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# Mechanisms of action for the biological differences in vitamin $D_2$ and vitamin $D_3$: further analysis of the D2-D3 study cohort

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Vitamin D plays an important role in musculoskeletal health due to its involvement in the regulation of calcium and phosphorus[1]. The two forms of vitamin D – vitamin $D_2$ and vitamin $D_3$ – are both metabolised into 25-hydroxyvitamin D (25OHD) in the liver[2], which is the marker of vitamin D status. 25OHD is then transported to the kidney via the serum, bound to vitamin D binding protein (VDBP)[3], where 25OHD is converted to 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) which is the active form of vitamin D[4]. VDBP has a greater affinity for vitamin $D_3$ than vitamin $D_2$ due to the differences in their side chains[4]. The aims of this analysis were to examine the effect of vitamin D supplementation on VDBP & $1,25(OH)_2D$ and to assess differences in VDBP & $1,25(OH)_2D$ in those with suboptimal (<50 nmol/L) vitamin D status compared to those with optimal (>50 nmol/L) vitamin D status.

Healthy Caucasian (CA) and South Asian (SA) women, aged 20–64yrs, were recruited from the county of Surrey onto the D2-D3 study[4]. The D2-D3 study was run over two consecutive winters and the participants took 15µg vitamin $D_2$, 15µg vitamin $D_3$ or a placebo daily for 12 weeks. A sub-set of participants (*n*73*:* *n*54 CA and *n*19 SA) were used in this analysis to explore the effects on VDBP and $1,25(OH)_2D$. Anthropometric measurements and blood samples were taken at baseline, week 6 and week 12. Serum 25OHD was measured using liquid chromatography tandem mass spectrophotometry (ANSciex 5500 tandem mass spectrophotometer and MassChrom® 25OHD3/D2 kit), serum $1,25(OH)_2D$ was measured using an in vitro chemiluminescent immunoassay (DiaSorin Liaison XL 1,25-dihydroxyvitamin D), and VDBP was measured using a monoclonal Human VDBP Immunoassay Quantikine ELISA (R&D Systems Europe). Statistical analysis was carried out using IMB SPSS24® (2016).

There were no significant differences in VDBP or $1,25(OH)_2D$ between CA or SA women at baseline ($p > 0{\cdot}5$), therefore CA and SA were combined for these analyses. The data are presented in Table 1. In a mixed between-within analysis of variance, a significant interaction was found between time and intervention group on $1,25(OH)_2D$ ($p < 0{\cdot}001$, partial eta squared = 0·26). There was a significant main effect of time on $1,25(OH)_2D$ ($p = 0{\cdot}007$, partial eta squared = 0·14). No significant interaction was found between time and intervention group on VDBP ($p = 0{\cdot}295$). Within the placebo group, there was no significant interaction between time and baseline vitamin D status (categorised as suboptimal <50 nmol/L or sufficient >50 nmol/L) on VDBP or $1,25(OH)_2D$ ($p = 0{\cdot}275$, $p = 0{\cdot}766$ respectively).

**Table 1.** VDBP and $1,25(OH)_2D$ concentrations at each visit (mean ± SD)

| | Placebo | Vitamin $D_2$ | Vitamin $D_3$ |
|---|---|---|---|
| **VDBP** (nmol/L) | *n* = 24 | *n* = 24 | *n* = 24 |
| Baseline | 373·4 ± 152·6 | 332·7 ± 128·4 | 431·4 ± 169·5 |
| Week 6 | 388·7 ± 125·9 | 338·6 ± 151·5 | 424·0 ± 159·2 |
| Week 12 | 392·7 ± 137·7 | 321·2 ± 123·7 | 378·8 ± 133·9 |
| **$1,25(OH)_2D$** (pmol/L) | *n* = 18 | *n* = 18 | *n* = 18 |
| Baseline | 48·9 ± 13·8 | 44·4 ± 12·4 | 43·0 ± 14·7 |
| Week 12 | 43·1 ± 10·4 | 59·3 ± 18·3 | 51·2 ± 13·3 |

In conclusion, the 12-week vitamin D supplementation intervention had a significant effect on $1,25(OH)_2D$ concentrations, with concentrations increasing in those taking vitamin $D_2$ or $D_3$. There was no significant effect on VDBP concentrations, However there is some debate as to the reliability of the kit that was used to measure VDBP, which is important to consider[5] and further analysis is certainly warranted.

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