Summary of results

PROPRIETARY DRUG NAME® / GENERIC DRUG NAME:
Oral vitD3 (140 000 IU, Oleovit D3, Fresenius Kabi, Austria) and Placebo (almond oil)

PROTOCOL TITLE:
English title: Placebo-controlled study to investigate the effects of vitamin D supplementation in healthy women and men on immunological, endocrine and metabolic parameters

German title: Placebo kontrollierte Studie zur Untersuchung der Effekte einer Vitamin D Supplementierung bei gesunden Frauen und Männern auf immunologische, endokrine und metabolische Parameter

Research Article title:
The effect of vitamin D supplementation on peripheral regulatory T cells and β cell function in healthy humans: a randomized controlled trial

DIABETES/METABOLISM RESEARCH AND REVIEWS, Diabetes Metab Res Rev 2011; 27: 942–945.
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Sponsor Details:
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Study Centers:
1 center in Austria

Study Initiation and Final Completion Dates:
Initiation December 2009; Final Completion May 2010

Phase of Development:
Phase 4

Study Objectives:

The primary objective of the study was to evaluate whether vitamin D substitution under controlled conditions over 3 months has an effect on the percentage or function (including assessment of apoptosis and expression of vitamin D-associated genes) of regulatory T cells circulating in the blood.

The secondary study objectives included:

- To evaluate whether vitamin D substitution over 3 months has an effect on the frequency and activity of other important circulating immune cells (immunophenotyping)

- To evaluate whether there is an effect on insulin secretion, plasma concentrations of renin, aldosterone, HDL cholesterol, osteoprotegerin, ADMA and on cytokine levels of IL1-beta, IL2, IL4, IL6, IL10, MCP1, TNF-alpha, adiponectin, leptin, resist and TGF-beta
- To create a serum/plasma bank of samples from this study
- To analyse potential vitamin D-associated genes (DNA)

METHODS:

After approval from the Ethics Committee (Medical University of Graz) a single centre, double-blind, placebo controlled trial was performed in 59 healthy non-diabetic subjects (49% females, mean age: 34 ± 10 years, body mass index: 24 ± 4 kg/m2) without any concomitant medication and normal serum calcium levels. Subjects received monthly oral vitD3 (140 000 IU, Oleovit D3, Fresenius Kabi, Austria) or placebo (almond oil) for a period of 3 months and the effect on serum 25(OH)D, percentage of Tregs in peripheral blood, stimulated C-peptide during MMTT and safety were assessed at baseline and after 12 weeks. Serum 25(OH)D was measured by ELISA (IDS, Bolden, UK). The intraand interassay coefficients of variation were 5.6 and 6.4%. Tregs were defined as CD4+CD25^{high}FoxP3+ CD127^{dim} cells. For quantification, whole blood was stained with anti-CD4 FITC, anti-CD25 PE-Cy7 and anti- CD127 PE monoclonal antibodies (BD Biosciences, San Jose, CA, USA). After permeabilization, intracellular staining for the transcription factor FOXP3 was performed using anti FoxP3 AF-647 (BD Biosciences), followed by cell analysis on a FACS Canto II cytometer (BD Biosciences). All subjects underwent a MMTT (6 mL Isosource fibre/kg bodyweight, max. 360 mL) at baseline and at the end of the study. C-peptide secretion was assessed by calculating area under the curve (AUC 0–120 min). Descriptive statistics are presented as mean ± standard deviation if not stated otherwise. Responses of 25(OH)D, Tregs and responses of MMTT parameters to the two treatments were analysed using repeated measures analysis of variance and if appropriate a paired t-test. A p-value < 0.05 was considered statistically significant. All statistical analyses were performed on the total study population (n = 59) and a subgroup with a 25(OH)D level <30 ng/mL (n = 41) using SPSS version 18.0 (PASW Statistics, Chicago, IL, USA).

Table 1 – Baseline characterisics of study population:

	Vitamin D	Placebo
n	30	29
Females/Males	14/16	15/14
Age (years)	32 ± 10	35 ± 11
Weight (kg)	74.9 ± 17.3	71.2 ± 13.0
Height (cm)	174 ± 10	172 ± 9
BMI (kg/m^2)	24.5 ± 3.9	23.9 ± 3.4
Waist–hip ratio	0.82 ± 0.1	0.83 ± 0.1
Serum 25(OH)D (ng/mL)	25.5 ± 11.4	25.8 ± 10.4

Number of Subjects :
60 subjects were screened, 60 subjects were included in the analyses, and 58 subjects completed the trial.

Diagnosis and Main Criteria for Inclusion:

60 healthy volunteers male and female subjects, with an age of 18 years or older, have been randomized to vitamin D3 supplementation or placebo.

Safety assessments:

Safety assessments included adverse events, physical examination, vital signs and standard laboratory safety parameters.

Statistical analysis:

The collected parameters have been tested for normal distribution using the Kolmogorov-Smirnov test or descriptive statistics. Non-normally distributed parameters were logarithmized (log10). For the data evaluation of the primary study objective and the secondary study objectives a paired Student's T-test was used. Results with a P-value below 0.05 were evaluated as statistically significant and the SPSS program (version 16.0) was used for the statistical analysis.

Results:

The average baseline level of 25(OH)D was 25.6 ± 10.8 ng/mL, only 18 subjects showed a sufficient 25(OH)D level of ≥30 ng/mL. Baseline demographics did not significantly differ between the two groups (Table 1) and were also similar in the subgroup. Mean percentage of Tregs in the vitD group increased from 4.89 ± 0.93% to 6.35 ± 0.78% at month 3 ($p \leq 0.001$), but remained unchanged in the placebo group (5.35 ± 1.01% versus 5.46 ± 0.95%; $p > 0.05$; Figure 1). Fasting C-peptide concentrations and mean peak C-peptide values at baseline (1.4 ± 0.5 ng/mL versus 1.5 ± 0.6 ng/mL and 6.9 ± 3.1 ng/mL versus 6.5 ± 2.8 ng/mL) and month 3 (1.5 ± 0.6 ng/mL versus 1 ± 0.5 ng/mL and 7.4 ± 3.3 ng/mL versus 6.2 ± 2.3 ng/mL) in the vitD group and in the placebo group did not change significantly. Similarly, themean AUC C-peptide atmonth 3 (580 ± 231 versus 538 ± 174 ng/mL, 120 min) and the change in the mean values from baseline (538 ± 218 versus 541 ± 203 ng/mL, 120 min) to end of treatment (580 ± 231 versus 538 ± 174 ng/mL 120 min) were comparable in the treatment and the placebo group (Figure 2). Subjects with insufficient baseline 25(OH)D levels appeared to have a more pronounced effect of vitD3 on insulin secretion; however, the difference was not statistically significant (Figure 2). C-reactive protein and serum calcium were within normal range during the whole study and no clinically relevant adverse events were reported.

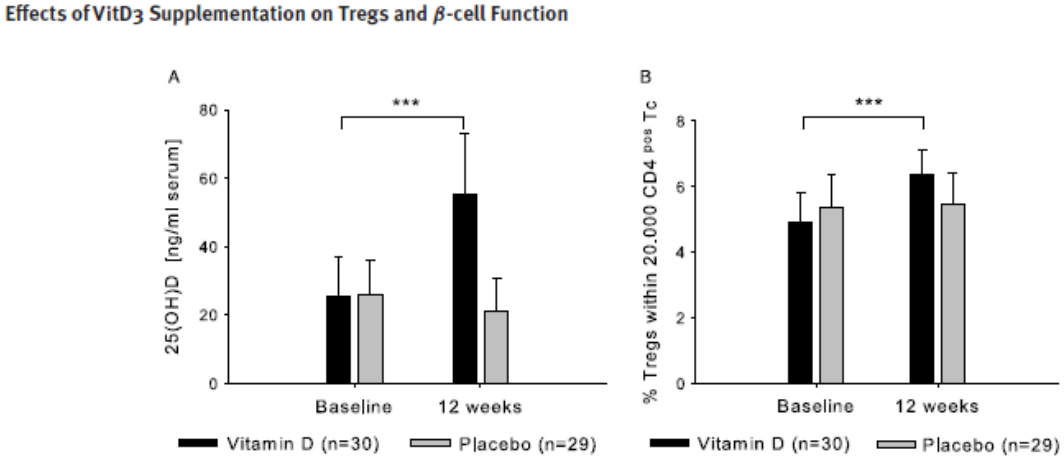

Figure 1. (A) Changes in serum 25(OH)D levels after 12 weeks of supplementation with 140 000 IU vitD3 monthly in the two investigated study groups. (B) With an increase in vitD, the percentage of peripheral $CD4^{pos}$ Tregs increased significantly in the treatment group after 12 weeks of supplementation. Data are given as mean ± standard deviation. $^{***}p \leq 0.001$

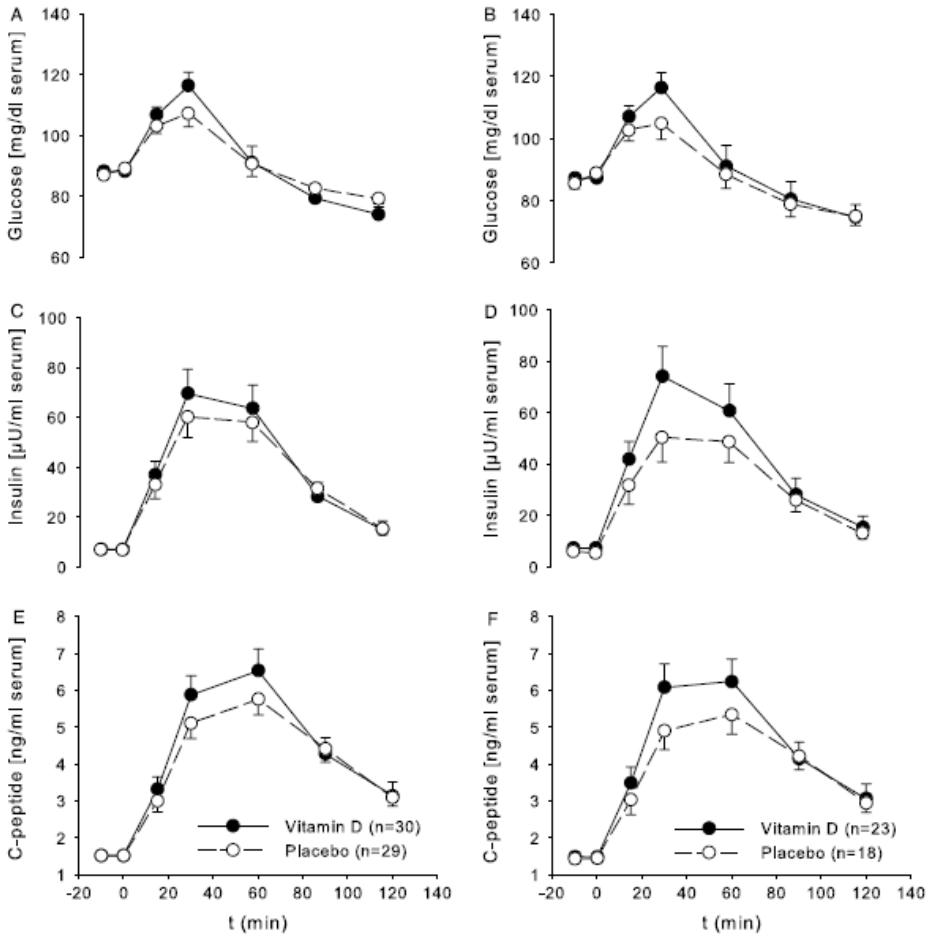

Figure 2. Glucose levels (A, B), insulin levels (C, D) and C-peptide levels (E, F) at week 12 in the $vitD_3$ treated group (filled circles) and in the control group (open circles). A, C, E show the result in the total investigated study population. B, D, F depict the analysis of the subgroup (vitD $n = 23$, placebo $n = 18$) with 25(OH)D baseline levels <30 ng/mL serum. Data are given as mean ± standard error of mean

Serious advers events/adverse events:

No serious adverse events have occured during the clinical trial and no clinically relevant adverse events were reported.

Conclusion:

A short time high dose vitD3 supplementation significantly increased the frequency of Tregs, but did not further improve β-cell function in apparently healthy subjects. The immunomodulatory potential of vitD might be an important mechanistic link for the association of vitD and T1D.