

**Study Title:**

Pilotstudie zur Untersuchung der Effekte einer Vitamin D Supplementierung bei gesunden Frauen und Männern auf die Anzahl der FOXP3+ regulatorischen T-Zellen in der gastrointestinalen Mukosa: Stufe 3 des Österreichischen Diabetes Präventions-Programmes (ADPP-003)

**Summary of Results**

Vitamin D receptors are found on several cells of the innate and adaptive immune system and the impact of vitamin D deficiency in the pathogenesis of immunomediated diseases such as diabetes type 1, multiple sclerosis and inflammatory bowel diseases have been highlighted. Animal and in vitro studies suggest that vitamin D is involved in reducing the risk of autoimmunity by modulating regulatory T cells, which are pivotal to maintain self-tolerance.

We have previously shown that cholecalciferol (vitamin D<sub>3</sub>-a precursor of the active metabolite) is able to increase frequency of peripheral regulatory T cells in the blood without negatively effecting suppressive capacity and apoptosis in healthy humans and in vitro exposure to cholecalciferol as well leads to increased percentage of regulatory T cells. We hypothesize that cholecalciferol may increase regulatory T cells in the intestinal mucosa, which harbors a large number of immune cells in the gut-associated lymphoid tissue. In a systematic assessment we determined the distribution of CD4+, CD8+ and Foxp3+regulatory T cells in several regions of the upper and lower gastrointestinal tract. Our data show a significant variation in the baseline T cell landscape along the human gastrointestinal tract and an immunomodulatory effect of cholecalciferol on T cells subpopulations in the intestinal mucosa of humans. These studies support the role of vitamin D as an immunomodulatory adjunct in the therapy of immunomediated diseases.

Cholecalciferol affects immune cells associated to the lamina propria

Cholecalciferol supplementation of young healthy participants (demographics shown in table 1) did not change the percentage of T cells (CD3pos cells) within lymphocytes but it caused significant changes in subtypes of T cells. In fact, a significant increase in the percentage of CD4pos T helper cells in all T cells (CD3pos) isolated from biopsies from the gastric antrum region was shown. The median baseline-value of 12.48% (IQR: 9.05-21.78%) increased to 16.46% (13.36-25.27%) after 8 weeks (p = 0.025, Fig. 1 A). CD8pos cytotoxic T cells within CD3pos T cells increased significantly in the gastric antrum region from a median baseline value of 30.25% (IQR: 22.64-43.33%) to 42.11% (IQR: 25.97-49.94%, p=0.037), in the appendix region from baseline 12.69% (IQR 11.49-16.67%) to 18.56% (IQR 12.17-24.46%, p = 0.005, Fig. 48 B) and in the right colon region from baseline 12.75% (IQR 9.34-18.27%) to 15.09% (IQR 12.45-19.96%, p = 0.035, Fig. 1 B). These changes were not reflected in the ratio CD4/CD8 as this didn't change significantly upon cholecalciferol intake (Fig. 48 C). The % of Treg within CD4pos T cells also remained unchanged upon cholecalciferol treatment for 8 weeks in all investigated regions of the human intestine, as shown in Fig. 48 D. The expression of the Helios marker in Tregs decreased especially in samples taken from the upper region of the intestine and this decrease reached significance in the Ileum (43.09% [22.68-55.55%] vs 20.44% [16.58-27.79%]; p=0.03, Fig. 1 E).

<b>ADPP003 Baseline</b>	
<b>n</b>	16
<b>Females (%)</b>	44
<b>Age (yrs)</b>	25 ± 4
<b>Height (cm)</b>	172 ± 8
<b>Weight (kg)</b>	69 ± 11
<b>BMI (kg/m<sup>2</sup>)</b>	23 ± 3
<b>Leukocytes (G/L)</b>	6.2 ± 1.3
<b>Serum C-reactive protein (mg/L)</b>	3.8 ± 9.2
<b>Serum 25(OH)D (ng/ml)</b>	22.3 ± 13.1
<b>NLR</b>	1.3 ± 0.5
<b>CD4/CD8 ratio</b>	2.0 ± 1.1
<b>Treg (% of CD4)</b>	6.2 ± 2.5

Table 1: Demographics and clinical baseline parameter of all participants included in the ADPP003 vitamin D supplementation study. BMI: body mass index, 25(OH)D: 25 -hydroxyvitamin D, NLR: neutrophil to lymphocyte ratio; Data are given in mean ± SD. G/L: giga/l = 10<sup>9</sup> /l blood

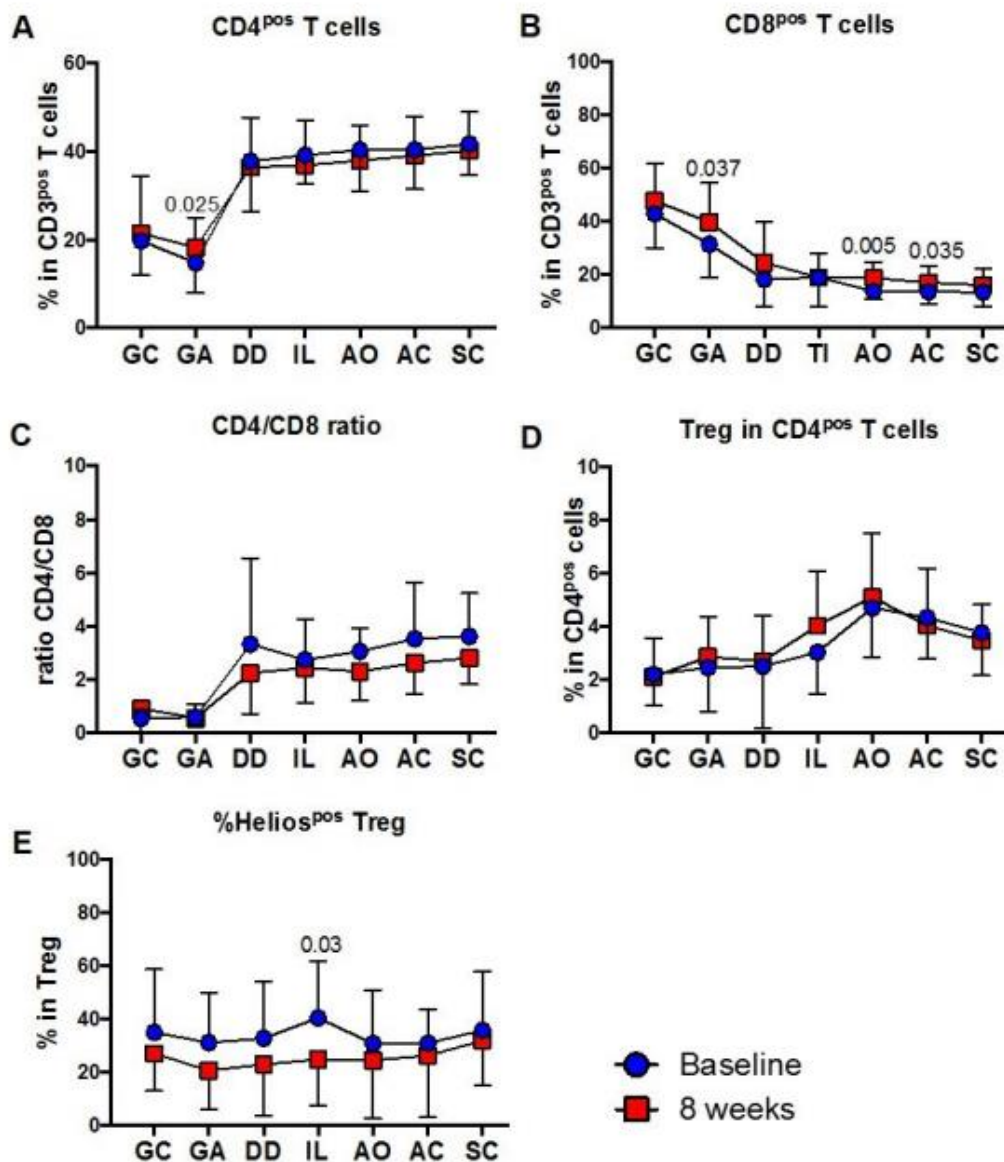


Figure 1: Changes in the percentages of T cell subtypes after 8 weeks of oral cholecalciferol supplementation in the investigated regions of the gastro-intestinal mucosa. A. Significant increased levels of CD4<sup>pos</sup> T helper cells in the gastric antrum region B. Significant changes in the % CD8<sup>pos</sup> cytotoxic T cells in gastric antrum, the appendiceal orifice and the ascending colon region C. No significant changes in the CD4/CD8 ratio D. no significant changes in the % Tregs E. Significant increased levels of Helios<sup>pos</sup> Tregs in the terminal ileum region. GC: gastric corpus region, GA: gastric antrum region, DD: duodenum, TI: terminal ileum region, AOR: appendiceal orifice region, AC: ascending colon region, SC: sigmoid colon region.