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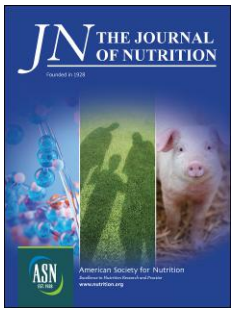

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The effect of vitamin D_3 supplementation on vitamin D status and associated health outcomes in children: a randomised controlled trial.

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Abbreviations: D-VinCHI, D vitamin in children; NDNS, National Diet and Nutrition Survey; 25(OH)D, 25-Hydroxyvitamin D; s-RTI, Simple reaction time; 5-RTI, 5-choice reaction time ; RVP, rapid visual information processing; SSP, spatial span

Data described in the manuscript, code book, and analytic code will be made available from corresponding author upon request pending [application and approval].

Abstract

Background: Vitamin D is a key regulator of musculoskeletal growth, immune regulation and cognitive function in children. As children in the UK and Ireland are at risk of vitamin D deficiency due to the northern latitude, supplementation is recommended. To our knowledge, the effect of supplementation on status and related health outcomes has not been investigated in children residing in the UK/Ireland.

Objective: To examine the effect of vitamin D_3 supplementation on vitamin D status, and related health outcomes in children.

Methods: The D-VinCHI study was a double-blind, randomised placebo-controlled trial, conducted among healthy children (aged 4–11 years). Children received 12 weeks of either 10µg/day vitamin D_3 or a placebo devoid of vitamin D (control) in the form of an oral spray. The primary outcome, plasma 25-hydroxyvitamin D [25(OH)D], and secondary outcomes including grip strength, balance, cognitive function, winter status, immune markers and bone turnover markers were assessed pre- and post-intervention.

Results: 118 participants completed the study (mean age 8.1±1.8y; 51% girls). Supplementation significantly increased 25(OH)D concentration (from 66.31±17.26 to 69.04±16.93 nmol/L) compared to a decline in the placebo group (63.67±19.48 to 56.29±18.58 nmol/L; $p<0.001$) and prevented deficiency during the extended winter months. No effects were observed on muscle function, cognitive function, immune function or bone turnover markers.

Conclusion: Vitamin D insufficiency is prevalent in UK/Irish children and supplementation in the form of an oral spray is effective in achieving/maintaining an

adequate status in most children. Further research is warranted to elucidate mechanisms underpinning non-response to supplementation and to further investigate the potential beneficial effects of supplementation on cognitive function.

This trial was registered with ClinicalTrials.gov (NCT05018988: https://clinicaltrials.gov/study/NCT05018988)

Keywords: Vitamin D Supplementation; 25(OH)D status; Muscle strength; Children; Balance; Immune Function; Cognitive Function; Bone turnover markers

Introduction

Vitamin D deficiency during childhood is associated with severe adverse skeletal health effects leading to an increased risk of developing rickets (1). The incidence of rickets in the United Kingdom (UK) is 0.48 per 100,000 in children under 16 years presenting to secondary care. Although evidence for an increasing incidence is limited, rickets persists in UK children, underscoring the importance of preventative strategies (2). Nutritional rickets represents the severe end of the vitamin D deficiency spectrum (3), but milder forms of deficiency (25-50 nmol/L) are more common and have been associated with adverse outcomes including impaired bone mineralisation, increased bone turnover (4,5) and reduced muscle strength in adolescents (6,7). Beyond, its role in calcium homeostasis and skeletal development, optimal vitamin D may also influence immune health and cognitive function in infants and adolescents (8, 9, 10, 11).

While global definitions of vitamin D status vary, in the UK vitamin D deficiency is defined as a circulating 25 hydroxyvitamin D (25(OH)D) concentration of ≤25 nmol/L (12). Concentrations of 25-50 nmol/L are considered insufficient and ≥50 nmol/L sufficient (13). At higher latitudes, vitamin D status is strongly influenced by season due to negligible UVB exposure throughout the extended winter (November – March) (14). When cutaneous synthesis is impeded, adequate vitamin D intakes must be met through diet and/or supplementation (15) yet consistently low dietary vitamin D intakes are reported in UK and Irish children (16,17,18). In the most recent National Diet and Nutrition Survey (NDNS) in the UK, 10% of children aged 4 – 10 years had low vitamin D status (18). Notably, within the NDNS dataset only 6 children aged 4 - 10 years were sampled for 25(OH)D concentrations in Northern Ireland, despite a relatively narrow latitude range (55-56ºN) which provides a distinct context for

examining vitamin D status given its mixed urban-rural characteristics. Previous research in children and adolescents at similar (51-55ºN) or higher latitudes (56-63ºN) has demonstrated that vitamin D supplementation can improve vitamin D status and reduce the risk of deficiency, including studies from the UK (19), Sweden (20) and Denmark (21). Most studies, however, have been limited to the winter months and narrow age ranges, restricting generalisability. In 2016, UK Government vitamin D recommendations were revised to include children (12), yet no comprehensive trial has evaluated whether supplementation is effective at maintaining or improving 25(OH)D concentrations across children aged 4 -11 years within the UK. Current guidelines of 10µg/day are based on supporting bone health and evidence for optimal vitamin D intakes to support other health outcomes, such as muscle function, remains limited especially in children.

The paucity of data investigating vitamin D status and effects of supplementation in healthy children shows that further research is warranted. Therefore, the primary aim of this study was to investigate the effect of 10µg/day vitamin D_3 supplementation over 12 weeks on 25(OH)D concentrations in healthy children aged 4-11 years. Secondary aims investigated the effect of vitamin D_3 supplementation on musculoskeletal function, sensorimotor function, and cognitive function, with subset analysis conducted in extended winter supplementation, immune markers, and bone turnover markers. By addressing these gaps, this study aims to provide novel evidence to inform child health policy in the UK, where supplementation is low, fortification is voluntary, and socioeconomic inequalities may exacerbate risk of deficiency.

Methods

Study design

The D vitamin in children (D-VinCHI) study (Phase 2) was conducted at the Nutrition Innovation Centre for Food and Health, Ulster University, Coleraine, Northern Ireland. Initial study recruitment and sampling commenced between November 2019 – March 2020 after which the study was postponed owing to the COVID-19 pandemic. Following recommencement of research, recruitment was completed between August 2021 – August 2023. Eligible children aged 4 to 11 years (n=118) were randomised to receive a spray containing either 10µg/day vitamin D_3 or a placebo (containing 0µg vitamin D_3) matched in taste, smell, appearance, and size of container. Children were randomly allocated in a 1:1 ratio stratified by age categories 4-6, 7-9, and 10-11 years old. Children from the same family were given the same allocation. All aspects of randomisation were carried out independently by the Human Interventions Studies Unit (HISU) Clinical Trials Manager at Ulster University. Participants were invited to attend two 90-minute appointments, at the beginning and end of the trial. Participants who had originally undertaken the study but were unable to complete the trial due to COVID-19 restrictions were invited to take part when the study recommenced in 2021. Ethical approval was granted from Ulster University's Research Ethics Committee (REC/19/0069; clinical trials registration: NCT05018988).

Participants

Recruitment occurred via social media, internal mailing listings, in-person educational health talks and school parent evenings. Parents contacted the researcher via email or

phone and either the researcher completed the screening questionnaire with the parent via phone, or the parent completed an online screening questionnaire via a QR link. Healthy participants free from chronic disease were invited to either enrol onto the trial if not taking a vitamin D supplement, or willing to abstain from starting vitamin D supplements. Children were excluded from participating if they were prescribed long-term medication that affected vitamin D metabolism; and/or diagnosed with a long-term or exacerbated health condition or disease (children with minor or mild health conditions were not excluded) or using sun beds for the duration of the intervention. All parents and children provided written consent and assent, respectively, at time of recruitment. Baseline appointments were held at either the HISU or a suitable off-site location with post-intervention appointments for eligible participants held 12 weeks later.

Anthropometry

Weight was measured using electronic SECA alpha flat scales (SECA ltd., Hamburg, Germany) and height (cm) was measured using a SECA 213 portable stadiometer (SECA Ltd, Germany). All children had measurements taken in triplicate wearing light clothing with items removed from pockets where necessary and without shoes. Body mass index was calculated (BMI, kg/m^2) and WHO AnthroPlus software for children and adolescents aged 5 - 19 years was used to convert into BMI z-scores (BMIz) (22).

Musculoskeletal

Grip strength was assessed by dynamometer with the participant standing, shoulder adducted and elbow parallel to thigh, to assess muscle strength (TKK 5001 Grip-A

analogue dynamometer (64080136); Takei Scientific Instruments Co. Ltd., Niigata, Japan). Three measurements were performed with short rests up to 30 seconds between each test. An age specific adapted grip strength of both dominant and non-dominant hand was calculated as average grip strength (Kg)/body mass index (23). Sensorimotor testing of participants included balance using single leg stance [SLS] and tandem stance [TS] with a method previously described by Condon and Cremin (24).

Cognitive function assessment

A battery of cognitive tests were conducted using the well validated Cambridge Neuropsychological Test Automated Battery (CANTABeclipse, Research Suite; Cambridge Cognition, UK) (25). Neurocognitive tests were designed to assess a range of cognitive domains at pre- and post-intervention through non-verbal stimuli and touch screen methodology. The following tests were performed: spatial span (SSP), simple and 5-choice reaction time (s-RTI, 5-RTI) and Rapid Visual Information Processing (RVP). Full details of each test procedure are reported elsewhere (26, 27). Briefly, all participants were asked to complete a motor screening test to familiarise themselves with the battery. Following this, the SSP task measured the capacity of immediate memory through the ability to recall the order of colour change of boxes in a series by pressing the highlighted boxes in the correct recall sequence. The dependent measure was the longest sequence recalled correctly. Attention was assessed by both simple and 5-choice reaction time; participants were required to react to a yellow dot appearing inside either one (simple RTI) or five (5-RTI) circles in the centre of the computer screen. Participants were instructed to release the press pad and touch the dot as quickly as possible. The dependent variable was mean latency

measured in milliseconds between the dot's appearance and the release of the press pad. The RVP is designed to assess sustained attention capacity through a 4-minute visual continuous performance task. Briefly, digits ranging from 2-9 are presented one at a time in a random order on the screen. Participants were required to respond when the target sequence was presented, the target sequence was modified for either age 4 – 6 or 7 – 11 years. The procedure for assessing RVP is described in full detail elsewhere (28).

Questionnaire measures

All questionnaires were self-reported except for the validated food frequency questionnaire (FFQ) (29) which was administered by the research team. Mean daily vitamin D intakes were calculated for each participant, based on dietary sources only and did not include vitamin D from the supplement given during the study. Parents provided information on child and parent demographics and lifestyle factors. The validated children's physical activity questionnaire was also completed at baseline (30,31). At the re-commencement of the study a COVID-19 lockdown questionnaire was introduced to document any known changes in vitamin D supplementation, vitamin D rich food intakes and outdoor activity which are all associated with 25(OH)D concentrations. To determine skin phototype, an adapted Fitzpatrick scale was utilised to determine skin pigmentation type (32).

Intervention

Both the supplement and the inactive placebo were provided in kind by BetterYou Ltd (BetterYou, Barnsley, UK) in oral spray form, 10mL. Each active vitamin D_3 spray consisted of xylitol, water, acacia gum, cholecalciferol (vitamin D_3), sunflower

lecithin, citric acid, preservative: potassium sorbate, peppermint oil. An inactive placebo spray identical but lacking cholecalciferol (vitamin D_3) served as the control. All sprays were packaged unlabelled with no distinguishing markings and were delivered to the clinical trials manager and distributed to the research team to ensure double blinding. Participants were provided with standardised verbal and written instructions on spray use, including priming the spray before use and a physical demonstration by the researcher with a placebo spray. Dose standardisation was achieved by instructing parents to administer one spray per day, corresponding with the manufacturer-labelled dose of 10µg per vitamin D_3 spray. The oral spray was administered daily either in the morning or evening to the mucosal buccal membrane, either by the child or assisted by the parent, depending on the child's ability. Participants were requested to return the spray bottles at the end of the study. Compliance was assessed through weighing the spray bottles pre- and post-use, as well as through a star chart completed by participants.

Blood collection and laboratory analysis

Pre- and post-intervention, a non-fasting 20ml venous blood sample was obtained from the antecubital fossa by a trained paediatric phlebotomist. Sample preparation and fractionation were undertaken within 4 hours of sampling and blood aliquots were stored at -80°C until batch analysis. EDTA 4mL samples were sent to the Clinical Biochemistry Laboratory, Altnagelvin Area Hospital for intact parathyroid hormone (iPTH) and analysed using a Cobas 800 clinical biochemistry analyser (e602 immunoassay module; Roche Diagnostics Ltd., UK) (CVs 1.7).

Plasma 25-OHD quantification

Plasma 25(OH)D concentrations were analysed by the gold standard liquid chromatography tandem mass spectrometry (HPLC and MS/MS). A Reverse Phase (phase A consisted of 50:50 (*v/v*) water: acetonitrile containing 0.1% formic acid as a modifier. Phase B consisted of acetonitrile with 0.1% formic acid) chromatography (Polar C18 2.6 μm (3.0 × 100 mm) column) (Phenomenex, Torrance, US). Mass spectrometry was performed using an API 4000 mass spectrometer (ABSCIEX, MA, UK) by means of turbo spray ionisation in positive ion mode. The multiple reaction monitoring transition for 25(OH)D_3 was used as an acquisition method. Quantification was performed by internal standard ratios using a linear regression model. Vitamin D status cut-off thresholds for 25(OH)D concentrations follow UK convention where ≤25nmol/L is deficient, 25-50nmol/L is insufficient and ≥50nmol/L is sufficient (12,13).

Blood Biomarker Analysis

To determine the effect of vitamin D supplementation on immune markers and bone turnover markers a subset of participants was selected based on baseline vitamin D status (n=54). Participants were categorised into either insufficient (≤50 nmol/L) or sufficient (≥50nmol/L) vitamin D status and were matched according to sex, age, BMI within the vitamin D or placebo group. Inflammatory markers including C-reactive protein (CRP), interferon gamma (IFN-γ), tumour necrosis factor-α (TNF-α) and interleukin (IL) -1 β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70 and IL13 were selected to explore changes in innate and adaptive cytokines that may be modulated by vitamin D. The inflammatory markers panel was analysed via electro chemi-luminescence-

based Meso Scale Discovery (MSD) immunoassay following manufacturer's instructions (MSD V-Plex Multi-Spot Assay System, K151A9H-1, and S-Plex Multi-Spot Assay System K151AKV-1 and K151Y9S-1, LLC, Merck & Co., Inc., Kenilworth, N.J., U.S.A.). Concentrations were expressed as mean ± SD in fg/ml. The lower limits of detection (LLOD) (pg/ml) for CRP and IL8 were 4.96 and 0.07, respectively. The lower limits of detection (fg/ml) for IFN-γ, TNFα, IL-1β, IL-2, IL-4, IL-6, IL-10, IL-12p70 and IL-13 were 10.8, 19.8, 180.0, 23.3, 33.8, 36.4, 44.8, 187.0 and 8.20, respectively. Sample concentrations below the lower limit of detection were inputted as LLOD. (Timepoint 1 CVs: 11.3%, 8.7%, 28.6%, 10.8%, 6.9%, 4.5%, 7.1%, 4.7%, and 12.1%. Timepoint 2 CVs: 4.6%, 4.8%, 15.3%, 5.2%, 7.3%, 3.5%, 5.3%, 5.3% and 7.5% respectively). Bone turnover markers were assessed via immunoassay C-terminal telopeptide of type 1 collagen (CTX) and procollagen type 1 intact N-terminal propeptide (P1NP), Roche; F. Hoffmann-La Roche Ltd.) (CVs: 28.1% and 22.2% Timepoint 1 and 34.9% and 24.9% Timepoint 2 respectively) (Belfast city hospital, Belfast, Northern Ireland, December 2023).

Statistical analyses

The sample size was estimated using data from Mortensen et al. (20). In that study in children, a daily dose of 10 µg/day vitamin D_3 resulted in a mean change in serum 25(OH)D concentration of 4.9 nmol/L, with a standard deviation of 8 nmol/L. Assuming a two-sided significance level of 0.05 and 90% power, a minimum of 47 participants per group was required to detect this difference. To account for an anticipated 20% dropout rate, an additional 12 participants were recruited per group, giving a total required sample size of 118. All secondary outcomes were exploratory, and the study was not powered to detect differences in these outcomes. All analysis

was conducted through the Statistical Package for the Social Sciences (SPSS), significance was set at $p<0.05$ (IBM SPSS Statistics for windows, version 29.0, IBM Corp, Armonk, NY). Following the Consolidated Standards of Reporting Trials (CONSORT) guidelines analyses were completed using intention-to-treat analysis. All data was double entered, data that was missed due to child refusal or not collected at time point two were imputed by last observation carried forward before analysis. Normality was tested using visual inspection of histograms and Kolmogorov-Smirnov normality test. Descriptive statistics were performed for baseline characteristics, with differences between groups compared using a chi-squared test for categorical data and an independent *t* test for continuous data.

The primary outcome was analysed using analysis of covariance (ANCOVA) with post-intervention concentration as the dependent variable, adjusting for, baseline 25(OH)D concentration, age, BMI, dietary vitamin D intake, physical activity (sedentary minutes), season, and skin pigmentation type. Children from the same family were included in the trial and the overall proportion of participants from the same family was modest and balanced between groups. Given the primary outcome was a biochemical measure and randomisation was balanced across groups, analyses were conducted at the individual level.

Due to violations of normality of secondary outcomes, between-group differences in change from baseline were analysed using ANCOVA with change score as the dependent variable. Furthermore, ANCOVA analyses, controlling for age, BMI, dietary vitamin D intake, physical activity (sedentary minutes) season, and skin pigmentation type were conducted to assess the effect of intervention on change in grip strength, sensorimotor function and cognitive function. In subgroup analyses, the

ANCOVA was repeated to assess the effect of intervention during the extended winter period. Model assumptions were checked, including normality of residuals, homogeneity of regression slopes and Levene's test of equality. Statistical significance was set at $p<0.05$ and 95% confidence intervals reported. Subgroup analysis of change (post- minus pre-intervention) in immune markers and bone turnover markers in insufficient and sufficient participants who were age, sex and BMI matched were conducted as exploratory analyses, not powered to detect statistically significant differences. To correct for multiple comparisons a Benjamini-Holberg false discovery rate (FDR) procedure was applied, adjusting the reported p-values accordingly to control for type I errors and reduce risk of false positives.

Results

Children's baseline characteristics are outlined in **Table 1.** A total of 229 child families were screened from which 118 children were recruited and randomised for the intervention. In total, 77 families participated in the trial. Inclusion of siblings was permitted to facilitate recruitment; distribution of siblings was similar between groups with 35 siblings in the placebo group and 37 siblings in the vitamin D group. A total of 102 children completed the intervention study in full, representing an 86% completion rate. A total of 16 children did not complete the study (intervention n = 13, placebo n=3). In the vitamin D group, reasons for withdrawal included fear of needles/refusal of second blood sample (n=3), illness (n=4 from one family), parent forgot to give the spray and lost interest (n=2 from one family) or were lost to follow up (n=4). In the placebo group, all three children withdrew without providing a reason (**Figure 1**). Although a larger number of participants withdrew from the vitamin D group compared with the placebo, withdrawals were due to a variety of reasons unrelated to the intervention and baseline characteristics of those lost to follow-up

were similar to participants who completed the study. Of the 118 children at baseline, 30 (25%) children had a serum 25(OH)D concentration <50 nmol/L indicative of insufficient status.

Effect of vitamin D supplementation on plasma 25(OH)D

The effect of intervention on plasma 25(OH)D concentrations at week 12, adjusted for baseline 25(OH)D, age, BMI, dietary intake, season, skin pigmentation score and physical activity (sedentary minutes), is shown in **Table 2**. Supplementation with 10µg/day significantly increased concentrations of 25(OH)D compared to no supplementation (P<0.001). Post-intervention, none of the supplemented children were deficient and only 6 (10%) remained insufficient. In contrast in the placebo group, 34% of the children were vitamin D insufficient, including, 2 (~4%) who were vitamin D deficient. Overall, there were no adverse effects of supplementation, and the oral spray was well tolerated.

Effect of vitamin D supplementation on plasma 25(OH)D during the extended winter

Secondary analyses showed that 10µg/day significantly increased concentrations of 25(OH)D compared to no supplementation (P<0.05) during the extended winter (November – March) (**Table 2**). Throughout this period of time, none of the supplemented children were vitamin D deficient and ~11% remained insufficient. In contrast in the placebo group, 50% were insufficient, of which, 1 child (~3%) was vitamin D deficient (<25nmol/L).

Effect of vitamin D supplementation on secondary outcomes

Intact PTH concentrations were within normal reference ranges at baseline and follow-up in both groups. There were no significant changes in iPTH between groups

(**Table 2**). There were no significant changes in absolute or relative grip strength between groups (Supplementary **Table 3**). There were no significant changes in time between groups in single leg balance with eyes open or eyes closed, nor when balancing in tandem stance (Supplementary **Table 4**). There were no significant changes in measures of cognitive function (SSP, RTI movement time, RTI reaction time, RVP) between groups (Supplementary **Table 5**).

In exploratory subgroup analyses, there was no significant change in any immune markers regardless of vitamin D status at baseline (**Supplementary Table 1**). There was no significant change in the bone turnover markers CTx or PINP post intervention regardless of baseline vitamin D status (**Supplementary Table 2**).

Discussion

The findings from this study demonstrate for the first time in Northern Ireland that supplementing with 10µg/day vitamin D_3 for 12 weeks leads to a mean increase in circulating 25(OH)D concentrations and, in the majority, prevents vitamin D insufficiency in children aged 4-11 years residing at a northerly latitude. During the extended winter, when the contribution of UVB exposure to vitamin D status is negligible, participants in the vitamin D group maintained or improved vitamin D status bar one participant who became insufficient, whereas within the placebo group 15% became insufficient. Beyond effects on vitamin D status, vitamin D_3 supplementation was associated with nominal improvements in measures of psychomotor speed; however, these effects did not remain statistically significant following correction for multiple comparisons. No effects, however, were observed on grip strength or sensorimotor function. These findings highlight that whilst modest supplementation is generally effective for maintaining sufficiency and supporting

neurocognitive outcomes, it may be inadequate to fully correct low baseline status or impact muscle and sensorimotor function in generally healthy children.

At baseline, the 25(OH)D concentrations were above the sufficiency threshold (>50nmol/L) however a quarter of children were vitamin D insufficient. Over the 12-week intervention, on average 25(OH)D concentrations increased in the supplementation group but declined in the placebo group, irrespective of season. Notably, without supplementation, insufficiency worsened over 12 weeks, with 34% in the placebo group vitamin D insufficient at endpoint including two children who fell below the cut-off for vitamin D deficiency. In contrast, following supplementation, in those children who were insufficient at baseline (n=10), vitamin D supplementation corrected insufficient status in six children. Nevertheless, ~40% receiving supplementation also experienced decreases in 25(OH)D concentrations, however, these changes rarely resulted in a shift to insufficient status with only six children dropping below sufficiency thresholds.

Beyond baseline status and ethnicity, the exact mechanism driving the variability in response to vitamin D supplementation has not yet been determined however, other biological factors, including genetic and metabolic differences (33) are known to contribute. Variants in the vitamin D binding protein genotype have been associated with vitamin D deficiency in adolescents (34) and with an improved response to supplementation in infants (35). Several vitamin D related genes contribute to individual differences in supplementation response in children. Single nucleotide polymorphisms in key genes including VDR, GC, CYP2R1 and RXRA have been associated with altered vitamin D metabolism, transport and receptor activity (36). These genetic factors, when considered alongside environmental (37) and societal factors (38) may help explain why some supplemented children did not increase their

25(OH)D concentrations. These findings suggest that while 10µg/day is broadly effective at maintaining vitamin D sufficiency for most children, it may be inadequate to fully correct low status in all children.

Seasonal effects were most evident during the extended winter (November -March), when the contribution of UVB exposure to vitamin D status is negligible and reliance upon dietary intake and vitamin D stores are essential. Among the 69 children enrolled during this period, baseline 25(OH)D concentrations did not differ between the supplementation and placebo groups. Following intervention, however, children who received supplementation achieved a sufficient status on average, whereas those not supplemented experienced a mean decline in 25(OH)D concentrations resulting in a mean insufficient status. Although this subgroup was not powered to detect statistically significant differences, the results highlight seasonal vulnerability of children's vitamin D status and reinforce the importance of consistent strategies to maintain sufficient status. Comparable intervention studies in European children have also reported mean increases in 25(OH)D concentrations of 5–25nmol/L following supplementation with up to 20µg/day (20,21), reinforcing that inter-individuality is common and higher doses may be required to optimise status across the population.

Generalisability of these findings to the broader NI population should be considered in the context of the socioeconomic and ethnic profile of Northern Ireland. Recent NI census results indicate that approximately 17% of individuals live in relative poverty, with child poverty rates higher than the population average (39). The region is also relatively ethnically homogenous compared with other parts of the UK, with approximately 96.6% of residents identifying as Caucasian and 3.4% belonging to minority groups (40). Consistent with this population profile, the study sample was

predominantly Caucasian and skewed towards more socioeconomically advantaged families. While this reflects the local demographic context, it may limit generalisability to more ethnically diverse or socioeconomically disadvantaged populations, in whom, vitamin D status, sun exposure behaviours and dietary patterns may differ and further research in these populations is warranted.

Although no cognitive outcomes remained statistically significant following adjustment for multiple testing, observed trends are biologically plausible given the known role of vitamin D in brain development and function. Vitamin D supplementation potentially stimulated improved motor execution speed, in contrast, the placebo group showed a decline, this divergent trajectory suggests a protective and enhancing role of vitamin D on neuromotor function in children. To our knowledge, this is the first time this has been shown in children as assessed using the well validated CANTAB battery. Micronutrient deficiencies are linked to brain health and cognitive function (41). Vitamin D is thought to exert its effects through broad expression of vitamin D receptors and metabolites in brain regions related to learning, memory and motor control (42,43,44). Hydroxylating enzymes in neurons and glia cells further enable metabolism, supporting a regulatory role in neuroplasticity within motor-related brain domains such as the cerebellum and basal ganglia (45). Furthermore, these findings are consistent with previous research conducted in adolescents aged 12-18 years, suggesting improved attention, memory and inhibitory control following supplementation (46) and improved gross motor function in infants aged 3-6 months following modest supplementation (47). These findings suggest that cognitive and psychomotor domains may be particularly sensitive to improvements in vitamin D status, even over relatively short intervention periods. The observed

improvement may therefore reflect the role of vitamin D in neurodevelopmental processes, particularly during ongoing maturation of the prefrontal cortex during late childhood (48, 49).

Vitamin D supplementation had no significant effect on grip strength and balance, which remained following correction for multiple comparisons. This may reflect the relatively short intervention duration and the differing physiological mechanisms underlying these outcomes (50). Grip strength is a proxy for muscle hypertrophy and contractile force, which may require longer supplementation periods or higher doses to demonstrate measurable improvements (51). Furthermore, grip strength and balance measures are subject to developmental and measurement variability in child populations, which makes small changes harder to detect (52). A previous study reporting improvements in muscle performance, included jumping mechanography and higher doses provided to a more deficient population, suggesting sensorimotor outcomes may require greater stimulus to show measurable change (6). Our findings, therefore, align with the idea that vitamin D status may positively influence cognitive and psychomotor functions before measurable changes in gross motor function are observed in generally healthy children.

Dietary intake was limited, with children obtaining only ~30% of the recommended vitamin D from diet. Only three children met the national guidelines (10 µg/d), and fortified (voluntary in the UK) cereals were the greatest contributor to their vitamin D intakes. It is worth noting that all three children were in the vitamin D supplement group. Notably similar low intakes have been reported in other child cohorts (16,20,21,53, 54), reflecting the limited contribution of vitamin D rich foods to children's diets. Supplementation with 10µg/day, although below the recommended dietary allowance of 15µg/day, helps bring total vitamin D intake (from diet and

supplements) closer to the typical 15µg/day recommended dietary allowance for this age group. These findings reinforce the need for supplementation in this age group in the absence of (bio)fortification policies (55).

In the subgroup that were vitamin D sufficient at baseline, nominal differences were observed between groups, with those receiving supplements having higher IL -4 and TNF- α when compared to placebo. Notably, in the vitamin D sufficient group, supplementation raised mean 25(OH)D concentrations from ~74 nmol/L to ~82 nmol/L, whilst the placebo group decreased from ~72 nmol/L to ~58 nmol/L. Although, these differences did not remain significant following correction for multiple comparisons and should be cautiously interpreted. Higher IL-4 and TNF-α in those with better vitamin D status may be indicative of their ability to maintain a more balance Th1/Th2 profile. Thus, suggesting that maintaining 25(OH)D >75 nmol/L may contribute to immune function homeostasis and prevent fluctuations that occur with marginal decline in vitamin D status (56). Evidence for vitamin D's effect on inflammatory markers in children remains mixed, with some studies reporting no effects on IL-6 or IL-4 (56, 57) but overall supporting a role for vitamin D in modulating immune responses.

In subset analysis, vitamin D supplementation had no significant effect, compared to placebo, on the bone turnover markers CTx and P1NP, irrespective of baseline vitamin D status, indicating no beneficial effect of supplementation on bone metabolism. However, the interpretation of these results is constrained by the limited sample size. Similarly, a previous meta-analysis found no effect of vitamin D supplementation on bone markers in children albeit the data were too limited to determine if bone markers are a robust vitamin D biomarker in children (58). Nonetheless, these data are consistent with findings from a recent systematic review and individual participant

data meta-analysis of randomized controlled trials, which collectively indicate that vitamin D supplementation is not warranted for improving bone health in children in the absence of severe vitamin D deficiency (59).

Alongside bone turnover markers, non-fasting plasma iPTH was assessed to provide additional insight into calcium-vitamin D homeostasis. In the overall study population, a modest increase in iPTH was observed in both the vitamin D and placebo group albeit non-significant, which was also observed among participants recruited throughout the winter months. Given that baseline iPTH were largely within normal reference range and iPTH was not a primary outcome, these results should be interpreted cautiously. The observed seasonal variation may reflect the influence of limited UVB exposure and other physiological factors on PTH regulation rather than a direct effect of supplementation alone.

Strengths and limitations

Strengths and limitations of the current study are noteworthy. To our knowledge, this is the first randomised controlled trial that examined the effects of 10µg/day vitamin D supplementation in children with study enrolment throughout the year within the province of Ulster. The current results were observed in children who were on average vitamin D sufficient at baseline, which may have influenced the response to supplementation. Future research is required to confirm if this dose/duration of supplementation is adequate for those with lower vitamin D status at baseline. Children from the same family were included in the trial, with siblings assigned to the same treatment group to avoid within-household cross-contamination and reflect real-world supplementation practices. Analyses were conducted at the individual level, and family-level factors, including shared environment and individual behaviours may have

influenced outcomes. The randomised controlled trial design and high compliance (>85%) support the internal validity of the findings. The use of an oral spray formulation, previously shown to be as effective as capsules (60), also represents a practical and well-tolerated method of supplementation that may be particularly attractive for younger children that have difficulty in taking capsules/tablets. The vitamin D_3 spray was a commercially manufactured product with a labelled dose of 10µg per spray. Products were supplied in sealed containers, stored according to manufacturer recommendations and used within their stated expiry date. While no formal laboratory verification of overage was conducted, use of a commercially available product reflects real-world supplementation practices, which is important in this study. Recruitment throughout the year allowed seasonal patterns in vitamin D status to be observed, enhancing the generalisability of the results to populations at a northern latitude. Furthermore, while the current study was only powered to detect changes in vitamin D status as the primary outcome, the simultaneous assessment of multiple secondary outcomes, whilst exploratory in nature, provide an integrative view of vitamin D's effects during childhood. Data reported here on health outcomes will help to inform future research in this area, in which studies may need to be longer in duration to detect changes in outcomes that adapt more slowly, such as grip strength or bone metabolism. Moreover, future research investigating the effect of vitamin D on functional measurements should consider a smaller age range of children to minimise developmental variability. Future research could consider screening at baseline for vitamin D deficiency and/or insufficiency to inform on the effects of supplementation in those children at risk of deficiency. Importantly, it should also be noted that this study was carried out pre and post the COVID-19 pandemic lockdown where media attention

was focused upon vitamin D and may have influenced health knowledge and behaviours.

Conclusion

Current public health strategies for vitamin D supplementation are insufficient to ensure adequacy in all children. Our findings show that 10µg/day vitamin D_3 maintains vitamin D sufficiency for most, but some children remained insufficient, particularly during winter. Supplementation was effective at maintaining vitamin D sufficiency, while small improvements in attention and psychomotor speed were observed highlighting potential broader neurodevelopmental benefits. Diet alone is inadequate, with most children only achieving a fraction of recommended levels. These results highlight the urgent need for targeted research in a population with insufficiency at baseline, over the extended winter months, to determine solely the effect of supplementation upon non-skeletal health outcomes. More broadly, these findings call for consideration of higher or tailored doses for at-risk children in the absence of mandatory fortification to safeguard vitamin D status across child populations.

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EMM, LKP, DJA, RRI, JMM, NG, and PJM designed the research; JEM, LG, COH, MMS, DUG, and ER conducted the research; ER analysed data; ER, EMM, LKP, and

PJM wrote the paper. PJM had primary responsibility for final content. All authors read and approved the final manuscript.

References:

1. Wacker M, Holick MF. Vitamin D—effects on skeletal and extraskeletal health and the need for supplementation. Nutrients. 2013 Jan 10; 5(1):111-148.
2. Julies P, Lynn RM, Pall K, Leoni M, Calder A, Mughal Z, et al. Nutritional rickets under 16 years: UK surveillance results. Archives of Disease in Childhood. 2020 Jun 1;105(6): 587-592.
3. Chanchlani R, Nemer P, Sinha R, Nemer L, Krishnappa V, Sochett E, et al. An overview of rickets in children. Kidney international reports. 2020 Jul 1; 5(7):980-990.
4. Lieben L, Masuyama R, Torrekens S, Van Looveren R, Schrooten J, Baatsen P, et al. Normocalcemia is maintained in mice under conditions of calcium malabsorption by vitamin D–induced inhibition of bone mineralization. The Journal of clinical investigation. 2012 May 1;122(5):1803-1815.
5. Marwaha RK, Garg MK, Mithal A, Gupta S, Shukla M, Chadha A. Effect of vitamin D supplementation on bone turnover markers in children and adolescents from North India. Indian Journal of Endocrinology and Metabolism. 2019 Jan 1;23(1): 27-34.
6. Ward KA, Das G, Roberts SA, Berry JL, Adams JE, Rawer R, et al. A randomized, controlled trial of vitamin D supplementation upon musculoskeletal health in postmenarchal females. The Journal of Clinical Endocrinology & Metabolism. 2010 Oct 1;95(10):4643-4651.
7. Foo LH, Zhang Q, Zhu K, Ma G, Hu X, Greenfield H, et al. Low Vitamin D Status Has an Adverse Influence on Bone Mass, Bone Turnover, and Muscle Strength in Chinese Adolescent Girls. The Journal of nutrition. 2009 May 1;139(5):1002-1007.

8. Hazell TJ, DeGuire JR, Weiler HA. Vitamin D: an overview of its role in skeletal muscle physiology in children and adolescents. Nutrition reviews. 2012 Sep 1;70 (9):520-33.
9. Saggese G, Vierucci F, Boot AM, Czech-Kowalska J, Weber G, Camargo Jr CA, et al. Vitamin D in childhood and adolescence: an expert position statement. European journal of pediatrics. 2015 May;174(5):565-76.
10. Mailhot G, White JH. Vitamin D and immunity in infants and children. Nutrients. 2020 Apr 27;12(5):1233.
11. Mutua AM, Mogire RM, Elliott AM, Williams TN, Webb EL, Abubakar A, et al. Effects of vitamin D deficiency on neurobehavioural outcomes in children: a systematic review. Wellcome open research. 2020 Jun 11;5:28.
12. Scientific Advisory Committee on Nutrition (SACN) (2016). Vitamin D and Health. London, UK: Public Health England
13. National Institute for Health and Care Excellence (NICE) (2022). Vitamin D deficiency in children: Definition. NICE Clinical Knowledge Summaries. Available online: https://cks.nice.org.uk/topics/vitamin-d-deficiency-in-children/background-information/definition/
14. Cashman, KD, Kehoe L, Kearney J, McNulty B, Walton J, Flynn A. Adequacy of calcium and vitamin D nutritional status in a nationally representative sample of Irish teenagers aged 13–18 years. European Journal of Nutrition. 2022 Dec;61(8):4001-4014.
15. Voortman T, van den Hooven EH, Heijboer AC, Hofman A, Jaddoe VW, Franco OH. Vitamin D deficiency in school-age children is associated with sociodemographic and lifestyle factors. The Journal of nutrition. 2015 Apr 1;145(4):791-798.

16. Irish Universities Nutrition Alliance (IUNA) (2019) The National Children's Food Survey II (NCFS II) Summary Report. Cork: IUNA. Available online: https://www.iuna.net/surveyreports

17. NDNS National Diet and Nutrition Survey. (2021). National Diet and Nutrition Survey. GOV.UK. Available online: https://www.gov.uk/government/collections/national-diethttps://www.gov.uk/government/collections/national-diet-and-nutrition-surveyand-nutrition-survey

18. NDNS National Diet and Nutrition Survey. (2025). National Diet and Nutrition Survey. GOV.UK. Available online: https://www.gov.uk/government/statistics/national-diet-and-nutrition-survey-2019-to-2023/national-diet-and-nutrition-survey-2019-to-2023-report

19. Smith TJ, Tripkovic L, Damsgaard CT, Mølgaard C, Ritz C, Wilson-Barnes SL, et al. Estimation of the dietary requirement for vitamin D in adolescents aged 14– 18 y: a dose-response, double-blind, randomized placebo-controlled trial. AJCN. 2016 Nov 1; 104(5):1301-1309.

20. Mortensen C, Damsgaard CT, Hauger H, Ritz C, Lanham-New SA, Smith TJ, et al. Estimation of the dietary requirement for vitamin D in white children aged 4–8 y: a randomized, controlled, dose-response trial. AJCN. 2016 Nov 1;104(5):1310-1317.

21. Öhlund I, Lind T, Hernell O, Silfverdal SA, Karlsland Åkeson P. Increased vitamin D intake differentiated according to skin color is needed to meet requirements in young Swedish children during winter: a double-blind randomized clinical trial. AJCN. 2017 Jul 1;106(1):105–112.

22. World Health Organization (WHO) WHO; Geneva: 2009. WHO AnthroPlus for Personal Computers Manual: Software for Assessing Growth of the World's Children and Adolescents. Available at http://www.who.int/growthref/en/.

23. Najafi F, Darbandi M, Rezaeian S, Hamzeh B, Moradinazar M, Shakiba E, et al. Relative Handgrip Strength and Incidence of Hypertension: A Case-Cohort Study from Ravansar Non-Communicable Diseases Cohort. Journal of Physical Activity and Health. 2022 Oct 1;19(10):666–672.

24. Condon C, Cremin K. Static balance norms in children. Physiotherapy Research International 2014 Mar;19(1):1–7.

25. CANTAB® [Cognitive assessment software] Cambridge Cognition (2015). All rights reserved. http://www.cantab.com.

26. Simpson EE, Maylor EA, Rae G, Meunier N, Andriollo-Sanchez M, Catasta G, et al. Cognitive function in healthy older European adults: the ZENITH study. European Journal of Clinical Nutrition. 2005 Nov;59(2):S26-30.

27. Furlong ON, Parr HJ, Hodge SJ, Slevin MM, Simpson EE, McSorley EM, et al. Consumption of a soy drink has no effect on cognitive function but may alleviate vasomotor symptoms in post-menopausal women, a randomised trial. European Journal of Nutrition. 2020 Mar;59(2):755-66.

28. Gau SF, Huang WL. Rapid visual information processing as a cognitive endophenotype of attention deficit hyperactivity disorder. Psychological Medicine. 2014 Jan;44(2):435-46.

29. Weir RR, Carson, EL, Mulhern MS, Laird E, Healy M, Pourshahidi LK. Validation of a food frequency questionnaire to determine vitamin D intakes using the method of triads. Journal of Human Nutrition and Dietetics. 2016 Apr;29(2):255-261.

30. Aini JN, Poh BK, Chee WSS. Validity of a children's physical activity questionnaire (cPAQ) for the study of bone health. Pediatrics International. 2013 Apr;55(2):223-228.

31. Sliter KA, Sliter MT. The Concise Physical Activity Questionnaire (CPAQ): Its development, validation, and application to firefighter occupational health. International Journal of Stress Management. 2014 Aug;21(3):283–305.

32. Fitzpatrick, T.B. The Validity and Practicality of Sun-Reactive Skin Types I Through VI. Archives of dermatology. 1988 Jun 1;124(6):869–871.

33. Hyppönen E, Vimaleswaran KS, Zhou A. Genetic determinants of 25-hydroxyvitamin D concentrations and their relevance to public health. Nutrients. 2022 Oct 20;14(20): 4408.

34. Kelishadi R, Heidari-Beni M, Akbarian SA, Tajadini MH, Javanmard SH. Genetic variation in cytochrome P450 2R1 and Vitamin D binding protein genes are associated with Vitamin D deficiency in adolescents. International Journal for Vitamin and Nutrition Research. 2019 Nov 15;90(3-4):339-345. https://doi.org/10.1024/0300-9831/a000632

35. Enlund-Cerullo M, Koljonen L, Holmlund-Suila E, Hauta-Alus H, Rosendahl J, Valkama S, et al. Genetic variations of the vitamin D binding protein affects vitamin D status and response to supplementation in infants. The Journal of Clinical Endocrinology & Metabolism. 2019 Nov;104(11):5483-98.

36. Iriani A, Rachman A, Fatina MK, Gemilang RK, Trisnandi A, Muskananfola FV. Vitamin D status, vitamin D receptor, CYP2R1, and CYP24A1 profiles in children. Frontiers in nutrition. 2024 Jun 7;11:1394367.

37. Shraim R, MacDonnchadha C, Vrbanic L, McManus R, Zgaga L. Gene-environment interactions in vitamin D status and sun exposure: A systematic review with recommendations for future research. Nutrients. 2022 Jun 30;14(13):2735.

38. Scully H, Laird E, Healy M, Crowley V, Walsh JB, McCarroll K. Low socioeconomic status predicts vitamin D status in a cross-section of Irish children. Journal of Nutritional Science. 2022 Jan;11:61.

39. Northern Ireland Statistics and Research Agency (NISRA) (2024). Poverty and Income Inequality in Northern Ireland 2023–24. Belfast: NISRA. Available online: https://www.nisra.gov.uk/statistics/household-income/poverty-and-income-inequality-northern-ireland

40. Northern Ireland Statistics and Research Agency (NISRA) (2022). Census 2021: Ethnic Group – Northern Ireland. Belfast: NISRA. Available online: https://www.nisra.gov.uk/statistics/census/census-2021-ethnic-group

41. Lam LF, Lawlis TR. Feeding the brain–The effects of micronutrient interventions on cognitive performance among school-aged children: A systematic review of randomized controlled trials. Clinical Nutrition. 2017 Aug 1;36(4):1007-1014.

42. Eyles DW, Smith S, Kinobe R, Hewison M, McGrath JJ, Distribution of the vitamin D receptor and 1α-hydroxylase in human brain. Journal of chemical neuroanatomy. 2005 Jan 1;29(1):21-30.

43. Eyles D, Burne T, McGrath J. Vitamin D in fetal brain development. In Seminars in cell & developmental biology. 2011 Aug 1;22(6):629-636.

44. Stephenson A, Mamo JC, Takechi R, Hackett MJ, Lam V. Genetic, environmental and biomarker considerations delineating the regulatory effects of vitamin D on central nervous system function. British Journal of Nutrition. 2020 Jan;123(1):41-58.

45. Landel V, Stephan D, Cui X, Eyles D, Feron F. Differential expression of vitamin D-associated enzymes and receptors in brain cell subtypes. The Journal of Steroid Biochemistry and Molecular Biology. 2018 Mar 1;177:129-134.

46. Bahrami A, Rezaeitalab F, Farahmand SK, Khorasani ZM, Arabi SM, Bahrami-Taghanaki H, et al. High-dose vitamin D supplementation and improvement in cognitive abilities, insomnia, and daytime sleepiness in adolescent Girls. Basic and Clinical Neuroscience. 2021 May 1;12(3):339.

47. Wicklow B, Gallo S, Majnemer A, Vanstone C, Comeau K, Jones, G. Impact of vitamin D supplementation on gross motor development of healthy term infants: a randomized dose-response trial. Physical & occupational therapy in pediatrics. 2016 Jul 2;36(3):330-342.

48. Anderson, P. Assessment and development of executive function (EF) during childhood. Child neuropsychology. 2002 Jul 1;8(2):71-82.

49. Romine CB, Reynolds CR. A model of the development of frontal lobe functioning: findings from a meta-analysis. Applied neuropsychology. 2005 Dec 1;12(4):190-201.

50. Ganmaa D, Hemmings S, Jolliffe DA, Buyanjargal U, Garmaa G, Adiya U, et al. Influence of vitamin D supplementation on muscle strength and exercise capacity in Mongolian schoolchildren: secondary outcomes from a randomised controlled trial. BMJ Open Sport & Exercise Medicine. 2024 Sep 26;10(3).

51. Bischoff-Ferrari HA, Dawson-Hughes B, Staehelin HB, Orav JE, Stuck AE, Theiler R, et al. Fall prevention with supplemental and active forms of vitamin D: a meta-analysis of randomised controlled trials. BMJ. 2009;339:843.

52. Buck R, Lambert MI. Getting a Grip on Strength Measurement in Children (6–13 Y): Impact of Typical Error of Measurement. Pediatric Exercise Science. 2022 Feb 2; 34(3):141-147.

53. Carroll A, Onwuneme C, McKenna MJ, Mayne PD, Molloy EJ, Murphy NP. Vitamin D status in Irish children and adolescents: value of fortification and supplementation. Clinical pediatrics. 2014 Dec;53(14):1345-1351.

54. Ní Chaoimh C, McCarthy EK, Hourihane JOB, Kenny LC, Irvine AD, Murray DM, et al. Low vitamin D deficiency in Irish toddlers despite northerly latitude and a high prevalence of inadequate intakes. European journal of nutrition, 2018 Mar;57(2):783-794.

55. Neill HR, Gill CI, McDonald EJ, McRoberts WC, Pourshahidi LK. Vitamin D biofortification of pork may offer a food-based strategy to increase vitamin D intakes in the UK population. Frontiers in nutrition. 2021 Dec 3;8,995.

56. Brett NR, Lavery P, Agellon S, Vanstone CA, Goruk S, Field CJ et al. Vitamin D status and immune health outcomes in a cross-sectional study and a randomized trial of healthy young children. Nutrients. 2018 May 27;10(6):680.

57. Hauger H, Ritz C, Mortensen C, Mølgaard C, Metzdorff SB, Frøkiær H et al. Winter cholecalciferol supplementation at 55°N has little effect on markers of innate immune defense in healthy children aged 4–8 years: a secondary analysis from a randomized controlled trial. EJN. 2019 Jun 1;58(4):1453–1462.

58. Cashman KD, Ritz C, Carlin A, Kennedy, M. Vitamin D biomarkers for Dietary Reference Intake development in children: a systematic review and meta-analysis. AJCN. 2022 Feb 1;115(2):544-558.

59. Wu F, Fuleihan GEH, Cai G, Lamberg-Allardt C, Viljakainen HT, Rahme M, et al. Vitamin D supplementation for improving bone density in vitamin D–deficient children and adolescents: systematic review and individual participant data meta-analysis of randomized controlled trials. AJCN. 2023 Sep 1;118(3):498-506.

60. Todd JJ, McSorley EM, Pourshahidi LK, Madigan SM, Laird E, Healy M, et al. Vitamin D3 supplementation in healthy adults: a comparison between capsule and oral spray solution as a method of delivery in a wintertime, randomised, open-label, cross-over study. BJN. 2016 Oct:116(8):1402-1408.

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Figure 1 CONSORT flow diagram. A total of 229 families were assessed for eligibility with 111 excluded due to not meeting eligibility criteria and not wishing to participate. Remaining children were randomly assigned to receive either a 10 μg/day oral spray or matched placebo.

Table 1. Characteristics of D-VinCHI study participants at baseline (n=118).

Treatment group	**Total (n=118)**	**Placebo (n=59)**	**Vitamin D (n=59)**	
Variable		***Baseline***	***Baseline***	***P***
Age (years)	8.2 ± 1.8	8.4 ± 1.9	8.3 ± 1.9	0.572
Sex				0.581[a]
Male	58 (49.2%)	31 (52.5%)	27 (45.8%)	
Female	60 (50.8%)	28 (47.5%)	32 (54.2%)	
Height (cm)	133.3± 12.7	135.9 ± 11.6	134.3 ± 11.6	0.312
Weight (kg)	31.8 ± 9.2	33.1 ± 9.9	31.3 ± 8.5	0.339
BMI (kg/m^2)	16.8 ± 2.19	17.5 ± 2.9	17.0 ± 2.2	0.570
BMI-for-age z-scores (kg/m^2)[b]	0.47 ± 1.04	0.49 ± 1.04	0.45 ± 1.05	0.819
BMI Categories				<0.001[a]
Thinness	2 (1.7%)	0 (0%)	2 (3.4%)	
Normal	82 (69.5%)	40 (67.8%)	42 (71.2%)	
Risk of overweight	24 (20.3%)	15 (25.4%)	9 (15.3%)	
Risk of obesity	10 (8.5%)	4 (6.8%)	6 (10.2%)	
Biomarkers				
25(OH)D concentration (nmol/L)	64.99 ± 18.37	63.67 ± 19.48	66.31 ± 17.25	0.436
25(OH)D concentration (min-max) (nmol/L)	(25.16 – 114.01)	(25.16 – 106.53)	(29.95 – 114.01)	
Vitamin D status				0.056[a]
Insufficient	30 (25.4%)	20 (33.9%)	10 (17.0%)	
Sufficient	88 (74.6%)	39 (66.1%)	49 (83.0%)	
PTH (ng/L)	31.31 ± 13.28	32.95 ± 15.22	32.95 ± 15.22	0.176
Season				0.937[a]
Spring	16 (13.6%)	9 (15.3%)	7 (11.9%)	
Summer	19 (16.1%)	10 (16.9%)	9 (15.3%)	
Autumn	37 (31.4%)	18 (30.5%)	19 (32.2%)	
Winter	46 (39.0%)	22 (37.3%)	24 (40.7%)	
Dietary vitamin D (µg/day)	3.70 (2.65, 5.20)	3.34 (2.25, 4.80)	4.13 (2.80, 6.10)	0.358

Parent Education[c]				0.110[c]
Secondary education	11 (9.3%)	1 (1.7%)	10 (16.9%)	
FE College	12 (10.1%)	8 (13.6%)	4 (6.8%)	
Undergraduate (BSc)	29 (24.6%)	15 (25.4%)	14 (23.7%)	
Postgraduate (MSc, PhD)	52 (44.1%)	29 (49.1%)	23 (39.0%)	
Other	2 (1.7%)	2 (3.4%)	0 (0%)	

Abbreviations: SD, standard deviation; BMI, body mass index; BMIz, BMI z-score; All anthropometry measured in triplicate, mean obtained. All values are mean ± SD. [a] chi^2 test for categorical variables. P values are significant at $p<0.05$. [b]Calculated using WHO AnthroPlus software. [c]Parent Education data available for n=106 . Vitamin D status total population: insufficient = 30; sufficient = 88; placebo group: insufficient = 20; sufficient = 39; vitamin D group insufficient = 10; sufficient = 49. Vitamin D intakes from food sources only.

Table 2. The effect of vitamin D supplementation on post-intervention biomarker status.

Biomarker	**Placebo (n=59)**		**Vitamin D (n=59)**			***P***
	Baseline	***Week 12***	***Baseline***	***Week 12***	***Between-group differences (95% CI)***	
Total 25(OH)D(nmol/L)	63.67 ± 2.75	57.67 ± 1.97	66.31 ± 2.44	69.35 ± 2.02	11.72 (5.96, 17.75)	**<0.001**
PTH (ng/L)	32.95 ± 15.22	35.81 ± 15.58	29.60 ± 10.73	30.24 ± 14.26	-2.46 (-7.75, 2.82)	0.357
Extended winter	***Placebo (n=34)***		***Vitamin D (n=35)***			
Total 25(OH)D (nmol/L)	55.15 ± 14.66	49.49 ± 14.19	61.07 ± 15.08	66.16 ± 16.60	15.81 (6.94, 24.68)	***<0.001***
PTH (ng/L)	36.18 ± 18.30	39.50 ± 16.34	30.92 ± 9.83	32.26 ± 14.28	-1.98 (-9.9, 5.9)	0.618

Baseline and week 12 data are presented descriptively; all inferential statistics are based on adjusted between-group comparisons. Results are presented as adjusted estimated marginal means with 95% confidence intervals. Between group differences were assessed using ANCOVA with post-intervention as the dependent variable, adjusting for baseline 25(OH)D, BMI, Dietary intake, age, physical activity (sedentary minutes), season, and skin pigment type. P-values represent adjusted between-group treatment effects. Subgroup analyses were analysed using the same ANCOVA framework.

Between group differences were assessed using ANCOVA with post-intervention as the dependent variable, adjusting for baseline PTH, BMI, Dietary intake, age, season, and skin pigment type. P-values represent adjusted between-group treatment effects. Subgroup analyses were analysed using the same ANCOVA framework. Missing data for 25(OH)D and PTH at week 12: Last observation carried forward n= 16 (13.6%) for total study population. Equality of variances across groups were verified using Levene's test ($p>0.05$), supporting the assumption of homogeneity of variances.