MUSCULOSKELETAL HEALTH IN AMBULATORY PHYSICALLY ACTIVE MEN WITH CEREBRAL PALSY: SEASONAL VARIATIONS AND THE ROLE OF VITAMIN D

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Thesis Abstract

Background: Individuals with Cerebral Palsy (CP) show impairments in muscle strength and power. Impairments in strength, in typically developed controls (TDC) low levels of force production and poor bone health are associated with low levels of vitamin D, and a high falls prevalence. It is possible that the severity of musculoskeletal impairments in individuals with CP is exacerbated by living in northern latitudes such as the UK and may contribute to high falls prevalence. **Methods:** Forty-eight participants were split into two groups of 24 active, ambulant men with CP (Gross Motor Function Classification Score I-II) and 24 healthy TDC completed one *in vivo* assessment of musculoskeletal health during the winter[1], including: *Vastus Lateralis* anatomical cross-sectional area (VL ACSA), isometric knee extension maximal voluntary contraction (KE iMVC), 10 m sprint, vertical jumps, radius and tibia bones T_{us} and Z_{us} scores. Plus falls frequency questionnaires to measure fear of falling and risk-taking behaviour were completed by 19 men with CP and 19 TDC. 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. 16 of the ambulant men with CP and 16 healthy TDC repeated these measures to assess seasonal variations on two separate occasions (the winter and in the summer).

[1] The data collection from this thesis occurred between March 2019 and March 2020 corresponding with the start of the COVID pandemic. Although winter and summer data were not directly impacted by the pandemic the original plan to complete four time points, and subsequent validation data from DEXA and PQCT was impossible as the FA placed a moratorium on training camps until March 2021.

Results: Men with CP had 40.5% weaker KE iMVC, 23.7% smaller VL ACSA, 22.2% lower vertical jump, 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_{us} and Z_{us} scores were 1.75 and 1.57 standard deviations lower than TDC, respectively ($p<0.05$). 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 ($r = 0.500$, $p=0.020$). Men with CP and TDC showed a 70.5% and 85.7% increase in serum 25(OH)D from winter to summer months ($p<0.05$) respectively, yet the mean of men with CP was below the adequate threshold of 30 $ng \cdot mL^{-1}$ in the summer. PTH decreased with increased levels of 25(OH)D during the summer months in both groups ($p<0.05$). Men with CP showed an increase in radius T_{us} and Z_{us} scores in the summer ($p<0.05$). 47% of men with CP were classified as fallers, fear of falling was 26.2% greater and risk-taking behaviours was 14.5% lower compared to TDC ($p<0.05$). Lower levels of KE iMVC in men with CP were associated with higher falls prevalence. **Conclusion:** A higher falls prevalence in men with CP, was associated with muscle weakness (chapter 3), where men with CP also showed large decrements in both bone and muscle outcomes compared to TDC (chapter 4). There was a high prevalence of vitamin D insufficiency presented in men with CP and TDC, and as a result was associated with muscle weakness in men with CP but did not contribute to other musculoskeletal decrements shown in men with CP compared to TDC (Chapter 4). Similarly, these vitamin D insufficiencies were inadequately overcome with an increase in TSE from winter to summer months, showing almost no benefit of large seasonal increases in vitamin D with no musculoskeletal improvement in

either men with CP (Chapter 5). The findings of this thesis suggest a greater sensitivity to low vitamin D in men with CP with regards to bone and muscle content and functional outcomes and emphasise the importance for future research into vitamin D supplementation in populations with musculoskeletal impairments.

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Declaration

I declare that this thesis is my own work, and no portion of the work has been submitted in support of an application for another degree or qualification of this university or any other learning institution. To the best of my knowledge, this thesis contains no material written or distributed previously by any other parties, apart from where I have otherwise stated.

List of Publications and Conference Presentations

1) Langley, C. K., Onambélé-Pearson, G. L., Sims, D. T., Hussain, A., Buffey, A. J., Bardwell, H. L., & Morse, C. I. (2021). Musculoskeletal Health in Active Ambulatory Men with Cerebral Palsy and the Impact of Vitamin D. *Nutrients*. 13(7). https://doi.org/10.3390/nu13072481

2) Langley, C. K., Onambélé-Pearson, G. L., Sims, D. T., Hussain, A., Buffey, A. J., Bardwell, H. L., & Morse, C. I. (2021). Musculoskeletal Health in Active Ambulatory Men with Cerebral Palsy and the Impact of Vitamin D. (Accepted - BASES Conference abstract).

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List of Abbreviations

Abbreviations	Description
25(OH)D	25-hydroxyvitamin D (Calcidiol)
1,25$(OH)_2$D	1,25-dihydroxyvitamin D (Calcitriol)
BF	Body fat
BM	Body mass
BMI	Body mass index
CP	Cerebral Palsy
CV	Coefficient of variation
FES-1	Falls Efficacy Scale - International
ELISA	Enzyme linked immunosorbent assay
EQ	Eligibility questionnaire
GMFCS	Gross motor function classification score
IFCPF	International Federation of Cerebral Palsy Football
KE iMVC	Knee extensor isometric maximal voluntary contraction
LBM	Lean body mass
IPAQ	International physical activity questionnaire
PA	Physical activity
PTH	Parathyroid hormone
SB	Sedentary behaviour
SPF	Sun protection factor
SEQ	Sun exposure questionnaire
TDC	Typically developed controls
TSE	Total sun exposure
UK	United Kingdom
UV b	Ultraviolet beta
vertical jump	Vertical jump
VL ACSA	Vastus Lateralis anatomical cross-sectional area

Thesis Outline

Chapter 1; a literature review that discusses the neuromuscular and skeletal factors that are associated with cerebral palsy and the role of vitamin D on musculoskeletal health.

Chapter 2; study aims, objectives and hypotheses.

Chapter 3; a cross sectional observation study investigating the prevalence of falls in young physically active ambulatory men with CP. The impact of muscle strength, fear of falling and risk-taking behaviours are discussed.

Chapter 4; a cross sectional observation study investigating the *in vivo* assessment of neuromuscular performance, bone outcomes and the impact of vitamin D during the winter months.

Chapter 5; a repeated measures within and between group study using similar procedures discussed in chapter 4 to assess the impact of seasonal variations in vitamin D; **1)** on neuromuscular performance and **2)** on parathyroid hormone and bone ultrasound T_{us} and Z_{us} scores.

Chapter 6; a summary of the findings from the preceding chapter are discussed and suggestions for future research are provided.

Chapter 1 – Literature Review

1.1 Introduction

Disability football within the UK represents a means to undertaking structured physical activity (PA) for those with numerous physical impairments. Cerebral Palsy (CP) an acquired musculoskeletal condition characterised by a velocity dependent resistance to stretch, represents one disability that would be positively improved through physical exercise such as football despite condition dependent impairments in muscle strength and power (Damiano and Abel, 1998). Impairments in strength, or low levels of force production, are also associated with low levels of vitamin D (Bischoff-Ferrari, 2007). It is possible that the severity of strength impairments in individuals with CP is exacerbated by living in northern latitudes such as the UK; this has however, not been investigated. The subsequent thesis outlined the potential implications for physical function, bone health and vitamin D deficiency in footballers with CP.

1.2 Para-Football in the UK

There are 9.4 million people in UK with a long-standing limiting disability, illness or condition that effects quality of life, equating to 18% of the population (The Football Association, 2019). It is now becoming more apparent that quality of life in individuals with disabilities can be significantly improved through PA (Durstine et al., 2000). As more individuals with disabilities are involved in PA and competitive sport, para-athletes (athletes with disabilities) develop bringing attention to para-sport including para-football, in which 23,876 individuals with varying disabilities participate in para-football in England (The Football Association, 2019).

Currently, the FA funds seven elite para-football squads: Blind men, CP men, Deaf men, and women Partially Sighted men, Powerchair and Amputee squads. Of the ambulatory disabilities supported by the FA, it is the CP athletes that may benefit directly from mobility and activity, as the spasticity that defines the condition, may directly impact their ability to play football (Scholtes et al., 2006). The FA have therefore created a development pathway that provides "opportunity for all" and facilitates the identification of talented CP footballers as young as five years old and allows them an opportunity to represent their country at the elite level.

At the elite level (≥16 years old), CP athletes are classified based on the level of their physical impairment. The current classification system was developed in 2018 by the International Federation of CP Football (IFCPF), in order to provide a robust structure and make competition as fair as possible (Figure 1-1). The IFCPF classification is based on the type of impairment, namely bilateral spasticity (A), athetosis/dystonia or ataxia (B) or unilateral spasticity (C) (Figure 1-1) and the severity of the impairment from least severe (FT3), moderate (FT2) to most severe (FT1). The current classification for athletes with CP, reflects to some degree the clinical classifications that are used within CP. The FA classifications reflect the more ambulatory range of the Gross Motor Function Classification System (GMFCS) classification adopted in the international, clinical classification of CP impairments. The GMFCS focuses on the functional impairments associated with CP rather than the severity of spasticity that has previously been shown to have limited impact on muscle function (Rethlefsen et al., 2010). For example, FT1-3 would be considered GMFCS I-II, with the more severe GMFCS III-V more likely to be considered for frame football or non-ambulatory sports such as powerchair football. Topographic distribution of muscle spasticity and type

of CP is also identified in the IFCPF classification process. All of the above methods are used synonymously within the IFCPF and the FA to classify individuals as (A) diplegic, (bilateral involvement of the lower limbs), (B) ataxic CP (coordination impairments or lack of ability to control muscle tone) (Hou et al., 2006) or (C) hemiplegic (unilateral involvement) (Figure 1-1) (O'Shea, 2008).

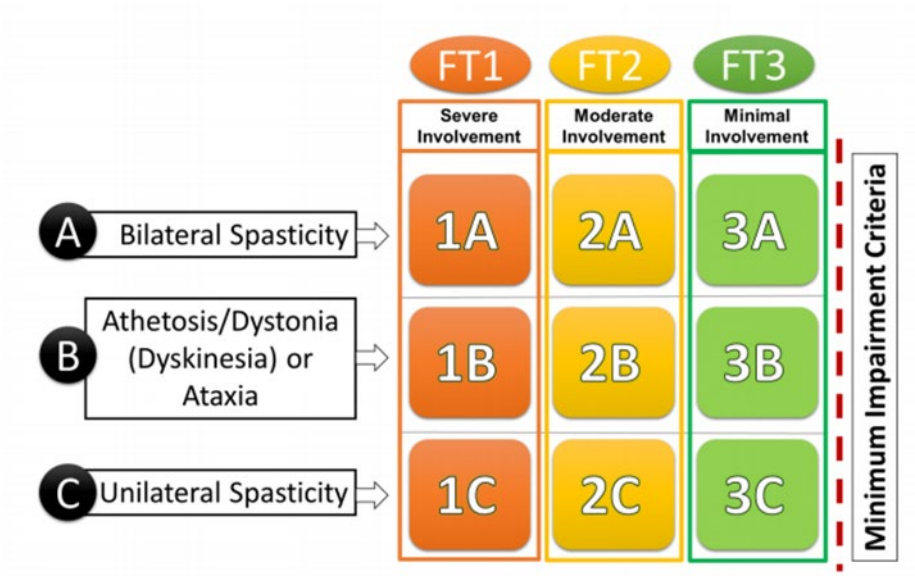

Figure 1-1. Structure of CP classification system (IFCPF, 2018). A, B, C represent the type of CP (A- Diplegia/ Bilateral spasticity; B- Ataxia/ Dyskinesia or Athetosis; C- Hemiplegia/ Unilateral spasticity). FT1, 2 and 3 represent the severity of involvement. FT1 = most severe, FT2 = moderate and FT3 = minimal). Players must meet the minimal level of impairment to compete at an international level.

1.3 The Neuromuscular Basis of Musculoskeletal Impairment in Adults with CP

The aetiology of CP is largely attributed to a brain lesion *in utero,* often caused by hypoxic-ischemic encephalopathy or asphyxia, or during infancy and occurs in about 2.1 per 1,000 live births (Oskoui et al., 2013). CP describes a group of permanent, but

non-progressive conditions that effect the development of mobility and posture and subsequently leads to activity limitations (Bax et al., 2005). The motor pathways associated with the affected region are often accompanied by muscle stiffness, disturbances of sensation, cognition, coordination and seizures (Bax et al., 2005). Increasing muscular stiffness, otherwise known as spastic CP, is diagnosed in upwards of 70% of all cases of CP (Stanley et al., 2000). Ataxic CP observed in 5-10% of all cases and athetoid or dyskinetic CP is apparent in 15-20% of individuals with CP (McHale et al., 2000). Approximately 60% of people with CP can walk independently or with aids (Morgan et al., 2014). It should be noted that although CP is non-progressive, the nature of CP often causes impaired ambulation when compared to children and controls, resulting in diminished musculoskeletal related fitness (McGinley et al., 2014).

The decline in musculoskeletal function described previously in adults with CP (Graham and Selber, 2003) is likely to be a combination of condition induced factors such as physical inactivity (Mitchell et al., 2015) and by the neural impairment to the muscle (Lieber and Fridén, 2019). Lower limb muscle size is a determinant of reduced isometric maximal voluntary contraction torque (iMVC) in both children and adults with CP (Moreau et al., 2009). Children with CP have a 31% smaller *Vastus Lateralis* (*VL)* anatomical cross sectional area (ACSA) than typically developing children (Moreau et al., 2009). Hussain et al. (2014) measured plantar flexion iMVC in 11 ambulatory CP footballers with spastic hemiplegia and observed that plantar flexion iMVC torque was 52% less than controls and 42% less than the non-paretic limb, but no difference in plantar flexion iMVC torque was identified between the control group and non-paretic limb of the CP group.

It is now known that PA and exercise is pivotal in not only managing CP, but also in the improvement of musculoskeletal size, strength and function particularly in the paretic limbs (Damiano et al., 2006). McNee et al. (2009) identified the size of the *medial* and *lateral Gastrocnemius* increased 23.2% and 23.6%, respectively, after a 10-week strength-training programme in children aged 7-16 years old with spastic CP (5-hemiplegic, 8-diplegic). The training induced improvements in muscle size and strength have been shown to improve gait symmetry and overall mobility of children with spastic hemiplegic CP (McNee et al., 2009). After a six-week strength-training programme in five children with spastic hemiplegic CP there was an average increase in lower limb iMVC of 20.3% and a 16% decrease in asymmetry, which contributed towards an improvement in gait pattern in the paretic and non-paretic leg (Damiano and Abel, 1998). Nevertheless, athletic populations with CP (e.g., cyclists and footballers) still show significantly smaller muscle size, strength and function in the paretic limbs, compared to healthy age matched controls (de Groot et al., 2012).

1.4 Risks Associated with Poor Neuromuscular Health in Cerebral Palsy

An under reported consequence of muscle weakness in CP could be an increased risk of falls (Morgan and McGinley, 2013a). Numerous studies have shown that muscle weakness is part of a multifaceted group of risk factors, which can increase risk of injurious falls (Ambrose et al., 2013, Moreland et al., 2004, O'Loughlin et al., 1993), this is particularly prevalent in elderly populations, individuals with greater levels of muscle weakness being more likely to experience reoccurring falls (Moreland et al., 2004). In those who experience falls an increased fear of falling is often elicited, which can consequently reduce PA and quality of life (Li et al., 2003). The known

weakness of the muscles in the lower limbs (Hussain, 2013) and altered gait pattern (Andersson et al., 2003) likely predisposes ambulatory individuals with CP to increased risk of injurious falls. Even, physically active men with CP have substantially reduced muscle strength of the plantar flexors compared to physically active men without CP (Overend et al., 1992), with the extent of the strength deficit being greater for the affected vs. unaffected limb (Hussain et al., 2013). This unilateral gait imbalance and weaker lower limb muscle strength has previously been associated with fall prevalence in the elderly, with knee extension (KE) weakness being associated with poor balance, slower walking speed and multiple falls per year (Lord et al., 1994, Shimada et al., 2011). There is no study currently, which investigates the impact of low muscle strength on falls in CP, despite studies showing that between 52-97% self-reported a fall in the previous 12-months (Morgan and McGinley, 2013b). With the high prevalence of falls in adults with CP, the occurrence of injuries such as fracture, dislocations and muscle tears from a fall have also been highlighted as a concern (Morgan et al., 2015). It is therefore of no surprise that adults with CP, including those who did not fall, had a higher fear of falling than elderly non-fallers scoring 55.1% higher on the Falls Efficacy Scale–International (FES-I), suggesting they perceive a greater risk of falling still (Morgan and McGinley, 2013a, Butler et al., 2015, Kempen et al., 2008). Because of the high prevalence of falls and the psychophysiological falls risk factors that are present in other CP populations (Morgan et al., 2015) methods to further reduce risk of falls even in physically active adults with CP should be investigated.

1.5 Skeletal Implications of Cerebral Palsy

The lower limb muscle strength in people with CP, particularly during puberty, will likely have a negative influence on the development of the bone matrix due to a decrease in loading on the bone (Rauch et al., 2004). Where studies have shown children and adults with CP to have a 2.3-5.5 fold greater risk of experiencing acute fractures and the decrease in quality of life associated with fracture (Sheridan, 2009, Mergler et al., 2009). This follows in accordance with the 'Mechanostat theory' Finbråten et al. (2015) provided evidence that reduced levels of ambulation in 51 children with CP (ages 8-18 yrs.) reduces bone health, with 11/15 (71%) of non-ambulatory children with CP having low bone mineral density ((BMD), BMD Z-score <-2), whereas low BMD was only observed in 12/36 (31%) ambulatory children with CP. Although BMD is higher in ambulatory children with CP compared to non-ambulatory children, it remains lower than age matched controls. Unay et al. (2003) showed, for example, lower BMD of 0.438 ± 0.17 $g \cdot cm^{-2}$ in 18 ambulatory children with spastic CP, compared to 0.502 ± 0.15 $g \cdot cm^{-2}$ in healthy controls. Other risk factors in CP that accompany differences in joint mobility and the frequency of weight bearing on the lower limbs are: poor diet (Chad et al., 2000), insufficient exposure to sunlight (Unay et al., 2003), and as a side effect of anticonvulsant medication (Weinstein et al., 1984). However, consistent with the Mechanostat theory, people with CP do show some association between PA and markers of bone health. Chad et al. (2000) found that after an 8-month PA intervention in nine children (n=6 ambulatory, 3 non-ambulatory) bone mineral content increased by 11.5% from 8.55 ± 1.32(g) at baseline to 9.53 ± 1.43(g) in the proximal femur.

Presently there is bone health data presented in children (Finbråten et al., 2015), adolescents (Henderson et al., 2002) and adults (Sheridan, 2009) with CP, but only adult swimmers in the para-sports populations (Blauwet et al., 2017). However, given the multifaceted contributors to lower bone health in CP, it may be assumed that bone health will be lower in the paretic limbs of athletes with CP when compared to controls. It is vital to identify modifiable risk factors during the peak bone mass accrual stage during maturation as a greater fracture risk in adults with CP than in children could be assumed, as the expected aging decline in bone mass is superimposed upon the already compromised bone health in CP (Mergler et al., 2009, Sheridan, 2009). However, the presence of lower bone health in athletic populations with CP could increase the risk of developing injures such as stress fractures or more acute traumatic fractures from falls and impact. Based on the finding of Finbråten et al. (2015), Chad et al. (2000) and de Groot et al. (2012), an increase in PA or structured force development through exercise programmes would aid in the development of bone health in populations with CP, where it has been estimated that a 10% increase in bone mass can decrease fracture risk by up to 50% (Sheridan, 2009).

As well as PA and exercise, another method that is often used to combat poor musculoskeletal health in adult and elderly populations is vitamin D supplementation (Bischoff-Ferrari et al., 2004) and more recently vitamin D is being used to optimise performance in athletic populations (Close et al., 2013). There is however, scant information on the level of impact that vitamin D can have on musculoskeletal health and performance within ambulatory para-sport, and is yet to be explored within physically active ambulatory men with CP.

1.6 Vitamin D

Vitamin D is a group of fat-soluble secosteroid (pro)-hormone, primarily responsible for the homeostasis of intestinal absorption of calcium and phosphate (Cesari et al., 2011). In vertebrates, the most notable compounds of vitamin D are vitamin D_3 (cholecalciferol) and vitamin D_2 (ergocalciferol) (Cesari et al., 2011). Vitamin D_3 is predominantly synthesised endogenously in the skin via solar ultraviolet beta (UV b) radiation exposure (wavelength 290-315 nm), it normally accounts for over 80% of circulating vitamin D (Larson-Meyer and Willis, 2010). Whereas vitamin D_2 is derived from plant origin and obtained through the diet (Boland, 1986). In terms of physiological impact, there have been links in typically developed adults between low vitamin D intake and impairments in numerous musculoskeletal outcomes (Girgis et al., 2015, Girgis et al., 2013), with endogenous levels dependent on the aforementioned external and environmental factors such as latitude, season and diet.

1.7 Guidelines for Dietary Vitamin D Intake

The guidelines for recommended dietary vitamin D intake are variable across the general adult population, these values are in addition to adequate sun exposure (see Table 1-2) in order to prevent vitamin D insufficiency (21-29 $ng \cdot mL^{-1}$) and deficiency (<20 $ng \cdot mL^{-1}$) (Table 1-1) (Ogan and Pritchett, 2013a). These guidelines are set out by the Institute of Medicine (IOM) and suggest that the estimated average requirement (EAR) is 400 $IU \cdot d^{-1}$ (10 µg) for males and females of any age and who are not in demographics that preclude them to be risk of vitamin D deficiency (Holick et al., 2012). On the other hand, the Endocrine Practice Guidelines Committee (EPGC) have

created guidelines for patients at risk of vitamin D deficiency (those with illness or diseases that inhibit vitamin D absorption, synthesis and metabolism) so that the suggested recommended daily amount (RDA) for adults ages >19 years old at risk of vitamin D deficiency is 1500-2000 $IU \cdot d^{-1}$ (37.5- 50 µg) (Holick et al., 2011).

Table 1-1. Major circulating metabolite 25(OH)D values to corresponding vitamin D status. Values in $ng \cdot mL^{-1}$ and $mmol \cdot L^{-1}$. **(Holick, 2007, Bischoff-Ferrari, 2007)**.

25(OH)D Status	$ng \cdot mL^{-1}$	$mmol \cdot L^{-1}$
Deficient	<20	<50
Insufficient	21-29	51-74
Adequate	30-40	75-100
Optimal	>40	>100

1.8 Dietary Sources of Vitamin D

Vitamin D can most commonly be found in oily fish, dairy products, meat and mushrooms (Holick, 2007). However, vitamin D dosage from food is relatively low and it is difficult to consume enough from diet alone, as food sources that contain vitamin D tend to only have small dosages (Bischoff et al., 1999) (Table 1-2), consequently, leading to individuals requiring over-the-counter vitamin D_2 and D_3 supplements.

Table 1-2. Examples of vitamin D content from natural sources (Bischoff et al., 1999, Holick, 2007).

Source	Vitamin D content
Natural sources	
Salmon	
Fresh, wild (3.5oz)	Approx. 600-1000 IU of vitamin D_3
Fresh, farmed (3.5oz)	Approx. 100-250 IU of vitamin D_3 or D_2
Canned (3.5oz)	Approx. 300-600 IU of vitamin D_3
Sardines, canned (3.5oz)	Approx. 300 IU of vitamin D_3
Mackerel, canned (3.5oz)	Approx. 250 IU of vitamin D_3
Tuna, canned (3.6oz)	Approx. 230 IU of vitamin D_3
Cod liver oil (1 tsp)	Approx. 400-1000 IU of vitamin D_3
Shitake mushrooms	
Fresh (3.5oz)	Approx. 100 IU of vitamin D_3
Sun-dried (3.5oz)	Approx. 1600 IU of vitamin D_3
Egg yolk	Approx. 20 IU of vitamin D_3 or D_2
Exposure to sunlight, ultraviolet B radiation (0.5 minimal erythemal dose) (I.e. 15 minutes).	Approx. 3000 IU of vitamin D_3

1.9 Vitamin D Synthesis and Metabolism

Given that sources of dietary vitamin D are limited, exposure to sunlight and thus UV b should be considered before the supplementation of vitamin D, as just 15 minutes of direct exposure can provide sufficient vitamin D to exceed the daily recommended amount (Table 1-2) (Holick et al., 2012). Vitamin D from sunlight exposure is formed when solar UV b radiation penetrates the skin and converts 7-dehydrocholesterol to pre-vitamin D_3 (Holick, 2007). With heat, pre-vitamin D_3 is rapidly converted to vitamin D_3, solar UV b destroys any excess pre-vitamin D_3 and vitamin D_3 so that excessive exposure to sunlight cannot cause hypervitaminosis D (Christakos et al., 2012). Dietary vitamin D_2 is manufactured through the UV b irradiation of ergosterol from yeast and transported via chylomicrons. Vitamin D_3 and D_2 (henceforth D_3 and D_2 is represented by D) from endogenous synthesis and diet is initially biologically

inactive (Dahlquist et al., 2015). Vitamin D binds to vitamin-D-binding proteins to circulate within the blood stream (Haussler, 1986).

Circulating inactive vitamin D is metabolised in the liver by the enzyme vitamin D-25-hydroxylase to become 25-hydroxyvitmain D (25(OH)D) the major circulating metabolite of vitamin D which is used to assess vitamin D status (Gallagher et al., 1979). 25(OH)D is then further metabolised in the kidneys by 1-α-hydroxylase to become vitamin D's active form, 1,25-dihydroxyvitamin D (1,25 $(OH)_2$ D) (Lips et al., 2006). 1,25$(OH)_2$D is then able to directly bind to vitamin D receptors found within the body, including on skeletal muscle (Ogan and Pritchett, 2013b), whereas bone is impacted by 1,25$(OH)_2$ D indirectly via the endocrine pathway (see further details below). Vitamin D receptors exist in human skeletal muscle tissue, signifying that 1,25$(OH)_2$ D has a direct impact on skeletal muscle activity (Dahlquist et al., 2015). The interaction of 1,25$(OH)_2$ D with vitamin D receptors, such as protein synthesis, cell proliferation and differentiation signify that vitamin D may be an important factor regarding muscle function and size (Figure 1-3).

The endocrine (indirect) pathway of 1,25$(OH)_2$ D mediates the regulation of the intestinal absorption of calcium from 15% to 40% and phosphorous to 60% from 80% in the presence of vitamin D (Holick, 2007). Parathyroid hormone (PTH) increases 25(OH)D synthesis into 1,25$(OH)_2$D when concentrations of active vitamin D are low and increases calcium reabsorption from bone. Note however that higher concentrations of PTH are often harmful to bone health (Kuchuk et al., 2009). A high concentration of 1,25$(OH)_2$D will lead to sufficient intestinal calcium absorption. As absorption of calcium is not required, 1,25$(OH)_2$D will reduce the rate of its own

synthesis via negative cutaneous feedback. This occurs via a decrease in the production and secretion of PTH, to then stop unnecessary metabolism of 25(OH)D into 1,25$(OH)_2$ D (Vieth et al., 2001) (Figure 1-2). A reduction of PTH, inhibits calcium reabsorption from bone matrix as calcium is not needed to be sourced from bone, therefore bone density remains unaffected whilst vitamin D concentrations are adequate (>30 ng·mL^{-1}) (Figure 1-2 and 1-3).

The different pathways in which vitamin D directly and indirectly interacts within the musculoskeletal system means that there is not a linear relationship between vitamin D and both muscle function and bone health (Sai et al., 2011). Serum 25(OH)D level of 30 ng·mL^{-1} is often used as the defining threshold for vitamin D insufficiency as serum PTH has shown a plateau at serum 25(OH)D of approximately 30 ng·mL^{-1} (Halfon et al., 2015) and therefore bone health is not compromised from elevated PTH. However, there is currently no physiological marker (such as PTH) that denotes a threshold for improvements in muscular performance. It has however been suggested by Janssen et al. (2013) that associations between higher 25(OH)D and lean muscle mass, size and performance were most pronounced below 60 mmol·L^{-1} (24 ng·mL^{-1}) and absent above 60 mmol·L^{-1} (24 ng·mL^{-1}), indicating both a bottoming and a ceiling effect. This notion suggests that there is likely differing thresholds for 25(OH)D to have an impact on muscle and bone.

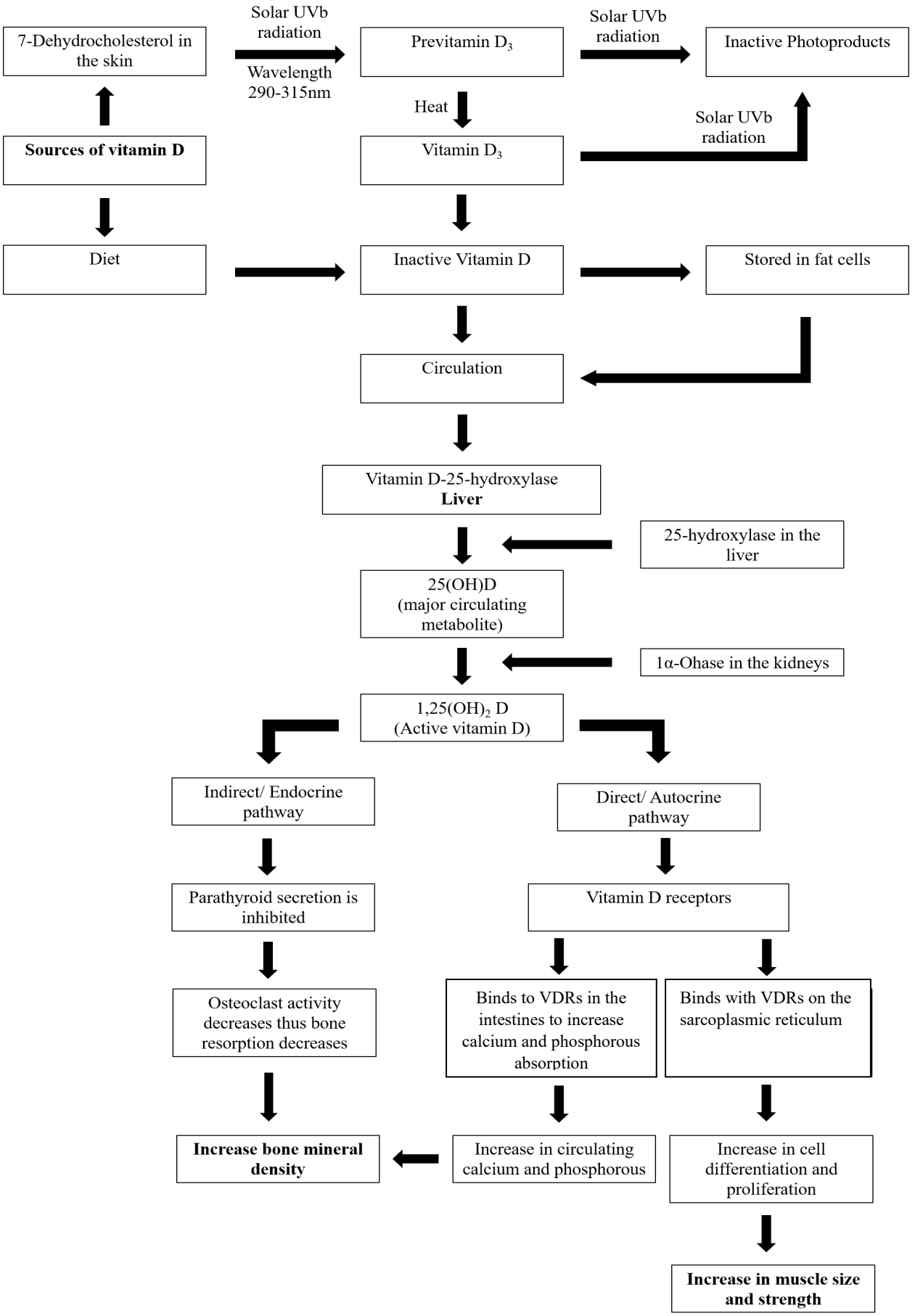
7-Dehydrocholesterol in the skin
Solar UVb radiation
Wavelength 290-315nm
Previtamin D_3
Solar UVb radiation
Inactive Photoproducts
Heat
Solar UVb radiation
Sources of vitamin D
Vitamin D_3
Diet
Inactive Vitamin D
Stored in fat cells
Circulation
Vitamin D-25-hydroxylase
Liver
25-hydroxylase in the liver
25(OH)D
(major circulating metabolite)
1α-Ohase in the kidneys
1,25$(OH)_2$ D
(Active vitamin D)
Indirect/ Endocrine pathway
Direct/ Autocrine pathway
Parathyroid secretion is inhibited
Vitamin D receptors
Osteoclast activity decreases thus bone resorption decreases
Binds to VDRs in the intestines to increase calcium and phosphorous absorption
Binds with VDRs on the sarcoplasmic reticulum
Increase bone mineral density
Increase in circulating calcium and phosphorous
Increase in cell differentiation and proliferation
Increase in muscle size and strength

Figure 1-2. The pathway in which vitamin D is sourced, metabolised, and used within the musculoskeletal system for normal physiological functioning. (Dahlquist et al., 2015, Holick, 2007, Cannell et al., 2009, Vieth et al., 2001).

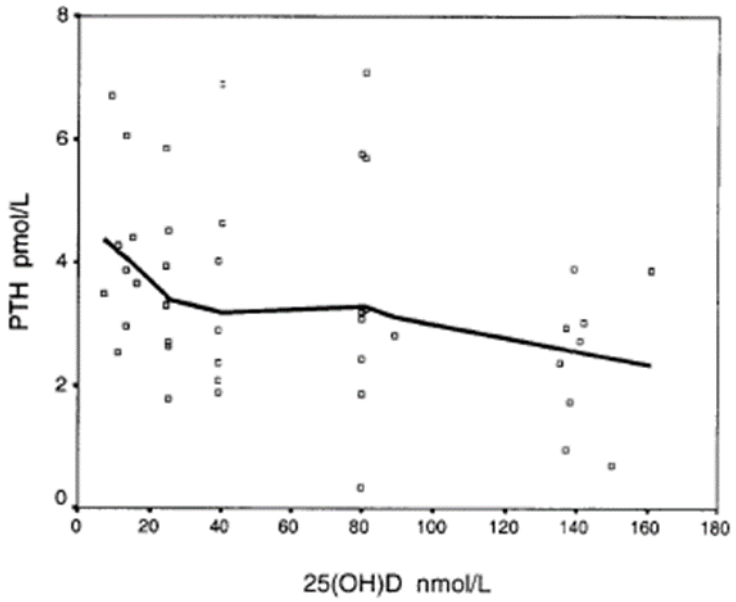

Figure 1-3. The relationship between PTH pmol·L^{-1} and serum 25(OH)D mmol·L^{-1} (p<0.05) (Vieth et al., 2001).

1.10 Vitamin D Deficiency and Seasonal Variations in the UK

Endogenous vitamin D_3 synthesis is severely decreased at latitudes over 35ºN during winter months (December-February in the northern hemisphere) and individuals at these latitudes are susceptible to vitamin D deficiency (Morton et al., 2012). Indeed 74.5% of the UK population is impacted by low vitamin D levels (the UK ~53ºN), comprising of 33.7% who are vitamin D insufficient (25(OH)D levels 29-21 ng·mL^{-1}) and 40.8% who are vitamin D deficient (25(OH)D <20 ng·mL^{-1}) (Lips, 2010). Cannell et al. (2009) highlighted that a UK cohort of males and females with a mean age of 45 years, were only sufficient in vitamin D concentrations (25(OH)D levels >30 ng·mL^{-1})

during their average peak in September. Unsurprisingly, vitamin D concentrations were low for all other months and during February and March concentrations were at their nadir (~15 ng·mL^{-1}, Figure 1-4). Predominant reasons for reduced vitamin D levels are due to insufficient dietary intake and restricted outdoor exposure to sun light (Lips, 2001).

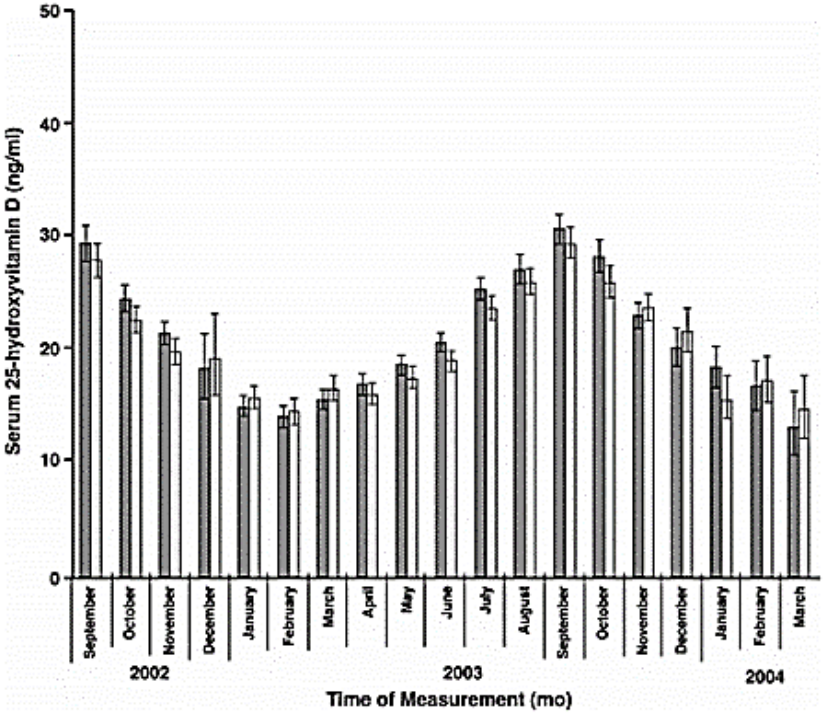

Figure 1-4. Average monthly variations in serum 25(OH)D concentrations.
Men (dark shade) and women (light shade) in a British cohort at ages 45yrs (Cannell et al., 2009).

At enhanced risk of Vitamin D insufficiency/deficiency-populations include those with disability, as shown by Oleson et al. (2010) and Flueck et al. (2016). Oleson et al. (2010) found that there is a high prevalence of vitamin D insufficiency (96.4% during

the winter and 81% in the summer) in Swiss individuals living at a latitude of 47ºN with spinal cord injuries (n=96). Even those with disabilities who undertake regular PA show high incidence of vitamin D insufficiency. For example, 83.7% of 72 Swiss elite wheelchair athletes (living at a latitude of 47ºN above the 35ºN threshold) were vitamin D insufficient during winter months (Flueck, 2016). This is comparable to research by Zürcher et al. (2018) who showed insufficiency in 51% of 603 Swiss Olympic athletes with no physical impairment. The prevalence of vitamin D deficiency is summarised below by latitude (Figure 1-5) and by population (Appendix 1-1). Although it is impossible to clearly delineate the impact of disability on vitamin D, where studies have presented vitamin D levels in controls compared to disability, of these seven studies, six show those with disability to have a higher incidence of insufficiency that control. None of the studies in this comparison researched if low vitamin D is prevalent in a population with CP.

The likely factors for lower vitamin D in athletes with disabilities could be attributed to differences in the main determining factors; 1) outdoor exposure to sunlight (2) dietary vitamin D intake (3) Level of PA. Although not directly related to outdoor activity, measures of PA are lower and sedentary behaviour is higher in adults with disabilities (Longmuir and Bar-Or, 2000). Jacques et al. (2018) reported that ambulant individuals with limb-girdle muscular dystrophy spent 29.6% less physically active during their waking hours, compared to healthy age matched controls. It could be hypothesised that low levels of PA may reflect less outdoor time, hence putting them at a higher risk of vitamin D insufficiency; however, this remains to be confirmed experimentally. It is clear that wheelchair athletes who undertake indoor sports have significant vitamin D deficiency compared to those who compete in outdoor sports

(Magee et al., 2013). In addition, measures of PA, sedentary behaviour and exercise contribute to improving musculoskeletal health and function, potentially offsetting some of the impairments associated with lower vitamin D.

Dietary vitamin D intake is the second determinant of endogenous vitamin D that needs to be considered when discussing vitamin D deficiencies. The current recommendations for dietary intake of vitamin D in 19-50 year olds is 400-600 IU·day^{-1} to benefit musculoskeletal function (Holick et al., 2012). Only a few foods, such as oily fish, naturally contain vitamin D and these tend to be relatively low dosages, consequently it is difficult to meet these recommendations. Within the general UK population, dietary intakes of vitamin D range from 168-291 IU·d^{-1} (3.5-6.39 µg·d^{-1}) and 140-255 IU·d^{-1} (4.2-7.28µg·d^{-1}) in adult males and females respectively, contributing to the 40.8% of the UK population who are vitamin D deficient (Lips et al., 2006). The data concerning vitamin D insufficiency and deficiency are taken from control populations around the world (Figure 1-5) which shows that there is no particular pattern but shows a prevalence of low vitamin D in the population mean data in either disabled populations or typically developed controls. Given the effect of low PA levels, poor diet and lack of sunlight, it is likely that the effect is similar in disabled populations, however this is unreported.

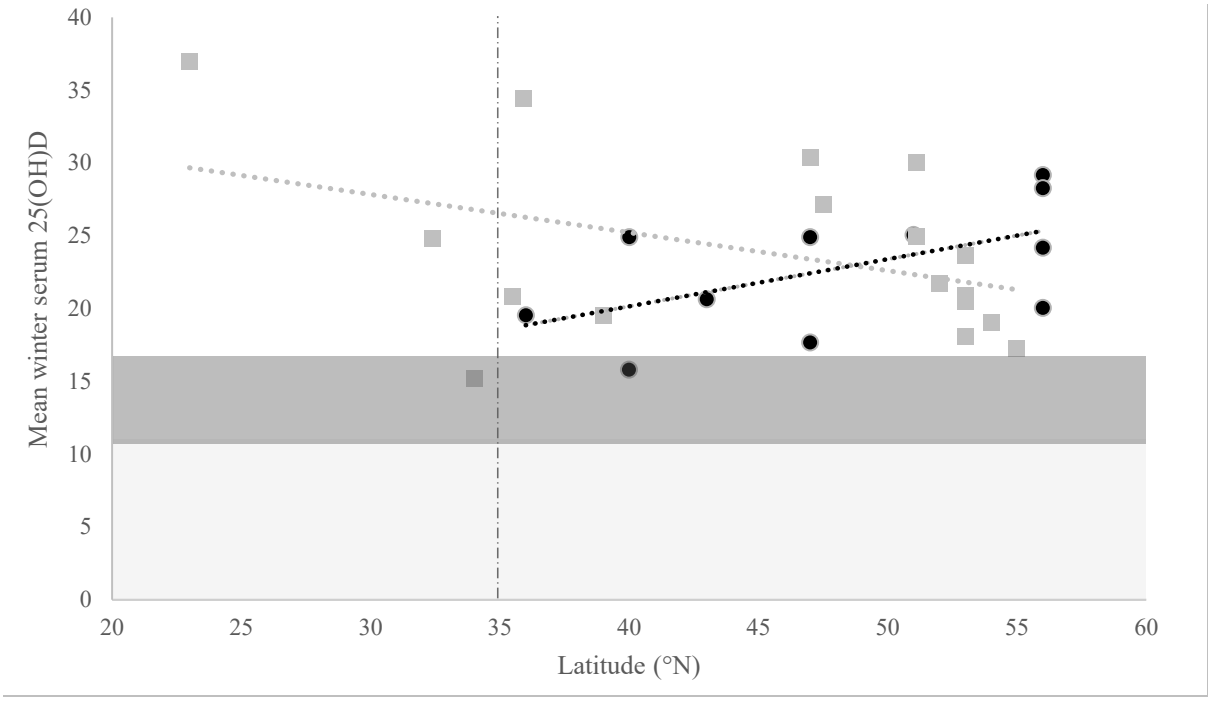

Figure 1-5. Serum 25(OH)D concentrations during the winter months as a factor of latitude (See individual study data in Appendix 1-1). Typically developed controls (grey square) and groups with disabilities (Black circle). Each data point represents the mean from a single study as described in Appendix 1-1. The grey dotted trend line denotes the means of TDC group, and the black dotted trend line denotes the group with disabilities. The dark grey shaded area denotes the threshold for vitamin D insufficiency and the light grey shaded area denotes threshold for deficiency. The vertical line denotes 35°N threshold, where any latitude above is impacted by seasonal variations in UV b.

1.11 Musculoskeletal Impact of Vitamin D and Low Vitamin D

Vitamin D levels account for observed variance in musculoskeletal outcome measures, with known implications for injury in otherwise healthy adults (Ogan and Pritchett, 2013b). Low levels of 25(OH)D are associated with low muscle strength

(Visser et al., 2003), muscle size (Girgis et al., 2013) and reduced bone mass (Mezquita-Raya et al., 2001) with known seasonal variations in these musculoskeletal measures and fluctuations in vitamin D (Cannell et al., 2009). The direct relationship between vitamin D and VDRs found on the sarcoplasmic reticulum in muscle is likely to account for decreases in muscle size and strength, when vitamin D is found to be low (Ceglia, 2008). Gilsanz et al. (2010) measured the size of the thigh muscle in 90 post pubertal who were separated into a serum 25(OH)D deficient (n=53) group and adequate (n=37) group. The deficient women showed a lower thigh muscle size of 83.7±34.0 cm^2 compared to 93.0±33.5 cm^2 in the adequate group. Likewise, Foo et al. (2009) examined the relationship between 25(OH)D status and muscle strength in Chinese adolescent women (n=301) and found that low 25(OH)D status (<20 $ng \cdot mL^{-1}$) was associated with reduced forearm strength, when compared to individuals with adequate 25(OH)D levels.

The inverse relationship of PTH and serum 25(OH)D is likely to have an adverse impact on bone health, if an individual has low vitamin D (Vieth et al., 2001). The increased secretion of PTH facilitates bone reabsorption to correct low concentrations of circulating calcium, that decrease from poor intestinal absorption from low vitamin D (Vieth et al., 2001). As a consequence of low vitamin D, BMD is likely to be diminished (Lappe et al., 2013). Sato et al. (1997) studied a cohort of 71 Japanese adults with Parkinson's disease, split in groups depending on vitamin D status. The insufficient group (n=20) showed higher Z scores -0.648±1.197, when compared to the deficient group (n=51) who exhibited significantly lower Z scores -1.610±1.624. With reduced BMD the prevalence of injuries such as stress fractures and breaks are increased, particularly in elderly and disabled populations who

experience acute impact from falls (Greenspan et al., 1998) and PA individuals who experience repetitive loading on the lower limb (Lappe et al., 2008). In 22 navy recruits that had lower limb stress fractures, 81.8% had low vitamin D (<30 $ng \cdot mL^{-1}$) and the risk of stress fracture in the 734 recruits with low serum 25(OH)D levels had increased the risk of developing fractures by 3.6 fold (Lappe et al., 2008). Increased risk of acute bone breaks from reduced BMD is a particular issue in populations who are at risk of experiencing falls such as the elderly (Nordström et al., 2011) and individuals with CP (Morgan and McGinley, 2013b), where injurious falls are associated with a greater fear of falling and reduced PA impacting quality of life (Li et al., 2003). There are also established links between lower vitamin D and injury occurrence within Paralympic athletes (Magee et al., 2013). Combined with the potential synergistic influence of reduced PA on these outcome measures, the low vitamin D associated with reduced UV b during winter months may accentuate physical impairments in athletes with disabilities and could increase their risk of musculoskeletal injury. The multifaceted nature of injury, musculoskeletal function and vitamin D within athletes with disabilities is unreported, particularly considering low vitamin D that may be present within this population.

Chapter 2 – Aims, Objectives and Hypotheses

2.1 Thesis Aim:

The aim of this present study is to increase our understanding of musculoskeletal outcomes in men with CP as it relates to the role of vitamin D and falls prevalence.

2.2 Objectives:

1) Investigate falls in young men with CP who are otherwise healthy and physically active compared to physical activity matched typically developed controls (TDC) without neuromuscular impairments.

2) Distinguish what outcome measures account towards falls in men with CP.

3) Identify if physically active young men with CP have a higher fear of falling compared to TDC.

4) Address if injuries are more prevalent in physically active men with CP who fall compared to non-fallers with CP.

5) To determine the impact of diet, time spent outdoors, PA and vitamin D status in men with CP compared to TDC without neurological disabilities.

6) To determine the role of vitamin D on musculoskeletal health in men with CP compared to TDC.

7) To determine differences in seasonal variations in vitamin D between men with CP and TDC.

8) To investigate if there are seasonal variation in vitamin D in physically active, ambulatory men with CP on neuromuscular performance outcomes.

9) To investigate if there are seasonal variation in vitamin D in physically active, ambulatory men with CP on PTH and bone ultrasound T_{us} and Z_{us} Scores.

2.3 Hypotheses:

1) Men with CP experience more falls compared to TDC.

2) Weaker knee extensor strength will increase risk of falls in men with CP.

3) Men with CP will have higher fear of falling compared to TDC.

4) Men with CP who fall will experience more injuries than non-fallers with CP

5) Men with CP will have lower levels of vitamin D compared to TDC.

6) Lower vitamin D will lead to reduced musculoskeletal health in men with CP.

7) Men with CP will experience a smaller magnitude of change of seasonal variations in vitamin D.

8) Men with CP will see improvements in neuromuscular performance outcomes during the summer months.

9) Men with CP will see improvements in tibia and radius T_{us} and Z_{us} scores during the summer months.

Chapter 3 - Falls, Fear of Falling and Muscle Strength in Physically Active Ambulatory Men with Cerebral Palsy

Abstract

Purpose: To assess and describe differences in lifestyle factors, neuromuscular outcomes, and the psychological impact of falls in men with cerebral palsy (CP). **Materials and Methods:** Nineteen, ambulant, physically active men with CP aged 21.9±2.9 (mean±SD) years (Gross Motor Function Classification System I-II) and 19 activity-matched typically developed controls (TDC), without neuromuscular impairment aged 25.4±2.7 years, completed knee extension isometric maximal voluntary contractions (KE iMVC), plus falls frequency questionnaires to measure fear of falling and risk-taking behaviour. **Results:** Men with CP had a 24.6% weaker KE iMVC, fear of falling was 26.2% greater and risk-taking behaviours was 14.5% lower compared to TDC (all $p<0.05$). 47% of CP were classified as fallers. CP fallers had similar KE iMVC and KE iMVC/BM compared to CP non-fallers ($p=0.117$ and $p=0.296$ respectively). CP fallers had 44% lower KE iMVC ($p<0.05$) but similar KE iMVC/BM compared to TDC ($p=0.545$). KE iMVC and KE iMVC/BM strength was similar between CP non-fallers and TDC ($p=0.458$). **Conclusion:** Young, physically active men with CP showed a high prevalence of falls and a weakness of the knee extensors, fear of falling was higher than that of TDC but was lower compared to previous reports in CP fallers. Despite this, fear of falling scores reported would still be defined as clinically high (>23), like that of older fallers. The incidence of falls and fear of falling in this highly active group suggests that the issue of falls in CP is currently underrepresented by previous research predominantly in adults aged 30+.

Key words – Cerebral palsy, Fallers, Strength, Non-fallers, Fear of falling

3.1 Introduction

Adults with cerebral palsy (CP) show earlier declines in of gait and balance quality compared to their typically developed counterparts, despite the underlying pathology being non-progressive (Michael-Asalu et al., 2019, Morgan and McGinley, 2014). These declines in balance and functional tasks are linked to a high prevalence of falls in adults with CP aged 30-65 yrs. (Morgan and McGinley, 2013b). Yet it is not the diagnostic symptom of muscle spasticity that underlies this fall prevalence, as falls in adults with CP are not associated with the severity of lower limb spasticity (Morgan et al., 2016). In contrast, muscle weakness, rather than spasticity, is associated with gross motor function impairment in adults with CP (Hong et al., 2012). It is possible that this disassociation between falls and spasticity in CP is due to muscle weakness, particularly unilateral weakness, which has long been linked to higher fall prevalence in the elderly (Perry et al., 2007). There are at present no such data for adults with CP despite between 52-97% self-reporting a fall in the previous 12-months (Morgan and McGinley, 2013a, Morgan et al., 2016, Opheim et al., 2012, Morgan et al., 2015, Morgan and McGinley, 2013b). This is a particular concern as Whitney et al. (2018) found that adults with CP aged between 18-30 years are seven times more likely to experience musculoskeletal morbidities including osteopenia, osteoporosis, and sarcopenia compared to TDC. Where these issues become apparent in (Morgan et al., 2015) who highlighted a high prevalence of falls in adults with CP and consequently the occurrence of injuries such as fracture, dislocations and a torn muscle from these falls. Alarmingly, individuals who suffer from reoccurring falls are at higher risk of experiencing more severe injuries (Ward et al.,

2015). Therefore, it is important to identify physiological and psychological contributors to the occurrence of potentially serious injurious falls in adults with CP, to identify appropriate interventions.

Muscle weakness is prevalent in adults with CP (Hussain et al., 2013), thus gait patterns are altered as a consequence of spasticity and muscle weakness, compared to healthy adults without neuromuscular impairment (Moreau et al., 2009, Andersson et al., 2003). Physically active (PA) men with CP have substantially reduced muscle strength of the plantar flexors compared to physically active men without CP (Overend et al., 1992), with the extent of the strength deficit being greater for the affected vs. unaffected limb (Hussain et al., 2013). This unilateral imbalance and weaker lower limb muscle strength has previously been associated with fall prevalence in the elderly, with knee extension (KE) weakness being associated with poor balance, slower walking speed and multiple falls per year (Lord et al., 1994, Shimada et al., 2011). Despite CP being a "non-progressive" condition (Shevell and Bodensteiner, 2004), it is of concern that longitudinal declines in walking ability, likely a result of muscle weakness, are observed in younger adults with CP aged 22-43 yrs old (Morgan and McGinley, 2014).

Numerous studies have shown that muscle weakness is part of a multifaceted group of risk factors, which can increase risk of falling (Ambrose et al., 2013, Moreland et al., 2004, O'Loughlin et al., 1993). In elderly populations, individuals with greater muscle weakness are more likely to experience reoccurring falls (Moreland et al., 2004) while adults with Parkinson's disease have a higher fear of falling (Mak et al., 2012). Older adults who report greater fear of falling, as indicated by a higher score

on the Falls Efficacy Scale–International (FES-I), often show avoidance behaviours of day-to-day tasks and reduced risk-taking behaviours based on the everyday risk-taking scale (Butler et al., 2015). Kempen et al. (2008) found that compared to non-fallers, single fallers and individuals who fell often, scored 49.5 and 73.1% higher on the FES-I, respectively. Unlike the elderly, adults with CP who had experienced falls scored only 6.65% higher on the FES-I scale compared to CP non-fallers (Morgan and McGinley, 2013a). Adults with CP however, including those who did not fall, had a higher fear of falling than elderly non-fallers scoring 55.1% higher on the FES-I, suggesting they perceive a greater risk of falling still, without the experience of a fall in the last 12-months (Morgan and McGinley, 2013a, Butler et al., 2015, Kempen et al., 2008). These data however, are from sedentary adults with CP, not physically active adults with CP. It is possible therefore that differences in lifestyle factors such as PA levels in adults with CP will impact muscle strength and risk of experiencing falls (Ross and Engsberg, 2007, Gianoudis et al., 2015, Moreira et al., 2018).

Due to the complex relationship between fear of falling and physiological contributors of falls in adults with CP, it is important to gain an understanding that could help the development of fall prevention interventions, as adopted by other populations who experience falls (McClure et al., 2005). Therefore, the aims of this study are to: 1) investigate falls in young men with CP who are otherwise healthy and physically active compared to physical activity matched typically developed controls (TDC) without neuromuscular impairments; 2) distinguish what outcome measures account towards falls in men with CP; 3) identify if physically active young men with CP have a higher fear of falling compared to TDC; and 4) address if injuries are more prevalent in physically active men with CP who fall compared to non-fallers with CP.

3.2 Materials and Methods

Nineteen, ambulatory men with spastic CP were recruited via The Football Association[2]; 6/19 were diplegic and 13/19 were hemiplegic (Table 3-1). Nineteen men without neurological impairment were recruited as the TDC group (Table 3-1). All participants provided written informed consent, after a local ethics committee approved the study.

Table 3-1. Classification and impairment details of participants.

		CP Diplegic n=6	**CP Hemiplegic n=13**	**CP Total n=19**	**TDC n=19**
GMFCS	I	-	13	18	-
	II	6	-	6	-
Side measured	Left	5	4	9	4
	Right	1	9	10	15

CP, Cerebral Palsy; TDC, typical developed controls; GMFCS, Gross motor function classification score.

3.2.1 Protocol

Participants completed two forms of assessment, the first was an *in vivo* assessment of muscle function, which was completed on a single data collection session. The second was a series of online surveys to assess falls, fear of falling and fall-related injuries. Muscle function and falls surveys were completed within 1 month of each

[2] Although there is overlap within the recruitment of CP participants between chapters, not all participants contributed to every chapter, each chapter therefore represents a CP population from a broad sample of those players who were available for testing.

other between January 2020 and March 2020, where the muscle function tests were completed before the falls screening.

3.2.2 Physiological Measures

Height (m) was measured in a standing position using a stadiometer (Seca 213, portable stadiometer, Hamburg, Germany). Body mass (BM in kg) was measured via a set of digital scales (Seca, Hamburg, Germany). Knee extensor isometric maximal voluntary contraction (KE iMVC) was recorded using a custom-made portable load cell. Prior to the tests, all participants were taken through a standardised warm up, which aimed to increase heart rate to over 120 bpm and included dynamic and static stretches of the lower limbs. Participants were given two attempts at each test, with a one-minute rest in between and the best result was recorded. Participants were seated with their arms across their chest and the load cell attached around the dominant leg (or the most impaired side for individuals with CP) with the knee at 90° flexion. Participants maximally contracted their KE for approximately three seconds until a visible plateau was observed on the force-time trace. The force (N) produced was digitised using an analogue-to-digital converter, displayed by a self-displayed and coded program using My LabVIEW (National Instruments, Berkshire, UK).

3.2.3 Falls Screening

Using online surveys (JISC, Portwall Lane, Bristol, UK) two falls screening surveys were distributed to all participants. Participants were provided with an individualised code to use in their surveys to ensure anonymity (e.g., EXP-01). These surveys were a 12-month falls recall screening survey and a 1-month falls recall screening survey that were designed to align with Morgan et al. (2015), where a fall was defined to the

participants as "*an event which resulted in you losing your balance and coming into contact with the ground or a lower level that could not have been prevented*" (Lamb et al., 2005). In line with previous studies in elderly populations (Peel, 2011) and to allow comparison to the only other CP falls data from Morgan et al. (2015), a 'faller' was defined as "an individual who had fallen more than twice in a 12 month period", if not, they were referred to as a 'non-faller'.

3.2.4 12-Month Falls Screening Survey

This survey (totalling 62 questions) was designed using: 1) Falls diary and injuries, 12-month recall; 2) the fear of falling questionnaire (i.e., FES-I); 3) Risk-taking behaviours questionnaires; 4) self-perceived mobility change since 18; and 5) habitual PA (Appendix 3-5).

(1) The falls diary and injuries, 12-month recall – incorporated 27 questions based on the recall of falls and near falls over the last 12 months prior to completing this survey. Questions were developed consistent with previous falls studies in the elderly and CP (Morgan and McGinley, 2013b, Morgan et al., 2015, Delbaere et al., 2010a). These questions included: frequency of falls or near falls, how the most recent occurred (i.e. trip, slip or stumble), perceived causes (i.e. uneven floor, a pet, slippery surface), where the most recent fall(s) occurred (i.e. indoors and outdoors at home or in the community) and whether alcohol was a contributing factor. This is followed by questions on injuries caused by falling or near falls and if so, where on the body was injured (i.e. arms, shoulders, wrists etc.), in what way (i.e. cut,

bruise or break) and finally what kind of medical attention was required (i.e. self-treated, hospital or no treatment).

(2) The FES-I is a 16-item questionnaire of fall-related self-efficacy and is a measure of fear of falling based on the FES (10 items) but extended by 6 additional items to include higher functional issues and social aspects of falls. The FES-I has 4 answer options of: 1 -not at all, 2- somewhat, 3- fairly, 4- very concerned, with the scores ranging between 16 to 56. Higher values indicate a greater concern of falling. Hauer et al. (2010) showed that ICCs for subsamples of persons with and without cognitive impairment ranged from moderate (ICC=0.58) to high (ICC=0.92) reliability for the FES-I.

(3) The risk-taking behaviours questionnaire was an 18-item questionnaire. Four of the questions were added from the health/safety domain of the Domain-Specific Risk-Taking (Adult) Scale (DOSPERT) (Blais and Weber, 2006) and 10 items from the everyday risk-taking scale used by Butler et al. (2015), with the addition of a four bespoke items for this study. Answers were scaled from: 1-Never, 2- Occasionally, 3-Mostly and 4-Always. The 18-item risk-taking behaviours questionnaire is scored as a whole between 18 and 72, with 72 being the highest risk-taking behaviours. The questions were also split into three subcategories which included: 1) Risk taking when performing day-to-day tasks, which require balance, 7-items scored between 7-28 (Inc. putting shoes and sock on, using a handrail going up and down stairs, the use of escalators, climbing up on furniture and ladders, moving around the house without lights on at night). 2) Daringness when crossing roads and using

public and private transport, 4-items scored between 4-16 (Inc. Catching a bus if you have to stand, crossing against the traffic lights, wearing seat belts and helmets on bikes). 3) Lifestyle choices, 7-items scored between 7-28 (Inc. drinking at social functions, sunbathing without sun cream, buying slippery shoes, running to answer a phone, and walking home in unsafe areas). Test-retest reliability of the 10-item everyday risk-taking scale was considered excellent in a subgroup of 40 adults (ICC=0.85; 95% CI=0.71–0.92) (Butler et al., 2015).

(4) Self-perceived mobility change was one single question to assess perceived walking ability compared to when they were 18 years old, individuals reported either unchanged, improved or worse walking ability (Morgan and McGinley, 2013b).

3.2.5 1-Month Falls Recall Screening Survey

The 1-month recall questionnaire was included to obtain more accurate information on recent injuries. This 20-question survey was designed using: 1) falls diary and injuries (1-month recall); 2) alcohol and lifestyle questionnaire; and 3) self-perceived and habitual (IPAQ) physical activity levels (Appendix 3-4).

(1) Falls and injury. The falls diary and injuries, 1-month recall – incorporated of 15 questions based on the recall of falls and near falls that were experienced in the one month prior to completing this screening. Questions included frequency of falls or near falls, how these occurred (i.e. trip, slip or stumble), perceived causes (i.e. uneven floor, a pet, slippery surface) and where the fall(s) occurred (i.e. indoors and outdoors at home or in the community). This

was followed by questions on injuries caused by falling or near falls and if so, where on the body was injured (i.e. arms, shoulders, wrists etc.), in what way (i.e. cut, bruise or break) and finally what kind of medical attention was required (i.e. self-treated, hospital or no treatment).

(2) Alcohol and lifestyle questionnaire. This included two questions that asked about consumption of alcohol in the last month and estimated weekly average of units the individual drank each week.

(3) Self-perceived physical activity level. This was one single question where individuals chose the level of physical activity, which best reflected them from the following options: sedentary (walking less than 20 minutes of PA per day), very active (VA) (undertake 40 minutes of moderate intensity PA per day) or athlete (high intensity exercise 5+ days a week). Schechtman et al. (1991) found this single question to be a valid means of categorising physical activity status within young adults. Due to limited participants within the 5 PA categories, the two highest and two lowest activity levels were combined. Therefore, PA groups are referred to as 'low sedentary', 'active' and 'VA athlete'. As only one participant was classed as sedentary, the low activity and sedentary classifications were omitted from analysis.

(4) Physical activity levels. Habitual PA was recorded through the international physical activity questionnaire-long form (IPAQ) (Appendix 3-2) 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. The long form IPAQ was seen to be valid showing a Cronbach's alpha of α=0.73 in adults ages 18-65 years (Mannocci et al., 2012) and had acceptable levels of reliability when compared to accelerometers in adults with neuromuscular disabilities, showing a strong correlation between the two methods for total PA time (p=0.55, p<0.001) (Ruescas-Nicolau et al., 2021).

(5) Behaviour changes from falls. This included two questions that asked individuals if they were worried about falling again and did they feel that they may change their behaviours or activities because of a fall.

3.2.6 Statistics

All quantitative analyses were performed using IBM SPSS Statistics software (v25). Where appropriate, parametric assumptions of normal distribution were confirmed using Shapiro-Wilk test's test (p<0.05) in all dependant variables. Comparisons of all men with CP and TDC were made using independent t-test. Group comparisons of CP fallers, CP non-fallers and TDC were made using one-way ANOVA with equal variance using Levene's test (p>0.05) in all dependant variables (fear of falling, and total risk-taking behaviours were p<0.05). For group difference in anthropometric and neuromuscular measures for CP fallers, CP non-fallers and TDC, Kruskal-Wallis tests were performed. Nominal data was assessed using Chi-square associations to highlight relationships between specific variables (e.g., fallers and total fear of falling score; fallers and total risk-taking behaviours score; fallers and KE iMVC; and total

fear of falling and total risk-taking behaviours). For the nominal variables, a significant outcome of Chi square is described as a significant association rather than a group difference based on previous recommendations (McHugh, 2013). Data are presented as mean±SD (95% confidence intervals [CI]) without subjective terminology and alpha levels are reported as exact *p* values, without dichotomous interpretation of 'significant' or 'non-significant' as advised by the American Statistical Association (Hurlbert et al., 2019). To acknowledge a low statistical power (See limitations 3.4.4) effect size was presented alongside *p* values using Cohens D (*d)* from independent t-tests and partial eta squared (η^2) taken from one way ANOVA's.

3.3 Results

3.3.1 Population Comparisons for all Men with CP and TDC

There was no statistical difference in stature (*p*=0.815, *d*=0.076), BM (*p*=0.266, *d*=0.367) or BMI (*p*=0.112, *d*=0.529) between men with CP or TDC (Table 3-2). Men with CP were 3.5 years younger compared to TDC (*p*<0.001, *d*=1.294, Table 3-2). Compared to TDC, the KE iMVC was 24.6% weaker in men with CP (*p*=0.008*, d*=1.001), whereas KE iMVC relative to BM (KE iMVC/BM) was not statistically different between the two groups (*p*=0.204, *d*=0.098, Table 3-2).

Based upon the falls classifications from Morgan et al. (2015), 47.4% of men with CP and 21.1% of TDC were classified as fallers (i.e. fell >2 times in the last 12 months). In the last 12 months, 84% of CP and 26% of TDC reported a near fall. The cause, obstacles, activities, and location of the individual's most recent fall are show in detail (Table 3-3). Of the four TDC who reported falls, none reported an injury, whereas

44.4% of CP fallers reported fall-related injuries (see Table 3-3 and Figure 3-1). One man with CP required a visit to accident and emergency after bumping their head. All other injuries either were self-treated or did not require medical attention.

Men with CP and TDC were PA matched based on IPAQ scores (p=0.253, d=0.377, Table 3-2). A chi squared test showed no relationship between IPAQ score and falls in men with CP ($\chi^2_{(14,19)}$=0.14 p=0.326) or TDC ($\chi^2_{(18,19)}$=19.00, p=0.392). The self-perceived PA levels reported in men with CP showed 15/19 (79%) were VA athletes and 4/19 (21%) were active. Of the TDC group, 7/19 (36.8%) were VA athletes, 11/19 (57.9%) were active and 1/19 (5.3%) were low sedentary. The proportion of men in the highest classification of PA was higher in CP than in the TDC ($\chi^2_{(2,\ 37)}$=7.18, p=0.028). In men with CP, walking ability was reported to be unchanged since high school in 10/19 (52.6%), with 6/19 (31.6%) reporting an improvement and 3/19 (15.8%) reporting walking ability to have worsened. In the TDC group, walking ability was unchanged in 16/19 (84.2%) and improved in 3/19 (15.8%).

In men with CP, fear of falling was 26.2% higher compared to TDC (p=0.01*,* Table 3-4). Total risk-taking behaviours score was 14.5% higher in CP compared to TDC (p=0.013*,* Table 3-4). A Pearson's correlation determined that fear of falling was not associated with KE iMVC in men with CP or TDC (r=-0.164, p=0.558 and r=-0.060, p=0.818, respectively), nor was fear of falling associated with KE iMVC/BM in men with CP or TDC (r=-0.288, p=0.298 and r=0.050, p=0.839, respectively). Men with CP scored 17.8% higher in risk-taking behaviours, day-to-day balance tasks (p<0.036, Table 3-4). There were no statistical differences in risk-taking behaviours public transport and roads and risk-taking behaviours lifestyle choices between men with

CP and TDC (p=0.132 and p=0.070, Table 3-4). There was no relationship between falls and fear of falling (r=0.475, p=0.129), nor was there a relationship between falls and risk-taking behaviours score in all men with CP and TDC (r=0.379, p=0.278).

3.3.2 Comparisons of CP Fallers, CP Non-Fallers and TDC

There was no difference in GMFCS between CP fallers and CP non-fallers (p=0.720, d=-0.168). Despite no significance CP fallers showed a 31.1% smaller KE iMVC compared to CP non-fallers (p=0.117, η^2=0.320), CP fallers had 44.0% lower KE iMVC and were weaker compared to TDC (p=0.003, η^2=0.320 Table 3-2). CP non-fallers showed no statistical difference in KE iMVC compared to TDC (p=0.458, η^2=0.320, Table 3-2). Despite lack of statistical significance, CP fallers showed a 35.1% and a 26.3% weaker KE iMVC/BM compared to CP non-fallers or TDC (p=0.296 and p=0.545, η^2=0.088 respectively), whereas, CP non-fallers and TDC had just a 9.0% difference in KE iMVC/BM which was not statistically significant (p=1.000, η^2=0.088, Table 3-2).

Table 3-2. Anthropometric and physiological measure for typically developed controls and men with cerebral palsy. Vales presented as means±SD.

	TDC (n=19)	**CP (n=19)**	**CP Fallers (n=9)**	**CP Non-fallers (n=10)**
Age (years)	25.4±2.7[a]	21.9±2.9	20.4±1.5[c]	23.2±3.2
Stature (m)	1.77±0.09	1.76±0.10	1.78±0.89	1.73±0.81
Body Mass (kg)	73.1±12.3	68.5±12.6	71.7±14.6	65.7±10.5
BMI ($kg \cdot m^{-2}$)	23.4±2.8	22.0±2.4	22.3±2.9	21.8±1.9
GMFCS (median(range))	-	1 (1-2)	1 (1-2)	1 (1-2)

IPAQ score	7899±5804	8171±4399	7352±4161	9573±4762
KE iMVC (N)	523.5±106.3[a]	408.7±123.4	334.7±86.1[c]	457.9±123.2
KE iMVC/BM ($N \cdot kg^{-1}$)	6.46±2.72	6.23±1.99	4.96±1.19	7.07±2.00

TDC, typically developed controls; CP, cerebral palsy; IPAQ, International physical activity questionnaire; BMI, body mass index; GMFCS, gross motor function classification score; KE iMVC, knee extensor isometric maximal voluntary contraction. [a] $p<0.05$ between TDC and CP; [b] $p<0.05$ between CP fallers and non-fallers; [c] $p<0.05$ between CP fallers and TDC, and [d] $p<0.05$ between CP non-fallers and TDC.

In CP fallers 7/9 (77.7%) were VA athletes and 2/9 (22.3%) were active. Of the CP non-fallers 8/10 (80%) were VA athletes and 2/10 (20%) active. Of the nine CP fallers 2/9 (22.2%) reported worsened walking ability since leaving high school, 4/9 (44.4%) reported an unchanged walking ability, 2/9 (22.2%) reported improved walking ability and 1/9 (11.2%) reported fluctuations in walking ability. In non-fallers, there was no individual reporting worsened walking ability, 6/10 (60%) reported unchanged walking ability and 4/10 (40%) reported improved walking ability. Of all fallers in both CP and TDC groups, none reported that they are afraid of falling again, nor do they feel they will change their behaviour to avoid a fall.

The CP fallers' fear of falling was 23.2% higher compared to TDC ($p<0.01$). There was no statistical difference between CP faller and non-fallers fear of falling ($p=0.062$, $\eta^2=0.313$). There was no statistical difference in fear of falling scores between CP non-fallers and TDC ($p=0.619$, $\eta^2=0.313$, Table 3-4). Risk taking behaviours day-to-day task scores were lower in CP fallers compared to TDC ($p<0.05$). There was no statistical difference in total risk-taking behaviours, risk-taking behaviours, public transport and roads or risk-taking behaviours, lifestyle choices between CP fallers

and TDC (p=0.097-1.000, η^2=0.063-0.201, Table 3-4). There was no statistical difference between CP fallers and non-fallers or CP non-fallers and TDC in total risk-taking behaviours or risk-taking behaviours subgroups, (p=0.197-1.000, η^2=0.165, Table 3-4).

Table 3-3. Results for falls and near falls in the past 12 months with descriptions for most recent falls in TDC and men with CP.

		TDC (n=19)	**CP (n=19)**
Falls in past 12 months (%)	0-1 falls	15 (79.2%)	10 (52%)
	2-3 falls	2 (10.4%)	0 (0%)
	4-6 falls	1 (5.2%)	1 (5.2%)
	7-19 falls	1 (5.2%)	5 (26.2%)
	20-60 falls	0	3 (15.6%)
	>60 falls	0	0
Near-falls	None	14 (74%)	3 (15.8%)
	Weekly	0	5 (26.3%)
	Monthly	3 (15.8%)	4 (21.1%)
	Less frequently than monthly	2 (10.2%)	7 (36.8%)
Cause of most recent fall	Balance loss	0	2
	Trip	0	6
	Slip	3	1
	Other	1	0
	Dog or other pet	0	0
	Indoor object	1	0

Obstacles contributing to most recent fall	Slippery surface	2	1
	Uneven path or flooring	1	5
	Steps or stairs	0	1
	Loss of balance	0	2
	Other	0	0
Activities at time of most recent fall	Walking	2	3
	Climbing or descending stairs	1	1
	Playing sport or undertaking sport	1	5
Location of most recent fall	Indoor at home	2	1
	Outdoors at home	1	2
	In the community	1	6
Injury sustained from most recent falls	None	4	2
	Minor, no medical attention	0	4
	Minor, self-treated	0	2
	Severe (GP or accident and emergency admission)	0	1

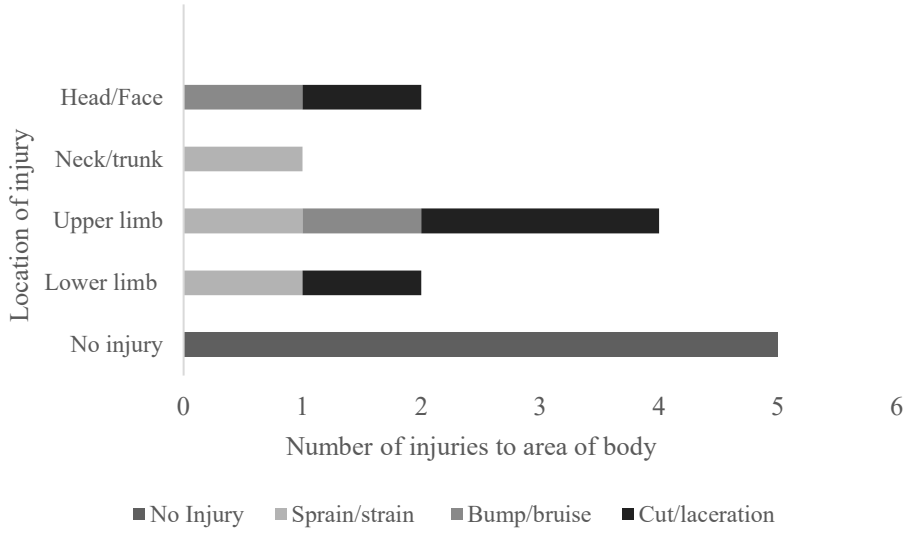

Figure 3-1. Distribution and type of injury sustained by men with CP after their most recent fall. Note: more than one injury may be resultant from most recent fall).

Table 3-4. Falls efficacy scale and risk-taking behaviour scores for typical developed controls and men with cerebral palsy.

	TDC (n=19)	CP (n=19)	CP fallers (n=9)	CP non-fallers (n=10)
Fear of Falling-FES-I total score	18.3±2.0[a]	23.1±6.7	25.8±7.1[c]	20.6±5.6
Risk taking behaviours, total score	47.5±0.7[a]	41.5±0.6	41.1±10.0	41.8±7.1
Risk taking behaviours, day-to-day balance tasks	19.0±3.3[a]	15.9±4.0	14.7±4.5[c]	17.0±3.4
Risk taking behaviours, public transport, and roads	12.3±2.4	10.8±3.4	10.7±3.7	11.0±3.3
Risk taking behaviours, lifestyle choices	16.2±2.2	14.7±2.5	15.9±2.8	13.8±2.1[d]

TDC, typical developed controls; CP, cerebral palsy; FES-I, Falls Efficacy Scale International; risk-taking behaviours, risk-taking behaviours. [a] $p<0.05$ between TDC

and CP; [b] $p<0.05$ between CP fallers and non-fallers; [c] $p<0.05$ between CP fallers and TDC, and [d] $p<0.05$ between CP non-fallers and TDC.

3.4 Discussion

The aims of the study were to: 1) investigate falls in young men with CP who are otherwise healthy and physically active compared to physical activity matched TDC without neuromuscular impairments; 2) to distinguish what outcome measures account towards falls in men with CP; 3) identify if physically active young men with CP have a higher fear of falling compared to TDC; and 4) address if injuries are more prevalent in physically active men with CP who fall compared to non-fallers with CP. The findings from the present study show 43% of physically active men with CP are classified as fallers, with 44% of these fallers experiencing an injurious fall. Compared to men without CP, men with CP had a higher level of fear of falling and lower total risk-taking behaviours, especially day-to-day balance risk-taking behaviours.

3.4.1 Physiological Factors and Falls

The results from this current study showed that severity of CP, at least for those classed as GMFCS I-II, were not different between CP fallers and CP non-fallers, a likely consequence of only ambulant, physically active participants being included. There is however, precedent for GMFCS severity to not be linked with other physical impairments. For example GMFCS severity is not associated with muscle strength variance in men with CP (Ross and Engsberg, 2002). It is likely therefore, that despite GMFCS being higher in the more prevalent fallers in previous data (Morgan et al., 2015), it could be linked to a corresponding weaker KE iMVC, as it was observed that

CP fallers had a 44% weaker KE iMVC than TDC and, whilst CP fallers had a 31.1% weaker KE iMVC compared to CP non-fallers this was statistically not different. Despite this, prior to this chapter there was currently no other research investigating whether KE strength is a predictor of falls in CP populations. The data presented above, therefore represents the biggest single physiological measure that has been linked to falls occurrence with the only other reported falls risk factor in CP, showing no difference between CP fallers and non-fallers (i.e. Standing balance, Morgan et al 2013). In terms of investigating the multifaceted determinants of falls in men with CP it should be noted that the present men with CP are well above the current KE MVC strength threshold which indicates high falls risk in the elderly (e.g. elderly fallers show KE MVC of 1.2 Nm/Kg, in contrast to 4.96 Nm/Kg in the present data)(Ding and Yang, 2016). Thus although the 31% lower KE MVC was not significant, given the wealth of evidence linking KE MVC to falls risk in other populations (OYA et al), and as part of the multi-faceted factors contributing to higher falls (Lord et al., 2003), it can be argued that the KE MVC weakness reported here is a contributory factors to falls in men with CP and of meaningful clinical significance. Whilst there is currently no other research investigating whether KE strength is a predictor of falls in CP populations, the 31.1% weaker KE iMVC in CP fallers compared to their non-falling counterparts in this present study shows similarities to the 31% reported previously de Groot et al. (2012). This KE iMVC weakness is consistent with studies that have previously linked KE strength and falls risk, such as 27% of elderly fallers have a weaker KE iMVC compared to elderly non-fallers (Ding and Yang, 2016). The data presented in the present chapter is the first, to the authors' knowledge, that

identifies a potential relationship between KE iMVC weakness and increased risk of falls in CP populations.

3.4.2 Falls in Men with CP

Although this current study shows a high falls rate over the last 12-months in men with CP (48%), experiences of falls were still lower compared to Morgan et al. (2015) who found that 82.4% of ambulatory men with CP experienced falls more than twice over a 12-month period. The main cause of falls in the adults with CP within Morgan et al. (2015) was general loss of balance at home, whereas, the most common cause in the present study was tripping on uneven flooring and occurred most often when being physically active (i.e., playing sport). Whilst it could be criticised that several of the CP fallers in the present study sustained falls from playing sport, it should be noted that population-based falls statistics have shown that fall-related injuries in young healthy men (aged 18-44) most typically occur during vigorous PA (Timsina et al., 2017) and there is an increasing call to include such falls in fall prevention research, in order to mitigate the significant economic cost of fall-related injuries. Despite the large difference in falls rate between this current study and Morgan et al. (2015), it should be noted that men with CP in the present study were, on average, 18.8 yrs younger, where in previous studies more elderly individuals without CP fall more frequently than younger counterparts (Peel, 2011). In this current study the men with CP also had less severe CP, where more serve presentations of CP show increased falling prevalence (Morgan et al., 2014), and were likely to have been more physically active, due to the nature of the recruitment process, than the adults with CP within the study by Morgan et al. (2015); PA was however not reported by Morgan

et al. (2015). That said, it is important to note that the current men with CP were PA matched to the TDC group, so falls cannot be explained by PA alone. Despite their active status, the current men with CP showed falls prevalence rates greater than the average falls prevalence (30-40%) reported in healthy older adults aged 65-75 (Scheffer et al., 2008), and fear of falling (FES-I) scores >23, which indicate high concern about falling, similar to that of older adult fallers without CP (Delbaere et al., 2010b). As such, our results suggest that falls in CP is likely a significant problem throughout the lifespan and should be studied further in younger and older demographics.

3.4.3 Psychological Factors and Falls

In men with CP, fear of falling was found to be higher and total risk-taking behaviours were lower compared to TDC, which is in line with previous literature in ambulatory adults with CP (Morgan and McGinley, 2013b, Morgan et al., 2015). This is likely to be because of a self-awareness of mobility and balance issues. FES-I scores did not differ between CP fallers and CP non-fallers, which suggests a generally high concern about falling within CP, similar to previous literature (Morgan and McGinley, 2013b, Morgan et al., 2015). Despite this, when asked in the falls questionnaire, CP fallers reported that they were not afraid of falling again, nor would they change their behaviour. This lack of change in fear of falling and behaviours is unusual given previous research suggesting falling increases fear of falling (Jamison et al., 2003). It is possible that fear of falling was not greater in fallers here, because injuries from a fall can elicit a greater fear of falling (Larson and Bergmann, 2008); in the current study, a majority of injuries sustained in the CP fallers were minor except for only

one faller who required emergency medical attention. Total risk-taking behaviours scores were not different between CP fallers and non-fallers, this suggests that risk-taking behaviours were not a primary factor contributing to falls in men with CP. It is important to note that within the active men with CP, there are limited reports of the impact of falls, injurious falls, and fear of falling on their quality of life. There is certainly no description of how this progresses, potentially at an accelerated rate, compared to TDC into old age, which already presents challenges and implications for fallers in terms of lower quality of life and mental health (Suzuki et al., 2002).

3.4.4 Strengths and Limitations

The present data represent the biggest single description of falling and fall related determinants in men with CP. Despite this there are a number of limitations, although not reaching statistical significance in outcome measures such as KE iMVC/BM this study showed large physiological differences of 44%. Because of this, a post hoc power analysis was performed via G*Power analysis [Frankfurt, Germany] using KE iMVC/BM to identify the appropriate sample size to see a large effect size (1-β=0.8). To see a large effect 21 CP fallers, 21 CP non-fallers and 21 TDC would be required to complete this current study. It should be noted that within the present FA structure there are only 24 men with CP attending training camps, recruiting 42 to reach the required power would have introduced heterogeneity into physical activity and athlete status of participants as they would have been recruited outside of elite football, and beyond the scope of the participant focus in this thesis. Furthermore, a major impact limiting this study was data collection occurring between March 2019 and March 2020 corresponding with the start of the COVID

pandemic. Although not all data collection was not directly impacted by the pandemic the original plan to collect subsequent data to meet the required sample size was ceased as the FA placed a moratorium on training camps until March 2021. Therefore, due to the large number of outcome measures and multiple group comparison using underpowered sample size there is the potential risk of type 1 error occurring. To overcome these limitations, the non-significant effect of lower KE MVC is discussed above in context of it being the only physiological determinant of falls that has been reported. As the first description of falls in physically active men with CP, the present data also serves as an important point of reference for powering subsequent studies.

The participants in the present study represent a physically active and highly functional proportion of men with CP (GMFCS I-II). The high levels of PA in these men with CP may mean that the findings may not be reflected in other more impaired populations with CP. Although a broader population would of course be relevant for generalisation, this current studies population of lower GMFCS impairment likely reduces the contribution of PA variance to the group differences and suggest that by regularly undertaking PA, there are benefits to muscle health and a possible reduction in falls risk. As the men with CP performed regular exercise and sport, some falls that were reported during these occasions may have been because of unavoidable contact during sport and not due to muscle weakness or balance deficits. In the current study, questionnaires were not modified to allow group comparisons with previous data, future falls research within physically active/athletic adults with CP should consider the nature of sporting activity in the falls prevalence more completely.

The recruitment of CP participants through sporting associations although appearing niche, is in fact a common route for accessing groups of ambulatory adults with CP (Hussain et al., 2014, O'Brien et al., 2021, Barber et al., 2011, de Groot et al., 2012), and although far from the most impaired representation of CP, presents a valid overview of those adults with CP who are categorised as GMFCS I-II. The data is consistent with lower falls associated with 1) higher PA, 2) younger age and potentially, 3) stronger KE. Further research in such physically active groups should also investigate whether there may be some protection offered by being physically active and having greater proprioception from their training which allows them to reduce the impact of a fall (Groen et al., 2010), hence the lower prevalence of fall-related injuries to previous studies. This could help identify the direct benefits of physical activity in men with CP in terms of falls avoidance, particularly in the type of activity, as alternative lower fall risk activities may be more beneficial to those with more severe impairments than assessed in the present study. In addition, it could also identify sport-related training that could aid fall recovery in specific groups.

3.5 Conclusion

The aim of the study was to assess and describe differences in lifestyle factors, neuromuscular outcomes and the psychological impact of falls in men with CP. The current study demonstrated a higher incidence of falls and fear of falling in men with CP, compared to a TDC group. Lastly, it was found that KE iMVC strength is likely to contribute to falls in men with CP. The present data suggests that falls may be a significant health concern throughout the lifespan in CP and as such, should be investigated further in younger demographics. Further investigation into

neuromuscular predictors of falls in physically active and sedentary men with CP should be considered to help identify appropriate falls prevention strategies in this population.

Chapter 4 - Musculoskeletal Health in Active Ambulatory Men with Cerebral Palsy and the Impact of Vitamin D

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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 PA 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), 10 m sprint, vertical jumps, radius and tibia bones T_{us} and Z_{us} scores. Assessments of vitamin D status through quantification of venous 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 vertical jump, 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_{us} and Z_{us} scores were 1.75 and 1.57 standard deviations lower than TDC, respectively ($p<0.05$), whereas neither tibia T_{us} nor Z_{us} scores showed any difference compared to TDC ($p>0.05$). Unexpectedly, not only was 25(OH)D not different between groups, but also 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.

Key words: Cerebral Palsy, Vitamin D, Strength, Bone, Sun Exposure.

4.1 Introduction

In the previous chapter, it was identified that knee extension strength through isometric maximal voluntary contractions (KE iMVC) which contributed to falls prevalence in men with CP. Any factor that could accentuate the neuromuscular impairments associated with CP, would likely worsen functional abilities of those with CP. 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 (Holick, 2007). Predominant reasons for reduced vitamin D levels are due to insufficient dietary intake and restricted outdoor exposure to sun light (Lips et al., 2006). 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 (Holick, 2007) 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 $ng{\cdot}mL^{-1}$) and 40.8% who are vitamin D deficient (25(OH)D <20 $ng{\cdot}mL^{-1}$).

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 (Holick et al., 2011). 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 (Lips et al., 2006).

Only a few foods, such as oily fish, naturally contain vitamin D and these tend to be relatively low dosages (Holick, 2007) 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 (Holick, 2007), 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 (Brett et al., 2016) and outdoor sunlight exposure are heavily reported with typically developed, able-bodied adults and children (hereafter termed TDC, typically developed controls) (Docio et al., 1998, Rajakumar et al., 2011b). Interestingly, individuals with disabilities, such as cerebral palsy and spinal cord injuries appear to have poorer micronutrient compared to age-matched TDCs Oleson et al. (2010), (Kalra et al., 2015). Furthermore, although not directly related to outdoor activity, physical activity (PA) is 48 minutes 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 Nooijen et al. (2014), 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. (2012) 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 $ng \cdot mL^{-1}$) during the winter months, while Flueck et al. (2016) 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 (Hussain et al., 2014, Finbråten et al., 2015). Lower levels of 25(OH)D are associated with lower musculoskeletal health outcomes including, but not limited to: lower strength (Bischoff et al., 1999), muscle size (Foo et al., 2009), reduced bone mass (Farrar et al., 2016), increased bone turnover marker parathyroid hormone (PTH) (Foo et al., 2009) and higher levels of adiposity in TDC (Rajakumar et al., 2011a). 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 (Carriero et al., 2009, Papageorgiou et al., 2019). These already impaired musculoskeletal outcomes are compromised further in CP through modifiable risk factors including poor dietary micronutrient intake (Hillesund et al., 2007), reduced sun exposure (Halliday et al., 2011), low PA levels (Seth et al., 2017) and as a side effect of anticonvulsant medications (Stallings et al., 1993). 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 physically active groups with CP nor its effects on musculoskeletal measures.

Therefore, the aim of the present study is to increase our understanding 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) Lower vitamin D will lead to reduced musculoskeletal health in men with CP.

4.2 Materials and Methods

4.2.1 Study Protocol

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 analyses.

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).

4.2.2 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 4-1). 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 (Morton et al., 2012, Cannell et al., 2009).

Table 4-1. 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	1	4	-	4	-
(FT)	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.

4.2.3 Anthropometric Measures

Height (m) was measured using a stadiometer (Seca 213, portable stadiometer, Hamburg, Germany) following the stretch-stature method (Voss and Bailey, 1997) and body mass (BM) (kg) via a set of digital scales with minimal clothing (underwear

only)(Seca, Hamburg, Germany). Percentages (%) of body fat and lean body mass (LBM) were measured using bioelectrical impedance (BIA) (Omron, body fat monitor, BF306, Kyoto, Japan). Participants stood upright with their arms out on front and gripping the electrodes on each handle. BIA has been shown to be valid (r^2=0.96) in comparison with Dual Energy X-ray Absorptiometry in adult TDC (De Lorenzo et al., 1998), and children with CP (concordance correlation coefficient, 0.75–0.82) (Oeffinger et al., 2014). To assess the validity of body fat% using BIA in 10 physically active men with CP from this current study, BIA was taken and compared to 7 site bilateral skin folds taken from both paretic and non-paretic sides were taken (14 skin folds in total) using the Jackson & Pollock method, which has previously been validated in adults with cerebral palsy by Hildreth et al. (1997). Skin folds showed no bilateral differences between paretic (10.9±3.1%) and non-paretic sides (11.5±3.2%) in physically active men with CP (p>0.05). A Bland-Altman presented a standardised error of 1.78, with an estimate ±95% limits of agreement (LoA) of 9.97 showing that BIA is a valid method to measure BF% in physically active men with CP.

4.2.4 Physical Activity

Habitual PA was recorded through the international physical activity questionnaire-long form (IPAQ) (Appendix 3-2) 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.

4.2.5 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 Reeves et al. (2004), the VL's proximal insertion and the myotendinous junction were marked to identify 50% of muscle length. Strips of tape, utilised as echo-absorptive markers, spaced equally apart, were 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 (Esformes et al., 2002). Reeves et al. (2004) 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.

4.2.6 Muscle Function

To assess muscle function, vertical jump 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. vertical jump height (m) was measured using a jump mat (Probotics Inc., Esslinger court, Huntsville, Alabama) in two conditions; with and without arm swing. Nuzzo et al. (2011) reported the jump mat to be a reliable piece of equipment to measure vertical jump height in men (ICC=0.93, coefficient of variation (CV)=2.3%) and women (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, $p< 0.01$) (Shalfawi et al., 2012).

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 as the participant's performance. The current study showed a high test-retest reliability in men with CP and TDC (ICC=0.996-0.998, both p<0.001).

Knee extensor isometric maximal voluntary contraction was collected as described in detail in Chapter 3 (Section 3.2.2). In this current chapter KE iMVC values were also presented relative to VL ACSA (KE iMVC/ACSA), BM (KE iMVC/BM) and LBM (KE iMVC/LBM).

4.2.7 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 cm from the condyle) was performed to obtain T_{us} and Z_{us} 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 T_{us} and Z_{us} scores were provided. Knapp et al. (2001) 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).

4.2.8 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. Day et al. (2001) found that food diary recall was a reliable method for micronutrient intake (r=0.75) when compared to urinary markers.

4.2.9 Sun Exposure Measurement

To estimate the level of endogenous skin synthesis of vitamin D_3 from sun exposure, a sun exposure questionnaire (SEQ) (Appendix 3-3) 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 (McCarty, 2008). 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 (Figure 4-1) (Fitzpatrick, 1988).

Figure 4-1. The Fitzpatrick scale used to determine skin colour (Fitzpatrick, 1988).

4.2.10 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 from erythrocytes, leukocytes, and platelets. Serum was aliquoted 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.

4.2.11 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) (Zerwekh, 2004). 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 and was lower at 2%. A four-parameter logistic curve also showed a reliable calibration curve (Optical density at 450nm vs. concentration (ng·ml^{-1}) r^2=0.982).

4.2.12 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·mL^{-1}, with a sensitivity of 0.761 pg·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 in our hands was 7.5%. A four parameter polynomial calibration curve (with Optical density end read point of 450nm) also showed a strong reliability score of r^2=0.999 in this current study.

4.2.13 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 'Greenhouse Geisser corrected *p* value was applied if variance was non-homogenous. Group differences for height, BM, BMI, BF%, LBM%, dietary intake, TSE, vertical jump no arms, vertical jump with arms, 10 m 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_{us} and Z_{us} scores, radius T_{us} and Z_{us} 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.

4.3 Results

4.3.1 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 4-2). 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 4-2). Pearson's correlations showed that age was not associated with any other outcome measure (all p>0.05) and as such need not be adjusted as a covariant in follow-up analyses.

Table 4-2. 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 ($kg \cdot m^{-2}$)	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 4-3). 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 4-3). The CP group has a 22.2% lower vertical jump with no arm swing and 21.8% lower vertical jump with arm swing compared to TDC (p<0.001 for both, Table 4-3). There were no differences in

handgrip strength (p=0.280) or 10 m sprint time between CP and TDC (p=0.302, Table 4-3).

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 vertical jump (with arm swing) (r=0.351, p=0.029), but KE iMVC/LBM was not associated with 10 m 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 10 m 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 4-3. 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 ($N \cdot cm^{-2}$)	15.2±3.1	17.6±4.2	0.048
KE iMVC/BM ($N \cdot kg^{-1}$)	6.07±1.41	7.60±1.65	0.004
KE iMVC/LBM ($N \cdot kg^{-1}$)	7.11±1.80	9.15±2.06	0.002
Vertical jump no arms (m)	0.40±0.04	0.50±0.05	<0.001
Vertical jump 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
10 m 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.

The CP group had a radius T_{us} score that was -1.75 SDs less and radius Z_{us} score that was -1.57 SDs less when compared to TDC (p<0.001, Table 4-4). There was no difference between tibia T_{us} scores (p=0.158, Table 4-4) and tibia Z_{us} scores between groups (p=0.143, Table 4-4). A Pearson's correlation showed that there were no significant relationships between 25(OH)D and any tibia or radius Z_{us} and T_{us} 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_{us} and T_{us} scores for both groups (r=0.012-0.205, all p>0.05).

Table 4-4. Tibia and radius bone ultrasound (T_{us} and Z_{us} score) in men with cerebral palsy (CP) and PA matched controls (TDC). Reported as mean±SD and p values.

	CP	**TDC**	***p***
Radius T_{us} score	-1.32±1.12	0.43±0.79	<0.001
Radius Z_{us} score	-0.93±1.12	0.64±0.79	<0.001
Tibia T_{us} score	0.50±1.62	-0.03±0.80	0.158
Tibia Z_{us} score	0.55±1.60	-0.03±0.82	0.143

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 4-5). 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 4-5). 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 4-5). 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 (Figure 4-2), but there was no association between 25(OH)D and KE iMVC/LBM in TDC (r= 0.281, *p*=0.103 (1-tailed), Figure 4-2).

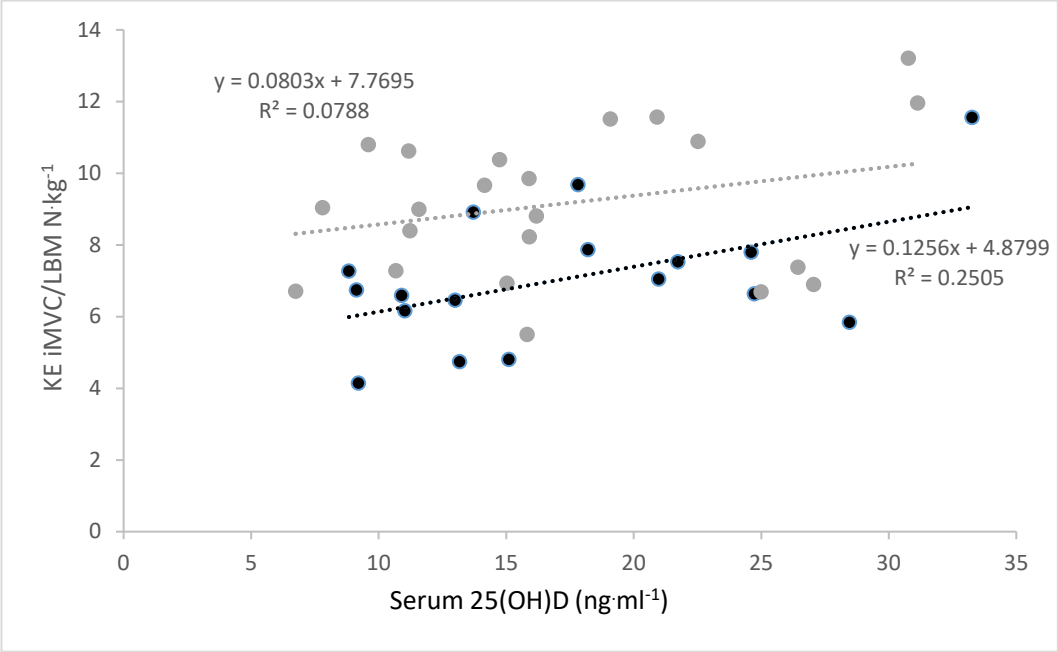

Figure 4-2. Pearson correlations between serum 25(OH)D and KE iMVC/LBM in men with CP (black filled dots) and TDC (grey filled dots).

Table 4-5. 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.

4.4 Discussion

The aim of this study was to increase the understanding the status of, and relationship between, 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 vertical jump 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 weakness and size in adults with CP (Eek and Beckung, 2008) most studies in CP measure plantar flexor strength and plantar flexor ACSA (Hussain et al., 2014, Noble et al., 2014, McNee et al., 2009). However, the KE were deemed an important muscle group to investigate as they play a dominant role in sports performance (Magalhaes et al., 2004), falls prevalence (Larson and Bergmann, 2008), as shown in chapter 3 and are limited in their description within CP (de Groot et al., 2012). The 40.5% lower KE iMVC in the present study is consistent with the 52% weaker plantar flexors (Damiano and Abel, 1998), and the likely consequence of the plantar flexors being more directly impacted by the CP condition (Hussain et al., 2014). In contrast to this current study's more modest levels of KE weakness, the previous report of a 69% weaker KE in CP (de Groot et al., 2012), 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 plantar flexor ACSA and 27.9% smaller in VL ACSA in men with CP compared to TDC Noble et al. (2014). 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% (Hildreth et al., 1997). 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 3,996 steps more/day on average when compared to other young ambulatory CP (Bjornson et al., 2007). Despite the higher levels of LBM in men with CP in this current study compared to other CP populations (Hildreth et al., 1997), LBM was still lower in men with CP when compared to TDC, which is consistent with other studies on body composition and muscle size (Hildreth et al., 1997). It should be noted, that it is very common for CP athletes to be recruited for musculoskeletal investigations (de Groot et al., 2012, Hussain et al., 2014, O'Brien et al., 2021), (see limitations below).

A lower knee extensor strength relative to LBM has several 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 this current studies 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 and women (Rolland et al., 2004, Erskine et al., 2017), postmenopausal women (Lebrun et al., 2006) and individuals with degenerative muscle impairments (Miscione et al., 2013). 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 10 m 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 (Saether et al., 2014) which could contribute to effective ground reaction force vectors when sprinting (Wang and Watanabe, 2012). Similarly, de Groot et al. (2012) 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 (Reina et al., 2017) 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 4-1), and running gait patterns are shown to have increased symmetry with increasing running speeds (Davids et al., 1998). Therefore, peak power output and increased sprinting gait symmetry may explain why there is no difference in 10 m 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_{us} scores of -1.07 and -0.38, respectively (Hartman et al., 2004). In the current study, the T_{us} 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 (Houlihan and Stevenson, 2009). 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 (Sai et al., 2011, Valcour et al., 2012), it is of surprise to find that both participants in the CP and TDC group who are classed as deficient in 25(OH)D, in fact 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 (Valcour et al., 2012). A potential reason for the CP and TDC showing similar tibia but lower radius T_{us} and Z_{us} scores, could be their matched activity levels. In adolescents with CP, tibia density was previously reported to be higher with increasing ambulation levels (Al Wren et al., 2011). Indeed, football is specifically effective at preserving age related bone health in those without CP (Vicente-Rodriguez et al., 2003). It is therefore likely that the 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 PAlevels 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 vitamin D deficiency in disabled populations, let alone athletes with disabilities, despite low vitamin D concentrations being documented in several nonathletic populations (Farrar et al., 2016, Bischoff et al., 1999, Foo et al., 2009). Notwithstanding the 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 (Webb et al., 2018). 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 there were 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 (Holick et al., 2011). Furthermore, dietary vitamin D intake accounts for <20% of circulating vitamin D, the observations of no association between dietary vitamin D intake and 25(OH)D is of no surprise (Holick, 2007). 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 which 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 (Webb et al., 2018).

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 (Montenegro et al., 2019). 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.

4.5 Strengths and Limitations

The participants in the present study represent a highly functional proportion of men with CP (GMFCS I-II). This current studies participant groups are however similar to the only other description of KE iMVC and sprint outcomes (de Groot et al., 2012), and are consistent with populations of CP athletes investigated by others (O'Brien et al., 2021). Comparisons with population based studies suggest that the proportion of GMFCS I: GMFCSII participants is around n= 3:2 (Michelsen et al., 2009). In contrast, the strength comparisons made in the present study, de Groot et al. (2012) and (O'Brien et al., 2021) 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 O'Brien et al. (2021). 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, the

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 that no data from women was included in this current study. Although not adverse to presenting sex disaggregated musculoskeletal outcomes such as, KE strength and tendon stiffness (Hicks et al., 2016, McMahon et al., 2018), the recruitment of CP participants in this current study 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, (Barber et al., 2011, de Groot et al., 2012). Given the known sex differences in bone measures, and the greater risk for osteoporosis (Bonnick, 2006), future research should strive to uncover the particular risks and implications of low vitamin D in women with CP.

4.6 Conclusion

The aim of this study was to increase the understanding 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.

Chapter 5 – The Impact of Seasonal Variations on Vitamin D in Active Ambulatory Men with Cerebral Palsy

Abstract

Purpose: To investigate if there are seasonal variation in vitamin D in physically active, ambulatory men with CP on **1)** neuromuscular performance outcomes, and **2)** PTH and bone ultrasound Scores (T_{us} and Z_{us}).

Materials & Methods: A longitudinal observational study, in sixteen ambulant men with CP aged 21.0±1.3 years (Gross Motor Function Classification Score I-II) and 16 healthy physical activity matched typically developed controls (TDC) aged 25.4±2.6 years, completed *in vivo* assessment of seasonal variations in 25(OH)D on musculoskeletal health. Assessments of vitamin D status through venous samples of serum 25-hydroxyvitamin D (25(OH)D) were taken in winter and summer and total sun exposure via questionnaire was taken. Neuromuscular outcomes included the assessment of *Vastus Lateralis* anatomical cross-sectional area (VL ACSA), using B-mode ultrasonography, isometric knee extension maximal voluntary contraction (KE iMVC), 10 m sprint, vertical jumps, and grip strength. Bone health measures included the assessment of parathyroid hormone (PTH), bone ultrasound assessments to obtain radius and tibia T_{us} and Z_{us} scores.

Results: Men with CP and TDC showed a 70.5% and 85.7% increase in serum 25(OH)D from winter to summer months (p<0.05) respectively, yet the mean of men with CP was below the adequate threshold of 30 ng·mL^{-1} in the summer. In all participants there was no seasonal effect on neuromuscular outcomes KE iMVC, VLACSA or vertical jump, but 10 m sprint slowed from winter to summer in men with CP (p<0.05). PTH decreased with increased levels of 25(OH)D during the summer months

in both groups (p<0.05). Men with CP showed an increase in radius T_{us} and Z_{us} scores in the summer (p<0.05). A seasonal interaction effect was seen only in the tibia in both men with CP and TDC. Where there was a contrasting direction in tibia T_{us} and Z_{us} scores where men with CP saw a decrease and TDC saw increases (p=0.029).

Conclusions: Although similar seasonal increases in 25(OH)D were observed in men with CP and TDC, they were considered insufficient to increase bone or neuromuscular outcomes**.** Therefore, it is important to undertake strategies to increase vitamin D to adequate or even physiological optimal levels, to elicit potential improvements in musculoskeletal health and performance.

Key Words: Cerebral Palsy, Seasonal Variations, Vitamin D

5.1 Introduction

The very low levels of circulating serum vitamin D in both men with CP and TDC in Chapter 4 is expected given the latitudes of testing (53ºN). As discussed, a key contributing factor to this low vitamin D is sun exposure, which although not a factor in the previous chapter as it is negligible in the winter months in the UK, may impact musculoskeletal outcomes because of seasonal variations in sunlight exposure from winter to summer. Endogenous vitamin D_3 synthesis occurs in the skin when directly exposed to solar ultraviolet beta (UV b) radiation and is our predominant source of circulating vitamin D (Holick, 2007). There are however, many factors that impact cutaneous vitamin D production including, but not limited to: season, time of day, melanin levels and other methods of sun protection such as clothing coverage and sun protection factor (SPF) creams >8 (Cannell et al., 2009). A substantial factor affecting vitamin D status in humans is the latitude where we reside (Hall et al., 2010). Individuals living at latitudes of over 35ºN have been found to experience greater seasonal changes in climate and therefore experience fluctuations in sunlight exposure. At these latitudes, UV b radiation from sunlight during winter months (December, January and February) is negligible and consequently endogenous vitamin D_3 synthesis is severely decreased (Hall et al., 2010). As reported in the previous chapter, adults in the UK live at a latitude of ~53ºN resulting in them being highly susceptible to vitamin D deficiency during the winter months. Cannell et al. (2009) found that UK adults with an average age of 45 years old 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 to the winter months. This resulted in 25(OH)D peaking in September and steadily declining through the winter months where many individuals elicited 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 effect athletic populations, particularly those who participate in outdoor sports due to higher amounts of sun exposure (Halliday et al., 2011). Morton et al. (2012) found that 20 professional footballers doubled their serum 25(OH)D during the summer months compared to winter months, increasing to levels of 40.9 $ng \cdot mL^{-1}$ from 20.5 $ng \cdot mL^{-1}$ which are considered optimal (>40 $ng \cdot mL^{-1}$) for musculoskeletal health (Holick, 2007). The increased time spent performing PA outdoors is likely to be a major contributor to the large increase in 25(OH)D seen in Morton et al. (2012) when compared to other studies in non-athletic cohorts (Cannell et al., 2009). Subsequently, it is common to see less pronounced seasonal variations of 25(OH)D in individuals who are predisposed to lower PA levels (Nooijen et al., 2014, Schaefer et al., 2014), such as the elderly (Webb et al., 1990) and individuals with disabilities (Ascherio and Munger, 2007). Even, athletes with disabilities spend less time outdoors due to reduced ambulation compared to control populations and are likely to see reduced 25(OH)D levels throughout the year (Pritchett et al., 2016). For example, Flueck et al. (2016) 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 low 25(OH)D (<30 $ng \cdot mL^{-1}$) during the winter at 21.7 $ng \cdot mL^{-1}$ and even during their peak levels in summer with an average of 29.5 $ng \cdot mL^{-1}$. 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} increased reported by footballers without neuromuscular impairments (Morton et al., 2012). 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) and are possibly less likely to see associated improvements in musculoskeletal health and performance when compared to 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 (Hamilton et al., 2014, Koundourakis et al., 2014) and bone health (Kopeć et al., 2013, Finbråten et al., 2015). For example, in professional footballers higher levels of 25(OH)D at >30 ng·mL^{-1} were associated with stronger peak muscle torque of the knee extensors in the non-dominant leg (Hamilton et al., 2014) and improved vertical jump and sprint ability (Koundourakis et al., 2014). With these known benefits in athletic populations it is of particular importance that active ambulatory men with cerebral palsy (CP) who are predisposed to smaller muscle size (Bischoff et al., 1999) and muscle weakness (Foo et al., 2009), 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 (Rapuri et al., 2002) where higher levels of 25(OH)D corresponded with a decrease in parathyroid hormone (PTH) from 25.4 pg·mL^{-1} to 21.4 pg·mL^{-1} (Kopeć et al., 2013). The reduction of PTH in the summer months is likely to be beneficial for bone health, as persistent elevated levels in PTH can lead to greater bone resorption (Tam et al.,

1982), thus reducing bone mineral content (BMC) and increasing risk of fractures. Where adults with CP already present an 2.3 fold increased risk of experiencing a fracture compared to typically developed counterparts (Houlihan and Stevenson, 2009). Despite the previous observations of lower nadir to peak increases in 25(OH)D in spinal cord injured athletes (Flueck et al., 2016), there is as yet no data to suggest whether this could attribute 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 (Holick, 2007). The aims of this study are to therefore investigate if there are seasonal variation in vitamin D in physically active, ambulatory men with CP on; 1) neuromuscular performance outcomes, and 2) PTH and bone ultrasound T_{us} and Z_{us} Scores.

5.2 Materials and Methods

5.2.1 Participants and Recruitment

In all 28 individuals were screened to identify the sixteen male, ambulatory CP para-footballers aged 18-30 years old (Gross motor function classification score (GMFCS) I-II, Table 5-1) who were recruited via The Football Association, and all provided written informed consent to participate. All CP para-footballers were tested during two sessions at FA training camps (See Figure 5-1). In order to physical activity match TDC's with out neuromuscular disorders to the experimental group (men with CP) 24 individuals were screened and 16 were subsequently recruited from Manchester Metropolitan University and tested on site over two visits (Figure 5-1). All data for this current study was collected between 14th February - 13th March 2019 and 16th

August 2019 – 15th September 2019 to coincide with the data ranges presented by Cannell et al. (2009) 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 UK for the last three months, reported to have taken vitamin D supplements and used sun beds within the last 3 months prior to the study, 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 long term 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) (See Figure 5-1).

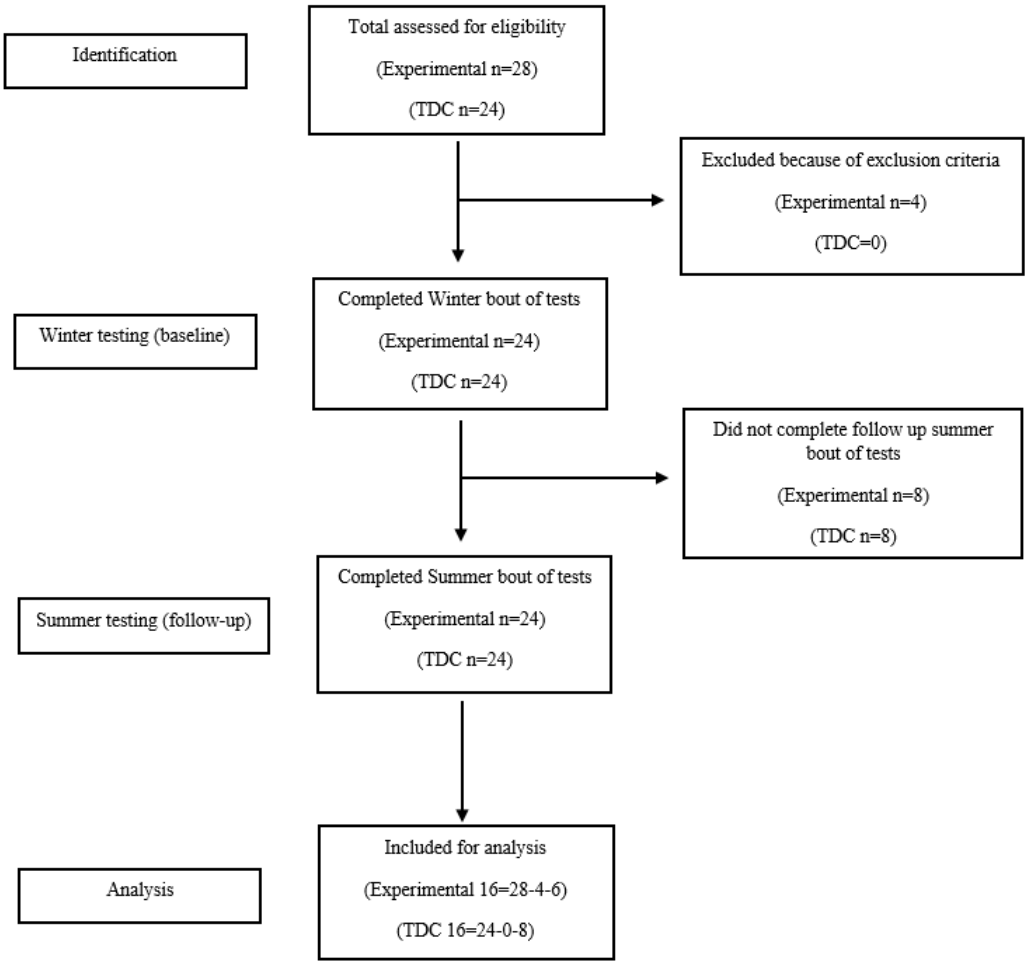

Figure 5-1. CONSORT style diagram, illustrating the recruitment process for the experimental group (men with CP) and TDC.

5.2.2 Study Protocol

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 below). All participants provided written informed consent, following approval from the local Ethics Committee, in accordance with the declaration of Helsinki.

Table 5-1. 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

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

5.2.3 Anthropometric Measures

Height (m) was measured using a stadiometer (Seca 213, portable stadiometer, Hamburg, Germany) following the stretch-stature method (Voss and Bailey, 1997), where and body mass (BM) (kg) via a set of digital scales with minimal clothing (Seca, Hamburg, Germany).

5.2.4 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·week^{-1}), duration of

each session (mins) and total time spent training (min·week^{-1}). Step count was also recorded through mobile phone accelerometers from those participants (n=32) with the iPhone Health Application (Apple Inc. Cupertino, California, US, version 13), as a daily average from the preceding 3 months.

5.2.5 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 participants spent exposed to direct sunlight in the spring and summer months only, as UV b exposure is negligible during the winter months the SEQ was used only once during the summer months (McCarty, 2008). 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 (Fitzpatrick, 1988).

5.2.6 Blood Sample Collection

Venous blood samples of 5 mL were taken from the antecubital region of the arm. Of the 16 men with CP, 15 provided blood samples and therefore provided serum 25(OH)D and PTH as one person had a fear of needles. Samples were collected via needle and eccentric luer tip syringe (Terumo corporation, Shibuya, Tokyo, Japan) and transferred into vacutainer plain tube (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.

5.2.7 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 (r^2=0.83) when compared to liquid chromatography mass spectrometry (LC-MS/MS) (Zerwekh, 2004). The manufacturer of the ELISA (Orgentec) provided intra- and inter-assay coefficient of variations (CV) of <14.6% and <11.7%. The intra assay CV for 25(OH)D presented in the current results section and was lower at 2%. A four-parameter logistic curve also showed a reliable calibration curve (Optical density vs. concentration ($ng \cdot mL^{-1}$) r^2=0.982).

5.2.8 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 Reeves et al. (2004), 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 (Esformes et al., 2002). Reeves et al. (2004) validated this technique against Magnetic Resonance Imaging (MRI) and showed an intraclass correlation (ICC) of 0.99 and mean typical error of 0.3 cm^2.

5.2.9 Neuromuscular Function

To assess muscle function, vertical jump height (m), 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 included dynamic stretches that focused on lower limb muscles. Participants were given two attempts at each test, with 1-minute rest in between and the best result was recorded. Vertical jump height was measured using a jump mat (Probotics Inc., Esslinger court, Huntsville, Alabama) in two conditions; with and without arm swing. Nuzzo et al. (2011) reported the jump mat to be a reliable measure of vertical jump height in men (ICC=0.93, CV=2.3%) and women (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 2 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) (Shalfawi et al., 2012).

Grip strength was assessed using an adjustable 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.

To record KE iMVC, participants were seated on a custom-made isometric 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 their fastened leg maximally while 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) (He et al., 2020). KE iMVC values were also presented relative to VL ACSA (KE iMVC/ACSA) and BM (KE iMVC/BM).

5.2.10 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. 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_{us} and Z_{us} score was provided. Knapp et al. (2001) 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).

5.2.11 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 $pg \cdot mL^{-1}$, with a sensitivity of 0.761 $pg \cdot mL^{-1}$. 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%.

5.2.12 Statistical Analysis

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 heterogenous. Group differences for age, height and BM was assessed via ANOVA and for TSE and IPAQ independent t-tests were used. Whereas vertical jump 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/LBM and KE iMVC/VL ACSA were assessed by 2-way repeated ANOVA. Where differences were observed, planned contrast t-tests were conducted to identify the level of effect. ANCOVA 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, the confidence interval was set at 95% with alpha set at ≤ 0.05.

5.2 Results

5.3.1 Descriptions of Men with CP and TDC

Men with CP were 4.4 years younger than TDC (p<0.001, Table 5-2). During the winter and summer men with CP had 16.6% and 17.2% lower body mass (both p<0.05). There was no difference in height between men with CP and TDC (p=0.195, Table 5-2).

Table 5-2. Anthropometric in men with cerebral palsy (CP) and typically developed controls (TDCs) data means±SD and % change from winter to summer.

	CP			TDC		
	Winter	Summer	% change	Winter	Summer	% change
Age (years)	21.0±1.3[b]	21.5±1.3[c]	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[b]	68.1±6.1[c]	-1.02%	80.2±11.2	79.8±10.8	-0.50%

[a] $p<0.05$ between winter and summer within in each group, [b] $p<0.05$ between in CP and TDC during Winter. [c] $p<0.05$ between CP and TDC during Summer.

5.3.2 Physical Activity

There were no differences in IPAQ scores PA frequency between men with CP and TDC (p>0.05), Physical activity duration and step count between men with CP or TDC during the winter or summer months (all p>0.05). There was no seasonal change in PA frequency, PA duration and step count between groups (all p>0.05, Table 5-3).

Table 5-3. Physical activity and lifestyle data for cerebral palsy (CP) and typically developed controls (TDC) during the winter and summer, presented as means±SD and % change between winter and summer.

	CP			TDC		
	Winter	Summer	% change	Winter	Summer	% change
TSE	-	27.4±2.2	-	-	29.4±1.3	-
IPAQ score	8737±3975	-	-	7128±4685	-	-
PA frequency (days·week^{-1})	4.0±1.9	3.4±2.0	-15.00%	3.9±1.6	4.1±1.3	4.60%
PA duration (mins·session^{-1})	70.1±32.2	74.4±29.1	6.13%	82.7±19.4	80.6±17.4	-2.54%
Step count (steps·day^{-1})	10696±3987	10010±2667	-6.41%	8932±3368	9408±4183	5.33%

TSE, total sun exposure; IPAQ, international physical activity questionnaire; PA, physical activity.

5.3.3 Vitamin D

There was a difference in 25(OH)D between CP and TDC (p=0.137). There was a main effect in seasonal measures 25(OH)D (p<0.001), such that men with CP had a 70.5% increase in serum 25(OH)D from winter to summer months (p=0.003), and TDCs had a 85.7% increase in serum 25(OH)D from winter to summer months (p<0.001, Figure 5-2). There was no interaction effect on 25(OH)D (p=0.169).

During the winter months the men with CP were classed as the following based on serum 25(OH)D levels; 0/15 (0%) were optimal or adequate, 4/15 (26.7%) were insufficient, 6/15 (40%) were deficient and 5/15 (33.3%) were severely deficient. During the winter in TDC, 0/16 (0%) were optimal, 2/16 (12.5%) were adequate, 2/16 (12.5%) were insufficient, 7/16 (43.75%) were deficient and 5/16 (31.25%) were severely deficient.

Based on serum 25(OH)D levels during the summer of the men with CP 1/15 (6.7%) was optimal, 4/15 (26.7%) were adequate, 7/15 (46.6%) were insufficient, 3/15 (20%) were deficient, none were severely deficient. During the summer months in TDC, 6/16 (37.5%) were optimal, 2/16 (12.5%) were adequate, 5/16 (31.25%) were insufficient, 2/16 (12.5%) were deficient and 1/16 (6.25%) were severely deficient. There were no significant differences between groups in the summer months (p=0.287, Figure 5-2).

Men with CP reported 7% less TSE then TDC during the summer (p<0.01). There was an association between TSE and 25(OH) D in both CP (r=0.465, p=0.041) and TDC (r=0.545, p=0.015).

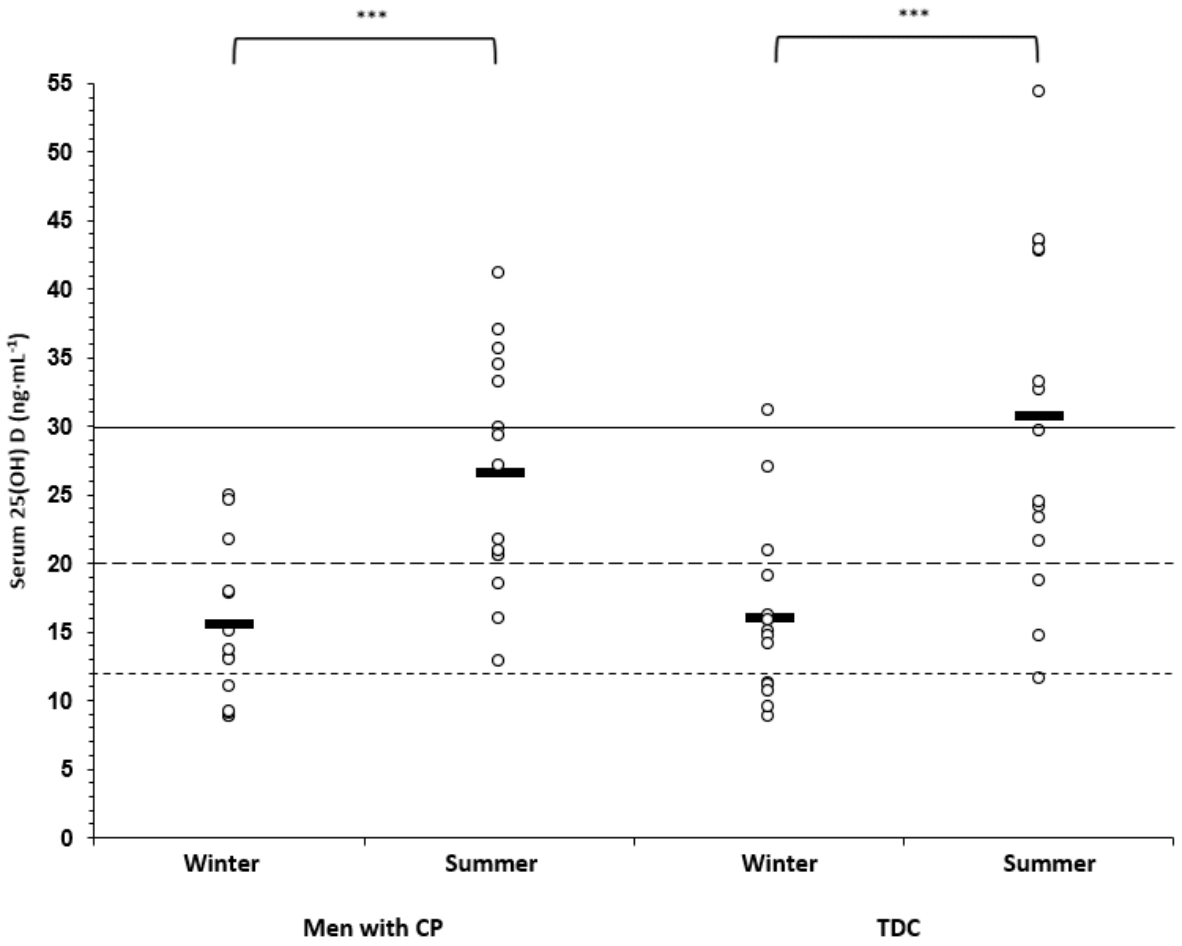

Figure 5-2. Serum 25(OH)D in men with CP and TDCs in the winter and summer months.

*** denotes significant difference between seasons, $p<0.001$. Below black line denotes serum 25(OH)D insufficiency threshold (20.1-30 ng·mL^{-1}) and below black dashed line denotes serum 25(OH)D deficiency (12.1-20 ng·mL^{-1}) and below the grey dashed line denotes severe deficiency (<12 ng·mL^{-1}).

5.3.4 Neuromuscular Performance Outcomes

Men with CP had 27% and 27.7% smaller VL ACSA in the winter and summer months respectively ($p<0.01$, Table 5-4), and 56.3% and 27.5% weaker KE iMVC in the winter and summer months respectively ($p<0.001$, Table 5-4). Men with CP had 34.2% weaker KE iMVC/BM compared to TDC during the winter ($p=0.012$, table 5-4). There was no group

difference in KE iMVC/VL ACSA (p=0.166) (Table 5-4). Men with CP had 29.2% and 21.4% smaller vertical jump no arm swing (p=0.001), and 26.8% and 14.3% smaller vertical jump with arm swing compared to TDC in the winter and summer months respectively (p=0.002). Men with CP had a 8.9% slower 10 m sprint time in the summer compared to TDC (p=0.021), but showed no difference in grip strength (p=0.072).

There was a season effect for 10 m sprint times (p=0.042) in men with CP where 10 m sprint times were 4.71% slower in the summer compared to winter (p=0.042, Table 5-4), there was no season effect on 10 m sprint in TDC (p>0.05). There was no seasonal effect on VL ASCA (p=0.979), KE iMVC (p=0.297), KE iMVC/VL ACSA (p=0.068) or KE iMVC/BM (p=0.170). There was also no season effect on vertical jump no arm swing (p=0.966), vertical jump with arm swing (p=0.288) or grip strength (p=0.418).

There was no interaction effect on VL ASCA (p=0.280), KE iMVC (p=0.317), KE iMVC/VL ACSA (p=0.444) or KE iMVC/BM (p=0.234). There were no interaction effects for vertical jump no arm swing (p=0.769), vertical jump arm swing (p=0.141), 10 m sprint (p=0.136) or grip strength (p=0.962). There were no correlations between 25(OH)D and VL ACSA, KE iMVC, KE iMVC/VL ACSA, KE iMVC/BM, vertical jump no arm swing, vertical jump with arm swing, 10 m sprint or grip strength when controlling for TSE in the summer months (all p>0.05).

Table 5-4. Neuromuscular outcomes means±SD and % change between winter and summer in men with cerebral palsy (CP) during and typically developed controls (TDC).

	CP			TDC		
	Winter	Summer	% change	Winter	Summer	% change
VL ACSA (cm^2)	27.1±3.3[c]	27.9±5.5[c]	-2.5%	35.3±9.0	34.6±8.51	-2.0%
KE iMVC (N)	400±95[c]	488±125[c]	22.0%	625±140	622±139	-0.5%
KE iMVC/VL ACSA ($N \cdot cm^2$)	15.2±3.4	18.2±4.8	19.7%	18.1±3.1	18.9±5.15	4.4%
KE iMVC/BM ($N \cdot kg^{-1}$)	5.87±1.34[c]	7.32±2.11[c]	24.7%	7.88±1.68	7.88±1.71	0.0%
Vertical jump no arm swing (m)	0.41±0.07[c]	0.42±0.09[c]	2.4%	0.52±0.09	0.51±0.08	-1.9%
Vertical jump with arm swing (m)	0.46±0.06[c]	0.49±0.09[c]	6.5%	0.57±0.08	0.56±0.08	-1.8%
10 m sprint (s)	1.91±0.15[b,c]	2.00±0.18[c]	4.7%	1.82±0.14	1.83±0.14	0.6%
Grip strength (kg)	40.7±12.5	41.2±12.6	1.2%	56.8±15.0	53.4±14.4	-6.0%

VL ACSA, Vastus Lateralis anatomical cross sectional area; KE iMVC, knee extensor isometric maximal voluntary contraction; BM, body mass. [a] for consistency with subsequent tables [a] denotes an interaction effect, none were found in the above outcome measures ; [b] denotes a difference from winter in each group $p<0.05$. [c] denotes a difference from TDC for corresponding season $p<0.05$.

5.3.5 Bone

There was no difference between CP and TDC for tibia T_{us} score (p=0.484) or tibia Z_{us} score (p=0.548). Men with CP had lower radius T_{us} scores than TDC in winter and summer months (p<0.001, Table 5-5). Men with CP had lower radius Z_{us} scores compared to TDC in the winter and summer months (p<0.001, Table 5). There was no difference in PTH (p=0.056) for winter or summer in CP and TDC.

There was no seasonal effect for tibia T_{us} score (p=0.309) or for tibia Z_{us} score (p=0.351). There was no seasonal effect for radius T_{us} score (p=0.141) or for radius Z_{us} scores (p=0.115). 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 TDC (p<0.001).

There was an interaction effect for tibia T_{us} scores (p=0.029). Comparisons showed that men with CP had similar tibia T_{us} scores to TDC during the winter months (p>0.05) but lower scores in the summer months (p<0.05, Table 5-5). There was an interaction effect (p=0.026) where men with CP showed similar tibia Z_{us} scores to TDC during the winter months (p>0.05) but lower tibia Z_{us} scores in the summer months (p<0.05). There was no interaction effect for radius T_{us} score (p=0.950) or for radius Z_{us} scores (p=0.916). There was no interaction effects for PTH (p=0.088). 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>0.05). 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>0.05). 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>0.05).

Table 5-5. PA 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. Presented as mean±SD.

	CP		TDC	
	Winter	Summer	Winter	Summer
Tibia T_{us} score	0.43±1.79	0.18±1.2[a]	0.24±0.80	0.90±0.79
Tibia Z_{us} score	0.47±1.77	0.20±1.2[a]	0.24±0.81	0.88±0.83
Radius T_{us} score	-1.30±1.06[c]	-0.91±1.5[c]	0.48±0.86	0.83±1.33
Radius Z_{us} score	-0.94±1.03[c]	-0.54±1.5[c]	0.74±0.92	1.19±1.37
PTH ($ng \cdot dL^{-1}$)	33.3±12.4[b]	17.4±0.6	25.6±9.9[b]	16.8±1.16

PTH, parathyroid hormone: [a] for consistency with subsequent tables [a] denotes an interaction effect; [b] denotes a difference from winter in each group $p<0.05$; [c] denotes a difference from TDC for corresponding season $p<0.05$.

5.3 Discussion

The main findings of this study show that there was consistent effect of increasing vitamin D in the summer from winter in both men with CP and TDC. With variations in the seasonal response of neuromuscular and skeletal outcomes such that neuromuscular performance outcomes showed no seasonal effect of 25(OH)D except for 10 m sprint time which slowed in CP, and improved tibia and radius T_{us} and Z_{us} scores in TDC and in the radius of men with CP.

This study found that 25(OH)D increased in the summer compared to the winter in men with CP and TDC and was consistent with other studies performed at similar latitudes (Cannell et al., 2009). The findings showed a 70.5% and 85.7% increase of 25(OH)D in men with CP and 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 (Maruyama-Nagao et al., 2016). The

similar increase in 25(OH)D in the present data compared to that from the more equatorial Japanese athletes, where a greater increase would be expected (Hagenau et al., 2009), is likely due to the Japanese athletes being from predominantly indoor sports.

The data presented in this current study is consistent with findings from typically developed athletes Wilson-Barnes et al. (2020), whereby no effects of 25(OH)D seasonal variation on VL ACSA, KE iMVC, KE iMVC/BM, KE iMVC/ACSA, vertical jump no arm swing, vertical jump with arm swing and grip strength were observed. It has been identified that 25(OH)D should be increased above the 'optimal' threshold (>40 ng·mL^{-1}) in TDC to elicit significant genomic and non-genomic effects such as increased myocyte proliferation or differentiation (Giuliani and Boland, 1984) and greater recruitment of type IIa muscle fibres (Ceglia, 2008) in skeletal muscle of physically active populations (Larson-Meyer and Willis, 2010).

In men with CP and TDC in present study and TD university athletes in Wilson-Barnes et al. (2020), despite a 25(OH)D increase in the summer (40% in Wilson-Barnes et al. (2020)), 25(OH)D levels below 40 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 <40 ng·mL^{-1}, 1 rep max squat and bench, vertical jump and 20 m sprint is unchanged (Close et al., 2013), whereas supplementation of D_3 raising 25(OH)D levels of 14 male footballers to >40 ng·mL^{-1} can improve 10 m sprint and vertical jump in physically active adults. As both groups in this current study had 25(OH)D levels well below 40 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, to increase their neuromuscular function and physical performance a supplementation strategy should be developed to raise 25(OH)D to optimal levels.

The lack of a seasonal variation in muscle function, likely due to 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, as there was no associations between KE iMVC and 10 m sprint in that group seen in Chapter 4. Nesser et al. (1996) 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. But is likely that the decrease in 10 m sprint 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 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 chapter shows a disparity between the upper and lower limb where a seasonal interaction was seen only in the tibia in both men with CP and TDC. Where there was a contrasting direction in tibia T_{us} and Z_{us} scores where men with CP saw a decrease and TDC saw increases. The increase seen in TDC tibia is consistent with other studies in healthy young adults (Vanderschueren et al., 1991, Krølner, 1983). This increase is most likely mediated by the decrease in PTH levels and therefore reduced levels of bone resorption with adequate levels of 25(OH)D (>30 ng·mL^{-1}) during the summer (Sai et al., 2011). Whereas the decrease in tibia T_{us} and Z_{us} score in men with CP does not fit with other existing literature, though 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 TDC is likely due to the tibia experiencing inconsistent loading patterns based on varying activity levels (Whalen et al., 1988). It was identified that PA frequency, average time and total time spent performing PA did not change throughout the seasons in either group in this study. 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 (Caldwell and Peters, 2009). 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 TDC (Overgaard et al., 1988). 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 adequate (<30 $ng \cdot mL^{-1}$) throughout the year. It has been shown that prolonged 25(OH)D insufficiency and deficiency leads to reduced bone health in the elderly (Ooms et al., 1995). Therefore, it may be pertinent to implement a 25(OH)D supplementation protocol in order to offset potential reductions in bone health that may not have yet manifested in younger men with CP.

5.5 Strengths and Limitations

It should be acknowledged that 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. Where during the winter months, despite not showing a difference in physical activity between time points (Table 5-3), it would be expected

that musculoskeletal performance based around vitamin D being at its nadir should also be at its poorest, yet 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 to the off season (Requena et al., 2017). 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.

A major impact limiting this study was data collection occurring between March 2019 and March 2020 corresponding with the start of the COVID pandemic. Although not all data collection was directly impacted by the pandemic the original plan to collect subsequent data over four time points was ceased as the FA placed a moratorium on training camps until March 2021. To determine if the current sample size used in this study was powered to see a large effect size (β=0.8) a post hoc G*power analysis was performed using 25(OH)D observed power (η^2) taken from the repeated measured ANOVA. The power analysis provided a suggested sample size of n=16 in each group for two repeated measures, suggesting that this study was powered appropriately. The seasonal data collected from the winter and summer months provided the most impactful insight into the greatest magnitude of change from the nadir to peak value of 25(OH)D and therefore it should be acknowledged that the data collected for this chapter is appropriate to show seasonal variations in vitamin D.

5.6 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 have low levels

of vitamin D throughout the entirety of the year, but 25(OH)D levels are not associated with any improvements in musculoskeletal outcomes in men with CP. Whereas TDC who had adequate levels of 25(OH)D in the summer months showed seasonal improvements in tibia T_{us} and Z_{us} scores. Therefore, in men with CP, it is important to undertake strategies to increase vitamin D to adequate or even physiological optimal levels in order to elicit potential improvements in musculoskeletal health and performance.

Chapter 6 - General Discussion, Study Limitations, Conclusions, Contribution of Thesis and Future Research

6. 1 General Discussion

The current thesis has investigated musculoskeletal outcomes in men with cerebral palsy and the impact of vitamin D. To the author's knowledge there is no extant data that addresses musculoskeletal health in physically active men with CP and the impact of vitamin D. Before the start of this current thesis, previous research on the impact of diminished musculoskeletal health on day-to-day life in CP has been performed in sedentary populations with CP (Morgan and McGinley, 2013a) but not in physically active populations with CP. Despite it becoming more apparent that vitamin D has a direct genomic and non-genomic impact on the sarcoplasmic reticulum of muscle (Ceglia, 2008), prior to this thesis, investigations into whether already impaired neuromuscular function in CP is exacerbated by low levels vitamin D or improved with seasonal increases in vitamin D levels has not yet been investigated. Where currently, studies have only primarily investigated absolute vitamin D status and the impact of seasonal variations in vitamin D on bone health in sedentary children with CP (Finbråten et al., 2015, Tosun et al., 2017, Henderson et al., 2002), but there is no literature to show the impact of vitamin D on bone health in physically active men with CP.

Following the findings from chapter 3 show that 43% of physically active men with CP are classified as fallers, with 44% of these fallers experiencing an injurious fall, where most of these injuries were minor. Although chapter 3 showed a high falls rate over the last 12-months in men with CP (43%), experiences of falls were still lower compared to Morgan et al. (2015) who found that 82.4% of ambulatory adults with CP experienced falls more than twice over a 12-month period. Prior to the findings from this study, the focus had been on the plantar flexors, here it can be confirmed that as with sprinting in able bodied (Newman et al., 2004), the KE extensors play a role in determining falls status, such that those 43% of men

with CP from chapter 3 who were fallers had 44% weaker KE strength compared to TDC. This emphasis on muscle weakness as an important functional determinant is at odds with the classical focus on spasticity, and GMFCS as primary outcomes for people with CP (Ross and Engsberg, 2007). More recent perspectives do however support the role of plantar flexion iMVC as being a determinant of motor function in CP (de Groot et al., 2012), with the data from chapter 3 demonstrating an important contribution of the KE to functional outcomes in ambulatory men with CP.

Psychological outcomes such as fear of falling and risk-taking behaviours have been shown to be significantly impacted in those that experience falls, where men with CP in chapter 3 have shown higher fear of falling and lower risk-taking behaviours compared to TDC, yet show similar levels to elderly populations (Butler et al., 2015). An elevated fear of falling and lower risk-taking behaviours are often associated with increased sedentary behaviours and reduced levels of spontaneous PA (Doi et al., 2012). Which consequently lead accelerated decreases in muscle weakness and sarcopenia in elderly individuals. As CP has recently been described as a condition with an already accelerated risk of sarcopenia (Jeon et al., 2019), and an earlier onset of ageing associated decrements in bone mass and body composition (Peterson et al., 2013). The higher falls prevalence, linked to muscle weakness seems to reflect the likelihood that even physically active adults with CP are likely to experience an earlier onset of ageing induced co-morbidities. This is likely to be accelerated in those who are more impaired, and less active than the selectively active population recruited for this thesis. Overall, this emphasises the importance of the focus form this thesis on musculoskeletal outcomes and potential co-variates such as vitamin D.

Prior to this thesis there was no current literature that investigated absolute vitamin D levels during their nadir in the winter months or the impact of vitamin seasonal variation in UK based men with CP. Chapter 4 showed that during the winter months 25(OH)D levels were similar between men with CP and TDC groups. However, almost all men with CP in chapter 4 and in chapter 5 were below levels considered adequate (<30ng·mL^{-1}) during the winter (n=20/22, 90.1% and n=15/15, 100%, respectively), which agreed with other studies that showed a high prevalence of low vitamin D in healthy UK adults (Cannell et al., 2009, Kift et al., 2018, Webb et al., 2010). However, as vitamin D was so low in men with CP and TDC in chapter 4 as a result from poor diet and negligible sun exposure, it was difficult to draw meaningful conclusions or group comparisons as would be necessary to make firm conclusions about low vitamin D accentuating the CP condition's impairments. However, it did show that men with CP who had lower 25(OH)D, experienced weaker KE iMVC/LBM, but there was association between 25(OH)D and KE iMVC/LBM in the TDC group. This finding in chapter 4 suggests that 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. A lower knee extensor strength relative to LBM has a number of functional implications in both CP and other conditions of muscle weakness such as increased risk of falls. In contrast there were no associations between 25(OH)D and other outcomes in chapter 4 or chapter 5, a likely consequence of the low levels of 25(OH)D. Therefore, this thesis was essential in identifying an issue with low 25(OH)D on KE strength in men with CP, but it was unable to fully explore the full impact of vitamin D and the impact of seasonal variations on musculoskeletal health within CP, as the levels were so low in the winter (baseline) comparisons in both groups, and increased to non-threshold levels for muscle function adaptations (Close et al., 2013). To confirm what has been observed in TDC supplementation studies should be recommended in

populations such as men with CP that are likely to show greater prevalence of vitamin D deficiency.

Prior to this thesis, there has been previous research investigating bone health in sedentary and non-ambulating adolescents and children with CP (Finbråten et al., 2015), but there was no research in physically active adults with CP. The finding in chapter 5 that bone responded positively in TDC with the increase in 25(OH)D in the summer suggests that thresholds for improvement is lower in the bone compared to muscle and is consistent with suggested 25(OH)D levels >30ng·mL^{-1} at which PTH plateaus reducing risk of increased bone turnover (Sai et al., 2011). The significant increase in bone T_{us} and Z_{us} scores from winter to summer is particularly meaningful as it suggests a heightened risk for falls related bone fractures in the winter, in a group of men who show a high falls prevalence, and low radius T_{us} and Z_{us} scores, particularly in the winter. It has been shown that prolonged 25(OH)D insufficiency and deficiency leads to reduced bone health in elderly (Ooms et al., 1995). Despite the data in children with CP and TD elderly being more prevalent, it is likely that outside of our active population of men with CP there are going to be middle-aged people with CP who are at greater risk of falls related injury as they will not have the bone and muscle benefits of an active lifestyle. This is likely to be further emphasised in older women with CP as this age and sex are at greater risk of weakness, low bone health and therefore bone related fracture (Agostini et al., 2018). This suggests a pertinence to implement a 25(OH)D supplementation protocol in order to offset potential reductions in bone health that may not have yet manifested in younger populations with CP.

6. 2 Study Limitations

The participants in the thesis represent a highly functional proportion of men with CP (GMFCS I-II). The participant groups are however similar to the only other description of KE iMVC and sprint outcomes (de Groot et al., 2012), and are consistent with populations of CP athletes investigated by others (O'Brien et al., 2021). Comparisons with population based studies suggest that the proportion of GMFCS I: GMFCSII participants is around n=3:2 (Michelsen et al., 2009). In contrast, the strength comparisons made in the present study, de Groot et al. (2012) and (O'Brien et al., 2021) 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 O'Brien et al. (2021). 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 that no data from women was included. Although not adverse to presenting sex disaggregated musculoskeletal outcomes such as, KE strength and tendon stiffness (Hicks et al., 2016, McMahon et al., 2018), the 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, (Barber et al., 2011, de Groot et al., 2012).

6.3 Final Conclusion

This thesis shows that men with CP are at high risk of low 25(OH)D during the winter and should look to safely supplement on vitamin D_3 in order to offset low levels of 25(OH)D to avoid potential deficits in KE iMVC/LBM due to a positive association found between 25(OH)D and KE iMVC/LBM in chapter 4. Using supplements to safely raise 25(OH)D levels has the potential to not only increase sport performance, but also reduce risk of falls, as it was observed in chapter 3 CP fallers had 44% weaker KE iMVC. Chapter 5 has also shown that may also be pertinent to continue supplementation throughout the summer months with aim to raise 25(OH)D levels above the 'optimal' threshold of >40 $ng \cdot mL^{-1}$ to elicit possible improvements in musculoskeletal health and performance.

6.4 Contribution of the Thesis

The current thesis is the first, to the author's knowledge, to investigate the prevalence of low vitamin D in physically active ambulatory men with cerebral palsy. The present thesis identifies that physically active men with CP are still at risk of experiencing injurious falls with KE weakness being a potential contributor to these falls. In addition, this thesis 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. Finally, that men with CP experience similar absolute and seasonal variance in vitamin D but have low levels of vitamin D throughout the entirety of the year, but 25(OH)D levels are not associated with any improvements in musculoskeletal outcomes in men with CP.

6.5 Future Research

- Further investigation into neuromuscular predictors of falls in physically active and sedentary men with CP should be considered to help identify appropriate falls prevention strategies in this population.
- 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.
- It is important to acknowledge that chapter 4 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.
- Future studies should look to investigate appropriate vitamin D supplementation strategies in both men with CP and TDC to correct for low vitamin D during the winter.
- Implementation of an appropriate supplementation protocol to raise 25(OH)D to optimal levels and investigations into its impact on neuromuscular performance should also be performed in men with CP and TDC.
- Future research may also look to implement a 25(OH)D supplementation protocol compared to a placebo to offset potential reductions in bone health that may not have yet manifested in younger men with CP.
- Given the known sex differences in bone measures, and the greater risk for osteoporosis with prolonged low vitamin D (Bonnick, 2006), future research should strive to uncover the particular risks and implications of low vitamin D in women with CP.

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Appendix 1

Appendix 1.1- Serum 25(OH)D concentrations during the winter months from different countries and latitudes.

Citation	Population	Country	Latitude	Mean winter 25(OH)D status ng·mL^{-1} (Mean ± SD)	Vitamin D status
Aydın et al. (2019)	N=55 healthy adults	Turkey	39 ºN	19.5 ± 11.4	Deficient
Close et al. (2013)	N=30 Athletes	UK	53 ºN	20.9 ± 10.56	Deficient
Dubnov-Raz et al. (2015)	N=57 healthy adults	Saudi-Arabia	32.4 ºN	36.95 ± 4.78	Sufficient
Flueck et al. (2016)	N=72 elite wheelchair athletes	Switzerland	47 ºN	24.9 ± 2.57	Insufficient
Flueck (2016)	Elite wheelchair athletes with CP or spinal cord injury (SCI)	Switzerland	47 ºN	17.67 ± 7.23	Deficient
Galan et al. (2012)	N=34 professional footballers	Spain	34 ºN	15.16	Deficient
Jastrzębska et al. (2016)	N=36 healthy adults	Poland	54.2 ºN	19.03 ± 6.49	Deficient
Kopeć et al. (2013)	N=24 professional footballers	Poland	51.1 ºN	24.96 ± 9.91	Insufficient

Koundourakis et al. (2014)	N=67 professional male footballers	Greece	35.9 ºN	34.41 ± 7.08	Adequate
Morton et al. (2012)	N=20 professional footballers	UK	53 ºN	20.48 ± 7.63	Deficient
Owens et al. (2015)	Young adults	UK	53 ºN	18.07 ± 8.03	Deficient
Pfeifer et al. (2009)	N=242 ambulatory adults aged >65	Germany	52 ºN	21.69 ± 7.23	Insufficient
Pritchett et al. (2016)	N=39 young outdoor and indoor athletes in wheelchairs	Canada	56ºN	Outdoor Athletics - 28.27 ± 8.14 Tennis - 20.04 Indoor Basketball - 24.18 ± 11.85 Rugby-29.95 ± 11.04	Insufficient Deficient Insufficient Insufficient
Rossini et al. (2010)	N=1191 adults with rheumatoid arthritis (RA)	Italy	40 ºN	24.9	Insufficient
Semba et al. (2000)	N=228 adults with disabilities	USA	36 ºN	19.55 ± 1.5	Deficient
Shanely et al. (2014)	N=34 Healthy adults	USA	35.5 ºN	20.83 ± 2.24	Insufficient
Smolders et al. (2008)	N=267 adults with MS	Netherlands	51 ºN	25.05 ± 13.47	Insufficient
Todd et al. (2016)	N=42 healthy adults	UK	53 ºN	17.27 ± 8.8	Deficient
van der Mei et al. (2007)	N=136 adults with multiple sclerosis (MS)	Australia	43 ºS	20.64	Insufficient
Wyon et al. (2018)	N=84 healthy adults	UK	51.5 ºN	23.96 ± 4.2	Insufficient
Zamboni et al. (2002)	N= 175 adults with RA	Italy	40 ºN	15.79 ± 10.0	Deficient
Zeitler et al. (2018)	N=581 healthy adults	Austria	47.52 ºN	27.17 ± 10.89	Insufficient

Appendix 2 - Ethical approval

Appendix 2.1 – Ethical approval letter for chapter 4 and 5 studies.

Manchester Metropolitan University

20/02/2019
Project Title: Vitamin D and muscle health

EthOS Reference Number: 2780

Ethical Opinion

Dear Christina Kate Langley,

The above application was reviewed by the Science and Engineering Research Ethics and Governance Committee and, on the 20/02/2019, was given a favourable ethical opinion. The approval is in place until 01/10/2021 .

Conditions of favourable ethical opinion

Application Documents

Document Type	File Name	Date	Version
Consent Form	Informed Consent Form	19/11/2018	1
Recruitment Media	Social Media Ad -updated	15/01/2019	2
Information Sheet	ISP form Experimental-Updated	15/01/2019	2
Project Proposal	CLangley_Project-proposal (1)	15/01/2019	2
Information Sheet	ISP form Controls-updated	15/01/2019	2
Additional Documentation	pre assessment questionnaire (1)	17/01/2019	1

The Science and Engineering Research Ethics and Governance Committee favourable ethical opinion is granted with the following conditions

Adherence to Manchester Metropolitan University's Policies and procedures

This ethical approval is conditional on adherence to Manchester Metropolitan University's Policies, Procedures, guidance and Standard Operating procedures. These can be found on the Manchester Metropolitan University Research Ethics and Governance webpages.

Amendments

If you wish to make a change to this approved application, you will be required to submit an amendment. Please visit the Manchester Metropolitan University Research Ethics and Governance webpages or contact your Faculty research officer for advice around how to do this.

We wish you every success with your project.

Science and Engineering Research Ethics and Governance Committee

Appendix 2.2 – Ethical approval letter for chapter 3 falls study.

29/04/2020
Project Title: Vitamin D and muscle health

EthOS Reference Number: 2780

Ethical Opinion

Dear Christina Kate Langley,

The above amendment was reviewed by the Science and Engineering Research Ethics and Governance Committee and, on the 29/04/2020, was given a favourable ethical opinion. The approval is in place until 01/10/2021 .

Conditions of favourable ethical opinion

Application Documents

Document Type	File Name	Date	Version
Additional Documentation	CP falls survey 12-month	28/04/2020	1
Additional Documentation	CP falls survey 1-month	28/04/2020	1
Additional Documentation	amended ISP form for CP footballers	28/04/2020	1

The Science and Engineering Research Ethics and Governance Committee favourable ethical opinion is granted with the following conditions

Adherence to Manchester Metropolitan University's Policies and procedures

This ethical approval is conditional on adherence to Manchester Metropolitan University's Policies, Procedures, guidance and Standard Operating procedures. These can be found on the Manchester Metropolitan University Research Ethics and Governance webpages.

Amendments

If you wish to make further changes to this approved application, you will be required to submit an amendment. Please visit the Manchester Metropolitan University Research Ethics and Governance webpages or contact your Faculty research officer for advice around how to do this.

We wish you every success with your project.

Science and Engineering Research Ethics and Governance Committee

Science and Engineering Research Ethics and Governance Committee

For help with this application, please first contact your Faculty Research Officer. Their details can be found here

3 Appendix - Questionnaires

3.1 Appendix - Eligibility questionnaire

Pre-assessment questionnaire.

Musculoskeletal health and injury in para footballers: Seasonal variations and the role of vitamin D.

MMU Ethics number: 2019-2780-3614

Participant study ID_____

1) Over the last 12 months have you experienced any illness that has resulted in you not being able to undertake physical activity? Yes ☐ No ☐

2) Over the last 3 months have you used a sunbed? Yes ☐ No ☐

If yes how often per week? _____

3) Over the last 3 months have you travelled abroad? Yes ☐ No ☐

If yes where and for how long? _____

_____

4) Are you currently taking any form of corticosteroid medication? Yes ☐ No ☐

5) Females only - are you currently taking any form of oral contraceptive? Yes ☐ No ☐

6) Are you currently taking any form of anticonvulsant medication? Yes ☐ No ☐

7) Over the last 3 months have you changed your diet? Yes ☐ No ☐

If so how? _____

8) Do you have any specific dietary requirements e.g. vegan, halal, or gluten free? Yes ☐ No ☐

Please state_____

9) Have you been exposed to any ionising radiation in the last 12 months (E.g. from X-rays, CT scans and DEXA etc.) Yes ☐ No ☐

Please state_____

10) Which skin tone from the scale below best represents yours? ☐

Appendix 3.2 - International physical activity questionnaire; long form

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** and **moderate** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal.

PART 1: JOB-RELATED PHYSICAL ACTIVITY

The first section is about your work. This includes paid jobs, farming, volunteer work, course work, and any other unpaid work that you did outside your home. Do not include unpaid work you might do around your home, like housework, yard work, general maintenance, and caring for your family. These are asked in Part 3.

1. Do you currently have a job or do any unpaid work outside your home?

   ☐ Yes

   ☐ No → ***Skip to PART 2: TRANSPORTATION***

The next questions are about all the physical activity you did in the **last 7 days** as part of your paid or unpaid work. This does not include traveling to and from work.

2. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, heavy construction, or climbing up stairs **as part of your work**? Think about only those physical activities that you did for at least 10 minutes at a time.

   _____ **days per week**

   ☐ No vigorous job-related physical activity → ***Skip to question 4***

3. How much time did you usually spend on one of those days doing **vigorous** physical activities as part of your work?

   _____ **hours per day**
   _____ **minutes per day**

4. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads **as part of your work**? Please do not include walking.

   _____ **days per week**

   ☐ No moderate job-related physical activity → ***Skip to question 6***

5. How much time did you usually spend on one of those days doing **moderate** physical activities as part of your work?

_____ **hours per day**
_____ **minutes per day**

6. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **as part of your work**? Please do not count any walking you did to travel to or from work.

_____ **days per week**

☐ No job-related walking → ***Skip to PART 2: TRANSPORTATION***

7. How much time did you usually spend on one of those days **walking** as part of your work?

_____ **hours per day**
_____ **minutes per day**

PART 2: TRANSPORTATION PHYSICAL ACTIVITY

These questions are about how you traveled from place to place, including to places like work, stores, movies, and so on.

8. During the **last 7 days**, on how many days did you **travel in a motor vehicle** like a train, bus, car, or tram?

_____ **days per week**

☐ No traveling in a motor vehicle → ***Skip to question 10***

9. How much time did you usually spend on one of those days **traveling** in a train, bus, car, tram, or other kind of motor vehicle?

_____ **hours per day**
_____ **minutes per day**

Now think only about the **bicycling** and **walking** you might have done to travel to and from work, to do errands, or to go from place to place.

10. During the **last 7 days**, on how many days did you **bicycle** for at least 10 minutes at a time to go **from place to place**?

_____ **days per week**

☐ No bicycling from place to place → ***Skip to question 12***

11. How much time did you usually spend on one of those days to **bicycle** from place to place?

_____ **hours per day**
_____ **minutes per day**

12. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time to go **from place to place**?

_____ **days per week**

☐ No walking from place to place → ***Skip to PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY***

13. How much time did you usually spend on one of those days **walking** from place to place?

_____ **hours per day**
_____ **minutes per day**

PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY

This section is about some of the physical activities you might have done in the **last 7 days** in and around your home, like housework, gardening, yard work, general maintenance work, and caring for your family.

14. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, chopping wood, shoveling snow, or digging **in the garden or yard**?

_____ **days per week**

☐ No vigorous activity in garden or yard → ***Skip to question 16***

15. How much time did you usually spend on one of those days doing **vigorous** physical activities in the garden or yard?

_____ **hours per day**
_____ **minutes per day**

16. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, sweeping, washing windows, and raking **in the garden or yard**?

_____ **days per week**

☐ No moderate activity in garden or yard → ***Skip to question 18***

17. How much time did you usually spend on one of those days doing **moderate** physical activities in the garden or yard?

_____ **hours per day**
_____ **minutes per day**

18. Once again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, washing windows, scrubbing floors and sweeping **inside your home**?

_____ **days per week**

☐ No moderate activity inside home → ***Skip to PART 4: RECREATION, SPORT AND LEISURE-TIME PHYSICAL ACTIVITY***

19. How much time did you usually spend on one of those days doing **moderate** physical activities inside your home?

_____ **hours per day**
_____ **minutes per day**

PART 4: RECREATION, SPORT, AND LEISURE-TIME PHYSICAL ACTIVITY

This section is about all the physical activities that you did in the **last 7 days** solely for recreation, sport, exercise or leisure. Please do not include any activities you have already mentioned.

20. Not counting any walking you have already mentioned, during the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **in your leisure time**?

_____ **days per week**

☐ No walking in leisure time → ***Skip to question 22***

21. How much time did you usually spend on one of those days **walking** in your leisure time?

_____ **hours per day**
_____ **minutes per day**

22. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like aerobics, running, fast bicycling, or fast swimming **in your leisure time**?

_____ **days per week**

☐ No vigorous activity in leisure time → ***Skip to question 24***

23. How much time did you usually spend on one of those days doing **vigorous** physical activities in your leisure time?

_____ **hours per day**
_____ **minutes per day**

24. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like bicycling at a regular pace, swimming at a regular pace, and doubles tennis **in your leisure time**?

_____ **days per week**

☐ No moderate activity in leisure time → ***Skip to PART 5: TIME SPENT SITTING***

25. How much time did you usually spend on one of those days doing **moderate** physical activities in your leisure time?
_____ **hours per day**
_____ **minutes per day**

PART 5: TIME SPENT SITTING

The last questions are about the time you spend sitting while at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading or sitting or lying down to watch television. Do not include any time spent sitting in a motor vehicle that you have already told me about.

26. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekday**?

_____ **hours per day**
_____ **minutes per day**

27. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekend day**?

_____ **hours per day**
_____ **minutes per day**

This is the end of the questionnaire, thank you for participating.

3.3 **Appendix** – Sun Exposure Questionnaire

Musculoskeletal health and injury in para footballer: Seasonal variations and the role vitamin D

Sun exposure questionnaire

Participant ID_____

During spring and summer seasons how often are you exposed directly to the sun outside (not through glass, window or umbrella)?

- Daily
- 5-6 times per week
- 3-4 times per week
- 1-2 times per week
- 3-4 times per month
- 1-2 times per month
- Rarely
- Never

What time of the day are you exposed to the sun (not through glass, window or umbrella)? (Tick all that apply)

a) During the weekdays:

- 7-9am
- 9-11am
- 11-1pm
- 1-3pm
- 3-5pm
- 5-7pm
- none

b) During the weekend:

- 7-9am
- 9-11am
- 11-1pm
- 1-3pm
- 3-5pm
- 5-7pm
- none

During the spring and summer seasons how much time do you usually stay outdoors in daylight?

a) Typical weekdays

- Less than 10 mins
- 10-20 mins
- 20-30 mins
- 30-1 hours
- 1-2 hours
- 2-4 hours
- 4-6 hours
- 8 or more hours
- None

b) Typical weekends

- Less than 10 mins
- 10-20 mins
- 20-30 mins
- 30-1 hours
- 1-2 hours
- 2-4 hours
- 4-6 hours
- 8 or more hours
- None

What areas of your body are often exposed to sunlight? (Tick all that apply)

- Face
- Full arms
- Hands
- Half arms
- Feet
- Half legs
- Full legs
- Other (State) .....

What kind of sun protection do you use?

- Sun cream
- Umbrella
- Hat
- Head scarf
- Other (State) .....

During spring and summer seasons how often do you wear sun protection?

- Daily
- 5-6 times per week
- 3-4 times per week
- 1-2 times per week
- 3-4 times per month
- 1-2 times per month
- Never

If you wear sun cream what sun protection factor (SPF) number of the sun cream you wear most often?

- 10
- 15
- 20
- 30
- 40
- 50+

3. 4 Appendix – 1 month falls survey JISC online surveys

Cerebral Palsy 1-month survey 2020

Study information

Previously you have taken part in a research project about falls in adults with Cerebral Palsy. So far this has involved some testing of bone and muscle health and the completion of some questionnaires about falls and fall risk.

We would like to ask you to complete a follow up survey about falls and falls risk in the last month, our aim is to complete 6-months of 1-month surveys in total. This 1-month recall survey should a take 10-15 mins.

In the email invite you will have received a participant id code. We are committed to keeping your identity and responses private, your responses will be linked to your bone and muscle health only through this code, as we do not use names within our data collection. Based on this, there will be no way to identify you from your responses and your data will remain anonymous throughout the research process. There are no questions that will ask about identifying information.

In order to complete this survey you will need your **project id** that was sent with your email invite.

Project ID

Using the invitation email you received for this study, please enter your unique **PROJECT ID** below. You can find this at the bottom of the email you were sent and can copy and paste it into the space below.

Falls screening

Have you had a fall within the last **month**, which has resulted in you losing your balance and coming into contact with the ground or a lower level that could not have been prevented?

If you have fallen in the last month, was it just the once, or multiple times? If multiple times roughly how many?

What activities were you doing when you fell in the last month? Select as many as are relevant.

- [ ] Walking
- [ ] Carrying something
- [ ] Bending over
- [ ] Reaching
- [ ] Turning
- [ ] Climbing or descending stairs
- [ ] Playing sport or undertaking exercise
- [ ] Other

If you selected Other, please specify:

Which of the following obstacles contributed to when you fell in the last month? Select as many as are relevant?

- [ ] Dog or other pet
- [ ] Indoor object
- [ ] Slippery surface
- [ ] Uneven path or flooring
- [ ] Steps or stairs
- [ ] Other

If you selected Other, please specify:

Which of the following best describes when you fell in the last month? Select as many as are relevant.

- [ ] Trip/stumble
- [ ] Slip
- [ ] Bumped into something/someone
- [ ] Pushed/pulled over
- [ ] General loss of balance
- [ ] Loss of support
- [ ] Fainting
- [ ] Other

If you selected Other, please specify:

Whereabouts were you when you fell in the last month? Select as many as are relevant.

- [ ] Indoors (at home)
- [ ] Outdoors (at home)
- [ ] In the community

Was drinking alcohol a contributing factor when you fell in the last month?

Were you injured when you fell in the last month?

Where on your body were you injured when you fell in the last month? Select as many as are relevant.

- ☐ Head/face/neck
- ☐ Arms/shoulder/wrist/hands
- ☐ Torso/chest/abdomen/back
- ☐ Legs/knees/ankles/hips

Which of the following types of injuries did you sustain as a result of when you fell in the last month? Select as many as are relevant.

- ☐ Bruise
- ☐ Open wound/cut
- ☐ Strain/sprain (soft tissue injury)
- ☐ Break or fracture to a bone
- ☐ Bump
- ☐ Other

If you selected Other, please specify:

Did you seek any medical attention when you fell in the last month? Select as many as are relevant.

- [ ] No medical attention
- [ ] No medical attention but I self-treated the injury
- [ ] I went to my GP
- [ ] I went to Accident and Emergency (A&E/ER)
- [ ] I was treated by a physiotherapist
- [ ] Other

If you selected Other, please specify:

Are you worried about falling again?

Do you feel that you might change your behvaiour or activities based on having a fall?

Near falls

Have you had a near fall within the last **month**, a slip or trip that would have resulted in you coming into contact with the ground, if the fall was not broken by an object or person preventing you from falling?

If you have had a near fall in the last month, roughly how many have you had?

Near falls details

What activities were you doing when you had a near fall in the last month? Select as many as are relevant.

- ☐ Walking
- ☐ Carrying something
- ☐ Bending over
- ☐ Reaching
- ☐ Turning
- ☐ Climbing or descending stairs
- ☐ Playing sport or undertaking exercise
- ☐ Other

If you selected Other, please specify:

Which of the following obstacles contributed to a near fall in the last month? Select as many as are relevant.

- ☐ Dog or other pet
- ☐ Indoor object
- ☐ Slippery surface
- ☐ Uneven path or flooring
- ☐ Steps or stairs
- ☐ Other

If you selected Other, please specify:

Whereabouts were you when you had a near fall in the last month? Select as many as are relevant.

- ☐ Indoors (at home)
- ☐ Outdoors (at home)
- ☐ In the community

Alcohol

Have you consumed alcohol in the last **month**?

Over the last month what was your average **WEEKLY** alcoholic drink intake. Use the information below to make an estimate.

More info

Activity levels

How would you best describe your physical activity level over the last month?

- ○ Sedentary (Walking less than 20 mins a day)
- ○ Slightly active (walk over 20 mins per day)
- ○ Active (undertake at least 20 mins of moderate physical activity per day)
- ○ Very active (undertake 40 mins of moderate intensity physical activity per day)
- ○ Athlete (high intensity exercise 5+ days a week)

Key for selection options

2 - Have you had a fall within the last month, which has resulted in you losing your balance and coming into contact with the ground or a lower level that could not have been prevented?

Yes
No

7 - Was drinking alcohol a contributing factor when you fell in the last month?

Yes-all of the falls
Yes-some of the falls
No

8 - Were you injured when you fell in the last month?

Yes
No

12 - Are you worried about falling again?

Yes
No

13 - Do you feel that you might change your behvaiour or activities based on having a fall?

Yes
No

14 - Have you had a near fall within the last month, a slip or trip that would have resulted in you coming into contact with the ground, if the fall was not broken by an object or person preventing you from falling?

18 - Have you consumed alcohol in the last month?

Yes
No

19 - Over the last month what was your average WEEKLY alcoholic drink intake. Use the information below to make an estimate.

1 unit
2 units
3 units (e.g. one large glass of wine)
4 units
5 units
6 units (e.g. 3 cans of beer)
7 units
8 units
9 units
10 units
11 units
12 units
13 units
14 units (e.g. one standard glass of wine a night for the week)
15 units
16 units
17 units
18 units
19 units
20 units
21 units (one large glass of wine per night over the week)
22 units
23 units
24 units
25 units
26 units
27 units
28 units
29 units
30 units (e.g. 10 pints of 5.1% beer per week)
31 units
32 units
33 units
34 units
35 units
36 units
37 units
38 units
39 units
40 units
>40 units

3.5 Appendix – 12 months falls survey

Cerebral Palsy 12-month falls 2020

Study information

You are being invited to take part in a research study. Before you decide whether or not to take part, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. If you would like to ask us for more information you can contact either Chrissie Langley (christina.k.langley@stu.mmu.ac.uk) or Dr. Christopher Morse (c.morse@mmu.ac.uk).

Please take time to decide whether or not you wish to take part.

This project aims to determine whether physically active adults with cerebral palsy may be prone to falls and fall related injuries. In adults with cerebral palsy falls are commonplace, happening in over half of those surveyed. By completing this survey you are helping us to try and understand what can contribute to higher levels of falls in adults with cerebral palsy.

We are collecting survey data from people with and without cerebral palsy, and as long as you are aged over 18 you can take part in this survey.

You are under no obligation to take part in this study. If, after reading this study information and asking any additional questions, you do not feel comfortable taking part in the study you do not have to.

This study is composed of a series of questionnaires which should take no more than 30 minutes to complete. Some of the questions are about topics related to falls and habits that may lead to falls such as risk taking, and alcohol intake.

If you have received this survey from an email invite, you will have previously taken part in some data collection about your muscle and bone health. In the email invite you will have received a participant id code. We are committed to keeping your identity and responses private, your responses will be linked to your bone and muscle health only through this code, as we do not use names within our data collection. Based on this, **there will be no way to identify you from your responses and your data will remain anonymous throughout the research process**. There are no questions that will ask about identifying information.

In order to complete this survey you will need your **project id** that was sent with your email invite.

Project ID

Using the invitation email you received for this study, please enter your unique **PROJECT ID** below. You can find this at the bottom of the email you were sent and can copy and paste it into the space below.

Consent to participate

As this is a research project we need you to read the following statement and confirm that you are happy to proceed: I confirm that I have read the study information on the previous page. I confirm I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason. I therefore agree to participate in the study. * *Required*

Falls screening

Have you had a fall within the last **12 months**, which has resulted in you losing your balance and coming into contact with the ground or a lower level that could not have been prevented?

Falls frequency

Roughly how many times have you fallen in the last 12-months?

If you have fallen more than once in the last 12-months, roughly how many times have you fallen?

Over the last 12-months, when you had a fall which of the activities were you doing at the time of the falls? (select as many as are relevant)

- ☐ Walking
- ☐ Carrying something
- ☐ Bending over
- ☐ Reaching
- ☐ Turning
- ☐ Climbing or descending stairs
- ☐ Playing sport or undertaking exercise
- ☐ Other

If you selected Other, please specify:

In the last 12-months, which of the following obstacles has contributed to a fall? Select as many as are relevant.

- ☐ Dog or other pet
- ☐ Indoor object
- ☐ Slippery surface
- ☐ Uneven path or flooring
- ☐ Steps or stairs
- ☐ Other

If you selected Other, please specify:

Which of the following best describes the falls you have had in the last 12-months? Select as many as are relevant.

- ☐ Trip/stumble
- ☐ Slip
- ☐ Bumped into something/someone
- ☐ Pushed/pulled over
- ☐ General loss of balance
- ☐ Loss of support
- ☐ Fainting
- ☐ Other

If you selected Other, please specify:

Where abouts were you when you had a fall in the last 12-months? Select as many as are relevant.

- ☐ Indoors (at home)
- ☐ Outdoors (at home)
- ☐ In the community

During any of the falls in the last 12-months, was drinking alcohol a contributing factor in any of them?

As a result of falling in the last 12-months have you been injured?

Where on your body have you been injured from falling in the last 12-months? Select as many as are relevant.

- ☐ Head/face/neck
- ☐ Arms/shoulder/wrist/hands
- ☐ Torso/chest/abdomen/back
- ☐ Legs/knees/ankles/hips

Which of the following types of injuries have you sustained as a result of a fall in the last 12-months? Select as many as are relevant.

- ☐ Bruise
- ☐ Open wound/cut
- ☐ Strain/sprain (soft tissue injury)
- ☐ Break or fracture to a bone
- ☐ Bump
- ☐ Other

If you selected Other, please specify:

What medical attention did you seek following a fall in the last 12-months?

- ☐ No medical attention
- ☐ No medical attention but I self-treated the injury
- ☐ I went to my GP
- ☐ I went to Accident and Emergency (A&E/ER)
- ☐ I was treated by a physiotherapist
- ☐ Other

If you selected Other, please specify:

Your recent fall

In this section we are interested in **only the most recent fall**. If you have only fallen once in the last 12-months we are interested in that.

What activities were you doing at the time of your recent fall?

- ☐ Walking
- ☐ Carrying something
- ☐ Bending over
- ☐ Reaching
- ☐ Turning
- ☐ Climbing or descending stairs
- ☐ Playing sport or undertaking exercise
- ☐ Other

If you selected Other, please specify:

Which of the following obstacles contributed to your recent most fall?

- ☐ Dog or other pet
- ☐ Indoor object
- ☐ Slippery surface
- ☐ Uneven path or flooring

If you selected Other, please specify:

Which of the following best describes your most recent fall?

- ☐ Trip/stumble
- ☐ Slip
- ☐ Bumped into something/someone
- ☐ Pushed/pulled over
- ☐ General loss of balance
- ☐ Loss of support
- ☐ Fainting
- ☐ Other

If you selected Other, please specify:

Whereabouts did your most recent fall occur?

- ☐ Indoors (at home)
- ☐ Outdoors (at home)
- ☐ In the community

Was drinking alcohol a contributing factor to your most recent fall?

Were you injured in your most recent fall?

Where on your body were you injured from from your most recent fall? Select as many as are relevant.

- [ ] Head/face/neck
- [ ] Arms/shoulder/wrist/hands
- [ ] Torso/chest/abdomen/back
- [ ] Legs/knees/ankles/hips

Which of the following types of injuries did you sustain as a result of your recent fall? Select as many as are relevant.

- ☐ Bruise
- ☐ Open wound/cut
- ☐ Strain/sprain (soft tissue injury)
- ☐ Break or fracture to a bone
- ☐ Bump
- ☐ Other

If you selected Other, please specify:

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Did you seek any medical attention after your most recent fall?

- ☐ No medical attention
- ☐ No medical attention but I self-treated the injury
- ☐ I went to my GP
- ☐ I went to Accident and Emergency (A&E/ER)
- ☐ I was treated by a physiotherapist
- ☐ Other

If you selected Other, please specify:

Near falls

Have you had a near fall within the last **12 months**, a slip or trip that would have resulted in you coming into contact with the ground, if the fall was not broken by an object or person preventing you from falling?

If YES, roughly how frequently in the last 12-months have you experienced a near fall?

If you had more than one near fall in the last 12-months, roughly how many have you had?

Near falls details

Over the last 12-months, when you had a near fall which of the activities were you doing at the time? (select as many as are relevant)

- ☐ Walking
- ☐ Carrying something
- ☐ Bending over
- ☐ Reaching
- ☐ Turning
- ☐ Climbing or descending stairs
- ☐ Playing sport or undertaking exercise
- ☐ Other

If you selected Other, please specify:

In the last 12-months, which of the following obstacles has contributed to a near fall? Select as many as are relevant.

- ☐ Dog or other pet
- ☐ Indoor object
- ☐ Slippery surface
- ☐ Uneven path or flooring
- ☐ Steps or stairs
- ☐ Other

If you selected Other, please specify:

Which of the following best describes the near falls you have had in the last 12-months? Select as many as are relevant.

- ☐ Trip/stumble
- ☐ Slip
- ☐ Bumped into something/someone
- ☐ Pushed/pulled over
- ☐ General loss of balance
- ☐ Loss of support
- ☐ Fainting
- ☐ Other

If you selected Other, please specify:

Where abouts were you when you had a near fall in the last 12-months? Select as many as are relevant.

- ☐ Indoors (at home)
- ☐ Outdoors (at home)
- ☐ In the community

Falls efficacy Scale

Please select the statement that reflects how concerned you are of falling during each of the following activities. If you currently don't do the activity (example: if someone does your shopping for you), please answer to show whether you think you would be concerned about falling IF you did the activity.

	1 Not at all concerned	2 Somewhat concerned	3 Fairly concerned	4 Very concerned
Cleaning the house (e.g. sweep, vacuum, dust)	○	○	○	○
Getting dressed or undressed	○	○	○	○
Preparing simple meals	○	○	○	○
Taking a bath or shower	○	○	○	○
Going to the shop	○	○	○	○
Getting in or out of a chair	○	○	○	○
Going up or down stairs	○	○	○	○
Walking around in the neighborhood	○	○	○	○
Reaching for something above your head or on the ground	○	○	○	○
Going to answer the telephone before it stops ringing	○	○	○	○
Walking on a slippery surface (e.g. wet or icy)	○	○	○	○
Visiting a friend or relative	○	○	○	○
Walking in a place with crowds	○	○	○	○
Walking on an uneven surface (e.g. rocky ground, poorly maintained pavement)	○	○	○	○
Walking up or down a slope	○	○	○	○
Going out to a social event (e.g. religious service, family gathering, or club meeting)	○	○	○	○

Falls risk

For each of the following activities, please select an option that best describes how often you undertake or avoid each activity. If the activity is not applicable to you at the present, please provide an answer indicating what you would do if you were in the described situation.

	Always	Mostly	Occasionally	Never
1. Do you sit down to put on your shoes and socks?	○	○	○	○
2. Do you hold the handrail when you walk down the stairs if one is available?	○	○	○	○
3. Do you hold the handrail when you walk up the stairs?	○	○	○	○
4. Would you catch a bus if you had to stand?	○	○	○	○
5. At traffic lights, do you start crossing after the DO NOT WALK sign starts flashing?	○	○	○	○
6. Would you cross against the lights to catch a bus if you might miss it?	○	○	○	○
7. Would you run to catch a bus or cross the road if you had to?	○	○	○	○
8. Would you climb up on furniture to reach high shelves or change a light bulb?	○	○	○	○
9. Do you use escalators in shopping centres?	○	○	○	○
10. Do you turn a light on at night when going from one room to another?	○	○	○	○
11. Do you always follow the correct safety procedures when climbing a ladder?	○	○	○	○
12. Would you drink heavily at a social function?	○	○	○	○
13. Would you drive a car without wearing a seatbelt?	○	○	○	○

14. Would you ride a bicycle or motorcycle without wearing a helmet?	○	○	○	○
15. Would you sunbathe without wearing sunscreen?	○	○	○	○
16. Would you walk home alone at night in an unsafe area of town?	○	○	○	○
17. When you buy shoes, do you check the soles to see if they are slippery?	○	○	○	○
18. Do you hurry to answer the phone if need be to avoid missing a call?	○	○	○	○

If you are aged 18-20 yrs old, have you noticed a change in how you walk in the last two years?
OR If you are aged over 20, how has your walking changed since you were 18?

If you selected Other, please specify:

Key for selection options

2 - As this is a research project we need you to read the following statement and confirm that you are happy to proceed: I confirm that I have read the study information on the previous page. I confirm I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason. I therefore agree to participate in the study.

Yes I consent to participate in this research.
No I do not consent to participate in this research.

3 - Have you had a fall within the last 12 months, which has resulted in you losing your balance and coming into contact with the ground or a lower level that could not have been prevented?

Yes
No

4 - Roughly how many times have you fallen in the last 12-months?

Never
Once
Several times
Regularly

10 - During any of the falls in the last 12-months, was drinking alcohol a contributing factor in any of them?

Yes-all of them
Yes-some of them
No

11 - As a result of falling in the last 12-months have you been injured?

Yes
No

19 - Was drinking alcohol a contributing factor to your most recent fall?

Yes
No

20 - Were you injured in your most recent fall?

Yes
No

24 - Have you had a near fall within the last 12 months, a slip or trip that would have resulted in you coming into contact with the ground, if the fall was not broken by an object or person preventing you from falling?

Yes
No

31 - If you are aged 18-20 yrs old, have you noticed a change in how you walk in the last two years? OR If you are aged over 20, how has your walking changed since you were 18?

It is unchanged
It has improved
It has got worse
Other