

# No Effect of the $1\alpha,25$ -Dihydroxyvitamin $D_3$ on $\beta$ -Cell Residual Function and Insulin Requirement in Adults With New-Onset Type 1 Diabetes

MARKUS WALTER, MD<sup>1</sup>  
THOMAS KAUPPER, MD<sup>1</sup>  
KERSTIN ADLER, PHD<sup>1</sup>

JOHANNES FOERSCH<sup>1</sup>  
EZIO BONIFACIO, PHD<sup>2</sup>  
ANETTE-G. ZIEGLER, MD<sup>1</sup>

**OBJECTIVE** — To determine whether daily intake of  $1\alpha,25$ -dihydroxyvitamin  $D_3$  [ $1,25(\text{OH})_2\text{D}_3$ ] is safe and improves  $\beta$ -cell function in patients with recently diagnosed type 1 diabetes.

**RESEARCH DESIGN AND METHODS** — Safety was assessed in an open study of 25 patients aged 18–39 years with recent-onset type 1 diabetes who received 0.25  $\mu\text{g}$   $1,25(\text{OH})_2\text{D}_3$  daily for 9 months. An additional 40 patients were randomly assigned to 0.25  $\mu\text{g}$   $1,25(\text{OH})_2\text{D}_3$  or placebo daily for 9 months and followed for a total of 18 months for safety,  $\beta$ -cell function, insulin requirement, and glycemic control.

**RESULTS** — Safety assessment showed values in the normal range in nearly all patients, regardless of whether they received  $1,25(\text{OH})_2\text{D}_3$  or placebo. No differences in AUC C-peptide, peak C-peptide, and fasting C-peptide after a mixed-meal tolerance test between the treatment and placebo groups were observed at 9 and 18 months after study entry, with  $\sim 40\%$  loss for each parameter over the 18-month period. A1C and daily insulin requirement were similar between treatment and placebo groups throughout the study follow-up period.

**CONCLUSIONS** — Treatment with  $1,25(\text{OH})_2\text{D}_3$  at a daily dose of 0.25  $\mu\text{g}$  was safe but did not reduce loss of  $\beta$ -cell function.

*Diabetes Care* 33:1443–1448, 2010

Type 1 diabetes results from autoimmunity against the insulin-producing  $\beta$ -cells of the pancreatic islets (1). A treatment that could stop or decrease autoimmune destruction of  $\beta$ -cells would provide substantial progress in type 1 diabetes therapy and could potentially be effective in preventing type 1 diabetes in individuals at high risk of developing the disease (2).

A multinational case-control study and a birth cohort follow-up study from Finland with prerecorded exposure data (3,4) have concluded that vitamin  $D_3$  supplementation at birth protects individuals from type 1 diabetes later in life,

and these conclusions are supported by meta-analysis (5). Others report lower serum levels of  $1\alpha,25$ -dihydroxyvitamin  $D_3$  [ $1,25(\text{OH})_2\text{D}_3$ , calcitriol] in patients with recently diagnosed type 1 diabetes than in healthy control subjects (6), although this finding is inconsistent among studies (7). Mechanistic studies show that  $1,25(\text{OH})_2\text{D}_3$  modulates dendritic cell maturation in vitro and in vivo (8–12) and facilitates a shift from a Th1 to a Th2 immune response (13). Furthermore, studies in the nonobese diabetic (NOD) mouse show that  $1,25(\text{OH})_2\text{D}_3$  reduces the incidence of insulinitis and diabetes (14). Vitamin  $D_3$  has also been shown to

have beneficial effects on insulin action (15), although a recent large study found no association between serum vitamin  $D$  concentration and insulin secretion or action (16).

$1,25(\text{OH})_2\text{D}_3$  was introduced to the clinic as a therapy to increase intestinal calcium resorption and serum levels of calcium, e.g., in patients with renal insufficiency. For type 1 diabetes, there is one report of its use in a pilot open study in which patients received intensive insulin therapy and either 0.25  $\mu\text{g}$  calcitriol on alternate days or nicotinamide (25 mg/kg daily) with up to 1-year follow-up (17). The results of this study were somewhat inconclusive, showing no difference between the two arms with respect to  $\beta$ -cell reserve, but a modest and transient reduction in insulin requirement in the group receiving calcitriol at the dose used. To determine whether  $1,25(\text{OH})_2\text{D}_3$  could be used at higher overall doses in patients with type 1 diabetes and whether this could reduce  $\beta$ -cell loss after type 1 diabetes onset, we performed a two-phase study assessing safety as well as efficacy.

## RESEARCH DESIGN AND METHODS

Patients with recent-onset type 1 diabetes were referred for participation in the study from hospitals or outpatient clinics in Bavaria, Germany, between November 2000 and 2006. They were selected according to the following criteria: they were 18–39 years of age, had been treated with insulin for less than 2 months (62 days), had a positive result on testing for islet autoantibodies (anti-GAD antibodies or anti-IA-2 antibodies), had plasma levels of calcium, phosphate, alkaline phosphatase, and creatinine within the normal ranges, and were compliant with insulin treatment. Exclusion criteria were disorders in calcium metabolism, kidney diseases, malignancy, and arterial hypertension. Pregnant or lactating women were excluded, and female patients with child-bearing potential had to practice an acceptable contraceptive technique from enrollment until 30 days after the last dose of study drug. Written in-

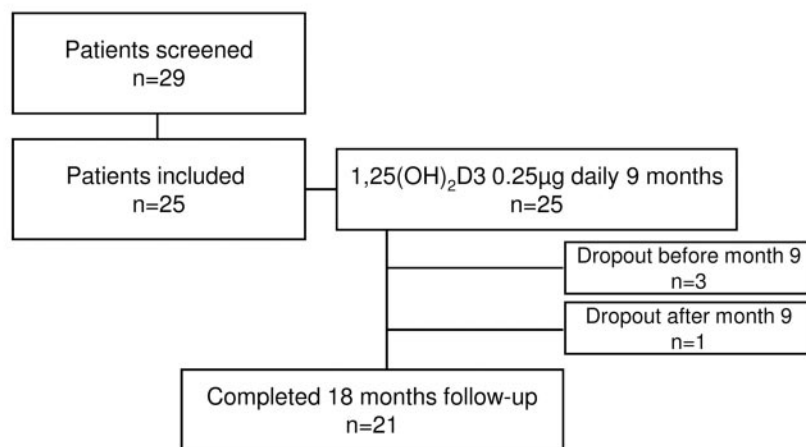
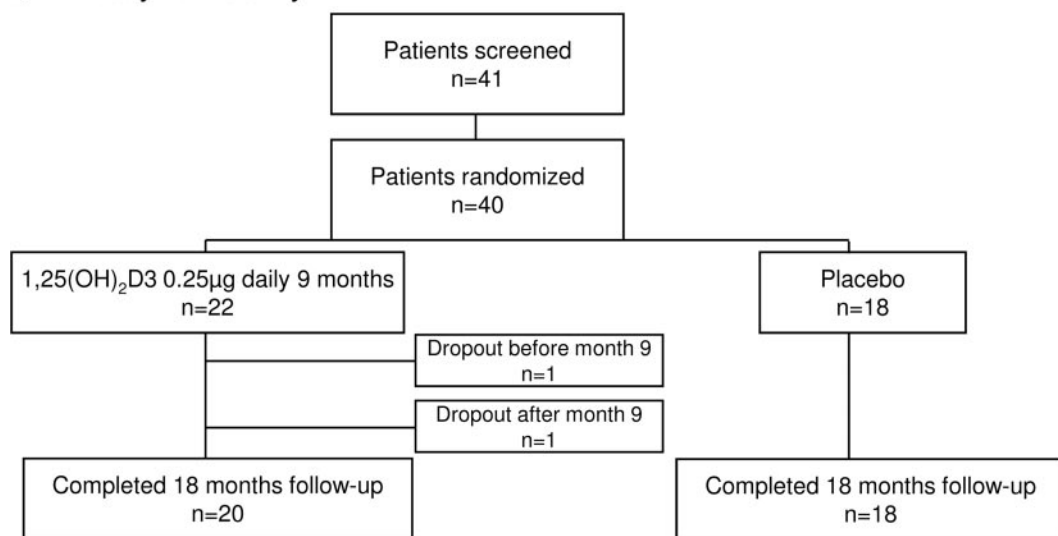
From the <sup>1</sup>Diabetes Research Institute, Forschergruppe Diabetes e.V., Munich, Germany; and the <sup>2</sup>Deutsche Forschungsgemeinschaft Center for Regenerative Therapies, Dresden, Germany.

Corresponding author: Anette-G. Ziegler, anziegler@lrz.uni-muenchen.de.

Received 16 December 2009 and accepted 18 March 2010. Published ahead of print at <http://care.diabetesjournals.org> on 31 March 2010. DOI: 10.2337/dc09-2297. Clinical Trials reg. no. NCT00960635, clinicaltrials.gov.

© 2010 by the American Diabetes Association. Readers may use this article as long as the work is properly cited, the use is educational and not for profit, and the work is not altered. See <http://creativecommons.org/licenses/by-nc-nd/3.0/> for details.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

**A Safety****B Efficacy and Safety**

**Figure 1**—Flow of participants. A: Participation in the open study designed to assess safety of 1,25(OH)<sub>2</sub>D<sub>3</sub> (n = 25). B: Random assignment into the phase II, randomized, double-blind, placebo-controlled study to assess efficacy of study drug (n = 22 study drug, n = 18 placebo).

formed consent was obtained from each patient. The study was conducted at the Diabetes Research Institute, Munich, Germany, and was approved by the ethics committee of the Medical Faculty at the Ludwig-Maximilians University, Munich, Germany.

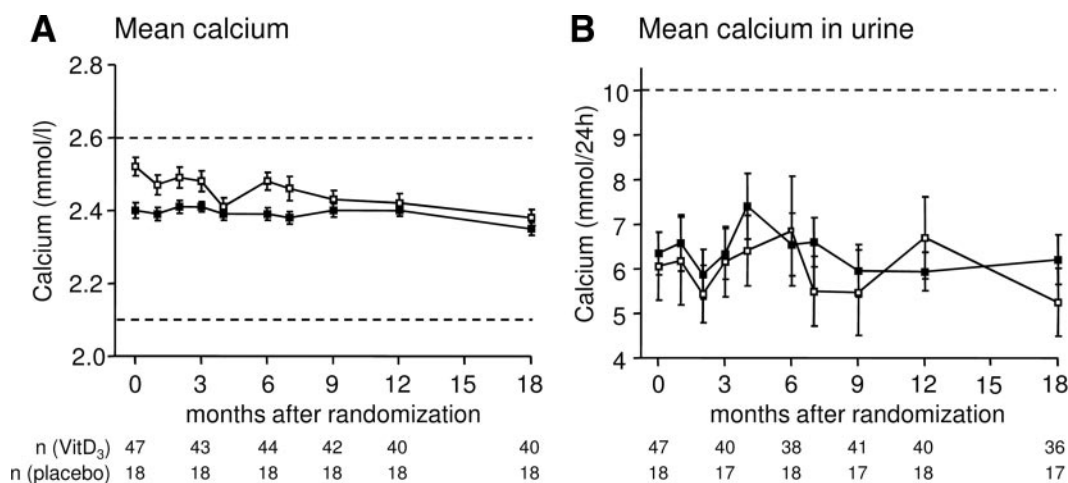
The study had two phases. The first was designed to assess safety at the dose selected for investigation. It was an open study that included 25 patients (median age  $\pm$  SD, 31.2  $\pm$  7.3 years; 14 men) who received treatment with 0.25  $\mu$ g 1,25(OH)<sub>2</sub>D<sub>3</sub> as Rocaltrol (F. Hoffmann-La Roche, Basel, Switzerland) daily at breakfast for 9 months and were followed for a total of 18 months (9 additional months after treatment). Safety parameters (plasma levels of calcium, phosphate, alkaline phosphatase, and creatinine and urinary calcium excre-

tion) were measured at study entry and at 1, 2, 3, 4, 5, 6, 7, 8, 9, 12, and 18 months. Kidney sonography was performed at study entry and at 9 and 18 months.

The second phase was subsequently performed to assess the effect of the study drug on residual  $\beta$ -cell function via a phase II, monocenter, randomized, double-blind, placebo-controlled study. Forty-one patients were screened, and 40 of these were randomly assigned to oral 0.25  $\mu$ g 1,25(OH)<sub>2</sub>D<sub>3</sub> (n = 22; median age  $\pm$  SD 31.4  $\pm$  6.8 years; 16 men; median days after diagnosis 35.0) or oral placebo (n = 18; age 24.0  $\pm$  6.0 years; 13 men; median days after diagnosis 40.0) daily at breakfast over a 9-month period. Randomization was performed by an independent pharmacy (Bastei-Apotheke, Munich, Germany), and the study medi-

cation was sent coded to the Diabetes Research Institute. Patients returned to the study center to receive their study drug supply and have efficacy and safety assessments performed at 0, 1, 2, 3, 4, 6, 7, 9, 12, and 18 months for safety, A1C, and insulin dose, and at 0, 9, and 18 months for a mixed-meal tolerance test (MMTT) and kidney ultrasound.

In both phases of the study, patients continued their normal insulin regimen as intensive insulin therapy, unless changes were clinically indicated. To avoid possible confounding through differences in glycemic control among the groups, diabetes management and glycemic targets were standardized as much as possible in all patients. Patients with A1C levels >8% had additional contacts with the study investigator to improve their metabolic control.



**Figure 2**—Safety parameters. Plasma calcium concentrations (A) and 24-h urinary calcium excretion values (B) for patients who received study drug ( $n = 47$ , ■) and patients who received placebo ( $n = 18$ , □) over 18 months of follow-up. Data are means  $\pm$  SEM. The reference calcium plasma levels (2.1–2.6 mmol/l) and urine secretion ( $<10$  mmol/24 h) are indicated by the dotted line. VitD<sub>3</sub>, 1 $\alpha$ ,25-dihydroxyvitamin D<sub>3</sub>.

### Safety parameters

Safety parameters included laboratory tests (plasma calcium, phosphate, creatinine, and alkaline phosphatase and urinary calcium excretion in 24 h), ultrasound of the kidneys at baseline, month 9 and month 18, and documentation of adverse events and concomitant medication. Laboratory tests were performed centrally at the Institute for Clinical Chemistry of the Klinikum Schwabing using accredited methods. 1,25(OH)<sub>2</sub>D<sub>3</sub> concentration was measured in an accredited laboratory by radioimmunoassay (MVZ Prof. Seelig, Karlsruhe, Germany).

### End point assessment during second phase

The primary efficacy variable was the area under the curve (AUC<sub>0–120 min</sub>) C-peptide (AUC C-peptide) of a MMTT at 18 months. The MMTT (Boost High Protein from Novartis in an amount of 360 ml) was administered to collect stimulated C-peptide samples (0, 30, 60, 90, and 120 min) at baseline, at month 9 immediately after the end of treatment, and at the final visit at month 18. Secondary efficacy variables were the peak C-peptide after MMTT at 18 months, daily insulin intake, and glycemic control, assessed by A1C levels. C-peptide concentrations were measured in Trasylol-stabilized EDTA plasma samples using an automated immunoassay analyzer (AIA 360; Tosoh, San Francisco, CA). The interassay coefficient of variation of the C-peptide assay is 4.2% at a concentration of 0.71 nmol/l and the lower limit of detection is

0.07 nmol/l. A1C was measured centrally by high-pressure liquid chromatography. Antibodies to insulin, GAD65, IA-2, and ZnT8 were measured by radiobinding assay as described previously (18). All measurements were performed with the operator blinded to group assignment.

### Statistical methods

For the analysis of safety, data from the first (safety) and second (efficacy) component of the study were combined, and all subjects who had at least one baseline and postbaseline measurement of safety parameters were included. For the analysis of efficacy, all randomly assigned patients who had taken at least one dose of study drug and had some postbaseline data for the primary efficacy parameter (AUC C-peptide) were included. Comparisons between groups and between baseline and follow-up time points were made using parametric tests (*t* test). With the assumption that the AUC C-peptide values would decrease by 40% over 18 months in the placebo-treated group, the efficacy phase of the study had 50% power to detect 50% preservation of AUC C-peptide after 18 months. All hypotheses testing was two-sided and performed at the 5% significance level.

## RESULTS

### Safety assessments

Forty-one of 47 patients who received study drug and all 18 patients receiving placebo completed the 18 months of follow-up (Fig. 1). Plasma and urinary excretion calcium values did not change

significantly during treatment in both groups (Fig. 2). Plasma calcium and 24-h urinary calcium excretion values were above the reference range in 1 and 16.6% of samples, respectively, from the treatment group and 3.7 and 15.9% of samples from the placebo group. During the safety phase, the study drug dose was temporarily halved in some patients who had elevated urinary calcium excretion values, but this had no effect on urinary calcium excretion. Moreover, these patients did not have elevated serum calcium. There were no signs of renal calcification during the safety phase. All other safety parameters were within reference ranges and did not differ between the treatment and placebo groups (Table 1). Serum concentrations of 1,25(OH)<sub>2</sub>D<sub>3</sub> increased after 9 months of treatment (24.6 vs. 30.3 pg/ml;  $P = 0.02$ ;  $n = 13$  patients in the safety recruitment phase only).

Adverse events reported by at least 10% of patients were upper respiratory tract infections and rhinitis. The frequencies of adverse events in the 1,25(OH)<sub>2</sub>D<sub>3</sub> treatment group (23 in 10 subjects) and in the placebo group (13 in 6 subjects) were comparable. One patient experienced a cholelithiasis with cholestasis, which was considered to be a serious adverse event but not drug related. No other clinically significant adverse event was reported.

### Efficacy phase

Of the 40 randomly assigned patients, 38 completed the study and two discontinued for private reasons (Fig. 1). Only

**Table 1—Safety parameters in treatment and placebo groups during study follow-up**

Parameter	1,25(OH) <sub>2</sub> D <sub>3</sub>			Placebo		
	Month 0	Month 9	Month 18	Month 0	Month 9	Month 18
<i>n</i>	47			18		
Calcium (mmol/l)	2.4 ± 0.1	2.4 ± 0.1	2.4 ± 0.1	2.5 ± 0.1	2.4 ± 0.1	2.4 ± 0.1
Alkaline phosphatase (IU/ml)	67 ± 19	62 ± 17	60 ± 17	65 ± 26	62 ± 16	63 ± 17
Phosphate (mmol/l)	1.2 ± 0.2	1.2 ± 0.2	1.1 ± 0.2	1.2 ± 0.2	1.2 ± 0.2	1.1 ± 0.2
Creatinine (mg/dl)	0.8 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1
24-h calcium urine excretion (mmol/24 h)	6.3 ± 3.2	6.0 ± 3.8	6.2 ± 3.3	6.1 ± 3.2	5.5 ± 4.0	5.2 ± 3.1

Data are means ± SD.

4.7% of dispensed tablets were returned (4.4% treatment and 5.1% placebo). The treatment and placebo groups were similar in terms of age, duration of disease, ethnicity, and baseline C-peptide concentrations (Table 2). The mean AUC C-peptide at month 9 and 18 and the change in the mean values from baseline to month 18 were comparable in the treatment and the placebo groups (Table 2 and Fig. 3). Both groups had ~40% decreases in mean AUC C-peptide from start of treatment to end of follow-up at 18 months. Eleven patients maintained their AUC C-peptide above baseline values at 9 months (6 treatment and 5 placebo) and 1 patient (treatment) had an AUC C-peptide above baseline at 18

months. Similarly, the fasting C-peptide and the mean peak C-peptide at baseline and at month 9 and 18 in the treatment and placebo groups were comparable with ~40% decreases over 18 months (Table 2).

The average insulin requirement per day and per kilogram body weight and A1C were similar between the treatment and placebo groups throughout follow-up (Table 2, Fig. 3). The mean A1C remained <6.5% from month 2 of follow-up in both groups, and the proportion of patients who reached an A1C <6.5% at 18 months was 55% in both groups.

No differences between treatment and placebo groups were observed for the

titer of insulin antibodies, GAD65, IA-2, and ZnT8 autoantibodies (not shown).

**CONCLUSIONS**— 1,25(OH)<sub>2</sub>D<sub>3</sub> at full dose was used for the treatment of autoimmune diabetes in a trial that included adult patients with recent-onset disease. 1,25(OH)<sub>2</sub>D<sub>3</sub> at a dose of 0.25 µg/day proved to be well tolerated. There were no significant safety issues and most adverse events were considered mild or moderate and unrelated or unlikely to be related to the study drug. Treatment with 1,25(OH)<sub>2</sub>D<sub>3</sub>, however, provided no protection against the decline of β-cell function after diabetes onset, as measured by fasting or stimulated C-peptide and did not improve insulin requirement or metabolic control. These data do not support the use of 1,25(OH)<sub>2</sub>D<sub>3</sub> to improve diabetes treatment in adult patients with type 1 diabetes.

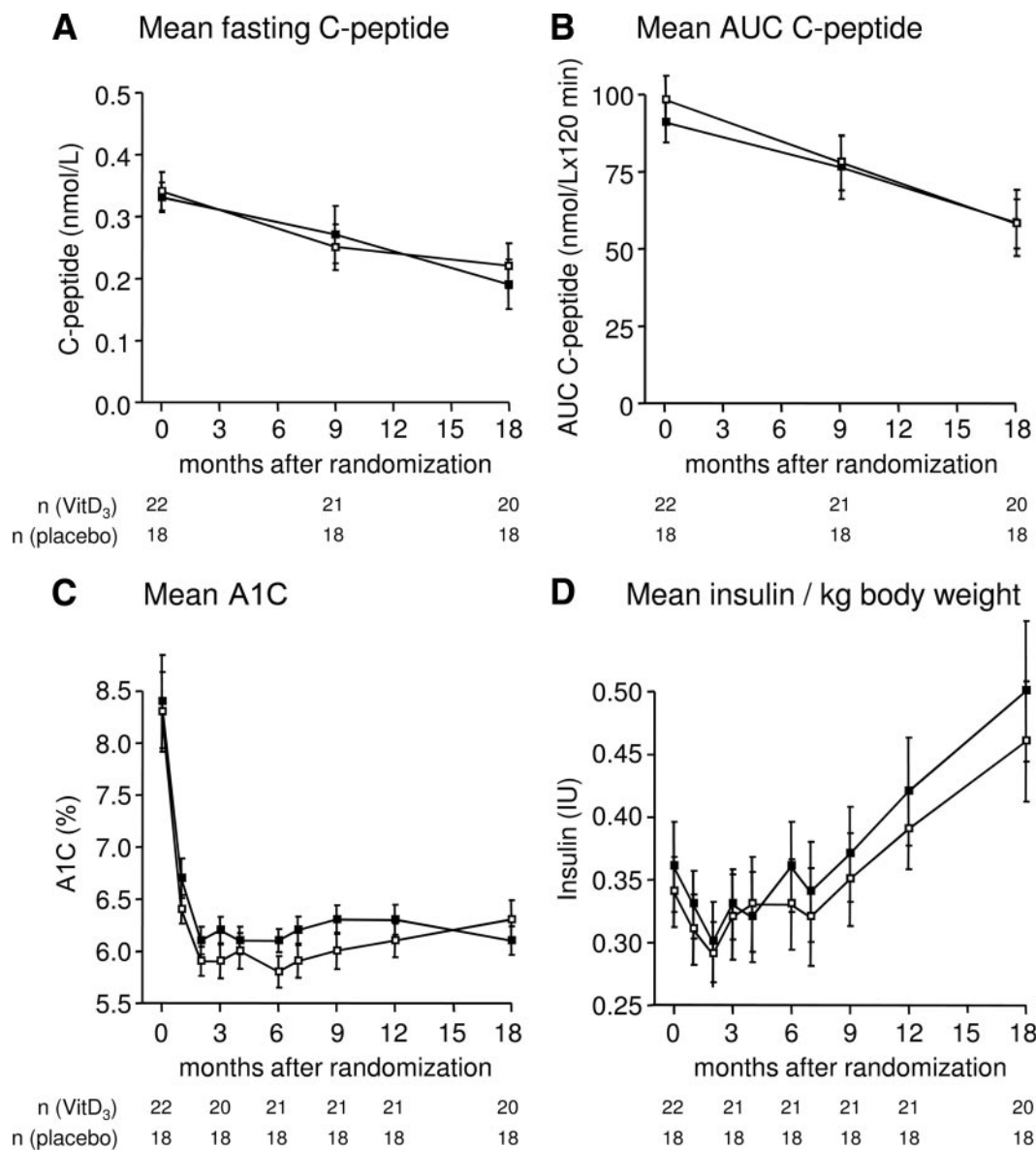
Previous observations in the NOD mouse showed beneficial effects of 1,25(OH)<sub>2</sub>D<sub>3</sub> in delaying autoimmune diabetes when the drug was given early (14) but not late during pre-diabetes (19). In humans, administration of the less active 1-α-hydroxyvitamin D<sub>3</sub> in infancy was reported to reduce type 1 diabetes risk (3,4,20). Administration of 1-α-hydroxyvitamin D<sub>3</sub> was also recently found to partially preserve β-cell function in patients with latent autoimmune diabetes (21). Finally, it was demonstrated that the beneficial effect of 1,25(OH)<sub>2</sub>D<sub>3</sub> on β-cell preservation was similar to that of nicotinamide when given soon after onset of type 1 diabetes at a lower dose than that used in our study (17). Our data are in line with those for the NOD mouse in concluding no benefit when given late in the disease, i.e., at diabetes onset. Although numbers in the trial were relatively small, there was complete overlap for the primary and secondary outcome markers between treatment and placebo groups. Moreover, both groups were very similar in their decline in basal, peak, and AUC C-peptide after a MMTT as was recently reported for the NBI-6024 trial of 188 patients with new onset diabetes (22).

The dose tested in our study is likely to be the maximum tolerable dose. The serum 1,25(OH)<sub>2</sub>D<sub>3</sub> concentration increased after treatment in the open safety study. Nevertheless, it is possible that the overall homeostasis of active vitamin D<sub>3</sub> concentration in the body was only minimally and variably affected. The use of 1,25(OH)<sub>2</sub>D<sub>3</sub> together with other immu-

**Table 2—Efficacy parameters in treatment and placebo groups during study follow-up**

Outcome marker and time point	1,25(OH) <sub>2</sub> D <sub>3</sub>	Placebo	<i>P</i> value
<i>n</i>	22	18	
AUC C-peptide (nmol/l × 120 min)			
Baseline	90.8 ± 30.3	98.1 ± 33.0	0.47
Month 9	76.3 ± 46.6	77.9 ± 37.7	0.91
Month 18	58.4 ± 47.7	58.0 ± 33.7	0.98
Fasting C-peptide (nmol/l)			
Baseline	0.33 ± 0.12	0.34 ± 0.13	0.74
Month 9	0.27 ± 0.21	0.25 ± 0.16	0.77
Month 18	0.19 ± 0.18	0.22 ± 0.15	0.66
Peak C-peptide (nmol/l)			
Baseline	1.00 ± 0.37	1.10 ± 0.36	0.36
Month 9	0.79 ± 0.47	0.85 ± 0.39	0.71
Month 18	0.66 ± 0.52	0.63 ± 0.34	0.86
A1C (%)			
Baseline	8.4 ± 2.1	8.3 ± 1.6	0.82
Month 9	6.3 ± 0.6	6.0 ± 0.7	0.13
Month 18	6.1 ± 0.6	6.3 ± 0.8	0.51
Daily insulin dose (IU/day)			
Baseline	24.9 ± 12.6	24.6 ± 8.9	0.94
Month 9	26.5 ± 13.1	25.8 ± 13.9	0.87
Month 18	35.6 ± 19.2	34.9 ± 18.1	0.92

Data are means ± SD.



**Figure 3**—Efficacy parameters. Fasting (A) and AUC C-peptide after mixed-meal stimulation (B), mean A1C (C), and mean insulin requirement per kilogram body weight (D) in the 1,25(OH)<sub>2</sub>D<sub>3</sub> treatment group (■) and the placebo group (□). Data are means ± SEM. VitD<sub>3</sub>, 1,α,25-dihydroxyvitamin D<sub>3</sub>.

nomodulators has been more effective for diabetes prevention in NOD mice. Our results do not rule out such combinations, but we suggest that such trials should show convincing mechanistic action in patients such as alteration of dendritic cell numbers/function (8–12) or T cell phenotype (13) before efficacy evaluation. It has also been reported that less calcemic analogs of 1,25(OH)<sub>2</sub>D<sub>3</sub> could be used at higher concentrations and that these are more effective in delaying diabetes onset in NOD mice (23–25). Thus, although our findings do not completely rule out the use of vitamin D<sub>3</sub> for type 1 diabetes treatment after diabetes onset

and need to be supported by mechanistic studies to determine the immunomodulatory effect of treatment in our patients, they suggest that 1,25(OH)<sub>2</sub>D<sub>3</sub> treatment in adult type 1 diabetic patients at the stage of overt disease will have limited potential for preserving β-cell function.

**Acknowledgments**—The study was supported by funds of the German Diabetes Association and the Charlotte Fievet Foundation.

No potential conflicts of interest relevant to this article were reported.

We thank Axel Nuber and Peter Banholzer for performing the kidney sonography, Christiane Winkler, Annette Knopff, Ulrike Mollen-

hauer, Stephanie Krause, and Marina Zwilling for expert technical assistance, all physicians who conducted study visits locally, and all patients who participated in the study.

#### References

1. Atkinson MA, Eisenbarth GS. Type 1 diabetes: new perspectives on disease pathogenesis and treatment. *Lancet* 358:221–229
2. Staeva-Vieira T, Peakman M, von Herrath M. Translational mini-review series on type 1 diabetes: immune-based therapeutic approaches for type 1 diabetes. *Clin Exp Immunol* 2007;148:17–31
3. Vitamin D supplement in early childhood and risk for type 1 (insulin-dependent) dia-

- betes mellitus. The Eurodiab Substudy 2 Study Group. *Diabetologia* 1999;42:51-54
4. 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;358:1500-1503
  5. Zipitis CS, Akobeng AK. Vitamin D supplementation in early childhood and risk of type 1 diabetes: a systematic review and meta-analysis. *Arch Dis Child* 2008;93:512-517
  6. Baumgartl HJ, Standl E, Schmidt-Gayk H, Kolb HJ, Janka HU, Ziegler AG. Changes of vitamin D<sub>3</sub> serum concentrations at the onset of immune-mediated type 1 (insulin-dependent) diabetes mellitus. *Diabetes Res* 1991;16:145-148
  7. Bierschenk L, Alexander J, Wasserfall C, Haller M, Schatz D, Atkinson M. Vitamin D levels in subjects with and without type 1 diabetes residing in a solar rich environment. *Diabetes Care* 2009;32:1977-1979
  8. D'Ambrosio D, Cippitelli M, Cocciolo MG, Mazzeo D, Di Lucia P, Lang R, Sinigaglia F, Panina-Bordignon P. Inhibition of IL-12 production by 1,25-dihydroxyvitamin D<sub>3</sub>. Involvement of NF-κB down-regulation in transcriptional repression of the p40 gene. *J Clin Invest* 1998;101:252-262
  9. Piemonti L, Monti P, Sironi M, Fraticelli P, Leone BE, Dal Cin E, Allavena P, Di Carlo V. Vitamin D<sub>3</sub> affects differentiation, maturation, and function of human monocyte-derived dendritic cells. *J Immunol* 2000;164:4443-4451
  10. Penna G, Adorini L. 1α,25-Dihydroxyvitamin D<sub>3</sub> inhibits differentiation, maturation, activation, and survival of dendritic cells leading to impaired alloreactive T cell activation. *J Immunol* 2000;164:2405-2411
  11. Griffin MD, Lutz W, Phan VA, Bachman LA, McKean DJ, Kumar R. Dendritic cell modulation by 1α,25 dihydroxyvitamin D<sub>3</sub> and its analogs: a vitamin D receptor-dependent pathway that promotes a persistent state of immaturity in vitro and in vivo. *Proc Natl Acad Sci USA* 2001;98:6800-6805
  12. Penna G, Amuchastegui S, Giarratana N, Daniel KC, Vulcano M, Sozzani S, Adorini L. 1,25-Dihydroxyvitamin D<sub>3</sub> selectively modulates tolerogenic properties in myeloid but not plasmacytoid dendritic cells. *J Immunol* 2007;178:145-153
  13. Boonstra A, Barrat FJ, Crain C, Heath VL, Savelkoul HF, O'Garra A. 1α,25-Dihydroxyvitamin D<sub>3</sub> has a direct effect on naive CD4<sup>+</sup> T cells to enhance the development of Th2 cells. *J Immunol* 2001;167:4974-4980
  14. Mathieu C, Gysemans C, Giulietti A, Bouillon R. Vitamin D and diabetes. *Diabetologia* 2005;48:1247-1257
  15. Teegarden D, Donkin SS. Vitamin D: emerging new roles in insulin sensitivity. *Nutr Res Rev* 2009;22:82-92
  16. Gulseth HL, Gjelstad IM, Tierney AC, Lovegrove J, Defoort C, Blaak EE, Lopez-Miranda J, Kiec-Wilk B, Risérus U, Roche HM, Drevon CA, Birkeland KI. Serum vitamin D concentration does not predict insulin action or secretion in European subjects with the metabolic syndrome. *Diabetes Care* 2010;33:923-925
  17. Pitocco D, Crinò A, Di Stasio E, Manfrini S, Guglielmi C, Spera S, Anguissola GB, Visalli N, Suraci C, Matteoli MC, Patera IP, Cavallo MG, Bizzarri C, Pozzilli P, IM-DIAB Group. The effects of calcitriol and nicotinamide on residual pancreatic beta-cell function in patients with recent-onset type 1 diabetes (IMDIAB XI). *Diabet Med* 2006;23:920-923
  18. Achenbach P, Lampasona V, Landherr U, Koczwara K, Krause S, Grallert H, Winkler C, Pflüger M, Illig T, Bonifacio E, Ziegler AG. Autoantibodies to zinc transporter 8 and SLC30A8 genotype stratify type 1 diabetes risk. *Diabetologia* 2009;52:1881-1888
  19. Gysemans CA, Cardozo AK, Callewaert H, Giulietti A, Hulshagen L, Bouillon R, Eizirik DL, Mathieu C. 1,25-Dihydroxyvitamin D<sub>3</sub> modulates expression of chemokines and cytokines in pancreatic islets: implications for prevention of diabetes in nonobese diabetic mice. *Endocrinology* 2005;146:1956-1964
  20. Stene LC, Joner G. Use of cod liver oil during the first year of life is associated with lower risk of childhood-onset type 1 diabetes: a large, population-based, case-control study. *Am J Clin Nutr* 2003;78:1128-1134
  21. Li X, Liao L, Yan X, Huang G, Lin J, Lei M, Wang X, Zhou Z. Protective effects of 1-α-hydroxyvitamin D<sub>3</sub> on residual β-cell function in patients with adult-onset latent autoimmune diabetes (LADA). *Diabetes Metab Res Rev* 2009;25:411-416
  22. Walter M, Philotheou A, Bonnici F, Ziegler AG, Jimenez R. No effect of the altered peptide ligand NBI-6024 on β-cell residual function and insulin needs in new-onset type 1 diabetes. *Diabetes Care* 2009;32:2036-2040
  23. van Halteren AG, van Etten E, de Jong EC, Bouillon R, Roep BO, Mathieu C. Redirection of human autoreactive T-cells upon interaction with dendritic cells modulated by TX527, an analog of 1,25 dihydroxyvitamin D<sub>3</sub>. *Diabetes* 2002;51:2119-2125
  24. Gysemans C, Van Etten E, Overbergh L, Verstuyf A, Waer M, Bouillon R, Mathieu C. Treatment of autoimmune diabetes recurrence in non-obese diabetic mice by mouse interferon-beta in combination with an analogue of 1α,25-dihydroxyvitamin-D<sub>3</sub>. *Clin Exp Immunol* 2002;128:213-220
  25. Gregori S, Giarratana N, Smiroldo S, Uskokovic M, Adorini L. A 1α,25-dihydroxyvitamin D<sub>3</sub> analog enhances regulatory T-cells and arrests autoimmune diabetes in NOD mice. *Diabetes* 2002;51:1367-1374