Musculoskeletal Health in Active Ambulatory Men with Cerebral Palsy and the Impact of Vitamin D

Christina Kate Langley (13141478@stu.mmu.ac.uk)
Manchester Metropolitan University Faculty of Science and Engineering https://orcid.org/0000-0002-3572-467X

Gladys Leopoldine Onambélé-Pearson
Manchester Metropolitan University - All Saints Campus: Manchester Metropolitan University

David Thomas Sims
Manchester Metropolitan University - All Saints Campus: Manchester Metropolitan University

Ayser Hussain
Manchester Metropolitan University - All Saints Campus: Manchester Metropolitan University

Aidan John Buffey
University of Limerick

Holly Leigh Bardwell
Manchester Metropolitan University - All Saints Campus: Manchester Metropolitan University

Christopher Ian Morse
Manchester Metropolitan University - All Saints Campus: Manchester Metropolitan University

Research

Keywords: Cerebral Palsy, Vitamin D, Strength, Bone, Sun Exposure.

Posted Date: June 17th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-603173/v1

Abstract

Purpose: 1) To determine the contribution of diet, time spent outdoors and habitual physical activity (PA) on vitamin D status in men with cerebral palsy (CP) compared to age matched controls (TDC) without neurological impairment. 2) To determine the role of vitamin D on musculoskeletal health, morphology and function in men with CP compared to TDC.

Materials and methods: A cross-sectional comparison study where, twenty-four active, ambulant men with CP aged 21.0±1.4 years (Gross Motor Function Classification Score I-II) and 24 healthy TDC aged 25.3±3.1 years completed *in vivo* assessment of musculoskeletal health, including: *Vastus Lateralis* anatomical cross-sectional area (VL ACSA), isometric knee extension maximal voluntary contraction (KE iMVC), 10m-sprint, vertical jumps (VJ), radius and tibia bones T and Z scores. Assessments of vitamin D status through venous samples of serum 25-hydroxyvitamin D (25(OH)D) and parathyroid hormone, dietary vitamin D intake from food diary and total sun exposure via questionnaire were also taken.

Results: Men with CP had 40.5% weaker KE iMVC, 23.7% smaller VL ACSA, 22.2% lower VJ, 14.6% lower KE iMVC/VL ACSA ratio, 22.4% lower KE iMVC/body mass (BM) ratio and 25.1% lower KE iMVC/lean body mass (LBM) ratio (all $p<0.05$). Radius T and Z scores were 1.75 and 1.57 standard deviations lower than TDC, respectively ($p<0.05$), whereas neither tibia T nor Z scores showed any difference compared to TDC ($p>0.05$). 25(OH)D was not different between groups, and 90.9% of men with CP and 91.7% of TDC had low 25(OH)D levels when compared to current UK recommendations. 25(OH)D was positively associated with KE iMVC/LBM ratio in men with CP ($r = 0.500$, $p=0.020$), but not in TDC ($r = 0.281$, $p=0.104$).

Conclusion: Musculoskeletal outcomes in men with CP were lower than TDC and despite there being no difference in levels of 25(OH)D between the groups, 25 (OH)D was associated with strength (KE iMVC/LBM) in the CP group, but not TDC. The findings suggest a greater sensitivity to low vitamin D in men with CP with regards to bone and muscle content and functional outcomes.

Introduction

Circulating vitamin D levels *in vivo* can account, in part, for observed variance of musculoskeletal outcome measures, with known implications for musculoskeletal impairments resulting from low vitamin D levels in otherwise healthy adults [1]. Predominant reasons for reduced vitamin D levels are due to insufficient dietary intake and restricted outdoor exposure to sun light [2]. Ultraviolet beta (UV b) radiation from sun exposure represents the primary source of endogenous vitamin D (serum 25-hydroxyvitamin D, 25(OH)D) and as a result, endogenous vitamin D_3 synthesis is severely decreased at latitudes over 35ºN during winter months (the UK is ~ 53ºN). Accordingly, individuals at these latitudes are susceptible to vitamin D deficiency [1] with 74.5% of the UK population exhibiting low vitamin D levels, comprising of 33.7% who are vitamin D insufficient, (25(OH)D levels of 30 – 20 $ng.ml^{-1}$) and 40.8% who are vitamin D deficient (25(OH)D < 20 $ng.ml^{-1}$).

The current recommendations for dietary intake of vitamin D for UK based 19–50 year olds is 400–600 IU per day to benefit musculoskeletal function [3]. Within the general UK population, dietary intakes of vitamin D range from 168–291 IU/d (3.5–6.39 µg/d) in men and 140–255 IU/d (4.2–7.28µg/d) in women, contributing to the high levels of the UK population who are vitamin D deficient [2]. Only a few foods, such as oily fish, naturally contain vitamin D and these tend to be relatively low dosages [1] meaning it is difficult to meet these recommendations through diet alone. As approximately 80% of circulating 25(OH)D comes from UV b radiation from spending time outdoors in direct sunlight [1], it is important that individuals perform outdoor activities in order to accumulate vitamin D in replacement of the lack of endogenous vitamin D. Measures of both dietary vitamin D analysis [4] and outdoor sunlight exposure are heavily reported with typically developed, able-bodied adults and children (hereafter termed TDC, typically developed controls) [5, 6]. Interestingly, individuals with disabilities, such as cerebral palsy and spinal cord injuries appear to have poorer micronutrient compared to age-matched TDCs [7, 8]. Furthermore, although not directly related to outdoor activity, physical activity (PA) is 48 mins lower and sedentary behaviour 80 minutes higher per day in men with CP with Gross Motor Function Classification Systems (GMFCS) of I-II compared to TDC [9], suggesting a lower level of UV b exposure in individuals with CP. Despite physically active individuals reporting higher sun exposure than more sedentary individuals, there are many studies that report low 25(OH) D in athletic populations with and without disabilities. For example, Morton et al. [10] found that elite English Premier League footballers (living at a latitude of 53 ºN) had low vitamin D (mean ± standard deviation (SD), 20.5 ± 7.63 ng•ml^{-1}) during the winter months, while Flueck et al. [11] showed 83.7% of 72 Swiss elite wheelchair athletes (living at a latitude of 47ºN) were vitamin D insufficient.

The presence of low vitamin D levels in physically impaired groups, such as those with CP, is a particular concern given their diminished musculoskeletal health measures, such as muscle force production [12, 13]. Lower levels of 25(OH)D are associated with lower musculoskeletal health outcomes including, but not limited to: lower strength [14], muscle size [15], reduced bone mass [16], increased bone turnover marker parathyroid hormone (PTH) [15] and higher levels of adiposity in TDC [17]. Individuals with CP are predisposed to reduced muscle size, strength and bone mass primarily due to reduced mechanical loading from altered gait patterns as a consequence of increased muscle tone and lower range of motion [18, 19]. These already impaired musculoskeletal outcomes are compromised further in CP through modifiable risk factors including poor dietary micronutrient intake [20], reduced sun exposure [21], low PA levels [22] and as a side effect of anticonvulsant medications [23]. As described, the observed lower vitamin D levels in groups with CP could be a risk factor to exacerbated musculoskeletal health, but there appears to be no data pertaining to the measurements of vitamin D in PA groups with CP nor its effects on musculoskeletal measures.

Therefore, the aim of this present study is to increase our understating of the role of vitamin D on musculoskeletal health in men with CP and compare it to TDC. The objectives of this study were to; 1) To determine the impact of diet, time spent outdoors, PA and vitamin D status in men with CP compared to

TDC without neurological disabilities. 2) To determine the role of vitamin D on musculoskeletal health in men with CP compared to TDC. The hypotheses of this study were 1) Men with CP will have lower levels of vitamin D compared to TDC. 2) Vitamin D will lead to reduced musculoskeletal health in men with CP.

Materials And Methods

Study protocol

This study followed 'Strengthening the Reporting of Observational Studies in Epidemiology' (STROBE) reporting guidelines (see supplementary file). The study took place in the UK at Manchester and Derby. A total of 48 volunteers participated in this study consisting of 24 ambulatory males with CP, and 24 male TDC (Table 1). All participants provided written informed consent, following approval from the local Ethics Committee. Participants were assessed for anthropometric measures, muscle size, bone health, muscle function, dietary vitamin D, total sun exposure and PA (described below). All participants provided venous blood samples for subsequent total serum 25(OH)D and serum PTH analysis.

All participants were aged 18–50 years old and UK residents having lived above 35ºN for at least three months prior to this study. The CP group were ambulatory male footballers with cerebral palsy, playing in the development or elite disability football teams from The Football Association, playing football more than twice per week. Typically developed control participants were free of any neuromuscular disorder and played football at least twice per week. Participants were excluded from the study if they: used sun beds more than once per week, went on regular holidays (defined as a destination between latitudes of 35ºN and 35°S with a duration > 7 days at a frequency > 2 per year), had any illnesses (e.g. chronic kidney disease), or were known to be using any medication that may have affect the metabolism of vitamin D (e.g. corticosteroids).

Participants and recruitment

Men with CP (diplegic = 6, hemiplegic = 18) were recruited via The Football Association and were classified as FT3 (n = 3), FT2 (n = 17) and FT1 (n = 4) with GMFCS between I (n = 18) and II (n = 6, Table 1). All participants were tested on a single testing session with the same equipment. Testing commenced on the 14th February 2019 and ended on the 13th March 2019, for all groups as total 25(OH)D levels were most likely to be near their nadir in the UK population based at a latitude of approximately 53°N [10, 24].

Table 1
Classification and impairment details of participants

		CP Diplegic n = 6	CP Hemiplegic n = 18	CP Total n = 24	TDC n = 24
GMFCS	I	-	18	18	-
	II	6	-	6	-
IFCPF classification (FT)	1	4	-	4	-
	2	2	15	17	-
	3	-	3	3	-
Side measured	Left	5	7	12	4
	Right	1	11	12	20

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

Anthropometric measures

Height (m) was measured using a stadiometer (Seca 213, portable stadiometer, Hamburg, Germany) following the stretch-stature method [25] and body mass (BM) (kg) via a set of digital scales with minimal clothing (Seca, Hamburg, Germany). Percentages (%) of body fat and lean body mass (LBM) were measured using bioelectrical impedance (BIA) (Bodystat Ltd, Douglas, Isle of Man). Participants lay supine without their limbs touching and their hands palm down. Four electrodes were placed on the: dorsal surface of the wrist between the bony process of the *radius* and *ulnar*, dorsal surface of the hand, 1 cm proximal from the peak of the knuckle of the middle finger; midway point of the bony process between the *tibia* and *fibula*; and 1 cm proximal to the knuckle of the middle toe (in accordance with manufacturers guidelines). BIA has been shown to be valid (R^2 = 0.96) in comparison with Dual Energy X-ray Absorptiometry in adult TDC [26], and children with CP (concordance correlation coefficient, 0.75–0.82) [27]. It should be noted that where greater variance is reported between BIA and DEXA in CP populations, this has been attributed in part to errors associated with the use of estimated standing height [27, 28], whereas in the present study all CP participants were able to provide a standing height measure.

Physical activity

Habitual PA was recorded through the international physical activity questionnaire-long form (IPAQ) and presented as IPAQ score. The IPAQ consisted of 27 questions asking about the amount of time spent performing sedentary behaviours, light intensity physical activities and moderate to vigorous physical activity around travel, work and free time. In addition to PA questions, participants were also asked to answer questions on PA around occupation, transport, home, yard/garden and leisure/sports. To assess habitual exercise, football training data was logged using 7-day diaries. Data collected included

frequency of training (days per week), duration of each session (mins) and total time spent training (min per week). Step count was also recorded through mobile phone accelerometers from those participants (n = 46) with the iPhone Health Application (Apple Inc. Cupertino, California, US, version 13), as a daily average from the preceding 3 months.

Muscle size

Images of the *Vastus Lateralis* (VL) of the impaired leg of hemiplegic CP or 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). As described by [29], the VL's proximal insertion and the myotendinous junction were marked to identify 50% of muscle length. A strip of echo-absorptive markers, spaced equally apart was placed horizontally around the VL to project a shadow onto the ultrasound image to provide a positional reference. With the probe in the transverse-plane, a recording of the probe moving from the medial border on the VL to the lateral border of the VL was obtained. Individual images were extracted from the recording and used to construct the muscle by overlapping anatomical landmarks and external markers using Microsoft PowerPoint. ImageJ software (Version 1.41, National Institutes of Health, Maryland, USA) was used to measure the cross-sectional area of the constructed VL to determine VL ACSA [30]. [29] validated this technique against Magnetic Resonance Imaging (MRI) and showed an inter class correlation (ICC) of 0.99 and mean typical error of 0.3 cm^2.

Muscle function

To assess muscle function, vertical jump (VJ) height (m), maximum sprint time (s), grip strength (kg) and isometric knee extension maximal voluntary contraction (KE iMVC, N) were measured. Prior to the tests, all participants were taken through a standardised warm up which aimed to increase heart rate to over 120 bpm (Polar H10 chest heart rate monitor, Polar Electro, Kempele, Finland) and dynamic stretches with focus on the muscles around the hips, knees and ankles. Participants were given two attempts at each test, with 1-minute rest in between and the best result was recorded. VJ height (m) was measured using a jump mat (Probotics Inc., Esslinger court, Huntsville, Alabama) in two conditions; with and without arm swing. [31] reported the jump mat to be a reliable piece of equipment to measure VJ height in males (ICC = 0.93, coefficient of variation (CV) = 2.3%) and females (ICC = 0.90, CV = 6%) over two separate days.

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 1 m apart at either end of a 10 m distance. Participants performed two sprints with a standing start 0.60 m behind the first set of gates and has be shown to be a reliable method when measured on two separate days (ICC = 0.912, $p < 0.01$) [32].

Grip strength was assessed using a handgrip dynamometer (Jamar plus, Sammons Preston Rolyon, Bolingbrook, IL). Participants chose their most comfortable grip position, and two maximal grip efforts were performed while standing with the elbow as extended as possible, and the arm raised in front of the body level with the shoulder. Both tests were separated by 1 minute and the highest value was recorded.

This current study showed a high test-retest reliability in men with CP and TDC (ICC = 0.996–0.998, both $p < 0.001$).

To record KE iMVC, participants were seated on a custom-made isokinetic chair fitted with a portable load cell (Manchester Metropolitan University). 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) with their knee at 90° flexion. The tested leg was fastened to a force transducer placed 5 cm above the lateral malleolus. Participants were instructed to extend the fastened leg maximally, and verbal encouragement was given during the measurement. Two trials were performed with 1-minute break between each trial. The highest force produced was digitised using an analogue-to-digital converter, displayed by a self-displayed and coded program (MyLabView, National Instruments, Berkshire, UK) [33]. KE iMVC values were also presented relative to VL ACSA (KE iMVC/ACSA), BM (KE iMVC/BM) and LBM (KE iMVC/LBM).

Bone ultrasound

To assess bone health, ultrasonic bone densitometry (Sunlight, BeamMed Ltd., Israel) of the distal radius (~ 5 cm from the condyle) and the distal tibia (~ 12 cmfrom the condyle) was performed to obtain T and Z scores. Participants lay supine for both measures. Ultrasound gel was applied to the skin surface at the measurement site to facilitate acoustic coupling. To assess the distal radius, the handheld probe was placed in the sagittal plane on the distal third of the radius. The probe was rotated ~ 70° laterally and ~ 70° distally in the horizontal axis around the radius slowly without lifting the probe from the skin surface. The distal third of the tibia was measured by placing the probe in the sagittal plane on the anterior portion of the tibia. The probe was moved back and forth ~ 4 cm in the transverse plane across the bone, without uncoupling the probe from the skin surface. The measurements for each procedure were repeated 3–5 times depending on scan quality. After the signal was digitised and stored, the data was transferred to a computer for automated analysis and a T and Z score was provided. [34] reported that Sunlight ultrasound systems are reliable intra-operator precision at distal radius: 0.36% (after 10 consecutive scans) and precise *in vivo* precision: 0.4–0.8% (scans were performed every 2 months for 2 years).

Dietary vitamin D assessment

To assess habitual dietary vitamin D intake, participants completed a 7-day food diary using a mobile phone application (Libro beta, Nutritics, Co. Dublin, Ireland). Participants logged the weight (g) of food used in all meals and snacks they consumed. This was then analysed in Nutritics Software which provided dietary vitamin D in µg. [35] found that food diary recall was a reliable method for micronutrient intake (R = 0.75) when compared to urinary markers.

Sun exposure measurement

To estimate the level of endogenous skin synthesis of vitamin D_3 from sun exposure, a sun exposure questionnaire (SEQ) was used to assess the frequency, time of day and amount of time that they spent exposed to direct sunlight in the spring and summer months [36]. Questions also included the type of sun

protection that participants habitually use that were likely to inhibit vitamin D_3 synthesis (i.e., SPF Sun cream and clothing worn). To obtain a sunlight exposure score, a coded model was used based around the sun exposure questions and Fitzpatrick scale to give a total sun exposure (TSE) score for each participant [37].

Blood sample collection

Venous blood samples of 5 ml were taken from the antecubital region of the arm for all participants. Samples were collected via needle and eccentric luer tip syringe (Terumo corporation, Shibuya, Tokyo, Japan), transferred into vacutainer plain tubes (BD Vacutainer Plus® plastic serum tube, Bristol Circle Oakville, ON) and immediately centrifuged at 4500 G (Hermle, Model Z380, Countertop Centrifuge, Gosheim, Germany) for 10 minutes to separate the serum. Serum was removed via a micropipette calibrated to 100 µl (Pipetman pipette 10–100 µl, Gilson Scientific Ltd, UK) into two Eppendorf tubes (Eppendorf Tubes® 3810X, Eppendorf, Hamburg) and stored at -20°C.

Measurement of serum 25(OH)D

Total 25(OH)D concentrations were measured using Enzyme-Linked Immune-Sorbent Assay (ELISA) (Orgentec Diagnostika GmbH, Germany). The Orgentec ELISA showed a good correlation of $R^2 = 0.83$ when compared to Liquid chromatography mass spectrometry (LC-MS/MS) [38]. The manufacturer of the ELISA (Orgentec) provided intra- and inter-assay CV's of < 14.6% and < 11.7%. The intra assay CV for 25(OH)D in our hands was in fact lower at 2%. A four parameter logistic curve also showed a reliable calibration curve (Optical density vs. concentration ($ng \cdot ml^{-1}$) $R^2 = 0.9819$.

Measurement of Parathyroid hormone

Serum PTH (PTH) was measured using a 90 minute, one-wash ELISA (Abcam, Cambridge, UK). The ELISA had a range of 4.69–300 $pg \bullet ml^{-1}$, with a sensitivity of 0.761 $pg \bullet ml^{-1}$. The manufacturer of the ELISA (Abcam, Cambridge, UK) provided intra and inter-assay CVs of 1.5% and 3.8% respectively; the intra-assay CV for PTH from this current study was 7.5%. A four parameter polynomial curve also showed a strong reliability score of $R^2 = 0.9991$ in this current study.

Statistical analyses

Statistics were performed using SPSS statistics (SPSS Statistics 25, IBM Chicago, IL, USA). Data was assessed for normal distribution using a Shapiro-Wilks test ($p > 0.05$). Homogeneity of variance was assessed using Levene's test, a corrected p value was applied if variance was non-homogenous. Group differences for height, BM, BMI, BF%, LBM%, dietary intake, TSE, VJ no arms, VJ with arms, 10m-sprint, grip strength, KE iMVC/BM, KE iMVC/LBM and KE iMVC/VL ACSA were assessed by independent T tests. Non parametric group differences for age, tibia T and Z scores, radius T and Z scores, VL ACSA and KE iMVC were assessed using Mann-Whitney U. Pearson correlations were performed to determine any relationships that exists between 25(OH)D and diet, UV b exposure and bone health All data are presented at mean ± SD unless otherwise stated, the confidence interval was set at 95% with alpha set at ≤ 0.05.

Results

Population comparisons for CP and TDC

The CP group were 4.3 years younger ($p < 0.001$), had 14.1% lower BM ($p = 0.001$), a 12.1% lower BMI ($p < 0.001$), a 20% lower BF% ($p = 0.23$) and 20% higher LBM% ($p = 0.023$) compared to TDC (Table 2). Although not specifically matched for PA, there were no group differences in height, IPAQ, step count, PA frequency, PA average minutes per session or PA total time per week between groups ($p > 0.05$, Table 2). Pearson's correlations showed that age was not associated with any other outcome measure (all $p > 0.05$).

Table 2
Anthropometric and physical activity data in men with cerebral palsy (CP) and PA matched controls (TDC).

	CP	TDC	*p*
Age (yrs)	21.0 ± 1.4	25.3 ± 3.1	< 0.001
Height (m)	1.74 ± 0.07	1.76 ± 0.08	0.335
BM (kg)	66.4 ± 10.1	76.5 ± 10.2	0.001
LBM (kg)	57.5 ± 9.8	64.0 ± 9.3	0.025
BMI ($kg \bullet m^{-2}$)	21.9 ± 2.1	24.7 ± 2.3	< 0.001
BF%	13.5 ± 4.4	16.5 ± 4.4	0.023
LBM%	86.5 ± 4.4	83.5 ± 4.4	0.023
GMFCS (mean (range))	1.2 (1–2)	-	-
IFCPF classification (mean (range))	2 (1–3)	-	-
IPAQ score	8384 ± 4720	7178 ± 5626	0.484
PA frequency (days/week)	4.00 ± 1.84	4.33 ± 1.86	0.535
PA duration (mins/session)	65.2 ± 28.3	77.9 ± 17.4	0.066
PA total time (mins/week)	251.3 ± 135.0	320.2 ± 149.5	0.100
Step count (steps/day)	8218 ± 3292	6943 ± 2295	0.126

Reported as mean ± SD and *p* values; BMI, BM index; BF%, body fat percentage; LBM, lean body mass percentage; GMFCS, gross motor function classification score; IFCPF, International federation of cerebral palsy football; IPAQ, international physical activity questionnaire; PA, physical activity.

The CP group had a 23.7% smaller VL ACSA ($p = 0.001$) and KE iMVC was 40.5% lower compared to TDC ($p < 0.001$, Table 3). KE iMVC/VL ACSA, KE iMVC/BM and KE iMVC/LBM were 14.6% ($p = 0.048$), 22.4% ($p = 0.004$) and 25.1% ($p = 0.002$) lower respectively in CP compared to TDC (Table 3). The CP group has a 22.2% lower VJ with no arm swing and 21.8% lower VJ with arm swing compared to TDC ($p < 0.001$ for

both, Table 3). There were no differences in handgrip strength ($p = 0.280$) or 10m-sprint time between CP and TDC ($p = 0.302$, Table 3).

In both groups combined, there was a positive relationship between KE iMVC/LBM and jump height (no arms) (R = 0.368, $p = 0.021$), and VJ (with arm swing) (R = 0.351, $p = 0.029$), but KE iMVC/LBM was not associated with 10m-sprint time (R = 0.024, $p = 0.881$). There was however, no relationship between KE iMVC/LBM and jump height (no arms), jump height (with arm swing) or 10m-sprint time in either men with CP (R = 0.232–0.379, $p > 0.05$) and TDC (R = 0.026–0.087, $p > 0.05$) when grouped separately.

Table 3
Neuromuscular outcome measures in men with cerebral palsy (CP) and PA matched controls (TDC).

	CP	TDC	*p*
VL ACSA (cm^2)	27.1 ± 5.4	34.4 ± 8.3	0.001
KE iMVC (N)	398.8 ± 94.3	601.4 ± 152.4	< 0.001
KE iMVC/ VL ACSA ($N \bullet cm^{-2}$)	15.2 ± 3.13	17.6 ± 4.15	0.048
KE iMVC/BM ($N \bullet kg^{-1}$)	6.07 ± 1.41	7.60 ± 1.65	0.004
KE iMVC/LBM ($N \bullet kg^{-1}$)	7.11 ± 1.80	9.15 ± 2.06	0.002
VJ no arms (m)	0.40 ± 0.04	0.50 ± 0.05	< 0.001
VJ with arms (m)	0.45 ± 0.04	0.56 ± 0.05	< 0.001
Grip strength (kg)	39.8 ± 11.9	45.6 ± 7.4	0.280
10m-sprint (s)	1.90 ± 0.14	1.86 ± 0.12	0.302

Reported as mean ± SD and *p* values; VL ACSA, Vastus Lateralis anatomical cross-sectional area; KE iMVC, knee extensor isometric maximal voluntary contraction; BM, BM; VJ, vertical jump.

The CP group had a radius T score that was − 1.75 SDs less and radius Z score that was − 1.57 SDs less when compared to TDC ($p < 0.001$, Table 4). There was no difference between tibia T scores ($p = 0.158$, Table 4) and tibia Z scores between groups ($p = 0.143$, Table 4). A Pearson's correlation showed that there were no significant relationships between 25(OH)D and any tibia or radius Z and T scores for both Groups (R = 0.162–0.253, all $p > 0.05$). There were also no significant relationships between PTH and any tibia or radius Z and T scores for both Groups (R = 0.012–0.205, all $p > 0.05$).

Table 4
Tibia and radius bone ultrasound (T and Z score) in men with cerebral palsy (CP) and PA matched controls (TDC).

	CP	TDC	*p*
Radius T score	-1.32 ± 1.12	0.43 ± 0.79	< 0.001
Radius Z score	-0.93 ± 1.12	0.64 ± 0.79	< 0.001
Tibia T score	0.50 ± 1.62	-0.03 ± 0.80	0.158
Tibia Z score	0.55 ± 1.60	-0.03 ± 0.82	0.143
Reported as mean ± SD and *p* values.			

There were no differences in 25(OH)D ng•ml^{-1} ($p = 0.381$), PTH ($p = 0.710$), dietary intake ($p = 0.540$) or TSE score between groups ($p = 0.790$, Table 5). Of the men with CP, 5/22 (22.7%) classed as severely deficient, 7/22 (31.8%), deficient, 8/22 (36.4%) insufficient and 2/22 (9.1%) adequate in 25(OH)D, while 8/24 (33.3%) of the TDC classed as severely deficient, 9/24 (37.5%) deficient, 5/24 (20.8%) insufficient and 2/24 (8.3%) adequate in 25(OH)D. A Pearson's correlations showed no relationship between 25(OH)D and dietary vitamin D in men with CP (R = 0.079, $p = 0.798$) or TDC (R = 0.165, $p = 0.607$, Table 5). Nor was there a relationship between 25(OH)D and TSE in men with CP (R = 0.041, $p = 0.857$) or TDC (R = 0.167, $p = 0.447$, Table 5). A Pearson's correlation showed that 25(OH)D levels were associated with stronger KE iMVC/LBM (R = 0.500, $p = 0.020$ (1-tailed)) in men with CP (Fig. 1), but there was no association between 25(OH)D and KE iMVC/LBM in TDC (R = 0.281, $p = 0.103$ (1-tailed), Fig. 1).

Table 5
Vitamin D outcome measures in men with cerebral palsy (CP) and PA matched controls (TDC).

	CP	TDC	*p*
25(OH)D ng·ml^{-1}	18.7 ± 7.3	16.9 ± 7.1	0.381
PTH pg·ml^{-1}	25.1 ± 10.2	31.8 ± 14.2	0.710
Dietary intake IU·day^{-1}	166 ± 186	205 ± 124	0.540
TSE score	27.4 ± 2.4	28.6 ± 2.1	0.790
Reported as mean ± SD and *p* values; 25(OH)D, 25-hydroxyvitamin D; PTH, parathyroid hormone; TSE, total sun exposure.			

Discussion

The aim of this study was to increase the understating of vitamin D and musculoskeletal health in men with CP. Our findings show that 1) the CP participants had lower KE strength, smaller VL ACSA, lower VJ

and sprint performance compared to TDC, 2) bone health group comparisons were site specific to upper and lower limbs, with the CP group showing lower radius scores, but no difference in the tibia from TDC, 3) 25(OH)D levels were similar between CP and TDC groups, however almost all participants were below levels considered sufficient, and 4) there was a positive association between 25(OH)D and KE iMVC/LBM in the CP group, but no association in the TDC group.

Due to the previous associations between impaired walking gait pattern and plantar flexors (PF) weakness and size in adults with CP [39] most studies in CP measure PF strength and PF ACSA [12, 40, 41]. However, the KE were deemed an important muscle group to investigate as they play a dominant role in sports performance [42], falls prevention [43] and are limited in their description within CP [44]. The 40.5% lower KE iMVC in the present study is consistent with the 52% weaker PF [45], and the likely consequence of the PF being more directly impacted by the CP condition [12]. In contrast to our more modest levels of KE weakness, the previous report of a 69% weaker KE in CP [44], likely reflects the sex difference in strength between their mixed sex CP participants, compared to their male only TDC. In the present study, men with CP had 23.7% smaller VL ACSA in men with CP, which is consistent with the 20% smaller PF ACSA and 27.9% smaller in VL ACSA in men with CP compared to TDC [40]. The men with CP in this current study have a higher LBM% of 86.5% compared to other studies which used BIA in young ambulatory males with CP who had a LBM% of 74.8% [28]. The high levels of PA in the men with CP in this current study is likely to be the prominent factor accounting to the 11.7% higher LBM%, as the men with CP in this study performed 3996 steps more/day on average when compared to other young ambulatory CP [46]. Despite the higher levels of LBM in men with CP in this current study compared to other CP populations [28], LBM was still lower in men with CP when compared to TDC, which is consistent with other studies on body composition and muscle size [28]. It should be noted, that it is very common for CP athletes to be recruited for musculoskeletal investigations [12, 44, 47], (see limitations below).

A lower knee extensor strength relative to LBM has a number of functional implications in both CP and other conditions of muscle weakness. It is likely that this is related to the increased impact of a maintained BM and a reduced muscle mass and quality on the affected limb, and suggests that the strength of this limb may influence how well those with CP can move their BM. Beyond our population of men with CP, a lower KEMVC/BM is an observation that has been made in a number of conditions such as: obese-aging men [48], postmenopausal women [49] and individuals with degenerative muscle impairments [50]. Due to the non-progressive nature of CP on neuromuscular properties, strategies to train and improve the quality (contractile properties, size and strength) of the effected muscles, particularly the lower limbs need to be considered alongside body fat management to contribute to improved neuromuscular function.

Surprisingly, there was no difference in 10 m sprint time between groups in the current study. A combination of factors may account for lack of 10m-sprint difference. One could be attributed to 18/24 (75%) of the current CP group classifying as GMFCS I. In higher GMFC scoring adults with CP there is increased gait symmetry to TDC [51] which could contribute to effective ground reaction force vectors

when sprinting [52]. de Groot et al. [44] also found no difference peak power output in a CP group with a GMFCS of I when compared to TDC, yet peak power output was strongly correlated with sprint speed (R^2 = 0.94). Therefore, due to the majority of men with CP being classed as GMFCS I in this current study, it is possible that improved 10 m sprint times in the CP group when compared to previous studies in CP [53] are due to the low number of GMFCS II classifications in the current study. It should also be considered that the majority of men with CP were hemiplegic in this study (Table 1), and running gait patterns are shown to have increased symmetry with increasing running speeds [54]. Therefore, peak power output and increased sprinting gait symmetry may explain why there is no difference in 10m-sprint.

The bone health group differences showed site dependence, with the distal radius being lower in the CP group compared to TDC, and no difference in the distal tibia between groups. This is similar to bone health data presented previously from adolescents with CP who had radius and tibia T scores of -1.07 and − 0.38, respectively [55]. In the current study, the T score of -1.32 suggested some bone loss (osteopenia) and a bone fracture risk factor of 2.3 fold greater when compared to normative values [56]. In contrast to the radius, the distal tibia showed no difference between groups. Serum PTH levels supported this finding and were not different in CP and TDC. However, despite current literature showing that serum PTH is elevated with decreasing 25(OH)D [57, 58], it is of surprise to find that both participants in the CP and TDC group who are classed as deficient in 25(OH)D, have normal levels (< 65 $pg{\cdot}ml^{-1}$) of serum PTH of 25.1 $pg{\cdot}ml^{-1}$ and 31.8 $pg{\cdot}ml^{-1}$ respectively. The normal PTH levels reflect the observations in this study of similar tibia bone health [58]. A potential reason for the CP and TDC showing similar tibia but lower radius T and Z scores, could be their matched activity levels. In adolescents with CP, tibia density was previously reported to be higher with increasing ambulation levels [59]. Indeed, football is specifically effective at preserving age related bone health in those without CP [60]. It is therefore likely that our observations of preserved tibia bone health, may be a consequence of regular lower limb bone loading through activities such as football and associated exercise. For future research, it is important to acknowledge that the present study does not show that football alone improves bone health in men with CP and more work is required to show this association with the sport. It is more probable that the general activity levels of the CP group, which included large elements of football, matched the bone health of their tibia to TDC. Certainly though, clinicians and physical trainers should incorporate exercises that load the upper limbs to ensure bone turnover and development occurs in groups with CP.

There is a paucity of data on the vitamin D concentration in disabled populations, let alone athletes with disabilities, despite low vitamin D concentrations being documented in several nonathletic populations [14–16]. Despite our findings that there are no differences in 25(OH)D between the men with CP and TDC, both groups were on average classified as deficient, with an insufficiency prevalence of ~ 90%. These low vitamin D levels relative to summer values appear to be the norm in the UK during the winter months [61]. Yet, in the present study, Low vitamin D levels may exacerbate the condition specific weakness as 25(OH)D explained 26% of the variance in KE iMVC/LBM in the CP participants. In contrast we saw no associations between 25(OH)D and other outcomes, a likely consequence of the very low levels of

25(OH)D. This was particularly noticeable in the lack of association between 25(OH)D and either dietary vitamin D or TSE in both participant groups, likely as all three could be considered very low. The participants all had low dietary vitamin D intake (185.5 ± 155 IU/d) not coming close to meeting the Institute of Medicine's recommendations of 400-600IU/d in adults 19–50 years old [3]. Furthermore, dietary vitamin D intake accounts for < 20% of circulating vitamin D, our observations of no association between dietary vitamin D intake and 25(OH)D is of no surprise [1]. Similarly, that there was no association between TSE and 25(OH)D in this current study, is consistent with negligible UV b radiation from sun exposure is during the latter winter months in the UK (i.e., February-March). Thus, even with the highest TSE scores, 25(OH)D would likely not have been high [61]. Despite the seasonal contribution to negligible 25(OH)D variance between groups, the role of 25(OH)D to KE MVC/BM is consistent with well-established role of 25(OH)D on skeletal muscle myogenesis, cell proliferation, differentiation, regulation of protein synthesis and mitochondrial metabolism [62]. With this knowledge (and the prevalent insufficiency in all participants), interventions such as vitamin D supplementation should be sought by both men with CP and TDC to correct for low vitamin D. In future, it is important that seasonal variations in vitamin D are measured to identify if increased UV b radiation from sun exposure improves 25(OH)D to adequate levels and if musculoskeletal health outcome measures are impacted in men with CP and other para-athletes.

Strengths and Limitations

The participants in the present study represent a highly functional proportion of men with CP (GMFCS I-II). Our participant groups are however similar to the only other description of KE iMVC and sprint outcomes [44], and are consistent with populations of CP athletes investigated by others [47]. Comparisons with population based studies suggest that the proportion of GMFCS I: GMFCSII participants is around n = 3:2 [63]. In contrast, the strength comparisons made in the present study, [44] and [47] are made in ambulatory participants with CP at a ratio of GMFCS I:GMFCS II, n = 3:1, with more severe impairments also included in [47]. The high levels of PA in these active CP participants will however mean that the findings may not be generalisable to other more impaired populations with CP. Although a broader population would of course be relevant for generalisation, our population of lower GMFCS impairment likely reduces the contribution of PA variance to the group differences and suggest that by regularly undertaking football and other forms of PA, there is some benefit to the musculoskeletal health of men with CP, particularly in the bone health of the lower limbs. As addressed throughout these limitations, and consistent with the caveat of all studies, the outcomes reflect the population under investigation. To this end it is important to note we include no data from women. Although not adverse to presenting sex disaggregated musculoskeletal outcomes such as, KE strength and tendon stiffness [64, 65], our recruitment of CP participants was driven by the prevailing opportunities for those with CP, and noticeably a much more limited development pathway for women's para-sport. In previous studies including active CP participants, where women are presented, it is as a minority (men: women, 2:1 and 4:1, respectively, [44, 66]. Given the known sex differences in bone measures, and the greater risk for osteoporosis [67], future research should strive to uncover the particular risks and implications of low vitamin D in women with CP.

Conclusion

The aim of this study was to increase the understating of vitamin D and musculoskeletal health in men with CP. It has illustrated that men with CP have lower KE iMVC force, smaller VL ACSA, reduced muscle function and site specific reduced bone health of the radius than TDC. Men with CP and TDC are both also at high risk of low vitamin D during the winter months, which could contribute to weakness of the KE iMVC/LBM in men with CP. Therefore, it is important that men with CP undertake strategies to amend low vitamin D during the winter months such as vitamin D supplementation to ensure that decreased muscular performance and potential risk of falls from exacerbated knee extensor weakness are reduced.

Abbreviations

25(OH)D
25-hydroxyvitamin D
BM
Body Mass
BMI
Body Mass Index
CP
Cerebral Palsy
GMFCS
Gross Motor Function Classification System
IFCPF
International Federation of Cerebral Palsy Football
IPAQ
International Physical Activity Questionnaire
KE iMVC
Knee Extensor Isometric Maximal Voluntary Contraction
LBM
Lean Body Mass
PA
Physical Activity
PF
Plantar Flexor
PTH
Parathyroid Hormone
TDC
Typically Developed Controls
TSE
Total Sun Exposure

UV
Ultraviolet
VL ACSA
Vastus Lateralis Anatomical Cross-Sectional Area
VJ
Vertical Jump

Declarations

Ethics Approval and Consent to Participate

All participants provided written informed consent, following approval from the Manchester Metropolitan University ethics committee, in accordance with the declaration of Helsinki.

Consent for Publication

Not applicable

Availability of data and materials

Data can be made available on request.

Competing Interests

The authors declare that they have no competing interests.

Funding

This research was funded through a joint PhD studentship by The Football Association and Manchester Metropolitan University

Author contributions

CL, CM and AH conceptualised the study, CL, AB, HB collected the data, CL interpreted and analysed the data, CL was the major contributor in writing the manuscript. All authors read and approved the final manuscript.

Acknowledgements

Thank you to all of the individuals at Manchester Metropolitan University and The Football Association who helped to make this study possible.

Authors Information

N/A

References

1. Holick MF, Vitamin D. *New England Journal of Medicine* 2007, *357* (3), 16.
2. Lips P, Van Ginkel FC, Jongen MJ, Rubertus F, Van Der Vijgh WJ, Netelenbos JC. Vitamin D physiology. Progress in biophysics molecular biology. 2006;92(1):4.
3. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, Murad MH, Weaver CM. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. The Journal of Clinical Endocrinology Metabolism. 2011;96(7):1911–30.
4. Brett NR, Lavery P, Agellon S, Vanstone CA, Maguire JL, Rauch F, Weiler HA. Dietary vitamin D dose-response in healthy children 2 to 8 y of age: a 12-wk randomized controlled trial using fortified foods. Am J Clin Nutr. 2016;103(1):144–52.
5. Docio S, Riancho JA, Pérez A, Olmos JM, Amado JA, González-Macías J. Seasonal deficiency of vitamin D in children: a potential target for osteoporosis-preventing strategies? J Bone Miner Res. 1998;13(4):544–8.
6. Rajakumar K, Holick MF, Jeong K, Moore CG, Chen TC, Olabopo F, Haralam MA, Nucci A, Thomas SB, Greenspan SL. Impact of season and diet on vitamin D status of African American and Caucasian children. Clin Pediatr. 2011;50(6):493–502.
7. Oleson CV, Patel PH, Wuermser L-A. Influence of Season, Ethnicity, and Chronicity on Vitamin D Deficiency in Traumatic Spinal Cord Injury. The Journal of Spinal Cord Medicine. 2010;33(3):202–13.
8. Kalra S, Aggarwal A, Chillar N, Faridi MMA. Comparison of Micronutrient Levels in Children with Cerebral Palsy and Neurologically Normal Controls. *The Indian Journal of Pediatrics* 2015, *82* (2), 140–144.
9. Nooijen CFJ, Slaman J, Stam HJ, Roebroeck ME, Berg-Emons RJ. v. d.; Learn2Move Research, G., Inactive and sedentary lifestyles amongst ambulatory adolescents and young adults with cerebral palsy. *Journal of NeuroEngineering and Rehabilitation* 2014, *11* (1), 49.
10. Morton JP, Iqbal Z, Drust B, Burgess D, Close GL, Brukner PD. Seasonal variations in vitamin D status in professional soccer players of the English premier league. Applied physiology nutrition metabolism. 2012;37:5.
11. Flueck JL, Hartmann K, Strupler M, Perret C. Vitamin D deficiency in Swiss elite wheelchair athletes. Spinal cord. 2016;54:(11).
12. Hussain AW, Onambele GL, Williams AG, Morse CI. Muscle size, activation, and coactivation in adults with cerebral palsy. *Muscle & nerve* 2014, *49* (1), 76–83.
13. Finbråten AK, Syversen U, Skranes J, Andersen GL, Stevenson RD, Vik T. Bone mineral density and vitamin D status in ambulatory and non-ambulatory children with cerebral palsy. Osteoporos Int. 2015;26(1):10.
14. Bischoff HA, Stahelin HB, Urscheler N, Ehrsam R, Vonthein R, Perrig-Chiello P, Tyndall A, Theiler R. Muscle strength in the elderly: its relation to vitamin D metabolites. *Archives of physical medicine and rehabilitation* 1999, *80* (1), 54–58.

15. Foo LH, Zhang Q, Zhu K, Ma G, Hu X, Greenfield H, Fraser DR. 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, *139* (5), 6.
16. Farrar MD, Mughal MZ, Adams JE, Wilkinson J, Berry JL, Edwards L, Kift R, Marjanovic E, Vail A, Webb AR. Sun exposure behavior, seasonal vitamin D deficiency, and relationship to bone health in adolescents. The Journal of Clinical Endocrinology Metabolism. 2016;101(8):3105–13.
17. Rajakumar K, de Las Heras J, Chen TC, Lee S, Holick MF, Arslanian SA. Vitamin D status, adiposity, and lipids in black American and Caucasian children. The Journal of Clinical Endocrinology Metabolism. 2011;96(5):1560–7.
18. Carriero A, Zavatsky A, Stebbins J, Theologis T, Shefelbine SJ. Determination of gait patterns in children with spastic diplegic cerebral palsy using principal components. Gait Posture. 2009;29(1):71–5.
19. Papageorgiou E, Simon-Martinez C, Molenaers G, Ortibus E, Van Campenhout A, Desloovere K. Are spasticity, weakness, selectivity, and passive range of motion related to gait deviations in children with spastic cerebral palsy? A statistical parametric mapping study. PLOS ONE. 2019;14(10):e0223363.
20. Hillesund E, Skranes J, Trygg KU, Bøhmer T. Micronutrient status in children with cerebral palsy. Acta Paediatr. 2007;96(8):1195–8.
21. Halliday TM, Peterson NJ, Thomas JJ, Kleppinger K, Hollis BW, Larson-Meyer DE. Vitamin D status relative to diet, lifestyle, injury, and illness in college athletes. *Medicine and science in sports and exercise* 2011, *43* (2), 8.
22. Seth A, Aneja S, Singh R, Majumdar R, Sharma N, Gopinath M. Effect of impaired ambulation and anti-epileptic drug intake on vitamin D status of children with cerebral palsy. *Paediatrics and International Child Health* 2017, *37* (3), 193–198.
23. Stallings VA, Charney EB, Davies JC, Cronk CE, NUTRITIONAL STATUS AND GROWTH OF CHILDREN WITH DIPLEGIC OR HEMIPLEGIC CEREBRAL PALSY. Developmental Medicine Child Neurology. 1993;35(11):997–1006.
24. Cannell JJ, Hollis BW, Sorenson MB, Taft TN, Anderson JJ. Athletic performance and vitamin D. Med Sci Sports Exerc. 2009;41(5):1102–10.
25. Voss L, Bailey B. Diurnal variation in stature: is stretching the answer? *Archives of disease in childhood* **1997**, *77* (4), 319–322.
26. De Lorenzo A, Sorge S, Iacopino L, Andreoli A, De Luca PP, Sasso G. Fat-free mass by bioelectrical impedance vs dual-energy X-ray absorptiometry (DXA). Appl Radiat Isot. 1998;49(5–6):739–41.
27. Oeffinger DJ, Gurka MJ, Kuperminc M, Hassani S, Buhr N, Tylkowski C. Accuracy of skinfold and bioelectrical impedance assessments of body fat percentage in ambulatory individuals with cerebral palsy. Developmental Medicine Child Neurology. 2014;56(5):475–81.
28. Hildreth HG, Johnson RK, Goran MI, Contompasis SH. Body composition in adults with cerebral palsy by dual-energy X-ray absorptiometry, bioelectrical impedance analysis, and skinfold anthropometry

compared with the 18O isotope-dilution technique. *The American journal of clinical nutrition* 1997, *66* (6), 1436–1442.

29. Reeves ND, Maganaris CN, Narici MV. Ultrasonographic assessment of human skeletal muscle size. *European journal of applied physiology* 2004, *91* (1), 116–118.
30. Esformes JI, Narici MV, Maganaris CN. Measurement of human muscle volume using ultrasonography. European journal of applied physiology. 2002;87(1):90–2.
31. Nuzzo JL, Anning JH, Scharfenberg JM. The reliability of three devices used for measuring vertical jump height. The Journal of Strength Conditioning Research. 2011;25(9):2580–90.
32. Shalfawi S, Enoksen E, Tønnessen E, Assessing test-retest reliability of the portable Brower speed trap II testing system. 2012.
33. He L, Khanal P, Morse CI, Williams A, Thomis M. Associations of combined genetic and epigenetic scores with muscle size and muscle strength: a pilot study in older women. *Journal of cachexia, sarcopenia and muscle* 2020, *11* (6), 1548–1561.
34. Knapp K, Blake G, Spector T, Fogelman I. Multisite quantitative ultrasound: precision, age-and menopause-related changes, fracture discrimination, and T-score equivalence with dual-energy X-ray absorptiometry. Osteoporos Int. 2001;12(6):456–64.
35. Day NE, McKeown N, Wong M-Y, Welch A, Bingham S. Epidemiological assessment of diet: a comparison of a 7-day diary with a food frequency questionnaire using urinary markers of nitrogen, potassium and sodium. Int J Epidemiol. 2001;30(2):309–17.
36. McCarty CA. Sunlight exposure assessment: can we accurately assess vitamin D exposure from sunlight questionnaires? *The American journal of clinical nutrition* 2008, *87* (4), 1097S-1101S.
37. Fitzpatrick TB. The validity and practicality of sun-reactive skin types I through VI. *Archives of dermatology* 1988, *124* (6), 869–871.
38. Zerwekh JE. The measurement of vitamin D: analytical aspects. Ann Clin Biochem. 2004;41(4):272–81.
39. Eek MN, Beckung E. Walking ability is related to muscle strength in children with cerebral palsy. Gait Posture. 2008;28(3):366–71.
40. Noble JJ, Fry NR, Lewis AP, Keevil SF, Gough M, Shortland AP. Lower limb muscle volumes in bilateral spastic cerebral palsy. *Brain and Development* 2014, *36* (4), 294–300.
41. McNee AE, Gough M, Morrissey MC, Shortland AP. Increases in muscle volume after plantarflexor strength training in children with spastic cerebral palsy. *Developmental Medicine & Child Neurology* 2009, *51* (6), 429–435.
42. Magalhaes J, Ascensao A, Soares J. Concentric quadriceps and hamstrings isokinetic strength in volleyball and soccer players. Journal of sports medicine physical fitness. 2004;44:119–25.
43. Larson L, Bergmann TF. Taking on the fall: The etiology and prevention of falls in the elderly. Clinical Chiropractic. 2008;11(3):148–54.

44. de Groot S, Dallmeijer AJ, Bessems PJ, Lamberts ML, van der Woude LH, Janssen TW. Comparison of muscle strength, sprint power and aerobic capacity in adults with and without cerebral palsy. *Journal of rehabilitation medicine* 2012, *44* (11), 932–938.
45. Damiano DL, Abel MF. Functional outcomes of strength training in spastic cerebral palsy. *Archives of physical medicine and rehabilitation* 1998, *79* (2), 119–125.
46. Bjornson KF, Belza B, Kartin D, Logsdon R, McLaughlin JF. Ambulatory physical activity performance in youth with cerebral palsy and youth who are developing typically. Physical therapy. 2007;87(3):248–57.
47. O'Brien SM, Carroll TJ, Barber LA, Lichtwark GA. Plantar flexor voluntary activation capacity, strength and function in cerebral palsy. *European Journal of Applied Physiology* 2021, 1–9.
48. Rolland Y, Lauwers-Cances V, Pahor M, Fillaux J, Grandjean H, Vellas B. Muscle strength in obese elderly women: effect of recreational physical activity in a cross-sectional study. Am J Clin Nutr. 2004;79(4):552–7.
49. Lebrun CE, van der Schouw YT, de Jong FH, Grobbee DE, Lamberts SW. Fat mass rather than muscle strength is the major determinant of physical function and disability in postmenopausal women younger than 75 years of age. Menopause. 2006;13(3):474–81.
50. Miscione MT, Bruno F, Ripamonti C, Nervuti G, Orsini R, Faldini C, Pellegrini M, Cocchi D, Merlini L. Body composition, muscle strength, and physical function of patients with Bethlem myopathy and Ullrich congenital muscular dystrophy. *The Scientific World Journal* 2013, 2013.
51. Saether R, Helbostad JL, Adde L, Brændvik S, Lydersen S, Vik T. Gait characteristics in children and adolescents with cerebral palsy assessed with a trunk-worn accelerometer. *Research in developmental disabilities* 2014, *35* (7), 1773–1781.
52. Wang Y, Watanabe K. Limb dominance related to the variability and symmetry of the vertical ground reaction force and center of pressure. *Journal of applied biomechanics* 2012, *28* (4), 473–478.
53. Reina R, Sarabia JM, Caballero C, Yanci J. How does the ball influence the performance of change of direction and sprint tests in para-footballers with brain impairments? Implications for evidence-based classification in CP-Football. *PloS one* 2017, *12* (11), e0187237.
54. Davids JR, Bagley AM, Bryan M. Kinematic and kinetic analysis of running in children with cerebral palsy. Developmental Medicine Child Neurology. 1998;40(8):528–35.
55. Hartman C, Brik R, Tamir A, Merrick J, Shamir R. Bone quantitative ultrasound and nutritional status in severely handicapped institutionalized children and adolescents. Clin Nutr. 2004;23(1):89–98.
56. Houlihan CM, Stevenson RD. Bone density in cerebral palsy. Physical Medicine Rehabilitation Clinics. 2009;20(3):493–508.
57. Sai AJ, Walters R, Fang X, Gallagher J. Relationship between vitamin D, parathyroid hormone, and bone health. The Journal of Clinical Endocrinology Metabolism. 2011;96(3):E436–46.
58. Valcour A, Blocki F, Hawkins D, Rao SD. Effects of age and serum 25-OH-vitamin D on serum parathyroid hormone levels. The Journal of Clinical Endocrinology Metabolism. 2012;97(11):3989–95.

59. Al Wren T, Lee DC, Kay RM, Dorey FJ, Gilsanz V. Bone density and size in ambulatory children with cerebral palsy. *Dev Med Child Neurol* 2011, *53* (2), 137–41.
60. Vicente-Rodriguez G, Jimenez-Ramirez J, Ara I, Serrano-Sanchez J, Dorado C, Calbet J. Enhanced bone mass and physical fitness in prepubescent footballers. *Bone* **2003**, *33* (5), 853–859.
61. Webb AR, Kazantzidis A, Kift RC, Farrar MD, Wilkinson J, Rhodes LE. Meeting vitamin D requirements in white Caucasians at UK latitudes: providing a choice. Nutrients. 2018;10(4):497.
62. Montenegro KR, Cruzat V, Carlessi R, Newsholme P. Mechanisms of vitamin D action in skeletal muscle. *Nutrition research reviews* 2019, *32* (2), 192–204.
63. Michelsen SI, Flachs EM, Uldall P, Eriksen EL, McManus V, Parkes J, Parkinson KN, Thyen U, Arnaud C, Beckung E. Frequency of participation of 8–12-year-old children with cerebral palsy: A multi-centre cross-sectional European study. *european journal of paediatric neurology* 2009, *13* (2), 165–177.
64. Hicks K, Onambélé G, Winwood K, Morse C. Muscle damage following maximal eccentric knee extensions in males and females. PloS one. 2016;11(3):e0150848.
65. McMahon G, Morse CI, Winwood K, Burden A, Onambélé GL. Gender associated muscle-tendon adaptations to resistance training. *PLoS One* **2018**, *13* (5), e0197852.
66. Barber L, Barrett R, Lichtwark G. Passive muscle mechanical properties of the medial gastrocnemius in young adults with spastic cerebral palsy. *Journal of biomechanics* 2011, *44* (13), 2496–2500.
67. Bonnick SL. Osteoporosis in men and women. Clin Cornerstone. 2006;8(1):28–39.

Figures

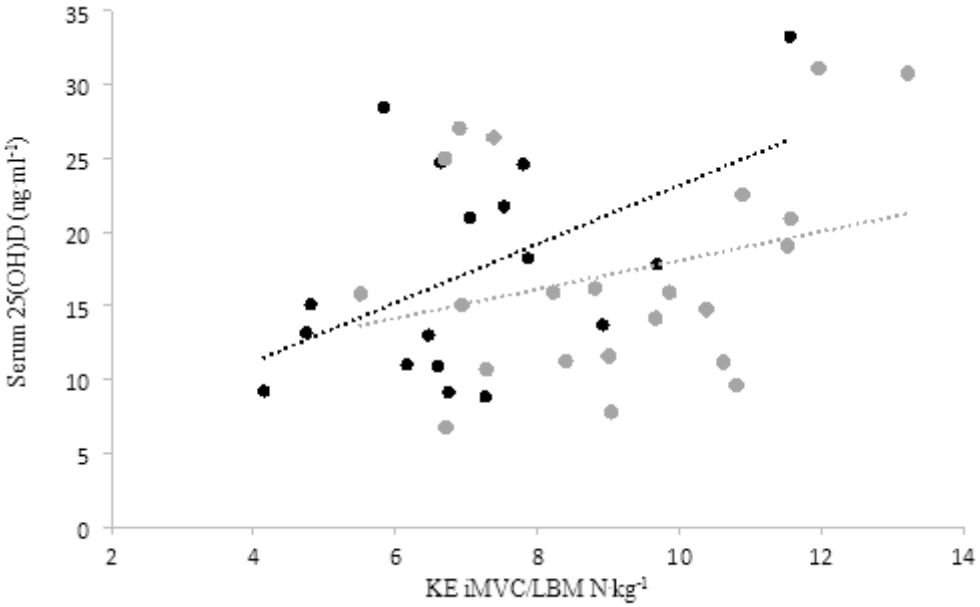

Figure 1

Pearson correlations between serum 25(OH)D and KE iMVC/LBM in men with CP (black filled dots) and TDC (grey filled dots).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- STROBEchecklistcrosssectional.docx