.69	1.36	4.41	2.98	0.37
	Month 6	7.82	16.07	8.27	15.51	0.94
	Month 9	4.79	4.24	3.55	1.14	0.28
	Month 12	7.84	10.93	3.28	0.93	0.09
TNF-α (pg/mL)	Baseline	1.28	0.27	1.22	0.35	0.56
	Month 3	1.26	0.29	1.19	0.39	0.58
	Month 6	1.30	0.26	1.21	0.36	0.50
	Month 9	1.41	0.76	1.13	0.33	0.17
	Month 12	1.32	0.26	1.12	0.13	**0.03**

Note: Median test was also performed to ensure that results provided in this Table were not affected by outliers. LSE=least square estimate, based on generalized linear model with repeated measures; IL=interleukin; IFN=interferon; TNF=tumor necrosis factor; SD=standard deviation; significant p value is bolded

Table 7: Adverse events

Adverse events	Vitamin D arm	Placebo arm
Upper respiratory tract complaints	1	5
Sinusitis	1	1
Otitis media	0	1
Hyperglycemia	3	1
Diabetic ketoacidosis	0	1
Mild hypoglycemia	3	1
Moderate hypoglycemia	1	0
Diarrhea	1	0
Stomachache	0	1
Vomiting	1	0
Transient weight loss	0	1
Skin rash	1	0
Mild transient hair loss	1	1
Confirmed COVID-19 infection	0	1

Figure Legends

Figure 1:

CONSORT Flow Diagram

Figure 2

A graph of the changes in 25-hyroxyvitamin D concentration in a 12-month RCT of ergocalciferol in youth with new-onset type 1 diabetes. The graph shows significantly higher 25-hydroxyvitamin D concentration in the ergocalciferol group compared to the placebo at 6 months (p=0.01) and at 9 months (p=0.02). All p-values were adjusted for multiple comparisons.

Figure 3a

Trend analysis of the least square estimates (LSE) of the means for fasting C-peptide showing no significant difference in the changes in fasting C-peptide concentration between the ergocalciferol- and placebo-treated patients with type 1 diabetes during the 12-month trial, (p=0.72).

Figure 3b

Trend analysis of the least square estimates of the means for stimulated C-peptide showing no significant difference in the change in stimulated C-peptide concentration at 90 minutes between the ergocalciferol- and placebo-treated patients with type 1 diabetes during the 12-month trial, (p=0.31).

Figure 3c

Least square estimates of the means for hemoglobin A1c (HbA1c) showing the change in HbA1c between the ergocalciferol and placebo groups during the trial

Trend analysis shows a rise in HbA1c value for the combined groups ($p<0.0001$). There was evidence of a faster rate of increase in HbA1c in the placebo compared to the vitamin group ($p=0.044$).

Figure 3d

Least square estimates of the means for insulin dose adjusted A1c (IDAA1c) showing the changes in IDAA1c between the ergocalciferol and placebo groups during the trial

Trend analysis shows a rise in IDAA1c value for the combined groups ($p<0.0001$). Though IDAA1c was significantly lower in the placebo group at 3 months, it subsequently rose sharply when compared to the vitamin group ($p=0.015$), suggesting that subjects in placebo group had greater residual β-cell function at the beginning of the study but lost this function at a faster rate than the individuals in the ergocalciferol group.

Figure 4

Least square estimates of the means for serum TNF-α concentration showing the changes in TNF-α between the ergocalciferol and placebo groups during the trial. The mean of rates of change in TNF-α was 0.03 per 3 months for the placebo group, and 0.01 per 3 months for the ergocalciferol group. Serum TNF-α concentration was significantly lower in the ergocalciferol group at 12 months.

Figure 1

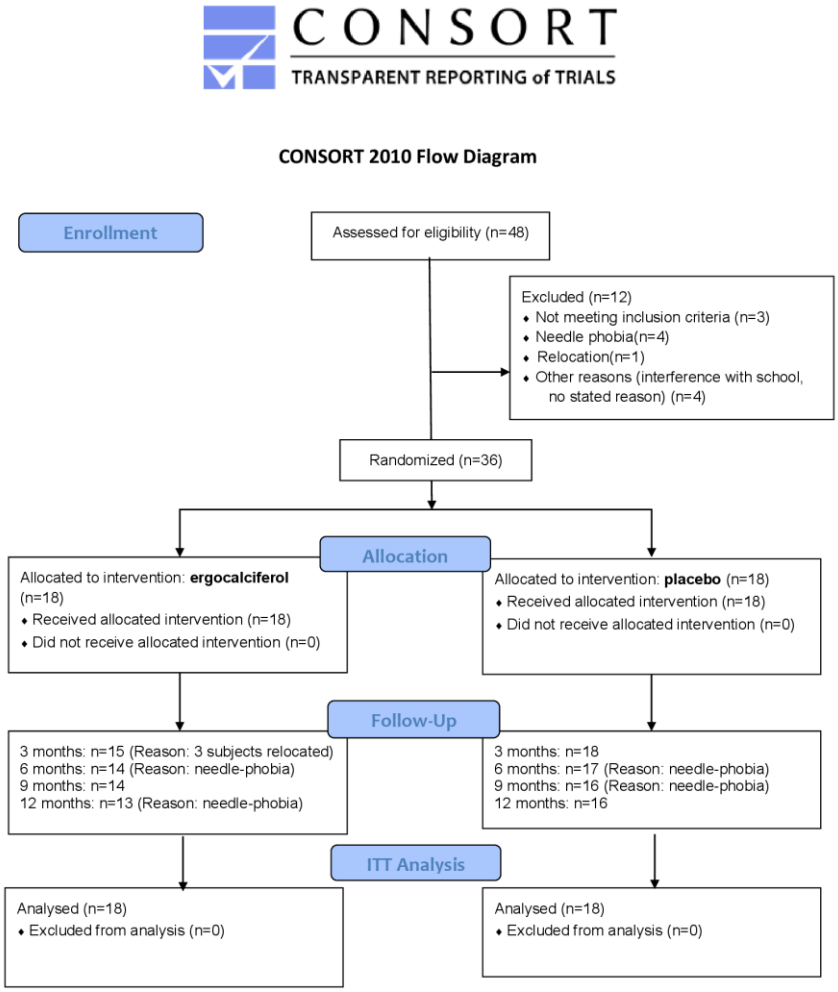

Figure 2

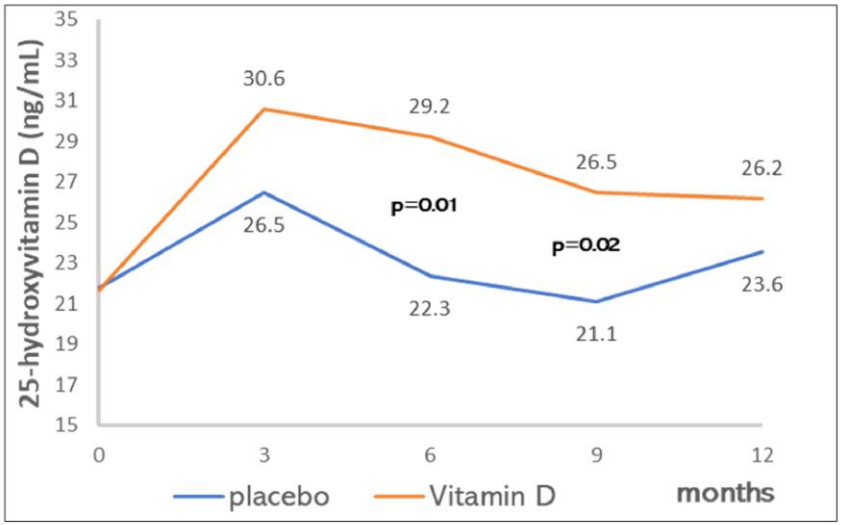

Figure 3

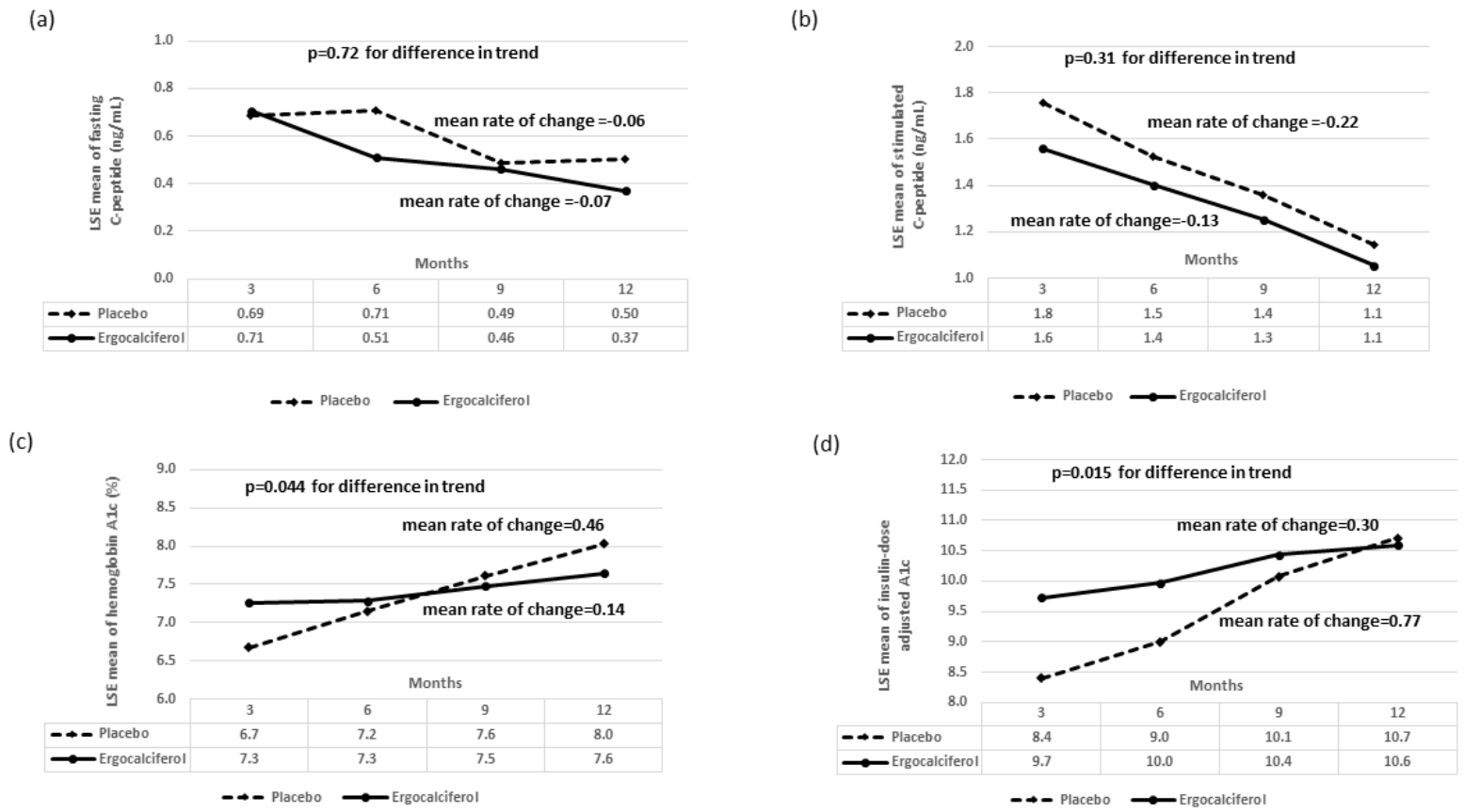

Figure 4