Effect of vitamin D supplementation on inflammation and nuclear factor *kappa*-B activity in overweight or obese adults

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Background

- Chronic low-grade inflammation is common in obesity and its related conditions, including type 2 diabetes.
- Experimental studies have shown that vitamin D regulates inflammation via inhibiting the nuclear factor-kappa B (NFκB) pathway; however clinical trials to support these findings are lacking.

Aims

To examine the effect of vitamin D supplementation (100,000 IU bolus + 4,000 IU daily cholecalciferol for 16 weeks) on inflammation markers and NFκB activity, compared to placebo, in vitamin D-deficient and overweight or obese adults.

Hypothesis

We hypothesized that vitamin D supplementation would reduce inflammation markers and NFκB activity, compared to placebo.

Methods

Inclusion Criteria:

- healthy, non-diabetic adults (WHO guidelines); age 18-60 years with body mass index (BMI) ≥ 25 kg/m².
- vitamin D deficient: 25-hydroxyvitamin D (25(OH)D) ≤50 nmol/L
- non-smokers; no heavy alcohol consumption, not using illicit drugs, medications, vitamins, supplements
- no clinical or laboratory signs of acute or chronic inflammation
- no hypercalcemia, allergies, cancer in last 5 years, menopause, pregnancy, or lactation

Intervention/Control:

- double-blind random allocation to 100,000 IU single bolus + 4,000 IU daily oral cholecalciferol or identical placebo capsules for 16 weeks

Outcome measures:

- anthropometry: BMI, waist-to-hip ratio (WHR), and % body fat by dual X-ray absorptiometry (DEXA)
- serum 25(OH)D: chemiluminescent assay
- high-sensitivity C-reactive protein (hsCRP): rate turbidimetry
- tumor necrosis factor (TNF), monocyte chemoattractant protein-1 (MCP-1), interleukins (IL)-18, 6, 8, 10, 12, 18, 23 and 33: multiplex assay; flow cytometry
- NFκB activity in peripheral blood mononuclear cells: DNA-binding assay

Ethics & Registration:

- Monash University Ethics (CF13/3874-2013001988); clinical trials.gov (ID: NCT02112721).

Forty-eight participants completed the study (30M/19F; age=31.7±8.5 years). Age and sex did not differ at baseline (p>0.3). Other characteristics are shown in Table 1.

Fig 1. Serum 25(OH)D levels increased significantly in the vitamin D group

- Serum 25-hydroxyvitamin D levels increased in the vitamin D group from 31.4 to 88.8 nmol/L (p<0.001), with no change in the placebo group (35.0 to 33.4, p=0.5) (Fig 1; Table 1).
- Change in 25(OH)D was not associated with change in NFκB activity (Fig 2) or inflammatory markers before or after adjustment for age, sex and % body fat (all p>0.05).

Table 1. Baseline, follow up, and change in outcomes in both groups:

Variable	Vitamin D group (n=25)				Placebo group (n=23)				P ₃	P ₄
	Baseline	Follow Up	P ₁	Change	Baseline	Follow Up	P ₂	Change		
BMI (kg/m ²)	31.8 ± 4.9	31.9 ± 4.9	0.8	0.04 ± 0.9	30.7 ± 3.9	30.6 ± 4.5	0.7	-0.1 ± 1.2	0.4	0.6
Body fat (%)	41.4 ± 8.0	40.9 ± 8.0	0.1	-0.5 ± 1.5	40.2 ± 9.0	39.7 ± 9.0	0.2	-0.5 ± 1.7	0.6	0.9
25(OH)D (nmol/l)	31.4 ± 12.5	88.8 ± 21.5	<0.001	57.4 ± 22.5	35.0 ± 10.4	33.4 ± 13.4	0.5	-1.6 ± 10.9	0.3	<0.001
NFκB (pg/μg protein)	47.7 ± 30.2	29.9 ± 12.5	0.01	-17.8 ± 31.9	45.6 ± 29.5	32.4 ± 18.7	0.06	-13.2 ± 32.4	0.8	0.6

P-values represent t-tests for differences within vitamin D group (P₁) or placebo group (P₂), or t-tests for differences at baseline (P₃) or differences in change scores (P₄) between groups.

Fig 2. Change in serum 25(OH)D was not associated with change in NFκB activity

Fig 3. No difference in NFκB activity between vitamin D and placebo groups

- NFκB activity decreased significantly within the vitamin D group (with a non-significant trend within the placebo group); however there was no difference in change in NFκB between groups (p=0.6; Fig 3).
- There were no differences between groups in change in any inflammation markers (all p>0.05).
- All results remained non-significant after adjustment for baseline values, age, sex and % body fat, and after further adjustment for dietary vitamin D intake, sun exposure and physical activity.

Conclusions

- Although experimental studies show that vitamin D reduces inflammation by inhibiting NFκB activity, data from our RCT shows no effect of vitamin D supplementation on inflammation markers or NFκB activity in vitamin D-deficient and overweight or obese, otherwise healthy adults.
- Larger trials are needed to confirm these findings, particularly in participants with diseases associated with low-grade inflammation, such as type 2 diabetes.

References

- Yu et al.(1995) *Immunology*, 92: 10990-994.
- Lundqvist et al. (2014). *Steroids*, 85: 30-35.