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Original Research

Seasonal variations in vitamin D do not change the musculoskeletal health of physically active ambulatory men with cerebral palsy: a longitudinal cross-sectional comparison study



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ABSTRACT

Increased levels of vitamin D in the summer months from natural seasonal variations in sun exposure have been linked to improvements in musculoskeletal health and function in UK populations; however, studies have shown that differences in lifestyles because of disability can inhibit the natural vitamin D increase in these populations. We hypothesized that men with cerebral palsy (CP) will experience smaller increases in 25-hydroxyvitamin D (25(OH)D) from winter to summer and men with CP will not experience any improvements in musculoskeletal health and function during the summer. A longitudinal observational study in 16 ambulant men with CP aged 21.0 ± 1.3 years and 16 healthy, physical activity matched, typically developed controls aged 25.4 ± 2.6 years, completed assessments of serum 25(OH)D and parathyroid hormone during winter and summer. Neuromuscular outcomes included *vastus lateralis* size, knee extensor strength, 10-m sprint, vertical jumps, and grip strength. Bone ultrasounds were performed to obtain radius and tibia T and Z scores. Men with CP and typically developed controls showed a 70.5% and 85.7% increase in serum 25(OH)D from winter to summer months, respectively. Neither group showed seasonal ef-

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; ANOVA, analysis of variance; BM, body mass; CP, cerebral palsy; IPAQ, International Physical Activity Questionnaire; KE iMVC, knee extensor isometric maximal voluntary contraction; PA, physical activity; PTH, parathyroid hormone; TDC, Typically developed controls; TSE, total sun exposure; T_{US}, ultrasound T score; UVB, ultraviolet beta; VL ACSA, *vastus lateralis* anatomical cross-sectional area; Z_{US}, ultrasound Z score.

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fect on neuromuscular outcomes muscle strength, size, vertical jump, or tibia and radius T and Z scores. A seasonal interaction effect was seen in the tibia T and Z scores ($P < .05$). In conclusion, there were similar seasonal increases in 25(OH)D observed in men with CP and typically developed controls, but serum 25(OH)D levels were still considered insufficient to improve bone or neuromuscular outcomes.

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1. Introduction

It is commonly known that the body's primary source of vitamin D comes via ultraviolet radiation beta (UVB) radiation from direct sun exposure on skin to facilitate endogenous vitamin D₃ synthesis, rather than dietary intake [1]. There are, however, many factors that affect cutaneous vitamin D production including, but not limited to, season, time of day, melanin levels, and methods of sun protection such as clothing coverage and sun protection factor creams >8 [2]. A substantial factor affecting vitamin D status in humans is latitude [3]. Individuals living at latitudes of above 35°N experience greater seasonal changes in climate and therefore experience larger fluctuations in sunlight exposure. At these latitudes, UVB radiation from sunlight during winter months (December, January, and February) is negligible and consequently endogenous vitamin D₃ synthesis is severely decreased [3]. Adults in the United Kingdom live at a latitude of ~53°N resulting in susceptibility to vitamin D insufficiency and deficiency (<20 ng/mL⁻¹) during the winter months.

Cannell et al. [2] found that UK adults with an average age of 45 years had significantly higher levels of 25-hydroxyvitamin D (25(OH)D), the major circulating metabolite of vitamin D accumulation in the summer months (June, July, and August) compared with the winter months. This resulted in 25(OH)D peaking in September and steadily declining through the winter months, where many individuals had their 25(OH)D nadir in March. These known seasonal variations in 25(OH)D have been linked to fluctuations in musculoskeletal health and have been shown to affect athletic populations, particularly those who participate in outdoor sports because of higher amounts of sun exposure [4]. Morton et al. [5] found that 20 professional footballers doubled their serum 25(OH)D during the summer months compared with winter months, increasing to levels of 40.9 ng/mL⁻¹ from 20.5 ng/mL⁻¹, which are considered optimal (>32 ng/mL⁻¹) for musculoskeletal health [1]. The increased time spent performing physical activity (PA) outdoors is likely to be a major contributor to the large increase in 25(OH)D seen in Morton et al. [5], when compared with other studies in nonathletic cohorts [2]. Subsequently, it is common to see less pronounced seasonal variations of 25(OH)D in individuals who are predisposed to lower PA levels [6,7], such as the elderly [8] and individuals with disabilities [9].

Even athletes with disabilities spend less time outdoors because of reduced ambulation compared with control populations and are likely to see reduced 25(OH)D levels throughout the year [10]. For example, Flueck et al. [11] assessed 25(OH)D

in 72 Swiss athletes with spinal cord injuries in summer and winter (latitude of 47°N). The athletes with spinal cord injuries had 25(OH)D levels of <32 ng/mL⁻¹, during the winter at 21.7 ng/mL⁻¹, and even during their peak levels in summer with an average of 29.5 ng/mL⁻¹, which are values associated with biochemical abnormalities such as increased parathyroid hormone (PTH), suggesting that bone health may be affected throughout the year [1]. Despite similar latitudes, this increase in 25(OH)D of just 7.8 ng/mL⁻¹ from winter to summer is less than half of the 20.4 ng/mL⁻¹ increase reported by footballers without neuromuscular impairments [5]. This smaller change and prolonged insufficiency in 25(OH)D in athletes with spinal cord injuries may also present in other athletic cohorts with poorer levels of ambulation such as footballers with cerebral palsy (CP) who are possibly less likely to see associated improvements in musculoskeletal health and performance when compared with athletes without disabilities because of seasonal variations.

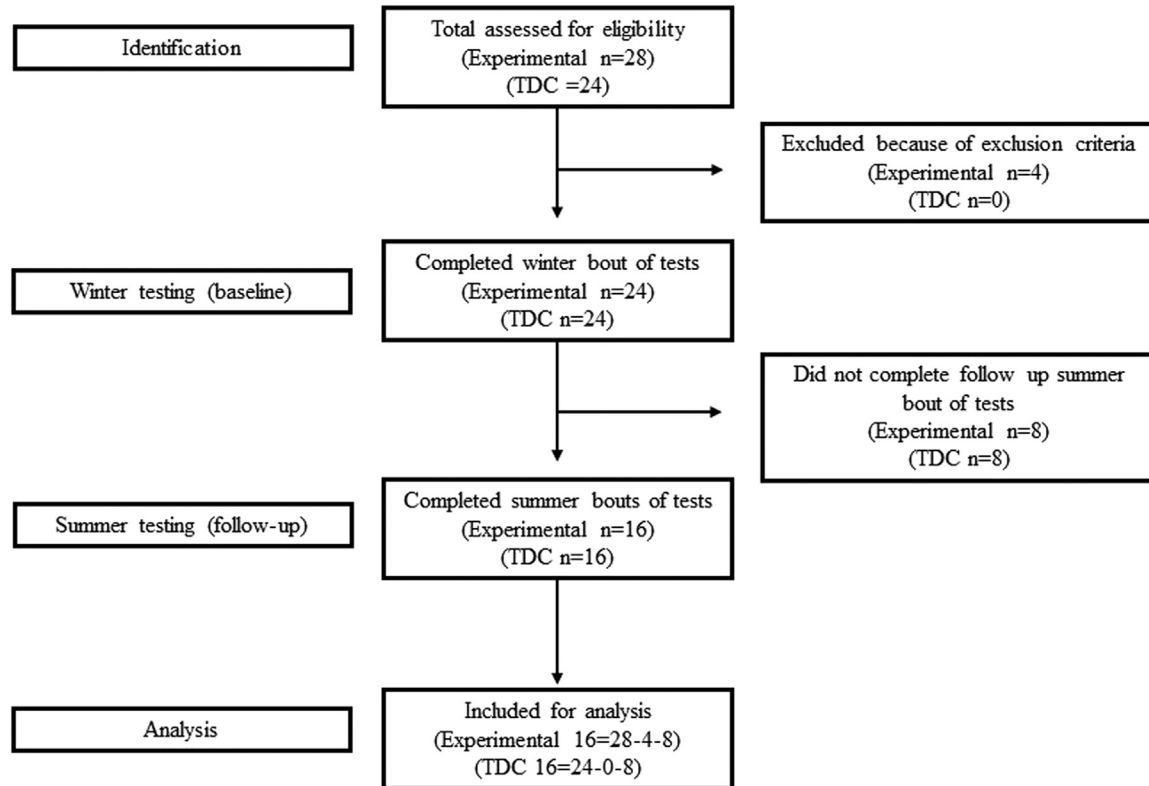
The natural increase in 25(OH)D during the summer months has been linked to improvements in neuromuscular function [12,13] and bone health [14,15]. For example, in professional footballers higher levels of 25(OH)D at >32 ng/mL⁻¹ were associated with stronger peak muscle torque of the knee extensors in the nondominant leg [12] and improved vertical jump and sprint ability [13]. With these known benefits in athletic populations, it is of particular importance that active ambulatory men with CP, who are predisposed to smaller muscle size [16] and muscle weakness [17], consider the potential benefits to their neuromuscular health that increasing levels of 25(OH)D may elicit. Seasonal variations of 25(OH)D can be linked to fluctuations in bone health [18]; higher levels of 25(OH)D corresponded with a decrease in PTH from 25.4 pg/mL⁻¹ to 21.4 pg/mL⁻¹ [14]. The reduction of PTH in the summer months is likely to be beneficial for bone health because persistent elevated levels in PTH can lead to greater bone resorption [19], reducing bone mineral content and increasing risk of fractures. Adults with CP already present a 2.3-fold increased risk of experiencing a fracture compared with typically developed counterparts [20]. Despite the previous observations of lower nadir to peak increases in 25(OH)D in spinal cord-injured athletes [11], there are as yet no data to suggest whether this could be attributed to a lower seasonal variations in musculoskeletal outcomes. If athletes with disabilities do not see natural increases in 25(OH)D during the summer months to elevate them to adequate or optimal status, their physical performance in training and competition may be inhibited [21].

The aims of this study are to therefore investigate if there are seasonal variation in vitamin D in physically active,

Table 1 – Classification and impairment details of participants

		CP Diplegic n = 4	CP Hemiplegic n = 12	CP Total n = 16	TDC n = 16
GMFCS	I	3	-	3	-
	II	1	12	13	-
IFCPF classification (FT)	1	3	-	3	-
	2	1	9	10	-
	3	-	3	3	-
Side measured	Left	3	7	10	4
	Right	1	5	6	12

FT, Classification score; GMFCS, Gross motor function classification score; IFCPF, International federation of cerebral palsy football.

**Fig. 1 – CONSORT style diagram, illustrating the recruitment process for the experimental group (men with CP) and the TDC. CP, cerebral palsy; TDC, typically developed controls.**

ambulatory men with CP on (1) neuromuscular performance outcomes and (2) PTH and bone ultrasound T and Z (T_{US} and Z_{US}) scores. We therefore hypothesized that (1) men with CP will experience smaller increases in 25(OH)D from winter to summer and (2) men with CP will experience improvements in musculoskeletal health and function during the summer.

2. Materials and methods

2.1. Participants and recruitment

Twenty-eight individuals were screened to identify the 16 male, ambulatory men with CP aged 21.0 ± 1.3 years old who participated in this study (Gross Motor Function Classification System I-II; Table 1, Fig. 1). Participants with CP were recruited via The Football Association. All men with CP were tested dur-

ing 2 sessions at Football Association training camps (Fig. 1). To physical activity match typically developed controls (TDCs) without neuromuscular disorders to the experimental group (men with CP), 24 individuals who played football competitively 2 to 3 times per week during a typical football season in the United Kingdom (August-May) were screened and 16 participants aged 25.4 ± 2.6 years were subsequently recruited from Manchester Metropolitan University and tested onsite over 2 visits (Fig. 1). All data for this current study were collected between February 21, 2019, and March 20, 2019, and August 16, 2019, and September 15, 2019. These dates coincide with the data ranges presented by Cannell et al. [2], who found total 25(OH)D levels were most likely to be near their nadir and peak, respectively, in UK populations during these months.

Participants were excluded from the study if they: had not lived in the United Kingdom for the past 3 months, reported having taken vitamin D supplements and used sun beds

within the 3 months before the study, went on regular holidays (defined as a destination between latitudes of 35°N and 35°S with a duration >7 days at a frequency >2 per year), had any long-term illnesses (e.g., chronic kidney disease), or were using any medication that may have affected the metabolism of vitamin D (e.g., corticosteroids) (Fig. 1). To determine if the current sample size used in this study was powered to see a large effect size ($\beta = 0.8$), a post hoc G*power analysis was performed using 25(OH)D observed power (η^2) taken from a repeated-measured analysis of variance (ANOVA). The power analysis provided a suggested sample size of $n = 16$ in each group for 2 repeated measures, suggesting that this study was powered appropriately.

2.2. Study protocol

This protocol is a longitudinal repeated-measures study following the methods described in detail from our previous cross-sectional comparison study in men with CP (Langley et al., 2021). The present study therefore describes in brief the measurements that have been described in detail previously. Participants were assessed for anthropometric measures, PA, sun exposure, 25(OH)D and PTH levels, muscle size, neuromuscular function, and bone ultrasound T_{US} and Z_{US} scores (described in the following section) over 2 occasions. All participants provided written informed consent following approval from the local Ethics Committee, Manchester Metropolitan University, in accordance with the declaration of Helsinki (Ethical approval number 2780).

2.3. Anthropometric measures

Height (m) was measured using a stadiometer (Seca 213, portable stadiometer, Hamburg, Germany) and body mass (BM) (kg) via a set of digital scales (Seca). Percentages of body fat and lean BM were measured using bioelectrical impedance (Omron, body fat monitor, BF306, Kyoto, Japan).

2.4. Physical activity

A demographical description of habitual PA was recorded through the International Physical Activity Questionnaire-long form (IPAQ) during initial testing in the winter months only and presented as IPAQ score. To assess habitual exercise, football training data was logged using 7-day diaries. Data collected included frequency of training ($\text{days}/\text{wk}^{-1}$), duration of each session (minutes), and total time spent training ($\text{minutes}/\text{wk}^{-1}$). Step count was also recorded through mobile phone accelerometers from those participants ($n = 32$) with the iPhone Health Application (Apple Inc. Cupertino, California; version 13), as a daily average from the preceding 3 months.

2.5. Sun exposure measurement

To estimate the level of endogenous skin synthesis of vitamin D_3 from sun exposure, a sun exposure questionnaire was used to assess the frequency, time of day, and amount of time that participants spent exposed to direct sunlight in the spring and summer months. Because UVB exposure is negligible during

the winter months, the sun exposure questionnaire was used only once during the summer months [22].

2.6. Blood sample collection

Venous blood samples were obtained from all 16 TDCs and from 15 of the 16 men with CP for analysis of 25(OH)D and PTH (1 person had a fear of needles). Total 25(OH)D concentrations were measured using enzyme-linked immunosorbent assay (Orgentec Diagnostika GmbH, Germany) and serum PTH was measured using a 90-minute, 1-wash enzyme-linked immunosorbent assay (Abcam, Cambridge, UK).

2.7. Muscle size and neuromuscular function

Images of the *vastus lateralis* (VL) of the impaired leg of those with hemiplegic CP or the most paretic leg of those with diplegic CP, and the dominant leg of TDC, were obtained using B-mode ultrasonography with a 7.5-MHz linear array probe (MyLabGamma Portable Ultrasound, Esaote Biomedica, Genoa, Italy) to estimate the anatomical cross sectional area (ACSA). Vertical jump height was measured using a jump mat (Probotics Inc., Huntsville, Alabama) in 2 conditions: with and without arm swing.

Maximum sprint speed was assessed over 10 m. Two sets of sensory timing gates (Brower timing system, Wireless Sprint System 2007, Brower, USA) were set up 2 m apart at either end of a 10-m distance. Participants performed 2 sprints with a standing start 0.60 m behind the first set of gates. Grip strength was assessed using an adjustable handgrip dynamometer (Jamar plus, Sammons Preston Rolyon, Bolingbrook, Illinois). Two maximal grip efforts were performed; the highest value was recorded.

To record knee extensor isometric maximal voluntary contraction (KE iMVC), participants were seated on a custom-made isometric chair fitted with a portable load cell (Manchester Metropolitan University, UK). Their arms were across their chest and the load cell attached around the dominant kicking leg (or the most paretic side in the CP group); their knees were at 90° flexion. Participants were instructed to extend their fastened leg maximally while verbal encouragement was given during the measurement. Two trials were performed, and the highest force produced was recorded. KE iMVC values were also presented relative to VL ACSA (KE iMVC/ACSA) and BM (KE iMVC/BM).

2.8. Bone ultrasound

Ultrasonic bone densitometry (Sunlight, BeamMed Ltd., Israel) of the distal radius (~5 cm from the condyle) and the distal tibia (~12 cm from the condyle) was performed to obtain T_{US} and Z_{US} scores. The measurements for each procedure were repeated 3 to 5 times depending on scan quality. After the signal was digitized and stored, the data were transferred to a computer for automated analysis and a T_{US} and Z_{US} score was provided.

2.9. Statistical analyses

Statistics were performed using SPSS statistics (SPSS Statistics 25, IBM Chicago, Illinois). Data were assessed for normal

Table 2 – Anthropometric outcomes from men with cerebral palsy (CP) and typically developed controls (TDCs)

	CP			TDC		
	Winter	Summer	% change	Winter	Summer	% change
Age (y)	21.0 ± 1.3 ^a	21.5 ± 1.3 ^b	2.38	25.4 ± 2.6	25.9 ± 2.6	1.97
Height (m)	1.76 ± 0.06	1.76 ± 0.06	0.00	1.79 ± 0.07	1.79 ± 0.06	0.00
Body mass (kg)	68.8 ± 6.7 ^a	68.1 ± 6.1 ^b	-1.02	80.2 ± 11.2	79.8 ± 10.8	-0.50

Data are presented as means ± SD and % change from winter to summer.
^a P < .05 between in CP and TDC during Winter.
^b P < .05 between in CP and TDC during Summer.

distribution using a Shapiro-Wilks test ($P > .05$). Homogeneity of variance was assessed using the Levene test. Where heterogeneity of variance was observed ($P > .05$ in the Levene test), the P value for “equal variances not assumed” is reported. Group differences for age, height, and BM was assessed via ANOVA and for total sun exposure (TSE) and IPAQ independent t tests were used. Whereas vertical jump with no arms, vertical jump with arms, 10-m sprint, grip strength, tibia T_{US} and Z_{US} scores, VL ACSA and KE iMVC, KE iMVC/BM, KE iMVC/lean body mass, and KE iMVC/VL ACSA were assessed by 2-way repeated-measure ANOVA. Analysis of covariance was performed to determine any relationships that exists between 25(OH)D, TSE, and musculoskeletal health outcomes. All data are presented as mean ± SD unless otherwise stated and the CI was set at 95% with alpha set at ≤ 0.05 .

3. Results

3.1. Descriptions of men with CP and TDC

Men with CP were 4.4 years younger than the TDC ($P < .001$; Table 2). During the winter and summer, men with CP had 16.6% and 17.2% lower BM (both $P < .05$). There was no difference in height between men with CP and the TDC ($P = .195$; Table 2).

3.2. Physical activity

IPAQ score, PA frequency, PA duration, and step count were not different between men with CP and the TDC when measured during winter ($P > .05$). PA frequency, PA duration, and step count were not different between men with CP and the TDC when measured during summer ($P > .05$). There was no seasonal change PA frequency, PA duration, and step count between groups (all $P > .05$; Table 3).

3.3. Vitamin D

There was no difference in 25(OH)D between CP and TDC during winter and summer ($P = .137$). 25(OH)D ($P < .001$) increased from winter to summer in men with CP by 70.5% ($P = .003$), and 85.7% in the TDC ($P < .001$; Fig. 2). There was no interaction effect on 25(OH)D ($P = .169$).

During the winter months, the men with CP were classed as follows based on serum 25(OH)D levels: 0/15 (0%) were optimal, 4/15 (26.7%) were adequate, 6/15 (40%) were insufficient,

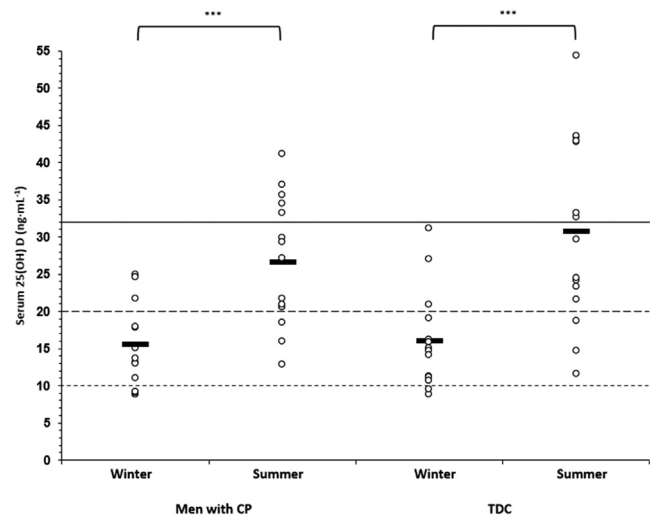


Fig. 2 – Individual plots (o) and means (-) of seasonal variations in serum 25(OH)D in men with CP and TDCs in the winter and summer months. Both groups show an increase in 25(OH)D from winter to summer, where * denote a significant difference between seasons, $P < .001$. There were no differences in winter or summer 25(OH)D values when comparing men with CP and the TDCs. Lower solid black line denotes serum 25(OH)D adequacy threshold (20–32 ng/mL⁻¹) and black dashed line (– –) denotes serum 25(OH)D insufficiency threshold (10–20 ng/mL⁻¹) and lower dashed line (– –) denotes serum 25(OH)D deficiency (<10 ng/mL⁻¹). 25(OH)D, 25-hydroxyvitamin D; CP, cerebral palsy; TDC, typically developed controls.**

and 5/15 (33.3%) were deficient. During the winter in the TDC, 0/15 (0%) were optimal, 3/15 (20%) were adequate, 10/15 (66.7%) were insufficient, and 2/15 (13.3%) were deficient.

Based on serum 25(OH)D levels during the summer of the men with CP, 5/15 (33.3%) were optimal, 7/15 (47.7%) were adequate, 3/15 (20%) were insufficient, and 0/15 (0%) were deficient. During the summer months in TDC, 7/15 (47.7%) were optimal, 5/15 (33.3%) were adequate, 3/15 (20%) were insufficient, and 0/15 (0%) were deficient.

Men with CP reported 7% less TSE than the TDC during the summer ($P < .01$). There was an association between TSE and 25(OH) D in both CP ($r = 0.465$, $P = .041$) and the TDC ($r = 0.545$, $P = .015$).

Table 3 – Physical activity and TSE for cerebral palsy (CP) and typically developed controls (TDC) during the winter and summer, presented as means \pm SD and % change between winter and summer

	CP			TDC		
	Winter	Summer	% change	Winter	Summer	% change
TSE	-	27.4 \pm 2.2	-	-	29.4 \pm 1.3	-
IPAQ score	8737 \pm 3975	-	-	7128 \pm 4685	-	-
PA frequency (days/wk ⁻¹)	4.0 \pm 1.9	3.4 \pm 2.0	-15.00	3.9 \pm 1.6	4.1 \pm 1.3	4.60
PA duration (minutes/session ⁻¹)	70.1 \pm 32.2	74.4 \pm 29.1	6.13	82.7 \pm 19.4	80.6 \pm 17.4	-2.54
Step count (steps/d ⁻¹)	10,696 \pm 3987	10,010 \pm 2667	-6.41	8932 \pm 3368	9408 \pm 4183	5.33

IPAQ, International Physical Activity Questionnaire; PA, physical activity; TSE, total sun exposure.

^aInteraction effect.

^bDifference from winter in each group, $P < .05$.

^cDifference from TDC for corresponding season $P < .05$. There were no significant differences shown in this table.

Table 4 – Neuromuscular outcomes means \pm SD and percent change between winter and summer in men with cerebral palsy (CP) and typically developed controls (TDC)

	CP			TDC		
	Winter	Summer	% change	Winter	Summer	% change
VL ACSA (cm ²)	27.1 \pm 3.3 ^b	27.9 \pm 5.5 ^b	-2.5	35.3 \pm 9.0	34.6 \pm 8.51	-2.0
KE iMVC (N)	400 \pm 95 ^b	488 \pm 125 ^b	22.0	625 \pm 140	622 \pm 139	-0.5
KE iMVC/VL ACSA (N/cm ²)	15.2 \pm 3.4	18.2 \pm 4.8	19.7	18.1 \pm 3.1	18.9 \pm 5.15	4.4
KE iMVC/BM (N/kg ⁻¹)	5.87 \pm 1.34 ^b	7.32 \pm 2.11 ^b	24.7	7.88 \pm 1.68	7.88 \pm 1.71	0.0
Vertical jump without arm swing (m)	0.41 \pm 0.07 ^b	0.42 \pm 0.09 ^b	2.4	0.52 \pm 0.09	0.51 \pm 0.08	-1.9
Vertical jump with arm swing (m)	0.46 \pm 0.06 ^b	0.49 \pm 0.09 ^b	6.5	0.57 \pm 0.08	0.56 \pm 0.08	-1.8
10-m sprint (s)	1.91 \pm 0.15 ^{a,b}	2.00 \pm 0.18 ^b	4.7	1.82 \pm 0.14	1.83 \pm 0.14	0.6
Grip strength (kg)	40.7 \pm 12.5	41.2 \pm 12.6	1.2	56.8 \pm 15.0	53.4 \pm 14.4	-6.0

BM, body mass; KE iMVC, knee extensor isometric maximal voluntary contraction; VL ACSA, Vastus Lateralis anatomical cross-sectional area.

^a Denotes difference from winter in each group, $P < .05$.

^b Difference from TDC for corresponding season, $P < .05$.

3.4. Neuromuscular performance outcomes

Compared with TDC, men with CP had 27% and 28% smaller VL ACSA in the winter and summer months, respectively ($P < .01$; Table 4), and 56.3% and 27.5% weaker KE iMVC in the winter and summer months, respectively ($P < .001$; Table 4). Men with CP had 34.2% weaker KE iMVC/BM compared with the TDC during the winter ($P = .012$; Table 4) and there was no difference in KE iMVC/BM between men with CP or the TDC in the summer months. There was no group difference in KE iMVC/VL ACSA ($P = .166$; Table 4). Men with CP had 29.2% and 21.4% shorter vertical jump no arm swing ($P = .001$), and 26.8% and 14.3% shorter vertical jump with arm swing compared with the TDC in the winter and summer months, respectively ($P = .002$). Men with CP had an 8.9% slower 10-m sprint time in the summer compared with the TDC ($P = .021$). There was no group difference in grip strength in winter or summer ($P = .072$).

There was a seasonal effect for 10-m sprint times ($P = .042$) in men with CP, where 10-m sprint times were 4.71% slower in the summer compared with winter ($P = .042$; Table 4) but there was no seasonal effect on 10-m sprints in the TDC ($P > .05$). There was no seasonal effect on VL ACSA ($P = .979$), KE iMVC ($P = .297$), KE iMVC/VL ACSA ($P = .068$), or KE iMVC/BM ($P = .170$). There was also no season effect on vertical jump without arm swing ($P = .966$), vertical jump with arm swing ($P = .288$), or grip strength ($P = .418$).

There was no interaction effect on VL ACSA ($P = .280$), KE iMVC ($P = .317$), KE iMVC/VL ACSA ($P = .444$), or KE iMVC/BM ($P = .234$). There were no interaction effects for vertical jump without arm swing ($P = .769$), vertical jump with arm swing ($P = .141$), 10-m sprint ($P = .136$), or grip strength ($P = .962$). There were no correlations between 25(OH)D and VL ACSA, KE iMVC, KE iMVC/VL ACSA, KE iMVC/BM, vertical jump without arm swing, vertical jump with arm swing, 10-m sprint, or grip strength (all $P > .05$).

3.5. Bones

There was no difference between men with CP and the TDC for tibia T_{US} score ($P = .484$) or tibia Z_{US} score in the winter ($P = .548$). There was no difference between men with CP and the TDC for tibia T_{US} score ($P = .444$) or tibia Z_{US} score in the summer ($P = .445$). Men with CP had lower radius T_{US} and Z_{US} scores than the TDC in winter and summer months (both $P < .001$; Table 5). There was no difference in PTH ($P = .056$) for winter or summer in the CP and TDC groups.

There was no seasonal effect for tibia or radius T_{US} score ($P = .309$ and $P = .141$, respectively) or for tibia or radius Z_{US} score ($P = .351$ and $P = .115$, respectively) in men with CP or the TDC. There was a seasonal effect on PTH with PTH decreasing from winter to summer by 47.8% in men with CP and by 34.4% in the TDC ($P < .001$).

Table 5 – Bone measures in men with CP during and TDCs during winter and summer time points within group and between group during the winter and summer months; mean \pm SD

	CP		TDC	
	Winter	Summer	Winter	Summer
Tibia T _{US} score	0.43 \pm 1.79	0.18 \pm 1.2 ^a	0.24 \pm 0.80	0.90 \pm 0.79
Tibia Z _{US} score	0.47 \pm 1.77	0.20 \pm 1.2 ^a	0.24 \pm 0.81	0.88 \pm 0.83
Radius T _{US} score	-1.30 \pm 1.06 ^b	-0.91 \pm 1.5 ^b	0.48 \pm 0.86	0.83 \pm 1.33
Radius Z _{US} score	-0.94 \pm 1.03 ^b	-0.54 \pm 1.5 ^b	0.74 \pm 0.92	1.19 \pm 1.37
PTH (ng/dL ⁻¹)	33.3 \pm 12.4 ^c	17.4 \pm 0.6	25.6 \pm 9.9 ^c	16.8 \pm 1.16

PTH, parathyroid hormone; T_{US}, ultrasound T score; Z_{US}, ultrasound Z score.

^a Denotes an interaction effect.

^b Denotes a difference from TDC for corresponding season, $P < .05$.

^c Denotes a difference from winter in each group, $P < .05$.

There was an interaction effect for tibia T_{US} scores ($P = .029$). Planned contrasts showed that men with CP had similar tibia T_{US} scores to the TDC during the winter months ($P > .05$) but lower scores in the summer months ($P < .05$; Table 5). There was an interaction effect ($P = .026$) in which men with CP showed similar tibia Z_{US} scores to the TDC during the winter months ($P > .05$) but lower tibia Z_{US} scores in the summer months ($P < .05$). There was no interaction effect for radius T_{US} score ($P = .950$) or for radius Z_{US} scores ($P = .916$). There were no interaction effects for PTH ($P = .088$). There was no correlation between 25(OH)D and tibia T_{US} or Z_{US} scores when controlling for TSE in the summer months for both groups (all $P > .05$). There was no correlation between 25(OH)D and radius T_{US} or Z_{US} scores when controlling for TSE in the summer months for both groups (all $P > .05$). A partial correlation showed that 25(OH)D in men with CP was not associated with any of the outcome measured when controlling for TSE in the summer months in either group ($r = 0.348$ - 0.449 , $P > .05$).

4. Discussion

The main findings of this study show that vitamin D increased by a similar amount from winter to summer in men with CP and TDC, leading to the rejection of hypothesis 1. Of the neuromuscular and skeletal outcomes, neuromuscular performance outcomes showed no positive seasonal effect of 25(OH)D in men with CP, leading to the rejection of hypothesis 2.

The present study found that 25(OH)D increased in the summer compared with the winter in men with CP and the TDC and was consistent with other studies performed at similar latitudes [2]. Our findings showed a 70.5% and 85.7% increase of 25(OH)D in men with CP and the TDC, respectively. The magnitude of 25(OH)D increase from winter to summer and absolute levels of 25(OH)D in both groups were similar to the 25(OH)D levels of athletes from winter to summer months living in Japan at latitudes of 36.2°N [23]. The similar increase in 25(OH)D reported in this study is comparable to that from the more equatorial Japanese athletes, where a greater increase would be expected [24], and is likely due to the Japanese athletes being from predominantly indoor sports.

The data presented in this current study are consistent with findings in KE strength, jump height, and grips strength

outcomes from typically developed athletes in Wilson-Barnes et al. [25]. In this current study, there were no effects of 25(OH)D seasonal variation on KE iMVC, KE iMVC/BM, KE iMVC/ACSA, vertical jump without arm swing, vertical jump with arm swing, and grip strength observed. It has been identified that 25(OH)D should be increased above the “optimal” threshold (>32 ng/mL⁻¹, reached by 11 of 28 participants) to elicit significant genomic and nongenomic effects such as increased myocyte proliferation or differentiation [1,26] and greater recruitment of type IIa muscle fibers [27] in skeletal muscle of physically active populations [28].

In men with CP and the TDC in the present study and TDC university athletes in Wilson-Barnes et al. [25], despite a 25(OH)D increase in the summer (40% [25]), 25(OH)D levels still fell below 32 ng/mL⁻¹, and no seasonal effect on KE strength, jump height, or grip strength were reported in either study. Similarly, where supplementation with vitamin D results in 25(OH)D <32 ng/mL⁻¹, 1 repetition maximum of squat and bench press, vertical jump, and 20-m sprint is unchanged [29]. However, supplementation of D₃ that raises 25(OH)D levels of 14 male footballers to >32 ng/mL⁻¹ can improve 10-m sprint and vertical jump in physically active adults. Because both groups in the present study had average 25(OH)D levels below 32 ng/mL⁻¹, it is unlikely that they experienced any significant increase in muscle size, strength, or vertical jump height. Therefore, given that physically active men with CP experience low vitamin D throughout the year, a supplementation strategy should be developed to raise 25(OH)D to optimal levels to increase their neuromuscular function and physical performance.

The lack of a seasonal variation in muscle function, likely resulting from 25(OH)D not reaching threshold levels, is not reflected by a decrease in CP sprint times in the summer season. The decreased 10-m sprint time in men with CP cannot be explained by other seasonal changes measured in this study, such as BM, PA frequency, PA durations, or any other neuromuscular outcomes. It is likely that knee extension strength alone is not enough to predict 10-m sprint ability in men with CP because there was no associations between KE iMVC and 10-m sprint in men with CP in Langley et al. [30]. Nesser et al. [31] found that 40-m sprint time can be predicted by the hip extensor and knee flexor strength in young male TDC; however, there are no equivalent investigations assessing different lower limb muscle groups to determine sprint ability in CP.

It is likely that the decrease in 10-m sprint time is due to an unmeasured change in the strength of other lower limb muscle groups in this current study. These findings suggest that to elicit any potential seasonal effect to improve neuromuscular outcomes and decrease the level of exhibited disability, men with CP and the TDC should aim to increase their levels of 25(OH)D to above the “optimal” threshold, either by method of supplementation or increased time spent outdoors.

The data from this study show a disparity between the upper and lower limbs, whereas a seasonal interaction was seen only in the tibia in both men with CP and the TDC. There was a contrasting direction in tibia T_{US} and Z_{US} scores, in which men with CP saw a decrease and the TDC saw increases. The increase seen in TDC tibia is consistent with other studies in healthy young adults [32,33]. In the present study, no association was seen between PTH and tibia or radius T_{US} and Z_{US} scores; this likely reflects the multifaceted determinants of bone, including markers such as osteocalcin [34]. The decrease in tibia T_{US} and Z_{US} score in men with CP does not fit with other existing literature, although no other research on seasonal variations in physically active CP populations currently exist. It is plausible that contrasting directions of tibia and radius T_{US} and Z_{US} scores in men with CP and the TDC is likely due to the tibia experiencing inconsistent loading patterns based on varying activity levels [35]. In the present study, PA frequency, average time, and total time spent performing PA did not change throughout the seasons in either group. It is possible, however, that a higher level of football match play in the winter could offer some bone health benefits to the tibia in winter, which are attenuated in the summer when the football season has finished [36]. Because of the variance in these loading patterns in the lower limbs throughout the year, measures of seasonal variations in bone of physically active men with CP may better be reflected in the radius, which in the present study showed no change in either group, consistent with previous data on minimal seasonal variation in upper limb bone health from the TDC [37]. Regardless of the lack of seasonal change in radius T_{US} and Z_{US} scores in men with CP from winter to summer in this current study, it was identified that in men with CP, 25(OH)D was below $<32 \text{ ng/mL}^{-1}$ throughout the year. Prolonged 25(OH)D below 32 ng/mL^{-1} leads to reduced bone health in the elderly because of elevated biological markers such as PTH [38]. Therefore, it may be pertinent to implement a 25(OH)D supplementation protocol to offset potential reductions in bone health that may not have yet manifested in younger men with CP.

A limitation of this study is that the seasonal increase in training during the winter seen in the men with CP may mask the seasonal decline in musculoskeletal health from low vitamin D. During the winter months, despite not showing a difference in physical activity between time points (Table 5), it would be expected that musculoskeletal performance based on vitamin D being at its nadir should also be at its poorest. However, higher intensity football training might attenuate some of the impact of low vitamin D in the present participants, as is observed in professional players with increased leg muscle mass compared with the off season [39]. This yet may highlight the importance of the engagement in sport and PA by individuals with physical disabilities as a way to preserve muscle function.

5. Conclusions

The aim of this investigation was to observe the effect on seasonal variation in vitamin D in physically active, ambulatory men with CP on neuromuscular performance outcomes, PTH, and bone ultrasound T_{US} and Z_{US} scores. This study has shown that men with CP showed a similar magnitude of 25(OH)D increase between winter and summer to the TDC, thus rejecting hypothesis 1. Men with CP showed low levels of vitamin D throughout the entirety of the year, likely contributing to the lack of association between 25(OH)D levels and any improvements in musculoskeletal outcomes and thus rejecting hypothesis 2. In contrast to men with CP, the TDC showed seasonal improvements in tibia T_{US} and Z_{US} scores. Future research should investigate whether increases in vitamin D, to adequate and above levels, in men with CP elicit potential improvements in musculoskeletal health and performance.

Declaration of Competing Interest

The authors have nothing to declare.

CRediT authorship contribution statement

Christina Kate Langley: Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft, Project administration. **Gladys Leopoldine Onambélé-Pearson:** Writing – review & editing, Supervision. **David Thomas Sims:** Writing – review & editing, Supervision. **Ayser Hussain:** Conceptualization, Supervision. **Reece Kumar Mohindra:** Investigation. **Bethany Louise Kershaw:** Investigation. **Christopher Ian Morse:** Conceptualization, Methodology, Writing – review & editing, Supervision, Project administration.

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