

COMPARATIVE EFFECTIVENESS RESEARCH

Effect of Cholecalciferol as Adjunctive Therapy With Insulin on Protective Immunologic Profile and Decline of Residual β -Cell Function in New-Onset Type 1 Diabetes Mellitus

Mônica A. L. Gabbay, MD, PhD; Maria N. Sato, MD, PhD; Claudia Finazzo, MS(Biomed); Alberto J. S. Duarte, MD, PhD; Sergio A. Dib, MD, PhD

Objective: To evaluate the effect of vitamin D₃ on cytokine levels, regulatory T cells, and residual β -cell function decline when cholecalciferol (vitamin D₃ administered therapeutically) is given as adjunctive therapy with insulin in new-onset type 1 diabetes mellitus (T1DM).

Design and Setting: An 18-month (March 10, 2006, to October 28, 2010) randomized, double-blind, placebo-controlled trial was conducted at the Diabetes Center of São Paulo Federal University, São Paulo, Brazil.

Participants: Thirty-eight patients with new-onset T1DM with fasting serum C-peptide levels greater than or equal to 0.6 ng/mL were randomly assigned to receive daily oral therapy of cholecalciferol, 2000 IU, or placebo.

Main Outcome Measure: Levels of proinflammatory and anti-inflammatory cytokines, chemokines, regulatory T cells, hemoglobin A_{1c}, and C-peptide; body mass index; and insulin daily dose.

Results: Mean (SD) chemokine ligand 2 (monocyte chemoattractant protein 1) levels were significantly higher (184.6 [101.1] vs 121.4 [55.8] pg/mL) at 12 months, as well as the increase in regulatory T-cell percentage (4.55% [1.5%] vs 3.34% [1.8%]) with cholecalciferol vs placebo. The cumulative incidence of progression to undetectable (≤ 0.1 ng/mL) fasting C-peptide reached 18.7% in the cholecalciferol group and 62.5% in the placebo group; stimulated C-peptide reached 6.2% in the cholecalciferol group and 37.5% in the placebo group at 18 months. Body mass index, hemoglobin A_{1c} level, and insulin requirements were similar between the 2 groups.

Conclusions: Cholecalciferol used as adjunctive therapy with insulin is safe and associated with a protective immunologic effect and slow decline of residual β -cell function in patients with new-onset T1DM. Cholecalciferol may be an interesting adjuvant in T1DM prevention trials.

Arch Pediatr Adolesc Med. 2012;166(7):601-607

TYPE 1 DIABETES MELLITUS (T1DM) is caused by a complex autoimmune process associated with altered humoral and cellular immunity that results in the specific immune destruction of insulin-producing β cells before and after the diagnosis.¹⁻³ There is clear evidence of clinical benefit resulting from preserved β -cell function⁴; in the past few years, intervention trials in patients with new-onset T1DM have been undertaken to silence and/or modulate this altered immune response, aiming to preserve endogenous insulin production.^{5,6}

*For editorial comment
see page 664*

A growing body of evidence from animal and in vitro studies suggests a functional role of vitamin D₃ as an adaptive immune system modulator.⁷ Most tissues in the body

have receptors for the active form of vitamin D₃, which are also expressed in activated T and B human lymphocytes. β Cells are able to produce 1,25 dihydroxyvitamin D₃ [1,25(OH)₂D₃], the biologically active form of vitamin D₃, with a marked increase in cytosolic calcium after acute application of this vitamin.⁸ Immunologic studies⁹⁻¹² in animal models showed that cholecalciferol (vitamin D₃ given therapeutically) not only acts to suppress lymphocyte proliferation but also can modify the helper T-cell subtypes 1 and 2 (T_H1/T_H2) cytokines profile. Cytokines are considered hormones of the immune system that have important functions related to cellular proliferation, differentiation, and survival, and it is widely recognized that T_H1 cytokine is associated with cell-mediated immunity and that T_H2 cytokine is associated with humoral immune responses.¹³ It is believed that T1DM appears to be triggered by T_H1 cells, while T_H2 cells may be protective.¹⁴

Author Affiliations: Diabetes Center of Endocrinology Division, Department of Medicine, São Paulo Federal University (Drs Gabbay and Dib), and LIM56-Immunology Laboratory, University of São Paulo (Drs Sato, Finazzo, and Duarte), São Paulo, Brazil.

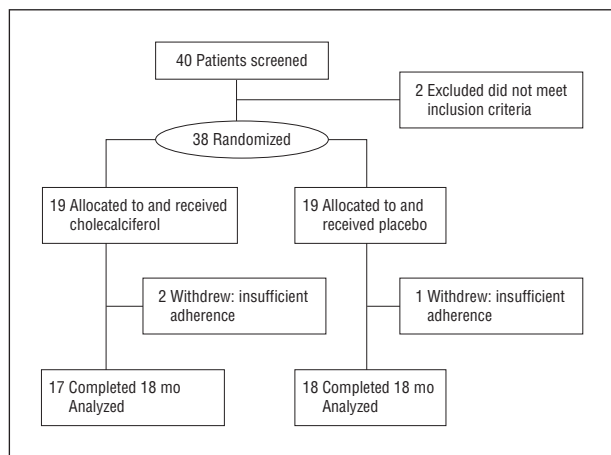


Figure 1. Flowchart of the 18-month randomized, parallel-group, double-blind, placebo-controlled trial in patients with new-onset type 1 diabetes.

It also has been reported¹⁵⁻¹⁷ that exposition of human β cells to $1,25(\text{OH})_2\text{D}_3$ protects them from death by reducing expression of major histocompatibility complex class I molecules, inducing expression of antiapoptotic A20 protein and decreasing expression of Fas, a transmembrane cell surface receptor transducing an apoptotic death signal and contributing to the pathogenesis of several autoimmune diseases.

There are data¹⁸ showing that supplementation with cholecalciferol in infants, as well as through dietary exposure to vitamin D_3 during pregnancy, is associated with reduced risk of human T1DM. In the clinical setting, 2 recent trials^{19,20} found no significant effect of $1,25(\text{OH})_2\text{D}_3$ on the preservation of β -cell function after T1DM onset. Nevertheless, the dose and mechanism of action of cholecalciferol in this condition are still under discussion.

Chemokines, chemotactic cytokines that possess the ability to attract and recruit distinct types of cells, have been implicated as recruiters of pathogenic and regulatory T cells to the pancreatic islets, highlighting their role in T1DM pathogenesis^{21,22} and in deceleration of disease onset.²³

In vivo studies²⁴ have shown that $1,25(\text{OH})_2\text{D}_3$ inhibits the expression of inflammatory cytokines in monocytes, such as interleukin 6 (IL-6), IL-8, IL-12, and tumor necrosis factor, in healthy individuals. The influence of this vitamin on cytokine production by lymphocytes may be another important link between vitamin D_3 and the immune system.²⁵

The aim of the present study was to evaluate the effect of vitamin D_3 [$25(\text{OH})\text{D}_3$], the main circulating metabolite of vitamin D_3 , on peripheral cytokine/chemokine levels, regulatory T cells, and residual β -cell function decrease when given as adjunctive therapy to insulin in patients with new-onset T1DM.

METHODS

DESIGN AND SETTING

We conducted a prospective 18-month randomized, double-blind, placebo-controlled study of cholecalciferol concomitant with intensive insulin therapy in patients with new-onset T1DM.

The research protocol (No. 0101/05) was approved by the São Paulo Federal University Ethics Committee, and written informed consent was provided by patients or their guardians. Assent was obtained from children. The study was conducted at the Diabetes Center of São Paulo Federal University, São Paulo, Brazil, from March 10, 2006, through October 28, 2010.

PARTICIPANTS

We screened 40 patients with T1DM (according to the American Diabetes Association standards²⁶), and 38 were randomized to receive cholecalciferol ($n=19$) or placebo ($n=19$); 2 individuals were excluded because they did not meet inclusion criteria. Three patients withdrew from the study, 1 in the placebo group and 2 in the cholecalciferol group because of insufficient adherence. A total of 17 participants in the cholecalciferol group and 18 in the placebo group completed the 18-month follow-up period (**Figure 1**).

Inclusion criteria were (1) age between 7 and 30 years, (2) disease duration less than 6 months (ie, from first insulin injection), (3) positive test results for islet cell autoantibodies (anti-glutamic acid decarboxylase 65 or antiprotein tyrosine phosphatase), and (4) fasting or 2-hour postmeal stimulated serum C-peptide level of 0.6 ng/mL or more (to convert to nanomoles per liter, multiply by 0.331) during a mixed meal tolerance test (6 mL/kg of body weight [maximum, 360 mL]; Sustagen, Mead Johnson). This test was performed between 7 and 10 AM after an overnight fast, during which only water was ingested. The patients received no short-acting insulin for at least 6 hours before the test. This C-peptide level was chosen according to the protocol of the TrialNet Study Group.²⁷ Exclusion criteria were severe systemic disease and disorders in calcium metabolism.

RANDOMIZATION

Randomization was performed by a pharmacist, without the researchers' knowledge. Patients were assigned to receive 2000 IU of cholecalciferol ($n=17$) or placebo ($n=18$) orally at breakfast daily throughout an 18-month period. We chose this dose because it was associated with reduced risk of type 1A diabetes in a Finnish birth-cohort study.¹⁸ The cholecalciferol and placebo were produced by an independent pharmacy in São Paulo (Millenium) and sent coded to the Diabetes Center.

INTERVENTION

After baseline evaluation, patients returned to the Diabetes Center at 1, 3, 6, 9, 12, 15, and 18 months for insulin dosage adjustment, hemoglobin (Hb) A_{1c} measurement, and safety assessment, and to receive their supply of cholecalciferol or placebo (adherence was assessed by drug container return). Evaluation was performed at 0, 6, 12, and 18 months for serum C-peptide, cytokines, and chemokines, as well as peripheral blood regulatory T-cell percentage. The insulin regimen for both trial arms was intensive (multiple [>3] daily injections associated with glucose self-monitoring [>3 checks of the glucose level]).

OUTCOME MEASURES

Complete blood cell count, aminotransferase levels, and phosphate and alkaline phosphatase levels were evaluated by automated chemistry analyzers (Advia 120 and 2400; Siemens, Germany).

The HbA_{1c} level was measured in whole blood using high-performance liquid chromatography (Tosoh Bioscience, normal value, 3.5%-6.0%). Serum C-peptide level was measured by an immunofluorometric assay (AutoDelfia) with a detec-

tion limit of 0.15 ng/mL (to convert to nanomoles per liter, multiply by 0.331). The intra-assay variation was 4.2% (0.52 ng/mL) and the interassay variations were 1.1% (0.52 ng/mL) and 3.4% (6.1 ng/mL).

Antiglucamic acid decarboxylase 65 and antiprotein tyrosine phosphatase were measured using a commercial radioimmunoassay kit (RSR Limited). Serum ionized calcium was measured using specific ion electrodes (AVL model 9180, Roche Diagnostics; normal value, 4.84-5.60 mg/dL [to convert to millimoles per liter, multiply by 0.25]).

The 25(OH)D₃ concentrations were determined by electrochemoluminescence (Roche Diagnostics). This immunoassay is specific for 25(OH)D₃. The intra-assay and interassay coefficients of variation for 25(OH)D₃ (39.9 ng/mL) were 5.7% and 7.3%, respectively (to convert to nanomoles per liter, multiply by 2.496).

Cytokines from undiluted patient serum were measured using inflammatory cytokine and chemokine bead array kits (Becton Dickinson). Analysis of the regulatory T cells (CD4⁺CD25⁺ regulatory T cells expressing FoxP3) was performed in peripheral blood mononuclear cells according to the manufacturer's instructions (eBioscience).

STATISTICAL ANALYSIS

Data that were not normally distributed were log transformed for analysis and back transformed for presentation. Data are expressed as mean (SD). We used repeated-measures analysis of variance to compare the cholecalciferol and placebo groups in terms of serum C-peptide, body mass index (calculated as weight in kilograms divided by height in meters squared), HbA_{1c}, and required dose of insulin. A paired 2-tailed *t* test or Mann-Whitney rank sum test was used for other comparisons. Pearson product moment correlation and multivariate regression analysis were used to assess correlation and association between and among variables. Kaplan-Meier life tables were constructed and compared by means of the log-rank χ^2 statistic to verify the percentage of individuals in both groups in whom serum C-peptide decreased to undetectable levels (<0.1 ng/mL) during monitoring. Statistical significance was assumed at *P* ≤ .05. Statistical analyses were performed using commercial software (SPSS version 17 for Windows, SPSS Inc).

RESULTS

The cholecalciferol and placebo groups were similar in terms of age (mean [SD], 13.5 [5.1] vs 12.5 [4.8] years), sex (male, 61% vs 59%), Tanner stage 1 (44% vs 38%), and duration of disease (2.2 [1.2] vs 2.7 [1.7] months).

Serum concentrations of 25(OH)D₃ in the cholecalciferol-treated group (basal, 26.34 [6.49] vs 6 months, 60.88 [21.64] ng/mL; *P* < .001) increased during this period and were maintained until the end of the study. In the placebo group, there was a nonsignificant (*P* = .38) variation in the 25(OH)D₃ serum levels during the same period (basal, 25.76 [5.68] vs 6 months, 28.57 [8.77] ng/mL). The 25(OH)D₃ serum levels exhibited a trend to be higher in the treatment group than in the placebo group (65.02 [25.95] vs 40.52 [10.35] ng/mL; *P* = .06) at 18 months. Plasma ionic calcium concentrations were similar in the cholecalciferol and placebo groups at basal conditions (1.31 [0.08] vs 1.33 [0.05] ng/mL) and at 18 months (1.35 [0.47] vs 1.29 [0.05] ng/mL). Other safety determinants (complete blood cell count and aminotransferase, phosphorus, and alkaline phosphatase lev-

Table 1. Changes in Clinical, Metabolic, and Endocrine Variables in Patients With New-Onset T1DM During the Study

Characteristic	Mean (SD)		<i>P</i> Value ^a
	Cholecalciferol (n = 17)	Placebo (n = 18)	
Fasting C-peptide, ng/mL			
Baseline	0.65 (0.45)	0.92 (0.83)	.25
Month 6	0.88 (0.85)	0.76 (0.71)	.66
Month 12	0.55 (0.42)	0.55 (0.35)	.99
Month 18	0.45 (0.34)	0.43 (0.49)	.91
Stimulated C-peptide, ng/mL			
Baseline	1.55 (0.91)	1.83 (1.03)	.40
Month 6	1.74 (1.57)	1.43 (0.97)	.47
Month 12	1.26 (1.03)	1.18 (1.04)	.84
Month 18	1.10 (1.03)	0.96 (1.08)	.73
Hemoglobin A _{1c} , % ^b			
Baseline	9.25 (2.17)	7.73 (2.16)	.05
Month 6	7.34 (1.56)	7.40 (2.14)	.93
Month 12	7.72 (1.96)	7.57 (1.65)	.81
Month 18	8.29 (2.06)	8.91 (2.71)	.53
Daily insulin dose, U/kg/d			
Baseline	0.52 (0.19)	0.43 (0.19)	.19
Month 6	0.57 (0.24)	0.56 (0.20)	.92
Month 12	0.72 (0.18)	0.69 (0.28)	.79
Month 18	0.81 (0.37)	0.80 (0.27)	.91
Body mass index ^b			
Baseline	18.5 (3.0)	18.6 (2.5)	.75
Month 6	18.7 (3.0)	19.0 (2.1)	.55
Month 12	18.7 (2.8)	19.3 (2.7)	.51
Month 18	19.5 (2.8)	20.5 (4.2)	.83

Abbreviation: T1DM, type 1 diabetes mellitus.

SI conversion factors: To convert C-peptide to nanomoles per liter, multiply by 0.331; to convert hemoglobin A_{1c} to a proportion of total hemoglobin, multiply by 0.01.

^aBetween-group comparisons.

^bCalculated as weight in kilograms divided by height in meters squared.

els) also were within the reference ranges in both groups. No clinical adverse events were reported during the study.

The groups were similar in terms of body mass index and insulin dosage (U/kg/d) at baseline and during the study (**Table 1**). The HbA_{1c} level was significantly higher in the cholecalciferol group than in the placebo group at the beginning of the study and fell significantly after 6 months in the cholecalciferol group (*P* = .05), with no significant difference in the other time points between the 2 groups.

There were no significant differences in sera concentrations of IL-12, IL-6, IL-1 β , IL-8, IL-10, chemokine IL-8, and chemokine IL-99 between the groups at baseline and during follow-up (**Table 2**). A significant decrease in IL-12 serum levels was detected in the first 6 months of study in both groups (*P* = .05).

In the cholecalciferol group, interferon gamma-inducible protein 10 (CXCL10) levels were significantly higher at baseline (148.1 [100.2] pg/mL vs 93.4 [47.4] pg/mL; *P* = .02) compared with the placebo group, as well as chemokine ligand 2 (CCL2) at 12 months (184.6 [101.1] pg/mL vs 121.4 [55.8] pg/mL; *P* = .04) and with a trend of higher values until the end of the study (*P* = .07) in the cholecalciferol group (Table 2). After regression analysis, IL-6 was the only independent factor

Table 2. Changes in IL-12, TNF, CXCL10, CCL2, and Regulatory T Cells in Patients With New-Onset T1DM During the Study

Characteristic	Mean (SD)		P Value ^a
	Cholecalciferol (n = 17)	Placebo (n = 18)	
IL-12, pg/mL			
Baseline	12.5 (17.5)	20.5 (36.3)	.25
Month 6	3.5 (7.3)	3.2 (4.3)	.66
Month 12	6.4 (10.4)	3.8 (8.9)	.99
Month 18	3.9 (5.0)	4.5 (6.3)	.91
TNF, pg/mL			
Baseline	9.3 (15.7)	17.4 (33.9)	.40
Month 6	1.9 (6.3)	1.1 (3.4)	.47
Month 12	4.6 (8.9)	2.2 (7.7)	.84
Month 18	2.2 (3.8)	2.3 (5.2)	.73
Chemokine IL-10, pg/mL			
Baseline	148.1 (100.2)	93.4 (47.4)	.05
Month 6	104.9 (57.1)	120.3 (91.7)	.93
Month 12	113.9 (99.2)	82.6 (38.3)	.81
Month 18	204.2 (311.9)	88.4 (59.1)	.53
IL-10, pg/mL			
Baseline	1.3 (0.5)	1.2 (0.5)	.19
Month 6	1.5 (0.6)	1.1 (0.6)	.92
Month 12	2.0 (3.1)	1.2 (0.5)	.79
Month 18	1.4 (1.0)	1.1 (0.6)	.91
CCL2, pg/mL			
Baseline	192.3 (92.0)	168.5 (87.8)	.75
Month 6	139.2 (45.2)	184.2 (87.1)	.55
Month 12	184.6 (101.1)	121.4 (55.8)	.051
Month 18	221.9 (257.2)	136.6 (69.1)	.83
Regulatory T cells, %			
Baseline	3.34 (1.8)	2.78 (1.7)	.75
Month 6	4.09 (2.3)	3.54 (1.5)	.55
Month 12	4.55 (1.5)	3.96 (2.1)	.51
Month 18	3.75 (1.6)	3.20 (1.5)	.83

Abbreviations: CCL2, chemokine ligand 2; CXCL, interferon gamma-inducible protein 10; IL, interleukin; T1DM, type 1 diabetes mellitus; TNF, tumor necrosis factor.

^aBetween-group comparisons.

associated with CCL2 ($\beta = 49.23$; $P = .02$). No association was found between chemokines/cytokines and serum C-peptide or metabolic variables during the monitoring period.

Percentages of regulatory T cells were not significantly different in either group at baseline ($P = .75$), but increased significantly at 12 months of the follow-up study in the cholecalciferol group ($P = .04$). The percentage of these cells in peripheral blood was not associated with any clinical or other immunologic variables studied.

During the follow-up period, there was a decrease in the antigitamic acid decarboxylase 65 titer of 60% (baseline vs 18 months; $P = .05$) in the cholecalciferol group, and antiprotein tyrosine phosphatase titers showed great variability, with a paradox increase of 160% ($P = .02$) up to 18 months with cholecalciferol vs placebo.

Interestingly, stimulated serum C-peptide was enhanced in the first 12 months (12% vs -35%; $P = .01$) and had less decay until 18 months (-14% vs -46%) in the cholecalciferol group compared with the placebo group ($P = .03$) (Figure 2).

The cumulative incidence of progression to undetectable (≤ 0.1 ng/mL) fasting serum C-peptide during the

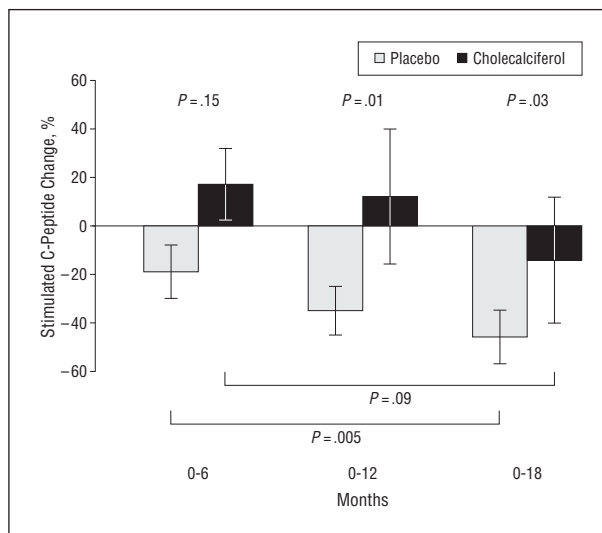


Figure 2. Analysis of the percentage of change in stimulated C-peptide level at 0, 6, 12, and 18 months in patients with new-onset type 1 diabetes treated with cholecalciferol or placebo. Limit lines indicate standard deviation.

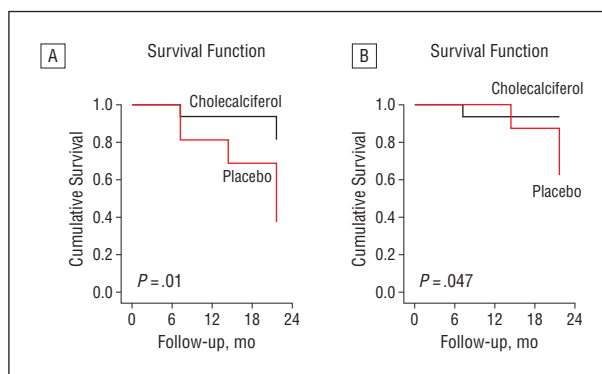


Figure 3. A cumulative incidence of progression to undetectable fasting C-peptide (A) and stimulated C-peptide (B) (≤ 0.1 ng/mL) in patients with new-onset type 1 diabetes treated with cholecalciferol or placebo. The number of patients was unchanged, with 17 in the cholecalciferol group and 18 in the placebo group.

18 months of monitoring reached 18.7% in the cholecalciferol group and 62.5% in the placebo group ($P = .01$), and stimulated C-peptide reached this level in 6.2% of the cholecalciferol group and 37.5% of the placebo group ($P = .047$) (Figure 3A and B).

Regression analysis showed that body mass index ($\beta = 0.09$, $P = .047$), insulin dose ($\beta = -1.20$, $P = .006$), and HbA_{1c} ($\beta = -0.16$, $P = .02$) were independently related to stimulated serum C-peptide.

COMMENT

This placebo-controlled trial of cholecalciferol, 2000 IU/d, involved patients with new-onset T1DM, including children, who had significant residual β -cell function, so that a possible effect of this vitamin could be detected. We found that stimulated serum C-peptide was enhanced during the first year after diagnosis and showed a significantly smaller decline after 18 months (the primary end point) with cholecalciferol compared with placebo.

Our results also suggest, in a clinical setting, a preliminary mechanism of action of cholecalciferol supplementation, demonstrating increased levels of peripheral CCL2 as well as the percentage of regulatory T cells in the cholecalciferol-treated group. Chemokine ligand 2 is a potent factor in the polarization of T_H0 cells toward a T_H2 phenotype.^{28,29} However, T_H1/T_H2 imbalance does not necessarily mean that T_H1 is only proinflammatory and T_H2 is only anti-inflammatory; instead, the interplay between those cytokines and defects in regulatory T cells probably determine the fate of β cells in T1DM.³⁰⁻³²

Taken together, the increase of CCL2 serum levels and regulatory T cells by cholecalciferol treatment in patients with new-onset T1DM may collaborate to delay the autoimmune destruction of the β cells.

Quantification of 25(OH)D₃ in serum is the best indicator of overall vitamin D status. The important forms of vitamin D are cholecalciferol (vitamin D₃) and ergocalciferol (vitamin D₂). However, the potency of ergocalciferol is less than one-third that of cholecalciferol, and more than 95% of 25(OH)D measurable in serum is usually 25(OH)D₃. The cholecalciferol and placebo groups had insufficient (<30 ng/mL) levels of 25(OH)D₃ at baseline. Low serum levels of vitamin D₃ have usually been reported in T1DM.^{33,34} The vitamin D₃ serum level increased in both groups during the study and was, as expected, significantly higher in the cholecalciferol-treated group. Normalization of 25(OH)D₃ levels in the placebo group after 1 year of the study may be related to a relationship between glycemic control and vitamin D₃ metabolism that is not yet understood. Higher 25(OH)D₃ and ionic calcium levels in the treatment group confirmed the participants' adherence to the cholecalciferol prescription. We did not observe any adverse events with this cholecalciferol dosage (2000 IU/d) during the study. Furthermore, it is an inexpensive, safe, and readily available drug, and it seems to be more correlated with regulatory T cells than with 1,25(OH)₂ D₃.³⁵

Immunotherapy targeting either B (anti-CD20) or T (anti-CD3) cells or induced regulatory T cells (GAD-alum) has been proposed³⁶ to preserve residual C-peptide secretion in new-onset T1DM.

Comparison of other studies with ours shows that stimulated serum C-peptide decreased approximately 38% in the anti-CD20 trial,³⁷ approximately 16% in the anti-CD3 trial,³⁸ approximately 8% in the GAD-alum trial,³⁹ and 14% in our (cholecalciferol study) at 18 months in the treatment group. In the placebo groups of these studies, decreases in the stimulated serum C-peptide levels were 56% in the anti-CD20 trial, 60% in the anti-CD3 trial, 75% in the GAD-alum trial, and 46% in our study. Therefore, our study has shown cholecalciferol to be one of the nontoxic approaches to preserve β -cell function in newly diagnosed T1DM.

Our results of cholecalciferol therapy in young patients with new-onset T1DM are in accordance with those of a study on latent autoimmune diabetes in adults.⁴⁰ One common aspect of these 2 studies was an inclusion criterion of new-onset T1DM with a baseline C-peptide level higher than 0.6 ng/dL. The fasting serum C-peptide level in our patients was higher than that in studies^{19,20} that had not shown a protective effect of cholecalciferol on β -cell function.

Similar to other cholecalciferol studies, our study did not find significant differences between the treatment and placebo groups in the daily insulin requirements or HbA_{1c} levels. These findings, however, are contrary to those of B-cell (anti-CD20) and T-cell (anti-CD3) studies^{37,38} that demonstrated improvement in HbA_{1c} levels and reduction of the daily insulin dose in the treatment group. It appears that ideal combination therapy would include 2 or more agents with complementary mechanisms, well-defined safety profiles in humans, and prolonged efficacy.⁴¹

Early glycemic control in T1DM is important to preserve β -cell function and inhibit progression of the disease.⁴² Because our study was randomized, we could not discard this possibility, since the cholecalciferol group had poorer metabolic control at baseline than did the placebo group (HbA_{1c}, 9.25% [2.17%] vs 7.73% [2.16%]; $P = .05$); however, both groups soon achieved similar glycemic control (at month 6) and remained similar throughout the study. Therefore, we suggest that glycemic control per se may not be responsible for the difference (cholecalciferol vs placebo) found in the evolution of residual β -cell function.

Many studies⁴³ demonstrate that glucotoxicity might also influence cytokine and chemokine production. Therefore, the higher levels of CXCL10 in the cholecalciferol group than in the placebo group may be explained by the higher HbA_{1c} level in the treatment vs placebo group at the beginning of the study.⁴⁴

To our knowledge, the influence of cholecalciferol as an adjunct to insulin therapy and glycemic control in cell-mediated immunity and proinflammatory cytokines has not been studied in T1DM. We analyzed the evolution of proinflammatory and anti-inflammatory cytokines/chemokines and regulatory T cells in our patients during cholecalciferol supplementation. In the first 6 months of the study, we saw overall diminished inflammatory cytokine secretion (IL-12, IL-6, IL-1 β , and tumor necrosis factor) in both groups. In general, it was related to improvement in metabolic control and anti-inflammatory effects of insulin.⁴⁵ However, after this period, we observed 3 benefits for the patients' immunologic profiles: (1) a tendency to increase IL-10, (2) a significant increase of CCL2, and (3) an increase in regulatory T cells, which might have contributed to the less-progressive β -cell decay in the cholecalciferol group.

There are data potentially involving IL-10-dependent regulatory T-cell pathways on recovery of β -cell function even after the clinical onset of T1DM.⁴⁶ An increase of CCL2 may suggest a shift toward helper T-cell immunity; however, this has been described⁴⁷ as occurring with routine insulin therapy during the first 2 years after diagnosis in a group of children with newly diagnosed T1DM. With respect to cholecalciferol supplementation associated with increase in the percentage of regulatory T cells, this was recently reported⁴⁸ in apparently healthy individuals who had taken 140 000 U of cholecalciferol monthly for 3 months.

Finally, we cannot rule out that the lesser decay of serum C-peptide shown in the cholecalciferol group could be mediated by its direct action on β -cell function, increasing the levels of cytosolic calcium that is important for the movement of insulin granules and insulin secretion.⁴⁹

Overall, we showed that cholecalciferol doses of 2000 IU/d concomitant with insulin therapy is safe and related to protective immunologic profile and slow decline of residual β -cell function in new-onset T1DM. Cholecalciferol can be an interesting adjuvant in combination with immunosuppressant drugs in T1DM prevention studies.

Accepted for Publication: February 2, 2012.

Correspondence: Mônica A. L. Gabbay, MD, PhD, Diabetes Center of Endocrinology Division, Department of Medicine, São Paulo Federal University, Rua Pedro de Toledo 910, Vila Clementino, CEP: 04032-001 São Paulo, SP, Brazil (monicagabbay@gmail.com).

Author Contributions: Dr Gabbay had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. **Study concept and design:** Gabbay, Duarte, and Dib. **Acquisition of data:** Gabbay, Sato, and Finazzo. **Analysis and interpretation of data:** Gabbay and Dib. **Drafting of the manuscript:** Gabbay, Sato, and Finazzo. **Critical revision of the manuscript for important intellectual content:** Gabbay, Duarte, and Dib. **Obtained funding:** Dib. **Administrative, technical, and material support:** Gabbay, Sato, and Dib. **Study supervision:** Dib.

Financial Disclosure: None reported.

Funding/Support: This study was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo and Coordenadoria de Aperfeiçoamento do Pessoal de Ensino Superior—Programas de Excelencia, Ministry of Education of Brazil.

Additional Contributions: Soraya Ogusuku, MBS, provided expert help with cytokine and chemokine analyses, and Maria do Rosário Dias de Oliveira Latorre, PhD, and Jose Italo Dias, MS, conducted statistical analyses. We are grateful to all the parents and patients who participated in the study.

REFERENCES

- Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care*. 2003;26(suppl 1):S5-S20.
- Atkinson MA, Eisenbarth GS. Type 1 diabetes: new perspectives on disease pathogenesis and treatment. *Lancet*. 2001;358(9277):221-229.
- van Belle TL, Coppieters KT, von Herrath MG. Type 1 diabetes: etiology, immunology, and therapeutic strategies. *Physiol Rev*. 2011;91(1):79-118.
- Palmer JP, Fleming GA, Greenbaum CJ, et al. C-peptide is the appropriate outcome measure for type 1 diabetes clinical trials to preserve β -cell function: report of an ADA workshop, 21-22 October 2001. *Diabetes*. 2004;53(1):250-264.
- Waldron-Lynch F, Herold KC. Advances in type 1 diabetes therapeutics: immunomodulation and β -cell salvage. *Endocrinol Metab Clin North Am*. 2009;38(2):303-317, viii.
- Ludvigsson J. Immune intervention at diagnosis—we treat children to preserve beta-cell function? *Pediatr Diabetes*. 2007;8(suppl 6):34-39.
- Hewison M. Vitamin D and the immune system: new perspectives on an old theme. *Endocrinol Metab Clin North Am*. 2010;39(2):365-379.
- Bland R, Markovic D, Hills CE, et al. Expression of 25-hydroxyvitamin D₃-1 α -hydroxylase in pancreatic islets *J Steroid Biochem Mol Biol*. 2004;89-90(1-5):121-125.
- Provedini DM, Manolagas SC. 1 α ,25-Dihydroxyvitamin D₃ receptor distribution and effects in subpopulations of normal human T lymphocytes. *J Clin Endocrinol Metab*. 1989;68(4):774-779.
- Boonstra A, Barrat FJ, Crain C, Heath VL, Savelkoul HFJ, O'Garra A. 1 α ,25-Dihydroxyvitamin D₃ has a direct effect on naive CD4⁺ T cells to enhance the development of Th2 cells. *J Immunol*. 2001;167(9):4974-4980.
- Gregori S, Giarratana N, Smioldo S, Uskokovic M, Adorini L. A 1 α ,25-dihydroxyvitamin D₃ analog enhances regulatory T-cells and arrests autoimmune diabetes in NOD mice. *Diabetes*. 2002;51(5):1367-1374.
- Gysemaans CA, Cardozo AK, Callewaert H, et al. 1,25-Dihydroxyvitamin D₃ modulates expression of chemokines and cytokines in pancreatic islets: implications for prevention of diabetes in nonobese diabetic mice. *Endocrinology*. 2005;146(4):1956-1964.
- Sia C. Imbalance in Th cell polarization and its relevance in type 1 diabetes mellitus. *Rev Diabet Stud*. 2005;2(4):182-186.
- Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor α -chains (CD25): breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *J Immunol*. 1995;155(3):1151-1164.
- Hahn HJ, Kuttler B, Mathieu C, Bouillon R. 1,25-Dihydroxyvitamin D₃ reduces MHC antigen expression on pancreatic beta-cells in vitro. *Transplant Proc*. 1997;29(4):2156-2157.
- Riachy R, Vandewalle B, Kerr Conte J, et al. 1,25-Dihydroxyvitamin D₃ protects RINm5F and human islet cells against cytokine-induced apoptosis: implication of the antiapoptotic protein A20. *Endocrinology*. 2002;143(12):4809-4819.
- Riachy R, Vandewalle B, Moerman E, et al. 1,25-Dihydroxyvitamin D₃ protects human pancreatic islets against cytokine-induced apoptosis via down-regulation of the Fas receptor. *Apoptosis*. 2006;11(2):151-159.
- Hyppönen E, Läärä E, Reunanen A, Järvelin MR, Virtanen SM. Intake of vitamin D and risk of type 1 diabetes: a birth-cohort study. *Lancet*. 2001;358(9292):1500-1503.
- Walter M, Kaupper T, Adler K, Foersch J, Bonifacio E, Ziegler AG. No effect of the 1 α ,25-dihydroxyvitamin D₃ on β -cell residual function and insulin requirement in adults with new-onset type 1 diabetes. *Diabetes Care*. 2010;33(7):1443-1448.
- Bizzarri C, Pitocco D, Napoli N, et al; IMDIAB Group. No protective effect of calcitriol on β -cell function in recent-onset type 1 diabetes: the IMDIAB XIII trial. *Diabetes Care*. 2010;33(9):1962-1963.
- Frigerio S, Junt T, Lu B, et al. β Cells are responsible for CXCR3-mediated T-cell infiltration in insulinitis. *Nat Med*. 2002;8(12):1414-1420.
- Flaishon L, Hart G, Zelman E, et al. Anti-inflammatory effects of an inflammatory chemokine: CCL2 inhibits lymphocyte homing by modulation of CCL21-triggered integrin-mediated adhesions. *Blood*. 2008;112(13):5016-5025.
- Shimada A, Oikawa Y, Yamada Y, Okubo Y, Narumi S. The role of the CXCL10/CXCR3 system in type 1 diabetes. *Rev Diabet Stud*. 2009;6(2):81-84.
- Almerighi C, Sinistro A, Cavazza A, Ciapriani C, Rocchi G, Bergamini A. 1 α ,25-Dihydroxyvitamin D₃ inhibits CD40L-induced pro-inflammatory and immunomodulatory activity in human monocytes. *Cytokine*. 2009;45(3):190-197.
- Willheim M, Thien R, Schratlbauer K, et al. Regulatory effects of 1 α ,25-dihydroxyvitamin D₃ on the cytokine production of human peripheral blood lymphocytes. *J Clin Endocrinol Metab*. 1999;84(10):3739-3744.
- American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care*. 2012;35(suppl 1):S64-S71. doi:10.2337/dc12-s064.
- Skyler JS, Greenbaum CJ, Lachin JM, et al; Type 1 Diabetes TrialNet Study Group. Type 1 Diabetes TrialNet—an international collaborative clinical trials network. *Ann N Y Acad Sci*. 2008;1150:14-24.
- Gu L, Tseng S, Horner RM, Tam C, Loda M, Rollins BJ. Control of Th2 polarization by the chemokine monocyte chemoattractant protein-1. *Nature*. 2000;404(6776):407-411.
- Roep BO. The role of T-cells in the pathogenesis of type 1 diabetes: from cause to cure. *Diabetologia*. 2003;46(3):305-321.
- Tang Q, Henriksen KJ, Bi M, et al. In vitro-expanded antigen-specific regulatory T cells suppress autoimmune diabetes. *J Exp Med*. 2004;199(11):1455-1465.
- Bettini M, Vignali DA. Regulatory T cells and inhibitory cytokines in autoimmunity. *Curr Opin Immunol*. 2009;21(6):612-618.
- Lindley S, Dayan CM, Bishop A, Roep BO, Peakman M, Tree TIM. Defective suppressor function in CD4⁺CD25⁺ T-cells from patients with type 1 diabetes. *Diabetes*. 2005;54(1):92-99.
- Littorin B, Blom P, Schölin A, et al. Lower levels of plasma 25-hydroxyvitamin D among young adults at diagnosis of autoimmune type 1 diabetes compared with control subjects: results from the nationwide Diabetes Incidence Study in Sweden (DISS). *Diabetologia*. 2006;49(12):2847-2852.
- Svoren BM, Volkening LK, Wood JR, Laffel LMR. Significant vitamin D deficiency in youth with type 1 diabetes mellitus. *J Pediatr*. 2009;154(1):132-134.
- Smolders J, Menheere P, Thewissen M, et al. Regulatory T cell function correlates with serum 25-hydroxyvitamin D, but not with 1,25-dihydroxyvitamin D,

- parathyroid hormone and calcium levels in patients with relapsing remitting multiple sclerosis. *J Steroid Biochem Mol Biol.* 2010;121(1-2):243-246.
36. Boettler T, von Herrath M. Immunotherapy of type 1 diabetes—how to rationally prioritize combination therapies in T1D. *Int Immunopharmacol.* 2010; 10(12):1491-1495.
 37. Pescovitz MD, Greenbaum CJ, Krause-Steinrauf H, et al; Type 1 Diabetes Trial-Net Anti-CD20 Study Group. Rituximab, B-lymphocyte depletion, and preservation of beta-cell function. *N Engl J Med.* 2009;361(22):2143-2152.
 38. Herold KC, Gitelman S, Greenbaum C, et al; Immune Tolerance Network ITN007A1 Study Group. Treatment of patients with new onset type 1 diabetes with a single course of anti-CD3 mAb teplizumab preserves insulin production for up to 5 years. *Clin Immunol.* 2009;132(2):166-173.
 39. Ludvigsson J, Faresjö M, Hjorth M, et al. GAD treatment and insulin secretion in recent-onset type 1 diabetes. *N Engl J Med.* 2008;359(18):1909-1920.
 40. Li X, Liao L, Yan X, et al. Protective effects of 1- α -hydroxyvitamin D₃ on residual β -cell function in patients with adult-onset latent autoimmune diabetes (LADA). *Diabetes Metab Res Rev.* 2009;25(5):411-416.
 41. Matthews JB, Staeva TP, Bernstein PL, Peakman M, von Herrath M; ITN-JDRF Type 1 Diabetes Combination Therapy Assessment Group. Developing combination immunotherapies for type 1 diabetes: recommendations from the ITN-JDRF Type 1 Diabetes Combination Therapy Assessment Group. *Clin Exp Immunol.* 2010;160(2):176-184.
 42. Shah SC, Malone JI, Simpson NE. A randomized trial of intensive insulin therapy in newly diagnosed insulin-dependent diabetes mellitus. *N Engl J Med.* 1989; 320(9):550-554.
 43. Foss-Freitas MC, Foss NT, Donadi EA, Foss MC. Effect of the glycemic control on intracellular cytokine production from peripheral blood mononuclear cells of type 1 and type 2 diabetic patients. *Diabetes Res Clin Pract.* 2008;82(3):329-334.
 44. Paroni F, Domsgen E, Maedler K. CXCL10—a path to β -cell death. *Islet.* 2009; 1(3):256-259. doi:10.4161/isl.1.3.9110.
 45. Ghanim H, Korzeniewski K, Sia CL, et al. Suppressing effect of insulin infusion on chemokines and chemokine receptors. *Diabetes Care.* 2010;33(5):1103-1108.
 46. Karges B, Durinovic-Belló I, Heinze E, Boehm BO, Debatin KM, Karges W. Complete long-term recovery of β -cell function in autoimmune type 1 diabetes after insulin treatment. *Diabetes Care.* 2004;27(5):1207-1208.
 47. Antonelli A, Fallahi P, Ferrari SM, et al. Serum Th1 (CXCL10) and Th2 (CCL2) chemokine levels in children with newly diagnosed type 1 diabetes: a longitudinal study. *Diabet Med.* 2008;25(11):1349-1353.
 48. Prietl B, Piiz S, Wolf M, et al. Vitamin D supplementation and regulatory T cells in apparently healthy subjects: vitamin D treatment for autoimmune diseases? *Isr Med Assoc J.* 2010;12(3):136-139.
 49. Bourlon PM, Faure-Dussert A, Billaudel B. Modulatory role of 1,25 dihydroxyvitamin D₃ on pancreatic islet insulin release via the cyclic AMP pathway in the rat. *Br J Pharmacol.* 1997;121(4):751-758.

Correction

Error in Acknowledgments. In the Article titled “Selective Protection Against Extremes in Childhood Body Size, Abdominal Fat Deposition, and Fat Patterning in Breast-fed Children” by Crume et al, published in the May issue of the *Archives* (2012;166[5]:437-443), an error occurred in the Acknowledgments section on page 442. A stray line of type (“had no role in the study.”) between the “Role of the Sponsor” and “Additional Contributions” sections should have been deleted.