MONASH University

The Optimisation of Bone Health in Chronic Neurological Conditions

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Contents

Thesis including published works declaration

I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

This thesis includes 5 original papers published in peer reviewed journals and 1 unpublished publication. The core theme of the thesis is bone health in chronic neurological disease. The ideas, development and writing up of all the papers in the thesis were the principal responsibility of myself, the student, working within the School of Clinical Sciences under the supervision of Associate Professor Frances Milat.

The inclusion of co-authors reflects the fact that the work came from active collaboration between researchers and acknowledges input into team-based research.

In the case of Chapters 3.2-4.3 my contribution to the work involved the following:

Thesis Chapter	Publication Title	Status *(published, in press, accepted or returned for revision)*	Nature and % of student contribution	Co-author name(s) Nature and % of Co-author's contribution*	Co-author(s), Monash student Y/N*
3.2	Musculoskeletal and endocrine health in adults with cerebral palsy: new opportunities for intervention	Published	60% Concept, collecting data, data analysis and interpretation, writing first draft	1. Phillip Wong: 10% data analysis, interpretation and manuscript writing 2. Michael Fahey 2.5%, input into data interpretation and manuscript writing 3. Justin Brown 2.5% input into data interpretation and manuscript writing 4. Andrew Churchyard: 2.5% data collection 5. Boyd Strauss 2.5% input into data collection, interpretation and manuscript writing 6. Peter Ebeling 5%, input into data interpretation and manuscript writing 7. Peter Fuller 5%, input into data interpretation and manuscript writing 8. Fran Milat 10%, input into data interpretation and manuscript writing	N
3.3	Trabecular bone score in adults with cerebral palsy	Published	60% Concept, collecting data, data analysis and interpretation, writing first draft	1. Phillip Wong: 10% data analysis, interpretation and manuscript writing 2. Michael Fahey 5%, input into data interpretation and manuscript writing 3. Peter Ebeling 5%, input into data interpretation and manuscript writing 4. Peter Fuller 10%, input into data	No

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The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the student's and co-authors' contributions to this work. In instances where I am not the responsible author I have consulted with the responsible author to agree on the respective contributions of the authors.

Main Supervisor signature: **Date:** **06/09/2019**

List of abbreviations

1,25$(OH)_2$D – 1,25 dihydroxy vitamin D
aBMD – areal bone mineral density
BMAD – bone mineral apparent density
BMC – bone mineral content
BMD – bone mineral density
BMI – body mass index
BMU – basic multicellular unit
BTM – bone turnover markers
BUA – broadband ultrasound attenuation
CIC – clean intermittent catheterisation
CKD MBD – chronic kidney disease bone and mineral disorder
COCP – combined oral contraceptive pill
CP – cerebral palsy
CSF – cerebrospinal fluid
CT – computed tomography
CTX – C-terminal telopeptide of type 1 collagen
CV – coefficient of variation
DKK1 – dickkopf1
DPD - deoxypyridinoline
DXA – dual energy x-ray absorptiometry
FGF 23 – fibroblast growth factor 23
FN – femoral neck
FRAX® – fracture risk assessment tool
FSH – follicle stimulating hormone
GH – growth hormone
GMFCS – gross motor function classification scale
GP – Greulich and Pye method
GTP – guanosine triphosphate
HSI – heel stiffness index
HUS – heel ultrasound
ICD – International Classification of Diseases
IGF-1 – insulin like growth factor 1
IOF – International Osteoporosis Foundation
ISCD – International Society for Clinical Densitometry
IV – intravenous
LH – lutenising hormone
LS – lumbar spine
LRP5/6 – low density receptor related protein 5/6
LTM – lean tissue mass
MMP9 – matrix metalloproteinase 9
MRI – magnetic resonance imaging
msV – millisievert
NTD – neural tube defect
OCN – osteocalcin
OHSS – ovarian hyperstimulation syndrome
P1NP – procollagen type 1 N propeptide
PBM – peak bone mass
PEG – percutaneous endoscopic gastrostomy
pQCT – peripheral quantitative computed tomography
PTH – parathyroid hormone
QCT – quantitative computed tomography
QUI – quantitative indec
RANK – receptor activator of nuclear factor kappa B
RANKL – receptor activator of nuclear factor kappa B ligand
RCT – randomised control trial
SB – spina bifida
SCPE – Surveillance of Cerebral Palsy in Europe
SMI – skeletal muscle index
TBS – trabecular bone score
TRAP – tartate resistant acid phosphatase
vBMD – volumetric bone mineral density
VP shunt – ventriculo-peritoneal shunt
WHO – World Health Organisation
Wnt – wingless signalling pathway
ZA – zoledronic acid

List of publications

1. **Trinh A,** Wong P, Fahey MC, Brown J, Churchyard A, Strauss BJ, Ebeling PR, Fuller PJ, Milat F
   Musculoskeletal and endocrine health in adults with cerebral palsy: new opportunities for intervention.
   J Clin Endocrinol Metab. 2016 Mar;101(3):1190-7.

2. **Trinh A**, Wong P, Brown J, Hennel S, Ebeling PR, Fuller PJ, Milat F.
   Fractures in spina bifida from childhood to young adulthood.
   Osteoporosis Int. 2017 Jan;28(1):399-406.

3. **Trinh A**, Wong P, Sakthivel A, Fahey MC, Hennel S, Brown J, Strauss B, Ebeling PR, Fuller PJ, Milat F
   Fat-bone interactions in adults with spina bifida.
   J Endocr Soc. 2017 Sep 27;1(10):1301-1311.

4. **Trinh, A.,** Wong, P., Fahey, M.C., Ebeling, P.R., Fuller, P.J. and Milat, F.
   The trabecular bone score in adults with cerebral palsy.
   Bone. 2018 Dec; 117:1-5

5. **Trinh A**, Wong P, Fahey MC, Brown J, Strauss BJ, Ebeling PR, Fuller PJ, Milat F.
   Longitudinal changes in bone density in adolescents and young adults with cerebral palsy: a case for early intervention.
   Clin Endocrinol (Oxf). 2019 Jun 27.

List of conference presentations/posters

1. *Australian and New Zealand Bone and Mineral Society ASM 2015*
   Poster: Musculoskeletal health in adults with cerebral palsy: new opportunities for intervention

2. *Australian and New Zealand Bone and Mineral Society ASM 2016*
   Presentation: Fractures in spina bifida from childhood to young adulthood (Finalist in New Investigator Session)

3. *Australian Academy of Cerebral Palsy and Developmental Medicine ASM 2016*
   Presentation: Musculoskeletal health in adults with cerebral palsy: new opportunities for intervention

4. *US Endocrine Society 2017*
   Poster: Characterisation of bone and body composition parameters in young adults with spina bifida

5. *Endocrine Society of Australia ASM 2018*
   Presentation: Muscle bone relationship in cerebral palsy

6. *Endocrine Society of Australia ASM 2019*
   Presentation: Longitudinal changes in bone density in adolescents and adults with cerebral palsy: a case for early intervention (Finalist in Bryan Hudson Award)

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6. ESA Travel grant 2019 ($400)

Appendices: other publications produced during candidature

1. **Trinh A**, Wong P, Ebeling PR, Fuller PJ, Milat F. Severe acute phase response after intravenous zoledronic acid in adult patients with cerebral palsy.
   Intern Med J. 2016 Apr;46(4):506-7.

2. **Trinh A**, Fahey MC, Brown J, Fuller PJ, Milat F.
   Optimizing bone health in cerebral palsy across the lifespan.
   Dev Med Child Neuro. 2017 Feb;59(2):232-233.

Abstract of thesis

Adults with chronic neurological disease are at high risk of developing osteoporosis and fracture. The two most common causes of childhood neurological disability are cerebral palsy (CP) and spina bifida (SB). CP results from a deficit or lesion of the immature brain and represents a group of disorders with problems in control of movement. SB represents a variety of congenital neural tube defects. As these individuals are now living longer, there is increasing recognition that bone health and preservation of mobility are crucial in maintaining independence and quality of life.

In adults with CP and SB, there is currently insufficient evidence to guide assessment and management of bone fragility. Paediatric studies in CP and SB have demonstrated the importance of functional status on bone health. In children, the distal lateral femur is the preferred site for measurement of bone mineral density (BMD), but the optimal site of BMD assessment has not been determined in adults with CP or SB. Changes in bone microstructure seen on quantitative computed tomography and high resolution magnetic resonance imaging are likely to contribute to bone fragility in children with these conditions. However, these findings have not been replicated in adults with CP or SB.

Therefore this thesis aimed to examine bone fragility changes in adolescents and adults with CP and SB as they age, to assess risk factors for low bone mass and whether fractures continue into adulthood; to determine the optimal method of measuring BMD in adults with CP and SB; to assess bone microarchitecture and its contribution to bone fragility; and to review longitudinal changes in BMD from adolescence to adulthood.

In adults with CP, the importance of function and ambulation on bone health was shown in both cross-sectional and longitudinal studies of BMD, and through its effects on bone microarchitecture. BMD at the femoral neck correlated with fracture risk, and measurements at the femoral neck and lumbar

spine allowed monitoring during the transition from adolescence to adulthood. Puberty was shown to be a critical time for bone accrual, and the presence of hypogonadism, which can be under-recognised and/or untreated, had adverse effects on bone and muscle mass. In adulthood, fractures that occurred were similar to typical osteoporotic fractures, albeit occurring at a younger age than expected.

In contrast, the risk of fragility fracture decreases in young adulthood in SB. The relationship between fracture and BMD is still unclear, but the high rates of obesity may explain the difference in fracture risk and bone mass seen between SB and CP.

In summary, this body of work demonstrates the multitude of factors negatively impacting bone health in adults with chronic neurological disease. In adults with CP and SB, the femoral neck (and to a lesser degree lumbar spine) should be the site of BMD measurement and follow-up. Attention to maintenance of muscle mass and ambulation is key to reducing bone loss in CP. Hypogonadism may play a role in bone health in CP through its effect on muscle, although further work needs to be done to determine the efficacy of sex-steroid replacement. The importance of early intervention to improve bone accrual in childhood and during puberty is emphasised by the longitudinal changes seen in BMD during this time in CP. In SB, further work is needed to address the management of obesity and associated cardiovascular comorbidities, as bone health is not as severely compromised.

Acknowledgements

This thesis would not be possible with the encouragement and support of my supervisors, colleagues, and family. It is my hope that all the hard work they have put in to help me will be able to change the lives of those with chronic neurological disabilities.

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To my colleagues at work, in particular Phillip. Thank you for all your invaluable advice and teaching. I would also like to acknowledge my fellow PhD students and other members of the bone research group who have seen me through this journey. Thank you to Ann Marie Stroud for always being willing to help with any DXA problems.

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Chapter 1 – Literature review and research aims

1.1 Bone structure and function

Bone is best known as a support structure for the body but is by no means a static or inert material. Approximately 10% of the human skeleton is undergoing remodelling at any point in time, allowing bone to adapt to the forces placed on it, repair damage and maintain mineral homeostasis. Bone is predominantly made of type I collagen strengthened by mineral in the form of calcium hydroxyapatite. The degree of mineralisation and collagen cross-linking influences the stiffness and flexibility of bone (1, 2). Interspersed in the collagen, there are bone cells which play a critical role in the modelling and remodelling process.

At a macroscopic level, a long bone can be divided up into the diaphysis (the shaft of the bone) and the epiphysis (the end of the bone). Bridging the two is the metaphysis which contains the growth plate, a layer of cartilage which is ossified once growth ceases. The diaphysis of long bones is composed predominantly of cortical (compact) bone and the epiphyses composed of trabecular (cancellous) bone. Vertebral bodies and flat bones such as the skull and pelvis, are comprised of trabecular bone encased by an outer layer of cortical bone. Cortical bone accounts for approximately 80% of bone mass and is much less porous than trabecular bone, allowing it to withstand loading. Trabecular bone is a lattice like structure providing it with flexibility.

The microscopic structural unit of cortical bone is the osteon, which contains a central cavity containing blood vessels/nerves/lymphatics surrounded by concentric circles of bone tissue. These concentric circles are known as lamellae. In contrast, the microscopic unit of trabecular bone is known as the trabecular packet, which does not contain a vascular channel. Lamellae are arranged longitudinally along trabeculae rather than concentrically.

There are 4 main types of bone cells: osteoblasts, osteoclasts, osteocytes and bone lining cells. Osteoblasts are derived from pluripotent mesenchymal stem cells and secrete collagen and minerals to form new bone. As the bone matrix mineralises, the osteoblast is trapped and changes into an osteocyte, which comprises 90-95% of all bone cells. Osteocytes live in small spaces known as lacunae and have long slender processes (canaliculi) that connect them to other bone cells. The osteocyte co-ordinates osteoblasts and osteoclasts in response to the mechanical and hormonal milieu. Osteoclasts are multinucleated cells that come from the monocyte-macrophage lineage. They are the cell responsible for bone resorption through the release of protons (acid) and enzymes including tartate resistant acid phosphatase (TRAP), matrix metalloproteinase 9 (MMP9) and cathepsin K. Bone lining cells are quiescent osteoblasts that cover the bone surfaces.

An area of bone that is being remodelled is called a basic multicellular unit (BMU) (3). This is thought to be triggered by damage to the bone which is conveyed by osteocyte deformation or apoptosis. Resorption is then undertaken by osteoclasts in a BMU, which takes approximately 3 weeks, before osteoblasts form new bone over 3-4 months. Bone mass can be lost either through increased rates of bone remodelling with activation of many BMUs, or if there is a negative balance in a BMU with the amount of bone resorbed greater than that formed (4).

There are two key pathways which regulate the activity of osteoclasts and osteoblasts in a BMU. The *wingless* (Wnt) signalling pathway plays a key role in osteoblastogenesis and differentiation. The binding of Wnt ligands to its receptor (a dual receptor composed of low density receptor related protein 5 (LRP5)/6 with frizzled) leads to inhibition of β-catenin degradation (Figure 1). β-catenin accumulates, translocates to the nucleus and activates transcription factors involved in osteoblastogenesis. Inhibitors of this process include sclerostin and dickkopf-1 (DKK-1) which are secreted by osteocytes and bind to LRP5/6 to prevent Wnt ligand binding. Indeed, this is thought to

be the predominant mechanism by which osteocytes respond to mechanical signals to regulate bone mass (5). Mechanical loading reduces osteocyte production of sclerostin and in turn increases bone formation.

Osteoclast activity is predominantly controlled by the receptor activation of nuclear factor κB (RANK)/receptor activator of nuclear factor κB ligand (RANKL) pathway. RANK is expressed on the osteoclast cell surface. Osteocytes secrete both RANKL, a pro-osteoclastogenic cytokine that stimulates this pathway, and osteoprotogerin (OPG), a decoy receptor for RANKL which acts as an antagonist. Binding of RANKL to RANK promotes osteoclast differentiation and survival. The central role of the osteocyte can be seen in Figure 1.

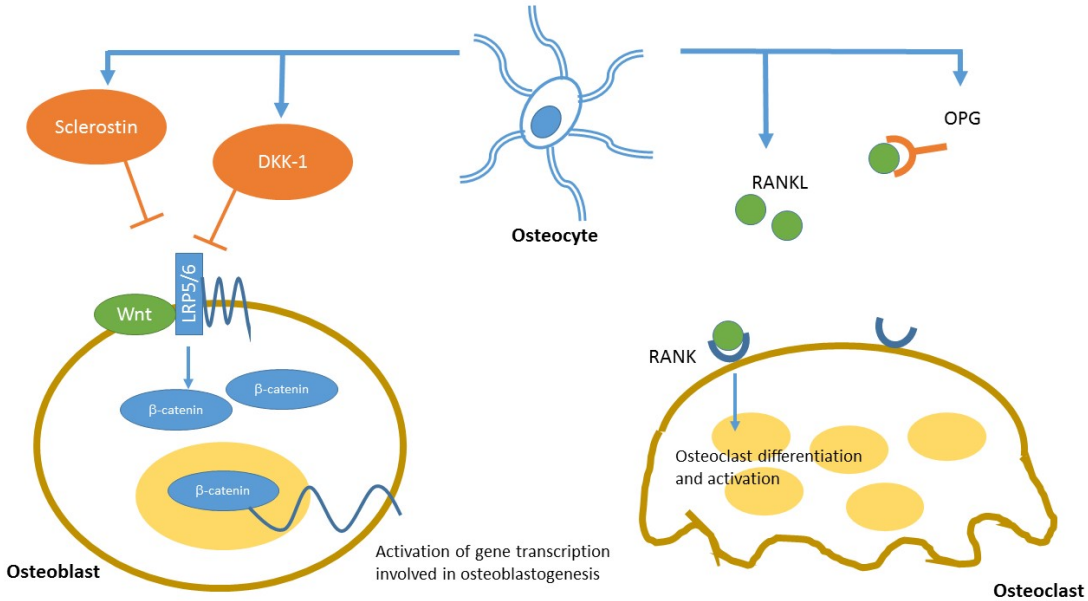


Figure 1 – Central role of osteocyte in regulating Wnt and RANKL/RANK pathways

1.2 Osteoporosis

Osteoporosis is a disease of low bone mass and disturbed bone microarchitecture with subsequent increased risk of fracture. In Australia, over 1 million people have osteoporosis and 6.3 million have osteopenia (precursor to osteoporosis) which will become increasingly prevalent as the population ages (6). Fractures are associated with significant morbidity and mortality and place a significant burden on health care utilisation and costs. In the US, it is estimated that one in two women and one in five men over the age of 50 will have an osteoporotic fracture in their lifetime (7). Increased mortality after an osteoporotic fracture is present for up to 10 years and is highest after hip fracture (8).

The diagnosis of osteoporosis can be made by the presence of a low trauma fracture or by radiological means using dual energy x-ray absorptiometry (DXA). DXA measures bone mineral density (BMD) in grams per centimetre squared (g/cm^2). An individual's bone density is then compared to that of young healthy adults and reported as a T-score. A T-score is the number of standard deviations above or below the BMD of young healthy Caucasian females. A T-score of <-2.5 on DXA is considered osteoporosis whilst a T-score of between -1 and -2.5 is defined as osteopenia according to the World Health Organisation (WHO) criteria (9). This definition was formulated for postmenopausal women after consideration of the prevalence of osteoporosis and risk of fracture with various cutoff values. In postmenopausal women, for every one standard deviation decrease in bone mineral density (BMD), fracture risk increases 1.5-3 fold (10). There are ethnic differences in BMD and the BMD-fracture relationship which is not fully captured in the definition of osteoporosis or the fracture risk assessment tool (FRAX®) (11).

Given that this definition of osteoporosis on DXA was initially formulated for postmenopausal women, it cannot be applied in a blanket fashion to other populations. In children and adolescents, it is more

appropriate to compare their measurements to healthy children of the same age and sex. In a DXA report, a Z-score is also calculated, which is the number of standard deviations of BMD above or below that of someone who is the same age and sex. In children and adolescents, the International Society of Clinical Densitometry (ISCD) uses the term 'low bone mass' rather than osteoporosis when the Z-score is <-2.0 (12). Osteoporosis should only be diagnosed if there is also a clinically significant fracture history which is defined as one or more of the following: 1) two or more long bone fractures by age 10 years; 2) three or more long bone fractures at any age up to age 19 years (13).

In young adults, the International Osteoporosis Foundation (IOF) working group proposed keeping the WHO definition of a T-score <-2.5 as long as growth has finished and peak bone mass (PBM) has been achieved (14). This is to maintain consistency; furthermore T-scores and Z-scores are very similar in this population. However, the (ISCD) 2016 guidelines recommend using Z-scores in premenopausal women and men <50 years of age, with a Z-score of <-2.0 as 'below the expected range for age' (15).

Bone density is only one determinant of bone strength, however, DXA-derived measures of BMD are easily obtainable and inexpensive. Notably, DXA does not measure true 'density'; BMD is calculated from a 2-dimensional picture and so is reported as areal bone mineral density (aBMD) to differentiate it from volumetric bone mineral density (vBMD). As children grow and the size of their bones increase, aBMD will increase yet vBMD will remain relatively unchanged.

There are a number of medical conditions and medications which lead to increased risk of fracture: reduced bone mass and strength in these cases is termed 'secondary osteoporosis'. This is to differentiate it from primary osteoporosis which is loss of bone mass due to ageing and or menopause. Secondary osteoporosis may account for up to 30% of osteoporosis in postmenopausal women and in 80% of men (16, 17). The most common secondary cause of osteoporosis in men is hypogonadism,

and may be seen in up to 16% of men with vertebral crush fractures (18) and greater than 50% of elderly men with hip fracture (19). Another important cause of bone loss is glucocorticoid use, which at supraphysiological doses can cause suppression and apoptosis of osteoblasts, and upregulation of osteoclastic bone resorption through stimulation of the RANK-L pathway (20). Other causes of secondary osteoporosis are listed in Table 1.

Table 1 – Secondary causes of osteoporosis

<table>
<tr>
<td>Endocrine
- Hyperparathyroidism
- Hyperthyroidism
- Diabetes
- Hypercortisolism
- Hypogonadism
- Growth hormone deficiency</td>
<td rowspan="2">Medications
- glucocorticoids
- lithium
- barbiturates
- anticonvulsants
- thiazolidinediones
- heparin, warfarin
- SSRIs, SNRIs
- affecting gonadal function: aromatase inhibitors, medryoxprogesterone acetate, gonadotropin releasing hormone agonists</td>
</tr>
<tr>
<td>Autoimmune
- rheumatoid arthirits
- systemic lupus erythematous
- ankylosing spondylitis</td>
</tr>
<tr>
<td rowspan="2">Haematological
- thalassaemia
- multiple myeloma
- monogammopathy of uncertain significance (MGUS)
- systemic mastocytosis</td>
<td>Renal
- chronic renal impairment
- idiopathic hypercalciuria
- renal tubular acidosis</td>
</tr>
<tr>
<td>Transplantation
- solid organ
- bone marrow</td>
</tr>
<tr>
<td>Gastrointestinal, nutritional and hepatic disorders
- malabsorption (coeliac)
- inflammatory bowel disease
- gastric bypass
- pancreatic insufficiency – cystic fibrosis
- chronic cholestatic disease
- chronic hepatitis
- haemochromatosis
- eating disorders</td>
<td>Immobilisation, neurological
- Cerebral palsy
- Spina bifida
- Rett syndrome
- Parkinson’s
- Duchenne’s muscular dystrophy and other muscular dystrophies
- Spinal cord injury
- Stroke
- Dementia
- Multiple sclerosis</td>
</tr>
</table>

1.2.1 Osteoporosis in young adults and adolescents

Low bone mass in young adults may reflect low peak bone mass (PBM) due to body size, genetics and the environment rather than true osteoporosis. This makes the diagnosis of osteoporosis in this population challenging, and may explain why the relationship between fracture and bone density is not as strong as that seen in postmenopausal women/men over 50.

Genetic factors account for up to 80% of the variance in PBM (21). However, modifiable factors such as calcium intake, weight bearing physical activity and hormonal status influence whether the genetically pre-determined PBM is obtained. Bone mineral content almost doubles during puberty between the age of 8 and 18 years (22). The Canadian CaMOS study found variations in timing of PBM dependent on site of measurement and sex. Areal BMD (aBMD) at the femoral neck peaked at ages 16-19 in females and 19-21 years in males, whereas lumbar spine aBMD peaked at ages 33-40 in females and 19-33 in males (23). If chronic illness is present in childhood during these important years of bone mass accrual, PBM will be severely affected and these individuals are at high risk of developing osteoporosis in adulthood.

1.3 Neurological disease and bone health

Many neurological diseases, whether congenital or acquired, have been associated with osteoporosis. These diseases include spinal cord injury, Rett syndrome, Parkinson's disease and multiple sclerosis (24-27). The most important mechanism underlying this is reduced mobility. Mechanical forces exerted on bones through physical activity result in maintenance of bone mass and adaptation of structure which has been described as the 'mechanostat' (28). It is well known that mechanical loading increases bone mass whilst disuse leads to loss of bone mass.

The effect of loading on bone is thought to be predominantly mediated by osteocytes as mentioned in Chapter 1.1. Mechanical strain signals are amplified through the osteocyte processes, causing osteocytes to release signaling molecules that alter the activity of osteoblasts and osteoclasts (29, 30). Unloading leads to upregulation of sclerostin and subsequent reduced bone formation (31, 32) (33). Bone resorption is also affected, as sclerostin increases expression of RANKL leading to increased osteoclast activity and numbers. Unloading in mice also increases osteocyte apoptosis, which leads to increased osteocyte RANKL production and cortical and trabecular bone resorption (34).

There is evidence in humans for the central role of sclerostin in mechanotransduction. High sclerostin levels have been found in adults who have been immobilised from stroke, with levels being positively associated with markers of bone resorption (35). In adults with CP, those who were non-ambulatory were found to have higher levels of sclerostin and lower BMD Z-scores than ambulatory adults with CP (36).

It is important to make a distinction between individuals in whom the onset of their neurological disease occurs during childhood from those who have an acquired neurological disease causing immobility. Those with early onset immobility have abnormal bone development in addition to

abnormal bone remodelling. A typical pattern of bone development has been described: long bones are slender with reduced bone density at the metaphyses (37). This is a consequence of reduced mechanical forces impacting the formation of cortical and trabecular bone. Fractures can therefore occur with minimal force such as with transfers from bed to chair etc., or with seizures.

When neurological disease is acquired, such as after spinal cord injury or stroke, there is a period of initial rapid bone loss followed by development of a new steady state after 2 years (38, 39). A greater than 20% loss of BMD has been described 4 months following spinal cord injury (40), with preferential loss of trabecular bone at the distal femur and proximal tibia (41).

Apart from causing deficits in bone metabolism, neurological disease also impacts on falls and imbalance. Diminished mobility and perception, coupled with impaired cognition, put patients with neurological disease at high risk of falls. Risk factors for falls have been found to overlap with risk factors for hip fracture in older women (42). In Grisso *et al*'s study, subjects with neurological impairment secondary to stroke or Parkinson's disease were found to have a 2 to 9-fold increased risk of hip fracture.

In conclusion, adults with neurological diseases are at increased risk of fractures through reduced bone mass and high falls risk. Those with congenital neurological disease have low peak bone mass compounded by ongoing loss of bone mass with ageing. Spina bifida and cerebral palsy are the two most common causes of chronic neurological disability in childhood and yet there are few studies examining bone health in adulthood. These two diseases and how they affect musculoskeletal health are discussed in detail in the next chapters.

2.1 Cerebral Palsy

Cerebral palsy (CP) is an umbrella term describing motor disorders that are due to a brain injury during central nervous system development. It is best seen as a clinical descriptive term rather than a diagnosis with a clear aetiology as such. The definition has been revised over the years to reflect the heterogeneity and the complexity of the problems faced by patients with CP. In 2004, the International Working Group on the Definition and Classification of Cerebral Palsy defined cerebral palsy as follows: "Cerebral palsy describes a group of permanent disorders of the development of movement and posture, causing activity limitation, that are attributed to non-progressive disturbances that occurred in the developing fetal or infant brain. The motor disorders of cerebral palsy are often accompanied by disturbances of sensation, perception, cognition, communication, and behaviour, by epilepsy, and by secondary musculoskeletal problems." (43, 44).

Associated impairments described in the definition are common and more likely if a severe motor impairment is present. These neurological disturbances can have a greater bearing on quality of life than the motor impairment itself (45).

It can thus be seen that the term 'CP' represents a wide spectrum of disability and is inadequate alone as a diagnosis in individual patients. Each individual with CP needs to have an assessment of underlying aetiology, motor type, severity of motor impairment and associated pathologies.

2.1.1 Diagnosis

The diagnosis of CP requires a multifaceted approach. Clues may include slow motor development, abnormal muscle tone or posture, and early hand preference due to hemiplegia. Imaging such as computed tomography (CT) or magnetic resonance imaging (MRI) of the brain may reveal structural abnormalities. In up to 85% of cases, abnormalities on MRI are seen and the absence of abnormalities

should lead clinicians to explore alternative diagnoses (46). MRI findings differ depending on the timing and severity of the cerebral insult. Cortical abnormalities such as polymicrogyria, where they are too many small folds in the cortex, occurs from an insult from 20 gestational weeks onwards. Periventricular white matter injury results from damage to white matter and can occur from mild-moderate insults at 24-34 weeks gestation (43).

Assessment for the presence of associated disabilities such as intellectual impairment, hearing and vision loss can help distinguish CP from other conditions. The main differential diagnoses include metabolic and genetic disorders, which progress over time. A key feature of CP is that it is a static neurological insult. Declining motor function in CP can occur later in life but in the context of degenerative processes affecting muscles, tendons and joints from chronic abnormal mechanical forces.

2.1.2 Pathogenesis

CP can result from brain injury occurring during the prenatal, perinatal or postnatal periods and for the majority the cause is not well understood (47). Predisposing factors include preterm delivery, intrauterine infections, intrauterine growth restriction, placental pathology and multiple pregnancy (48) (49) . Postnatal causes range from neonatal jaundice to meningitis, trauma and stroke.

Previously many cases were attributed to perinatal 'birth asphyxia' i.e. acute hypoxia during late labour and/or birth. However, it is now recognised that signs of fetal compromise at birth may be caused by chronic long-standing hypoxia and pathology, and asphyxia accounts for <10% of CP (50, 51). Supporting this is epidemiological evidence that despite increased Caesarian delivery rates, the incidence of CP is unchanged (52).

With the emergence of inexpensive new generation DNA sequencing technology there has been renewed interest into genetic causes of CP. It has been known for some time that there is increased prevalence of CP among twins, siblings and in children with a parent or other family member with cerebral palsy (53-55). In addition, congenital anomalies such as microcephaly, cardiac anomalies and facial clefts are much more prevalent in individuals with CP compared with the general population (56, 57).

From studying families with ⩾2 members with CP, at least six monogenetic forms of CP have now been described (58). The genes involved have known roles in neurotransmission, neuronal polarity and signalling. In sporadic cases of CP, other potential disease causing genes have been identified using whole exome sequencing, but require further validation (59). Chromosomal microarray is another technique that has been utilised in CP given that copy number variations have been implicated in a number of neurodevelopmental conditions such as autism and intellectual disability. In up to 31% of individuals with CP, pathogenic copy number variations have been found (60, 61) . In total, it is estimated there may be a potential genetic contribution in up to 34-45% of CP (52).

It therefore can be seen that there are multiple causality pathways to the development of CP, and many occur early in utero. This can explain the heterogeneity of CP and the challenges in finding successful interventions to reduce the prevalence of this condition.

2.1.3 Epidemiology

The prevalence of CP worldwide is 2-3.5 per 1000 live births (62-64) and appears stable over time despite increased survival of preterm infants (65). The Australian Cerebral Palsy Register found that between 1993-2006, the prevalence was similar being 2.1 per 1000 live births (95% CI 2-2.2) (66).

2.1.4 Life expectancy

The life expectancy of children with cerebral palsy has increased significantly due to advances in medical care. In a cohort of over 300 individuals with cerebral palsy, almost 85% survived to age 50 years, conditional on surviving to age 20 years (67). In a Western Australian cohort who were born between 1958 and 1994, the standardised mortality ratios dropped significantly after age 15 (68).

Respiratory causes of death are predominant in younger patients, a reflection of oropharyngeal dysfunction and aspiration risk. In older patients, Hemming and colleagues used the United Kingdom death registry to determine cause of death in 506 individuals with cerebral palsy from the Bristol area born between 1930-1960. They classified cause of death into broad groups from the International Classification of Diseases (ICD), 9th version. Hemming *et al* found increased mortality was predominantly due to cancer, circulatory system and digestive system diseases (67). Deaths from digestive system diseases were higher than what would be expected in the general population. Similarly, in a study of over 45,000 adults with CP of whom 10% died during the study period, standardised mortality ratios due to respiratory, circulatory, neoplastic and digestive diseases were high (69). The high rate of mortality due to circulatory diseases is somewhat surprising and it has been suggested that this may related to physical inactivity. Overall, circulatory diseases accounted for 46% of deaths in adults with CP with a 2-4 fold increased risk of dying from ischaemic heart disease in those aged 35-55 years of age with CP compared to the general population. Increased mortality from digestive diseases were predominantly due to diseases of the oesophagus and intestinal obstruction, which is unique to the CP population and accounted for 3% of adult deaths. Cancer mortality may be due to delayed diagnosis or treatment; notably, death from breast cancer in women with CP was 3 times more likely than a comparable age-matched group of the general population. The caveat to this is that both these studies were based on death registry data which can be unreliable: in the second study, 7% had CP listed as their underlying cause of death.

Predictors of increased mortality include severity of motor impairment, cognitive impairment and associated impairments such as visual impairment and epilepsy (68, 70, 71). In a large Swedish registry of CP patients, the motor type of CP also appeared to have an effect with tetraplegic and dyskinetic types associated with reduced survival compared to other CP types (hemiplegia, diplegia, ataxic types) (70).

With the increased survival of children with cerebral palsy, there needs to be a streamlined transition process into adult models of care. Adult physicians need to be aware of late onset secondary conditions associated with CP which contribute to the increased morbidity and mortality identified in adults with this condition. Epidemiological studies have shown adults with CP particularly have a higher prevalence of osteoporosis and fracture, as well as cardiometabolic complications such as diabetes, hypertension and myocardial infarction compared with age matched adults (72-74). Concerningly, as these individuals age, many become dependent on assistive devices (75) and up to 75% of individuals lose their ability to mobilise (76). Preserving bone and muscle health to maintain mobility and independence is therefore critical in adult life.

2.1.5 Classification

To aid medical documentation and discussion about the limitations in function patients with cerebral palsy experience, there have been a number of classification systems developed. These systems group individuals according to their functional and motor capacity as outlined below, which also allows standardisation for research purposes.

Motor classification

There is a wide spectrum of motor disability seen in cerebral palsy. Broadly, it is divided into spastic, dyskinetic, ataxic types or 'mixed' as described by the Reference and Training Manual for the

Surveillance of Cerebral Palsy in Europe (SCPE) (77). Dyskinetic CP can be further classified into dystonia or choreoathetosis. Topographic involvement of the limbs is also commonly used to describe the extent of motor impairment as tetraplegia, hemiplegia or diplegia.

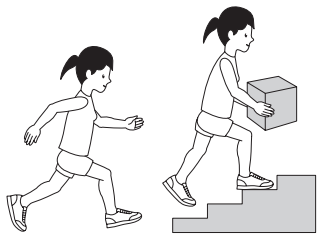
Spastic cerebral palsy is most common, seen in 70-80% of patients and is characterised by increased muscular tone and increased deep tendon reflexes. In contrast, 10-20% of patients have dyskinetic CP and experience involuntary uncontrolled movements with variable muscle tone. Ataxic CP is the least common and predominantly affects balance and co-ordination.

The predominant motor type may point to a specific anatomical location to the cerebral injury and provide clues to the underlying aetiology. For example, dyskinetic (athetoid) CP is associated with abnormalities of the basal ganglia and is caused by kernicterus from rhesus incompatibility. Tetraplegia and hemiplegia is predominantly associated with cortical abnormalities whereas diplegia (sparing of upper limbs) is usually associated with white matter changes (43).

Functional classification

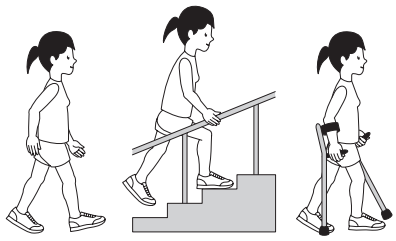
Classification of CP into motor type and number of limbs involved does not provide complete insight into functional limitations. This has led to the development of a functional classification system known as the Gross Motor Function Classification System (GMFCS) (78). This is the most validated and widely used classification system in CP. It allows an objective measure of gross motor function, particularly focused on the ability to sit and walk. GMFCS levels I-III are ambulatory, whilst levels IV-V are severe and wheelchair bound (see Figure 1). It has been shown that there is good agreement between carer reports and professional reports of GMFCS (79). GMFCS is usually stable over time and level as assessed at age 12 reflects functional level as an adult (80).

GMFCS E & R between 12th and 18th birthday: Descriptors and illustrations



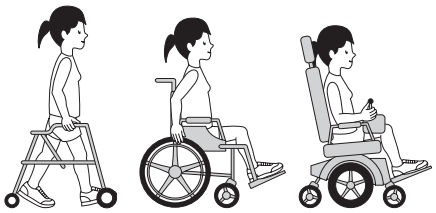
GMFCS Level I

Youth walk at home, school, outdoors and in the community. Youth are able to climb curbs and stairs without physical assistance or a railing. They perform gross motor skills such as running and jumping but speed, balance and coordination are limited.



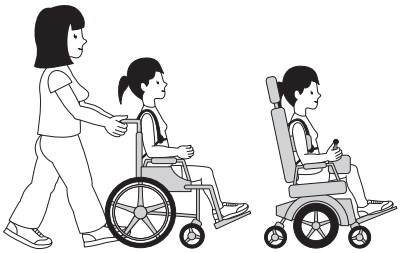
GMFCS Level II

Youth walk in most settings but environmental factors and personal choice influence mobility choices. At school or work they may require a hand held mobility device for safety and climb stairs holding onto a railing. Outdoors and in the community youth may use wheeled mobility when traveling long distances.



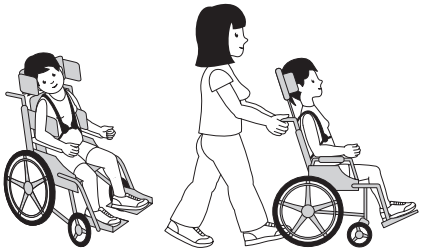
GMFCS Level III

Youth are capable of walking using a hand-held mobility device. Youth may climb stairs holding onto a railing with supervision or assistance. At school they may self-propel a manual wheelchair or use powered mobility. Outdoors and in the community youth are transported in a wheelchair or use powered mobility.



GMFCS Level IV

Youth use wheeled mobility in most settings. Physical assistance of 1–2 people is required for transfers. Indoors, youth may walk short distances with physical assistance, use wheeled mobility or a body support walker when positioned. They may operate a powered chair, otherwise are transported in a manual wheelchair.



GMFCS Level V

Youth are transported in a manual wheelchair in all settings. Youth are limited in their ability to maintain antigravity head and trunk postures and control leg and arm movements. Self-mobility is severely limited, even with the use of assistive technology.

GMFCS descriptors: Palisano et al. (1997) Dev Med Child Neurol 39:214-23
CanChild: www.canchild.ca

Figure 1 - GMFCS for children between 12-18 years old, adapted from Royal Children's Hospital Melbourne

2.1.6 Musculoskeletal complications

Scoliosis

Scoliosis develops secondary to weak and imbalanced paraspinal muscles and therefore is not present at birth but presents later in childhood. It often worsens with growth of the skeleton and is more commonly seen in those with poorer functional state (GMFCS IV and V) with rates up to 77% in adults with spastic quadriplegia (81). The presence of scoliosis can affect an individual's ability to sit upright. This has important implications for feeding with increased risk of gastric reflux and pulmonary infections, for wheelchair prescription, pain management and pressure care. In extreme cases restriction in pulmonary function can occur. Spinal fusion can reduce deformity but is associated with high complication rates in 40-80% of cases (81).

Hip dysplasia

Similar to scoliosis, hip dysplasia is not present at birth and develops secondary to spasticity or contractures due to weaker extensor and adductor muscles (82). Furthermore, lack of ambulation contributes to abnormal development of the femoral head with femoral anteversion and coxa valga (83). This affects growth of the hip, with subluxation and then dislocation of the femoral head if not appropriately monitored and treated. Consequences include pain, difficulty sitting and limitations in mobility. Incidence increases with worsening severity of GMFCS and is more common in those with spastic quadriplegia.

There are hip surveillance programs in Australia and Sweden for children with cerebral palsy. This allows for early identification and treatment of hip dysplasia which has reduced the incidence of hip dislocation in these countries (84, 85). Treatment can involve soft tissue lengthening via adductor tendon release, or realignment through femoral and pelvic osteotomies.

Spasticity and contractures

Spasticity occurs due to lack of descending inhibition of spinal tracts. Contractures may result, although the development of contractures cannot entirely be explained by spasticity (86). Common interventions to reduce spasticity include: 1. intrathecal baclofen (a muscle relaxant), 2. botulinum toxin A injections (botox) 3. selective dorsal rhizotomy where dorsal spinal rootlets are resected to reduce the spinal reflex (46).

These musculoskeletal complications of CP reduce muscle strength and mobility, and can worsen over time. Apart from their impact on bone health through the mechanostat theory, these musculoskeletal issues also can impede the measurement of bone quantity and the diagnosis of osteoporosis in adults with CP which will be covered in the next section.

2.2 Osteoporosis in cerebral palsy

Osteoporosis and fractures are well-recognised medical issues faced by children and adolescents with CP. Clinically, notable differences in this cohort in comparison to idiopathic osteoporosis in the general community include the young age of onset and the multiple risk factors for bone loss. Risk factors that have been described include severity of the motor impairment, malnutrition, use of anticonvulsants and growth hormone deficiency (87-89).

The prevalence of osteoporosis and fracture has been determined in children with CP, yet there is limited adult data and minimal longitudinal data. With increased life expectancy, this information is necessary to assist in the timing and selection of patients for treatment. Adults with CP are particularly vulnerable to osteoporosis and fracture due to their longer cumulative exposure to risk factors that lead to bone loss and declining bone mass with age (87). Critical gaps in our understanding include: the relative contribution of poor bone accrual and bone loss to osteoporosis in adults with CP, ongoing risk of fracture in adults with CP, and the optimal method of diagnosing and monitoring bone health in adults with CP.

2.2.1 Prevalence of osteoporosis in CP

Previous reports of low BMD in children with CP range widely between 27 and 77% (90). The broad range of values reflects differing methods of examining BMD and variability in the severity of CP in the cohorts examined. Adult data on prevalence of osteoporosis in CP is limited. In a large cohort of 435 adults aged 40-60yo with CP in Michigan, up to 58% of adults with GMFCS IV-V and 40% with GMFCS I-III had documented osteopenia/osteoporosis (91). In a prospective study of 48 adults with CP aged 25-46y, most of whom were ambulatory, 35% were found to have a Z-score of <-2.0 (92). The prevalence of osteoporosis in these studies is concerning given the young age of the patients.

The three longitudinal studies of BMD in CP provide some insight into the aetiology of low bone mass in adults with CP (93-95). In children, Z-scores declined with age over time despite absolute increases in BMD in the order of 2-5%/year, reflecting that bone accrual was less than that of healthy growing children. In older individuals with CP, BMD changes are minimal with up to 5 years of follow-up. Overall, these studies suggest that osteoporosis in adults with CP is largely a consequence of poor bone accrual. However, these studies predominantly used only two time points to assess longitudinal change, which is problematic when the yearly changes in BMD reported varied widely between -31% to 42%. This wide range is likely secondary to positioning difficulties in the presence of contractures and motion artefact.

2.2.2 Fracture rate and types

Fracture diagnosis in CP can be missed due to communication barriers, sensory deficits and difficulty obtaining confirmatory imaging. There have been a number of retrospective studies assessing fracture rate in CP, predominantly of non-ambulatory children reporting a fracture prevalence of 12%-23% (96-98). Despite the rate being similar to that of healthy children, these studies note that it is still significant as non-ambulatory children with CP are unlikely to experience the falls and injuries of typically developed children. Fracture incidence has been described by two studies using the person-years method and ranges from 2.7-4.5% in children with CP (96, 99).

The type of fractures in children vary in location and mechanism of injury in comparison to those seen in typically developed children. Most occur in the long bones, particularly in the lower extremities, thus causing immobility and the potential for even greater bone loss. Femoral fractures have been reported to occur without any documented trauma in up to 73% of cases (100) and are usually located in the distal femur (101). Risk factors for fracture include recent surgery or immobilisation, anticonvulsant use, PEG feeding and low weight for age (90, 99, 101, 102).

It is unclear whether adults with CP have ongoing high fracture rates and whether they have the same types of fractures as children. Fractures typically associated with osteoporosis (including neck of femur, vertebral or radial fractures) may become more prevalent in adults with CP as they age. Marciniak *et al.* described fractures in 8 of their 42 adult subjects with 3 of these fractures being spinal fractures (95). A study using a private health insurance database of over 5,000 adult patients with CP showed an increase prevalence of fracture compared with age and gender matched adults without CP (6.3% vs 2.7%) (74). When fractures were divided into location, there was a higher prevalence of fracture in adults with CP at all locations compared with matched adults, with most fractures occurring in the lower extremity although this was not assessed statistically.

2.3 Radiological assessment of bone fragility in CP

2.3.1 Dual energy x-ray absorptiometry (DXA)

In clinical practice, measurement of bone density is most commonly performed using DXA. DXA is readily available, relatively inexpensive, and there is minimal radiation exposure with an effective dose of 0.3-0.4 msV for a whole body scan (103). The coefficient of variation of DXA measurements are in the order of 0.5% at the lumbar spine and 0.7% at the femoral neck, allowing for accurate long-term monitoring of patients (104). The sites that are routinely measured include the hip, spine and 33% radius as recommended by the ISCD (105). In postmenopausal women, DXA has been shown to predict fracture risk. The risk of hip fracture increases 2.6 times for every standard deviation reduction in femoral neck bone density (106). BMD at the femoral neck is superior to measurements at the spine or radius in the prediction of hip fractures (106). This has led to the widespread use of DXA for bone density measurement and its incorporation into algorithms such as FRAX® (107) to calculate an individual's absolute risk of fracture.

To extrapolate the use of DXA to predict fracture in CP patients comes with some caveats. Firstly, aBMD is a 2-dimensional measure of a 3-dimensional structure and thus, can be affected by body size, which is particularly relevant in CP. Shorter people will falsely be reported to have a lower BMD (108, 109). There is no consensus how to correct for body size, with various groups proposing calculations such as bone mineral apparent density (BMAD) or dividing BMD by height (110). However, bone size may play an important role in bone strength and correcting BMD for size may reduce its usefulness in predicting fracture (111). Indeed, studies have shown that areal bone density is non-inferior to calculated volumetric bone density for fracture prediction or measures of bone strength (112, 113).

The utility of DXA in patients with CP is also limited by the musculoskeletal problems that arise in CP. Scoliosis at the spine and subsequent surgery to correct this may render the spine region of interest

uninterpretable. Femoral neck measurements can be limited by hip dislocation or metallic rods and implants used to improve alignment of the hip. Thus a technique of scanning the lateral distal femur using DXA has been developed for paediatric CP patients to circumvent these problems and take into account positioning difficulties from contractures (114). The distal femur is also a common site of fracture in the paediatric CP population making it a logical site for BMD measurement. There are established reference ranges in children (115, 116), and correlation between Z-scores at the distal femur and fracture history in a mixed cohort of children with CP or muscular dystrophy (117) has been established. For every 1.0 reduction in Z-score at the distal femur, the risk of fracture increases by 6-15%. The association between DXA-derived BMD and fracture in children with CP has also been described in two other studies (118, 119). However, it needs to be noted that the relationship between fracture and BMD in the general paediatric population is not well established.

In adults with CP, most studies assessing BMD have used the traditional regions of interest: the femoral neck, total hip and lumbar spine (92, 95, 120, 121). BMD Z-scores have not been consistently used in these studies; however, the young age of the cohorts renders this crucial. Low bone mass is defined as a Z-score of <-2.0, which equates to a BMD two standard deviations lower than the age- and sex-matched reference range. Henderson *et al*., has reported the use of the lateral distal femur site on a preliminary series of 32 adults with CP (122). Besides the reduced reproducibility of measurements reported by Henderson *et al*., the use of lateral distal femur readings in adults is limited by a lack of normative data and need for specialist training in the technique. Given this, it may be more appropriate at present to use the hip and spine regions of interest for monitoring in adults with CP. Treatment decisions based on BMD must be after careful consideration given that there is no clear relationship between fragility fracture and BMD that has been demonstrated in adults with CP. Low BMD in CP is predominantly secondary to reduced physical activity; reduced physical activity may in turn lower the risk of falls and injuries leading to fractures.

There has been one study using a Lunar PIXI densitometer to assess BMD using DXA at the calcaneus and forearm of wheelchair-bound individuals, many of whom had CP. However, this model of densitometer appears to be unavailable commercially at present, with heel ultrasound gaining prominence (see section 2.3.2 on 'Other imaging modalities').

In summary, DXA is still the method of choice in assessment of bone density in CP, however, clinicians need to be aware of the limitations specific to this cohort. Lateral distal femur measurements are ideal for assessing children, whilst proximal hip and spine measurements may be more appropriate for adults.

Body composition measurement with DXA

Besides measuring bone density, DXA is able to differentiate between bone, fat and lean tissue and thus enable the assessment of body composition. DXA has been shown to correlate strongly with other techniques such as underwater weighing, total body potassium and MRI (123, 124).

Assessment of body composition in CP is clinically important to guide optimal outcomes in feeding and exercise interventions. This may be particularly pertinent in view of the unexpected high mortality rate in adults with CP due to circulatory diseases (67, 69). A potential contributing factor is the excess body fat seen in children and adults with CP, more so in those with a higher GMFCS (125-127). Due to the growth restriction and reduced muscle mass in these patients, elevated body fat may not be clinically evident. In children with CP, there has been a recent study utilising DXA that found that all children with a low BMI had adequate or excess body fat (125). Distribution of body fat also appears altered in CP, with greater visceral and trunk adiposity, higher intra and intermuscular fat (127-129).

There are no validated adult CP-specific equations to estimate body fat using anthropometric measures and the common anthropometric measure of body composition, body mass index (BMI), is unable to distinguish fat from lean mass. In adults with CP, measures of body fat using DXA have been shown to be highly correlated to body fat estimated through the doubly labelled water technique (130).

2.3.2 Imaging of bone microarchitecture

Bone density is only one facet of bone strength; bone geometry, bone microarchitecture, the degree of mineralisation and turnover also determine fracture risk (131, 132). There are a number of modalities which can assess bone microarchitecture and geometry which are covered below.

Quantitative Computed Tomography

Quantitative Computed Tomography (QCT) is a cross-sectional imaging modality which can assess bone size and geometry. Its resolution allows differentiation between trabecular and cortical compartments in bone. QCT provides a true volumetric bone density, not affected by bone size. In CP, it has been used to determine the relative contribution of bone density and bone size to fragility to allow interventions to be better targeted.

The studies using either QCT or peripheral QCT (pQCT) in children with CP have shown conflicting results. Two groups (total of 50 children) found volumetric density at either the spine (L3) or distal tibia was no different when compared with typical aged and sex matched children (133, 134). They postulated that smaller bone size and reduced cortical thickness may therefore account for the reduction in areal BMD and fragility seen in CP. However, Tasdemir *et al.* found the volumetric BMD (vBMD) of L1-L3 was significantly lower in 24 children with CP compared with 19 healthy age and sex-matched controls, particularly those who were non-ambulant (135). Overall, these studies suggest

that smaller bone size and reduced cortical thickness account for the main differences in bone density on DXA seen in children with CP. However, true changes in density cannot be ruled out given there were differing sites of measurements between the studies and reduced study power due to the small number of participants. The participants with CP in the study by Al Wren *et al*. had a tendency to a lower spine (L3) volumetric density but this did not reach statistical significance, furthermore, there appeared to be changes in distal tibial vBMD but no control group was available for comparison.

Bone attenuation on CT images can also be used as a surrogate for bone density (136). Moon *et al*. measured bone attenuation retrospectively from hip CT scans in 126 children/adolescents with CP and 86 controls (137). Attenuation was lower in those with CP and was associated with both functional level and the degree of hip instability.

High resolution MRI

High resolution MRI is another imaging modality that can examine both trabecular and cortical envelopes in bone, with the advantage of not requiring ionising radiation. Using high resolution MRI, Modlesky *et al.* have shown abnormal microarchitecture in the distal femur of non-ambulatory children with CP (138, 139). Trabecular number and thickness were reduced whilst trabecular separation increased in children with CP compared with normal controls. The deficits in bone microarchitecture were more marked the greater the distance from the growth plate.

Cortical bone thickness and cortical bone cross sectional area can also be measured with MRI. Reduced cortical bone thickness and cross sectional area have been demonstrated at the femur and distal tibia in individuals with CP (140, 141).

These advanced imaging modalities are usually only found in research institutions limiting their use. In addition, the length of time required for acquisition of these scans and the precision required makes them susceptible to significant motion artefact. Custom splints, inflatable stabilising cushions, and positioning subjects in a wheelchair have been techniques used to attempt to minimise movement of subjects with CP during scans (142).

Trabecular bone score

Trabecular bone score (TBS) can be derived retrospectively from a lumbar spine (LS) DXA image to estimate trabecular architecture. It is not a direct measure; rather TBS is a textural index that examines pixel grey level variations in the LS image (143). A dense trabecular structure has a large number of small amplitude pixel variations and corresponds to a high TBS. Deteriorated bone microarchitecture has a small number of large amplitude variations and produces a low TBS (143, 144)

In *ex vivo* studies, TBS correlates with trabecular number, connectivity, thickness and separation (145, 146). Low TBS is associated with hip fracture in postmenopausal women and men >50yo, and can be complimentary to the FRAX® risk assessment tool (147, 148). Current ISCD guidelines propose that in postmenopausal women and older men, TBS can be used in association with BMD and FRAX® to guide treatment decisions, but should not be used alone (149). There currently is no role for TBS in monitoring treatment.

To date, no studies have investigated the use of TBS in CP to examine microarchitectural changes. The advantage of using TBS in CP is that no further imaging is required. Furthermore, the issues with motion artefact with pQCT and MRI are not so pronounced.

2.3.3 Other imaging modalities

Hand radiographs can be used to calculate metacarpal bone density, which is predominantly cortical bone. With the advent of DXA, it fell out of popularity. Hand radiographs may be easier to acquire than DXA in individuals with CP. Metacarpal bone density in adults with CP has been shown to be negatively associated with use of anticonvulsant drugs and higher ALP levels (150). However, the analysis of the radiographs used in this study is not routine and reference ranges are not available. In recent times, the development of a computer assisted diagnosis technique known as digital x-ray radiogrammetry has sparked renewed interest in assessing bone density from hand radiographs. Its utility appears to be greatest in the rheumatoid arthritis population where it is used to quantify periarticular osteoporosis associated with disease activity (151). This has potential to be applied to the CP population, particularly in children where hand radiographs are often obtained to determine bone age.

Heel ultrasound (HUS) is another method of examining bone quality, in this case without the need for ionising radiation. Bone tissue can alter the speed and intensity of ultrasonic waves. Measurements are expressed as speed of sound in metres per second (m/s) and broadband ultrasound attenuation (BUA) in decibels per megahertz (dB/MHz). These measurements are then combined into scores known as the quantitative index (QUI) or heel stiffness index (SI). These are not measurements of bone density per se but correlate with bone density and can predict fragility fracture in postmenopausal women and older men (152). In the current ISCD guidelines, the main utility of HUS is to identify those at low risk of fracture who do not require further investigations (153). It potentially can also be usedm, if DXA is unavailable, to identify high risk patients (in combination with clinical risk factors) who should commence treatment.

In 67 subjects with CP ranging from 5 to 25 years of age, QUI was lowest in those with anticonvulsant use and history of long bone fracture compared with those without anticonvulsant use and fracture

(154). In subjects with CP, the portable nature of HUS is attractive particularly in those who are non-ambulatory and could be used as an initial screening tool.

In summary, there have been multiple imaging modalities used in individuals with CP to assess bone density and bone microarchitectural changes. DXA is the most readily available technique to assess bone density and can also provide additional information regarding body composition and bone microarchitecture. Research based techniques such as QCT and MRI have shown reduced bone size, cortical thickness and abnormal bone microarchitecture contribute to the bone fragility seen in CP.

2.4 Bone turnover markers in CP

Bone turnover markers (BTM) are divided into bone formation markers and bone resorption markers. They are either bone matrix components that are released during bone remodelling or are enzymes that reflect osteoblastic or osteoclastic activity (155). Common bone formation markers include N-terminal type 1 collagen extension peptide (P1NP), alkaline phosphatase (ALP) and osteocalcin. Bone resorption markers include C-telopeptide (CTX), N-terminal cross-linking telopeptide of type I collagen (NTX-1) and deoxypyridinoline (DPD). The most recognised use of BTM at present is for monitoring treatment responses in osteoporosis, or as an indication of possible secondary osteoporosis when particularly elevated. Although higher BTM is associated with greater fracture risk in many studies (156), the role of BTM in assessment of individual fracture risk is not yet clear.

Kim *et al*. found in adults with CP that the bone resorption marker CTX was abnormally elevated in 74%, while the bone formation marker osteocalcin (OCN) was normal in 90% (121). However, in another small cross-sectional study of 28 adults with CP, there was no difference in OCN and CTX between non-ambulatory and ambulatory adults, and the median OCN and CTX fell within the normal range for both groups (36).

ALP is another commonly used surrogate marker of bone formation, although it is not specific to bone and is also produced by the liver. In children with CP, ALP levels are within the normal range and does not differ according to ambulatory status (135). Serum osteocalcin levels and ALP also did not correlate to BMD in a series of 139 children with CP (118). With such conflicting results, it is difficult to draw conclusions from BTM measurements in CP.

2.5 Risk factors for skeletal fragility in CP

To date, the main determinants identified for low bone mass in children with CP have been poor nutrition, reduced functional state, and use of anticonvulsants (88, 118). Many of these risk factors continue into adulthood and cause a cumulative effect on bone health.

2.5.1 Malnutrition

Malnutrition may lead to low bone mass and fractures via a number of different mechanisms, including micronutrient deficiency, reduced skeletal muscle mass and fat mass, and increased risk of falls from muscle weakness.

Malnutrition in cerebral palsy is predominantly caused by poor oropharyngeal function leading to inadequate caloric intake. There is no evidence of increased energy expenditure except in individuals with athetoid type cerebral palsy with involuntary movements (157, 158). It has been demonstrated through indirect calorimetry that individuals with cerebral palsy have reduced resting energy expenditure (159, 160).

Gastrostomy has been increasingly used to address nutritional deficiencies in cerebral palsy. There are no good quality randomised control trials to demonstrate benefit over oral feeding in cerebral palsy (161), however, there is evidence that body weight increases predominantly from fat deposition (162). There is no increase in height and children with CP remain growth restricted.

In children with CP, surrogate markers of nutritional state such as weight for age, triceps skinfold thickness, tube feeding, and caregiver reported feeding difficulties correlate with BMD Z-scores (88, 89). Body mass index (BMI) is a common surrogate marker to assess nutritional state in adults and is calculated by weight divided by height^2 (kg/m2). BMI is used as a measure of adiposity and correlates

to fat mass and percent fat mass. Yoon *et al*. in a study of 38 adults with CP found that BMI was positively correlated with T-scores of the lumbar spine and femur (120).

2.5.2 Functional status

Given the importance of mechanical loading in the maintenance of bone mass outlined in section 1.1, it is unsurprising therefore to see a correlation between ambulatory status and bone mass in patients with cerebral palsy. In a stepwise regression analysis, Henderson *et al.* showed that GMFCS level correlated to BMD Z-scores at the distal femur and lumbar spine in children with CP (88). In a study of 51 children with CP, non-walkers had lower BMD Z-scores (range -1.7 to -5.4) than walkers (-0.8 to -1.5) at both the distal femur and lumbar spine (163). In addition, in a subgroup analysis of 22 children with hemiplegia, there was a statistically significant difference in Z-scores at the femur of the affected compared with the unaffected limb.

In recent years, small studies in adults with CP have also shown the detrimental effect of decreased mobility on BMD (92, 120, 121) . In a cross-sectional study of 48 adults with CP (age range 25-46y), GMFCS correlated with Z-scores at the lumbar spine and total hip (92).

2.5.3 Muscle-bone relationship

The force exerted on bone from physical activity is not only through gravitational effects of loading but also from muscular contraction. The muscle-bone relationship has been demonstrated in young women and men using DXA measures of lean mass and bone mass (164, 165). Abnormalities of muscle in CP include reduced muscle volume, changes in muscle composition and strength. Ambulatory and non-ambulatory children and young adults with CP have increased inter and intramuscular fat on MRI (128, 129, 166).

As muscle has positive effects on bone mass through signals derived from muscle contraction, there have been interest as to whether muscle contraction from spasticity is different to that seen in dyskinesia. Kim *et al.* found lower femoral trochanter BMD Z-scores in adults with spastic CP compared with dyskinetic CP, although this was not seen at the femoral neck or lumbar spine (121). Another study found a trend to lower bone density in those with spastic CP compared with dyskinetic CP but this was not statistically significant (120).

Furthermore, muscle strength may be more important than measures of muscle function. Using stepwise regression, Chen *et al.* found that measures of muscle strength were more highly associated with distal femoral BMD and lumbar BMD than measures of muscle function in ambulatory children with CP (167).

2.5.4 Falls

Falls occur frequently in ambulant adults with CP, with one study showing 68% experience one or more falls within a 6 month period (168). The rate of falls are alarming with 7/16 adults in another study falling more than 10 times a year (169). Balance dysfunction appears to be the main contributor to falls risk in this cohort (169, 170). This is consistent with its contribution to falls risk in other adults with chronic neurological disease such as multiple sclerosis and muscular dystrophy (171).

Standardised screening tools to assess falls risk may not be appropriate in adults with CP as they are designed for much older cohorts with different comorbidities such as polypharmacy and incontinence (168). There are currently no disability specific risk assessment tools, and falls have not yet been confirmed to be associated with increased fracture risk in this cohort. The reason for this may be that the mechanism of injury is different in this population. What is known however, is that a history of falls is associated with reduced quality of life and physical function (172).

In those whom are wheelchair bound, even less is known about the frequency of wheelchair related accidents. In a mixed cohort of 577 wheelchair users, of which 9.3% had cerebral palsy, over half had fallen from their wheelchairs at least once (173). This resulted in injury in 47.1% of cases, of which 10.6% were fractures.

2.5.5 Anticonvulsant use

Epilepsy can affect up to 60% of patients with CP, and is more common in those with spastic tetraplegia (174-176). Fractures occur between two to six times more frequently in patients with epilepsy than the general population (177). Fractures occur secondary to trauma associated with seizures and falls, or as a result of lower BMD from anticonvulsant use and associated physical disabilities. The pathophysiology of bone loss induced by anticonvulsant medication is not completely understood. The most well characterised mechanism is induction of the hepatic cytochrome p450 enzyme system which increases degradation of vitamin D. This is seen with drugs such as carbamazepine and phenytoin. However, non-enzyme inducing antiepileptics are also associated with reduced bone density, with *in vitro* evidence that antiepileptics can have direct effects on osteoblasts, impair calcium absorption, reduce calcitonin levels and lead to resistance to parathyroid hormone (PTH) (178).

Use of anticonvulsant medication is independently associated with lower BMD Z-scores in the distal femur in children with CP (88). In those with CP and intellectual disability, BMD Z-score at the lumbar spine was lower in those who had epilepsy compared with those without (179). However, after multivariate analysis, epilepsy was not an independent risk factor for BMD Z-scores. The effect of anticonvulsants on BMD has not been consistently replicated in other studies, likely due to the heterogeneity of the anticonvulsants, use of varying DXA sites, and small number of participants. (92, 180, 181).

2.5.6 Hypogonadism

Disturbances in gonadal function in both males and females reduce BMD and increase fracture risk (182-184). Furthermore, gonadal steroids play a crucial role during puberty for accrual of peak bone mass, and altered pubertal development can have long lasting consequences on adult bone health (185). Altered pubertal progression in CP has been demonstrated in a cross-sectional study of 207 children with CP (186). Caucasian girls with CP entered puberty earlier with earlier breast development, but menarche occurred later compared with population data. Similarly, in boys with CP, pubic hair and genital development occurred earlier compared with population data, but completion of genital development occurred later. Early breast development and pubic hair development was also noted in girls with neonatal encephalopathy, of whom a subset had CP (187). The effect of altered puberty on bone density was not reported in either study. In addition, due to lack of longitudinal data it is unknown whether there are permanent adult deficits in gonadal function.

Gonadal function in CP may be compromised by a number of factors. Malnutrition can cause a functional hypothalamic hypogonadism such as that seen in anorexia nervosa (188). Hypopituitarism has also been found in children with CP with some cases being associated with abnormal MRI findings (189).

Identifying and treating hypogonadism in CP is complex in the context of disability. Puberty is a challenging time for caregivers, with rapid growth and weight gain, psychosocial sexual development, and the onset of menstruation in girls (190). For these reasons, caregivers may not raise concerns if there are no signs of pubertal progression in adolescents. Furthermore, various methods have been used to try and prevent the outcomes of puberty, for example, progestin to induce therapeutic amenorrhoea (191, 192). However, this practice leads to a hypo-oestrogenemic state which can

contribute to osteoporosis (193). Controversially, high dose oestrogen with hysterectomy has also been used to stunt final growth but no follow-up on bone health (194).

Despite this, adequate gonadal function is necessary for optimisation of bone health. The dose required and method of replacement of gonadal steroids in CP to preserve bone health is yet to be determined, and clinicians need to be mindful of the implications of replacement seen from the caregiver's perspective.

2.5.7 Vitamin D

Vitamin D plays an essential role in mineralisation of bone and the maintenance of calcium phosphate homeostasis. It does this through the action of 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) on the vitamin D receptor in the kidneys, small intestine and bone. Active $1,25(OH)_2D$ is produced by hydroxylation of vitamin D by the liver and the kidneys through specific p450 enzymes. The predominant source of vitamin D is through ultraviolet-B radiation acting on cholesterol in the skin to produce cholecalciferol (vitamin D_3). There are some foods with small amounts of vitamin D_3 but dietary sources do not provide more than 5-10% of vitamin D requirements (195). The optimum level of vitamin D for musculoskeletal health is not clearly defined. Current Australian Guidelines for adults and children define adequate 25(OH) vitamin D levels as $\geqslant$50nmol/L (195, 196).

Severe vitamin D deficiency can lead to diseases of mineralisation of bone, specifically osteomalacia in adults and rickets in children. Low vitamin D levels increase PTH through feedback loops, leading to accelerated bone loss.

Vitamin D deficiency in cerebral palsy may occur for a number of reasons: poor sunlight exposure, inadequate dietary sources of vitamin D, and the effect of anticonvulsant medication that increase vitamin D metabolism. The prevalence of vitamin D deficiency in CP is difficult to ascertain given the

varying definitions of deficiency in different countries and the change in definitions over time. In Australian children with CP of varying degrees of severity, 34% were found to have 25(OH) vitamin D levels of <50nmol/L with no relationship to GMFCS or use of anticonvulsants (197). Other studies of children have shown the prevalence of vitamin D deficiency to be anywhere between 33% to 53% (88, 118, 180). However, data regarding vitamin D levels in healthy children are lacking, so it is difficult to ascertain whether CP is an added risk for deficiency. Of note, an estimated 31% of Australian adults have inadequate vitamin D levels (198) so it is likely many adults with CP will also have vitamin D deficiency. Currently the only data available on vitamin D in adults with CP comes from Kim *et al.* who showed the mean 25(OH) vitamin D level in adults with spastic CP were higher than those with dyskinetic CP (33±13 nmol/L vs 23±11 nmol/L, p =0.027) suggesting that many would have been deficient (121). In a number of studies, Henderson and colleagues have shown that there is no relationship between vitamin D levels and bone mineral density in children with CP (88, 99, 118). This finding has been confirmed by other groups, although very low levels (<25nmol/L) may be associated with a reduction in BMD and an increase in PTH levels with likely osteomalacia (163, 180).

2.5.8 Growth hormone

Growth hormone (GH) is the most important hormone for postnatal longitudinal bone growth (199). In adult life, GH has also been shown to have stimulatory effects on bone remodelling in a biphasic manner. GH initially increases bone resorption with bone loss, followed by increased bone formation which leads to a net increase in bone mass after 12-18 months. Its action in bone is mediated through direct effects of GH on its receptor and through endocrine and autocrine/paracrine production of insulin-like growth factor 1 (IGF-1) (199). Treatment of both children and adults with GH deficiency using recombinant human GH has shown increases in BMD (200-203).

It is well known that children with CP have short stature. Malnutrition plays an important role in growth failure in CP, which can be partially ameliorated with percutaneous endoscopic gastrostomy (PEG) feeding (204-206). However, Stevenson and colleagues found that linear growth worsened with age, independent of nutritional status. Thus non-nutritional factors may also play a role, such as altered pubertal development, lack of weight bearing and neurological factors (207, 208). Given the important role of GH/IGF-1 axis in normal longitudinal growth, it has been postulated that GH deficiency can contribute to growth failure in children with CP.

Studies assessing the GH/IGF-1 axis in CP have used varying methods. Low basal GH and abnormal responses to insulin tolerance testing and arginine plus L-dopa stimulation have been shown in children with CP (209, 210). Furthermore, in a study of 30 children with CP ages 4.5-15 years of age, the average IGF-1 Z-score was -0.735±1.18 (211). These findings are very different to those found in malnutrition where high basal GH levels and low IGF-1 levels are seen (212). Coniglio and colleagues have thus suggested that it is possible that children with CP have anatomic or neurochemical abnormalities of the hypothalamic-pituitary axis resulting from the original neurological insult (209). Currently there is no data on whether GH deficiency continues into adulthood in CP.

In summary, nutrition and function are key factors in determining bone health in children and adolescents with CP. Gonadal function and growth hormone levels may also play a role but have not been fully elucidated. In adults with CP, issues with nutrition and function are likely to continue and in fact worsen; the effect of these factors on bone health requires further exploration as they are potentially reversible.

2.6 Treatment of bone disease in CP

Given the importance of nutrition and function on bone density in CP, both non-pharmacological and pharmacological approaches have been used to improve bone density. Conducting randomised control trials and accurately measuring bone density changes in this population comes with unique challenges. Most studies have low numbers of participants (n<50) and only have bone density as an end point rather than fracture risk.

2.6.1 Exercise interventions

The strong relationship between ambulatory state and bone density in CP has given rise to interest in improving muscle strength and mechanical loading in subjects with CP. However, typical exercise regimens would not be suitable for patients with CP. The interventions that have been studied in CP range from standing frames and vibrating platforms, to physiotherapy focusing on muscle strength. The skeleton is exposed to two main types of signals: few low-frequency large magnitude events, but many high frequency low magnitude signals (213). Vibration platforms utilise low magnitude, high frequency strains to mimic the events that the skeleton is usually exposed to from muscle contractions. Use of standing frames similarly would increase the low-frequency signals to the skeleton.

The studies are all of less than 12 months duration and use differing measures of bone mass/quality making comparisons difficult (Table 1). Overall, there appears to be a positive effect of these interventions on bone density, however, the interventions were not tolerated by many subjects and the results are conflicting as to whether femoral or lumbar bone density is most improved. In addition, subjects need to be ambulatory or at least able to stand for 10 minutes assisted for these interventions to be applied.

Table 1 – Exercise intervention studies to improve BMD in CP

Treatment	Design	N	Control	Intervention Period	Measurement	Outcome	Study
Increased routine static standing program by 50%	RCT	26	Regular static standing frame program	9 months	Quantitative computed tomography	6% increase in vertebral vBMD, no change in proximal tibia vBMD	Caulton *et al* (220)
Weightbearing lower limb program, 20 mins 2 time/week for 2 months then 3 times/week for 6 months	RCT	18	Normal Routine	8 months	DXA femoral neck	vBMD femoral neck increased by 5.6% compared with -6.3% in controls	Chad *et al* (219)
Physiotherapy routine consisting of energetic and resistance exercises 3times/week for 26 weeks	RCT	26	Normal routine	6 months	DXA lumbar spine, femoral neck	Femoral neck BMD increased by 7.6%, no change in lumbar spine BMD	Kitsios *et al* (218)
Low magnitude, high frequency vibration platform 10 mins/day, 5 days/week for 6 months	RCT	20	Placebo device	6 months	Quantitative computed tomography	Proximal tibial vBMD 17.7% higher than controls, lumbar vBMD 4.7% higher but not statistically significant	Ward *et al* (217)
Low magnitude, high frequency vibration platform 10mins/day for 6 months	RCT, crossover	31	Normal routine	6 months	Computed tomography of spine and tibia (proximal and midshaft)	Cortical bone area increases only in tibia, no effect on cancellous bone, no effect in spine	Wren *et al* (216)
Dynamic standing frame	Pilot study	4	Static standing frame	2 months	DXA	Increases in BMD at lumbar spine and distal femur, proximal femur in 3 children. All able to tolerate standing frame	Gudjonsdottir *et al* (215)
Whole body vibration three lots of 3min, r times/week. 12Hz increased to 20Hz	Interventional study	40	No control	20 weeks	DXA	Total body BMD +0.8%, lumbar spine 1.3%, lower limbs +2.2%	Gusso *et al* (214)

2.6.2 Bisphosphonates

Bisphosphonates are the mainstay of treatment of osteoporosis in postmenopausal women and older men. Oral and intravenous (IV) bisphosphonates have been shown to improve bone density as well as reduce vertebral and non-vertebral fractures in large trials of postmenopausal women and men (156, 221, 222). Bisphosphonates have a high affinity for bone due to their ability to bind to hydroxyapatite crystals. They are preferentially incorporated into sites of high bone remodelling and inhibit bone resorption through inhibiting the breakdown of hydroxyapatite (159).

There are two main classes of bisphosphonates: the first generation non-nitrogen containing and the newer nitrogen containing bisphosphonates. The newer generation bisphosphonates promote osteoclast apoptosis through inhibition of the farnesyl pyrophosphate synthase enzyme in the mevalonic acid pathway involved in the production of lipids and cholesterol (160). Consequently the modification of guanosine triphosphate binding proteins which are critical for osteoclast cellular activities is inhibited (157).

There have been a number of studies using bisphosphonates in children with cerebral palsy (Table 2). Only two of these studies were randomised control trials (RCT), one using IV pamidronate in 12 subjects and the other using oral risedronate in 20 subjects, both showing significant improvements in BMD (223, 224). Another RCT performed by Iwasaki et al did not provide adequate statistical analysis to interpret the effect of bisphosphonates on BMD (225). When combined with the observational studies, there appears to be a positive effect of bisphosphonates on BMD in CP.

Table 2 – Studies using bisphosphonates in children with CP

Design	N	Age(years)	Medication	Treatment duration	Outcome	Study
Retrospective review	20 (10 CP)	9.6±4.7	Zoledronic acid , initial dose 0.0125mg/kg, subsequent doses 0.025mg/kg at 12 weekly intervals; annualized dose 0.10±0.02mg/kg	1.7±0.7 years	Pre: TBBMD Z score -1.51±1.59 Post: -1.04±1.51 at 24 months Pre: LS Z-score -2.72±1.4 Post: -0.46±1.47 at 24 months	Simm *et al* (230)
RCT	12	6-16 years	Pamidronate 1mg/kg of body weight daily for 3 consecutive days every 3 months	1 year	Distal femur BMD increased 89% ± 21% vs placebo 9% ± 6% Z-scores –4.0 ± 0.6 to –1.8 ± 1.0 in the pamidronate group vs placebo (–4.2 ± 0.3 to –4.0 ± 0.3).	Henderson *et al* (223)
Retrospective review	9	Mean 10.5 (range 6-15.6)	Pamidronate 1mg/kg of body weight daily for 3 consecutive days every 3 months	15 doses, mean duration 12.5months	LS Z-score increased from -4.0 to -2.8. Distal femur metaphysis, Z-score increased -3.6 to-1.7 at 12 to 49 months.	Bachrach *et al* (229)
Prospective observational	23	10±5	Pamidronate 0.75mg/kg of body weight daily for 2 consecutive days every 4 months	1 year	LS Z-score -3.8±1.6 to -2.3±1.2 FN Z-score -4.5±1.2 to -2.6±0.9	Plotkin *et al* (228)
RCT	20	Mean 7.6 (range 1-16)	Oral risedronate (dose not given) and $1,25(OH)_2$ vitamin D	6 months	LS BMD changed by 0.04±0.035g/cm2 in risedronate group vs 0.01±0.02 in $1,25(OH)_2$ only group	Iwasaki *et al* (224)
Retrospective review	10 (4 CP)	10.9±5.8	Pamidronate 0.5-1.0mg/kg for 2 days every 3-4 months	6-15months	LS BMD -4.22±1.24 to -2.61±1.69, p=0.008	Moon *et al* (227)
Retrospective review	42: 10 Zol (6 CP) 32 APD (25 CP)	12.5±7 (Zol) 8.7±3.7 (P)	Pamidronate 1.0mg/kg for 3 days every 3 months Zoledronic acid 0.025-0.05mg/kg/dose every 3-4 months	1 year	Pre: LS Z score -3.45±1.0 Post: -2.4±0.9, p<0.001 Pre LS BMD 0.403±0.12 Post: 0.476±0.12 g/cm2 p<0.001	Bowden *et al* (226)

Table 2 (continued) – Studies using bisphosphonates in children with CP

Design	N	Age(years)	Medication	Treatment duration	Outcome	Study
Retrospective cohort study	27 (14 CP)	10.5 (range 6.2-13.3)	Zoledronic acid 0.05mg/kg/dose every 6 months	1 year	Pre : LS Z-score -1.74 Post : LS Z-score -0.37 (p<0.001)	Ooi *et al* (232)
Retrospective review	18 (11 CP)		Pamiodronate 1mg/kg for 3 days every 4 months	1 year		Allington *et al* (38)
Prospective observational	26	9.3±3.9	Alendronate 1mg/kg/week with 600mg calcium and 400U vitramin D per day	1 year	Pre: LS Z score -3.45±1.0 Post: -2.4±0.9, p<0.001 Pre LS BMD 0.403±0.12 g/cm^2 Post: 0.476±0.12 g/cm^2, p<0.001	Paksu *et al* (231)

Fracture data is sparse, with one follow-up study of 25 children showing a reduction in fracture rate post pamidronate treatment (233). However, it is not clear whether these children would have continued to experience fractures given limited epidemiological data regarding fracture incidence in children with CP.

The tolerability of bisphosphonates in this population must also be taken into account. As many patients with CP have gastrointestinal issues, or require PEG feeding, IV rather than oral bisphosphonates are an attractive option. The two available IV bisphosphonates are pamidronate and zoledronic acid (ZA). ZA is more potent and has a longer half-life than pamidronate, allowing for yearly dosing which is beneficial for patients with poor mobility. Furthermore, long term suppression of BTM and changes in BMD after one dose of ZA can be seen for up to 5 years (234).

ZA has been used in two studies of children with various neuromuscular disabilities including CP and was shown to improve BMD and be non-inferior to pamidronate (226, 230). A recent published retrospective review of young patients (median age 12 years) receiving ZA demonstrated that it was generally well tolerated (235). This included 7 patients with cerebral palsy. The most common adverse events were hypophosphataemia in 25%, acute phase reaction in 19% and hypocalcaemia in 16% of patients. However, there has been a case report of a severe acute phase reaction to ZA in a child with CP (236) and a case of febrile seizures secondary to zoledronic acid administration in a child with CP (237). There is insufficient data regarding the long term safety of bisphosphonate use on growing bone; reassuringly there have been two small studies (n<150) that suggest growth continues as expected in bisphosphonate treated children with osteogenesis imperfecta and osteoporosis (238, 239).

In summary, bisphosphonates are likely to improve BMD and reduce fracture risk in individuals with CP. Side effects however may be significant and must be considered when prescribing this to a population with difficulty communicating physical symptoms.

2.6.3 Other pharmacotherapy

There have been two studies examining the effect of supplementation with vitamin D on BMD in children with CP. An activated form of vitamin D, alfacalcidol, given as monotherapy to children with CP at a dose of 0.03ug/kg/day resulted in a statistically significant change in BMD at the lumbar spine (224). However, the absolute change in BMD of 0.01g/cm^2 (+3.8%) may fall within the range of the least significant change of the DXA scanner, which was not reported by the authors. Follow up of these patients was reported at the 3 year mark and there was no effect of alfacalcidol on BMD (225). A combination of calcitriol 0.25ug daily and 500mg calcium daily was shown to increase BMD at the lumbar spine in children with CP and epilepsy, compared with a reduction in BMD in those who were only observed (240). Baseline levels of calcium or vitamin D were not measured in either study.

In other institutionalised populations, intermittent dosing of vitamin D is convenient and has been shown to safely increase levels (241). Similarly, there has been one study using a once-off dose of 100,000 IU of vitamin D_3 in children with CP with no adverse effects on calcium levels (242).

With the evidence available, vitamin D supplementation in patients with CP to achieve the recommended target of $\geqslant$50nmol/L is sufficient. There is no clear role for vitamin D alone in the treatment of osteoporosis. Target levels can be achieved with daily or intermittent dosing(195).

Denosumab has been administered to 10 children with significant suppression of bone turnover markers CTX and OCN 3 months after one dose (243). There has been no follow up reported and no

bone density data available. The use of denosumab in children and young adults needs to be approached with caution given its effects are rapidly reversed with cessation of the medication (244). A rebound increase in bone turnover following cessation may result in severe hypercalcaemia in children (245) and multiple spontaneous vertebral fractures in adults (246, 247). As a result of the increased risk of vertebral fractures following denosumab cessation, current guidelines recommend a replacement drug (typically a bisphosphonate) to prevent bone loss and vertebral fracture after denosumab is stopped (248). The optimal choice of bisphosphonate to replace denosumab is not known. Further work is needed to elucidate optimal therapy following denosumab cessation as well as the role of denosumab in osteoporosis in young adults.

There has been one interventional study of 10 children with CP who were randomised to GH treatment or placebo over 18 months (249). GH-treated patients had statistically significant improvements in spinal BMD and linear growth compared with controls. Further larger studies are required to determine whether human recombinant GH should be used in CP for its positive effects on growth and bone density.

In conclusion, bisphosphonates have the most evidence of all the therapies to increase BMD in children with CP. Bisphosphonates are likely to also be effective in adults with CP, given that its use in reducing fracture risk in postmenopausal women and older men is well documented. However, the appropriate timing of bisphosphonate use and duration of use is still unclear at present.

3. Spina bifida

Spina bifida (SB) or meningomyelocele is a term that describes a subset of neural tube defects (NTD). NTD refer to congenital defects of the central nervous system where the neural tube fails to close during embryogenesis leading to exposure of the nervous system. Spina bifida is when there is involvement of the spinal cord, whilst anencephaly is exposure of the brain and craniorachischisis is exposure of the entire nervous system. The latter two are incompatible with life.

3.1.1 Diagnosis

Currently, the diagnosis of SB is usually made prenatally. Initially screening was performed with α-fetoprotein levels in maternal serum (250), however, the diagnosis is now often made during second trimester ultrasonography. Ultrasound can detect more than 90% of foetuses with SB (251).

3.1.2 Pathogenesis

In the embryo, the neural tube is the structure that develops into the spinal cord and brain. Formation of the neural tube begins with a process called primary neurulation which involves enlargement, shaping, folding and fusion of the neural plate. As the neural tube develops into a spinal cord, bone and muscle form a protective barrier around it. The more caudal elements of the spinal cord are formed by a process called secondary neurulation.

SB occurs from a defect in primary neurulation, and as a result bone and muscle are unable to grow over the developing spinal cord. Spinal cord and meninges then protrude through the defect with leakage of cerebrospinal fluid. This is in contrast to closed neural tube defects otherwise known as meningoceles which result from abnormalities in secondary neurulation. Often there is no abnormal neurology as the spinal cord resides in the spinal canal and only the meninges herniate out.

3.1.3 Epidemiology

The prevalence of SB varies between geographical location and ethnicity with rates of 0.20-2.92/1000 births reported (252). However, these figures can be distorted by pregnancy termination from antenatal screening. A large European registry, EUROCAT, includes data on terminations, stillbirth and livebirths and found the prevalence to be 0.51 per 1000 births during 2003-2007 (253).

Established risk factors for SB are family history, low maternal folate intake, maternal diabetes and the anticonvulsant drugs valproic acid and carbamazepine (252).

There is a 20-50 fold increased risk of neural tube defects in siblings of affected subjects compared with the general population (254). The genetic basis is complex given that more than 240 genes are required for neural tube closure in mice (255). In some of these genes, mutations have been found in human orthologues in patients with NTD (251). Interestingly, variants of genes involved in folate-homocysteine metabolism have also been associated with NTD (252), providing evidence for gene-environment interaction in this complex disease.

Folate supplementation has been shown to reduce the risk of NTD by up to 70% in randomised control trials (256, 257). The mechanism by which folate deficiency leads to NTD is unclear and may relate to the importance of folate in several metabolic pathways. Of note, besides altered folate metabolism through maternal dietary insufficiency and mutations in the pathway, there is also evidence that maternal autoantibodies against the folate receptor may be pathogenic in some cases of NTD (258).

3.1.4 Life expectancy

There are two prospective longitudinal studies that provide insight into the long-term prognosis in SB. The most complete study has 50-year longitudinal data from an original cohort of 117 infants born

with spina bifida between 1963 and 1971 (259). All of the 117 subjects were accounted for with the mean age of survivors being 46 years (range 43-49 years). Two-thirds of the cohort had died and the most common causes of death were cardiorespiratory, neurological and urological. Those with a neurological sensory level of L2 or below had significantly better survival (55%) than those with a sensory level of L1 or above (12%). From this cohort, the estimated mortality rate for those between age 5 and 30 was approximately 1% per year. Results from the 25 year follow-up of another cohort of infants born at a similar time showed that the death rate increases as age increases into early adulthood (260). The most common cause of death in adulthood in this cohort was unrecognised shunt malfunction, but this may reflect the recruitment of subjects from a neurosurgical clinic. 24% of the cohort had died during the 25 year follow-up. Although mortality was not compared to the general population in either of these studies, it is clear life expectancy is still significantly reduced in people with SB.

Over time, there have been improvements in survival in infants with SB. In a population based registry, 82.7% infants with SB survived to age 1 if born between 1979-1983 compared with 91% survival if born between 1989-1994 (261). It is unknown if these reduced mortality rates continue through to childhood and late adulthood.

3.1.5 Classification

There are a number of different classification scales for ambulation in SB but one of the most commonly used is the Hoffer ambulation scale (262). It grades ambulation into four functional levels:

1. community ambulator: walk indoors and outdoors, can use crutches/braces. Wheelchair only used for long trips in community
2. household ambulators: walk only indoors and with apparatus
3. non-functional ambulators: walk only during therapy, in school or in hospital

4. non-ambulators: wheelchair bound but can transfer independently

Spinal classification

Neurological level in SB is usually documented as the lowest motor level capable of antigravity function (263). Thus, thoracic patients have flaccid lower limbs, L1/2 have hip flexors, L3 knee extensors, L4 knee flexors and L5 ankle dorsiflexors. Patients are often then divided into thoracic/upper lumbar, mid lumbar (L3-4) and lumbosacral (L5-S1) although this is not a formal classification method.

Of note, the spinal level determined on imaging may not always reflect functional level (264) so is not commonly used as a classification method in the literature. However, motor level may also not reflect ambulatory status as this also is dependent on factors such as weight, spasticity and balance dysfunction (265).

3.1.6 Complications of spina bifida

Hydrocephalus and Chiari II malformation

The open neural tube leads to poor distension of the embryonic ventricular system and is thought to be responsible for the Chiari II malformation which is seen in most cases of myelomeningocele (266). The Chiari II malformation involves changes to the skull, cerebral hemispheres and the posterior fossa. One of the consequences of this is hydrocephalus due to the displacement of the brainstem downwards with blockage of cerebrospinal fluid (CSF).

The presence and severity of the Chiari II malformation with associated hydrocephalus determines the outcome for patients with SB (260, 267). Traditionally, hydrocephalus has been treated with a ventriculo-peritoneal (VP) shunt, however, there are now alternatives such as endoscopic third

ventriculostomy, conservative management of stable hydrocephalus, and prevention through prenatal surgical closure of the myelomeningocele (268, 269).

Neurogenic bladder and bowel

The level of the neurological lesion in spina bifida does not correlate with bladder and bowel function (17). Bowel continence is crucial for quality of life in those with SB. On the other hand, the management outcomes for neurogenic bladder are twofold: 1) to preserve renal function and 2) to achieve urinary continence. In the context of bone health, the preservation of renal function is critical in individuals with SB to avoid chronic kidney disease mineral and bone disorder (CKD-MBD) compounding low bone mass seen in this condition.

Patients with SB can either have an overactive sphincter with high bladder pressures or low sphincter resistance and are at risk of incontinence. High pressures can lead to hydronephrosis and is often treated with clean intermittent catheterisation (CIC) and anticholinergic medication such as oxybutynin. More recently, injections of botulinum toxin (Botox) into the bladder musculature can also be used to reduce pressures but need to be repeated due to its temporary nature (13). In those who cannot achieve continence with CIC and medication (30-50%), bladder augmentation using a piece of intestinal tract allows the bladder to store large amounts of urine.

In those with low sphincter resistance, bladder neck procedures to improve continence include creation of slings and artificial sphincters.

CIC can be difficult to perform when the patient's mobility is affected, and creation of a catheterisable channel using the appendix (Mitrofanoff) or ileum (Yang Monti) is performed (16). In a similar fashion, a catherisable channel can be created to the caecum to allow an enema to be administered (called a

MACE procedure). This improves faecal incontinence by allowing regular bowel evacuation if more conservative measures such as suppositories, laxatives and dietary changes fail.

Musculoskeletal

Individuals with SB can also develop hip dislocation and scoliosis from muscle imbalance similar to that seen in CP (see section 2.1.6). However, the sequelae of hip dislocation is different in spina bifida as these individuals do not experience pain. Reviews have shown that surgery to relocate the hip generally does not improve ambulation, and guidelines suggest surgery only be performed in unilateral hip dislocation in those with a neurological level of L4 or below (18). Function is more associated with range of motion at the hip which may be achieved through contracture release (19).

Similarly, treatment of scoliosis has not shown to improve ambulatory function or health related quality of life and may have a variable effect on sitting balance (5, 18, 270).

3.2 Osteoporosis in Spina Bifida

3.2.1 Prevalence

There are very few studies documenting prevalence of osteoporosis in SB. In a prospective study of 24 children, half of which were ambulatory, osteoporosis was found in 42.9% as defined by a Z-score of -2.5 or below (271). A higher prevalence of 64.9% (defined as a Z-score <-2) was found in a retrospective study of children with SB (272). However, these children were selected to have DXA scans for clinical reasons such as fracture, immobility etc.

There is only one study in 21 adults (mean age of 30 years) who were recruited from a rehabilitation service (273). Using a T-score of <-2.5 for the diagnosis of osteoporosis, 33% had osteoporosis in at least one of the measured sites being the L1 vertebrae, femoral neck or trochanter. The L1 vertebral site was chosen as L2-L4 is often involved in spina bifida; the missing posterior element of the vertebra can lower the BMD value. The reason for the use of the trochanter site was not stated in the study. Given the young age of subjects in these studies, these prevalence rates are significantly higher than that of the general population.

3.2.2 Fracture rates and types

Fractures are common in children with SB with a prevalence of up to 30%, and the most common fracture site being the distal femur (274-276). In particular, fracture diagnosis can be delayed in SB due to the associated sensory deficits, and patients may be instead investigated for infection or thrombosis as a cause of limb swelling (277). Increased fracture rates are seen in those who have already fractured, and in those with reduced mobility, including those who are acutely immobilised following surgery (278, 279).

Fracture risk in SB may be attenuated in adulthood, with one study showing a decline in annual incidence of fracture from 29/1000 patient years in adolescence to 18/1000 patient years in adulthood (280). There appeared to be no difference in fracture sites between children, adolescents and adults. This appears to be at odds with the results of another study of individuals with varying developmental disabilities, where femoral fractures were inversely associated with age but strongly associated with reduced mobility (281).

3.3 Radiological assessment of bone fragility in SB

3.3.1 Dual energy x-ray absorptiometry

The limitations of traditional DXA scanning seen in patients with CP are also present in the SB population. Patients with SB have growth restriction which may affect the measurement of bone density in 2 dimensions, can have metal implants from orthopaedic operations, and likewise have positioning difficulties due to scoliosis, contractures and hip dislocation. To circumvent these issues, the distal lateral femur has also been used as an alternative site in children with SB (272, 282). Both studies did not have sufficient power to demonstrate a relationship between fracture and BMD at the distal lateral femur. However, Haas *et al.* found ambulatory status and neurological level was associated with BMD in univariate but not multivariate analysis.

Furthermore, the absence of posterior elements in the vertebrae of SB patients can lower the BMD of the lumbar spine (283). Some groups have thus used the bone density of the L1 vertebra only, as this is rarely involved in a small number of patients with high level lesions (273, 284).

3.3.2 Other imaging modalities

Single photon absorptiometry has been used in older studies for the assessment of BMD in SB (285, 286). This technique has been largely superseded by the advent of DXA as it can only measure peripheral sites such as the radius or tibia which needed to be submerged under water. Single photon absorptimetry measurements of BMD are highly correlated to DXA measures at the same site.

There have been no studies using QCT, pqCT or MRI in subjects with SB. Horenstein *et al.* have used a novel technique of assessing bone quality by obtaining CT images of the entire length of the tibia (287). They were able to demonstrate in non-ambulatory children with SB the greatest deficit in bone

mass was seen in the proximal and distal tibial epiphysis, common sites of fracture in the SB population.

3.4 Bone turnover markers in SB

A matched case control study of 28 children with SB and 58 controls found lower ALP levels (187 vs 237U/L) and lower PTH levels (14.5 vs 18.4pg/ml) in SB children despite lower 25(OH) vitamin D levels (22.2 vs 26.4ng/ml) (288). Similarly, Kafadar *et al.* found lower urinary DPD levels (a marker of bone resorption) in SB children compared with controls (289) . This would be suggestive of low bone turnover but needs to be validated in further studies.

3.5 Risk factors for bone fragility in SB

Risk factors for low bone mass or fractures in SB are similar to those seen in CP with some important caveats. Lack of mobility, use of anticonvulsants and vitamin D deficiency are common risk factors seen in SB and CP. However, malnutrition and requirement for supplemental feeding is uncommon in SB. Conversely, obesity and its related complications are highly prevalent in children and adults with SB (290-292). In addition, a number of patients with SB may undergo urological intervention with intestinal segments for neuropathic bladder. This may result in reduced bone mass which is covered in the next section.

There have been a number of small studies in mostly children and adolescents examining the relationship between these risk factors and bone density (Table 3). Most conclude unsurprisingly that immobility has a detrimental effect on bone density. This is not only at weightbearing sites such as the femoral neck or distal femur (271, 272), but also at the lumbar spine (289, 293). Neurological level, which is associated with mobility level has also been shown to correlate with bone density in some studies. However, a clear relationship between fracture risk and bone density has not been defined.

Table 3 – Risk factors for low BMD in spina bifida

Study	**N (age)**	**Imaging modality and site**	**Risk factors identified** √ positive correlation, X no correlation
Apkon *et al* (271)	24 children (4-18yrs)	DXA femoral neck, whole body	√Ambulatory status × fracture
Okurowska-Zawada *et al* (294)	30 children (6-17yrs)	DXA lumbar spine, total body	√ fracture
Quan *et al* (285)	35 children (6-19 yrs)	Single photon absorptiometry distal radius	×Ambulatory status √ fracture
Haas *et al* (282)	44 children (6-18yrs)	DXA of distal lateral femur and lumbar spine	√Ambulatory status √neurological level × fracture × bladder augmentation × Tanner stage
Szalay *et al* (272)	37 children (4-22)	DXA distal lateral femur, lumbar spine	√Ambulatory status (femur)
Rosenstein *et al* (286)	80 patients (1.3-21.7yrs)	Single photo absorptiometry of tibia and distal radius	√Ambulatory status √neurological level × fracture
Martinelli *et al* (284)	49 patients (5-20)	DXA of L1 vertebra	√Ambulatory status
Ausili *et al* (293)	60 patients (5-14)	DXA femoral neck, lumbar spine	√Ambulatory status (LS) √ sport activity (LS, FN)
Kafada *et al* (289)	31 patients (4-18)	DXA lumbar spine L1-4, femoral neck	√Ambulatory status (LS)
Valtonen *et al* (273)	21 adults (20-46)	DXA L1 vertebra, L hip	×Ambulatory status
Akbar *et al* (278)	29 children	DXA femoral neck	√Ambulatory status √neurological level

3.5.1 Urological intervention

Urological intervention with intestinal segments can result in a hyperchloremic metabolic acidosis from reabsorption of urinary ammonium with chloride through the intestinal mucosa (295). To buffer the chronic acidosis, calcium carbonate is released from bone which can lead to bone demineralisation with increased risk of stones. In animal studies, chronic metabolic acidosis also suppresses bone-specific matrix protein synthesis and alkaline phosphatase activity in osteoblasts whilst activating osteoclasts through increased RANKL production (296, 297). It is unclear whether this is clinically significant with some studies showing no difference in serum pH and bicarbonate post urinary surgery (298, 299). However, the buffering from bone may conceal the metabolic acidosis and thus not show up on biochemical analyses.

There are no long term data to support the association between urological intervention (with intestinal segments) and an increased fracture risk or changes in bone histomorphometry (300, 301). Studies are conflicting as to whether BMD is reduced being limited by their retrospective nature and small study numbers (302-305). The diverse underlying reasons for the urological intervention can also lead to significant bias, as patients with SB have multiple risk factors for low BMD compared with patients with bladder exstrophy.

3.5.2 Renal stones

The association between urolithiasis and reduced bone density has been documented in large epidemiological studies and is thought to be due urinary losses of calcium (306, 307). In SB, urolithiasis is more prevalent than the general population with rates between 4 and 20% (308-310). Given the potential for hypercalciuria in patients with urological intervention as outlined earlier, it is important to know the composition of these stones. If these stones are indeed calcium based, it would support

the hypothesis that subjects who have had urological intervention can develop hypercalciuria leading to both bone loss and stone formation.

Confounding this is that bladder stones account for 50-90% of cases in these series of SB patients. The pathogenesis of bladder stones differs to that of upper tract stones as they are predominantly caused by urinary stasis from intestinal reservoirs/neuropathic bladder or recurrent urinary tract infections. Furthermore, indwelling catheters have been found to be a risk factor for upper tract stones in SB.

There have been small case series describing stone type and urinalysis in patients with SB. In one study, 10 of 14 patients with bladder stones had struvite stones or mixed struvite/carbonate apatite stones, and four patients had urinalysis showing hypercalciuria in only one (309). In the same study, 16 patients had upper tract stones, 2 of which had stone composition showing struvite stones. Urinalysis in two patients with upper tract stones demonstrated hypercalciuria and hypocitraturia respectively. In a retrospective review of 40 patients who had undergone augmentation cystoplasty in SB, a decrease rather than increase in serum chloride was associated with stone development (22).

In an paediatric cohort of SB patients, Quan *et al.* found non-ambulatory children had a higher mean urinary calcium excretion of 3.9mg/kg/day compared with ambulatory children (1.9mg/kg/day) (285). They did not comment on whether urological intervention had been performed or whether renal stones were present, however these values border on hypercalciuria in healthy children and adolescents (311).

Looking at the data as a whole, there is no compelling evidence that urological intervention with intestinal segments plays a major role in bone disease in SB. Those who have had such interventions

can be screened with urinalysis and further investigations undertaken if significant hypercalciuria is found.

3.5.3 Renal impairment

Renal impairment is a major cause of mortality in adults with SB, along with infection, neurological and cardiorespiratory causes (259, 312, 313). It occurs predominantly due to inadequate treatment of a neurogenic bladder. High pressures in the bladder due to detrusor overactivity and sphincter dysfunction can lead to pressure on the kidneys. Recurrent urinary tract infections and urolithiasis can contribute to worsening renal impairment (314).

The prevalence of renal impairment in adults with SB was 25.7% in a recent systematic review of 13 studies comprising 1128 patients (74). This figure may be an underestimate of the true extent of the issue in SB due to survival bias. A subset of these studies described the stage of chronic kidney disease, with 1.3% on dialysis (CKD stage 5). The prognosis of SB patients with renal impairment appear no different to that of patients with renal impairment secondary to other causes (149, 315).

Renal impairment leads to bone metabolism abnormalities through a complex interplay of factors including secondary hyperparathyroidism, impaired hydroxylation of vitamin D, metabolic acidosis, and resistance to PTH and fibroblast growth factor 23 (FGF-23) (316). Collectively the abnormalities are described as chronic kidney disease mineral and bone disorder (CKD-MBD). CKD increases the risk of hip fracture 4-fold and mortality and morbidity after a fracture is considerably higher than that of the general population (21, 72, 73). There have been no studies looking at the effect of CKD on fracture risk or bone density in either children or adults with SB.

3.5.4 Endocrine dysfunction

Precocious/early puberty is common in patients with spina bifida who have hydrocephalus, with reported rates of 10-50% (317, 318). It is more frequently seen in girls than boys with SB which mirrors the prevalence in the general population (319). Precocious puberty leads to early bone maturation and shorter final height, with apparent higher bone mass for chronological age but no difference if adjusted for bone age. (320-322). After treatment with gonadotrophin releasing hormone agonists, studies have shown inconsistent effects on bone density (322-324). Currently there is no data describing the effects of precocious or early puberty on bone health in SB. Hypogonadism appears to be uncommon in SB with only a few cases of male hypogonadism described in the literature (325, 326). The cases varied from adolescents to adults, and a subset had gonadotropin levels consistent with hypergonadtrophic hypogonadism i.e. primary gonadal failure.

3.6 Treatment of bone disease in SB

3.6.1 Pharmacological treatment

The role of specific bone-preservation therapy in SB patients is currently unclear. There have only been two children with SB who have received bisphosphonates in case series of children who received treatment for osteoporosis of varying causes (155, 327). Treatment in these cohorts were well tolerated but there was insufficient power to comment on fracture prevention and BMD changes.

Quan *et al.* conducted a randomised placebo controlled trial of hydrochlorothiazide (328) based on their earlier finding of hypercalciuria in non-ambulatory children with SB (285). Urinary calcium reduced but bone density did not improve after one year of 12.5mg (patient weight <25kg) or 25mg (patient weight >25kg) of hydrochlorothiazide.

Further research is needed into whether bisphosphonates or other bone-preservation therapies are efficacious in both the paediatric and adult population with SB.

3.6.2 Exercise interventions

All studies to date examining exercise interventions are retrospective case control studies. Children with SB who are seen in institutions that encourage mobility with walking frames and orthoses, have been compared with those that are encouraged to use a wheelchair for independence (329) (330). These two studies found conflicting findings about the role of walking on reducing fracture risk, and neither had bone density data.

4. Aims and Objectives of thesis

Bone disease is emerging as a major co-morbidity in adults with CP and SB as their life expectancy improves. Low bone density and increased fracture risk has been well documented in children with these conditions and is primarily a result of immobility. However, the natural history of bone disease in these conditions in adulthood is incompletely understood due to the paucity of data on fracture prevalence in adults and longitudinal clinical and bone mineral density data.

The following hypotheses were formulated:

1. Reduced BMD in CP and SB is due to multiple factors beginning from childhood. The use of DXA at traditional sites such as femoral neck and lumbar spine have their limitations particularly in children, but may be more clinically relevant in the adult cohort and are more readily available.
2. The relationship between skeletal muscle and bone mass is paramount in chronic neurological disease and therefore interventions to maintain muscle mass are crucial to improve skeletal health.
3. Fractures continue to occur in adulthood creating significant morbidity and mortality. The fractures seen may be different to those seen in childhood and be more similar to low trauma fractures seen in older women and men.
4. Bone fragility in chronic neurological disease is likely a combination of reduced BMD and altered bone microarchitecture.
5. Low BMD in chronic neurological disease is predominantly due to reduced peak bone mass, but early loss in young adults may play a role.
6. Gonadal function is likely compromised in chronic neurological disease and may contribute to poor bone health.

To address these hypotheses, the aims of this thesis are:

Cerebral palsy

1. To investigate the relationship between skeletal muscle mass, fat mass, BMD and fracture in adult subjects with CP through a retrospective cross-sectional study.
    - To determine the relationship between BMD at both the proximal hip and spine and fragility fracture in adults with CP.
    - To determine whether muscle or fat is most associated with BMD to guide nutritional and exercise interventions.
    - To determine the risk factors for low BMD in adults with CP including nutritional, pharmacological, endocrinological and functional risk factors.
2. To assess bone microarchitectural parameters in adults with CP.
    - To determine the relationship between bone microarchitecture, BMD and fracture.
3. To assess longitudinal changes in bone density in CP over childhood, adolescence and adulthood to improve understanding of pathophysiology of bone fragility in this condition.
    - To determine if reduced peak bone mass or early loss of bone in young adulthood is the predominant cause of low BMD.
    - To assess the impact of puberty and growth on BMD in CP.
4. To describe the aetiology and treatment of hypogonadism and delayed puberty in CP, and its effect on bone density.

Spina bifida

1. To investigate the relationship between skeletal muscle mass, fat mass, BMD and fracture in adult subjects with SB through retrospective cross-sectional study.
    - To determine the relationship between BMD at proximal hip and spine and fragility fracture in adults with SB.

- To determine whether muscle or fat is most associated with BMD to guide nutritional and exercise interventions.
- To determine the risk factors for low BMD in adults with SB including nutritional, endocrinological, renal and functional risk factors.

2. To investigate the rates of fractures across the lifespan in SB.
    - To determine whether fractures continue into adulthood and whether fracture type differs between children and adults.

Chapter 2 – Methodology

Introduction

Methodology specific for each study is described in the Methods section of each chapter. In this chapter an overview is provided of the patient cohort, and the biochemical and radiological techniques used in the studies.

Patient Cohorts

Monash Health is a tertiary teaching hospital in Victoria, Australia that provides care across the lifespan with obstetric, newborn, paediatric and adult services. Children with CP and SB are seen in specialist medical clinics and the Victorian Paediatric Rehabilitation Service. As adolescents, they are linked into the Young Adults Transition Clinic which takes referrals state-wide, and then referred on to adult services. One such service is the Metabolic Bone Clinic at Monash Health, which is staffed by both paediatric and adult endocrinologists to allow the smooth transition of care of these patients and is uniquely placed to understand changes in bone health in this group.

Definition of cerebral palsy and spina bifida

In 2004, the International Working Group on the Definition and Classification of Cerebral Palsy defined cerebral palsy as follows: "Cerebral palsy describes a group of permanent disorders of the development of movement and posture, causing activity limitation, that are attributed to non-progressive disturbances that occurred in the developing fetal or infant brain. The motor disorders of cerebral palsy are often accompanied by disturbances of sensation, perception, cognition, communication, and behaviour, by epilepsy, and by secondary musculoskeletal problems." (43, 44). This definition was used to identify patients for the studies.

Spina bifida or meningomyelocele is a term that describes a subset of neural tube defects (NTD). NTD refer to congenital defects of the central nervous system where the neural tube fails to close during embryogenesis leading to exposure of the nervous system. SB is when there is involvement of the spinal cord, whilst anencephaly is exposure of the brain and craniorachischisis is exposure of the entire nervous system. The latter two are incompatible with life. In our studies, SB is defined as open neural tube defects of the spinal cord and does not include patients with spina bifida occulta or caudal regression syndrome.

Biochemical Assessments

All biochemical measurements were performed through the Chemical Pathology Department at Monash Health. These comprised serum creatinine, 25(OH) vitamin D, calcium, phosphate, PTH, bicarbonate and ALP.

Dual Energy X-ray Absorptiometry

aBMD was measured by DXA at the lumbar spine, femoral neck and total body on all participants unless limited by previous scoliosis surgery, femoral surgery or difficulty positioning. Total lean tissue mass and fat mass were derived from the whole body scan. All measurements were obtained using a GE Lunar Prodigy (Madison, Wisconsin, software version 12) at Monash Health. The majority of scans were performed by one technician, and were given 1 hour timeslots to allow for positioning difficulties and the need to acquire multiple scans if motion artefact was present. I assessed all scans to determine whether there was significant motion artefact or large variations in the region of interest and excluded these sites from analysis.

Subjects were scanned from L1-L4 in all cases. However, when scans were reviewed individually, in subjects with scoliosis, L1 was often involved in the inflection of the curve making it difficult to reliably reproduce measurements between scans. L2-4 was therefore chosen for analysis in patients with CP.

In SB, the presence of vertebral arch deficits can falsely lower the L1–L4 lumbar spine BMD. The L1 vertebra was therefore chosen, because it is rarely involved in SB, in line with previous studies [21, 22].

BMD was expressed as grams per centimeter squared (g/cm^2). Z-scores were used due to the young age of the cohorts, which compares subjects to age and gender matched controls. Low BMD was pre-specified and defined as a Z-score of ≤-2.0 as per the ISCD guidelines for adults < 50 years of age (15).

Trabecular Bone score (TBS)

TBS measurements of L2-L4 were retrospectively obtained from DXA spinal images using TBS iNsight Software (version 3.0.2.0, Medimaps). TBS values were classified as intermediate risk (TBS 1.23-1.31) or high risk (TBS <1.23) of fracture based on a recent meta-analysis of 14 population cohorts including women and men (147). Subjects with TBS that were consistent with intermediate or high risk of fracture were considered to have low TBS.

Statistical Analyses

The distribution of data was explored using the Shapiro-Wilk test. All normally distributed data were expressed as mean with SD, and non-parametric data as median with minimum and maximum ranges. A p-value of <0.05 was considered significant and analyses were conducted using SPSS 24 (IBM, Armonk, NY).

Differences between groups were determined using the independent t test for normally distributed variables, the Mann-Whitney U test for nonparametric variables, and the Fisher exact test for categorical variables. The relationship between continuous and categorical variables was determined

using linear and logistic regression. Potential confounder factors were adjusted for using multiple regression analyses. Mixed model analyses were used for longitudinal analysis to determine the significance of changes in aBMD and Z-score over time, with time defined as the number of years since baseline DXA.

Chapter 3 - Understanding bone fragility in adults with CP through DXA-derived measures of BMD, body composition and bone microarchitecture

3.1 Introduction

Individuals with CP are living longer and are now experiencing diseases associated with ageing. In Australia, even those with most severe impairments are living to young adulthood, and if they survive to age 25 their remaining life expectancy is another 30 years (39). A number of epidemiological studies have identified two main disease processes highly prevalent in adults with CP, namely bone fragility and cardiometabolic disease (72-74).

Adults with CP are at high risk of osteoporosis and fracture due to exposure to risk factors commencing in childhood such as immobility, anticonvulsant use and malnutrition. This is further compounded by the loss of bone mass that occurs with ageing. What is unclear is whether low bone mass in adults with CP is predominantly due to less bone accrual during childhood, earlier onset of bone loss or an accelerated rate of bone loss compared with the general population. Previous small longitudinal studies suggest the rate of bone accrual in CP is less than that of typically developed children, and in adults there may be a decline in Z-scores over time although statistically insignificant (93, 95). Rates of osteoporosis and fracture in adults with CP is poorly described, and it not known whether these individuals experience typical osteoporotic fractures or continue to have distal femoral fractures seen in children with CP.

In young men and women, lean mass accounts for greater variance in BMD than fat mass; this relationship between muscle and bone is likely to be important in adults with CP. The interplay between body composition and bone has not been previousy explored in CP and may guide therapeutic strategies. DXA allows for assessment of bone density and body composition concurrently. Furthermore, it can be used to derive TBS, a marker of bone microarchitecture. In

children, the distal lateral femur site was devised to overcome difficulties with measuring BMD in children with CP and correlates to fracture risk (114, 117). However, the optimal site of measurement of BMD has not yet been determined in adults and there have been no studies that have found an association between fracture and BMD.

The objective of this chapter was to better understand bone fragility in adults with CP through DXA-derived measures of BMD, body composition and bone microarchitecture. Chapter 3.2 is a cross-sectional study of adults with CP assessing the prevalence and type of osteoporotic fractures seen, and exploring the effect of functional, endocrine and nutritional factors on the relationship between BMD, fracture and body composition. Chapter 3.3 examines the longitudinal changes in BMD in adolescents and adults with CP focusing on the relative importance of bone accrual or bone loss in the pathogenesis of low bone mass. Chapter 3.4 uses TBS to elucidate bone microarchitectural changes in CP and its potential use in the assessment of bone health and fracture risk in adults with CP.

Chapter 3.2 – Musculoskeletal and Endocrine Health in Adults with Cerebral Palsy: New Opportunities for Intervention

Musculoskeletal and Endocrine Health in Adults With Cerebral Palsy: New Opportunities for Intervention

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Context: Cerebral palsy (CP) increases fracture risk through diminished ambulation, nutritional deficiencies, and anticonvulsant medication use. Studies examining bone mineral density (BMD) in adults with CP are limited.

Objective: To examine the relationship between body composition, BMD, and fractures in adults with CP. The effect of functional, nutritional, and endocrine factors on BMD and body composition is also explored.

Design: Retrospective cross-sectional study.

Setting and Participants: Forty-five adults with CP (mean age, 28.3 ± 11.0 years) who had dual-energy x-ray absorptiometry imaging at a single tertiary hospital between 2005 and 2015.

Results: Seventeen (38%) had a past history of fragility fracture; 43% had a Z-score of ≤ −2.0 at the lumbar spine (LS) and 41% at the femoral neck (FN). In nonambulatory patients, every one unit decrease in FN Z-score increased the risk of fracture 3.2-fold (95% confidence interval, 1.07–9.70; $P = .044$). Stepwise linear regression revealed that the Gross Motor Function Classification System was the best predictor of LS Z-score ($R^2 = 0.550$; $\beta = -0.582$; $P = .002$) and FN Z-score ($R^2 = 0.428$; $\beta = -0.494$; $P = .004$); 35.7% of the variance in BMD was accounted for by lean tissue mass. Hypogonadism, present in 20% of patients, was associated with reduced lean tissue mass and reduced LS BMD. Lean tissue mass positively correlated with BMD in eugonadal patients, but not in hypogonadal patients.

Conclusions: Low BMD and fractures are common in adults with CP. This is the first study to document hypogonadism in adults with CP with detrimental changes in body composition and BMD. (***J Clin Endocrinol Metab*** **101: 1190–1197, 2016)**

Cerebral palsy (CP) is the most common motor disorder among children, affecting 2 to 3.5 per 1000 live births (1), and it results from a static insult to the developing fetal or infant brain. The disorder of movement is often accompanied by disturbances of sensation, cognition, communication, and perception and by seizures (2). Given the limitations in mobility and associated risk of falls (3), the optimization of bone health is paramount because fractures further limit mobility. Ambulatory function declines in parallel with health-related quality of life measures (4).

Numerous studies document a high prevalence of low bone mineral density (BMD) in up to 77% of children and adolescents with CP (5, 6). Risk factors for low BMD in

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Abbreviations: BMD, bone mineral density; BMI, body mass index; CP, cerebral palsy; DXA, dual-energy x-ray absorptiometry; GMFCS, Gross Motor Function Classification System; LTM, lean tissue mass; PEG, percutaneous endoscopic gastrostomy; SMI, skeletal muscle mass index.

children with CP include nutritional status, anticonvulsant use, and functional status (6, 7). In children with CP, most fractures occur in the long bones of the lower limbs, particularly in the distal femur and proximal tibia (7–9). Femoral fractures may occur without any documented trauma in up to 73% of cases (10).

As CP life expectancy continues to improve, low BMD and fractures are emerging as health priorities for patients and clinicians alike. In a cohort of over 300 individuals with CP, of those who survived to age 20 years, almost 85% survived to age 50 years (11). Adults with CP are particularly vulnerable to fractures because they have a longer cumulative exposure to risk factors and declining bone mass with age (12). However, literature examining the prevalence of osteoporosis and fractures in adults with CP is limited. It is also unclear whether adults with CP experience the same types of fractures as children, or whether fractures typically associated with osteoporosis (including neck of femur, vertebral, or radial fractures) become more prevalent with increasing age.

In recent years, small studies in adults with CP have shown the detrimental effect of decreased mobility on BMD (13–15). A cross-sectional study of 48 adults with CP of variable functional status (age range, 25–46 years) reported a mean Z-score of -1.4 ± 1.1 at the lumbar spine and -1.36 ± 1.0 at the total hip. Functional status as measured by Gross Motor Function Classification System (GMFCS) correlated with Z-scores at the lumbar spine and total hip, and there was a positive relationship between BMI and Z-scores at both sites (13). Two studies in adults have confirmed the importance of weight and ambulation in regard to bone density (14, 15). However, there is a lack of literature examining the relationship between BMD and body composition in adults with CP. In the general population, lean tissue mass (LTM) accounts for a larger proportion of the variance in BMD than fat mass in young females and males (16, 17). Given the known nutritional issues in this cohort, determining the influence of LTM and fat mass on BMD in adults with CP is particularly important.

In addition, the prevalence and contribution of secondary causes of low bone mass such as hypogonadism have not been reported previously in adults with CP. If deficits in gonadal function are present, this can have significant implications for the musculoskeletal health of adults with CP. Only one study describes early commencement and late completion of puberty in 207 children with GMFCS III-IV (18), but the implication of this finding on bone health was not evaluated.

The aim of this study was to assess the prevalence and type of fractures in an adult population with CP and to investigate how functional, nutritional, and endocrine factors impact the relationship between body composition and BMD in these individuals.

Patients and Methods

Patients

A retrospective cross-sectional cohort study of 45 consecutive adults with CP who had dual-energy x-ray absorptiometry (DXA) imaging at a single tertiary hospital from 2005–2015 was analyzed. The study was approved by the local ethics committee (Monash Health Human Research Ethics Committee).

Data collection and clinical measures

GMFCS grades the severity of gross motor function impairments based on the ability to mobilize or the need for assistive devices (19). Levels range from I to V, with individuals at level V having the greatest impairment and being wheelchair bound. This was obtained from the medical records, and participants were divided into two groups: predominantly ambulatory (GMFCS I-III) and nonambulatory (GMFCS IV-V).

A history of hypogonadism was established from the medical record. This was defined in males as the use of androgen replacement therapy or low T levels (<8 nmol/L) documented on two separate occasions and in females as the use of hormone replacement therapy for induction of pubertal development, menopause before the age of 40, or low estradiol levels (<73 pmol/L) on two separate occasions.

Information on current or past use of anticonvulsant medication, vitamin D levels at the time of BMD measurement, and use of percutaneous endoscopic gastrostomy (PEG) feeding was obtained from the medical record. Minimal trauma fracture was defined as a self-reported or radiologically proven fracture occurring after a fall from standing height or less, or a minimal trauma incident other than a fall (eg, turning over in bed). Vertebral fracture was diagnosed from a lateral thoracolumbar spinal x-ray, when available.

BMD measurements

BMD was measured by DXA at the lumbar spine (L2–L4), femoral neck- and total body on all participants unless limited by previous scoliosis surgery, femoral surgery, or difficulty positioning. Low BMD was prespecified and defined as a Z-score of ≤ -2.0, as per the International Society of Clinical Densitometry guidelines for adults < 50 years of age (20).

Total LTM and fat mass were derived from the whole body scan. All measurements were obtained using a GE Lunar Prodigy (software version 12; GE Healthcare) at a single center. Anthropometric measures of age, weight, and height were documented. In cases where true height could not be obtained, length while lying flat was used or height was calculated using knee height using Stevenson's equation (21): estimated height = (knee height $\times$ 2.69) + 24.2 cm.

Adults were categorized into four body mass index (BMI) groups: underweight (BMI $<$ 18.5 kg/m^2), normal (BMI, 18.5–25 kg/m^2), overweight (BMI $>$ 25 kg/m^2), or obese (BMI $>$ 30 kg/m^2). Increased fat mass was defined as $>$ 35% in females and $>$ 25% in males as per American Association of Clinical Endocrinologists/American College of Endocrinology guidelines (22). Percentiles for fat mass were calculated from the National

Health and Nutrition Examination Survey 1999–2004 body composition data matched for age and gender (23). Low lean mass was defined as: skeletal muscle mass index (SMI) = appendicular lean mass divided by height^2 of < 7.26 kg/m^2 in men and < 5.5 kg/m^2 in women, as per the European Working Group on Sarcopenia in Older People (24).

Statistical analysis

The distribution of the data was explored by the Shapiro-Wilk test. All normally distributed data were expressed as mean ± standard deviation and nonparametric data as median with interquartile ranges. Differences between groups were determined using the Mann-Whitney *U* test for continuous variables and χ^2 for categorical variables. Group differences in GMFCS were determined using the Kruskal-Wallis test.

A univariate regression analysis was performed to examine the correlation between fat and LTM with BMD at the lumbar spine, femoral neck, and total body separately. This was followed by a multivariate analysis after adjusting for age, gender, and height in these models.

Univariate analysis was used to determine predictors of fracture and BMD Z-score at all three sites. Given the possibility of significant covariance between factors such as ambulatory status, use of antiepileptic medication, and PEG feeding, stepwise multiple regression analysis was performed to determine which factor was most important. Multicollinearity was determined for all multiple and stepwise regression models by calculating the variance inflation factor. Any regression model with a variance inflation factor > 10 was excluded. A P value of $< .05$ was considered to be statistically significant, and all tests were two-sided. Analyses were conducted using IBM SPSS statistics for Windows (version 22; SPSS Inc).

Results

Baseline characteristics

Clinical characteristics of patients are summarized in Table 1. The majority had severe functional limitations with a GMFCS of IV or V and had a mean height at or below the 10th centile. In 37 patients, total body BMD was obtained, enabling total and regional LTM and fat mass to be derived. Lumbar spine BMD was obtained in 35 patients, and femoral neck BMD was obtained in 37 patients. Mean lumbar spine Z-score was -1.61 ± 2.0, and mean femoral neck Z-score was -1.72 ± 1.42. Reduced BMD, as defined by a Z-score ≤ -2.0, was present in 15 of 35 patients (43%) at the lumbar spine and in 15 of 37 (41%) patients at the femoral neck.

The mean BMI was 22.3 ± 6.6 kg/m^2, which fell into the normal range for adults; however, this obscures the fact that a large proportion of patients fell into either the underweight (31%) or overweight/obese (27%) category. Of the patients with normal BMI, six of nine females (67%) and three of six males (50%) had an increased percentage of fat on DXA. Using age and gender-matched fat centiles from NHANES III, seven of 15 with normal BMI had a fat centile > 75%. Using linear regression, only 46% of the variance in BMI could be explained by percentage fat mass ($P < .001$). Reduced SMI was present in 10 of 18 males (56%) and 14 of 18 females (78%). PEG-fed patients had a trend to reduced SMI (4.52 ± 1.40 vs 5.55 ± 1.46 kg/m^2; $P = .054$) and increased percentage fat (40.2 ± 17.3 vs 30.0 ± 14.2%; $P = .054$) compared with non-PEG-fed patients with no difference in BMI (22.3 ± 7.1 vs 22.0 ± 2.7 kg/m^2; $P = .57$).

Table 1. Baseline Characteristics of Adults With CP

Demographic	
Age, y	28.3 ± 11.0
Male, n (%)	23 (51)
Anthropometric	
Weight, kg	54.7 ± 20.2
Height, cm	155.4 ± 13.6
BMI, kg/m^2	22.3 ± 6.6
Clinical variables, n (%)	
Anticonvulsant use	22 (49)
Nonambulatory	33 (73)
PEG feeds	8 (18)
GMFCS I-III	12 (27)
IV	7 (15)
V	26 (58)
Fracture	17 (38)
Hypogonadal	9 (20)
DXA variables	
LTM, kg	34.0 ± 11.0
Fat mass, kg	18.3 ± 13.2
Bone mineral content, kg	1.96 ± 6.71
Total body BMD, g/cm^2	1.04 ± 0.11
Total body Z-score	−1.12 ± 1.41
Lumbar spine BMD, g/cm^2	1.01 ± 0.28
Lumbar spine Z-score	−1.61 ± 2.0
Femoral neck BMD, g/cm^2	0.77 ± 0.18
Femoral neck Z-score	−1.72 ± 1.42

Values are expressed as mean ± SD or number (percentage) unless otherwise stated.

Fracture

A history of fragility fracture was noted in 17 of 45 patients (38%), with three experiencing multiple fractures. Of the 17 patients with a history of fracture, eight had a fracture during childhood, with all these fractures involving the lower limb. Fractures of the femur were present in five, and three patients had tibia/fibula fractures during childhood. Fractures occurred in 53% of patients in adulthood. Of the 20 patients who underwent lateral spinal x-rays during adulthood, four had vertebral crush fractures (20%). The remainder of the fractures in adulthood involved the ankle (n = 3), ribs (n = 2), and sacrum (n = 1).

Univariate analysis revealed that no clinical, anthropometric, or DXA parameters were significantly associated with fracture. In particular, BMD and Z-scores at the spine, femoral neck, and total body and the clinical vari-

ables (anticonvulsant use, hypogonadism, PEG feeding, ambulatory status, and GMFCS) were not associated with fracture. However, in nonambulatory patients, bone mineral content, areal BMD (spine, femoral neck, total body), and femoral neck Z-score were all associated with fracture ($P < .05$). For every one unit reduction in femoral neck Z-score, the risk of fracture increased 3.2-fold (95% confidence interval, 1.07–9.70; $P = .044$).

Hypogonadism

In this study, nine of 45 patients (20%) were hypogonadal. Of these, two had primary gonadal failure, six had hypogonadotropic hypogonadism, and one had a mixed picture (orchidectomy for undescended testis and hyperprolactinemia). Two patients (one male, one female) had elevated prolactin levels; one was able to have a magnetic resonance imaging of the pituitary that was normal. The remaining patients with hypogonadotropic hypogonadism were unable to have a magnetic resonance imaging of the pituitary for logistical reasons. In addition, three male patients had elevated LH with normal levels of T.

Differences between eugonadal and hypogonadal patients are summarized in Table 2. Hypogonadal patients were shorter and weighed less than their eugonadal counterparts. After adjustment for gender, LTM was significantly reduced ($P = .04$), whereas there was no difference in fat mass. In hypogonadal patients, lumbar spine BMD and lumbar spine Z-score were reduced. Hypogonadal patients were all nonambulatory and were more likely to have PEG feeds. There was a trend toward more fractures in hypogonadal patients, which was not statistically significant (67 vs 31%; $P = .055$).

To determine the clinical factors that correlated most to LTM, multiple regression analysis was performed with SMI as the dependent variable and the clinical variables as the independent variables after adjusting for age. Patients were stratified based on gender. In females, hypogonadism was the only clinical variable after adjustment for age to correlate with SMI ($R^2 = 0.595$, $P = .034$; $\beta = -0.570$, $P = .042$). In males, there were no variables that correlated with SMI.

Predictors of BMD

Using univariate analysis, ambulatory status and GMFCS correlated with Z-scores at the lumbar spine, femoral neck, and total body ($P < .05$) (Table 3). At the lumbar spine, hypogonadism was negatively correlated with Z-scores ($R^2 = 0.131$; $\beta = -0.362$; $P = .033$), and at the total body, PEG feeding was negatively correlated with Z-scores ($R^2 = 0.108$; $\beta = -0.329$; $P = .047$). Stepwise linear regression after adjusting for height showed GMFCS to be the best predictor of lumbar spine Z-score ($R^2 = 0.550$; $\beta = -0.582$; $P = .002$) and femoral neck Z-score ($R^2 = 0.428$; $\beta = -0.494$; $P = .004$). For every one unit increase in GMFCS, lumbar spine Z-score was reduced by 0.582 and femoral neck Z-score was reduced by 0.494.

Table 2. Differences in Body Composition and Clinical Variables Between Eugonadal and Hypogonadal Patients

	Eugonadal	Hypogonadal	*P* Value
n	36	9	
Demographic[a]			
Age, y	29.4 ± 11.8	24.0 ± 5.6	.20
Males, n	20 (56%)	3 (33%)	.207
Anthropometric[b]			
Weight, kg	58.3 ± 20.2	40.3 ± 13.2	**.044**
Height, cm	158.2 ± 12.1	143.9 ± 13.6	**.013**
BMI, kg/m^2	23.0 ± 6.9	19.4 ± 4.0	.181
DXA variables[b]			
LTM, kg	36.0 ± 10.7	25.2 ± 7.7	**.040**
Fat mass, kg	19.4 ± 13.7	13.6 ± 9.9	.232
Bone mineral content, kg	2.11 ± 0.64	1.43 ± 0.50	**.046**
SMI, kg/m^2	5.58 ± 1.34	4.39 ± 1.31	.118
Total body BMD, g/cm^2	1.06 ± 0.11	0.98 ± 0.07	.137
Total body Z-score	−1.01 ± 1.40	−1.63 ± 1.48	.203
Lumbar spine BMD, g/cm^2	1.04 ± 0.22	0.77 ± 0.20	**.025**
Lumbar spine Z-score	−1.25 ± 1.93	−3.06 ± 1.88	**.045**
Femoral neck BMD, g/cm^2	0.79 ± 0.17	0.70 ± 0.15	.272
Femoral neck Z-score	−1.69 ± 1.47	−1.90 ± 1.15	.673
Clinical variables, n (%)[a]			
Anticonvulsant use	18 (50)	4 (44)9	.530
Nonambulatory	24 (67)	(100)	**.044**
PEG feeds	3 (9)	5 (56)	**.004**
Fracture	11 (31)	6 (67)	.055

Data are expressed as mean ± SD or number (percentage). Bold indicates *P* values that are statistically significant.

[a] Mann-Whitney *U* test.

[b] Logistic regression adjusted for gender.

Relationship between body composition and bone mass

Using linear regression, the relationship between BMD with body composition parameters was examined (Table 4). Univariate analysis showed that 35.7% of the variance in BMD was accounted for by LTM. After multivariate analysis adjusting for age, gender, and height, only LTM had a positive association with BMD ($R^2 = 0.599$, $P < .01$; $\beta = 0.611$, $P = .045$). In eugonadal patients, LTM was correlated with BMD in both univariate and multivariate analysis. This relationship was lost in hypogonadal patients in whom neither LTM nor fat mass correlated with BMD.

Table 3. Clinical Predictors of Z-Scores at the Lumbar Spine, Femoral Neck, and Total Body

	Lumbar Spine Z-score			Femoral Neck Z-score			Total Body Z-score		
	R^2	β	*P* Value	R^2	β	*P* Value	R^2	β	*P* Value
Hypogonadism	0.131	−0.362	**.03**	0.003	−0.054	.765	0.031	−0.175	.299
PEG feeding	0.078	−0.279	.104	0.026	−0.161	.371	0.108	−0.329	**.05**
Nonambulatory	0.326	−0.571	**<.001**	0.296	−0.545	**0**	0.164	−0.404	**.01**
GMFCS	0.363	−0.602	**<.001**	0.295	−0.544	**0**	0.143	−0.379	**.02**
Anticonvulsant use	0.028	0.167	.337	0.002	−0.041	.821	0.033	0.181	.285

Bold indicates *P* values that are statistically significant.

Discussion

This study in adults with CP revealed that 38% had a prevalent fragility fracture, with 53% of these occurring in adulthood. The types of fractures are different from those described in children with CP, with our adult cohort experiencing predominantly ankle, vertebral, and rib fractures. These osteoporotic-related atraumatic fractures are occurring at a younger age in adults with CP than in the general population. Asymptomatic vertebral fracture in other populations is associated with an increased risk of subsequent osteoporotic fracture, including hip fracture independent of BMD (25).

The distal femur is the most common site of fracture in the pediatric CP population. A technique of scanning the lateral distal femur using DXA has been developed with this in mind and to allow for positioning difficulties and artifacts from scoliosis, hip dislocation, and metallic hardware (26). There are established reference ranges in children (27, 28), and correlation between Z-scores at the distal femur and fracture history in a mixed cohort of children with CP or muscular dystrophy (29) has been shown. Henderson et al (30) reported a preliminary series of 32 adults with CP using this technique with precision of duplicate scans of between 2.4 and 7.1% in various regions of the distal femur. Currently its use in adults is limited by a lack of normative data and the need for specialist training in the technique.

Given these limitations, using traditional scanning techniques validated in postmenopausal women and older men, we successfully obtained lumbar spine measurements in 35 patients and femoral neck measurements in 33 patients. Over 40% of patients had BMD below the expected range for age. We demonstrated that GMFCS and ambulatory status was a significant predictor of low BMD at all sites. Furthermore, in nonambulatory adults, femoral neck Z-score correlated with fractures. The importance of function and ambulation to BMD is consistent with the pediatric and adult literature. Of concern, therefore, is that a decline in mobility occurs in over 25% of adults with CP over time (3). Our findings support the use of standard lumbar spine and proximal hip DXA for adults with CP as advocated by Fowler et al (13). In ambulatory patients with CP, we were unable to demonstrate a statistically significant relationship between BMD and fracture. This may be due to the small cohort of ambulatory patients, where six of the 12 ambulatory patients sustained fractures. We thus postulate that an increased risk of falls in ambulatory patients compared with nonambulatory patients may account for their higher risk of fracture.

The use of DXA enables the assessment of body composition. In adults with CP, measures of body fat using DXA have been shown to be highly correlated to body fat estimated through the doubly labeled water technique, which is considered the "gold standard" (31). There are no validated adult CP-specific equations to estimate body fat using anthropometric measures, and the common anthropometric measure of body composition, BMI, is unable to distinguish fat from lean mass. Consistent with this, our cohort showed that only 46% of the variance in BMI could be explained by percentage fat mass. In addition, 60% of

Table 4. Relationship Between LTM and Fat Mass With Total Body BMD

	R^2	*P* Value	β	*P* Value
All				
Fat mass	0.188	**.004**	0.458	**.004**
LTM	0.359	**<.001**	0.614	**<.001**
Model[a]	0.599	**<.001**		
Fat mass			0.147	.269
LTM			0.611	**.045**
Hypogonadal				
Fat mass	0.278	.224	0.527	.224
LTM	−0.036	.414	0.370	.414
Model[a]	−0.0839	.803		
Fat mass			0.751	.306
LTM			0.949	.537
Eugonadal				
Fat mass	0.152	**.019**	0.425	**.019**
LTM	0.327	**.001**	0.592	**.001**
Model[a]	0.646	**<.001**		
Fat mass			−0.004	.977
LTM			1.011	**.007**

Bold indicates *P* values that are statistically significant.

[a] Multivariate analysis adjusted for age, gender, and height.

those with a normal BMI had an increased percentage of fat mass. A recent study utilizing DXA to assess body composition in 47 children with CP found that all children with a low BMI had adequate or excess body fat (32). The use of DXA for assessment of body composition in this cohort is thus clinically important to guide optimal outcomes in feeding and exercise interventions. This may be particularly pertinent in view of the unexpected high mortality rate in adults with CP due to circulatory diseases (11, 33).

We also found reduced SMI in > 50% of males and almost 80% of females. Sarcopenia has been associated with increased risk of falls and fractures, impaired functional state, and increased risk of death in the older population (34, 35). Because this was a retrospective study, we were unable to obtain functional measures of muscle strength to complement our body composition data. However, patients with severe functional disability (GMFCS IV-V) cannot be assessed with conventional techniques such as handgrip strength or knee flexion/extension strength.

This is the first study to use DXA in adults with CP to examine the relationship of LTM and fat mass to BMD. Previous studies in adults with CP have demonstrated a positive relationship between BMI and BMD (13, 15). We have shown that LTM is the most powerful positive predictor of BMD. This is consistent with the literature showing that LTM accounts for a larger variance in BMD than fat mass in young females and males, whereas in postmenopausal women, fat mass is relatively more important (36–38). LTM may exert its effect via mechanical stress through muscle contraction, whereas it has been proposed that fat has positive effects on bone through secretion of hormones such as insulin, amylin, and leptin and through the increased aromatization of T to estrogen (39, 40).

However, this positive correlation between LTM and BMD is attenuated when hypogonadism is present. This has a number of possible clinical implications: first, nutritional interventions that increase weight through fat deposition may not translate to improvements in BMD. Moreover, interventions that increase weight bearing or muscle mass may be preferable, and finally our data suggest that treatment of hypogonadism will be paramount before such interventions.

Altered pubertal progression was found in a cross-sectional study of 207 children with CP (18). In particular, menarche commenced late in girls with CP. There are no follow-up studies describing gonadal status of adults with CP. Hypogonadism is a risk factor for osteoporosis, and our finding that 20% of adults had hypogonadism is of concern. Hypogonadal patients with CP had reduced LTM and lower lumbar spine BMD, which has been well described in other hypogonadal populations. The significant functional differences between eugonadal and hypogonadal subjects along with the body composition changes suggest a long-standing and more permanent deficit in gonadal status.

Most patients had hypogonadotropic hypogonadism on endocrine evaluation. The etiology of this is unclear, and possible explanations include: poor nutrition and chronic illness leading to a functional hypothalamic hypogonadism, or injury to the immature brain in CP leading to hypothalamic-pituitary axis dysfunction. Common causes of hypogonadotropic hypogonadism in other populations such as untreated obstructive sleep apnea, glucocorticoid therapy, or narcotic pain medication were not present in this cohort. Whether early detection and treatment of hypogonadism is of benefit from a functional and musculoskeletal perspective remains unanswered. We recommend screening for hypogonadism in adolescents and adults with CP presenting with low BMD and fracture to ensure that this important diagnosis is not missed. Challenges with treatment include addressing the caregiver's concerns regarding sexual behavior, growth, and psychosocial changes accompanying puberty.

This study represents one of the largest adult cohorts with CP in the literature. We are the first to confirm hypogonadism to be highly prevalent in adults with CP, particularly in those with more severe motor dysfunction and requiring PEG feeding. Our findings highlight the need to screen for hypogonadism, low BMD, and vertebral fractures in patients who are nonambulatory and/or on PEG feeds. The presence of these clinical features is associated with poor musculoskeletal outcomes. The emphasis of management in those with less severe functional limitations (GMFCS I-III) should be on minimizing the risk of falls while encouraging ambulation.

We are mindful of a number of limitations to this study. BMD is a two-dimensional measure of a three-dimensional structure and can be affected by body size, which is particularly relevant in our cohort who has restriction in growth. To reduce the effect of body size on BMD, we adjusted for height in our multivariate and stepwise analyses. This is a cross-sectional study, and therefore causation cannot be established between hypogonadism and the body composition changes we have described. Longitudinal monitoring and reporting of BMD, falls, and fractures is needed in this cohort. Fracture recall may be biased, and fractures were not systematically confirmed on imaging. Our small sample size increases the risk of type I error. We were also unable to collect sufficient data on vitamin D status and therefore were unable to examine the effect of vitamin D on BMD.

In conclusion, adults with CP experience prevalent fragility fractures at a young age. Poor mobility and hypogonadism are important factors contributing to low BMD in this cohort. LTM has a significant positive association with BMD, but its effect is attenuated by the presence of hypogonadism. Early recognition and treatment of hypogonadism in patients with CP may have beneficial effects on musculoskeletal health, and this warrants further study.

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Chapter 3.3 – Trabecular bone score in adults with cerebral palsy

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Full Length Article

Trabecular bone score in adults with cerebral palsy

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ABSTRACT

Context: Bone fragility in cerebral palsy (CP) is secondary to a complex interplay of functional, hormonal, and nutritional factors that affect bone remodelling. A greater understanding of bone microarchitectural changes seen in CP should assist therapeutic decision making.
Objective: To examine the relationship between trabecular bone score (TBS), BMD and fractures in adults with CP; the influence of clinical factors and body composition on bone microarchitecture were explored.
Design: Retrospective cross-sectional study.
Setting and Participants: 43 adults (25 male) with CP of median age 25 years (interquartile range 21.4–33.9) who had evaluable dual-energy X-ray absorptiometry imaging of the lumbar spine from a single tertiary hospital between 2005-March 2018.
Results: 24/43 (55.8%) of patients had TBS values indicating intermediate or high risk of fracture (< 1.31). TBS correlated with areal BMD at the lumbar spine, femoral neck and total body. TBS was significantly associated with arm and leg lean mass, with adjustment for age, gender and height (adjusted $R^2 = 0.18$, $p = 0.042$ for arm lean mass; adjusted $R^2 = 0.19$, $p = 0.036$ for leg lean mass). There was no difference in TBS when patients were grouped by fracture status, anticonvulsant use, gonadal status or use of PEG feeding. TBS was lower in non-ambulatory patients compared with ambulatory patients (1.28 vs 1.37, $p = 0.019$).
Conclusions: Abnormal bone microarchitecture, as measured by TBS, was seen in > 50% of young adults with CP. TBS correlated with both areal BMD and appendicular lean mass. Maintaining muscle function is likely to be important for bone health in young adults with CP and needs to be confirmed in further studies.

1. Introduction

The insult to the brain in cerebral palsy (CP) can occur during the prenatal, perinatal or postnatal period and is non-progressive. However, the resulting motor impairment progresses significantly over time. Up to 75% of individuals lose their ability to mobilise [1] and many become dependent on assistive devices [2] as they progress to adulthood. As life expectancy continues to improve in CP [3], preserving bone and muscle health to maintain mobility and independence is critical. However, assessment and maintenance of musculoskeletal health in CP is limited by function, joint contractures, scoliosis, and cognitive impairment.

Bone mineral density (BMD) as measured by dual X-ray absorptiometry (DXA) is widely available and used as the main surrogate of bone strength. The diagnosis of osteoporosis is based on either BMD criteria or the presence of minimal trauma fracture. Bone strength, however, is determined not only by bone density but other factors such as bone geometry, bone microarchitecture, bone turnover and bone mineralisation [4,5]. Using high-resolution MRI, Modlesky et al. have shown abnormal microarchitecture in the distal femur of non-ambulatory children with CP [6,7]. Trabecular number and thickness were reduced while trabecular separation was increased in children with CP compared with normal controls. The deficits in bone microarchitecture were more marked the greater the distance from the growth plate. The changes in cortical bone that have been demonstrated include a reduction in cortical thickness and cross-sectional area [8]. However, cortical volumetric bone density as measured by peripheral quantitative computed tomography (pQCT) is not different from typically developing children [9]. The smaller bones found in children with CP may account for these findings. In adults with CP, using thoracoabdominal CT scans, trabecular and cortical bone density were found to be reduced compared to age, sex and BMI matched controls [10].

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Advanced imaging modalities such as high-resolution MRI and high-resolution pQCT are usually only found in research institutions and these measurements are susceptible to significant motion artefact. The trabecular bone score (TBS) was developed to estimate trabecular microstructure from a 2-dimensional lumbar spine (LS) DXA image. TBS is a textural index that examines pixel grey level variations in the LS image without the need for further imaging [11]. Low TBS is associated with hip fracture in postmenopausal women and men aged > 50 years and can be complementary to the FRAX risk assessment tool [12,13].

To date, no studies have investigated the use of TBS in CP. This study aimed to evaluate whether TBS can provide further insights into bone microarchitecture in CP and its use in the clinical setting. To do this, the correlation between TBS and areal measures of spinal, femoral neck and total body BMD and body composition in young adults with CP was determined. The relationships of TBS with fractures and clinical factors relating to function, nutrition and endocrine status were also explored.

2. Methods

2.1. Subjects

A retrospective cross-sectional cohort study of 43 consecutive adults with CP who had DXA imaging of the lumbar spine at a single tertiary hospital from 2006-March 2018 was analysed. Medical indications for DXA imaging included suspected low BMD in the setting of immobility, fracture, anticonvulsant use and hypogonadism. The study was approved by the institutional ethics committee (Monash Health HREC).

2.2. Data collection and clinical measures

GMFCS grades the severity of gross motor function impairments based on the ability to mobilise or need for assistive devices [14]. Levels range from I to V with individuals at level V having the greatest impairment and are wheelchair bound. GMFCS was obtained from the medical records, and participants divided into two groups: predominantly ambulatory (GMFCS I–III) or non-ambulatory (GMFCS IV-V).

A history of hypogonadism was established from the medical record. Hypogonadism was defined in males as the use of androgen replacement therapy or low testosterone levels (< 8 nmol/L) documented on two separate occasions and in females as the use of hormone replacement therapy for induction of pubertal development, menopause before the age of 40 or low oestradiol levels (< 73 pmol/L) on two separate occasions.

Current or past use of anticonvulsant medication and use of percutaneous endoscopic gastrostomy (PEG) feeding was obtained from the medical record. Minimal trauma fracture was defined as a self-reported or radiologically proven fracture occurring after a fall from standing height or less, or a minimal trauma incident other than a fall (e.g. turning over in bed). Vertebral fracture was diagnosed from a lateral thoraco-lumbar spinal X-ray, where available.

2.3. BMD measurements

BMD was measured by DXA at the lumbar spine (L2–L4), femoral neck and total body on all participants unless limited by previous scoliosis surgery, femoral surgery or difficulty positioning the subject. L1 was excluded as it is often involved in the scoliosis of the spine seen in CP making BMD difficult to interpret at this site.

Low BMD was pre-specified and defined as a Z score of ≤ -2.0 as per the International Society of Clinical Densitometry guidelines for adults < 50 years of age [15]. The coefficient of variation (CV) for BMD of a Hologic anthropomorphic lumbar spine phantom measured daily over the period from mid 2004 to end 2011 was 0.51%. The CV for percentage body fat of a total body phantom measured weekly was 3.11%.

Total lean tissue mass (LTM) and fat mass were derived from the whole-body scan. All measurements were obtained using a GE Lunar Prodigy (Madison, Wisconsin, software version 12) at a single centre. Anthropometric measures of age, weight and height were documented. In cases where true height could not be obtained, length while lying flat was used or height was calculated using knee height using Stevenson's equation [16]: Estimated Height = (Knee Height $\times 2.69$) + 24.2 cm.

Low lean mass was defined as a skeletal muscle mass index (SMI) = appendicular lean mass divided by height^2 of < 7.26 kg/m^2 in men and < 5.5 kg/m^2 in women, as per the European Working Group on Sarcopenia in Older People (EWGSOP) [17].

2.4. TBS

TBS measurements of L2-L4 were retrospectively obtained from DXA spinal images using TBS iNsight Software (version 3.0.2.0, Medimaps). TBS values were classified as intermediate risk (TBS 1.23–1.31) or high risk (TBS < 1.23) of fracture based on a recent meta-analysis of 14 population cohorts including women and men [12]. Subjects with TBS that were consistent with intermediate or high risk of fracture were considered to have low TBS.

2.5. Statistical analysis

The distribution of the data was explored by the Shapiro-Wilk test. All normally distributed data were expressed as mean with standard deviation and non-parametric data as median with interquartile ranges. Outliers were identified by Box and Whisker plots (beyond ± 1.5 interquartile range). The association with TBS with DXA and anthropometric measures was determined using Pearson and Spearman correlation for variables with a parametric and non-parametric distribution respectively. Multivariate regression analysis was performed to adjust these analyses for age, gender and height. Student *t*-tests was used to compare TBS between subgroups of participants. Analyses were conducted using IBM SPSS Statistics for Windows (Version 25. Armonk, NY).

3. Results

The clinical characteristics of patients are summarised in Table 1. The majority had severe functional limitations with a GMFCS of IV or V, and nearly half required anticonvulsant therapy. Lumbar spine, total body and femoral neck BMD were obtained in 43, 35 and 30 patients respectively. Of the 43 patients, 22 (51.2%) had low BMD (Z score ≤ -2) at the lumbar spine and 12/30 (40%) had low BMD at the femoral neck. 16 (37.2%) of 43 CP adults had a TBS of 1.23–1.31, regarded as having an intermediate risk of fracture and 8 (18.6%) had a TBS of < 1.23, indicating a high risk of fracture. Of the 22 patients who had a LS *Z*-score > −2, 6 (27.2%) had TBS values indicating intermediate or high risk of fracture.

3 of the 15 prevalent fractures were vertebral fractures. 1 of these 3 patients (33.3%) had a low TBS but also had a Z score of < −2. Of the remaining 12 patients who experienced non-vertebral fractures, 6 (50%) had a low TBS. One of these patients who fractured with a low TBS had a Z score of > −2.

Lumbar spine TBS correlated positively with weight and BMI as well as areal BMD at all three sites (lumbar spine, femoral neck, total body) (Table 2). The strength of the correlation between TBS and BMI was not significantly attenuated even after excluding those subjects with low BMI of < 15 kg/m^2 (n = 7). There was no association between TBS and total body lean or fat mass. When body compartments were considered separately, a significant positive association between TBS and arm and leg lean mass was identified. After adjustment for age, gender and height, the associations remained significant (adjusted $R^2 = 0.18$, $p = 0.042$ for arm lean mass; adjusted $R^2 = 0.19$, $p = 0.036$ for leg lean

Table 1
Clinical characteristics of patients.

Demographic	
Age, y	25 (21.4–33.9)
Male, n (%)	25 (58.1%)
Anthropometric	
Weight, kg	48.5 (35.8–68.5)
Height, cm	157.6 ± 12.7
BMI, kg/m2	18.6 (13.3–25.1)
Clinical variables, n (%)	
Anticonvulsant use	21 (48.8%)
PEG feeds	4 (9.3%)
Fracture	15 (34.9%)
Hypogonadal	9 (20.9%)
Nonambulatory	29 (67.4%)
GMFCS I-III	17 (39.5%)
IV	6 (14%)
V	20 (46.5%)
DXA variables	
LTM, kg (n = 35)	34.6 ± 12.4
Fat mass, kg (n = 35)	13.5 ± 11.2
Bone mineral content, kg (n = 35)	1.86 ± 0.64
Total body BMD, g/cm^2 (n = 35)	1.010 ± 0.111
Total body *Z*-score (n = 35)	−1.32 ± 1.20
Lumbar spine BMD, g/cm^2	0.92 ± 0.16
Lumbar spine Z-score	−1.81 ± 1.20
Microarchitectural variables	
Trabecular bone score	1.32 ± 0.14
Low risk TBS	19 (44.2%)
Intermediate risk TBS	16 (37.2%)
High risk TBS	8 (18.6%)

Table 2
Correlation of TBS with anthropometric and body composition measures, and BMD.

Characteristic	Correlation with TBS (R)	p
Age*	0.020	0.90
Height	0.183	0.241
Weight*	0.352	**0.021**
BMI*	0.343	**0.024**
BMD		
Lumbar spine	0.482	**0.001**
Femoral neck	0.552	**0.002**
Total body	0.361	**0.033**
Total body BMC	0.353	**0.037**
Lean mass	0.273	0.113
Fat mass	0.237	0.171
Fat mass, %	0.105	0.547
Arms lean mass	0.335	**0.049**
Legs lean mass	0.353	**0.037**
Arms fat mass	0.247	0.159
Legs fat mass	0.335	0.053
Android lean mass	0.140	0.422
Android fat mass	0.124	0.477

*Spearman correlation.

mass). Despite the android region overlaying the TBS region of interest, there was no association between android lean mass and TBS (R = 0.140, p = 0.422) or android fat and TBS (R = 0.124, p = 0.477).

There was no difference in TBS when patients were grouped by fracture history, anticonvulsant use, PEG feeding, or gonadal status (Table 3). TBS was significantly lower in non-ambulatory vs ambulatory patients (1.28 vs 1.37, p = 0.019). The difference in TBS seen in patients with or without low lean mass (as measured by skeletal mass index) approached statistical significance (p = 0.052). Similarly, non-ambulatory patients and patients with low lean mass had lower lumbar spine BMD values. Hypogonadism was associated with lower spine and femoral neck BMD but not lower TBS. To check for confounders, Student *t*-tests was used to assess differences between groups for continuous variables and the chi square test was used for categorical variables. In cases where there were significant differences between the two groups, multiple linear regression was used to adjust for the covariate. This did not affect the significance of the analyses.

4. Discussion

TBS is a surrogate measure of trabecular architecture and has been shown in ex vivo studies to correlate with trabecular number, connectivity, thickness and separation [18,19]. This is the first study to investigate TBS in adults with CP. We found over 50% of our cohort had an intermediate or high risk TBS, supporting the notion that abnormal bone microarchitecture in CP contributes to bone fragility. Our findings are consistent with previous MRI studies showing trabecular changes in non-ambulatory children with CP [6,7]. The prevalence of poor bone microarchitecture in this cohort is unsurprising given the multitude of risk factors contributing to bone disease in CP. According to the mechanostat regulatory mechanism of bone remodelling initially proposed by Frost [20], load-bearing bones adapt to changes in their mechanical environment by forming or resorbing bone. In CP, less trabecular bone may be formed in response to reduced force placed on bone during skeletal development as a result of impaired mobility. Additional factors such as hypogonadism, anticonvulsant use and malnutrition are also likely to adversely affect trabecular bone formation. It is unclear whether adults with CP also experience accelerated bone remodelling comparable to postmenopausal women with thinning and loss of trabeculae. Further loss of mobility over time and ongoing exposure to factors such as anticonvulsant use and malnutrition may lead to accelerated remodelling at an earlier age. There has been a lack of longitudinal studies in adults with CP to test this hypothesis.

TBS was higher in ambulatory patients and was weakly associated with appendicular lean tissue mass. This association between lean mass and TBS may be a reflection of the relationship between BMD and TBS in this cohort. We have previously shown in adults with CP lean mass correlates with BMD [21]. Given the relationship between TBS and BMD, the utility of TBS may lie in situations where TBS and BMD are discordant. For example, 27% of patients in our cohort with a Z score > −2 had a low TBS. This may represent a group who despite their non-osteoporotic BMD, are at higher risk of fracture and thus require more intensive monitoring.

The association between ambulatory function and TBS has not been previously reported in the literature. Our findings suggest function may be involved in the development and maintenance of bone health in CP. Muscle deficits seen in CP include reduced muscle size, abnormal fatty infiltration and changes in fibre type [22–24]. Changes can be seen even in ambulatory patients compared with normal controls [25] and in children from 2 to 5 years of age [26]. There has been increasing interest in applying the sarcopenia framework to CP to explore therapeutic interventions [27] which will have flow on effects for improving mobility and bone strength. We did not have functional measures of muscle strength to assess for sarcopenia formally. However, patients with severe functional disability (GMFCS IV–V) cannot be evaluated with conventional techniques such as handgrip strength or knee flexion/extension strength.

We were unable to find the association between fracture and TBS which has been demonstrated in postmenopausal women and older men. Most fragility fractures occur in patients who have normal or osteopenic BMD [28]; TBS can improve fracture prediction by identifying these patients for treatment. However, we were limited by our small sample size with few fractures. Out of the 15 patients who fractured, there was only one who had normal BMD but low TBS. Given the retrospective study design all fractures may not have been documented in the medical records. Moreover, the sample size required to enable adequate power to examine fracture endpoints would almost certainly require international collaboration. Indeed, the relationship between bone density and fracture in CP is limited to three cross-sectional studies in children and one in adults, each utilising different DXA regions of interest and with cohorts with varied clinical characteristics

Table 3
Differences in TBS according to clinical subgroups.

	n	TBS	95% confidence interval of the difference	p value	LS BMD (g/cm^2)	95% confidence interval of the difference	p value	FN BMD (g/cm^2)	95% confidence interval of the difference	p value
Fracture*										
Yes	15	1.31	−0.07 to 1.11	0.680	0.90	−0.08 to 0.21	0.357	0.80	−0.14 to 0.14	0.984
No	27	1.33			0.97			0.80		
Anticonvulsant use*										
Yes	20	1.32	−0.08 to 0.09	0.899	0.98	−0.20 to 0.08	0.367	0.78	−0.10 to 0.16	0.604
No	22	1.33			0.92			0.82		
PEG feeding										
Yes	4	1.19	−0.21 to 0.50	0.291	0.78	−0.05 to 0.41	0.125	0.49	−0.02 to 0.67	0.062
No	39	1.33			0.96			0.81		
Non-ambulatory										
Yes	26	1.28	0.02 to 0.17	**0.019**	0.86	0.09 to 0.34	**0.001**	0.75	−0.01 to 0.24	0.073
No	17	1.37			1.08			0.86		
Low lean mass*										
Yes	26	1.33	−0.001 το 0.18	0.052	0.92	0.01 το 0.37	**0.040**	0.80	−0.08 το 0.22	0.342
No	8	1.42			1.11			0.87		
Hypogonadism										
Yes	9	1.27	−0.05 το 0.17	0.257	0.78	0.05 to 0.37	**0.011**	0.63	0.07 to 0.36	**0.004**
No	34	1.33			0.99			0.84		

*Outlier (1 patient) was removed from these analyses.

[21,29–31]. The weak relationship may reflect the difficulty of obtaining measurements in this population, falls risk that differ between ambulatory and non-ambulatory patients, and the lack of prospective studies in this area.

One of the advantages of using TBS is that it is unaffected by height, unlike DXA-derived BMD. This point is particularly relevant in CP, with some researchers concerned that low bone mass has been over-diagnosed in this population due to inadequate correction of BMD for body size [32,33]. TBS is also an attractive tool in CP as it can be analysed retrospectively without the need for additional radiation. It provides a measure of bone microarchitecture which otherwise would be challenging to obtain in patients with neurological impairment. Groups that have used high resolution MRI or pQCT to image bone microarchitecture in CP have reported the need for custom splints, inflatable stabilising cushions, and positioning while in a wheelchair [34].

We are mindful that this study is exploratory given the limitations of using TBS in this population. In particular, there is currently no reference range for TBS in those < 50 years of age. We used TBS thresholds for fracture risk derived from a meta-analysis of 14 population cohorts including postmenopausal women and older men [12]. It is unclear whether such TBS cut-offs would reflect fracture risk in our population of patients; ideally we would like to compare our data to a similar age cohort analogous to the use of Z scores. However, it is encouraging that the proportion of patients with a low BMD as defined by Z score < −2.0 (51%) was similar to the proportion of patients with abnormal TBS (56%). Furthermore, the cut-offs correspond to TBS values for L1–L4 whereas we used TBS values for L2–L4. L1 has a lower bone density than L2–L4 thus TBS is likely to be lower in L1. This may falsely increase the values for TBS in our patients and underestimate fracture risk. However, we chose L2–L4 as L1 is often involved in the scoliosis of the spine, making BMD and TBS difficult to interpret at this site. In addition, TBS is only recommended for BMI from 15 to 35 kg/m^2. This is due to the adjustment that is made in the calculation by the TBS software which is based on BMI [35]. Increased adiposity and soft tissue thickness can reduce the TBS value in the same way it can interfere with DXA results [11], however this effect is less pronounced using GE-Lunar machines which is what was used in this study [36]. BMI in individuals with CP may also not reflect percentage body fat and it may be more accurate to adjust for soft tissue thickness.

In conclusion, > 50% of adults with cerebral palsy have reduced TBS. TBS was higher in ambulatory patients. The established notion that maintenance of muscle function is required for skeletal health is therefore also likely to be applicable in adults with CP. Further prospective studies are needed to validate the use of TBS in young adults with CP and determine whether it is associated with fracture risk.

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Disclosure statement

The authors have nothing to disclose.

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Chapter 3.4 – Longitudinal changes in bone density in adolescents and adults with cerebral palsy: A case for early intervention

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ORIGINAL ARTICLE

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Longitudinal changes in bone density in adolescents and young adults with cerebral palsy: A case for early intervention

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Abstract

Context: Cerebral palsy (CP) is a motor disorder affecting movement, muscle tone and posture due to damage to the foetal or infant brain. The subsequent lack of ambulation, nutritional deficiencies, anticonvulsant use and hormonal deficiencies have been implicated in the low bone mass associated with this condition.

Objective: To assess changes in areal bone mineral density (aBMD) during adolescence and young adulthood in individuals with CP. The effect of ambulation, nutrition, hypogonadism on longitudinal changes in aBMD is also examined.

Design: Retrospective longitudinal study.

Setting and participants: Forty-five subjects with CP who had longitudinal dual-energy X-ray absorptiometry (DXA) scans at a single tertiary hospital between 2006 and 2018.

Results: Mean age at first DXA was 19.4 years (range: 10-36 years), 57.8% were male and 80% were nonambulatory. The mean Z-scores at baseline were <−2.0 at all sites – lumbar spine (LS), femoral neck (FN), total hip (TH) and total body (TB). The median change in aBMD was +1.2%-1.9% per year in all subjects but in those <20 years of age, the median change was 4%-8% per year. Z-scores across all sites remained stable over time. Reduced functional state as measured by the gross motor functional classification scale (GMFCS) had a small negative effect on aBMD over time.

Conclusion: In adolescents with CP, low bone mass was evident from the baseline DXA. However, significant bone accrual occurred during the second decade, followed by bone maintenance in young adulthood. Future studies should focus on optimizing bone health from early childhood.

KEYWORDS

bone density, cerebral palsy, fracture, longitudinal, osteoporosis

1 | INTRODUCTION

Cerebral palsy (CP) is the most common motor disorder among children, resulting from a static insult to the developing foetal or infant brain. As CP life expectancy continues to improve,[1] there is increasing recognition that a lifespan approach is needed to maintain both health and function. As these individuals age, many become dependent on assistive devices[2] and up to 75% of individuals lose their ability to mobilize.[3] Preserving bone and muscle health to maintain mobility and independence is therefore critical.

Adults with CP are particularly vulnerable to osteoporosis and fracture due to their longer cumulative exposure to risk factors that lead to bone loss and declining bone mass with age.[4] In a large cohort of 435 adults aged 40-60 years with CP in Michigan, up to 58% of

adults with Gross Motor Function Classification Scale (GMFCS) IV-V and 40% with GMFCS I-III had documented osteopenia/osteoporosis.[5] In a small study of predominantly nonambulatory adults with CP, prevalent fractures were present in 38%, with 53% of fractures occurring in adulthood.[6] However, it is unclear whether low bone mass in adults with CP is predominantly due to less bone accrual during childhood, earlier onset of bone loss or an accelerated rate of bone loss in adulthood compared with the general population.

In recent years, small cross-sectional studies in adults with CP have demonstrated the importance of ambulation on bone mineral density (BMD) as measured by dual X-ray absorptiometry (DXA).[7-9] Other factors associated with low BMD in these studies included type of CP (dyskinetic vs spastic), low BMI, presence of hypogonadism and decreased lean mass.[6-8,10]

Small longitudinal studies of bone density in CP have inconsistent findings. In 69 children with CP aged 2-17 years (followed for 2-4 years), an increase in BMD of 2% per year at the distal femur and 5% per year at the lumbar spine was shown but distal femur Z-scores decreased with age.[11] This suggests that the rate of bone accrual in CP was less than typically developed children. In a cohort of 40 subjects with CP aged 6-26 years (followed for up to 6 years), the yearly change in distal femur BMD was 0.7%-1.0% and was positively correlated with weight.[12] However, in both studies, it was noted that the yearly changes in BMD varied widely between −31% and 42%. This is likely to be secondary to positioning difficulties in the presence of contractures and motion artefact. In a study of 15 predominantly nonambulatory adults, using lumbar spine and femoral neck as the region of interest, there were nonsignificant declines in BMD and BMD Z-scores over time.[9]

In view of the lack of studies of longitudinal bone density in adolescents and young adults with CP, the aim of this study was to assess bone accrual in the second decade of life and the changes in BMD in adulthood. We also investigated the effect of ambulation, functional state, nutrition, hypogonadism and weight on BMD longitudinally.

2 | METHODS

2.1 | Subjects

Monash Health is a tertiary hospital in Victoria, Australia that provides care across the lifespan with obstetric, newborn, paediatric and adult services. Children with CP are seen in specialist medical clinics and the Victorian Paediatric Rehabilitation Service (VPRS). As an adolescent, they are linked into the Young Adults Transition Clinic which takes referrals state-wide, and then referred on to adult services. One such service is the Metabolic Bone Clinic at Monash Health, which is staffed by both paediatric and adult endocrinologists to allow the smooth transition of care of these patients; it is uniquely placed to understand longitudinal changes in bone health this group.

A retrospective longitudinal analysis of 45 individuals with CP who had more than one DXA study from 2006 to March 2018 was performed. Individuals had DXA imaging for suspected low BMD in the setting of immobility, fracture, anticonvulsant use or hypogonadism. Routine practice in the clinic was to order repeat scans every 1-2 years; individuals did not have repeat scans only if they declined or were lost to follow-up. Subjects were included if they were 10 years of age or older during their first DXA scan in order to assess changes in BMD in the second decade. This cut-off was based on the lower end of the normal range of pubertal onset in North America and Europe, which is 8 years in girls and 9 years in boys.[13] The study was approved by the institutional ethics committee (Monash Health HREC).

2.2 | BMD measurements

Bone quantity was measured as bone mass in grams per anterior posterior projected area of measured bone in cm squared or areal BMD (aBMD), not true bone density. Sites of measurement by DXA included lumbar spine (L2-L4), proximal hip and total body (including head) on all participants unless limited by previous scoliosis surgery, femoral surgery or difficulty positioning the subject. Subjects were scanned from L1-L4 in all cases. However, when scans were reviewed individually, in subjects with scoliosis, L1 was often involved in the inflection of the curve making it difficult to reproduce reliable measurements between scans. L2-4 was therefore chosen for analysis. The majority of scans were performed by one technician, and were given 1 hour timeslots to allow for positioning difficulties and the need to acquire multiple scans if motion artefact was present. One researcher (AT) assessed all scans to determine whether there was significant motion artefact or large variations in the region of interest and excluded these sites from analysis.

All measurements were obtained using a GE Lunar Prodigy (Madison, Wisconsin) and retrospectively analysed with one software version (version 17) at a single centre. Anthropometric measures of age, weight and height were documented. In cases where true height could not be obtained, length while lying flat was used. In those aged <20 years, gender- and age-specific height Z-scores were calculated using the Centre for Disease Control (CDC) 2000 growth charts.

Low BMD was prespecified and defined as a Z-score of ≤−2.0 as per the International Society of Clinical Densitometry guidelines for adults <50 years of age.[14] The coefficient of variation (CV) for BMD on our DXA using an Hologic anthropomorphic lumbar spine phantom measured daily over the period from 2004 to 2011 was 0.51%.

2.3 | Data collection and clinical measures

Gross motor functional classification scale grades the severity of gross motor function impairments based on the ability to mobilize or need for assistive devices.[15] Levels range from I to V with individuals at level V having the greatest impairment and are wheelchair bound. Gross motor functional classification scale was obtained from the medical records, and participants divided into two groups: predominantly ambulatory (GMFCS I-III) or nonambulatory (GMFCS IV-V).

A history of gonadal dysfunction including delayed puberty was established from the medical record. Hypogonadism was defined in

males as the use of androgen replacement therapy or low testosterone levels (<8 nmol/L) documented on two separate occasions; and in females as the use of hormone replacement therapy, menopause before the age of 40 or low oestradiol levels (<73 pmol/L) on two separate occasions. Where possible, timing of puberty, age of menarche and bone age was documented. Bone age was estimated from a hand X-ray using the Greulich and Pyle (GP) method. Delayed puberty was defined as the absence of testicular enlargement in boys or breast development in girls at an age that is more than 2 standard deviations later than the population mean (age of 14 years in boys and 13 years in girls).

Current or past use of anticonvulsant medication and use of percutaneous endoscopic gastrostomy (PEG) feeding was obtained from the medical record. Minimal trauma fracture was defined as either a self-reported or radiologically proven fracture occurring after a fall from standing height or less, or a minimal trauma incident other than a fall (eg turning over in bed). Bisphosphonate use was defined as any exposure to bisphosphonate prior to first DXA or during follow-up. Vitamin D levels that were done within 6 months of DXA were collected.

2.4 | Statistical analysis

The distribution of the data was explored by the Shapiro-Wilk test. All normally distributed data were expressed as mean with standard deviation and nonparametric data as median with interquartile ranges. Univariate analysis was used to determine the relationship between variables and baseline aBMD. Mixed model analyses were used to determine the significance of changes in aBMD and Z-score over time, with time defined as the number of years since baseline DXA. All models were analysed as random intercept with time as a fixed variable. The effect of variables on longitudinal changes in aBMD was calculated and reported as parameter estimates. Subjects were divided into <20 years old and ≥20 years old to assess for differences in changes in BMD and Z-score, as the rapid increases in BMD seen in puberty should have largely occurred by age 20.[16] Subjects who had measurements that fell into both categories were included in one age category only and the other measurements were excluded. Independent samples *t* test was used to compare baseline aBMD and Z-scores between those aged <20 years and those ≥20 years, and chi-square used for categorical variables. Mann-Whitney *U* test was used to compare annualized median change in aBMD and Z-score between those aged <20 years and those ≥20 years. A two-sided *P*-value of 0.05 was chosen to indicate statistical significance. Analyses were conducted using IBM SPSS Statistics for Windows (Version 24, Armonk, NY).

3 | RESULTS

Forty-five patients with cerebral palsy who had more than one DXA scan were included in the study. 30 patients (66.7%) had more than two scans for analysis, whilst 16 patients (35.6%) had five or more scans for analysis. 50% of patients had at least 4 years of follow-up, with the mean interval between scans 1.6 ± 1.1 years. Baseline characteristics of the patients and baseline DXA-derived aBMD and Z-scores are shown in Table 1. Mean age (range) at first DXA was 19.4 years (10 - 36), 12 patients (26.7%) were less than 20 years old at their first DXA scan. For subjects aged <20 years at their first DXA, the mean age of initial DXA was 13.9 ± 3.1 years. 57.8% were male and 80% were nonambulatory. Anticonvulsant use was common (n = 27, 60%), most (n = 20) were on multiple agents with the most common agents being sodium valproate, lamotrigine and carbamazepine. Apart from three patients, there was a long duration of anticonvulsant use of >10 years as seizures presented shortly after birth or developed in childhood. Mean BMI of subjects was 18.3 ± 4.5 kg/m^2 and 31.1% required PEG feeding. Minimal trauma fracture was documented in 42.2% of subjects, the majority of these (76.2%) involved the femur or tibia/fibula.

Fifteen subjects (33.3%) were hypogonadal; 9 were female. All female patients had a history of delayed puberty; mean age of menarche for these patients was 18 ± 3 years. Of the 6 male patients with hypogonadism, 2 presented with delayed puberty and the remainder developed hypogonadism as an adult. Overall, 9 of the 15 patients received hormone induction/hormone replacement therapy.

Eleven subjects (24.4%) received bisphosphonates, one patient received bisphosphonate for avascular necrosis while the remainder received bisphosphonates for osteoporosis and fracture. All patients received zoledronic acid, one patient initially received pamidronate followed by zoledronic acid. Doses of zoledronic acid were 4-5 mg intravenously if given to adult patients, and doses in children were weight-adjusted (0.05 mg/kg). The number of doses and dosing interval varied upon the clinical situation; adults were given between 1-4 doses of zoledronic acid at 12 monthly to 24-monthly intervals whereas children were given a total of 2-6 doses of intravenous zoledronic acid at 6 monthly intervals. Vitamin D3 was supplemented to aim for serum levels >50 nmol/L and often involved the use of high dose intermittent dosing (typically 50 000 IU/dose) for convenience.

At first baseline scan, mean Z-scores at the lumbar spine (LS), femoral neck (FN), total hip (TH) and total body (TB) were all ≤−2.0. Subjects who were aged <20 years at baseline scan had a trend to a lower FN and TH Z-score than those aged ≥20 years at baseline scan, but this did not reach statistical significance (Table 1). Bone age X-ray was performed in 10 of the 12 subjects aged <20 years, with a mean delay in bone age of 1.1 ± 1.6 years. Mean age- and gender-specific height Z-score in those aged <20 years was −1.7 ± 1.4. There was no significant change in height Z-score per year (0.15 per year, 95% CI −0.02 to 0.31, $P = 0.084$). In those aged ≥20 years, mean height was for females was 144.5 ± 5.5 cm and for males was 161.0 ± 13.5 cm.

Total body bone mineral content (TBBMC), LS aBMD, and FN aBMD, TH aBMD was plotted against the age of the patient to gain an approximation of when peak bone mass was achieved (see Figure 1). Linear, cubic and quadratic line of best fit was calculated and the line with the greatest R^2 was chosen ($R^2 = 0.330$, $P = 0.02$). Peak bone mass appeared to be achieved late in the third decade of life/early in the fourth decade and plateaued thereafter. FN aBMD appeared to peak earlier than LS aBMD (Figure 1B,D).

TABLE 1 Baseline characteristics (n = 45)

	All	<20 y (n = 12)	≥20 y (n = 33)	*P* value[#]
Age (y)	19.4 ± 5.2	13.9 ± 3.1	22.1 ± 3.8	<0.001
Male (%)	57.8	41.7%	63.6%	0.306
Body mass index (kg/m^2)	18.3 ± 4.5	15.8 ± 3.1	19.2 ± 4.6	0.019
Anticonvulsant use	60%	78.6%	51.5%	0.084
Ambulatory	20%	21.4%	18.2%	0.796
GMFCS				0.272
I-II	6.7%	16.6%	3%	
III	11.1%	0%	15.2%	
IV	17.8%	16.7%	18.2%	
V	64.4%	66.7%	63.6%	
Fracture	42.2%	50.0%	42.4%	0.633
Bisphosphonate exposure	24.4%	28.6%	27.3%	0.927
Hypogonadal	33.3%	50.0%	27.3%	0.133
PEG feeding	31.1%	35.7%	27.3%	0.563
Serum calcium (mmol/L)	2.38 ± 0.12	2.36 ± 0.14	2.38 ± 0.12	0.728
Serum phosphate (mmol/L)	1.26 ± 0.21	1.34 ± 0.26	1.23 ± 0.19	0.214
PTH (pmol/L)	3.89 ± 2.41	4.60 ± 3.25	3.81 ± 2.41	0.673
ALP (U/L)	110.0 ± 49.0	151.5 ± 77.6	97.6 ± 30.9	0.016
25-OH Vitamin D (nmol/L)	76.8 ± 29.1	88.7 ± 35.0	73.7 ± 27.3	0.230
Lumbar spine aBMD (g/cm^2)	0.820 ± 0.229	0.659 ± 0.203 (n = 9)	0.908 ± 0.201 (n = 20)	0.004
Lumbar spine Z-score	−2.0 ± 1.4	−3.1 ± 1.3	−2.2 ± 1.6	0.150
Femoral neck aBMD (g/cm^2)	0.681 ± 0.215	0.560 ± 0.210 (n = 7)	0.711 ± 0.159 (n = 20)	0.060
Femoral neck Z-score	−2.5 ± 1.6	−3.4 ± 1.7	−2.2 ± 1.2	0.059
Total hip aBMD (g/cm^2)	0.662 ± 0.200	0.550 ± 0.200	0.697 ± 0.142	0.046
Total hip Z-score	−2.7 ± 1.5	−3.6 ± 1.6	−2.4 ± 1.1	0.052
Total body aBMD (g/cm^2)	0.872 ± 0.183	0.769 ± 0.130 (n = 12)	0.921 ± 0.127 (n = 30)	<0.001
Total body Z-score	−2.0 ± 1.5	−2.5 ± 1.1	−1.8 ± 1.4	0.162
Total body BMC (g)	1518.8 ± 575.2	1035.8 ± 371.1	1718.6 ± 526.4	<0.001

Note: Data are presented as mean ± standard deviation or percentages.
Abbreviation: GMFCS = gross motor function classification scale.
[#]*P* value for differences between <20 y and ≥20 y were calculated using independent sample *t* test for continuous variables and chi-square for categorical variables.

Total body bone mineral content, aBMD and Z-score at the LS, total hip (TH), and total body (TB) increased slightly in the overall analysis and was statistically significant (Table 2). There were wide variations in changes in aBMD ranging between losses of −22% and gains of +38% per year. Subjects were then divided into those aged <20 years and ≥20 years. At baseline, the only difference in clinical variables between the two age groups was BMI (<20 years mean BMI 15.8 ± 3.1 kg/m^2 vs ≥20 years 19.2 ± 4.6 kg/m^2, $P = 0.019$; see Table 1). There was a significant difference in the change in TBBMC and aBMD per year between the two groups, with those aged <20 years having a 4%-8% increase in aBMD per year compared with minimal changes in aBMD in those aged ≥20 years (Table 3). Bisphosphonate exposure was similar in both groups, 28.6% in those <20 years vs 27.3% in those ≥20 years, $P = 0.927$ and this did not have a significant impact on changes in Z-scores over time in either age group.

The effect of sex, ambulatory status and functional status, hypogonadism, bisphosphonate use and weight on change in aBMD and TBBMC over time was assessed using mixed model analysis (Table 4). Reduced functional status as measured by GMFCS had a negative effect on aBMD of the order of −0.01 g/cm^2/y at the LS, TH and TB ($P < 0.01$). There was a statistically significant effect of vitamin D and weight on change in aBMD at all sites, although the magnitude of change was clinically unimportant being 0.001-0.0001 g/cm^2/y. Females had a greater increase in aBMD at most sites compared with males, by 0.007-0.011 g/cm^2/y ($P < 0.05$). The other assessed variables had an inconsistent or nonsignificant effect on changes in aBMD and TBBMC over time. A subgroup analysis was performed in subjects aged <20 years and ≥20 years of age which did not demonstrate a clinically significant association between any variables with aBMD or TBBMC.

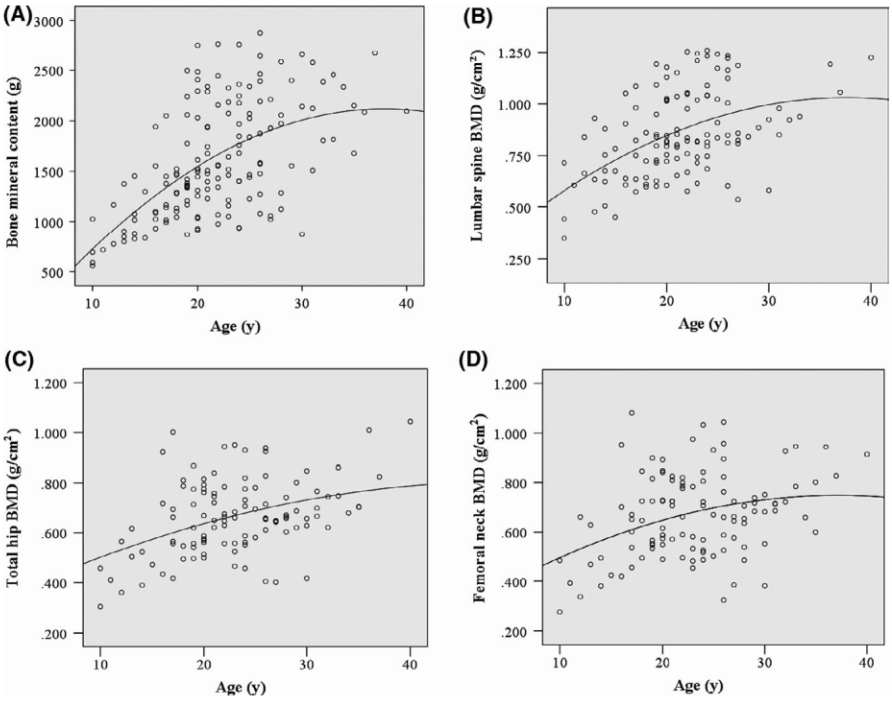

FIGURE 1 Estimation of peak bone mass. Panel A, bone mineral content plotted over age, B, lumbar spine aBMD plotted over age, C, total hip aBMD plotted over age, D, femoral neck aBMD plotted over age. Quadratic line of best fit shown

TABLE 2 Change in aBMD, BMC and Z-score per year of follow-up

DXA measurement	N	Change/y	95% CI	*P* value
Lumbar spine aBMD	29	0.012 g/cm^2	0.008, 0.017	**<0.001**
Lumbar spine Z-score	29	0.060	0.020, 0.101	**0.004**
Femoral neck aBMD	27	0.001 g/cm^2	−0.005, 0.006	0.830
Femoral neck Z-score	27	0.012	−0.032, 0.055	0.604
Total hip aBMD	27	0.004 g/cm^2	−0.002, 0.009	0.058
Total hip Z-score	27	0.045	0.008, 0.086	**0.020**
Total body aBMD	42	0.010 g/cm^2	0.006, 0.013	**<0.001**
Total body Z-score	42	0.060	0.015, 0.106	**0.009**
Total body BMC	42	22.4 g	14.2, 30.7	**<0.001**

Note: Bold values indicate $P < 0.05$.

TABLE 3 Change in areal BMD/BMC according to age (median % change/y)

	<20 y (n = 12)	>20 y (n = 33)	*P* value
Skeletal site	Median % change/y		
LSaBMD	8.5 (1.9, 13.7)	0.9 (−1.2, 3.1)	**0.001**
FNaBMD	8.5 (−4.4, 17.9)	-0.5 (−5.2, 3.3)	**0.026**
THaBMD	3.9 (−3.3, 14.1)	0.0 (−3.2, 6.3)	0.256
TBaBMD	4.3 (1.3, 8.1)	0.1 (−2.2, 2.3)	**<0.001**
TBBMC	7.8 (3.2, 14.2)	0.3 (−3.1, 2.7)	**<0.001**

Note: Data are expressed as percentages (25th, 75th percentiles). Comparisons using Mann-Whitney *U*.
Bold values indicate $P < 0.05$.

4 | DISCUSSION

In our study, adolescents with CP gain aBMD during puberty with a median increase of 4%-8% per year, followed by a period of consolidation in young adulthood. This finding is in keeping with longitudinal data from typically developing children in the second decade of life and is reflected in the stability of Z-scores over time. Our observed change in aBMD/y is comparable to that seen by Henderson et al,[11] who found a 5.0%-6.4% mean change in aBMD/y in their 12-19-year-old group. A key strength of our study is that we had repeated measurements in the same subject. aBMD is challenging to obtain in this population due to contractures, scoliosis and movement artefact. Technically, this is reflected in the wide variation in aBMD between two scans (ranging from −22 to +38%) in our study, as was also observed in other longitudinal studies. As a result of these variations, repeated measurements allowed the recognition of these inconstancies in aBMD over time. In our clinical experience, given these disparities, we do not base treatment decisions in this cohort on the change in aBMD between two scans but rely on the overall trend.

By choosing the lumbar spine and proximal hip as sites of measurement, we could calculate Z-scores for this cohort. Of note, the mean baseline Z-scores at all sites measured were <−2.0, suggesting that the deficit in bone mass had occurred in the first decade of life. In those <20 years, even after taking into consideration mean height Z-scores of

TABLE 4 Effect of variables on bone density per year of follow-up

Predictor	ΔLSaBMD/y	ΔFNaBMD/y	ΔTHaBMD/y	ΔTBaBMD/y	ΔTBBMC/y
Female	0.011 **P = 0.047**	0.007 P = 0.206	0.011 **P = 0.030**	0.009 **P = 0.014**	14.4 P = 0.101
Nonambulatory	−0.022 **P < 0.001**	−0.006 P = 0.628	−0.011 P = 0.316	−0.012 P = 0.082	−36.6 **P = 0.037**
GMFCS	−0.009 **P < 0.001**	−0.006 P = 0.172	−0.011 **P = 0.007**	−0.009 **P < 0.001**	−18.9 **P = 0.003**
Hypogonadal	0.003 P = 0.512	0.002 P = 0.709	0.003 P = 0.586	0.009 **P = 0.007**	7.8 P = 0.396
Anticonvulsant use	−0.016 **P < 0.001**	0.003 P = 0.487	−0.003 P = 0.598	−0.006 P = 0.072	12.6 P = 0.131
Bisphosphonate use	0.001 P = 0.836	0.005 P = 0.394	0.001 P = 0.805	−0.004 P = 0.354	0.19 P = 0.985
Fracture	0.021 **P < 0.001**	0.005 P = 0.380	0.004 P = 0.440	0.001 P = 0.988	14.1 P = 0.169
Weight	−0.0001 **P = <0.001**	−0.001 **P = <0.001**	−0.001 **P < 0.001**	−0.0001 **P = 0.014**	−1.5 **P < 0.001**
Vitamin D	−0.001 **P < 0.001**	0.001 **P < 0.001**	0.001 **P < 0.001**	−0.001 **P < 0.001**	−0.6 **P = 0.001**

Abbreviations: ΔLSaBMD/y, change in lumbar spine areal BMD/y; FN, femoral neck; GMFCS, gross motor function classification scale; TB, total body; TH, total hip.
Bold values indicate $P < 0.05$.

−1.7 ± 1.4, mean Z-scores of −3.6 at the FN and −3.7 at the LS are concerning. Height Z-score did not change significantly over time, suggesting that growth failure occurs earlier in childhood. This is consistent with cerebral palsy-specific growth charts which show linear growth failure present from age 2, with only slight worsening over time.[17]

It is reassuring that aBMD Z-scores are stable over time, suggesting bone accrual and maintenance occur in parallel to typically developing adolescents albeit at a lower set-point. This result requires validation as it is in contrast to the findings of Henderson et al[11] which showed that Z-scores declined at the distal femur over time but increased at the lumbar spine. Possible explanations may be that their cohort included younger children from the age of 2 years, as well as difference in the DXA sites measured. The lateral distal femur may be preferred over the proximal hip in childhood CP and other neurodisabilities,[18] with improved positioning in the setting of metallic hip implants and contractures, and validation against fracture outcomes.[19] However, reference data are only available for Hologic scanners, and there is no adult normative data to allow for calculation of Z-scores. Therefore, measurements of the proximal femur should be considered in adolescence to allow monitoring through to adulthood.

We postulate given the stability of Z-scores in our cohort, deficits in bone accrual earlier in life rather than bone loss in adolescence or early adulthood is the predominant cause of low bone mass in adults with CP. Treatments to optimize bone health in CP need to be tailored to this new understanding. To determine the optimal timing of intervention, we estimated peak bone mass in CP by plotting BMC and aBMD as a function of patient age. Peak bone mass in this cohort of individuals with CP occurred in the late third/early fourth decade of life, with aBMD at the femoral neck peaking earlier than the lumbar spine. The Canadian CaMOS study revealed similar findings although at an earlier age, with aBMD at the femoral neck peaking at ages 16-19 in females and 19-21 in males, whereas lumbar spine aBMD peaked at ages 33-40 in females and 19-33 in males.[20] This difference may be due to the high rates of delayed puberty and subsequent delayed bone accrual in our cohort. Early intervention in childhood to improve nutrition and mobility or use of pharmacological therapy may be crucial in the prevention of low bone mass and fractures in adulthood. Timely pubertal induction is likely to be important to optimize bone health into adulthood. Bisphosphonates produce much larger changes in aBMD and BMC in children compared with adults; consideration as to whether to administer bisphosphonates earlier in CP is an important clinical question. Current guidelines suggest that bisphosphonates be given in children with CP in the presence of either a vertebral fracture, or Z-score ≤−2.0 and 2 or more long bone fractures.[21]

Reduced functional status as measured by GMFCS had a small negative effect on change in aBMD over time in our cohort of −0.01 g/cm^2/y. It was surprising that functional status as measured by GMFCS or ambulation status did not have a greater effect as cross-sectional studies in both children and adults with CP have shown a consistent positive effect of ambulation on aBMD. By excluding subjects <10 years of age, we may not have captured the impact of ambulation on bone accrual given the onset of motor deficits in CP at birth. Unfortunately, we had insufficient numbers to assess how the loss of ambulation affects aBMD. Only one subject in our cohort went from walking daily with a frame to being a wheelchair user; other patients who had a change in mobility status went from using a standing frame to nonweight bearing.

We were unable to find any other variables that had a significant effect on changes in BMD over time in mixed model analyses.

Bisphosphonate use did not appear to affect aBMD; this is surprising but may be partly explained by the variability in the age of the patient when the medication was given, the type of bisphosphonate administered, the dose administered and the duration of treatment. Furthermore, the variations in aBMD year to year of a given subject may mask any effect of bisphosphonate on aBMD. Treatment of hypogonadism was inconsistent, and so the real effect of sex-steroid deficiency on bone accrual could not be ascertained. Baseline BMI for our cohort was 18.3 ± 4.5 kg/m^2 with those <20 years having a mean BMI of only 15.8 ± 3.1 kg/m^2. This is likely to be an important risk factor for low bone mineral accrual due to the consequences of reduced load bearing, nutritional deficiencies and effects on gonadal function. This was not borne out by our longitudinal findings, with no correlation between weight and BMD in either the <20 or >20-year-old groups.

The main limitation of our study is the small number of patients and the lack of control group for our study cohort. Our study subjects already had risk factors for low BMD and does not represent the CP population as a whole. 72% of our cohort had a GMFCS level of IV or V whereas 34% of a birth cohort of Victorian CP cases obtained through a statewide register between 1990 and 1992 had a GMFCS of IV or V.[22] There were insufficient measurements in adults >30 years to determine when adults with CP begin to lose bone. The heterogeneity in duration of follow-up may also affect our estimation of peak bone mass. The wide variations in changes in aBMD per year reduce the certainty of our observations and may explain the lack of variables associated with longitudinal changes in aBMD in our cohort. This reflects the problems of DXA scanning in CP. However, no viable, readily available alternative quantitative technique to assess BMD exists at present. We are reassured that our findings are similar to the previous work in this field. The Australian BMD reference range for the Lunar Prodigy was derived from different cohorts for those <20 years to those ≥20 years which may affect comparisons between the two groups.

An inherent limitation of using DXA is that it does not measure true volumetric bone density (vBMD). aBMD will increase during growth predominantly due to increases in size rather than true density. For example, as the size of the vertebral body increases during growth, the amount of bone within it (BMC) also increases so vBMD remains the same although aBMD increases.[23] DXA BMC may therefore be superior to DXA aBMD for assessing bone mass accrual in children.[24] Volumetric bone density measurements would be advantageous but these can be difficult to obtain in this population, as they are particularly susceptible to movement artefact. We have therefore also reported Z-scores and BMC values to try and address these issues of size and growth.

In conclusion, deficits in bone mass in CP are seen before puberty. Individuals with CP still have significant bone accrual during pubertal years with a median increase of 4%-8% in aBMD per year. The low bone mass seen in adults with CP is likely a reflection of poor bone accrual in early childhood rather than being due to rapid bone loss. Interventions during childhood such as improving muscle strength and mobility, optimizing nutritional and hormonal status, and judicious use of bisphosphonates are likely key to achieving optimal adult bone health.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

DATA ACCESSIBILITY

The data that support the findings of this study are available from the corresponding author, AT, upon reasonable request.

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Hypogonadism and delayed puberty in adolescents and young adults with cerebral palsy

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Abstract

Objective: To describe our experience of delayed puberty and hypogonadism in cerebral palsy (CP), with specific reference to aetiology, treatment, adverse effects of treatment and bone phenotype.

Design: Retrospective cohort study of CP patients attending two tertiary paediatric hospitals.

Patients and Measurements: 27 individuals with CP and a history of delayed puberty or hypogonadism were identified. Clinical, biochemical and radiological data were collected from the medical records. Individuals who received sex-steroid treatment were invited to participate in a telephone interview exploring the issues of bone and sexual health, medication compliance, tolerance of medication effects and its influence on quality of life.

Results: The median age of assessment of gonadal status was 18 years (interquartile range 16.5 - 20.5) and 17 (63%) were female. The mean BMI was 17.4kg/m^2 (IQR 13.7 - 23.4). Most patients were non-ambulatory (81.5%) with a gross motor function classification scale (GMFCS) of IV or V. Delayed puberty was documented in 23 patients (85.2%); mean age of menarche in 15 females was 17.3±2.1 years. Sex-steroid replacement was given to 16 patients (69.6%) with varying routes of administration and doses. Treatment was generally well tolerated; however one episode of ovarian hyperstimulation was recorded. Despite sex-steroid replacement, changes in bone mineral density between subjects were inconsistent.

Conclusions: Delayed puberty and hypogonadism is not uncommon in severely affected individuals with CP, and may be diagnosed late. Further work is required to determine the appropriate sex-steroid replacement dose and route of administration for optimal bone and general health in this population.

Cerebral palsy is the most common disorder of movement seen in children, affecting 2-3.5/1000 live births (1). With improved life expectancy, there has been an increased focus on musculoskeletal morbidity in adults with CP. The prevalence of osteoporosis in a large North American cohort of adults with CP was 10% in those aged 31-40 years, increasing to 25% in those over 50 years of age (2). Fracture prevalence was found to be 38% in a cohort of 45 young adults with CP, with over half of fractures occurring in adulthood (3). Low bone mass leading to fractures can impair mobility and quality of life.

The abnormal bone phenotype in cerebral palsy commences in childhood, with less accrual of bone due to reduced biomechanical forces exerted on bone. Compounding this issue is anticonvulsant use, inadequate nutrition, vitamin D deficiency and growth hormone deficiency (4-6). One factor requiring further exploration is the role of sex-steroids, with altered pubertal progression and hypogonadism being described in CP. Central precocious puberty (CPP) in CP is thought to be secondary to the insult to the central nervous system as seen in other neurodevelopmental disabilities (7). CPP may progress more rapidly in CP than in typically developing children, and has been associated with a poorer response to gonadotropin-releasing hormone agonist therapy (8). Delayed progression through puberty was described by Worley *et al.,* with girls with CP having late menarche despite entering puberty earlier (9). The aetiology of delayed puberty or hypogonadism in CP is varied; there has been a case series of hypopituitarism in four children with CP with multiple hormone deficiencies (10), and both hyper- and hypogonadotrophic hypogonadism identified in young adults with CP (3).

There is some evidence that these alterations in the hypothalamic-pituitary-gonadal axis function may impact bone health. Our previous study in adults with CP showed an association between hypogonadism and lower lumbar spine bone mineral density (BMD) and reduced

muscle mass (3). Studies assessing bone age in CP are conflicting, showing both advanced (11) and delayed skeletal age which is influenced by the degree of functional impairment and fat stores (12-14). Importantly, no studies to date examine the tolerability of pubertal induction and/or sex steroid replacement in this population specifically and its effects on bone health. Much of the literature in females with chronic neurological disability including CP focus on contraception or the suppression of menses, and in both sexes concerns regarding mood, aggression, or altered sexual behaviour may discourage treatment (15).

In this study, we aimed to describe our experience of delayed puberty/hypogonadism in CP. We describe the aetiology of delayed puberty/hypogonadism in our cohort, the treatments and their tolerability, the effects on bone density and side effects.

Methods

Participants

A retrospective cross-sectional cohort study of 27 adolescents and young adults with CP who had pubertal delay or hypogonadism was analysed. Participants were identified from existing hospital databases until December 2018. Both institutional ethics committees approved the study (Monash Health HREC, Royal Children's Hospital HREC).

Data collection and clinical measures.

Delayed puberty was defined as the absence of testicular enlargement in boys or breast development in girls at an age that is more than 2 standard deviations later than the population mean (age of 14 years in boys and 13 years in girls). Hypogonadism was defined in males as the use of sex-steroid therapy or low testosterone levels (<8nmol/L) documented on two separate occasions, and in females as the use of sex-steroid therapy for induction of pubertal development, menopause before the age of 40 (hypergonadotrophic), or low oestradiol concentrations (<73pmol/L) on two separate occasions.

The following variables were collected from the medical record at the time of diagnosis if available: FSH, LH, oestradiol/testosterone, Tanner stage, bone age and dual energy x-ray absorptiometry (DXA). Age of commencement of sex-steroid therapy, the medication used, dosage and side effects was collected if treatment was undertaken. For females, the age of menarche was collected. Bone age was estimated by a radiologist from a hand x-ray using the Greulich and Pyle (GP) method.

Gross Motor Function Classification Scale (GMFCS) grades the severity of gross motor function impairments based on the ability to mobilise or need for assistive devices (16).

GMFCS was obtained from the medical records, and participants divided into two groups: predominantly ambulatory (GMFCS I-III) or non-ambulatory (GMFCS IV-V).

Current or past use of anticonvulsant medication and use of percutaneous endoscopic gastrostomy (PEG) feeding was obtained from the medical record. Minimal trauma fracture was defined either as a self-reported or radiologically proven fracture occurring after a fall from standing height or less, or a minimal trauma incident other than a fall (e.g. turning over in bed).

Bone density

DXA scans were performed on a GE Lunar Prodigy (Madison, Wisconsin, software version 17) or Hologic QDR 4500 or Horizon DXA scanner (Hologic Inc., Bedford, MA).

Interview

Participants and/or their families identified from existing databases were sent a letter of invitation to participate in a telephone interview. Participants posted a signed consent form and consent was also confirmed verbally at the time of the interview. The same study team member (AT) interviewed all participants. All interviews were audio recorded and transcribed into a dataset. Interview questions mainly comprised of bone and sexual health, medication compliance, tolerability and quality of life (See Supplementary Information).

Statistics

The distribution of the data was explored by the Shapiro-Wilk test. All normally distributed data were expressed as mean with standard deviation and non-parametric data as median with interquartile ranges. Differences between groups were determined using the Mann-Whitney U test for continuous variables with non-parametric distribution.

Results

Baseline characteristics

Twenty seven patients were found to have delayed puberty or hypogonadism. Clinical characteristics of the patients are shown in Table 1. Median age of assessment of gonadal status was 18 years (IQR 16.5 – 20.5). Nine patients (33.3%) were assessed due to concerns regarding gonadal status, the remainder were assessed due to concerns regarding bone health. The majority of patients were non-ambulatory (81.5%) with a GMFCS of IV or V.

Seventeen (63%) were female. The mean age of menarche (documented in 15 patients) was 17.3 ± 2.1 years. Two patients had not yet had menarche at the ages of 18 and 20. Eleven patients had a hand x-ray performed to estimate bone age, with the mean delay in bone age being 4.1 ± 2.2 years. Eleven (40%) of patients had a prevalent fracture, with seven patients experiencing multiple fractures. All fractures occurred with minimal trauma. The fractures predominantly involved the lower limb (55%), with the remainder involving the upper arm (n=3), clavicle (n=3) and spine (n=2).

Cause of delayed puberty or hypogonadism

All 17 female patients had delayed puberty; two had unexplained primary ovarian insufficiency and the remainder were hypogonadotrophic on biochemical assessment. Of the ten males, six had delayed puberty and four had secondary hypogonadism. Two cases of male hypogonadism were hypergonadotrophic.

Hypogonadotrophic hypogonadal (HH) patients had screening for other pituitary hormone abnormalities. Two male patients had panhypopituitarism requiring thyroxine and cortisone replacement with no pituitary abnormality identified on magnetic resonance imaging (MRI). Two other patients had elevated prolactin concentrations between 2-3 times the upper limit of

normal; one had an MRI which did not demonstrate pituitary pathology, the other patient was on domperidone to increase gastrointestinal motility. The remaining patients had not been imaged to exclude structural hypothalamic or pituitary causes of hypogonadism as their presumptive diagnosis was of functional HH deduced from their low body weight. HH patients had a lower median BMI of 16.9kg/m^2 (IQR 13.2-21.9) compared with hypergonadotrophic patients 24.0kg/m^2 (IQR 13.2-24.7), although, given the small numbers, the difference was not statistically significantly different.

Treatment

Seventeen patients (63%) received treatment (Table 2), 16 with sex-steroid replacement therapy and one male patient received treatment with cabergoline for an elevated prolactin. Some patients trialled multiple treatment regimens.

Of the females who underwent pubertal induction, three received oral oestradiol valerate which was commenced at 1mg alternate days with gradual up-titration to 2mg daily. Progesterone was subsequently added two years after oestrogen initiation in the form of oral medroxyprogesterone acetate. One patient passed away on treatment due to unrelated causes, another ceased therapy due to side effects and one changed to the combined oral contraceptive pill (COCP) after induction. Four received oestradiol in the form of a transdermal patch, three commenced at a dose of 12.5mcg twice/week which was then uptitrated to 25mcg twice/week, the remaining patient commenced at a dose of 25mcg twice/week. Progesterone was also added later in the form of oral medroxyprogesterone acetate. Two patients continue on this treatment, one is deceased, and another patient ceased treatment as she developed spontaneous menses after weight gain. In all patients requiring progesterone, the dose necessary for regulation of menses was no higher than 5mg of oral medroxyprogesterone acetate daily.

Three female patients received sex-steroids after puberty for oligomenorrhoea with low bone density. Two patients were prescribed the COCP and another prescribed a combined continuous transdermal patch which later changed to transdermal oestradiol and oral progesterone. One patient on the COCP ceased this due to side effects.

Male patients treated for delayed puberty received oral testosterone undecanoate at a dose of 40mg daily which was gradually up-titrated. The final dose varied between 80mg and 240mg daily. One patient was changed from oral testosterone undecanoate to mixed testosterone esters (Sustanon™), testosterone implants and then back to oral testosterone due to side effects. Of the three male patients who received hormone therapy for secondary hypogonadism, two received 1% transdermal testosterone gel (50mg/5g daily) and one patient used a transdermal testosterone patch (5mg/24hours). All male patients were documented to still be on treatment.

Side effects

Three of eleven female patients experienced issues with menses (breakthrough bleeding, menorrhagia or difficulty managing menses). These patients received either the COCP or the combined transdermal patch.

One female patient (aged 20) receiving oestradiol for pubertal induction had the unexpected occurrence of spontaneous ovarian hyperstimulation syndrome (OHSS) on 2mg oestradiol valerate. In addition to a history of severe CP (GMFCS level V) and low BMI (16.2kg/m^2), she had a family history of Kallmann syndrome (father) and was conceived through in-vitro fertilisation. The patient's sense of smell was unable to be reliably assessed. At baseline, her oestradiol level was undetectable with a follicle stimulating hormone (FSH) of 0.5IU/L and lutenising hormone (LH) of 0.3IU/L. Before presentation, she had been slowly up-titrated from a dose of 0.5mg oestradiol valerate over 18 months. She presented with a four-day history of

severe abdominal pain and PV spotting. On examination, she was normotensive with a heart rate of 108 and temperature of 37.4° C. C-reactive protein was elevated at 367mg/L and WCC was 24.6×10^9/L. Computed tomography (CT) of the abdomen and abdominal ultrasound demonstrated grossly enlarged ovaries with cystic changes (Figure 1). This imaging finding contrasted to an ultrasound done before treatment in which the uterus was small and ovaries could not be identified. A diagnostic laparoscopy identified bilaterally enlarged ovaries with pelvic pus and adhesions. Following surgical washout, she was treated with intravenous antibiotics and required a further CT guided drainage of an intra-abdominal collection (Figure 1B). Oestradiol valerate was ceased. She continues to be amenorrhoeic with undetectable oestradiol levels. Her mother has refused further treatment with sex-steroids for her, and her bone health is being monitored.

Two male patients had side effects documented in the medical records or on questioning the carer through interviews. One patient who had changed from oral testosterone to Sustanon™ and testosterone implants found the parental administration route to be highly distressing so was changed back to oral testosterone. One patient developed worsening acne and irritability when the dose of oral testosterone undecoate was increased from 160mg to 240mg daily.

Interview

Of the 17 patients who received treatment, seven families consented to an interview (five female, two male). All the interviewees were mothers of the patients. Five patients who had received treatment were deceased at the time of the study. A worsening mood was described by two carers of a male and female patient, although they felt it was not severe enough to require cessation of treatment. Possible improvement in cognition was described by the carer of only one female patient. None of the carers of the five female patients who were interviewed

noted any difficulty with managing menses after treatment was commenced. The carers of the two male patients denied any issues with aggression with testosterone replacement.

Bone density

Seven patients had longitudinal bone density data for evaluation. Results are shown in Table 3. Only two patients (Patient 4 and 7) had significant increments in bone density on treatment, these patients both received treatment for pubertal induction.

Discussion

Puberty is an important time for accrual of bone, with bone mass almost doubling during this time (17). The amount of bone gained during puberty determines peak bone mass (PBM) and PBM is a significant determinant of risk of osteoporosis and fracture later in life (18). Delayed puberty is associated with lower BMD and increased fracture risk (19). Hypogonadism in adults also results in low BMD, with sex-steroid replacement ameliorating these effects (20-22). The importance of sex-steroids for bone accrual and maintenance in the general population is likely to also apply to those with CP, although this has not formally been demonstrated.

Delayed puberty or hypogonadism in CP may be under-recognised or poorly reported by carers and health professionals who may not raise concerns regarding psychosexual function in disability. In our cohort, most patients were diagnosed after a referral to endocrinologists because of concerns regarding bone health. This represents an opportunity for screening for sex steroid deficiency and provides a rationale for treatment. Tanner staging should be part of routine paediatric assessment in CP and in females, the lack of menses can alert carers and clinicians to delayed puberty or hypogonadism.

Hypogonadal patients in our cohort mostly had a GMFCS level of IV or V and were PEG fed, which also predisposes them to poor bone health. Furthermore, the predominance of HH and the tendency to low BMI in these individuals suggest that the aetiology may be related to poor nutritional status. In a multivariable analysis, Henderson *et al.* also found low body fat independently predicted delayed skeletal age in children with CP (14). Optimising nutrition therefore may impact positively on bone health in CP in many ways: maintenance of muscle mass, adequate calcium and vitamin D intake, and promoting a eugonadal state.

The doses of sex-steroid used are generally lower than standard sex-steroid replacement therapy. These doses were chosen to take into account the weight of these patients and to ensure tolerability. Future studies are required to determine whether these doses are sufficient for bone accrual and maintenance. This may explain the inconsistent BMD changes in our cohort. Furthermore, the formulation of sex-steroid may be important, with previous studies in premature ovarian insufficiency and anorexia nervosa showing oestradiol increased BMD more than ethinyloestradiol in the combined COCP (10,12,13). In males, the transdermal and oral route were most commonly chosen to avoid the distress of parental administration, as well as avoid peaks and troughs of testosterone levels which has the potential to alter mood or behaviour.

Overall sex-steroid replacement was well tolerated in our series of patients with some important caveats. Menstrual issues were a concern for some female patients necessitating cessation or switching of treatments. No patients received a progesterone containing intrauterine device or depot progestogen combined with oral/transdermal oestrogen, which are suggested options for menstrual management (23). Adverse changes to mood or behaviour with hormone replacement were uncommon and mild and should not be a reason to withhold treatment.

Of concern, one patient developed unexpected OHSS on 2mg oestradiol daily. This side effect is unusual in women who are neither pregnant nor undergoing ovulation induction. However, it has been reported to occur spontaneously in the setting of an FSH-producing pituitary adenoma, mutations of the FSH receptor, polycystic ovarian syndrome and hypothyroidism (8). Of note, our patient had a family history of Kallmann syndrome; there are no known associations of spontaneous OHSS with the condition, and mechanistically this is difficult to

reconcile. In addition, before treatment with oestradiol, our patient had normal thyroid function, low FSH, and her ovaries were not seen on ultrasound.

There are some limitations to our study. We were unable to obtain MRI imaging in all patients with HH to exclude pituitary pathology. However, we did have biochemical assessment of the other pituitary axes. Five families did not consent or could not be contacted for an interview to confirm the documented medical history. Our bone density data in this cohort is limited and cannot adequately assess the efficacy of sex-steroid replacement on improving or maintaining bone density in CP.

In summary, delayed puberty and hypogonadism may be poorly recognised in CP leading to a late diagnosis. Assessment of bone health in CP should include screening for sex-steroid deficiency, which is more common in those with a poor functional status. Optimisation of nutritional status and cautious replacement of sex-steroids or use of pubertal induction needs to be considered. Further larger studies are required to determine the efficacy of sex-steroid replacement on bone health in individuals with CP.

What is already known on this topic:

- Altered pubertal progression in the form of both central precocious puberty and delayed puberty can occur in cerebral palsy
- Aetiology of delayed puberty and hypogonadism in CP is varied
- Sex-steroid deficiency may impact bone health in cerebral palsy

What this study adds

- Delayed puberty and hypogonadism is more common in those with a poor functional status and may be related to nutritional status
- Sex-steroid replacement in CP is generally well tolerated
- The effect of sex-steroid replacement in CP on bone density is inconsistent and requires further study

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Table 1 – Baseline characteristics of subjects with CP with hypogonadism or delayed puberty

Female	17 (63%)
BMI kg/m^2	17.4 (13.7-23.4)
Anticonvulsant use	16 (61.5%)
Nonambulatory	22 (81.5%)
PEG fed	11 (42.3%)
GMFCS	
II-III	5 (18.5%)
IV	7 (25.9%)
V	15 (55.6%)
Fracture	11 (40.7%)

All expressed as n (%) unless otherwise stated

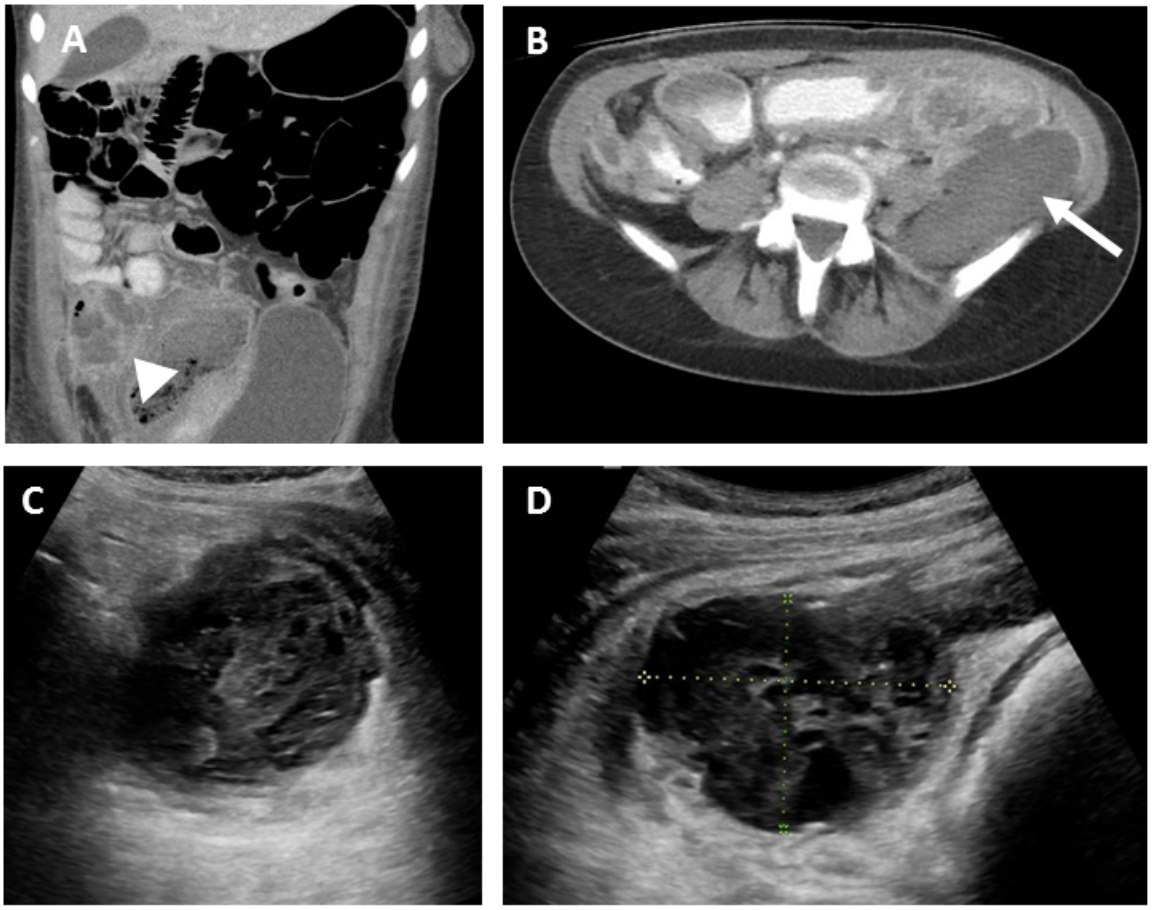
Table 2 – Treatments used and side effects

Treatment	N	Side effects
Oral oestradiol valerate and oral medroxyprogesterone acetate	3	1 Possible ovarian hyperstimulation syndrome
Transdermal Oestradiol and oral medroxyprogesterone acetate	4	
Combined oral contraceptive pill	3	1 Menorrhagia 1 Difficulty managing menses
Combined continuous transdermal patch	1	1 Breakthrough bleeding
Oral testosterone undecanotate	4	1 Worsening acne and irritability
1% transdermal testosterone gel	2	
Transdermal testosterone patch	1	1 Erythema site of patch
Sustanon, testosterone implants	1	1 Difficulty with administration

Table 3 - % change in BMD/year on treatment

Patient	Treatment	Age (years) commenced	Years of followup	TBBMD %Δ/yr	LS BMD %Δ/yr	FNBMD %Δ/yr
1	COCP	21	3	-	0.54%	-1.87%
2	Transdermal E, oral P	18	10	0.94%	1.61%	2.45%
3	COCP	14	3	1.35%	-	-
4	Transdermal E, oral P	15	3	-	5.76%	8.99%
5	Testosterone gel	25	5	-0.1%	-4.83%	0.25%
6	Testosterone patch	21	5	0.36%	0.43%	-
7	Testosterone gel	18	4	-	36.4%	9.3%

Figure 1 – Patient with ovarian hyperstimulation syndrome with oestradiol valerate



Panel A – CT abdomen demonstrating grossly enlarged ovary (triangular arrow). Panel B – CT abdomen demonstrating intra-abdominal collection (arrow). Panel C and D – Left and right ovary as depicted on ultrasound respectively.

3.6 Conclusion

In the cross-sectional study of adults with CP (chapter 3.2), 38% had a prevalent fragility fracture, with 53% of fractures occurring in young adulthood. Despite the young mean age of patients (28.3 years), fractures occurring in adulthood were predominantly osteoporotic related fractures of the ankle, vertebra and ribs. The distal femur fracture is not seen in adulthood and may be due to changes in size and geometry of bone with growth and changes to the growth plate. Non-ambulation, higher GMFCS, the presence of hypogonadism and PEG feeding were found to be risk factors for low BMD. Furthermore, femoral neck Z-scores were associated with fractures in non-ambulatory patients. In support of the important role function plays in bone health, lean tissue mass had a greater effect on BMD than fat mass. This relationship was attenuated by the presence of hypogonadism, which led us to investigate in more detail the aetiology of sex-steroid deficiency in CP and the effects of replacement (chapter 3.5).

Patients with gonadal dysfunction were identified from referrals to the Endocrine unit at two major paediatric hospitals in Victoria. The majority of referrals were in fact for bone health and represent an opportunity to screen for and treat gonadal dysfunction. Gonadal dysfunction was common in those with poorer functional state (GMFCS IV or V) and in those who were PEG fed. The aetiology in most cases were hypogonadotrophic hypogonadism and presented as delayed puberty. Those with hypogonadotrophic hypogonadism tended to have a lower BMI (16.9 kg/m^2 vs 24.0kg/m^2), suggestive of a functional hypothalamic cause of hypogonadism similar to that seen in anorexia and other conditions of malnutrition. Treatment with sex-steroid replacement was well tolerated in most individuals, although I was unable to demonstrate significant improvements in BMD due to the small number of patients.

Longitudinal changes in BMD during puberty were significant with an increase of 4-8% in aBMD per year (chapter 3.3). Deficits in bone mass in CP are seen even prior to puberty. Z-scores were stable during adulthood, suggesting low bone mass in adults with CP is likely due to poor bone accrual in early childhood rather than rapid bone loss later in life. PBM occurred late in the third/early in the fourth decade of life and may be due to the high rates of delayed puberty and subsequent delayed bone accrual in our cohort. The early onset of deficits in bone mass highlight the need for interventions during childhood. Given the importance of muscle, mobility, endocrine health and nutrition on BMD seen in our cross-sectional work and that of other groups, optimising these factors are likely key to achieving potential PBM and improve adult bone health. The effect of hypogonadism/delayed puberty on bone health is likely amplified in our cohort and reflects referral patterns to our centre. In those with poor functional state and suboptimal nutrition, the identification of hypogonadism/delayed puberty is important; the role of sex-steroid deficiency in bone health in those with GMFCS I-III is less clear.

Abnormal bone microarchitecture as measured by TBS (chapter 3.4) was found in over 50% of adults with CP. TBS was higher in ambulatory patients and was weakly associated with lean mass, again confirming the importance of muscle and function in CP. TBS may be a useful adjunct to assess bone health in CP as it can be determined retrospectively from a lumbar spine DXA without need for further scans or radiation. A relationship between TBS and fracture was not seen. The use of TBS may be in identifying adults with CP who have normal BMD but at risk of fracture.

In summary, DXA-derived BMD at traditional sites of the lumbar spine and femoral neck may be used in adults with CP to identify individuals at risk of fracture and requiring treatment. Adults with CP appear to have typical osteoporotic fractures albeit at a younger age, and x-rays should be used to screen for spinal fractures. Our studies confirmed the importance of function and nutrition on bone

health in CP, and identified a novel risk factor of gonadal dysfunction in this cohort. Intervention early in childhood addressing these risk factors is likely to have greatest impact as impaired bone accrual appears to be the primary driver of low bone mass in this condition. The potential use of hormonal treatment requires further investigation in prospective randomised studies.

Chapter 4 – Bone health in SB

4.1 Introduction

Fractures are common in children with SB with a prevalence of up to 30% (278, 279). Most of these fractures involve the distal femur, similar to the fractures seen in CP (274, 275). In children with SB, immobility following major surgery, prior fracture, higher spinal level and non-ambulatory status have been shown to be associated with fracture (275, 278, 279).

The gaps in knowledge pertaining to bone health in adults with SB is similar to those in CP. The relationship between low BMD and fracture is unclear in the SB cohort due to the small numbers of patients in previous studies (271, 272, 282). Fracture risk appears to decline in adulthood although no differences in fracture site has been reported (280). Given our findings in CP, we would expect more typical osteoporotic fractures in adults with SB rather than the ongoing occurrence of distal femur fractures.

There are differences between the SB and CP cohort that may alter fracture risk and are worthy of exploration. In SB, urological intervention with intestinal segments can lead to metabolic acidosis, and bladder dysfunction can lead to chronic kidney disease. These factors have not been studied systematically in relation to BMD or fracture risk. The relationship between body composition and bone health may also be quite different in SB as obesity is highly prevalent in adults with spina bifida (292). Mortality and morbidity in adults with SB are now predominantly due to cardiometabolic diseases such as stroke and acute myocardial infarction (331). Obesity may have protective effects on bone, with previous studies documenting a positive association between BMI and BMD (332, 333). Secretion of hormones such as insulin, amylin, and leptin as well as through the increased aromatisation of testosterone to estrogen may account for the protective effects of fat on bone (334, 335).

The objective of this chapter was to characterise the risk of fracture and low bone mass in adults with SB. Chapter 4.2 is a retrospective cohort study of adults with SB assessing the risk factors for, prevalence and type of osteoporotic fractures seen compared with children with SB. Chapter 4.3 is a cross-sectional study of adults with SB assessing how changes in body composition may affect BMD and metabolic risk factors in these individuals.

Chapter 4.2 – Fractures in spina bifida from childhood to young adulthood

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ORIGINAL ARTICLE

Fractures in spina bifida from childhood to young adulthood

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Abstract
Summary This study assessed the prevalence and types of fractures in spina bifida and examined risk factors for fracture. Fracture prevalence was highest in childhood and reduced in adolescence and young adulthood. The importance of maintaining mobility is highlighted by the increased risk of fracture in those who are non-ambulatory.
Introduction The aims of this study are to study the prevalence and types of fractures according to age group in spina bifida and examine risk factors associated with fracture.
Methods This is a retrospective cohort study of 146 individuals with spina bifida aged 2 years or older who attended the paediatric or adult spina bifida multidisciplinary clinic at a single tertiary hospital.
Results Median age at which first fracture occurred was 7 years (interquartile range 4–13 years). Fracture rates in children (ages 2–10), adolescents (ages 11–18) and adults (age > 18) were 10.9/1000 (95 % confidence interval 5.9–18.3), 5.4/1000 (95 % CI 1.5–13.8) and 2.9/1000 (95 % CI 0.6–8.1) patient years respectively. Childhood fractures predominantly involved the distal femur and femoral shaft; these fractures were rarely seen in adulthood. Non-ambulatory status was associated with a 9.8 times higher risk of fracture compared with ambulatory patients (odds ratio 9.8, $p = 0.016$, 95 % CI 1.5–63.0). Relative risk of re-fracture was 3.1 (95 % CI 1.4–6.8). Urological intervention with intestinal segments was associated with renal calculi ($p = 0.037$) but neither was associated with fracture.
Conclusions The risk of fracture is lower in adults compared with children with spina bifida. The predominant childhood fracture affects the distal femur, and immobility is the most significant risk factor for fracture. Clinical factors contributing to fracture risk need to be elucidated to enable selection of patients who require investigation and treatment of osteoporosis.

Keywords Adult · Fracture · Immobility · Spina bifida · Urological intervention

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Introduction

Spina bifida describes a wide range of congenital malformations with failure of fusion of the neural tube. The prevalence of spina bifida varies between geographical location and ethnicity with rates between 0.20 and 2.92/1000 births reported [1]. Supplementation with folate has significantly reduced the prevalence of spina bifida. Spina bifida is often accompanied by other malformations of the nervous system such as the Arnold-Chiari malformation. Long-term complications of spina bifida relate to reduced mobility and sensation, neurogenic bladder and bowel and orthopaedic abnormalities (scoliosis, hip dislocation). As life expectancy

increases [2, 3], effective fracture prevention in spina bifida should lead to improvements in morbidity and mobility.

Fractures are common in children with spina bifida with a prevalence of up to 30 %, and the most common site is the distal femur [4–7]. Moreover, the sensory deficits associated with spina bifida can lead to a delay in the diagnosis of fractures with disastrous consequences. Risk factors for fracture include immobility following major surgery, prior fracture, higher spinal level involvement and non-ambulatory status [5–7]. Other potential causes of low bone mass in spina bifida include loss of sympathetic and sensory nerve innervation of bone, urological intervention with intestinal segments leading to metabolic acidosis, anticonvulsant use, vitamin D deficiency and renal dysfunction. These factors have not been studied systematically in relation to bone mineral density (BMD) or fracture risk. The relationship between low BMD and fracture is well established in the post-menopausal population, but is still unclear in the spina bifida cohort due to small numbers of patients [8–10].

It appears that this high risk of fracture does not continue into young adulthood, consistent with the bimodal distribution of fractures in the general population where there are peaks in childhood/early adolescence and in women over the age of 55 and in men over 80 [11]. Dosa et al. followed 221 patients with spina bifida over a year and documented 42 prevalent and 5 incident fractures. Using the incident fracture data, they calculated the fracture risk to be highest in adolescence with an annual incidence rate of 29/1000 declining to 18/1000 in adulthood [12], with no difference in fracture sites. There have been no other studies looking at fracture rates in adults with spina bifida and no data on whether anticonvulsants, urological intervention or renal calculi are associated with fractures in this population. Given the difficulties with transition to adult care for spina bifida adolescents, identifying those who need follow-up in regard to their bone health is important [13].

Monash Health is a large health service serving an ethnically diverse population in the suburbs of Melbourne, Australia which has both paediatric and adult spina bifida services. We sought to investigate the risk factors, rate and types of fracture seen in adults compared with children with spina bifida.

Methods

Patients

A retrospective cross-sectional cohort study was conducted of 146 individuals with spina bifida aged 2 years or older who attended the paediatric or adult spina bifida multidisciplinary clinics at a single tertiary hospital between 2005 and 2015. The study was approved by the local Institutional Ethics Committee (Monash Health HREC).

Data collection and clinical measures

Demographic data, together with the medical and surgical history, were extracted from the medical records. Patients were excluded if they had spina bifida occulta or isolated caudal regression syndrome. The fracture site and cause were identified with the cause divided into: trauma, fall, transfer, acute immobilisation or unknown. Fractures were considered secondary to recent surgery or acute immobilisation if the fracture had taken place within 12 weeks of the event.

In those patients with a clinical suspicion of vertebral fracture, this was investigated with lateral thoracolumbar radiography. This was followed by detailed imaging including computed tomography or a three-phase bone scan given the difficulty of assessment of vertebral morphology secondary to scoliosis and/or surgery involving the spine in many patients.

Age at last follow-up visit (or age of death) and age at fracture were recorded. For fracture rate analysis, patients were divided into three groups according to age at first fracture or last follow-up: children (ages 2–10), adolescents (ages 11–18) and adults (over 18 years). Ambulatory status was collected according to the Hoffer ambulation scale [14]: community ambulator, household ambulator, functional ambulator or non-ambulator. Patients were then further divided into predominantly ambulators (community/household) or non-ambulators (functional and non-ambulators). Spinal level was obtained from the medical record or when unavailable, from medical imaging investigations and divided into high (above L3), mid (L3–4), or low (L5 and below). A history of hypogonadism or precocious puberty was established from the medical record.

Hypogonadism was defined in males as the use of androgen replacement therapy or low testosterone levels (<8 nmol/L) documented on two separate occasions and in females as the use of hormone replacement therapy for induction of pubertal development and menopause before the age of 40 or low estradiol levels (<73 pmol/L) on two separate occasions. Precocious puberty was defined as the appearance of Tanner stage B2 in girls before 8 years or testicular volume of ≥4 ml in boys before 9 years and elevated luteinizing hormone levels.

The presence of upper urinary tract calculi (renal/ureteric) was determined from the medical record with confirmation from imaging where available. Bladder calculi were not included given that these calculi occur in spina bifida predominantly due to urinary tract infections and urinary reservoirs with incomplete emptying. The presence of renal dysfunction and metabolic acidosis was assessed through serum creatinine, bicarbonate and chloride levels obtained from medical records. Data regarding urological intervention, which involved intestinal segments (bladder augmentation with intestinal segments or ileal conduit), was also collected.

Statistical analysis

The distribution of the data was explored by the Shapiro-Wilk test. All normally distributed data were expressed as mean with standard deviation and nonparametric data as median with interquartile ranges. Differences between groups were determined using the Mann-Whitney *U* test for continuous variables and Fisher's exact test for categorical variables.

Fracture rates were calculated using patient years with the age of the patient determined at the time of fracture or last follow-up. For analysis according to age group, the fracture rate was calculated by the number of fractures in that age group divided by the number of patient years lived in the age group. Confidence intervals were based on the Poisson assumption. If a patient sustained multiple fractures, time to first fracture was used. For re-fracture analyses, time to subsequent fracture was calculated as the time between the first and second fracture. Relative risks were calculated by comparing re-fracture rate to rates of initial fracture.

Univariate analysis was used to determine predictors of fracture. Clinical variables examined were age, sex, ambulatory status, spinal level, hydrocephalus, urinary diversion, anticonvulsant use, renal calculi, hypogonadism and precocious puberty. Biochemical variables were serum creatinine, bicarbonate and chloride levels.

Given the possibility of significant covariance between factors such as ambulatory status, use of antiepileptic medication and hydrocephalus, multiple regression analysis was performed to determine which factor was most important. Multicollinearity was determined for regression models by calculating the variance inflation factor (VIF). Any regression model with a VIF greater than 10 was excluded. A P value of <0.05 was considered to be statistically significant and all tests were two-sided. Analyses were conducted using IBM SPSS statistics for Windows (Version 22. Armonk, NY).

Results

Baseline characteristics

Clinical characteristics of the patients are summarised in Table 1. In total, 146 patients aged between 2 and 52 years were identified. The median patient age was 19 years and 49 % were male. Fifty-three percent were adults ($n = 77$) with a mean age of 31.6 years (range 19–51). Almost half the patients were non-ambulatory (43 %). Spinal level was L2 or above in 22 % of patients, L3–L4 in 30 % and L5 and below in 48 %. Precocious puberty was confirmed in one girl and one boy, with 4 girls being assessed as having early puberty. None were treated with gonadotrophin releasing hormone due to late presentation or loss to follow-up. However, 5 male patients were found to be hypogonadal, with 2 of these occurring following orchidectomy. Of the remaining three patients, one had delayed puberty and was commenced on testosterone at age 14, and two had hypogonadotrophic hypogonadism, one after pituitary surgery and the other in the context of obstructive sleep apnoea. Apart from the patient with delayed puberty, all other cases were detected and treated in adulthood. Previous urological intervention with intestinal segments (either bladder augmentation or formation of ileal conduit) was present in 36 % of patients.

Table 1 Baseline characteristics

Male (*n*)	71 (49 %)
Age (years)*	19 (9–31)
Children (2–10 years)	41 (28 %)
Adolescents (11–18 years)	28 (19 %)
Adults (>18 years)	77 (53 %)
Spinal level	
High (above L3)	32 (22 %)
Mid (L3–L4)	43 (30 %)
Low (L5 and below)	70 (48 %)
Non-ambulatory	63 (43 %)
Fractures	21 (14 %)
Hydrocephalus	95 (65 %)
Urinary diversion	53 (36 %)
Anticonvulsant use	21 (14 %)

Fracture rates and sites

There were 32 fractures in 21 patients with 6 patients having multiple fractures. The median age at which first fracture occurred was 7 years (interquartile range (IQR) 3.5–14.5 years). The overall fracture rate was 6.9/1000 patient years (95 % CI 4.0–9.8) with 3063 patient years of follow-up. Fracture rates in children, adolescents and adults were 10.9/1000 (95 % CI 5.9–18.3), 5.4/1000 (95 % CI 1.5–13.8) and 2.9/1000 (95 % CI 0.6–8.1) patient years respectively. The proportion of children who were non-ambulatory for fracture rate analysis was 36 %, compared to 43.8 % of adolescents and 50 % of adults. Of the 14 children with fracture, 10 were non-ambulatory (71.4 %). Chi square analysis revealed that, in children, the difference in fracture rate according to ambulatory state remained significant ($p = 0.023$).

Six of the 21 patients with fractures had re-fractured over 280 years of follow-up. The characteristics of these 6 patients who sustained multiple fractures are shown in Table 2. Median follow-up from initial fracture to subsequent fracture or last clinic follow-up/death was 5 years (IQR 3–21.5). The absolute risk of re-fracture was 21.4/1000 patient years (95 % CI 4.3–38.5/1000). The relative risk of re-fracture was 3.1 compared to population rates of initial fracture (95 % CI

Table 2 Characteristics of patients with multiple fractures

Age at last follow-up, sex M/F	Age when fracture occurred and types of fractures	Spinal level; ambulation status	Other clinical characteristics
13F	9: Elbow 10: Metatarsal stress 10: Humerus	L5/S1; Ambulatory	Early puberty, hydrocephalus
40F	4: L Distal femur 5: L Proximal tibia/fibula 10: L Femoral neck 12: L Proximal tibia	T12; Non-ambulatory	Ileal conduit, anticonvulsant use, hydrocephalus, upper tract calculi
10 M	4: L Distal femur 4: L Distal femur 9: L Distal femur	Thoracic; Non-ambulatory	Obstructive sleep apnoea, PEG fed, anticonvulsant use, hydrocephalus
36 M	7: R Proximal tibia 32: R femoral neck	L5/S1; Ambulatory	Hydrocephalus
46 M	3: L Distal femur 21: L Distal femur 30: R Distal femur	L4; Non-ambulatory[a]	Ileal conduit, hydrocephalus
12 M	3: R Distal femur 9: R ankle	Thoracic; Non-ambulatory	Precocious puberty, hydrocephalus

L left, *R* right

[a] Patient was ambulatory prior to first fracture; however, fracture occurred after 6 weeks of immobilisation with bilateral hip spica for ilio-psoas transplants

1.4–6.8). In patients with multiple fractures, 8 fractures occurred in the same leg as the previous fracture.

The types of fractures that occurred are shown in Table 3. Most involved the lower limb, and three fractures occurred in the setting of immobilisation (hip spica or immobility from another fracture). In children, the preponderance of fractures occurred around the knee joint; however, this was not seen in adulthood. The aetiology of fracture was a fall in 28 % ($n = 9$), spontaneous in 25 % ($n = 8$), secondary to immobilisation post-surgery in 16 % ($n = 5$), from transfers in 3 patients and unknown in 22 % ($n = 8$). Of the fractures that occurred in the context of a fall, 3 were secondary to falls from wheelchairs, and the remainder were from standing height or less.

Risk factors for fracture

Univariate analysis showed that non-ambulatory status and hydrocephalus were significantly associated with fracture (Table 4). No other clinical or biochemical variables were associated with fracture. Using multiple logistic regression after adjusting for age, gender and creatinine, only non-ambulatory state was significantly associated with fracture (OR 9.8, $p = 0.016$, 95 % CI 1.5–63.0). Fracture-free probability according to ambulatory status is shown as a Kaplan-Meier plot (Fig. 1).

Urological intervention with intestinal segments were significantly associated with upper tract calculi ($p = 0.037$) but neither were associated with fracture. There was no difference in bicarbonate or chloride levels in those who had urological intervention compared to those who did not.

Discussion

In 146 patients with spina bifida, 14 % had a history of fracture which is similar to the reported rates of 11–30 % [4–6, 12]. Most fractures occurred in children, with the fracture rate significantly decreasing from 10.9/1000 in childhood to 2.9/

Table 3 Location of fractures

Site	No. of fractures	Childhood	Adolescence	Adulthood
Lower extremities				
Distal femur/femoral shaft	16	10	4	2
Femoral neck	2	1		1
Tibia/fibula	7	6	1	
Other	3	2		1
Upper extremities	3	2		1
Vertebral	1			1
Total	32	21	5	6

Table 4 Risk factors for fracture

Characteristic	Fractures $n = 21$	No fractures $n = 125$	P value
Age	26 (13–43)	21 (12–32)	0.101
Sex (M)	10 (48 %)	61 (49 %)	1.00
Non-ambulatory	16 (76 %)	45 (36 %)	0.001[a]
Hydrocephalus	18 (86 %)	77 (62 %)	0.046
Urinary diversion	10 (48 %)	43 (34 %)	0.327
Anticonvulsant use	6 (29 %)	15 (12 %)	0.084
Renal calculi	3 (14 %)	7 (6 %)	0.158
Hypogonadism	0 (0 %)	5 (4 %)	0.455
Precocious puberty	2 (9.5 %)	4 (3.2 %)	0.207
Creatinine (mmol/L)	48.0 (26.8–66.5)	51 (34.0–61.0)	0.401
Bicarbonate (mmol/L)	25.0 (23.3–26.8)	26.0 (25.0–28.0)	0.317
Chloride (mmol/L)	104.0 (102.0–107.0)	103.0 (101.0–106.5)	0.454
Spinal level			0.084
High	8 (38 %)	24 (19 %)	
Mid	7 (33 %)	36 (29 %)	
Low	6 (29 %)	64 (53 %)	

Results shown for univariate analysis [a] After multivariate analysis adjusting for age, gender, creatinine, only non-ambulatory status remained significant

1000 patient years in adulthood. This reduction in fracture rate with age is similar to that reported by Dosa et al.; however, they found the highest rate in adolescence [12]. Given the rarity of fracture in their population during 1 year of observation, the addition of one fracture can significantly change the annual incidence rate. Overall, both our and their findings are reflective of the general population risk of fracture with a peak in childhood/early adolescence then declining until late adulthood. The reduction in fracture rate in adulthood may reflect higher rates of non-ambulation, but notably the majority of fractures in children occurred in those who were non-ambulatory. Adults may have a lower fracture rate as they are more likely to take greater care with mobilising and transfers. Future studies with longer term follow-up would be of benefit to see if older adults with spina bifida develop typical osteoporotic fractures at a younger age.

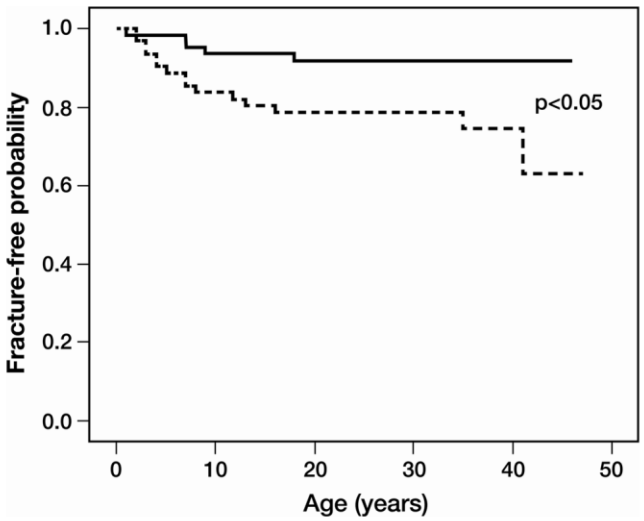

Fig. 1 Overall risk of fracture by ambulatory status. *Dashed line* non-ambulatory patients, *solid line* ambulatory patients

In the general population, rates of fracture in young adults vary between 9.8–14.4/1000 patient years [15, 16]. Young adults with spina bifida appear to be at lower risk of fractures than expected, likely due to lack of mobility. In contrast, adults with traumatic spinal cord injury have higher fracture rates compared to our cohort, at 18–21.8 fractures per 1000 patient years [17, 18]. Though the reasons for this are unclear, we postulate that adults with spina bifida have adapted to their chronic neurological deficit over time and may be less likely to experience falls. The population of spinal cord injury patients who have been studied also have had more severe and complete motor deficits than that seen in our population. Whether acute loss of mobility affects fracture risk differently to chronic immobility is unknown.

The distal femoral or femoral shaft fractures that occur in children with spina bifida have been well described previously [6, 19]. These femoral fractures are commonly seen in individuals with developmental disabilities and are inversely associated with age but strongly associated with reduced mobility [20]. The lack of neural and muscular signals in the lower limb in neurological disability is thought to lead to poor bone accrual and smaller bone size at these sites. Skeletal fragility may be related to smaller bone size rather than reduced bone density per se, as has been shown in a cerebral palsy cohort using peripheral quantitative computed tomography [21]. It is unclear why adults with spina bifida rarely fracture at the

distal femoral/femoral shaft site. It may be that the increase in the size and change in geometry of bone with ageing together with the completion of bone modelling reduces the risk of fracture. Thus, despite low bone mass being diagnosed in up to 33 % of young adults with spina bifida [22], the identification of patients at increased risk for fracture and who require treatment is complex.

Non-ambulatory state was associated with a 9.8 times higher risk of fracture compared with those who were ambulatory. We were unable to demonstrate a relationship between spinal level and fracture, although there was a trend to more fractures in the group with a higher spinal level involvement. This finding is consistent with the previous known association between spinal level and ambulatory status [14, 23]. The importance of immobility and risk of fracture is also highlighted by our finding that five fractures were in the setting of acute immobilisation post-surgery or after a recent fracture, and that eight of the fractures in patients with multiple fractures occurred in the ipsilateral leg. Those who fractured had a 3.1 times higher risk of further fracture than the general spina bifida population. Thus, spina bifida patients who have had a previous fragility fracture are non-ambulatory or are post-operative should be targeted for investigation and potentially treatment of osteoporosis. In the paediatric cohort, many have advocated for shortening the period of post-surgical immobilisation where feasible, as well as avoidance of hip spica casting [4, 7, 24].

Bladder augmentation with intestinal segments or ileal conduit in our cohort was not associated with changes in bicarbonate level, chloride level or risk of fracture. Intestinal segments incorporated into the urological tract can result in a hyperchloremic metabolic acidosis from reabsorption of urinary ammonium with chloride through the intestinal mucosa [25]. To buffer the chronic acidosis, calcium carbonate is released from bone which can lead to bone demineralisation and an increased risk of stones. The literature is conflicting as to whether this is clinically significant with some studies showing no difference in pH and bicarbonate post-urinary surgery [26, 27]. There are no long-term data to support the association of urinary surgery with an increased fracture risk or changes in bone histomorphometry [28, 29]; however, there are some small studies that suggest bone mineral density is reduced, particularly if acidosis is present [30, 31].

It is reassuring that we found no association between fracture and urological intervention or upper tract calculi. Further investigation is required to examine the presence of abnormal urinary calcium excretion, incidence of stones and bone density in adult spina bifida patients based on their history of urinary surgery. We recommend that spina bifida patients who have undergone urological intervention with intestinal segments undergo urinary calcium excretion studies. The presence of hypercalciuria should alert the clinician to the risk of osteomalacia and metabolic acidosis. A trial of bicarbonate therapy may be useful in these situations.

There were only five females and one male with precocious/early puberty compared to five males with documented hypogonadism on replacement with testosterone. Neither were associated with fracture, but our findings are limited by the small numbers of patients. Precocious/early puberty is well recognised in patients with spina bifida who have hydrocephalus, with reported rates of 10–50 % [32, 33]. It is more common in girls than boys with spina bifida which mirrors the prevalence in the general population [34]. Precocious puberty leads to early bone maturation and shorter final height, with apparent higher bone mass for chronological age, but no difference if adjusted for bone age [35–37]. After treatment with gonadotrophin releasing hormone agonists to delay the onset of puberty, studies have shown inconsistent effects on bone density [37–39]. Few reports examine the incidence of hypogonadism in spina bifida [40, 41]. Given that early pubertal development is common, we postulate that screening is not routinely performed; however, unrecognised hypogonadism can have major consequences for musculoskeletal health as well as psychosocial wellbeing. Consistent with previous reports, in our patients, hypogonadism was more common in males and only became apparent in adulthood. The aetiology of the hypogonadism was varied.

We are mindful of some limitations to this study. The medical records may not have captured all fractures that occurred in our patients. In addition, due to the sensory deficits in spina bifida, not all fractures may have been recognised. The spinal level was obtained from the medical record and when unavailable from medical imaging. The level determined on imaging may not always reflect functional level [42]. In addition, dual energy X-ray absorptiometry (DXA) data was not routinely obtained in our paediatric patients, limiting our ability to evaluate the relationship between bone density and fracture in this population. We were also unable to examine 25-OH vitamin D levels, anthropometric variables and fracture risk in this retrospective study as we had insufficient data from medical records.

Conclusion

Fracture rates decline as patients with spina bifida make the transition to adulthood, and young adults are less likely to experience the distal femoral fractures commonly seen in children with chronic neurological disability. Non-ambulatory patients were nine times more likely to sustain a fracture compared with ambulatory patients. This supports the concept that optimisation of muscle strength and balance may lead to a reduction in fractures. Future work requires an exploration of the relationship between vitamin D, bone density and

fracture in this cohort. In addition, given the high prevalence of renal calculi in spina bifida, further elucidation of the chemical composition of these stones and the relationship between calculi and bone health is warranted.

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Compliance with ethical standards

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Conflicts of interest None.

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Chapter 4.3 – Fat- bone interactions in adults with spina bifida

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Fat–Bone Interactions in Adults With Spina Bifida

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Context: Spina bifida (SB) can lead to changes in body composition and bone mineral density (BMD) through diminished ambulation, renal impairment, and anticonvulsant medication. With increased life expectancy, diseases such as obesity and osteoporosis are emerging comorbidities in SB, with limited data to guide management.

Objective: To examine the relationship between cardiometabolic factors, body composition, BMD, and minimal trauma fractures (MTFs) in adults with SB.

Design: Retrospective cross-sectional study.

Setting and Participants: Forty-nine adults with SB (median age, 32.7 years; interquartile range, 22.6 to 39.0) who had undergone dual-energy x-ray absorptiometry imaging at a single tertiary hospital from 2004 to 2015.

Results: The mean body mass index was 31.7 ± 7.5 kg/m2; 26 (53.1%) were obese. Using age- and sex-matched fat percentiles from the National Health and Nutrition Examination Survey III, 62.5% had a total body percentage fat greater than the 95th percentile. Low bone mass (defined as a Z-score of ≤ -2.0) was present in 21.9% at the L1 vertebra and in 35.1% at the femoral neck. Ten (20.4%) had a history of MTFs. A BMD or Z-score at L1, femoral neck, or total body site did not correlate with the occurrence of MTF. Fat mass was significantly and positively associated with BMD after adjustment for age, sex, and height and accounted for 18.6% of the variance in BMD ($P = 0.005$). The prevalence of metabolic comorbidities, such as hypertension (20.4%) and obstructive sleep apnea (16.3%), was high.

Conclusions: Obesity and low BMD are common in young adults with SB. An increased fat mass correlated significantly with BMD. The prevalence of metabolic complications in patients with SB is increased and deserves further study.

Freeform/Key Words: body composition, bone density, myelomeningocele, obesity, osteoporosis, spina bifida

Spina bifida (SB) occurs when the neural tube fails to fuse during embryonic development, leading to neurologic deficits below the level of the lesion. Motor, sensory, and autonomic function can be affected, leading to reduced mobility, the risk of pressure sores, orthopedic

Abbreviations: BMD, bone mineral density; BMI, bone mineral index; DXA, dual-energy x-ray absorptiometry; FM, fat mass; LTM, lean tissue mass; MTF, minimal trauma fracture; OSA, obstructive sleep apnea; SB, spina bifida.

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deformities, and neurogenic bladder and bowel. With the increasing life expectancy [1, 2], chronic diseases of adulthood, including obesity, osteoporosis, cardiovascular disease, and renal failure, are also emerging as health problems in this vulnerable group. However, limited data for adults with SB are available to guide the clinical management of these conditions.

Dual-energy x-ray absorptiometry (DXA) imaging allows for the simultaneous assessment of body composition and bone density. Obesity has been reported as early as 6 years of age in children with SB [3, 4] and becomes an increasing health issue with adulthood [5]. The mortality and morbidity of SB have been shifting from renal failure to those associated with metabolic derangements, such as stroke and acute myocardial infarction [6]. The distribution of fat is pathologically altered in those with SB, with a greater distribution in the lower limbs [7] and predominantly within the muscle compartment [8].

Obesity, however, might have protective effects on bone, with previous studies documenting a positive association between body mass index (BMI) and bone mineral density (BMD) [9, 10]. The reduction in fracture risk with an increased BMI is likely site-specific, with reduced hip and pelvis fractures but increased extremity fractures [11, 12]. This can be explained, in part, by soft tissue padding at these proximal sites. Fat might also exert positive effects on bone through the secretion of hormones such as insulin, amylin, and leptin and through the increased aromatization of testosterone to estrogen [13, 14].

A low bone mass is common in children with SB, with a prevalence of ≤65% [15] and has been strongly associated with nonambulatory status and higher spinal level deficits [16, 17]. Other factors likely to play a role include vitamin D deficiency, anticonvulsant use, renal dysfunction, and urological intervention with intestinal segments, leading to metabolic acidosis. Fractures are prevalent in ≤30% of children [18, 19] and predominantly involve the lower limbs. Measurement of bone density in those with SB is hampered by positioning difficulties and artifacts from metal implants from orthopedic operations. Furthermore, the absence of posterior elements in the vertebrae of patients with SB can spuriously lower the BMD of the lumbar spine [20]. Accordingly, some groups have measured the bone density of the L1 vertebra only, because this will be affected in only a few patients with high-level lesions [21, 22].

To the best of our knowledge, only one study has reported on the bone density in adults with SB [21]. That study included 21 participants with a mean age of 30 years. They had recruited from a rehabilitation service [21]. Using a T-score of <-2.5 for the diagnosis of osteoporosis, 33% had osteoporosis in at least one of the sites measured (L1 vertebra, femoral neck, or trochanter), and 47% had osteoporosis at either the femoral neck or trochanter. Given the young age of subjects in these studies, these prevalence rates were significantly greater than those of the general population; however, the significance of this finding is unclear, given the fracture rates in young adults with SB appear to be lower than those of unaffected adults [23, 24].

The aim of the present study was to characterize the changes seen in body composition and bone density in a young adult population with SB using DXA and to examine how the changes in body composition might affect the BMD and metabolic risk factors in these individuals.

1. Methods

A. Patients

A retrospective cross-sectional cohort study of 49 adults with SB who had undergoing DXA imaging at a single tertiary hospital from 2005 to 2015 was undertaken. The medical indications for DXA imaging included a suspected low BMD in the setting of immobility, fracture, renal disease, anticonvulsant use, and hypogonadism. The Monash Health human research ethics committee approved the present study.

B. Data Collection and Clinical Measures

Patients were excluded if they had SB occulta or isolated caudal regression syndrome. Demographic data, the prevalence of hydrocephalus, current or previous use of anticonvulsant

medication, and minimal trauma fracture (MTF) were obtained from the medical record. A MTF was defined as a self-reported or radiologically proven fracture occurring after a fall from a standing height or less or a minimal trauma incident other than a fall (*e.g.,* turning over in bed). Fractures of the skull, hands, and toes were excluded.

Ambulatory status was defined using the Hoffer ambulation scale [25] as community, household, functional, or nonambulators. The patients were then further divided into predominantly ambulators (community/household) and nonambulators (functional and nonambulators). The spinal level was obtained from the medical record and divided into high (above L3), mid (L3-L4), and low (L5 and below).

Data regarding urological intervention, which involved intestinal segments (bladder augmentation with intestinal segments or ileal conduit), were collected. The presence of renal dysfunction and metabolic acidosis was assessed from the estimated glomerular filtration rate, serum creatinine, bicarbonate, and chloride levels obtained from the medical records. Hypogonadism was defined in males as the use of androgen replacement therapy or low testosterone levels (<8 nmol/L) documented on two separate occasions and in females as the use of hormone replacement therapy for induction of pubertal development, menopause at age ≤ 40 years, or low estradiol levels (<73 pmol/L) on two separate occasions. Other biochemical variables included serum 25(OH) vitamin D, alkaline phosphatase, and γ-glutamyl transpeptidase at BMD measurement.

The medical conditions associated with obesity were documented and included cardiovascular disease, deep venous thrombosis, pulmonary embolism, obstructive sleep apnea (OSA), cerebrovascular disease, type 2 diabetes mellitus, and hypertension. If patients died during the study period, the age at death and the cause of death were recorded.

B-1. BMD measurements

BMD was measured using DXA at the L1 vertebra, femoral neck, and total body for all participants, unless limited by previous scoliosis surgery, femoral surgery, or difficulty with positioning. The presence of vertebral arch deficits can falsely lower the L1–L4 lumbar spine BMD. The L1 vertebra was therefore chosen, because it is rarely involved in SB, in line with previous studies [21, 22]. Patients were excluded if the entire lumbar spine was involved. A low BMD was defined as a Z-score of ≤ -2.0, in accordance with the International Society of Clinical Densitometry guidelines for adults aged <50 years [26]. The coefficient of variation for BMD of a Lunar anthropomorphic lumbar spine phantom measured daily from mid-2004 to the end of 2011 was 0.51%. The coefficient of variation for the percentage body fat of a total body phantom measured weekly was 3.11%.

The total lean tissue mass and fat mass were derived from the whole body scan. All measurements were obtained using a General Electric Lunar Prodigy, software version 12 (Madison, WI) at a single center. The anthropometric measures of age, weight, and height were documented. In cases in which the true height could not be obtained, the patient's length lying flat was used for the BMI calculations.

The adults were categorized into four BMI groups: underweight (BMI, <18.5 kg/m^2), normal (BMI, 18.5 to 25 kg/m^2), overweight (BMI, >25 kg/m^2), or obese (BMI, >30 kg/m^2). The percentiles for fat mass were calculated from the National Health and Nutrition Examination Survey 1999 to 2004 body composition data matched for age and sex [27]. The fat mass was further divided into the trunk, arms, and legs, and the percentage of fat per segment was calculated by dividing the segment fat mass by the total mass of the segment. An increased fat mass was defined as $>35\%$ for females and $>25\%$ for males in accordance with the American Association of Clinical Endocrinologists/American College of Endocrinology guidelines [28].

C. Statistical Analysis

The distribution of data was explored using the Shapiro-Wilk test. All normally distributed data are expressed as the mean $\pm$ standard deviation and the nonparametric data as the

median and interquartile range (IQR). Differences between groups were determined using the independent *t* test for normally distributed variables, the Mann-Whitney *U* test for nonparametric variables, and the Fisher exact test for categorical variables. Univariate analysis was used to determine the predictors of fracture.

Univariate regression analysis was performed to examine the correlation between the fat mass (FM) and lean tissue mass (LTM) with BMD at L1, the femoral neck, and total body separately. This was followed by a multivariate analysis after adjusting for age, sex, and height in these models. Multicollinearity was determined for multivariate regression models by calculating the variance inflation factor. Any regression model with a variance inflation factor >10 was excluded.

A *P* value of <0.05 was considered statistically significant, and all tests were two-sided. The analyses were conducted using SPSS statistics for Windows, version 22 (IBM Corp., Armonk, NY).

2. Results

A. Baseline Characteristics

The clinical characteristics of the 49 patients are summarized in Table 1. The median age of the patients was 32.7 years (IQR, 22.6 to 39.0), 40.8% were male, and more than one-half were nonambulatory. Spinal level involvement was above L3 in 9 patients (18.4%), at L3-L4 in 16 patients (32.7%), and at L5 and below in 24 patients (49%). Also, 27 patients (55.1%) had a history of urological intervention with either bladder augmentation or formation of the ileal conduit. The mean duration of urological intervention was 24.8 ± 12.5 years. Hypogonadism was found in 5 patients, all male, of varying etiology. All received testosterone replacement. For 40 patients, the total body BMD was obtained, enabling the total and regional LTM and FM to be derived.

B. Body Composition

The mean BMI was 31.7 ± 7.5 kg/m^2, in the obese range for adults. Only 10 patients (20.4%) had a normal weight or were underweight, with 13 patients (26.5%) overweight and 26 (53.1%) obese. Of the 8 patients with a normal BMI, 7 (87.5%) had an increased percentage of body fat on DXA (defining as body fat >25% in men or >35% in women), which would classify these patients as obese in accordance with the American Association of Clinical Endocrinologists/American College of Endocrinology guidelines [28]. The prevalence of obesity according to total body fat percentage was 87.5%. Using age- and sex-matched fat percentiles from National Health and Nutrition Examination Survey III [27], 25 of 40 (62.5%) had a DXA total body fat percentage greater than the 95th percentile. When the regional fat percentiles were calculated, the percentage of tissue mass that was fat by site was at the 50th percentile for arms (IQR, 12.5% to 75%), the 95th percentile for legs (IQR, 90% to 95%), and 95th percentile for trunk (IQR, 50% to 95%).

The BMI was not significantly different statistically between the ambulatory and nonambulatory patients (30.3 ± 6.8 kg/m^2 vs 32.0 ± 8.7 kg/m^2, respectively; $P = 0.58$). The distribution of fat and lean tissue according to ambulatory status is shown in Table 2. Before adjustment for age, sex, and height, those who were nonambulatory had a greater percentage of leg mass that was fat than did the ambulatory patients (56.8% vs 46.7%; $P = 0.006$). No relevant difference was found in the percentage of arm or trunk mass that was fat between the ambulatory and nonambulatory patients. Similar results were found after adjusting for covariates. A trend was found toward a greater percentage of trunk fat and total body fat in those who were nonambulatory, but this did not reach statistical significance. The relationship between LTM and ambulatory status showed substantially greater leg, trunk, and total lean tissue for the ambulatory patients; however, the difference failed to maintain statistical significance after adjustment for age, sex, and height.

Table 1. Baseline Characteristics (n = 49, Unless Specified Otherwise)

Variable	Patients
Demographic	
Age, y	
Median	32.7
IQR	22.6 to 39.0
Male sex, n (%)	20 (40.8)
Anthropometric	
Weight, kg	71.1 ± 19.6
Height, cm	151.2 ± 11.0
BMI, kg/m^2	31.0 ± 7.5
Clinical, n (%)	
Spinal level	
High	9 (18.4)
Mid	16 (32.7)
Low	24 (49.0)
Nonambulatory	27 (55.1)
Anticonvulsant use	10 (20.4)
Hydrocephalus	36 (73.5)
Fracture	10 (20.4)
Urological intervention	27 (55.1)
Upper tract calculi	2 (4.1)
Renal impairment (eGFR <90 mL/min/1.73 m^2; n = 42)	9 (18.4)
Hypogonadism	5 (10.2)
DXA	
LTM, kg	36.9 ± 9.0
FM, kg	30.4 ± 13.8
Bone mineral content, kg	2.40 ± 0.51
L1 BMD (n = 32), g/cm^2	1.04 ± 0.15
L1 Z-score	
Median	−1.1
IQR	−1.9 to −0.1
FN BMD (n = 37), g/cm^2	0.81 ± 0.13
FN Z-score	
Median	−1.5
IQR	−2.4 to −0.7
TB BMD (n = 40), g/cm^2	1.14 ± 0.10
TB Z-score	
Median	−0.3
IQR	−1.1 to 0.5
Radius BMD (n = 27), g/cm^2	0.86 ± 0.11
Radius Z-score	
Median	−0.6
IQR	−1.3 to 0.4
Biochemical	
Vitamin D (n = 23), nmol/L	58.0 ± 25.3
Creatinine, mmol/L	
Median	51
IQR	38 to 84
Bicarbonate, mmol/L	24.4 ± 3.4
Chloride, mmol/L	105.5 ± 3.3
ALP (U/L)	
Median	92.5
IQR	75 to 112.5
GGT (U/L)	
Median	20.5
IQR	14.5 to 50

Abbreviations: ALP, alkaline phosphatase; eGFR, estimated glomerular filtration rate; FN, femoral neck; GGT, γ-glutamyl transpeptidase; TB, total body.

Table 2. DXA and Body Composition Parameters According to Ambulatory Status

Region	Nonambulatory	Ambulatory	*P* Value
Lumbar spine Z-score (n = 32)	−1.46 (0.3 to −3.2)	−0.72 (1 to −3.7)	0.096
Femoral neck Z-score (n = 37)	−1.97 (−0.2 to −3.9)	−1.36 (−0.1 to −3.5)	0.097
Lean tissue (n = 40), %			
Arm	0.08 ± 0.04	0.08 ± 0.02	0.82
Leg	0.11 ± 0.03	0.14 ± 0.02	0.001
Trunk	0.24 ± 009	0.29 ± 0.06	0.06
Total	0.48 ± 0.17	0.56 ± 0.11	0.08
Fat (n = 40), %			
Arm	33.93 ± 14.53	33.01 ± 13.20	0.84
Leg	56.82 ± 9.97	46.65 ± 11.66	0.003[a]
Trunk	47.94 ± 13.21	41.99 ± 12.19	0.051
Total	46.83 ± 12.03	40.76 ± 11.65	0.095

[a]Statistically significant after adjusting for sex, age, and height.

C. Morbidity and Mortality

The patients had a mean follow-up duration of 6.54 ± 3.54 years, with four patients (8.1%) dying during the study period. The cause of death in one patient each was intra-abdominal sepsis (age, 21 years), acute hydrocephalus (age, 24 years), aspiration pneumonia (age, 41 years), and metastatic transitional cell carcinoma of the bladder in an ileal conduit (age, 50 years). The prevalence of metabolic comorbidities was high, including hypertension in 20.4%, OSA in 16.3%, deep vein thrombosis or pulmonary embolism in 8.2%, and type 2 diabetes mellitus requiring medication in 6.1%. In terms of cardiovascular disease, only one patient had a documented cerebrovascular accident, and none had documented ischemic heart disease.

D. DXA Parameters

L1 BMD was obtained in 32 patients (65.3%) and femoral neck BMD in 37 patients (75.5%). The median lumbar spine Z-score was −1.05 (IQR, −1.85 to 0.1), and the median femoral neck Z-score was −1.50 (IQR, −2.4 to −0.7). A reduced BMD, as defined by a Z-score of ≤−2.0, was present in 7 of 32 patients (21.9%) at L1 and in 13 of 37 patients (35.1%) at the femoral neck.

E. DXA and Fracture

Of the 49 patients, 10 (20.4%) had a history of fragility fracture, with one patient experiencing four fractures. Six patients had fractures that had occurred during childhood and four had experienced fractures in adulthood. None of those with fractures in childhood experienced additional fractures in adulthood. All fractures involved the lower limb except for two fractures of the shoulder.

Fractures were not significantly associated with the clinical risk factors for osteoporosis or anthropometric or DXA parameters on univariate analysis. The BMD and Z-scores at the L1 vertebra, femoral neck, and total body were not associated with the occurrence of fracture. In addition, BMI, height, weight, and DXA parameters of FM and LTM were not associated with the occurrence of fracture. Clinical variables, including sex, hydrocephalus, ambulatory status, urological intervention, presence of renal calculi, renal impairment, hypogonadism, and anticonvulsant use, were not associated with either BMD (femoral neck or lumbar spine) or fracture status.

F. Relationship Between Body Composition and Bone Mass

The relationship between BMD and body composition parameters was examined using linear regression modeling. Univariate analysis showed that 18.6% of the variance in total body

BMD was accounted for by the FM and 14% by the LTM ($P < 0.05$; Table 3). After adjustment for age, sex, and height, only FM had a statistically significant positive association with total body BMD ($R^2 = 0.399$; $P = 0.019$, $\beta = 0.427$, $P = 0.014$). The BMI also correlated extensively with the total body BMD on both univariate and multivariate analysis after adjusting for age and sex.

3. Discussion

To the best of our knowledge, the present study included the largest cohort of young adults with SB to date in whom bone density and body composition were examined. More than 50% of our cohort was obese using BMI criteria, and 87.5% were obese according to the total percentage of body fat, as calculated using DXA. This percentage is greater than the previously reported prevalence of 35% to 37% in young adults with SB [5, 29]. In a similar study of 18 adults with SB, Liu *et al.* [30] also found that the BMI calculated using either the length or arm span grossly underestimated the presence of obesity compared with that determined by trunk fat recorded using DXA. Coupled with the high prevalence of obesity-related complications found in this cohort, optimizing the cardiometabolic health of young adults with SB is important.

Obesity is a well-recognized problem for patients with SB and is thought to results primarily from reduced energy expenditure [3]. Other causes of weight gain can include hydrocephalus and cerebral abnormalities, leading to neurohormonal and appetite disturbances, and epigenetic factors, with maternal obesity and diabetes both risk factors for neural tube defects [4, 5, 31, 32]. In our study, leg fat was substantially increased, as expected; however, of greater concern was that the percentage of trunk fat was also at the 95th percentile. This has implications for cardiovascular health in this young population [33]. The lack of ambulation was associated with increased fat in the legs; however, only a trend was found toward reduced total body fat and trunk fat. This might have been because of our small sample size and the lack of functional measures; however, 50% of the ambulatory patients had a BMI within the obese range. The changes in body composition seen in our adult cohort could not be satisfactorily explained by ambulatory status. Other small studies of adults with SB have similarly shown no correlation between obesity and ambulatory status or physical activity levels [5, 30]. It could be that interventions to increase physical activity should be implemented earlier, considering that young children with SB (mean age, 9 years) were found to have no differences in trunk fat compared with their peers [7].

In a previous study of 225 subjects with SB, 43% had hypertension, 7.5% had diabetes, and 6.6% had renal impairment [34]. In a 40-year longitudinal study, the mortality rate per decade was 9% (7 of 77) for those aged 5 to 14 years, 13% (9 of 70) for those aged 15 to 24 years, and 15% (9 of 61) for those aged 25 to 34. The cause of death was mainly unexpected and included epilepsy, pulmonary embolus, acute hydrocephalus, and acute renal sepsis [35]. We found high rates of obesity-related diseases in our population, including hypertension (20.4%), OSA requiring continuous positive airway pressure (16.3%), and deep vein thrombosis

Table 3. Relationship Between Body Composition and Total Body BMD (n = 40)

Variable	R^2	*P* Value	β	*P* Value
Fat	0.186	0.005[a]	0.432	0.005[a]
Muscle	0.140	0.016[a]	0.374	0.016[a]
Model[b]	0.310	0.019[a]		
Fat	NA	NA	0.427	0.014[a]
Muscle	NA	NA	0.258	0.291

Abbreviation: NA, not applicable.
[a]Statistically significant.
[b]Linear regression model.

or pulmonary embolism (8.2%). From these data, deep vein thrombosis prophylaxis should be considered for nonambulatory young adults with SB who require surgery. Medications that can increase the risk of thrombosis such as oral contraceptive medication should also be used with caution in this population.

Obesity, however, can have positive effects on bone through fat–bone interactions. A greater BMD in those with a higher BMI could result from increased LTM or FM. In previous studies of body composition, LTM accounted for a larger variance in BMD than did FM in young females and males, although in postmenopausal women, the FM was relatively more important [39–41]. In our cohort, we demonstrated a relevant and positive correlation between FM and total body BMD, even after adjustment for age, sex, and height. The unusual loss of the muscle–bone relationship in young adults might result from the alterations in body composition seen in adults with SB. Whether this can explain why adults with SB appear to have a lower than expected risk of osteoporosis and fracture requires validation in other cohorts.

We were unable to demonstrate a relationship between BMD and the occurrence of fracture, in line with previous studies [15, 16, 42]. This was likely because of the small sample sizes in these studies and the inherent limitations of using DXA in this population. Overall, a low bone mass was seen in 21.9% of our patients at the L1 vertebra and in 35.1% of patients at the femoral neck. This was lower than the only other study of BMD in adult patients with SB, in which 47% of patients demonstrated low BMD at the femoral site [21]. Compared with the study by Valtonen *et al.* [21], our participants were of similar age and had an equivalent prevalence of obesity, ambulation, renal impairment, and anticonvulsant use. Also, more fractures were documented in their group than in ours (38.1% vs 20.4%); however, it is unclear whether the fractures identified by Valtonen *et al.* [21] were MTFs. The lower than expected rates of fracture described previously in young adults with SB suggests that treatment based on a low BMD alone should be approached with caution. Other risk factors such as a history of a fragility fracture or rapid bone loss might signal the need for intervention. Longitudinal studies of BMD are required, especially in older adults with SB, who will experience further bone loss with age.

Adults with SB have a high burden of chronic medical conditions and are at risk of bone disease. Despite the young age of our cohort (median age, 32.7 years), more than one-half of all patients were nonambulatory, one in five patients required anticonvulsant medications, and one in five patients had renal impairment. In addition, 1 in 4 men (5 of 20 men) had hypogonadism. We recommend DXA screening for adults with SB who have sustained a fragility fracture, have developed a chronic disease associated with bone loss, or require medications associated with bone loss [26]. This includes patients treated with antiepileptic medication and patients with hypogonadism and renal disease. In the absence of evidence-based guidelines to guide clinical practice, the treatment of young adults with low BMD needs to be individualized. Ensuring vitamin D levels are replete, treating hypogonadism in those without contraindications, encouraging weightbearing exercise (where possible), and optimizing general health are all appropriate. The use of specific osteoporosis medications should be assessed on a case-by-case basis by the treating physician but should be limited to individuals with fracture and/or a severe reduction in BMD, after discussion of the risks and benefits with the patient.

From the perspective of body composition, it is not surprising that obesity in adults with SB is common, given the high prevalence of childhood obesity described with this condition [3, 4]. In adults with SB and obesity, we recommend screening for hypertension, dyslipidemia, impaired glucose tolerance, and diabetes mellitus and for the associated comorbidities, including OSA [43]. Strategies are urgently needed to reduce the incidence and severity of childhood obesity and to effectively treat adult obesity, especially in this vulnerable group.

To the best of our knowledge, ours is the first study to assess both BMD and body composition, using DXA in adults with SB. Our findings highlight the importance of screening for obesity and its related complications. DXA allows for performance of detailed body composition analysis, overcoming the limitations with traditional anthropometric measurements

such as BMI. Patients with SB can experience growth restriction and scoliosis or joint contractures, which can further skew measurements of height. It also allows for the assessment of fat distribution, which is important when considering cardiovascular risk. However, the present study had a number of limitations, in particular, the small sample size, especially when reporting medical comorbidities in this group. We were unable to fully adjudicate or confirm death of our subjects who were lost to follow-up. In addition, adult patients with SB who continue to attend outpatient clinics at a tertiary referral center are more likely to have more severe neurologic disease and medical comorbidities than those in the general community. These medical comorbidities include renal disease, hypogonadism, and anticonvulsant use, all of which can contribute to bone disease and increase fracture risk. Furthermore, insufficient data were available regarding vitamin D status. Therefore, we were unable to examine any effect of vitamin D levels on BMD. As described previously, BMD measurements can be difficult to interpret in patients with SB and, furthermore, will be affected by body size. We have described an association between FM and BMD. However, owing to the cross-sectional nature of our study, this should be explored further.

4. Conclusions

Adults with SB have a high risk of both low BMD and obesity. Obesity could play a role in the skeletal phenotype of this population. The clinical assessment of bone health (in particular, in those with risk factors for bone disease) and screening for obesity with its related metabolic complications is recommended for adults with SB. The high prevalence of metabolic risk factors is of particular concern and requires further study.

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4.4 Conclusion

The retrospective cohort study (chapter 4.2) found that the risk of fracture is lower in adults with SB than children. Fracture rates in children (ages 2-10), adolescents (age 11-18) and adults (age >18) were 10.0/1000, 5.4/1000 and 2.9/1000 years respectively. Type of fracture also varied, with childhood fractures involving the distal femur and femoral shaft rarely seen in adults. A higher risk of fracture was seen in non-ambulatory patients with an odds ratio of 9.8, and those who had fractured had a relative risk of refracture of 3.1. Clinicians should concentrate on monitoring bone health in those who have had a previous fracture or who are non-ambulatory.

Obesity was highly prevalent (chapter 4.3), present in over 50% of young adults in the cohort with a mean BMI of 31.7kg/m^2. Multiple metabolic comorbidities were common with a high rate of hypertension and obstructive sleep apnoea despite the young age of patients. Low bone mass was seen in up to 35%, but did not predict minimal trauma fracture. Fat, rather than lean mass was found to correlate with BMD which needs to be confirmed in further studies. Urological intervention or renal impairment were not associated with bone fragility in this cohort.

In summary, obesity rather than low bone mass appears to be of greater concern in adults with SB and efforts need to be made in childhood to prevent future cardiometabolic complications. DXA-derived measures of BMD were not associated with fracture in our cohort but allows for the simultaneous assessment of body composition. The reduced risk of fracture and low bone mass seen in SB compared to CP may be due to the interaction between fat and bone which requires validation in future studies.

Chapter 5 – Conclusions and future directions

With improved life expectancy in both CP and SB, individuals are now experiencing diseases of ageing. Osteoporosis and fracture are increasingly recognised as important comorbidities in adults with SB and CP, leading to loss of independence, reduced mobility and pain. In children with SB and CP, there has been a body of research demonstrating the early occurrence of low bone mass, primarily a result of lack of ambulation. However, the natural history of bone disease in these conditions is incompletely understood due to the paucity of longitudinal clinical, bone mineral density and fracture data. In addition, knowledge of clinical risk factors for low BMD and fractures is limited.

The objective of the work described herein was to investigate the risk factors associated with reduced BMD and fractures in adults with CP and SB. The major themes that have emerged from my thesis are: i) the relationship between BMD, body composition and fracture in CP and SB; ii) considerations for transitional care to adult services: differences in measurement, diagnosis and consequences of low bone mass with ageing in individuals with CP and SB iii) novel risk factors or protective factors for bone health in CP and SB including gonadal dysfunction in CP and renal disease in SB.

Relationship between BMD, body composition and fracture

The importance of muscle function and ambulation on bone strength has been demonstrated in both human and animal models of unloading or disuse. This is mediated by the osteocyte, which senses deformation and responds through altering the secretion of factors such as sclerostin, DKK-1, RANKL and OPG. This in turn acts on osteoblasts and osteoclasts to change bone formation and resorption respectively. The system is designed to allow bones to adapt to changes in use. However, in CP and SB, the lack of mobility from childhood leads to a pathological state of low bone mass and fragility.

The importance of the muscle-bone relationship was clearly seen in my cross-sectional and longitudinal study of CP. 35.7% of the variance in BMD was accounted for by lean tissue mass (336). After adjusting for age, gender and height, lean tissue mass continued to have a positive association with BMD whereas fat mass did not. Apart from bone mass, bone microarchitecture is also affected by muscle changes in CP. TBS, a measure of trabecular microarchitecture was significantly lower in non-ambulatory patients (337). Furthermore, TBS was positively associated with arm and leg lean mass. In my longitudinal study, reduced functional status as measured by GMFCS was the only clinical variable that had a consistent effect on longitudinal changes in BMD. GMFCS had a negative effect on longitudinal changes in BMD on the order of -0.01g/cm^2/year at all sites measured (338), which was a novel finding. In addition, low Z-scores were demonstrated at baseline (mean age 13.9 years) and stability of Z-scores were noted during the third and fourth decade of life. This suggests that poor bone accrual in early childhood is the major cause of low bone mass in CP, driven by reduced mobility.

These findings support work by other groups which have also found that measures of muscle strength, ambulatory status and GMFCS are the strongest predictors of BMD (88, 92, 167). Interventional studies which have targeted improving muscle strength and function or the ability to stand and mobilise, have generally found positive, albeit small improvements in BMD (218-220). Given the difficulties with conducting interventional trials in this cohort, our work and the work of the other studies cited, confirm the validity of using this interventional approach to improving bone health in CP.

Interestingly, the muscle-bone relationship does not appear to be as strong in adults with SB. My cross-sectional study in SB found that although lean tissue mass was associated with BMD, accounting for 14% of its variance, there was a stronger relationship between fat mass and BMD (243). This may be a result of the significant alterations in body composition in adults with SB but it requires further

study. We found that 87.5% of our cohort were obese according to total percentage of body fat calculated by DXA, and that 62.5% had a total body fat percentage over the 95th centile when age and sex matched to percentiles from the National Health and Nutrition Examination Survey III (NHANES III).

Considerations during transition of care to adult services

Much of the literature to date has focused on bone health in children with CP and SB. During the transition from childhood to adult services, bone or joint problems were one of the areas of highest unmet need in CP identified by a collaborative research group into transitional care (245). This is compounded by the large decrease in the use of rehabilitation services and use of standing devices in those transitioning to adult services (339).

Understanding the differences in assessment and management of bone health between adults and children with CP and SB will better inform transitional care. Firstly, the measurement of bone mass in children is affected by growth delay and pubertal delay. This is due to an inherent limitation of DXA, which is a two-dimensional image so does not capture differences in bone size in the calculation of bone density. The ISCD paediatric guidelines recommend adjusting BMD/BMC results for height Z-score in situations of short stature or growth delay (12). Although there is no formal recommendation in adults with short stature, it is widely recognised that short stature can falsely lower BMD results. Our adult cohort of CP individuals had a mean height of 155.4 ± 13.6 cm and our SB cohort had a mean height of 151.2 ± 11.0cm. Adult physicians need to take this into account before diagnosing pathological low bone mass in CP and SB. Conversely, smaller thinner bones are more fragile and correcting DXA for size may negate the contribution of bone geometry to fracture risk.

The site of measurement of BMD also varies between children and adults. Work by Henderson and colleagues has established the lateral distal femur as the site of choice for measuring BMD in non-ambulatory children with CP (117). Traditional sites of measurement in adults are the proximal hip and lumbar spine. My study was the first in adults to show a relationship between proximal hip BMD and fracture in non-ambulatory adults with CP (336). This validates the use of these sites, which have been utilised by other studies to assess BMD in adults with CP (92, 95, 120).

The other benefit of using the proximal hip and lumbar spine in adolescents and adults with CP is to ensure comparability of DXA measurements over time. There have been concerns using the proximal femur in the past due to variability in skeletal development at that site (12). Longitudinal changes in the proximal hip and lumbar spine during puberty in adolescents with CP are in the range of 4-8% per year (338) and mirror the changes seen in healthy adolescents of between 5-15% per year (340, 341). Recently, the ISCD have updated their paediatric guidelines to recognise the importance of continuity of DXA measurements across the transition period. They have therefore suggested the use of proximal femur measurements in adolescents with reduced weight bearing if reference data are available (342). This is important as I have shown Z-scores are stable over time in this cohort, and if there are decline in Z-scores, attention needs to be given to nutrition, function and gonadal status.

There are also significant differences in types of fracture and rates of fracture in adolescents and adults with CP and SB compared with children. Young adults with CP can develop vertebral fractures (95, 336) and screening spinal x-rays should be done to guide treatment. Distal femur fractures are uncommon in adults with CP and SB (336, 343). Rates of fracture decline in adulthood in SB (343); a focus on cardiometabolic health may be more critical than bone health (243).

Novel factors affecting bone health in CP and SB

In CP, the presence of gonadal dysfunction may have significant implications for bone health. Hypogonadal patients had lower lumbar spine BMD compared with eugonadal patients (336) . The relationship between muscle mass and BMD in hypogonadal patients was lacking, suggesting that correction of hypogonadism is required prior to physiotherapeutic interventions. The delay in peak bone mass accrual to the late third decade/early fourth decade of life in CP may also be explained by the high rates of gonadal dysfunction in our cohort (338).

To examine the issue of gonadal dysfunction in more detail, I sought to examine cases identified from the two major paediatric hospitals in the state of Victoria. I found that hypogonadism is more common in females and those with a poor functional state (GMFCS IV or V). The mean age of menarche documented in 15 females was 17.3 years. Most cases were of hypogonadotrophic hypogonadism and these patients had a lower median BMI of 16.9kg/m^2 compared with hypergonadotrophic hypogonadal patients (24.0kg/m^2) (unpublished, Chapter 3.5). This suggests a functional hypothalamic hypogonadism caused by nutritional deficiencies. Treatment with sex-steroid replacement was well tolerated in most patients. I was unable to demonstrate the effect of sex-steroid replacement on BMD, limited by the small number of patients as well as the variability in the formulation, dose and duration of sex steroid replacement.

There were also a number of cases (n=5) of hypogonadism of varying aetiology in males in the SB cohort. However, there were also 6 cases of early puberty and precocious puberty. The issue of overnutrition is much more common than malnutrition in SB and may explain the differences in these two cohorts.

Unique to the SB cohort are the consequences of neurogenic bladder and their potential effect on bone health. Bladder augmentation with intestinal segments or creation of an ileal conduit can lead to a metabolic acidosis as a result of reabsorption of urinary ammonium through the intestinal mucosa. To buffer the acidosis, calcium carbonate is released from bone which can lead to osteomalacia and kidney stones. Urological intervention or the presence of renal calculi were not associated with fracture or bone density in my cross-sectional study in SB (343). This finding needs to be confirmed in larger studies as there were only 2 out of 49 patients with renal calculi. Over 50% did have urological intervention but this was not associated with any differences in bicarbonate or chloride levels.

Renal impairment in SB is a consequence of neuropathic bladder causing obstruction. This can be compounded by recurrent urinary tract infections and urolithiasis (314). In a recent systematic review, renal impairment was found in 25.7% of adults with SB, although only 1.3% had CKD stage 5 on dialysis (344). Renal impairment can affect bone health in a multitude of ways. I found no association between renal impairment (defined as eGFR <90ml/kg/min) and BMD or fracture. However, renal impairment was only present in 18% of our cohort, and may reflect improved management of neuropathic bladder. Indeed, renal impairment is becoming a less common complication of SB and less likely to contribute to mortality since the introduction of clean intermittent catheterisation and other urological procedures (331, 345). Prevention of renal disease will improve both bone health and cardiovascular risk in SB.

Clinical implications

DXA is an important means of evaluating bone health in CP. Both the proximal hip and lumbar spine sites can be used for monitoring changes in BMD in adolescents and adults. Not only does this allow for continuity of measurements over the transition period but femoral neck Z-score is also associated

with fracture risk in non-ambulant adults with CP. Lean mass is associated with BMD in CP, and maintenance of function through physical therapy is an important facet of treatment. However, gonadal function must be adequate prior to such interventions. Hypogonadism is common in CP and may present as delayed puberty in children or develop in adulthood. It is more prevalent in non-ambulatory individuals and those who are PEG fed. The aetiology of hypogonadotrophic hypogonadism in CP in some cases may be related to nutrition. Longitudinal changes in BMD in puberty in CP are appropriate with a 4-8% median increase in BMD per year. The deficits in bone mass observed in patients with CP occur prior to puberty with low Z-scores already present at first DXA. Z-scores remain stable over adolescence and adulthood. Fractures in adulthood differ to those in children with CP and are the more typical osteoporotic fractures seen in postmenopausal women and older men.

In light of the findings presented in this thesis, current recommendations for individuals with CP who are at high risk of low BMD or have a past history of fracture, should include 1-2 yearly DXA of the lumbar spine and femoral neck to monitor BMD and body composition. DXA images need to be assessed to ensure changes in BMD are not due to motion artefact or positioning difficulties. Risk factors for low BMD include those with a high GMFCS, hypogonadism and nutritional issues. Lateral thoracolumbar x-ray of the spine should be taken at baseline and if there are concerns regarding new back pain. During adolescence, Tanner staging should be performed routinely to assess pubertal development with biochemical monitoring if indicated. Given that low bone mass in adults with CP is predominantly an issue of lack of bone accrual earlier in life, intervention should not be purely based on BMD. The use of bisphosphonates should be considered if there has been a significant decline in BMD or a new minimal trauma fracture.

Similarly, in SB the types of fractures are different in adults compared to children. Distal femur fractures predominate in children but rarely occur in adults. Adults have a reduced rate of fracture compared to adolescents and children, which may reflect the changing geometry and size of bone with growth, as well as increased awareness of their environment and falls risk. In SB, obesity rather than low BMD is of greater concern which may be due to the fat-bone relationship although this needs confirmation in larger studies.

Current recommendations for individuals with SB therefore differ to those with CP. DXA should only be performed in those who have sustained a fragility fracture. The focus needs to be on cardiometabolic health with screening for hypertension, hypercholesterolemia and promotion of healthy weight targets.

Future directions

There are ongoing important gaps in our understanding of bone health in adults with chronic neurological disease. The role of bisphosphonates in fracture prevention has not been shown in randomised control trials in adults with CP or SB. The optimal timing of bisphosphonates needs to be explored; particularly given the childhood burden of fracture in CP and the occurrence of vertebral fractures in early adulthood. The importance of gonadal function for bone health in CP needs to be established using DXA-derived measures, with the dose and formulation of sex-steroid replacement determined. Maintenance of function and lean mass is difficult to achieve in this population, physiotherapy programs need to be designed and assessed for efficacy. Further longitudinal data in older adults with CP are required to determine at which age bone loss occurs and if it occurs at a more rapid rate than the general population. An evidence-based screening protocol for cardiometabolic health in adults with SB needs to be established to address increasing mortality secondary to its complications.

Chapter 6 - References

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Appendices

Appendix 1 – Severe acute phase response after zoledronic acid in adults with cerebral palsy

Severe acute phase response after intravenous zoledronic acid in adult patients with cerebral palsy

Osteoporosis is a recognised problem in patients with cerebral palsy (CP), with fractures having a devastating impact on already reduced mobility. Studies examining bisphosphonate treatment in children with CP are limited,[1] with no published data evaluating the use of intravenous zoledronic acid (ZA) in adults with CP. We describe our preliminary experience with intravenous ZA in six adults with CP. As many have gastrointestinal issues, or require percutaneous endoscopic gastrostomy feeding, intravenous ZA is an attractive option. In addition, the long half-life of ZA allows for less frequent dosing which has a logistic benefit for poorly mobile patients.

Clinical characteristics of the patients are shown in Table 1. Of six patients, four (all males) had significant acute phase response (APR) with fever, myalgias and arthralgias following ZA infusion. Only one patient had prophylactic paracetamol given (Patient 2). Two required hospitalisation (Patients 1 and 2) due to the severity of the reaction.

Patient 1 presented 12 h following 4 mg ZA with a fever of 39°C, nausea and generalised pain. He had an elevated white cell count of 16.5×10^9/L and C-reactive protein level of 129.2 mg/L (RR 0–5). Investigations, including blood and urine cultures and CT brain/abdomen/pelvis, were unremarkable. He was discharged Day 4 with improvement in his clinical state and no source of infection found. No glucocorticoids were administered.

Patient 2 presented 20 h following 2 mg ZA with a temperature of 37.8°C. He was in severe generalised pain despite regular post-infusion paracetamol and required opioid analgesia. He was discharged the following day after resolution of his pain. Both patients had normal serum calcium and were vitamin D replete with levels >80 nmol/L.

The two other patients who experienced APR (Patients 3 and 4) had low grade fever with mild myalgias and arthragias which resolved within 2 days. They both have had further doses of ZA with no recurrence of the APR.

APR has been reported in up to 32% of patients within 3 days of ZA in randomised clinical trials of postmenopausal women with osteoporosis.[2] Previous analyses of studies in postmenopausal women have found low vitamin D levels and younger age to be associated with increased risk of APR.[3] Of note, lower doses of 0.25–2 mg were not associated with reduced incidence of APR in a Phase 2 study of ZA.[4] The pathophysiology of the APR is yet to be fully understood, but thought to involve a subset of peripheral T cells, γδ T-cell receptor lymphocytes which on exposure to ZA can differentiate into effector memory T cells and release pro-inflammatory cytokines.[5] Patients with cystic fibrosis (CF) are particularly susceptible to APR following ZA, and prednisolone is routinely co-prescribed to reduce its likelihood.[6] In these patients, the increased baseline production in pro-inflammatory cytokines, such as tumour necrosis factor-α (TNF-α), may explain the increased risk of APR after intravenous bisphosphonates.

It is unclear why adults with CP appear to have more frequent and severe APR to ZA, and whether pre-treatment with glucocorticoids can attenuate this response. There are no post-marketing data regarding APR in adults with CP receiving ZA to confirm our findings. Although the numbers are small, we believe that attention should be drawn to this as assessment and management of APR in this population is particularly challenging where neurocognitive impairment exists. This finding has not been seen in other populations of young patients with bone disease or immobility, such as osteogenesis imperfecta, Duchenne muscular dystrophy or spinal cord injury. There is evidence of a chronic inflammatory state, which plays a role in muscle pathology and the ongoing neurological deterioration in CP. In school-age children with CP, increased plasma TNF-α expression, as well as increased TNF-α expression from stimulated peripheral blood mononuclear

Table 1 Clinical characteristics of the patients who received ZA

Patient	Age (years), sex	GMFCS	PEG	Anti-convulsant	Vitamin D (nmol/L)	Fracture	FN Z score	Change in BMD (%)†
1	21, M	IV	N	N	82	N	−4.3‡	+5.6
2	36, M	V	N	Y	137	N	−2.3	NA
3	24, M	V	Y	N	85	Y	−1.9	+2.9
4	24, M	V	N	N	54	N	−3.3	+9.5
5	66, M	III	N	N	112	Y	−2.4	NA
6	18, F	V	Y	N	91	Y	−2.6	+8.7

Shaded area represents patients who had infusion reactions. †Change in BMD = % change post zoledronic acid infusion during 18–24 month follow-up scan. ‡Lumbar spine Z score (FN unavailable). BMD, bone mineral density; F, female; FN, femoral neck; GMFCS, gross motor functional classification scale; M, male; N, no; NA, not available; PEG, percutaneous endoscopic gastrostomy; Y, yes; ZA, zoledronic acid.

cells, has been found.[7] Whether the underlying pro-inflammatory state in conditions such as CP and CF predisposes these patients to APR mediated by T cells requires further characterisation. Given the paucity of literature in prevention and treatment of bone disease in adults with CP, we believe our report has important clinical implications and informs a new research agenda.

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Appendix 2 – Optimising bone health in cerebral palsy through the lifespan

Optimizing bone health in cerebral palsy across the lifespan

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SIR–We read with interest the new osteoporosis care pathway from the American Academy of Cerebral Palsy and Developmental Medicine (AACPDM), highlighting the important issue of bone health in children with cerebral palsy (CP).[1] The focus of the guidelines is on optimizing nutrition, vitamin D supplementation, encouraging weight-bearing to promote skeletal development, and considering bisphosphonate administration as a preventive and therapeutic measure. We welcome the timely release of the guidelines, given that individuals with CP are living longer and are likely to develop osteoporosis as a consequence of factors beginning in childhood.

With recent studies documenting increased skeletal fragility in adulthood, we would like to raise a number of points that may impact the assessment and management of bone health in childhood and transition to adulthood.

GONADAL STATUS

Puberty plays a major role in accrual of bone strength in growing children and adolescents, so that delayed or disordered pubertal development may have detrimental effects.

In young adults with CP, we found that 20% were hypogonadal on clinical and biochemical assessment.[2] Adults with hypogonadism had lower lumbar spine bone density and lower lean tissue mass on dual-energy x-ray absorptiometry (DXA) assessment than eugonadal adults with CP. The majority had hypogonadotrophic hypogonadism, with possible explanations including poor nutrition and chronic illness likely leading to functional hypothalamic hypogonadism. Alternatively, hypothalamic–pituitary axis dysfunction may result from the early injury to the immature brain seen in CP. It is unclear when hypogonadism is manifesting in CP; it may be during late childhood, given a cross-sectional study of 207 children with CP by Worley et al.[3] that demonstrated altered pubertal progression. Timing of puberty will also be influenced by nutritional status, with overweight promoting earlier onset, and underweight delaying progression.

Given the negative effect of established hypogonadism on bone mineral density, clinical assessment of pubertal progression with Tanner staging is recommended. The onset of breast development marks the onset of puberty in young females; increase in testicular volume to 4mL marks puberty onset in young males. Puberty is delayed if pubertal change has not begun by age 13 in young females, or by age 14 in young males. If there is concern regarding pubertal delay, further investigations should include bone age x-ray with biochemistry for gonadotrophins and testosterone/estradiol. Consideration of sex hormone replacement should be made if deficits are identified, bearing in mind that pubertal growth, psychosocial sexual development, and menarche are recognized as challenging for caregivers, and concerns about aberrant pubertal progression may not be raised with health care professionals.

BONE DENSITY MEASUREMENTS

In the recent guidelines by Ozel et al.,[1] the lateral distal femur or the total body is the site of choice for bone density measurement using DXA. The distal femur is clinically relevant, being a common site of fracture in children, and has established reference ranges in children. It has been validated with a correlation noted between z-scores and fracture history in children with CP.

In adults however, there is no normative data for lateral distal femoral assessment and this technique is not readily available. A number of studies in adults with CP have used the lumbar spine, femoral neck, or total hip for measurement,[2,4,5] with z-scores at these sites correlated to fracture risk. Furthermore, this site may be more relevant to the more typical fragility fractures seen in adults with CP, which include vertebral, ankle, and rib fractures. In adolescents transitioning to adult care, it may be worth considering using these sites in addition to the distal lateral femur to prepare for ongoing monitoring in adulthood.

BODY COMPOSITION

The use of whole body DXA scanning also allows the assessment of body composition. This is an invaluable tool which allows differentiation between fat and lean tissue mass, with relevance to those caring for patients with nutritional concerns. Nutritional deficits can lead to poor muscle mass and failure of skeletal growth. Given that body composition can be calculated from a whole body scan with no further radiation exposure, it is worthwhile requesting this assessment in addition to bone density.

TRANSITION TO ADULT CARE

In summary, osteoporosis in CP is a disease that begins in childhood but manifests throughout life as fractures. Ensuring follow-up is paramount in maintaining quality of life and mobility of those who have had previous fragility fractures, as well as those with multiple risk factors for reduced bone density. This is a shared task for both paediatricians and adult physicians.

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