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Original research

Effect of high dose vitamin D_3 on the HIV-1 reservoir: A pilot randomised controlled trial

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ABSTRACT

Introduction: Antiretroviral therapy for people living with HIV-1 must be taken lifelong due to the persistence of latent virus in long-lived and proliferating $CD4^+$ T cells. Vitamin D_3 is a steroidal gene transcription regulator which exerts diverse effects on immune and epithelial cells including reductions in $CD4^+$ T cell proliferation and improvement in gut barrier integrity. We hypothesised that a high dose of vitamin D_3 would reduce the size of the HIV-1 reservoir by reducing $CD4^+$ T cell proliferation.

Methods: We performed a randomised placebo-controlled trial evaluating the effect of 24 weeks of vitamin D_3 (10,000 international units per day) on the HIV-1 reservoir and immunologic parameters in 30 adults on antiretroviral therapy; participants were followed for 12 weeks post-treatment. The primary endpoint was the effect on total HIV-1 DNA at week 24. Parameters were assessed using mixed-effects models.

Results: We found no effect of vitamin D_3 on the change in total HIV-1 DNA from week 0 to week 24 relative to placebo. There were also no changes in integrated HIV-1 DNA, 2-long-terminal repeat (2-LTR) circles or cell-associated HIV-1 RNA. Vitamin D_3 induced a significant increase in the proportion of central memory $CD4^+$ and $CD8^+$ T cells, a reduction in the proportion of senescent $CD8^+$ T cells and a reduction in the natural killer cell frequency at all time points including week 36, 12 weeks after the study drug cessation. At week 36, there was a significant reduction in total HIV-1 DNA relative to placebo and persistently elevated 25-hydroxyvitamin D levels. No significant safety issues were identified.

Conclusions: Vitamin D_3 administration had a significant impact on the T cell differentiation but overall effects on the HIV-1 reservoir were limited and a reduction in HIV-1 DNA was only seen following cessation of the study drug. Additional studies are required to determine whether the dose and duration of vitamin D_3 can be optimised to promote a continued depletion of the HIV-1 reservoir over time.

Trial registration: ClinicalTrials.gov NCT03426592.

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1. Introduction

Antiretroviral therapy (ART) in people living with HIV-1 infection (PLHIV) can potently suppress HIV-1 RNA in plasma; however, ART cannot eliminate HIV-1 which persists as integrated provirus in long-lived and proliferating $CD4^+$ T cells. HIV-1 replicates efficiently within $CD4^+$ T cells expressing CCR6, a marker of Th17 cells which line the gastrointestinal tract, and causes a profound depletion of $CD4^+$ T cells in the gastrointestinal tract following infection.[1,2] The loss of $CD4^+$ T cells impairs the gut barrier integrity leading to bacterial translocation across the gut wall and chronic immune activation.[3,4] This does not completely resolve on ART[5–7] and may contribute to HIV-1 persistence on ART by stimulating the proliferation of latently infected $CD4^+$ T cells.[8–11]

Vitamin D_3 is a steroidal regulator of gene transcription and exerts multiple immunomodulatory effects which may be beneficial in targeting HIV-1 persistence. Vitamin D_3 supplementation in PLHIV has been shown to increase the $CD4^+$ T cell percentage and, in viraemic individuals, to reduce HIV-1 viral load.[12,13] In multiple sclerosis, high dose vitamin D_3 has been shown to reduce T cell proliferation[14] and to shift $CD4^+$ T cells to a less differentiated phenotype.[15] Vitamin D_3 has also been shown to reduce the frequency of both Th1 and Th17 cells, which are known to be preferentially infected in PLHIV on ART.[15–19] Furthermore, vitamin D_3 promotes the gut barrier integrity in animal models and *in vitro*[20,21] and has been shown to enhance bacterial diversity and reduce the bacterial pathogen colonisation in the upper gastrointestinal tract in healthy humans.[22]

We hypothesised that a high dose vitamin D_3 would reduce HIV-1 persistence on ART through its effects on immune cells and the gut. To address this hypothesis, we performed a pilot randomised placebo-controlled trial evaluating the effect of 24 weeks of high dose vitamin D_3 on HIV-1 persistence in PLHIV on suppressive ART. Dose and duration were based on a multiple sclerosis study demonstrating clear immunologic effects of vitamin D_3 including a shift towards less differentiated $CD4^+$ T cell subsets.[15] We assessed the pharmacokinetics and safety of vitamin D_3 and its effects on a range of virologic and immunologic parameters.

2. Materials and methods

2.1. Study design and participants

This pilot randomised double-blind placebo-controlled trial was conducted between February 5, 2018 and May 9, 2019. Thirty PLHIV over 18 years of age on ART with plasma HIV-1 RNA below 40 copies/ml for at least 3 years were recruited at three sites in Melbourne, Australia: Royal Melbourne Hospital, the Alfred Hospital and Melbourne Sexual Health Centre. Participants were required to have a screening 25-hydroxyvitamin D level between 50 nM and 125 nM, the lower limit being to avoid deficiency within the placebo arm. Complete inclusion and exclusion criteria are listed in the Supplementary Material pp 53–55.

2.2. Randomisation and blinding

Participants were randomly assigned 1:1 to vitamin D_3 or placebo with randomisation stratified by time-of-enrolment cohorts. Each such cohort comprised ten participants sequentially enrolled across all sites who were randomised and commenced on study drug simultaneously to facilitate similar seasonal endogenous vitamin D production over time. Details of the random allocation sequence generation, storage and use are outlined in the Supplementary Material p 44. Vitamin D_3 and placebo were over-encapsulated with identical hydroxypropylmethyl cellulose capsules to maintain blinding. Participants, study investigators, dispensing pharmacists and statisticians remained blinded to the treatment allocation until all data had been analysed.

2.3. Procedures

Participants received either 10,000 IU vitamin D_3 capsules (Healthy Origins, Pennsylvania, USA) or placebo capsules (Swiss Caps Romania SRL, Cornu, Romania) which they were instructed to take orally once every morning for 24 weeks. The vitamin D_3 capsules contained olive oil while the placebo capsules contained a mixture of palm oil, sunflower oil, rapeseed oil and tuna oil. All participants were also requested to consume at least 1g of dietary calcium per day throughout the trial using written and online resources (Supplementary Material p 63 and www.calorieking.com.au). Participants were advised to remain on ART throughout the trial. Study site visits occurred at weeks 0 (baseline), 12, 24 and 36 (Fig. 1) while phone visits occurred at weeks 2, 6, 18 and 28. Details of procedures and specimens collected at each visit are outlined in the Supplementary Material pp 47–50 while specimen processing is outlined in the Supplementary Material pp 42–43.

2.4. Laboratory assays

Details of nested real-time PCRs for total HIV-1 DNA, integrated HIV DNA, 2-long terminal repeat (2-LTR) circles and unspliced HIV-1 RNA, cellular immunophenotyping using flow cytometry and 25-hydroxyvitamin D and high sensitivity C-reactive protein (hsCRP) assays are described in the Supplementary Material pp 2–3. For flow cytometry analysis, T cell maturation subsets were distinguished using CD45RA, CCR7 and CD27 as previously described.[8,23] Fluorescent antibodies used are shown in Table S1 while the gating strategy is shown in Fig. S1.

2.5. Outcomes

The primary outcome was the fold difference between the vitamin D_3 and placebo arms in the mean fold change in frequency of total HIV-1 DNA (copies/10^6 $CD4^+$ T cells) from week 0 to week 24. Secondary outcomes included fold differences between groups in fold change over time from week 0 to weeks 12, 24 and 36 in frequency of total HIV-1 DNA (other than week 24), integrated HIV-1 DNA, 2-LTR circles and unspliced HIV-1 RNA and absolute differences between groups in absolute change over time from baseline to weeks 12, 24 and 36 in frequency of $CD4^+$ T cells, $CD8^+$ T cells and NK cells, their maturation subsets, activation status, exhaustion marker and chemokine receptor expression and serum hsCRP and 25-hydroxyvitamin D levels. Other secondary endpoints included study drug and dietary calcium adherence and incidence and severity of adverse events.

2.6. Statistics

No data was available to estimate the size or standard deviation of the effect of vitamin D_3 on HIV-1 DNA to perform a power calculation. This was a pilot study to generate these estimates to inform a power calculation for future studies. Sample size was determined based on cost and feasibility. All analyses were by intention-to-treat; no imputation was made for missing data. A 2-sided 5% significance threshold (and corresponding 95% confidence interval) was used as an indication of association. Thus 95% confidence intervals around differences between groups that excluded 1 for fold differences or 0 for absolute differences were considered significant. The statistical analysis plan is detailed in the Supplementary Material pp 17–21 while details of the statistical

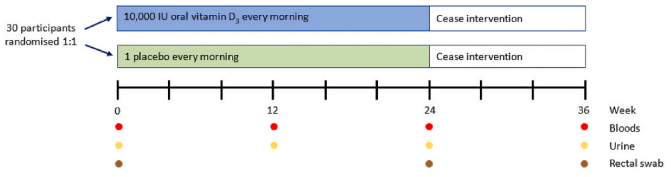

Fig. 1. Study protocol.

approach used for each assay are outlined in the Supplementary Material p 2.

2.7. Study approval

The study was approved by the Human Research Ethics Committee of Melbourne Health, Melbourne, Australia and carried out in accordance with the National Health and Medical Research Council National Statement on Ethical Conduct in Human Research 2007 (updated 2018) and the Note for Guidance on Good Clinical Practice (CPMP/ICH-135/95). Written participant consent was obtained prior to any study related activities. The trial is registered at clinicaltrials.gov (NCT03426592).

3. Results

3.1. Participants

Of 42 potential participants screened for trial eligibility, 30 were enrolled and randomised to the vitamin D_3 arm (n = 15) or the placebo arm (n = 15). Three participants in the vitamin D_3 arm withdrew from the study early for personal reasons (two prior to starting study drug and one prior to week 12). In the placebo arm, one participant withdrew from the study for personal reasons after week 12 and another withdrew from the study drug after week 12 due to an adverse event (grade 2 constipation considered possibly related to study drug) but remained on the study and had all study procedures as per protocol (Fig. 2). Therefore, 12 participants in the vitamin D_3 arm and 14 in the placebo arm were evaluated for the primary endpoint. Baseline characteristics were similar between groups (Table 1).

3.2. Pharmacokinetics and adherence

The 25-hydroxyvitamin D levels were similar at baseline between groups and increased in the vitamin D_3 arm to a mean of 228 nM (95% CI 187 nM–279 nM) at week 12 and 248 nM (95% CI 203 nM–304 nM) at week 24 (Fig. 3A). Relative to the placebo arm, 25-hydroxyvitamin D levels changed from baseline by 170 nM (95% CI 131 nM–209 nM) at week 12 and 185 nM (95% CI 142 nM–228 nM) at week 24. Levels remained slightly elevated relative to the placebo arm at week 36 (difference 45 nM (95% CI 23 nM–66 nM)) (Fig. 3B). Adherence to study drug and dietary calcium was high with no differences seen between study arms (Fig. S2).

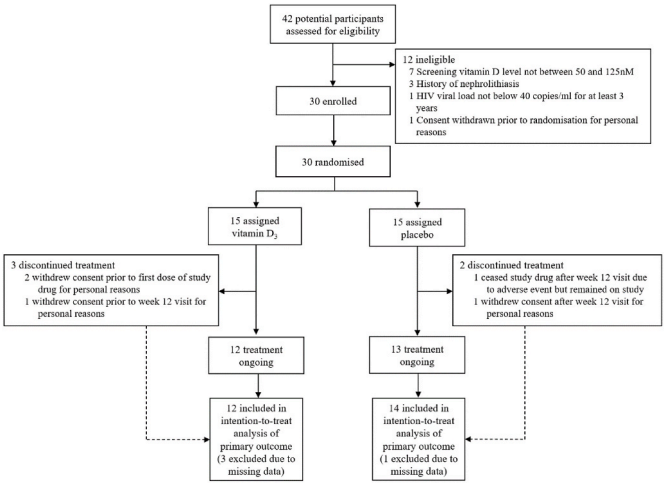

Fig. 2. Trial profile.

Table 1
Baseline characteristics.

	Placebo (n = 15)	Vitamin D_3 (n = 15)	Total (N = 30)
Age, median (IQR), years	49 (38–54)	50 (35–52)	49 (38–52)
Gender and sex – cisgender male, no. (%)	15 (100)	15 (100)	30 (100)
Race			
Indigenous Australian, no. (%)	0 (0)	1 (7)	1 (3)
White, no. (%)	14 (93)	13 (87)	27 (90)
Black, no. (%)	1 (7)	0 (0)	1 (3)
Pacific Islander, no. (%)	0 (0)	1 (7)	1 (3)
Duration of HIV-1 infection, median (IQR), years	12 (9–15)	11 (6–18)	11 (7–16)
CD4 T cell count nadir, median (IQR), cells/μL	320 (180–476)	265 (52–499)	280 (109–484)
Time since CD4 T cell count nadir, median (IQR), years	9 (4–12)	7 (5–11)	8 (5–12)
Most recent CD4 T cell count, median (IQR), cells/μL	700 (630–1154)	830 (701–910)	782 (635–1052)
ART containing:			
NNRTI, no. (%)	9 (60)	4 (27)	13 (43)
Integrase inhibitor, no. (%)	5 (33)	10 (67)	15 (50)
Protease inhibitor, no. (%)	1 (7)	3 (20)	4 (13)
Systolic BP, median (IQR), mmHg	120 (110–133)	125 (115–130)	120 (115–130)
Diastolic BP, median (IQR), mmHg	74 (70–85)	80 (72–89)	80 (70–85)
BMI, median (IQR), kg/m^2	27 (25–28)	25 (23–29)	26 (24–28)
No. of comorbidities, median (IQR)	2 (1–8)	2 (1–4)	2 (1–5)
Comorbidities			
Smoking, no. (%)	2 (13)	3 (20)	5 (17)
Alcohol misuse, no. (%)	1 (7)	2 (13)	3 (10)
Intravenous drug use, no. (%)	1 (7)	1 (7)	2 (7)
Previous hepatitis B, no. (%)	1 (7)	1 (7)	2 (7)
Previous hepatitis C, no. (%)	3 (20)	2 (13)	5 (17)
Hypertension, no. (%)	2 (13)	3 (20)	5 (17)
Hypercholesterolaemia, no. (%)	4 (27)	3 (20)	7 (23)
Diabetes mellitus, no. (%)	1 (7)	0 (0)	1 (3)
Class 1 obesity, no. (%)	0 (0)	2 (13)	2 (7)
Ischemic heart disease, no. (%)	1 (7)	0 (0)	1 (3)
Congestive cardiac failure, no. (%)	0 (0)	0 (0)	0 (0)
COPD, no. (%)	0 (0)	1 (7)	1 (3)
Malignancy, no. (%)	1 (7)	0 (0)	1 (3)

Abbreviations: ART = antiretroviral therapy; NNRTI = non-nucleoside reverse transcriptase inhibitor; COPD = chronic obstructive pulmonary disease.

3.3. Safety

One serious adverse event occurred during the trial; this was hospitalisation for community acquired pneumonia complicating influenza A infection in a placebo arm participant. Non-serious adverse events were evenly balanced between groups and were all grade 1 or 2 with the exception of one grade 3 event (Table S2); no grade 4 events occurred. One case of hypercalcemia occurred; this was asymptomatic and occurred in a participant receiving vitamin D_3 at week 24. The level was minimally elevated at 2.65 mM and returned to normal one week later following routine cessation of study drug. No cases of symptomatic hypercalciuria occurred; asymptomatic hypercalciuria in the vitamin D_3 arm was 0.58 (95% CI 0.09 to 2.49) fold that in the placebo arm (2 of 13 participants in the vitamin D_3 arm and 4 of 15 in the placebo arm).

3.4. Virology

Our primary goal was to determine the effect of high dose vitamin D_3 on markers of HIV-1 persistence (total and integrated HIV-1 DNA, 2-LTR circles and unspliced HIV-1 RNA, Fig. 4A–D). For the primary endpoint, there was no significant difference in total HIV-1 DNA between participants in the vitamin D_3 and placebo arms (1.15 (95% CI 0.93 to 1.40) fold difference in the change in frequency of total HIV-1 DNA per 10^6

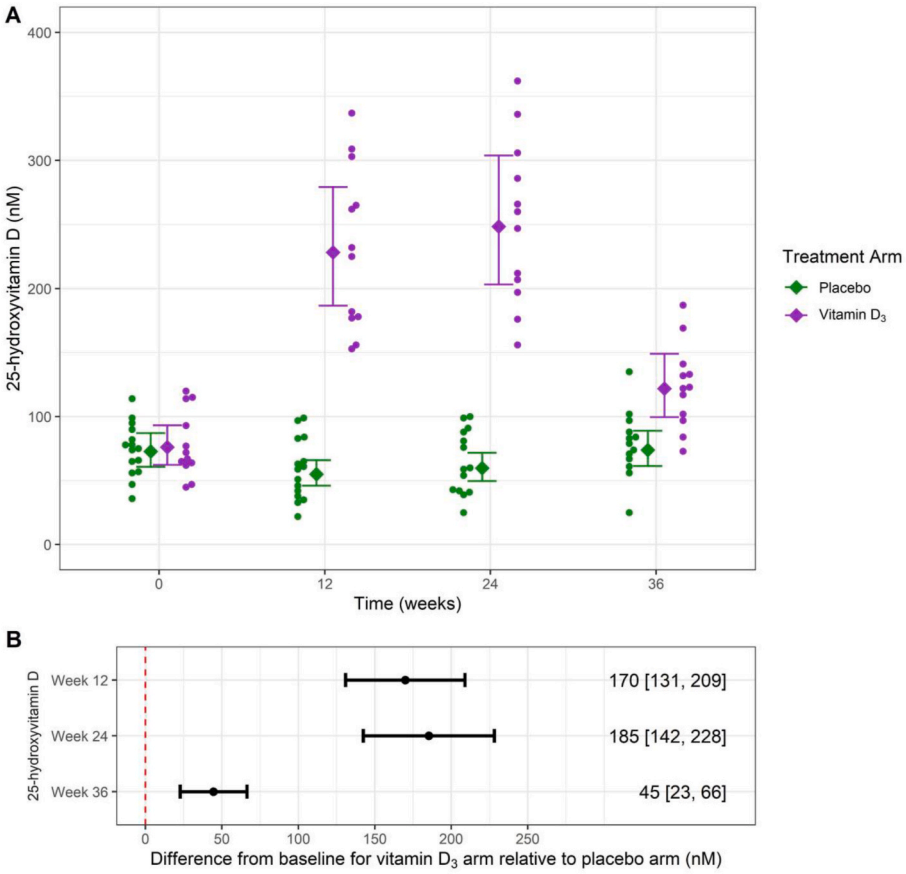

Fig. 3. Pharmacokinetics.
(A) 25-hydroxyvitamin D levels within each group over time; means and 95% confidence intervals are depicted. n = 12, 12, 12, 12 for vitamin D_3 arm and n = 15, 15, 14, 13 for placebo arm at weeks 0, 12, 24 and 36 respectively (sample missing in one placebo arm participant at week 36). (B) Effect of vitamin D_3 on change in 25-hydroxyvitamin D levels from baseline relative to placebo. Means and 95% confidence intervals are shown as determined using mixed effects linear models. n = 12, 12, 12 for vitamin D_3 arm and n = 15, 14, 13 for placebo arm at weeks 12, 24 and 36 respectively.

$CD4^+$ T cells from baseline to week 24 (p = 0.19)). From baseline to week 12 there was a 1.24 (95% CI 1.01 to 1.51) fold increase (p = 0.039) and from baseline to week 36 there was a 0.76 (95% CI 0.62 to 0.94) fold decrease (p = 0.009) in frequency of total HIV-1 DNA relative to those in the placebo arm (Fig. 4E). The other virologic endpoints showed no significant effect of vitamin D_3 (Fig. 4E). All participants maintained a plasma viral load below 40 HIV-1 copies/ml throughout the trial.

3.5. *$CD4^+$ and $CD8^+$ T cell maturation subsets*

Our secondary goal was to determine the impact of high dose vitamin D_3 on immune cell differentiation and other immunologic parameters that might be relevant to HIV reservoir size. There were no significant differences between arms in change over time in $CD4^+$ T cells, $CD8^+$ T cells or B cells as a proportion of viable cells (Table S3). We expected vitamin D_3 to shift $CD4^+$ T cells to a less differentiated phenotype.[15] Accordingly, relative to placebo, the vitamin D_3 arm was associated with an increased frequency of central memory (CM) $CD4^+$ T cells at each time point and a decrease in frequencies of effector memory (EM) and terminally differentiated (TD) $CD4^+$ T cell subsets which were significant at week 36 for EM $CD4^+$ T cells and at week 24 for TD $CD4^+$ T cells (Fig. 5A, Table S3 & Fig. S3). Similarly, $CD8^+$ T cells showed a clear shift towards less differentiated subsets in participants receiving vitamin D_3 relative to placebo with a significant increase in CM $CD8^+$ T cells and a decrease in effector memory re-expressing CD45RA (EMRA) $CD8^+$ T cells at all time points (Fig. 5B, Table S3 & Fig. S4). Naïve $CD8^+$ T cell frequency also increased at week 36 relative to placebo.

3.6. *$CD4^+$ and $CD8^+$ T cell activation, exhaustion and chemokine receptor expression*

Relative to the placebo arm, participants in the vitamin D_3 arm had an increase in frequency of activated CD38+HLA-DR+ $CD8^+$ T cells at week 12 and of exhausted PD-1+ $CD8^+$ T cells at week 24 (Table S3 & Fig. S5). At week 36, participants in the vitamin D_3 arm had an increase in frequency of activated CD38+HLA-DR+ $CD4^+$ T cells (Table S3 & Fig. S6). Relative to placebo, participants in the vitamin D_3 arm demonstrated an unexpected increase in frequency of Th17 (CCR6+CXCR3-) $CD4^+$ T cells at weeks 12 and 24 whereas there was a reduction in frequency of Th1 (CCR6-CXCR3+) $CD4^+$ T cells at week 12 (Table S3 & Fig. S6). Participants in the vitamin D_3 arm demonstrated a reduction in the frequency of senescent $CD57^+$ $CD8^+$ T cells at each time point relative to placebo (Table S3 & Fig. S5).

3.7. *Natural killer (NK) cells and high-sensitivity C-reactive protein (hsCRP)*

Relative to placebo, in the vitamin D_3 arm there was a significant decrease in the frequency of total NK cells at all time points (Fig. S7 & Table S3). There was no significant difference between arms in the change over time in the frequency of NK cell maturation subsets, including regulatory NK cells (CD56bright), cytotoxic NK cells (CD56dimCD16+) or cytotoxic NK cells that had shed CD16 (CD56dimCD16-[24]) (Table S3 & Fig. S8). We also evaluated NK cells expressing NKG2A and NKG2C, receptors for the non-classical MHC class Ib molecule HLA-E which appears to play an important role in immune control of HIV-1.[25] The NKG2A inhibits NK cell activation upon recognition of self-peptide derived from the leader sequence of MHC class Ia (HLA-A, -B or –C) presented on HLA-E; however, pathologic alteration of peptide expression on HLA-E can activate NK cells either through NKG2A disinhibition or NKG2C activation.[26–28] We found an increase in the frequency of NKG2A+NKG2C– NK cells and decrease in frequency of NKG2A-NKG2C+ NK cells at weeks 12 and 24 relative to placebo (Fig. S7 & Table S3). Participants in the vitamin D_3 arm had an

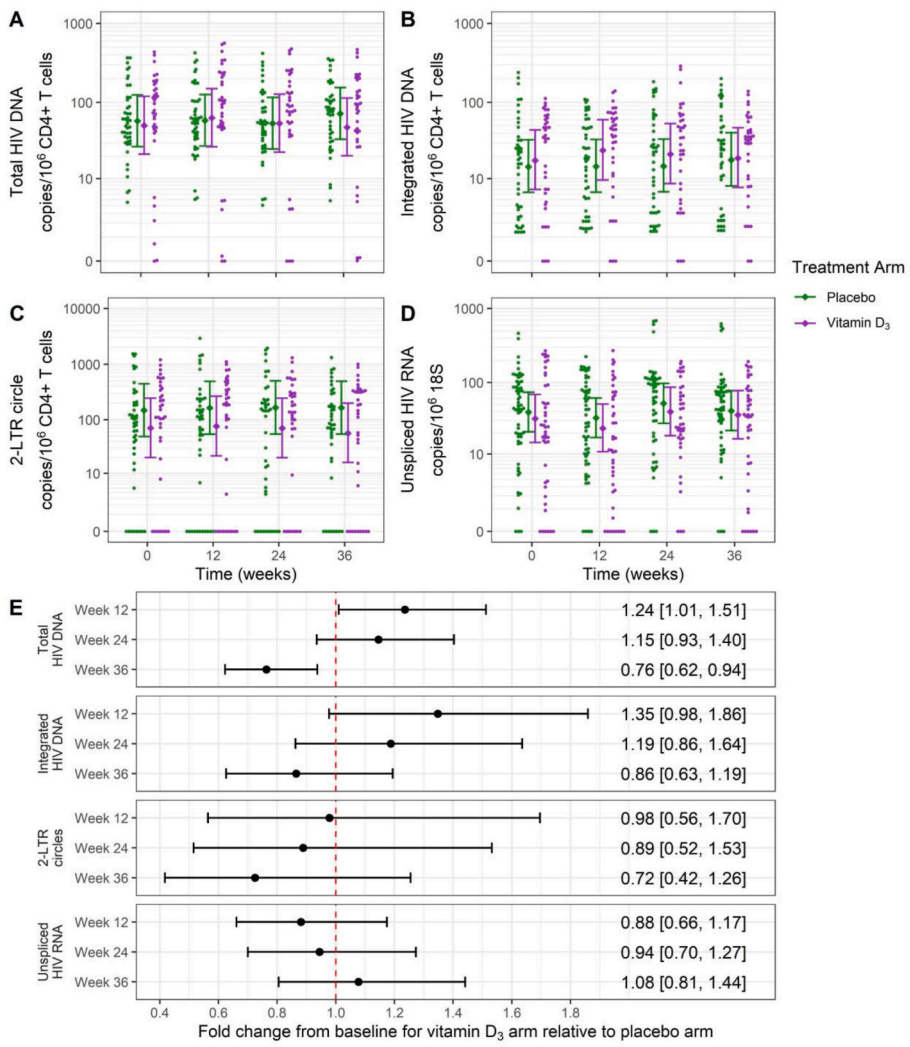

Fig. 4. Virology.
(A) Total HIV-1 DNA, (B) integrated HIV DNA, (C) 2-LTR circles and (D) unspliced HIV-1 RNA within each group over time; means and 95% confidence intervals are depicted. n = 12, 12, 12, 12 for vitamin D_3 arm and n = 15, 15, 14, 14 for placebo arm at weeks 0, 12, 24 and 36 respectively for each assay. (E) Effect of vitamin D_3 on change in frequency of HIV-1 DNA and RNA within $CD4^+$ T cells from baseline relative to placebo. Means and 95% confidence intervals are shown as determined using mixed effects negative binomial regression models. n = 12, 12, 12 for vitamin D_3 arm and n = 15, 14, 14 for placebo arm at weeks 12, 24 and 36 respectively for each assay.

increase relative to placebo in the frequency of activated CD38+HLA-DR+ NK cells at weeks 24 and 36 (Fig. S7 & Table S3). There was no difference between arms in the change in hsCRP level over time (Table S3).

4. Discussion

This is the first reported study to evaluate the effect of high dose vitamin D_3 on the HIV-1 reservoir in PLHIV on ART. Although no toxicity has ever been reported with doses of vitamin D_3 up to 10,000 IU/day,[29] this dose has not previously been evaluated in the context of ART. We found no difference between the vitamin D_3 arm and the placebo arm in the change in frequency of total HIV-1 DNA within $CD4^+$ T cells from baseline to week 24. However, relative to placebo, there was an increase in total HIV-1 DNA in the vitamin D_3 arm from baseline to week 12 and a decrease from baseline to week 36 (12 weeks after ceasing study drug) without any significant changes in integrated HIV-1 DNA, 2-LTR circles or cell associated HIV-1 RNA. We found an increase in the frequency of both $CD4^+$ and $CD8^+$ CM T cells, a reduction in senescent $CD8^+$ T cells and a reduction in NK cells at all time points, including following cessation of vitamin D_3. We also saw some changes in T cell activation and exhaustion although these were not maintained over the duration of the study drug administration. No significant safety issues were identified throughout the trial.

Our study demonstrated limited impact of high dose vitamin D_3 on the HIV-1 reservoir. The increase that we saw in total HIV-1 DNA at week 12 was of marginal significance and not maintained at week 24 whilst still on study drug. However, the reduction in total HIV-1 DNA at week 36 had a confidence interval well clear of 1.0 making a false positive result less likely. The 25-hydroxyvitamin D levels were still elevated at this time point relative to placebo, likely reflecting its long half-life *in vivo*.[30] We believe that our findings could have several interpretations: the effect of vitamin D_3 on HIV-1 DNA could be time-dependent (ie 24 weeks of exogenous vitamin D_3 exposure is insufficient but 36 weeks is sufficient for a beneficial effect), dose-dependent (ie vitamin D_3 only exerts a beneficial effect at lower levels) or withdrawal-dependent (ie exposure to a high dose was necessary for a beneficial effect to be seen at subsequent lower 25-hydroxyvitamin D levels). Further trials would be required to determine which of these explanations is correct.

Whilst the point estimates for the mean effect on integrated HIV-1 DNA were similar to those for total HIV-1 DNA at each time point, confidence intervals were wider in keeping with the greater coefficient of variation for the integrated HIV-1 DNA assay.[31] Despite the observed changes in HIV-1 DNA, it is important to note that more than 90% of total HIV-1 DNA detected by standard PCR methods is defective and therefore not capable of replicating and causing viral rebound upon ART cessation.[32] Whilst the frequency of total HIV-1 DNA in PLHIV on long-term suppressive ART correlates closely with the intact virus,[33] we cannot exclude a differing effect of vitamin D_3 on the intact and defective HIV-1 DNA. Quantification of the intact HIV-1 DNA and replication competent virus will be important in any future studies.[32]

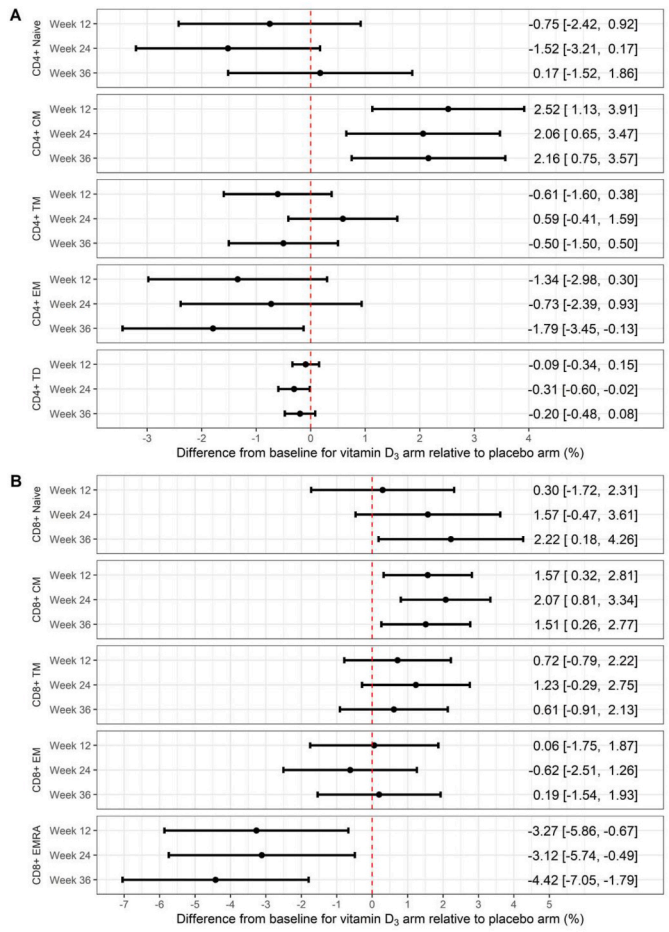

Fig. 5. $CD4^+$ and $CD8^+$ T cell maturation subsets.
Effect of vitamin D_3 on change in frequency of (A) $CD4^+$ and (B) $CD8^+$ T cell maturation subsets from baseline relative to placebo. Means and 95% confidence intervals are shown as determined using mixed effects linear models. n = 12, 12, 12 for vitamin D_3 arm and n = 15, 14, 14 for placebo arm at weeks 12, 24 and 36 respectively for each assay. CM = central memory; TM = transitional memory; EM = effector memory; TD = terminally differentiated; EMRA = effector memory re-expressing CD45RA.

The most striking findings in our study were immunological. Vitamin D_3 induced a clear increase in the proportion of both $CD4^+$ and $CD8^+$ CM T cells and a reduction in more differentiated T cells. This likely reflects the known effect of vitamin D_3 in reducing $CD4^+$ and $CD8^+$ T cell proliferation.[14,34] The $CD4^+$ T cell proliferation is an important mechanism for HIV-1 persistence on ART.[8,9] Previous work has shown a higher frequency of HIV-1 DNA and clonal expansion of infected cells within more mature $CD4^+$ T cell subsets such as EM compared to less mature CM $CD4^+$ T cells.[35] These changes in $CD4^+$ T cell subsets were observed at all time points, including at week 36 despite the reduction in 25-hydroxyvitamin D level, suggesting that an ongoing antiproliferative effect could have contributed to the reduction in total HIV-1 DNA seen at week 36.

In regard to T helper subsets, the reduction in Th1 cell frequency was not surprising[16] whereas we expected a decrease in the frequency of Th17 cells based on findings of high dose vitamin D_3 in multiple sclerosis.[15] However, the effect of vitamin D_3 on Th17 cells may not necessarily be the same for PLHIV as for people with multiple sclerosis. For instance, if HIV-1 selectively depletes Th17 cells in more differentiated $CD4^+$ T cell subsets, a vitamin D_3-induced shift towards less differentiated subsets (which was seen in both our study and the multiple sclerosis study[15]) could increase the total Th17 cell frequency, even if vitamin D_3 reduced the frequency of Th17 cells within each maturation subset. Irrespective, the increase in Th17 cells in our study may have contributed to the transient increase in total HIV-1 DNA at week 12 as $CD4^+$ T cells expressing CCR6 are also known to be enriched for HIV-1 DNA.[18,19,36]

We observed changes in $CD8^+$ T cells and NK cells induced by vitamin D_3 that may have favoured clearance of infected cells. For example, the reduction of senescent $CD8^+$ T cells by vitamin D_3 at all time points may have enhanced HIV-1 specific $CD8^+$ T cell function and therefore elimination of HIV-1-infected cells.[37,38] Further studies would be required to evaluate this possibility. The increase in frequency of NKG2A+NKG2C– NK cells could also have contributed to the depletion of total HIV-1 DNA. Infected $CD4^+$ T cells can present a conserved HIV-1 peptide on HLA-E which is not recognised by the inhibitory NKG2A/CD94 receptor complex leading to disinhibition and potent killing by NKG2A+ NK cells.[39] In contrast, NK cells expressing NKG2C do not exhibit enhanced degranulation in response to HIV-1-infected cells potentially due to co-expression by these NK cells of inhibitory receptors to HLA-C.[39]

The main strength of this study was its randomised double-blind placebo-controlled design providing a high degree of internal validity. The main limitation was the small sample size which increases the risk of false negative results. Additionally, multiplicity of testing with a small sample size increases the risk of false positive results. However, as a pilot trial, our analyses were exploratory and results will need to be confirmed in future studies. Other important limitations were that intact virus was not measured and that we only evaluated the HIV-1 reservoir in blood whereas the majority of the reservoir resides in lymphoid tissue. The predominance of white males in our trial limits generalisability of our findings to females and other racial groups. Finally, we cannot exclude the possibility of some of our results being due to differences in oil content between the vitamin D_3 and placebo capsules; however, this seems unlikely considering that these are commonly consumed in much higher quantities in the diet.

5. Conclusions

Vitamin D_3 had no effect on the change in frequency of total HIV-1 DNA in peripheral blood $CD4^+$ T cells from baseline to week 24. However, it had a number of immunologic effects which may be relevant to the HIV-1 reservoir size as there was a reduction in total HIV-1 DNA at week 36, 12 weeks after ceasing the study drug, with persistently elevated 25-hydroxyvitamin D levels at this time point compared to placebo. Additional studies are required to determine whether the dose and duration of vitamin D_3 can be further optimised to promote a continued depletion of the HIV-1 reservoir over time.

Authors' contributions

MCP: conceptualisation, methodology, validation, investigation, data curation, writing – original draft, writing – review and editing, visualisation, project administration; NM: formal analysis, writing – review and editing, visualisation; DJP: formal analysis, writing – review and editing, visualisation; AR: validation, investigation, writing – review and editing; JJC: writing – review and editing, supervision; BS: methodology, data curation, writing – review and editing, project administration; BA: resources, writing – review and editing; AS: resources, writing – review and editing; JHM: methodology, writing – review and editing; TAR: methodology, writing – review and editing; PUC: methodology, writing – review and editing, supervision; JFH: methodology, resources, writing – review and editing, supervision; SJK: methodology, resources, writing – review and editing, supervision, funding acquisition; SRL: conceptualisation, methodology, resources, writing – original draft, writing – review and editing, visualisation, supervision, project administration, funding acquisition.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jve.2023.100345.

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