Bone Health and Fracture Risk Factors in Children Living in New Zealand

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Abstract

Background: Fractures are common in childhood but are a neglected public health issue. A complex interplay of non-modifiable (e.g. genetics) and modifiable risk factors (e.g. obesity, physical activity, calcium intake, sugar-sweetened beverages (SSB), and vitamin D status) have been associated with childhood fractures. Early identification of risk factors during childhood could enable lifestyle changes to be instigated, thus enhancing bone mineralisation, preventing fractures, and improving adult bone health.

Vitamin D is an essential nutrient for the absorption of calcium from the intestine, regulation of serum calcium, and bone health. An adequate 25(OH)D concentration is considered important for ensuring bone health during childhood since there is a relationship between vitamin D deficiency and insufficiency and skeletal health problems such as rickets, metabolic bone disease, and hypocalcaemia during childhood. Unfortunately, there is limited data available regarding the vitamin D status and risk factors for vitamin D deficiency in New Zealand children.

Measuring paediatric bone mineral status can help us to find children who could be exposed to an increased risk of bone problems (e.g. osteopenia, osteoporosis) later in their life. Quantitative ultrasound (QUS) is a measuring method commonly employed in paediatric populations for assessing skeletal status. Childhood overweight and obesity are also associated with developing musculoskeletal problems, injuries, and fractures early in childhood. Recently, bioelectrical impedance analysis (BIA) has received much attention as a method for measuring body composition. However, the validity of these two devices needs to be investigated in a New Zealand paediatric population.

Aims: The main aim of this study is to explore fracture history and related risk factors in children living in Auckland, New Zealand. The secondary aims are to determine the wintertime vitamin D

status of children living in Auckland and its determinants and to validate the QUS and in-built algorithm of BIA measurements against dual-energy X-ray absorptiometry (DXA) in children.

Methods: This was an observational, cross-sectional study in a sample of school-age children (aged 8 – 13 years old) living in Auckland (during August 2016 and 2017 – late winter in the southern hemisphere). Six local primary schools across Auckland were selected. We originally approached schools through a collaboration of primary school science teachers and asked for expressions of interest. We then endeavoured to recruit schools specifically to include a wide range of socio-demographic levels and ethnicities. All school children within the specified age group were invited to participate.

Children were stratified by gender (2 groups), ethnicity (6 categories), and skin colour (4 groups), and logistic regression used to determine the contribution of risk factors for fracture and vitamin D deficiency. A sample of 10-15 per factor per group is the standard requirement for regression analysis, meaning that 480-720 participants would be required to investigate the above-mentioned factors. To validate the QUS and BIA, a sample of 128 children was calculated based on the G*Power program [version 3.1 software: medium effect size: 0.6; power: 95%; the level of significance: 5%]. Healthy children were recruited from primary schools. Children were excluded if they had 1) a history of any disease affecting vitamin D metabolism (e.g. cardiac, kidney or liver disease) or 2) a history of any long-term medication use (e.g. steroids) 3) had any surgical implants, metal screws or similar, or 4) had a cast.

Children received an envelope containing a study information sheet, consent form, and some questionnaires (e.g. fracture history, siblings' history of fractures, family osteoporosis history, physical activity (PA), ethnicity, skin colour, and sun exposure). A dairy and other calcium-containing foods food frequency questionnaire and SSB questionnaire were completed by the children with help from parents. Children who, together with their parents, gave written consent and returned the completed questionnaires, were measured at school.

Tests included anthropometric (weight and height) and body composition (bioelectrical impedance analysis, InBody720, Seoul, Korea) measurements, and finger-prick blood spot to measure capillary 25-hydroxyvitamin D (25(OH)D) concentrations. Some children were invited to the Nutrition Research Facility at Massey University, Albany on one occasion to test the validity of the QUS and BIA measurements against a DXA scan. Total body less head (TBLH), bone mineral content (BMC), bone mineral density (BMD), and body composition (fat-free mass, fat mass, and body fat percentage (%BF)) were measured with DXA (QDR Discovery A, Hologic, USA); calcaneal BMD and stiffness index (SI) with QUS (Sahara QUS, Hologic, USA), and total mass and %BF on the InBody 230 (Biospace Ltd., Seoul, Korea). Relative validity was assessed using Pearson's and Lin's concordance correlation coefficients (CCC), and Bland-Altman plots.

Results: A total of 647 children (354 girls) with the mean ± standard deviation (SD) age of 9.8 ± 0.7 years were recruited. New Zealand European (n = 252) (NZE) and South Asian (n = 68) children reported the lowest (20.2%) and highest (44.1%) fracture incidence, respectively. New Zealand European, compared to South Asian children, had higher 25(OH)D concentrations (74.6 ± 19.8 vs. 48.4 ± 19.3 nmol/L, $P < 0.001$), higher total calcium intake (764.0 ± 394.4 vs. 592.7 ± 266.3 mg/day, $P < 0.018$), and lower %BF (19.5 ± 6.6 vs. 23.4 ± 8.4, $P < 0.003$). The main determinants of fracture history for boys were high %BF, low 25(OH)D, low calcium intake, high SSB consumption, siblings' fracture history, family osteoporosis history, and being South Asian; and for girls were high SSB consumption, siblings' fracture history, and family osteoporosis history.

Five hundred and seven children agreed to do the finger prick test. Mean ± SD 25(OH)D concentration were 64.0 ± 20.8 nmol/L, with 30.8% of the population presenting with 25(OH)D ≥ 75 nmol/L, 41.4% 50-75 nmol/L, and 27.8% < 50 nmol/L. Capillary 25(OH)D was significantly higher in NZE compared to all other ethnic groups (75.0 ± 20.1 nmol/L, $P < 0.001$). Children with dark/brown skin colour had lower 25(OH)D concentration compared to other categories of skin colour (51.7 ± 18.0 nmol/L, $P < 0.001$). Using multiple logistic regression analysis, determinants of

25(OH)D were %BF and ethnicity.

In 124 healthy children, positive correlations between QUS SI and DXA (BMC and BMD) were observed (range = 0.30-0.45, $P < 0.01$). Results from Lin's CCC test showed that almost perfect correlations between BIA and DXA fat-free mass (0.96), fat mass (0.92), and substantial correlation for %BF (0.75) ($P < 0.05$).

Conclusion: Approximately one-quarter of our participants reported one or more fractures during their childhood. Our results showed that being of South Asian ethnicity was a significant risk factor for fracture in boys. Some children were at high risk of vitamin D deficiency during winter months, for whom vitamin D supplementation might be recommended. Good nutrition (especially good sources of calcium and reducing SSB intakes) should be recommended to children during growth and development to reduce their risk of fractures.

Among 507 children, approximately one-third had 25(OH)D < 50 nmol/L. Determinants of a 25(OH)D < 50 nmol/L included %BF and ethnicity. Wintertime serum 25(OH)D was highly variable. There are some children at high-risk of 25(OH)D < 50 nmol/L for whom supplementation may be considered.

The current study was the first to evaluate the validity of calcaneal QUS and BIA against DXA in a paediatric New Zealand population for measuring bone density and body composition, respectively. Although BIA results were not as accurate as DXA and DXA remains the gold standard method for clinical assessment, BIA can be an alternative method for investigating body composition among children in large cohort field studies. Calcaneal QUS and DXA are not interchangeable methods for measuring bone density in children similar to our study population.

Keywords: Bone, fracture, children, and determinants (e.g. milk consumption, SSB, physical activity, obesity, vitamin D).

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List of Abbreviations

%BF	Body Fat Percentage
1,25(OH)$_2$D	1α,25-dihydroxyvitamin D
24,25(OH)$_2$D	24,25-dihydroxyvitamin D
25(OH)D	25-hydroxy vitamin D
2D LC-MS/MS	Two-Dimensional Liquid Chromatography Tandem Mass Spectrometry
aBMD	areal Bone Mineral Density
ACC	Accident Compensation Corporation
AI	Adequate Intake
BA	Bone Area
BIA	Bioelectrical Impedance Analysis
BMC	Bone Mineral Content
BMD	Bone Mineral Density
BMI	Body Mass Index
BUA	Broadband Ultrasound Attenuation
C cells	Clear cells
CaR	Ca^{2+}-sensing Receptors
CCC	Concordance Correlation Coefficients
CI	Confidence Intervals
CLA	Conjugated Linoleic Acid
cm	Centimetre
CMPA	Cow's Milk Protein Allergy
CT	Calcitonin
CT	Computed Tomography
CYP24A1	Cytochrome P_{450}, family 24, subfamily A, polypeptide 1
CYP27B1	Cytochrome P_{450}, family 27, subfamily B, polypeptide 1
CYP2R1	Cytochrome P_{450}, family 2, subfamily R, polypeptide 1
D_2	Ergocalciferol
D_3	Cholecalciferol
dB/MHz	Decibel by megahertz
DBS	Dried Blood Spots
DXA	Dual-energy X-ray Absorptiometry
EAR	Estimated Average Requirement
FFM	Fat-Free Mass
FFQ	Food Frequency Questionnaire
FGF23	Fibroblast Growth Factor 23
FGFR-3	Fibroblast Growth Factor Receptor-3
FM	Fat Mass
g	Gram
GCs	Glucocorticoids
GH	Growth Hormone
GHR	Growth Hormone Receptors

GHRH	Growth Hormone-Releasing Hormone
Gla	γ-carboxyglutamic acid
Glu	Glutamate
HCl	Hydrochloric Acid
IGF-1	Insulin-like Growth Factor-1
IL	Interleukins
ILGF-1R	Insulin-like growth factor-1 receptor
IPAQ	International Physical Activity Questionnaire
ISAK	International Society for the Advancement of Kinanthropometry
ISCD	International Society for Clinical Densitometry
IU	International Unit
IUGR	Intrauterine Growth Restriction
K_1	Phylloquinone
K_2	Menaquinone
K_3	Menadione
kg	Kilogram
kHz	Kilohertz
LOA	Limits of Agreement
LS	Lumbar Spine
m/s	Metre per second
m^2	Square of metre
MBP	Milk Basic Protein
MET	Metabolic Equivalent
mg	Milligram
ml	Millilitre
mmol	Millimole
MRI	Magnetic Resonance Imaging
n	Number
nmol/L	Nanomoles per litre
NZ	New Zealand
NZE	New Zealand European
NZEO	New Zealand European and Other
OH	Hydroxyl radical
OR	Odds Ratio
PA	Physical Activity
PBM	Peak Bone Mass
PGs	Prostaglandins
Pi	Phosphate ion
PTH	Parathyroid Hormone
QUS	Quantitative Ultrasound
RANK-L	Receptor Activator of Nuclear factor-kB Ligand
RDA	Recommended Dietary Intake
ROI	Region of Interest
SD	Standard Deviation

SI	Stiffness Index
SOS	Speed of Sound
SPF	Sun Protection Factor
SSB	Sugar-Sweetened Beverages
T_3	3,5,3`-L- triiodothyronine
T_4	Thyroxine
TB	Total Body
TBLH	Total Body Less Head
TBW	Total Body Water
TH	Thyroid Hormone
TSH	Thyroid-Stimulating Hormone
UL	Upper Level of intake
UV	Ultraviolet
UVβ	Ultraviolet radiation beta
Vb	Velocity bone
VDBP	Vitamin-D-Binding Protein
VDR	Vitamin D Receptors
WB	Whole Body
WHO	World Health Organization
µg	Microgram

List of Manuscripts and Conference Presentations

Published Manuscripts

The following publications have been included in this thesis and incorporated as different chapters in manuscript format. Therefore, in some cases, there may be replication.

Paper I

Maryam Delshad, Kathryn L Beck, Cathryn A Conlon, Owen Mugridge, Marlena C Kruger, Pamela R von Hurst (2020). Fractures risk factors among children living in Auckland, New Zealand. Journal of Steroid Biochemistry and Molecular Biology **200**: 105655.

Incorporated as Chapter Three

Paper II

Maryam Delshad, Kathryn L Beck, Cathryn A Conlon, Owen Mugridge, Marlena C Kruger, Berit P Jensen, Jing Ma, Pamela R von Hurst (2019). Wintertime Vitamin D status and its related risk factors among children living in Auckland, New Zealand. New Zealand Medical Journal **132**(1504): 67-76.

Incorporated as Chapter Four

Paper III

Maryam Delshad, Kathryn L Beck, Cathryn A Conlon, Owen Mugridge, Marlena C Kruger, Pamela R von Hurst (2020). Validity of quantitative ultrasound and bioelectrical impedance analysis for measuring bone density and body composition in children. European Journal of Clinical Nutrition. In press: doi: 10.1038/s41430-020-00711-6.

Incorporated as Chapter Five

Conference Presentations

I Maryam Delshad, Kathryn L Beck, Cathryn A Conlon, Owen Mugridge, Marlena C Kruger, Pamela R von Hurst. Fracture risk factors among children living in New Zealand.

- Poster presentation at the Nutrition Society of New Zealand: Annual Scientific Meeting, Auckland, 2019, supported with a Nutrition Society of New Zealand conference grant
- Poster presentation at the 22nd vitamin D workshop, New York City, 2019

II Maryam Delshad, Kathryn L Beck, Cathryn A Conlon, Owen Mugridge, Marlena C Kruger, Berit P Jensen, Jing Ma, Pamela R von Hurst. Wintertime Vitamin D status and its related risk factors among children living in Auckland, New Zealand.

- Oral presentation at the UV workshop 2018 in Wellington, supported with a Massey University conference grant
- Poster presentation at the 22nd vitamin D workshop, New York City, 2019

III Maryam Delshad, Kathryn L Beck, Cathryn A Conlon, Owen Mugridge, Marlena C Kruger, Pamela R von Hurst. Validity of quantitative ultrasound (QUS) and bioelectrical impedance analysis (BIA) against dual-energy X-ray absorptiometry (DXA) for measuring bone density and body composition in children.

- Oral presentation at the Nutrition Society of New Zealand: Annual Scientific Meeting, Auckland, 2019

Chapter One

Introduction

1. Introduction

Bone health in children has drawn little attention and bone research is predominantly focused around peri- and post-menopausal women, and those with diagnosed osteoporosis, that is, when the problems manifest. Accumulation of bone density early in life is considered critical for the development of optimal peak bone mass (PBM). Peak bone mass is the maximum amount of bone acquired at skeletal maturity when accrual ceases or plateaus (Gordon, Zemel et al. 2017). Many experts believe that enhancing PBM early in childhood and stabilising it during young adulthood may delay the development of osteoporosis and fracture later in life (Bachrach 2001, Borges and Brandao 2006).

A partial or a complete break in the bone is called a bone fracture, which can be caused by a strong external pressure and force. Fractures do not only occur later in life but are also common in children, which can cause pain, lack of mobility, and loss of independence (Manias, McCabe et al. 2006). Despite this, determinants of fracture risk have largely been ignored. Insufficient consumption of calcium and inadequate exposure to sunlight resulting in low concentration of vitamin D during childhood can cause poor bone mineral density (BMD) and consequently lead to fractures in early life (Heaney, Abrams et al. 2000). Childhood fractures can affect daily activities and may be an indicator of increased risk of fracture in adulthood (Clark 2014). Approximately one-third of children have a bone fracture before the age of 17 years (Cooper, Dennison et al. 2004). In 1998, it was estimated that 7500 fractures occur annually in New Zealand children aged 3-15 years, an incidence of approximately 1% of this age group (Goulding, Cannan et al. 1998). In 2000, the annual incidence of distal forearm fractures in Dunedin children aged 3–15 years were estimated to be 10.4 per 1000 for both boys and girls (Jones, Cannan et al. 2000).

Several non-modifiable (e.g. age, gender, genetics, disease) and modifiable risk factors (e.g. obesity, physical activity, and nutrition) have been considered to affect skeletal mineralisation and PBM. Among modifiable risk factors for fractures, nutrition is a widely investigated and established

determinants. A well-balanced diet is a very important requirement for normal growth and puberty. Among all nutrients, calcium is well known for its critically important role in the acquisition of BMD (Stagi, Cavalli et al. 2013). The main sources of calcium intake in New Zealand are milk and dairy products (e.g. cheese, cream, and yoghurt) (Ministry of Health 2012). The Ministry of Health (2012) recommends that children (2-12 years) and young people (13-18 years) consume two to three servings per day of milk and dairy products in order to meet their calcium requirements. In the 2002 National Children's Nutrition Survey, approximately 40% of New Zealand children reported drinking milk (not flavoured) once a day. New Zealand European children were more likely to consume milk daily (41% boys; 39% girls) compared with Māori (39% boys; 33% girls) and Pacific children (32% boys; 23% girls) (Ministry of Health 2003). Furthermore, results of a survey of New Zealand children conducted in 2008/2009 showed that 62.5% of children and young people drank plain milk at least once a week, including 24.2% who drank plain milk seven or more times a week (Clinical Trials Research Unit 2010). Milk consumption in children is influenced by various factors, including such as age, gender, ethnicity, sugar-sweetened beverages (SSB), parental influence, and dietary patterns (Goulding, Rockell et al. 2004). Also, adverse reactions to milk consumption including side effects of lactose intolerance or milk protein allergy can unfavourably influence milk consumption. Avoiding milk consumption has been associated with a history of fractures in children (Goulding, Rockell et al. 2004, Black, Williams et al. 2002).

Vitamin D is another nutritional factor involved in musculoskeletal health. Vitamin D is an essential nutrient for the absorption of calcium from the intestine, regulation of serum calcium, and bone health (Lieben, Carmeliet et al. 2011). An adequate 25(OH)D concentration is considered important for ensuring bone health during childhood since there is a relationship between vitamin D deficiency and insufficiency and skeletal health problems such as rickets, metabolic bone disease, and hypocalcaemia during childhood (Wagner and Greer 2008, Moon, Harvey et al. 2014). In New Zealand, sunlight exposure is the major source of vitamin D for most people since diet alone is not adequate to meet vitamin D requirement (Ministry of Health and Cancer Society of New Zealand

2012). However, the efficacy of synthesised vitamin D through the skin can be influenced by various factors (e.g. geographic latitude, season, time of day, ethnicity, obesity, skin pigmentation, use of sunscreens, exposed body surface, and exposure duration) (Kimlin 2008, Rockell, Green et al. 2005, Vierucci, Del Pistoia et al. 2013, Godar, Pope et al. 2012).

Childhood overweight and obesity are associated with developing musculoskeletal problems, injuries, and fractures early in childhood (Adams, Kessler et al. 2013, Paulis, Silva et al. 2014). There are a variety of other methods available for measuring body composition (e.g. skinfold, thickness ultrasound, and hydro-densitometry (or underwater weighing)) however, dual-energy X-ray absorptiometry (DXA) is one of the most applicable techniques and is becoming increasingly available for both clinical and research purposes. Dual-energy X-ray absorptiometry can provide information about compartments of body composition including fat mass (FM), fat-free mass (FFM), and body fat percentage (%BF) (Ellis, Shypailo et al. 1994). Recently, bioelectrical impedance analysis (BIA) has received much attention as an alternative method for measuring body composition since it is a fast, safe, and easy to apply both in the field and in clinical settings for tracking of body composition changes in children (Noradilah, Ang et al. 2016).

Over the past decade, with the development of more bone measurement methods, the demand for evaluation of paediatric bone mineral status has increased (Baroncelli 2008). The main reason for measuring paediatric bone mineral status is to find children who could be exposed to an increased risk of bone problems (e.g. osteopenia, osteoporosis) later in their lives. One of the important osteoporosis risk factors in adulthood is the amount of bone gained during childhood growth and its subsequent rate of loss (Specker and Schoenau 2005). It is the gold standard method available for measuring BMD (Binkley, Berry et al. 2008) and bone mineral content (BMC) (Specker and Schoenau 2005). Quantitative ultrasound (QUS) is another measuring method, which is employed in paediatric populations for assessing skeletal status (Specker and Schoenau 2005). Two main variables can be measured by QUS devices, speed of sound (velocity of ultrasound waves inside of the bone) (m/s) and broadband ultrasound attenuation (slope of attenuation of mechanical waves travelling

through the bone) (decibels/mega-hertz). From these, a third parameter is calculated (stiffness index (SI)) (Baroncelli 2008). It is safe, easy to use, radiation-free, and portable. However, a major issue with the use of QUS is the lack of information regarding the validity of ultrasonic measurement of bone in a paediatric population living in New Zealand.

While DXA measurements are accurate and valid, the method is expensive, requires trained operators, and exposes children to ionising radiation (Binkovitz and Henwood 2007). Therefore, it is not suitable for routine paediatric research practice and is impractical for use in the field. On the other hand, although both QUS and BIA devices have been used in the assessment of BMD and body composition respectively, their validity against conventional reference methods (e.g. DXA) as a measure of BMD and body composition in children remains uncertain. Therefore, the validity of these two devices needs to be investigated in the paediatric population.

1.1 Aims and Objectives

Main Aim

The main aim of this study is to explore fracture history and related risk factors in children living in Auckland, New Zealand.

Secondary Aims

1. To determine the wintertime vitamin D status of children living in Auckland and its determinants.
2. To validate the QUS and in-built algorithm of BIA measurements against DXA in children.

The objectives of this research are:

1. To identify related risk factors for fracture history (e.g. intake of milk, calcium, and SSB, vitamin D status, physical activity (PA), ethnicity, body composition, SI, siblings' history of fractures, and family history of osteoporosis) in children.
2. To assess wintertime vitamin D status in children and to identify related risk factors for the deficiency (e.g. ethnicity, %BF, sun exposure, PA, skin colour, and gender).

3. To evaluate QUS derived measurements of bone density against DXA values in children.
4. To evaluate the in-built algorithm of BIA derived measurements of body composition against DXA values in children.

1.2 Hypothesis

1. Servings of milk, calcium, and SSB consumption, vitamin D status, PA, ethnicity, body composition, siblings' history of fracture, and family history of osteoporosis will increase or decrease the risk of fractures in children.
2. Some factors (e.g. ethnicity, skin colour, sun exposure behaviours, body composition, PA, gender, and age) will be significant predictors of vitamin D deficiency.
3. Quantitative ultrasound is a valid instrument for measuring bone density in children.
4. Bioelectrical impedance analysis is a valid instrument for measuring body composition in children.

1.3 Structure of the Thesis

This thesis starts with an introduction (chapter one), which highlights an overview of aims, objectives, and hypothesis. Chapter two highlights a review of the relevant literature. This chapter comprises of different sections, each dedicated to different topics. The first section highlights an overview of bone structure, growth, and metabolism. This section is followed by a review, focusing on bone fracture, the evidence for non-modifiable (e.g. genetic, age, and gender) and modifiable bone risk factors (e.g. diet and nutrition, PA, vitamin D), and obesity for bone fracture, followed by factors affecting bone density (including dietary factors and hormones). To meet the requirement of this thesis, vitamin D and bone health and the importance of milk/dairy products are discussed. As body composition is an outcome measure, methods measuring paediatric body composition (e.g. BIA) are discussed. Similarly, because there are some commonly used methods to assess bone density in children such as DXA and QUS, these tools are discussed.

Chapters three, four, and five address the primary and secondary aims of the study, each presented in

the form of a manuscript for publication in a peer-reviewed journal. All the details regarding the methodological procedures of the studies, recruitment, and statistical analysis were explained in each chapter. Chapter three highlights the primary outcome findings, the fracture risk factors among children living in Auckland, New Zealand. Chapters four and five report the secondary outcome findings. Chapter four reports the wintertime vitamin D status and its related risk factors among children living in Auckland. It is widely acknowledged that vitamin D is a key nutrient associated with bone health and its status is affected by seasonality. This chapter is followed by chapter five which consists of a validation study, the validity of QUS and BIA against DXA for measuring bone density and body composition in children. Both QUS and BIA machines have been used in the assessment of BMD and body composition, respectively. However, their validity against conventional reference methods such as DXA as a measure of BMD and body composition in children remains uncertain. As each study is presented in the form of a manuscript suitable for publication, there may be some repetition throughout the thesis.

Chapter six consists of the discussion of findings which highlights the main results observed including their significance and relevance, and methodological strengths and limitations, followed by a brief conclusion, and recommendations for future research. Appendices include each chapter's appendices as well as an information sheet and consent forms.

1.4 Researchers' Contribution

Researchers	Contributions
Maryam Delshad Siyahkaly	PhD student
	Involved in: participant recruitment and data collection
	Responsible for most aspects of the study including statistical analysis and writing the thesis report
	Responsible for all aspects of the manuscripts including conceptualisation and design of manuscripts, searching the literature, data extraction, data analysis, drafting manuscript, and manuscript submission

Associate Professor Pamela R von Hurst	Academic supervisor and assistance including: Conceptualisation and design of the study, acquisition of funding and ethics approval, recruitment, data collection, reviewing thesis, advising data analysis, and reviewing all manuscripts
Associate Professor Kathryn L Beck	Academic co-supervisor and assistance including: Recruitment, data collection, reviewing thesis, advising data analysis, and reviewing all manuscripts
Associate Professor Cathryn A Conlon	Academic co-supervisor and assistance including: Recruitment, data collection, reviewing thesis, advising data analysis, and reviewing all manuscripts
Prof Marlena Kruger	Academic co-supervisor and assistance including: Reviewing thesis, advising data analysis, and reviewing all manuscripts
Owen Mugridge, MSc	Responsible for: Project coordination, recruitment, and data collection
Dr Berit P Jensen	Responsible for: Measurement and analysis of the children's blood sample
Dr Jing Ma	Responsible for: Measurement and analysis of the children's blood sample

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I declare that my role in the study as indicated above is representative of my actual contribution.

Maryam Delshad Siyahkaly

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Chapter Two

Literature Review

2. Introduction

A fracture is a broken bone, which can be a partial or a complete break in the bone. Although bone is a stiff tissue, it bends and breaks under strong external pressure and force. Childhood fractures are common, and cause pain, lack of mobility, and loss of independence (Manias, McCabe et al. 2006). Landin (1983) was a pioneer who investigated the epidemiology of fracture among children. He ran a study in children (aged 0-16 years) and studied 8682 fractures, finding that incidence of fractures is more common in boys (42%) compared to girls (27%) (Landin 983). In a study by Mayo Clinic, the researchers compared the incidence of fracture in children in 1970s and 2000s and showed that there are 32% and 58% more fractures in boys and girls respectively (Khosla et al. 2003). In Dunedin (New Zealand), the incidence of fractures was estimated as 1% in a group of girls aged 3-15 years (Goulding, Cannan et al. 1998). Two years later in 2000, another author estimated the annual incidence of distal forearm fractures as 10.4/1000 (1.04%) for both boys and girls (Jones, Cannan et al. 2000). The same authors conducted another study in 2002 and investigated 601 New Zealand children up to 18 years old and found 172 cases had sustained a single fracture and 119 of children fractured more than once (Jones, Williams et al. 2002). Yeh et al. (2006) showed 468 fracture events with 212 (67.7%) sustaining a single fracture and 101 children with more than one fracture among Dunedin (New Zealand) children (n = 313, 145 girls and 168 boys, <13 years).

To bring about change in behaviour for health benefits, it is well acknowledged that the population of interest has to understand the severity of the potential risk, their susceptibility to it, the benefits of making change, and education alone is not sufficient (Worsley 2002). Some studies have attempted to identify the risk factors for poor bone health and fractures in children. There are non-modifiable (e.g. age, gender, and genetics) and modifiable risk factors (e.g. obesity, physical activity, nutrition, and vitamin D status) that can affect skeletal mineralisation and peak bone mass (PBM) (for the brief discussion on these factors refer to non-modifiable and modifiable determinates of bone fracture). Calcium intake is an important determinant for the accrual of bone mass during normal growth in

childhood. Milk and dairy products are good sources of important macronutrients and micronutrients (e.g. calcium) (Dror and Allen 2014), and bone anabolic growth factors (e.g. osteoprotegerin) (Kanezler, Bodamyali et al. 2001). Milk basic protein (MBP) consists of different components, which can promote bone formation and increasingly suppress bone resorption (Kanezler, Bodamyali et al. 2001, Yamamura, Aoe et al. 2002). Milk and dairy product intake are also associated with the stimulating of bone formation and leg length growth through increasing expression of insulin-like growth factor-1 (IGF-1) (Uenishi, Ishida et al. 2007). However, some factors can affect milk consumption. The importance of milk consumption and factors affecting milk consumption will be discussed in the section importance of milk/dairy products. There is a relationship between vitamin D deficiency, and insufficiency and skeletal health problems during childhood. In the literature, several factors have been postulated that can influence vitamin D absorption. This will be discussed in the section on vitamin D and bone health. When investigating body composition in children, it is important to use techniques that are as accurate and precise as possible. There are a variety of methods available for measuring body composition of paediatric populations. Recently, bioelectrical impedance analysis (BIA) has received much attention, which will be discussed in the section methods for measurement of paediatric body composition. Measuring paediatric bone mineral status will help us to identify children who are at risk of developing osteopenia and osteoporosis later in their life. There are different techniques, which can be used for measuring bone mineral density (BMD) in paediatric population including dual-energy X-ray absorptiometry (DXA) and quantitative ultrasound (QUS), which will be discussed in the section on methods for measurement of paediatric body health.

A literature search was conducted using MEDLINE, EMASE, PubMed electronic databases, and Google Scholar. Medical Subject Headings (MeSH), MeSH major topics, and free text terms were used to identify relevant publications and reference list were hand-searched for relevant articles. Due to limited evidence in the paediatric population, the evidence from adult studies is discussed.

Bone Structure, Growth and Metabolism

2.1 Bone

The human skeletal system is a composite of 206 separate bones along with their cartilage and provides a framework for the human body. The human bones have important roles in protecting vital and soft organs (e.g. heart and lung), storing and preserving 99% of the total body calcium, connecting muscles, tendons, and ligaments, supporting our movement, and producing blood cells (Marieb and Hoehn 2013).

2.2 Bone Structure

Bone is a composite organ made up of various tissues such as osseous tissues, nervous tissues, muscle, dense connective tissues, cartilage, epithelium, blood-forming tissues, and adipose tissues (Marieb and Hoehn 2013). There are two categories of bone structure including macrostructure and microstructure.

2.2.1 Macrostructure of the Bone

Textures of Bone

Macroscopically, there are two different types of bone; cortical bone (compact bone) and trabecular bone (spongy bone) (Djonic, Milovanovic et al. 2013). There are small spaces in the bone tissue for vessels and red bone marrow. These spaces are more in trabecular bone (cancellous or spongy bone) and less in cortical bone (compact bone) (Stagi, Cavalli et al. 2013). Cortical bone is located in the shafts and diaphysis region of long bones (e.g. femur, tibia, radius) but trabecular bone is found inside flat bones (e.g. rib cage) and the end of long bones (Stagi, Cavalli et al. 2013). The primary functions of these two bones cause the differences in their structure: cortical bone has a protective function, carries most of the mechanical load, and tolerates the burden of all muscles, while trabecular bone generally has metabolic functions (Brandi 2009).

Gross Anatomy of the Bone

The structure of a long bone shows the gross anatomical characteristics of a typical long bone. There are three structural parts in a long bone; diaphysis, epiphysis, and metaphysis as revealed in Figure 2.1.

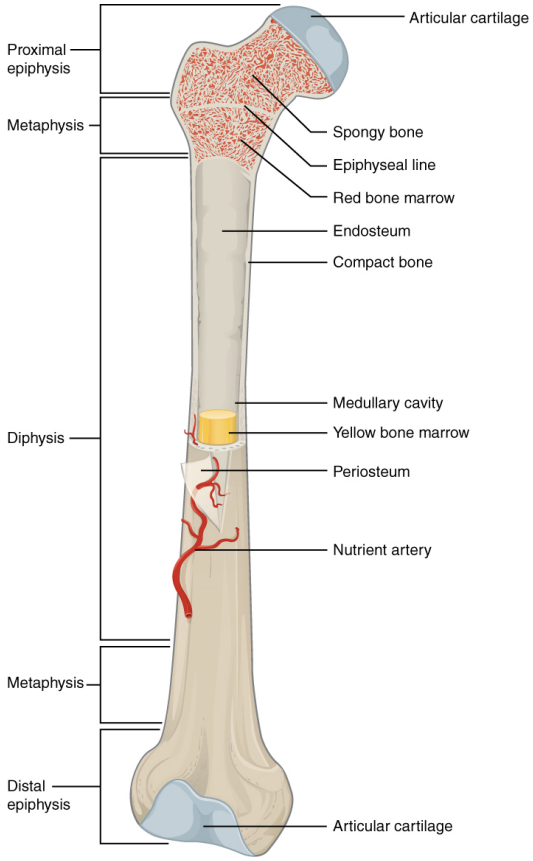


Figure 2.1. The structure of a long bone
Note. Adapted from Betts et al. (2013). Copyright 2020 by the Creative Commons Attribution License v4.0.

The diaphysis or the shaft is found between the proximal and distal bone ends and makes up the vertical axis of a long bone (Marieb and Hoehn 2013). The outer layer of the diaphysis is composed of dense and hard cortical bone, which surrounds a central space called medullary cavity (or marrow cavity) and is filled with yellow marrow (Marieb and Hoehn 2013). The epiphysis (plural = epiphyses) is the expanded portions and presents at the end of proximal and distal of the diaphysis (Marieb and

Hoehn 2013). The outer layer of the epiphysis is covered by cortical bone and inside of the epiphysis is the spongy bone, which is filled with red marrow (Stagi, Cavalli et al. 2013). Metaphysis (plural = metaphyses) is the area between the epiphysis and diaphysis and includes the epiphyseal plate in growing bones. This layer of a hyaline (transparent) cartilage is responsible for length growth. In early adulthood, when the bone growth stops, it is replaced by the bone tissue and turned into the epiphyseal line (Marieb and Hoehn 2013).

The outer surface of a bone is called the periosteum. The periosteum is composed of a fibrous membrane and a cellular layer. The endosteum is the inner surface of a bone, which covers the spongy bone, where bone growth, repair, and remodelling occur (Marieb and Hoehn 2013).

2.2.2 Microstructure of the Bone

Bone Composition

Bone (osseous) is a composite tissue made up of widely separated cells surrounded by a large amount of matrix. The matrix section contains inorganic salts (about 70%), predominantly hydroxyapatite (calcium phosphate) and some calcium carbonate (provide bone's hardness), organic collagen fibres (about 22% and provide bone's flexibility), and 8% of water by weight (Manolagas 2000). Calcification is a process by which the matrix plus a few other salts are deposited into the collagen fibre's framework. Growth factors and bone morphogenetic proteins are also found in the matrix component (Manolagas 2000).

Microstructure of Cortical Bone

The basic structure of cortical (compact) bone is a characteristic cylindrical structure called an osteon (also known as a Haversian system). Each Haversian system runs parallel to the long axis of the diaphysis. There is a Haversian canal in the centre of each Haversian system, which contains nerve fibres, blood vessels, lymph vessels, and loose connective tissue. The Haversian canal is surrounded by 4-20 concentric mineralised collagen fibres to form planar arrangements known as Lamellae (Rho, Kuhn-Spearing et al. 1998). Volkmann's canals are horizontal axes, which provide communication

between osteons, marrow cavity, and peritoneum (Marieb and Hoehn 2013). The Lacunae in a bone are small spaces within the bone matrix and between the lamellae rings containing osteocytes. Hair-like minute canals, the so-called canaliculi, provide interconnection between these lacunae and the Haversian canal as revealed in Figure 2.2 (Marieb and Hoehn 2013).

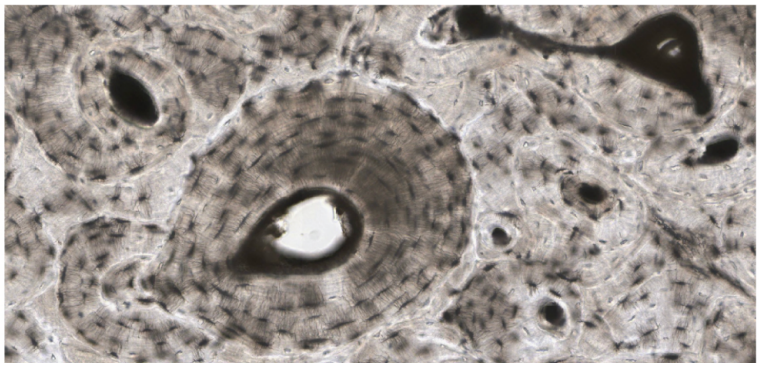


Figure 2.2. Microstructure of osteon
Note. Adapted from Betts et al. (2013). Copyright 2020 by the Creative Commons Attribution License v4.0.

Microstructure of Trabecular Bone

The structure of trabecular bone is less organised, lighter, and less dense than cortical bone. Trabecular bone is made of small interconnections known as trabeculae plates and small, irregular cavities that contain red bone marrow (Rho, Kuhn-Spearing et al. 1998). The adjacent cavities are connected through canaliculi and receive their own blood supply instead of Harversian canals. The trabecular plates consist of three basic cellular structures: rod–rod, rod–plate, or plate–plate (Rho, Kuhn-Spearing et al. 1998).

Bone Cells

Three types of bone cells are responsible for different metabolic functions of the bone. These cells are osteoblasts, osteocytes and osteoclasts. The bone structure is mainly supported and maintained by the interaction between these cells.

Osteoblasts

Osteoblast cells originate from multipotent and mesenchymal stem cells, which can differentiate into osteoblasts, adipocytes (fat cells), chondrocytes (cartilage-forming cells), myoblasts (muscle cells) or fibroblasts (Bianco, Riminucci et al. 2001). When pre-osteoblast cells reach the site of bone formation, they undergo a 3-stage differentiation with the expression of certain molecular markers specific for each stage. These three stages are characterised by cell proliferation, matrix maturation, and matrix mineralisation (Rutkovskiy, Stenslokken et al. 2016), which then change into mature osteoblasts (Katsimbri 2017). Osteoblasts are initially responsible for synthesis and mineralisation of bone and then for bone remodelling. They deposit collagen and produce osteoid and then provide the required enzymes (e.g. alkaline phosphatase and osteocalcin) for bone mineralisation (Hadjidakis and Androulakis 2006). Osteoblasts also produce prostaglandins (PGs), growth factors, and cytokines, which have an important role in regulating bone formation or bone resorption (Katsimbri 2017). Osteoblasts either can stay on the bone surface, becoming flat and lining cells or can undergo programmed cell death (Hadjidakis and Androulakis 2006). As the matrix is forming, mature osteoblasts become embedded in the matrix and differentiate into osteocytes (Bonewald 2011).

Osteocytes

Osteocytes are the most abundant type of cells (95%) in bone tissue (Katsimbri 2017). Osteocytes originate from the differentiation of osteoblasts once mineralisation of bone has occurred. During bone formation, mature osteoblasts are embedded by an un-mineralised osteoid (Katsimbri 2017). Following mineralisation, these trapped cells transit into osteocytes. Osteocytes are found in lacunae, between the lamellae rings (Bonewald 2011), and can sense mechanical stress to the bone during locomotion, and are therefore crucial to repairing micro-damage to the skeleton (Bonewald 2011). Osteocytes are responsible for detecting bone micro damages (microscopic fractures within the mineralised bone) and subsequently will control the remodelling process for repairing the damaged bone (Katsimbri 2017). There are several other functions that osteocytes are involved in, such as

sensing calcium, regulating the osteoid matrix maturation, and mineralizing bones (Kamioka, Honjo et al. 2001).

Osteoclasts

Osteoclasts, large multinucleated cells derived from haematopoietic progenitors in the bone marrow, are responsible for bone resorption by acidification and proteolysis of the bone matrix (Teitelbaum 2000). Bone resorption is crucial for normal skeletal growth, the maintenance of bone integrity throughout life, and bone remodelling cycle (Bar-Shavit 2007).

Bone resorption starts with binding of non-polarised and mature osteoclasts with the bone matrix (activation and polarisation of osteoclast) (Kamioka, Honjo et al. 2001). Subsequently, polarised osteoclasts produce podosomes and membrane domains. Podosomes contain an actin-rich structure in the core, which is surrounded by integrins and cytoskeletal protein rings (Kamioka, Honjo et al. 2001). The membrane domains include three distinct zones; a sealing zone, a ruffled border, and a functional secretory domain (Kamioka, Honjo et al. 2001). At the sealing zone, the plasma membranes of the osteoclasts are attached to the bone matrix through actin rings. After the attachment is finished, the ruffled border forms in the membrane (between the polarised osteoclast and mineralised bone matrix) (Kamioka, Honjo et al. 2001). A vacuolar-type H^+-ATPase pump in ruffled border membrane is involved in the acidification process; it releases hydrochloric acid (HCl) to dissolve minerals, and proteases to dissolve the Howship's lacuna (Nakamura 2007). After bone resorption, the degraded materials are endocytosed via osteoclasts and released at the functional secretory domain. Finally, osteoclasts undergo apoptosis (Vaananen and Laitala-Leinonen 2008).

Osteoclastogenesis is up-regulated by cytokines (e.g. receptor activator of nuclear factor-kB ligand (RANK-L), interleukins 1 and 6 (IL 1 and 6) (Boyle, Simonet et al. 2003), and hormones such as thyroid hormone (TH), parathyroid hormone (PTH), glucocorticoids (GCs), and 1α,25-dihydroxyvitamin D ($1,25(OH)_2D$) (Kanatani, Sugimoto et al. 2004, Rao, Lu et al. 2006). Osteoclasts express osteoprotegerin, which has an osteo-protective role by inhibiting the differentiation of osteoclasts (Boyle, Simonet et al. 2003).

2.3 Bone Modelling and Remodelling

2.3.1 Bone Modelling

The process of bone formation during development and growth, and adaption of its shape and size in response to mechanical loads is known as bone modelling (Manolagas 2000). During the modelling process, bone is removed from one site and deposited into another site by the action of osteoblasts and osteoclasts (Manolagas 2000, Brandi 2009). Unlike bone remodelling, the activities of osteoblasts (which leads to bone formation) and osteoclasts (followed by bone resorption) are independent and not coupled in bone modelling. Therefore, the activities of osteoblasts and osteoclasts happen at different locations and at different time intervals (Brandi 2009). Both anabolic and catabolic modelling cells are active throughout our life; while bone modelling occurs more than remodelling in children and adolescents to support the increased growth velocity (Clarke 2008), bone remodelling is more frequent during adulthood (when a skeleton is fully matured) to keep the integrity of bone matrix (Roberts, Huja et al. 2004). Bone modelling is a physiological mechanism allowing a growing skeleton to adapt to functional loads. This is addressed as Wolff's bone law, which states that bones change shape and size in response to stresses and forces placed on them (Clarke 2008).

2.3.2 Bone Remodelling

Once the skeleton becomes mature, old bone is constantly replaced by new bone at the same location (Manolagas 2000, Katsimbri 2017). This constant renewing bones process is called remodelling, which occurs through osteoblastic and osteoclastic cells as highlighted in Figure 2.3 (Katsimbri 2017). Unlike bone modelling, the bone remodelling cycle involves tightly coupled bone resorption by osteoclasts followed by bone formation by osteoblasts (Robling, Castillo et al. 2006). There are four distinct sequential phases: activation, reversal, formation, and termination (Brandi 2009, Katsimbri 2017).

Before the activation phase, bone is in a quiescent stage. Then, remodelling is initiated by either hormonal signals (from the action of oestrogen) or secretion of PTH in response to systemic changes

in calcium homeostasis, or mechanical loads (Katsimbri 2017). In the activation phase, following the detection of the signal, osteoclast precursors from the circulation are recruited and activated (Clarke 2008).

Following activation, osteoclastic resorption of bone begins, a process regulated by local cytokines and systemic hormones. In each remodelling cycle, bone resorption lasts approximately 2–4 weeks (Katsimbri 2017), starting with osteoclasts cells attaching to the bone matrix and then degrading the osteoid matrix and create irregular Howship's lacunae on the surface of the trabecular bone and cylindrical Haversian tunnels within the cortex (Brandi 2009). The resorption phase is completed when multinucleated osteoclasts undergo apoptosis and are followed by reversal (Clarke 2008, Brandi 2009, Katsimbri 2017).

The reversal phase occurs when bone resorption finishes and transitions to bone formation (Clarke 2008). Once bone resorption is completed, resorption cavities host a variety of mononuclear cells (e.g. monocytes, osteocytes and pre-osteoblasts) ready to begin new bone formation (Clarke 2008). During this phase, bone formation initiates when osteoclasts are replaced by osteoblast-lineage cells (Katsimbri 2017).

The bone formation process is the longest phase of the bone remodelling cycle and takes approximately 4-6 months to complete. There is a two-step formation process. First, osteoblasts synthesise the bone matrix to fill the cavities left behind by the osteoclasts (Clarke 2008). This new organic matrix (osteoid) consists of proteins such as type I collagen and composes 50% and 40% of our bone volume and our bone weight respectively. Then, osteoblasts regulate osteoid mineralisation and continue to form new bone until some osteoblasts are surrounded and buried within the matrix and become osteocytes (Katsimbri 2017). These osteocytes maintain close contact with one another, bone surface lining cells, and osteoblasts with an extensive canalicular network connection (Brandi 2009). Other osteoblasts differentiate into flattened 'lining cells' that covers the bone surface and between 50% and 70% undergo apoptosis (Katsimbri 2017).

The final phase, termination, is when bone mineralisation (or calcification) begins about 30 days after the bone formation (Lind, Deleuran et al. 1995). At this stage, osteoblastic differentiation is suppressed through sclerostin release by osteocytes (ten Dijke, Krause et al. 2008), inhibiting bone formation (Matsuo and Irie 2008), entering the quiescent phase, and finally terminating the bone remodelling cycle (Lind, Deleuran et al. 1995).

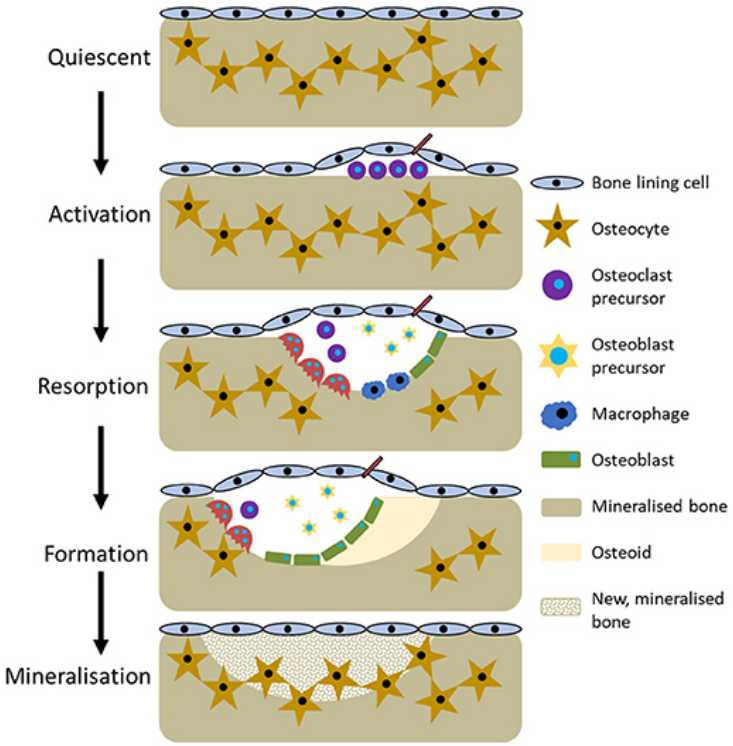


Figure 2.3. Bone remodelling
Note. Adapted from Owen et al. (2018). Copyright 2020 by the Creative Commons Attribution License v4.0.

2.4 Bone Mass Accrual and Peak Bone Mass

Bone mass accrual is defined as the accumulation of minerals in the skeletal system during normal growth. The timing of bone mass accrual varies due to gender-specific patterns of pubertal maturation (Zemel, Kalkwarf et al. 2011). As our body grows, our skeletal system grows in length, in breadth, and mass. In healthy individuals, the years immediately following fusion of the epiphyses are critical for the acquisition of peak bone mass (PBM) (Heaney, Abrams et al. 2000). Peak bone mass is the maximum amount of bone acquired at skeletal maturity when accrual ceases or plateaus (Gordon, Zemel et al. 2017). The maximum accrual in bone mass happens almost 6 months after the pubertal growth spurt, however, bone mass and BMD gain continue for some years afterwards (Bailey, McKay et al. 1999). Bone mineral density is the amount of bone tissue (calcium and other minerals) in a certain segment of the bone (volume) (Schoenau, Saggese et al. 2004). The exact age at which bone mass reaches a peak varies according to gender, type of measurement, skeletal site, maturational timing, and lifestyle factors (Gordon, Zemel et al. 2017). For instance, Boot et al. (2010) ran a longitudinal study demonstrating that PBM was attained between 18-20 years in females and between 18-23 years in males. This gender difference can be explained by the late puberty attainment, later growth spurt, and greater bone size in males (Boot, de Ridder et al. 2010). Genetic and environmental factors have been shown to contribute to PBM. Heredity has shown to be responsible for about three-fourths of the variance in PBM (Heaney, Abrams et al. 2000). A well-balanced diet rich in micronutrients (e.g. calcium, vitamin D, vitamin K, vitamin C, phosphorous, and magnesium) alongside weight-bearing exercise (moderate to vigorous intensity) are the most effective strategies to establish good bone health (McDevitt, McGowan et al. 2014). Smoking, alcohol consumption, caffeine intake, and oral contraceptive use are other potential factors, which influence bone health. Acquiring a high PBM early in life can be a good predictor of higher bone mass and subsequently better fracture protection later in life (Heaney, Abrams et al. 2000).

2.5 Bone Fracture in Children

2.5.1 Epidemiology of Fracture

In the middle of the 20th century, according to the World Health Organization (1957), the mortality rate of infants and children due to infections and nutritional disorders steadily decreased in European countries, while the rate of accidents as a cause of death remained high, or even increased. Therefore, it was of high importance to consistently monitor the rate of childhood accidents and investigate their risk factors to facilitate prevention programmes (Ehrenpreis 1957). One of the most common types of an accident during childhood is a bone fracture (Landin 1983), a partial or a complete break in the bone. Although bone is a stiff tissue, under external pressure and force it bends and breaks if this force is too strong. Approximately 9% of all childhood injuries are attributed to fracture, which has drawn the attention of Canadian (Spady, Saunders et al. 2004). Fractures can cause pain, lack of mobility and independence (Manias, McCabe et al. 2006).

Landin (1983) investigated 8682 fractures in children (aged 0-16 years) and found that the incidence of fractures is more common in boys (42%) compared to girls (27%). At least one fracture will occur in approximately a third of both genders before they reach age 17 years old (Cooper, Dennison et al. 2004). It has been estimated that 7,500 fractures occur annually in New Zealand children aged 3–15 years, an incidence of approximately 1% of this age group (Goulding, Cannan et al. 1998). Jones et al. (2000) estimated the annual incidence of distal forearm fractures in Dunedin children aged 3–15 years were 10.4/1000 (1.04%) for both boys and girls.

It is interesting to note that during growth, many healthy children suffer from sustained fractures (Jones, Williams et al. 2002). A study in Dunedin (New Zealand) children (n = 313, 145 girls and 168 boys, < 13 years) revealed 468 fracture events with 212 (67.7%) sustaining a single fracture and 101 children with more than one fracture (Yeh, Grant et al. 2006). A cohort study investigated 601 New

Zealand children up to 18 years old and found 172 cases who sustained a single fracture. In this study 119 of children fractured more than once (Jones, Williams et al. 2002).

2.5.2 Fracture Patterns

Previous studies have shown that the upper limb was a dominant site of fractures (68-75%) in children compared to lower limbs (Maasalu, Raukas et al. 2009, Jenkins, Nimphius et al. 2018). The most common sites of broken bones in children are wrist/forearm, making up at least a third of all fractures as revealed in Table 2.1(Landin 1983, Jones, Williams et al. 2002, Cooper, Dennison et al. 2004, Maasalu, Raukas et al. 2009), and with consistent proportionality for both boys and girls (Jenkins, Nimphius et al. 2018). For instance, Rennie et. al. (2007) found that 82.2% of all fractures were located in the upper limb, 17.3% in the lower limb, and only 0.5 in the pelvis or spine. They also found that distal forearm and hand were the most common sites of fractures (57.1%). However, the incidence of the upper leg and vertebral fractures in children is infrequent and bimodal in both gender groups (Cooper, Dennison et al. 2004, Rennie, Court-Brown et al. 2007).

Table 2.1. Overview of studies describing fracture incidence in children

First author (year) country	Time frame	Age range (years)	Fractures incidence (10^4)	Frequent fracture site
Landin (1983) Sweden	1950-1979	0-16	212	23% Distal forearm
Worlock (1986) England	1981	0-12	160	35.8% Distal forearm
Cooper (2004) UK	1988-1998	0-17	133	30% Forearm
Moon (2016) UK	1988-2012	0-17	169 boys 103 girls	43.5% Forearm 29.7% Forearm
Kopjar (1998) Norway	1992-1995	0-12	128	65% Arm
Tiderius (1999) Sweden	1993-19944	0-16	193	26% Distal forearm
Hedstrom (2010) Sweden	1993-2007	0-19	201	26% Distal forearm
Lyons (1999) Wakes	1996	0-14	361	36% Forearm
Moustaki (2001) Greece	1996-1998	0-14	120	43% Forearm
Brudvik (2003) Norway	1998	0-15	245	27% Distal forearm
Maasalu (2009) Estonia	1998-2007	0-14	689	68% Upper limb
Rennie (2007) Scotland	2000	0-15	202	33% Distal Forearm
Mayranpaa (2010) Finland	2005	0-15	163	30.4% Distal Forearm
Randsborg (2013) Norway	2010-2011	0-16	180	31.1% Distal radius
Jenkins (2018) Australia	2005-2015	0-16	85	75% Upper limb

2.6 Aetiology of Fracture During Growth

2.6.1 Accidents

Accidental trauma such as falls (Lyons, Sellstrom et al. 2000, Rennie, Court-Brown et al. 2007), slips, and trips during activities like walking, running, climbing, and playing (Lyons, Sellstrom et al. 2000) is the most common type of injury-causing paediatric fractures. Rubie-Davies and Townsend (2007) recruited over 25,000 students from 76 elementary schools (grades 1-6) and showed that 59 fractures from 118 sustained fractures occurred at schools due to falls from heights less than 150 cm. Sports-related fractures vary between countries and between different regions in one country. For example, Lyons et al. (2000) reported a higher risk from ball sports (e.g. soccer, rugby) in South Wales, and from ice and snow sports (e.g. skiing) in Scandinavian children.

Seasonal variations in fracture occurrence have been described in previous studies. Hedstorm et al. (2010) found that the lowest number of fractures occur in December (snow and ice are common between November and April) and the highest percentage of outdoor fractures occur in June in Swedish children. Lyons et al. (2000) reported a trend for sports-fractures. They found that sports-fractures increased in spring, the number of fractures dropped in mid-summer (school holidays), rose again in autumn, and decreased in winter. The rate of paediatric fractures caused by road traffic accidents is very low. Rennie et al. (2007) reported that only 6.7% of their study population had car accident fractures.

Landin (1983) categorised the severity of the trauma into three different levels: 1) slight trauma: falling to the ground from standing height on the same level, falling from less than 0.5 metre (e.g. stools, chairs or beds), and low energy sport type (e.g. ball sports, skiing, wrestling, judo, skateboard, and roller skating), 2) moderate trauma: falling from less than 0.5-3 metres (e.g. bunk-bed, stairs, bicycle, horse-back, swings, or similar playing equipment) 3) severe trauma: falling from more than 3 metres (e.g. from window or roofs) and all traffic accidents.

2.6.2 Risk Factors for Fracture

Risk factors for fracture can be categorised into two groups: modifiable (e.g. obesity, physical activity, nutrition, vitamin D) and non-modifiable (e.g. genetics) risk factors. These risk factors are as also well-established determinants of skeletal mineralisation and PBM. It is important to note that early identification of risk factors during childhood could enable changes to be instigated (e.g. lifestyle, nutritional, or behavioural), which would enhance BMD to prevent fractures, and improve adult bone health. A brief discussion of the factors affecting children's bone health is presented below.

Modifiable Determinants of Bone Fracture

Diet and Nutrition

During skeletal maturity, calcium deficiency can adversely affect PBM gain and increase the risk of fractures later in life (Heaney, Abrams et al. 2000). Therefore, adequate dietary intake of calcium is important for bone growth, development, and formation (Stagi, Cavalli et al. 2013). Alshamrani et al. (2019) in a cross-sectional study showed that every additional unit of calcium intake (mg/day) was associated with a significant (but a small) decrease in the likelihood of fracture risk in children 6-15 years old (n = 130). The small but significant relationship could be due to the small sample size in this study, and more significant results would have been observed with larger sample size.

According to New Zealand recommendations, children aged 9 to 11 years require 1000 mg/day calcium through diet (Ministry of Health 2006). However, based on the Ministry of Health (2003), Ministry of Health (2012) report the median intake of calcium in boys and girls, 7-10 years old was 788 mg and 628 mg, respectively. They also estimated that the prevalence of inadequate calcium intake would be as high as 65% (by overall) in children and young people aged 5–14 years. The main sources of dietary calcium in New Zealand are milk and dairy products (e.g. cheese, ice cream, and yoghurt) (Ministry of Health 2012). As shown by Goulding et al. (2004), pre-pubertal children who avoid drinking milk have more fractures than matched controls. Black et al. (2002) studied 50 milk

avoiders (aged 3-10 years old) and found that children who habitually avoided consumption of cow milk have sustained a high number of bone fractures compared to milk drinking children.

Vitamin D is another nutritional factor involved in the pathophysiology of skeletal health problems. Observational and clinical studies suggest that vitamin D deficiency and insufficiency can adversely affect childhood skeletal health, leading to conditions/diseases such as rickets, metabolic bone disease, and hypocalcaemia (Wagner and Greer 2008, Moon, Harvey et al. 2014). Inadequate vitamin D in circulation (due to low dietary intake or sun exposure) decreases the absorption of calcium from the intestine and results in an increased release of calcium from bone tissue to maintain calcium in circulation (Lips 2001). A decrease in serum calcium concentration leads to an increase in PTH secretion (secondary hyperparathyroidism) and bone turnover, and consequently a decrease in BMD (Lips 2001). This could ultimately result in the development of osteomalacia and osteoporosis. In a one-year prospective case-control study of the role of vitamin D in fracture, Thompson et al. (2017) recruited 120 (fractured and non-fractured) children, aged 2-14 years old. They found an association between 25(OH)D concentration between 50 to 75 nmol/L and higher fracture incidence, a finding confirmed by Saglam et al. (2017) who also reported a higher prevalence of 25(OH)D < 50 nmol/L or 25(OH)D = 50-75 nmol/L in children with distal radius fractures than healthy children. Ryan et al.'s findings (2012) also support the association between 25(OH)D < 50 nmol/L and increased odds of forearm fracture in African American children. Therefore, optimal vitamin D status is necessary for maximising bone mineralisation and minimising of fracture risk (Laird, Ward et al. 2010).

Physical Activity

During childhood and adolescence, weight-bearing physical activity, as a biomechanical force, can increase bone density and its strength (Ma and Gordon 2012). The osteogenic effects of exercise on bone density and strength is lost if children do not do exercise regularly (Ma and Gordon 2012). Repetitive mechanical loading during geometric changes in pre-pubertal and pubertal years can lead to biological bone mass gain (size and shape) (Bass, Saxon et al. 2002). Weight-bearing exercise also improves agility, coordination, posture, and balance through strengthening muscles, and may help to

prevent falls and fractures (Goulding, Jones et al. 2003). Low BMD and consequently increased fracture risk is prevalent in children with limited weight-bearing capacity (e.g. severe cerebral palsy) (Henderson, Lark et al. 2002). Manias et al. (2006) found that children with recurrent fractures had lower physical activity levels.

Obesity

In recent years, childhood overweight and obesity have become a global health problem. World Health Organization (2016) has estimated that over 41-million children under five years old could be overweight. According to the New Zealand Health Survey in 2018/19, 11.3% of children and adolescents (aged 2-14 years) living in New Zealand were obese (Ministry of Health 2019). There is an association between childhood overweight and obesity and developing musculoskeletal problems, injuries, and fractures early in childhood (Adams, Kessler et al. 2013, Paulis, Silva et al. 2014). Different theories have been postulated regarding a greater risk of bone fractures in children with obesity. Childhood obesity is associated with a higher propensity for falls with injuries or fractures due to poor musculoskeletal control and decline in motor skills (Cheng, East et al. 2016). In children who are overweight or obese, there is a structural adaptation in response to muscle forces and local mechanical strain, but not with static loads represented by body weight (Petit, Beck et al. 2005). Plausible explanations for a greater risk of bone fractures may be lower physical activity levels, inadequate intake of dietary calcium, and low vitamin D concentration (Wortsman, Matsuoka et al. 2000, Farr and Dimitri 2017). A positive relationship between physical activity and BMD has been observed in both adults (Cooper, Cawley et al. 1995) and children (Gunnes and Lehmann 1996).

Non-modifiable Determinants of Bone Fracture

Age

Khosla et al. (2003) reported that before 5 years old, there is little difference in fracture rates between gender groups, however, thereafter fracture rates rapidly increase and reach a peak in girls aged 8-11 years and boys aged 11-14 years old. It is suggested that the peak of fracture rates during childhood/adolescence can be related to asynchrony between bone mineralisation and linear growth (Bianchi 2007).

Data from the New Zealand Accident Compensation Corporation (ACC) on the number and cost of fractures (any accident reported at home, school, playgrounds, and sports events, except car-related injuries) for 8-12-year-old (boys and girls) from 2014 to 2019 is reported below in Tables 2.2 and 2.3. Although numbers of claims in New Zealand for fractures was stable between 2014 and 2019 the cost of treating fractures is rising and therefore improving bone health is important to reduce the financial burden on our health care system.

Table 2.2. Active claims count; 2014 to 2019

	Active claims count					
Age (years old)	2014	2015	2016	2017	2018	2019
8	2,491	2,692	2,883	2,760	2,793	2,727
9	2,856	2,996	3,194	2,976	2,994	3,334
10	3,526	3,419	3,738	3,369	3,514	3,695
11	3,628	3,853	3,018	3,758	3,788	3,055
12	3,471	3,875	3,132	3,129	3,561	3,823

Data provided by the New Zealand Accident Compensation Corporation (ACC) (2019)

Table 2.3. Overall cost of active claims; excluding GST; 2014 to 2019

	Active claims cost					
Age (years old)	2014	2015	2016	2017	2018	2019
8	$1,483,302	$1,600,501	$1,851,375	$1,908,728	$1,995,145	$1,900,615
9	$1,583,401	$1,794,677	$1,787,222	$1,849,718	$2,048,071	$2,362,739
10	$1,988,284	$2,041,537	$2,225,697	$2,107,735	$2,620,234	$2,710,275
11	$2,074,810	$2,195,313	$2,180,036	$2,732,630	$3,485,641	$3,091,560
12	$2,674,352	$2,854,415	$3,001,032	$3,127,131	$3,312,999	$3,459,811

Data provided by the New Zealand Accident Compensation Corporation (ACC) (2019)

Results from previous studies established that children who had inherently weak bones, experience their first fracture at a young age and then continue to fracture later in their life (Goulding, Grant et al. 2005, Goulding, Jones et al. 2005, Yeh, Grant et al. 2006). Interestingly, although the rate of adolescents' fractures is high, most adolescents with repeated fractures have their first fracture at a younger age. Goulding et al. (2005) conducted a birth cohort study and followed up 601 participants for 18 years in New Zealand. Their results demonstrated a positive relationship between first fracture and increased risk of further fractures during growth and showed that most children had sustained further fractures before they reached their teens. Similar to adults (Klotzbuecher, Ross et al. 2000, Kanis, Johnell et al. 2004), they found individuals who experienced a first fracture were approximately twice at risk of future fractures in comparison to those without previous fractures.

Gender

Fractures rate are found to vary by gender. Rubie-Davies and Townsend (2007) showed that fracture incidence in New Zealand primary school students were more common in boys than girls. Similar results were found by Cooper et al. (2004) who investigated the incidence of fractures among British children in a population-based cohort study. Their results revealed that fractures were more common among boys than girls. Lyons et al. (1999) investigated children aged 0-14 years old and they found the fracture rate is higher in boys than girls and it rose with age in both gender groups. Landin (1983)

investigated fracture patterns in children (aged 0-16 years) and also found that the incidence of fractures is more common in boys (42%) compared to girls (27%). The greater fracture rates in boys may be linked to greater participation in sports and recreational activities (Lyons et al. 1999).

Genetics

It has been shown that genetic factors (a combination of polygenic effects, gene-gene interactions, and gene-environment interactions) have a crucial effect on bone density throughout life (Clark 2014, Ho-Le, Center et al. 2017, Kim 2018), and are responsible for up to 80% of the inter-subject variance in bone mass (Guglielmi, Adams et al. 2009). It is important to note that it is unlikely that only one single genetic locus is the main factor resulting in fragile bone (Clark 2014), and genetics is only one of the many non-modifiable fracture risk factors.

Musculoskeletal Weakness

Children with musculoskeletal weakness either due to chronic diseases or genetic disorders have lower muscle mass, strength, and BMD, therefore they are more likely to fracture bones after minimal trauma (Goulding 2007). For example, fractures are more common in children with Duchenne's muscular dystrophy (Larson and Henderson 2000), cystic fibrosis (Braun, Bacchetta et al. 2017), cerebral palsy (Henderson, Lark et al. 2002), and sickle cell disease (Buison, Kawchak et al. 2005). However, the severity of the disease can influence fracture risk. For instance, Rovner et al. (2005) found that there is no association between mild to moderate cystic fibrosis with increased fracture risk in their participants (aged 6-25 years) compared to a healthy control group (aged 4-25 years). In contrast, results from a review which was conducted by Mergler et al. (2009), confirmed that BMD is a serious problem in children with severe cerebral palsy.

Medications

Low bone mass is common in many paediatric patients ranging from common conditions in childhood such as asthma to severe medical conditions such as arthritis, malabsorption syndromes, cancer, and organ transplantation due to a combination of imbalanced diet, low physical activity, muscle

weakness, medications, and radiotherapy (Harris, Lee et al. 2020, Maratova, Hradsky et al. 2017, Rohani, Arjmandi et al. 2017). Therefore, children with a range of medical conditions can be at heightened risk of fractures compared to healthy children. Evidence suggests a relationship between transplantation (e.g. lung) and lower BMD and fractures in children (Putman, Simoneau et al. 2018). Bloomhardt et al. (2020) evaluated children with leukaemia/lymphoma survivors who were treated. Their results showed that high risk of developing lumbar spine low BMD and a significantly increased risk of fracture in children who were older at diagnosis, white, and underweight. Evidence showed that systemic corticosteroids contribute to decrease BMD and increase risk of fractures in sick children but not using inhaled corticosteroids in children with asthma (Zieck, George et al. 2017, Loke, Gilbert et al. 2015).

Intrauterine Factors

Early in utero, a foetus skeleton forms as cartilage. Throughout growth and development (modelling and remodelling), bone mineral deposition (osteogenesis) is most intense and rapid during the third trimester of pregnancy (Fewtrell 2011). Thus, preterm infants (<37 week's gestation) has suboptimal mineralisation since they may have missed, in part or completely, the period of greatest bone mineral accrual in utero, and are prone to fracture (Michaud, Luu et al 2020). Maternal smoking and poor diet are associated with mineralised skeleton acquisition during intrauterine life (Brand, Hiyoshi et al 2020, Salles 2016). Being very preterm, having intrauterine growth restriction (IUGR), and poor childhood growth is also directly linked to lower adult bone mass and risk of fracture later in life (Kesavan and Devaskar 2019, Xie, Alos et al 2019). Since, any of these factors (being born preterm, maternal factors or anything which influences birth weight such as IUGR) are likely to influence bone health, therefore optimisation of mothers and their infants' nutrition and intrauterine growth should be considered as an important strategy to decrease subsequent fracture risk.

Lower Bone Mineral Density

Low BMD is an important predictor of fracture risk (Johnell, Kanis et al. 2005) in adults and it is suggested that low BMD can also be a risk factor for fractures in paediatric populations. For instance,

Skaggs et al. (2001) conducted a study in a group of 100 girls (aged 4-15 years). They found that girls who sustained distal radius fractures after low-energy impact trauma have smaller cross-sectional bone area compared to a fracture free control group. In another study, Goulding et al. (2000) conducted a prospective study and concluded that reductions in total body BMD increased risk of new fractures within a 4-year follow up in a group of 170 young girls. Ferrari et al. (2006) recruited 125 girls in Switzerland and followed them up for 8.5 years. Their observations indicated those girls who had fractures, had lower bone mass gain and smaller vertebral bone size in comparison with those who had no fractures during the study period. Their results suggested that paediatric fractures might be markers of inadequate bone mass acquisition and insistent bone fragility. Although fractures were self-reported and they did not use X-rays and medical records, recent data have shown that the accuracy of self-reported fractures is 80% or greater for forearm/wrist fractures (Chen et al. 2004). Other conditions such as poor diet, hormonal disorders, some congenital conditions (which have an adverse effect on bone development), and chronic disease, as well as broken bones after modest trauma in healthy children can cause reductions in BMD acquisition (Goulding 2007). The effect of hormones and diet regarding bone health and bone density will be discussed in the next section.

2.7 Factors Affecting Bone Density

2.7.1 Hormones and Bone Metabolism

Parathyroid Hormone (PTH)

Parathyroid hormone (PTH) has a crucial role in the maintenance of bloodstream calcium concentration (Khanal and Nemere 2008). In the parathyroid gland, blood calcium levels are constantly sensed through Ca^{2+}-sensing receptors (CaR) that are present in the chief cells, where PTH is produced and stored (Khanal and Nemere 2008). There is a strong relationship between PTH levels and calcium homeostasis, as when Ca^{2+} concentration decreases, PTH secretion is rapidly increased in the bloodstream and stimulates calcium resorption and reabsorption from bone and kidney, respectively (Perez, Picotto et al. 2008).

Parathyroid hormone acts on osteoblasts in bone, stimulates osteolysis, a process that can lead to the release of calcium into the extracellular fluid. It also stimulates resorption of mineralised bone and releases Ca^{2+} and phosphate ion (Pi) into the extracellular fluid (Khanal and Nemere 2008). In the kidney, PTH inhibits reabsorption of Pi and increases its excretion, therefore Pi is rapidly removed from the circulation. Moreover, PTH decreases calcium excretion through increasing Ca^{2+} reabsorption by enhancing the uptake of calcium by Ca^{2+}-ATPase and a Na^{+}-Ca^{2+}antiporter (Khanal and Nemere 2008). Also, PTH increases 1α-hydroxylase enzyme activity in the kidney, stimulates the hydroxylation of 25(OH)D, thereby indirectly stimulating Ca^{2+} absorption by the gut (Khanal and Nemere 2008). The effect of PTH on a bone can be either anabolic or catabolic, however, the catabolic action of PTH (stimulation of bone resorption) is better understood than its anabolic action (the process of bone formation) (Silva, Costa et al. 2011).

Calcitonin (CT)

Parafollicular cells or clear cells (C cells) of the thyroid gland secrete polypeptide hormone, which is known as calcitonin (CT) (Khanal and Nemere 2008). Calcitonin is one of the three main regulating hormones of plasma calcium concentration. The main function of CT is to decrease plasma calcium

concentration when it is high by decreasing osteoclast activity and inhibiting bone resorption, decreasing calcium absorption by enterocytes, and decreasing calcium reabsorption by the kidneys (Khanal and Nemere 2008). However, there is evidence that CT can promote intestinal calcium absorption by increasing active vitamin D levels in vivo when dietary calcium is low (Khanal and Nemere 2008).

Thyroid Hormone (TH)

Thyroid hormones, thyroxine (T_4) and 3,5,3`-L- triiodothyronine (T_3) have a key role in bone metabolism. In children, T_3 is necessary for skeletal development and linear growth (Gogakos, Duncan Bassett et al. 2010). It stimulates expression of fibroblast growth factor receptor-3 (FGFR-3), which is a protein in the fibroblast growth factor pathway and has an important role in the stimulation of cell growth (Shao, Wang et al. 2006).

Thyroid hormones enhance bone resorption since they have a direct effect on osteoclast activities (Cooper and Biondi 2012). Both hypothyroidism and hyperthyroidism have been shown to have a relationship with an increased fracture risk (Gogakos, Duncan Bassett et al. 2010). In children with hypothyroidism, thyroid-stimulating hormone (TSH) concentration is high due to abnormally low activity of the thyroid gland therefore low levels of T_3 and T_4 (Cooper and Biondi 2012), resulting in delayed growth and bone maturation (Setian 2007). In hyperthyroidism, serum TSH levels are very low, and the thyroid gland makes too much T_3 and T_4 (Cooper and Biondi 2012). In this situation, bone turnover is increased, which leads to an increased risk of osteoporosis and fracture (Vestergaard and Mosekilde 2003).

Although hyperthyroidism is a rare condition in childhood, it can be potentially a serious disorder if it is not controlled (Srinivasan and Misra 2015). Evidence suggests that during long-term hyperthyroidism, calcium balance can be changed through increased bone resorption. Results from animal studies showed that Ca^{2+} influx at the brush border membrane vesicles and Ca^{2+} efflux from the basolateral membrane of enterocytes were higher in hyperthyroid compared to hypothyroid states (Kumar and Prasad 2002, Kumar and Prasad 2003). Also, thyroid hormones can increase the activity

of the Na^+/Ca^+ exchanger pump within the enterocytes (Khanal and Nemere 2008). Moreover, a cooperative effect has been shown between thyroid hormones and vitamin D (Khanal and Nemere 2008).

Growth Hormone (GH)

Growth monitoring is important for the evaluation of nutritional status during child development. There are several hormones, which are important for normal growth and bone homeostasis, however, it is generally accepted that growth hormone (GH) is the major regulator of body growth. Growth hormone increases the cell number (e.g. bone, cartilage, and other tissue) rather than increasing cell size (Tritos and Klibanski 2016).

Several peripheral and central signalling mechanisms control the synthesis of GH (Bergan-Roller and Sheridan 2018). Growth hormone is produced and secreted by the anterior pituitary gland. However, its synthesis and secretion are under the control of hypothalamus hormones, promoted by growth hormone-releasing hormone (GHRH) and inhibited by somatostatin (Bergan-Roller and Sheridan 2018).

Binding GH to the growth hormone receptors (GHR) in the epiphyseal growth plate stimulates linear growth. Growth hormone also stimulates the production of IGF-1 within skeletal and other non-skeletal tissues. Then, IGF-1 binds to its receptors (insulin-like growth factor-1 receptor (ILGF-1R)), and increases bone cell proliferation and enhances growth (Gat-Yablonski, Yackobovitch-Gavan et al. 2009). The direct effect of GH on the cartilage is an important stimulator of chondrocyte proliferation in the epiphyseal growth plate, leading to IGF-1 production (Mackie, Tatarczuch et al. 2011). Therefore, GH/IGF-1 axis is responsible for long bone growth and skeletal size, and thus any reduction in this axis due to inflammatory cytokines can cause growth defects and low bone mass (Berryman, Christiansen et al. 2008).

Glucocorticoids (GCs)

Glucocorticoids (GCs) are used as medicine in clinical situations such as asthma since they have anti-inflammatory and immunosuppressive properties (Christakos, Dhawan et al. 2011). Long-term use of

GCs can lead to osteoporosis due to inhibition of the function of mature osteoblasts and IGF-1, and consequently a reduction in BMD (Canalis 2005). In addition to bone resorption, GCs can cause malabsorption of calcium in the intestine (De Nijs 2008). It is suggested that at pharmacological doses, long-term GCs treatment affects calcium transport and their effects may be minimal at physiological doses (Khanal and Nemere 2008).

The use of GC can be an issue since the rates of childhood asthma are high in New Zealand (Asher et al. 2008). However, a recent study conducted by Zieck et al. (2017) did not show any relationship between fracture risk and inhaled corticosteroids among children with asthma (aged 6-18 years). Loke et al. (2015) ran a systematic review and meta-analysis study and their results also showed there is no association between adults or children with asthma who are using inhaled corticosteroids and harmful effects on fractures or BMD. Although they did not assess differences between medications (e.g. dose or type of inhaler), they did a comprehensive search and included both observational and randomised control trials in adults and/or children with asthma.

Oestrogen

Oestrogen has a key function in the regulation of skeletal maturation and longitudinal bone growth (Simm, Bajpai et al. 2008). During the pubertal growth spurt, oestrogen is the principal mediator, not only in girls but also in boys. Oestrogen is needed for proper closure of the epiphyses in both gender groups, and higher estradiol levels in girls may explain the more rapid growth spurt in girls compared to boys (Frank 2003).

The effect of oestrogen on bone growth can be through direct or indirect mechanisms. Directly oestrogen prevents bone resorption (osteoclastogenesis) and stimulates osteoblastogenesis through its receptors (Chagin and Savendahl 2007). Oestrogen also inhibits the production of inflammatory cytokines (e.g. IL-1, and IL-6) therefore decreases bone loss, which has been used as a therapy for the treatment of low BMD (Syed, Modder et al. 2010). Oestrogen indirectly can influence the haematopoietic cell lineage therefore, its deficiency can cause osteoclast hyperactivity and also increase osteoclast production (Oursler 2003).

2.7.2 Dietary Factors

Calcium

Well-balanced nutrition is crucial for normal growth and puberty. Calcium is an essential nutrient, which is recognised for its critically important role in the acquisition of strong and healthy bones and can influence BMD by up to 20% (Stagi, Cavalli et al. 2013). According to the human body composition, calcium has a fifth place after oxygen, carbon, hydrogen, and nitrogen and makes up 1.9% of body weight (FAO 2002). Approximately 99% of this calcium is found as hydroxyapatite (calcium-phosphate complexes) in our bones and is responsible for the strength and rigidity of the skeletal system (Peacock 2010). Only small amounts are present in the extracellular fluid, plasma, and soft tissues in forms of calcium phosphate, calcium oxalate, and calcium carbonate (Greer and Krebs 2006). In addition to structural roles, calcium is responsible for other critical functions such as cellular signalling, nerve impulse transmission, muscle contraction, second messenger and protein activator, blood clotting, and regulator of blood pressure (Chung, Balk et al. 2009, Peacock 2010).

Calcium, either from diet or supplements, is in the form of complexes with other dietary components or micronutrients. So, to absorb calcium, these complexes must be broken down and calcium released into a soluble and ionised form (Amalraj and Pius 2015). Overall, about 90% of calcium is absorbed by the small intestine and the balance absorbed through the large intestine (Wasserman 2004).

The main sources of calcium intake for children in New Zealand are milk and dairy products (e.g. cheese and yoghurt) (Ministry of Health 2012). Because milk and yoghurt contain about 300 mg calcium per serving, consuming 2-3 servings of milk and dairy products per day are recommended for 2-12-year-old children to achieve the total calcium requirement (Ministry of Health 2012). Other non-dairy sources of calcium are legumes, nuts (e.g. almonds), tofu, vegetables (e.g. broccoli, kale), and fish with edible bones (e.g. sardines) (Ministry of Health 2012).

Phosphorus

More than half of the bone mass and teeth are composed of phosphorus (in the form of phosphate) and similar to calcium, phosphorus plays an important role in bone deposition (Peacock 2020). The main source of phosphorous is dairy products, meat, and its alternatives, such as beans, lentils, and nuts. It is also found in grains, especially whole-grain products and a smaller amount in vegetables and fruit (Peacock 2020). Phosphorous deficiency is unlikely unless specific medications are used (Linglart and Biosse-Duplan 2016).

Magnesium

In addition to calcium and phosphorous, another mineral, which has an important role in bone mineralisation is magnesium. About 59% of the total magnesium is located in our bones and affects hydroxyapatite crystal composition (Jahnen-Dechent and Ketteler 2012). A wide variety of food contains magnesium however, the highest amount of magnesium is found in green leafy vegetables, raw cacao, nuts, and seeds (Musso 2009). Magnesium deficiency is rare but can occur in bone disorders, malabsorption, diabetes or after prolonged vomiting, diarrhoea or alcohol abuse (Rude, Singer et al. 2009). During magnesium depletion, the bone structure is changed and because of this change, the bone structure becomes brittle and fragile (Tucker, Hannan et al. 1999).

Zinc

Zinc is an essential mineral for normal growth, development, and bones' maintenance (Yamaguchi 1998). Almost all body tissues require zinc but the highest proportion of total body zinc is accumulated in our bones. In the case of zinc deficiency bone homeostasis is altered (Yamaguchi 2010). The main physiological functions of zinc in bone metabolism include activation of osteoblastogenesis and prevention of osteoclastogenesis (Yamaguchi 2010). Zinc promotes bone growth and bone mass through IGF-1 (Heaney, Abrams et al. 2000). Zinc storage in our skeletal system may firstly lead to increase alkaline phosphatase activity and stimulation of collagen formation, which have an important role in bone calcification. Following that, the amount of calcium

may be significantly increased in bones (Yamaguchi 1998).

Sodium

A study in pre-adolescent girls showed that urinary sodium is one of the factors that can affect urinary calcium excretion (Matkovic, Ilich et al. 1995). The authors concluded that low dietary calcium consumption and high sodium intake during rapid growth, may increase urinary calcium loss, and decrease calcium retention in bones. In another study, Shi et al. (2012) showed the dominant role of sodium excretion on calcium excretion. Their results confirmed that 0.9 mmol urinary calcium increased when 100 mmol sodium excreted. High dietary sodium intake elevates calciuria due to an intimate association between renal tubular mechanisms involved in the reabsorption of these ions and consequently decreases calcium ion concentration within the extracellular fluid. Therefore, PTH and $1,25(OH)_2D$ secretions are increased, which can lead to an increase in bone turnover rate (Heaney 2006).

Other Nutrients

Fluoride is capable of delaying bone mineralisation and altering bone crystal structure. It can convert carbonated hydroxyapatite into carbonate fluorapatite, which is more stable and resistant to acid dissolution than hydroxyapatite (Everett 2011).

Other nutrients such as copper (cofactor for lysyl oxidase and important for collagen crosslink formation), manganese (important for cartilage formation), and iron (essential for bone formation and collagen synthesis) have important roles in the development of PBM and bone matrix (Heaney, Abrams et al. 2000).

Protein

Optimal protein intake should be maintained through diet because both high consumptions of protein and protein-energy malnutrition have an adverse effect on bone through altered calcium homeostasis, which can lead to hypercalciuria or hypocalciuria respectively. A high amount of dietary protein (especially animal sources) reduces pH levels (acidosis) consequently bone is used as a buffer. As a

result of this situation, urinary calcium excretion increases and bone demineralisation can occur (Jesudason and Clifton 2011). However, results from a review article showed that a diet with high protein has anabolic effects such as increasing serum IGF-1, decreasing PTH concentration, and enhancing calcium absorption which can partially or totally compensate protein-induced acid load negative effects on urinary calcium excretion and musculoskeletal system (Cao 2017).

Protein deficiency during childhood also leads to secondary hyperparathyroidism and decreases cortical bone formation and therefore interferes with PBM acquisition (Heaney, Abrams et al. 2000). Moreover, low protein intake can have adverse effects on collagen synthesis, which is the most abundant protein in our body (Katsimbri 2017). Adequate amounts of protein are necessary for both the production and action of IGF-1, which is an essential factor for bone length growth and bone formation. Insulin-like growth factor-1 stimulates proliferation and differentiation of chondrocytes in the epiphyseal plate (Bonjour, Ammann et al. 2001, Rizzoli, Bonjour et al. 2007). The Ministry of Health (2012) recommended the consumption of 40 g/day and 35 g/day protein for boys and girls (aged 9-13 years old), respectively. Their results showed that New Zealand children and young people have adequate protein intake (Ministry of Health 2012).

Fibre

Dietary fibre is a type of carbohydrate that cannot be digested by mammalian enzymes. It is found in plant-based sources (e.g. fruits, vegetables, legumes), and aids in the maintenance of bowel movements, and reduces the risk of cardiovascular disease and diabetes (Bosscher, Van Caillie-Bertrand et al. 2001). Despite these beneficial health effects, there are some disadvantages like decreasing the bioavailability of some essential minerals (e.g. calcium, zinc, and iron) and potentially impacting BMD. Various food components such as oxalic acid and phytates found in dietary fibre are responsible for the inhibition of minerals and trace elements absorption (Kennefick and Cashman 2000).

Vitamin K

The classic metabolic role of vitamin K in blood coagulation has an important role in bone

metabolism. Vitamin K is a fat-soluble vitamin, which has three groups including phylloquinone (K_1), menaquinone (K_2), and menadione (K_3) (Plaza and Lamson 2005). The major sources of vitamin K_1 are green leafy vegetables and some plant oils. Natural sources of vitamin K_2 are fermented food, meat, and milk products. Gut bacteria also produce vitamin K_2 (Shearer, Bach et al. 1996). Vitamin K_3 is not a natural form of vitamin K, but it is a synthetic analogue of vitamin K and acts as a pro-vitamin, which is converted in the liver to menaquinone and it is used as a supplement to help blood clot (Plaza and Lamson 2005).

During bone mineralisation, osteoblasts produce a calcium-binding protein, osteocalcin. Osteocalcin has three glutamate residues (Glu). A vitamin-K-dependent carboxylase enzyme converts glutamic acid to γ-carboxyglutamic acid residues (Gla). Then, carboxylated osteocalcin can bind to calcium ions in the hydroxyapatite molecule (Weber 2001, Plaza and Lamson 2005). The beneficial role of vitamin K in adult bone health and bone fracture prevention has been studied before (Iwamoto, Sato et al. 2009, Bultynck, Munim et al. 2020) however, there is limited data available regarding the importance of vitamin K in the bone mass acquisition of children. For example, a longitudinal study by van Summeren et al. (2008) showed that improvement of vitamin K status over two years can increase whole-body bone mass and BMC.

Vitamin C

Vitamin C plays a significant role in collagen formation through acting as a cofactor for prolyl and lysyl hydroxylases which are important enzymes for collagen formation and maturation (Marini, Cabral et al. 2007, Aghajanian, Hall et al. 2015). It also is an important regulator for osteoblast fate determination and proliferation, promoting the expression of genes involved in chondrocyte differentiation, and it has a positive effect on trabecular bone formation (Aghajanian, Hall et al. 2015).

Vitamin D

Vitamin D has an important role in bone health and is a focus of this research. Therefore, vitamin D will be discussed in detail compared to the other nutrients involved in bone health.

2.8 Metabolism of Vitamin D

Vitamin D is a secosteroid, fat-soluble vitamin, which is essential during life. There are two forms of vitamin D. The first one, which is a pro-hormone, known as vitamin D_2 (ergocalciferol) is found in plants (Zerwekh 2008). The second one is produced in humans and animals by skin exposure to sunshine (Zerwekh 2008) from 7-dehydrocholesterol (pro-vitamin D) and is known as pre-vitamin D_3. Pre-vitamin D_3 is then isomerised through sunlight for a few hours and transformed into vitamin D_3 (cholecalciferol) (DeLuca 2004). After vitamin D_2 or vitamin D_3 enter into the blood circulation, they are carried by the vitamin-D-binding protein (VDBP) (85-90%) and mainly transported to the liver, however, a small amount is deposited in the adipose tissue for storage (Blum, Dolnikowski et al. 2008). Less than 1% is free in the blood circulation and the rest (10-15%) (Zerwekh 2008) is bound to albumin (Jones, Redmond et al. 2017).

A hydroxyl radical (OH) is added to the carbon 25 by the cytochrome P_{450} (CYP2R1) vitamin D-25-hydroxylase in the liver and converted to 25-hydroxyvitamin D [25(OH)D], which is the main circulating form of vitamin D (DeLuca 2004). However, 25(OH)D is not an active metabolite and it must be hydroxylated to 1α,25-dihydroxyvitamin D [$1,25(OH)_2D$] in the kidney by CYP27B1 1α-hydroxylase (DeLuca 2004). Vitamin D acts via vitamin D receptors (VDR), which are found in many tissues including bone (DeLuca 2004).

In a phosphate loop, fibroblast growth factor 23 (FGF23) and in the calcium homeostasis loop, PTH down-regulates and up-regulates CYP27B1, respectively. When serum calcium decreases, the parathyroid gland is stimulated and produces more PTH (DeLuca 2004). The CYP27B1 is up-regulated by PTH secretion and therefore stimulates conversion of 25(OH)D to $1,25(OH)_2D$ in the kidneys. The $1,25(OH)_2D$ increases serum calcium concentrations through 1) increasing calcium mobilisation from bones, 2) increasing calcium re-absorption from kidneys (DeLuca 2004), and 3) increasing active calcium absorption from the intestine (Mihai and Farndon 2000).

When the concentration of $1,25(OH)_2D$ is high, the production and excretion of PTH are decreased, the activity of the CYP27B1 is inhibited, and activity of CYP24A1 (24-hydroxylase) is increased (Holick 2007). The $24,25(OH)_2D$ and $25(OH)D$–26, 23-lactone are catabolic products of $1,25(OH)_2D$ and $25(OH)D$ and are excreted in faeces and urine (Holick 2007). The schematic representation of vitamin D metabolism is presented in Figure 2.4.

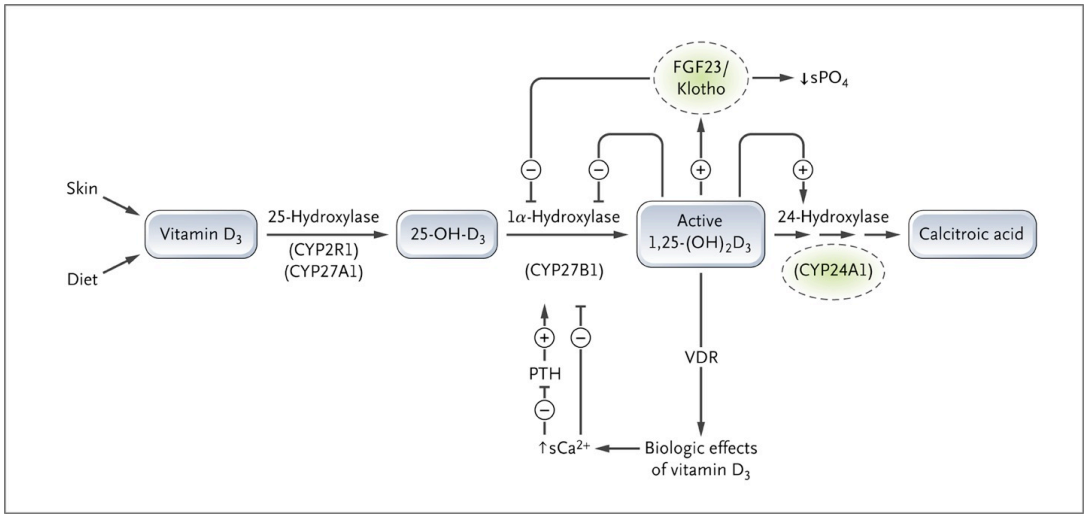


Figure 2.4. Vitamin D metabolism and some physiological actions
Note. Adapted from Schlingmann et al. (2011) Copyright 2020 by the Massachusetts Medical Society.

2.9 Vitamin D Measurement

There are some different vitamin D metabolites (more than 50), but two have been investigated routinely including $25(OH)D$ and $1,25(OH)_2D$ (Zerwekh 2008). There is a limited requirement for assessing blood concentrations of $1,25(OH)_2D$ and it is not measured as part of the routine vitamin D testing (DeLuca 2004, Zerwekh 2008) since $1,25(OH)_2D$ has a short half-life (≈ 4 hours) and our calcium requirement tightly regulates its production (Zerwekh 2008). Vitamin D_2 or vitamin D_3 also has a half-life of ≈ 24 hours, therefore the most recent sunlight exposure or vitamin D ingestion can affect its serum concentration. Therefore, measuring the blood concentration of vitamin D or $1,25(OH)_2D$ is not useful (Zerwekh 2008).

In contrast, measuring blood concentration of 25(OH)D produces a good evaluation of vitamin D values and whether people are vitamin D sufficient or vitamin D deficient since it has a longer half-life (≈ 3 weeks) (Zerwekh 2008). Thus its concentration provides an accurate indication of body stores from either cutaneous vitamin D synthesised from sunlight exposure or vitamin D ingested from a diet/supplement over a long period (Zerwekh 2008). Moreover, in the liver, 25(OH)D production is not significantly regulated and depends on substrate availability (Zerwekh 2008). Because of these reasons, measuring serum 25(OH)D provides the most reliable estimation of a person's vitamin D status.

2.10 Optimal Serum 25(OH)D

There is not a worldwide consensus about adequate serum 25(OH)D concentrations. Historically, for determining optimal levels of serum 25(OH)D, rickets/osteomalacia was used as an indicator. The threshold of rickets/osteomalacia is 20 nmol/L (Heaney 2004). Also, there is an inverse relationship between the optimal level of serum 25(OH)D and maximum suppression of PTH. The level of PTH is the main determinant of bone remodelling, reducing as 25(OH)D elevates (Heaney 2004, Bjorkman, Sorva et al. 2009). In previous studies, an inverse relationship between PTH and serum 25(OH)D has been reported (Ghergherechi, Hazhir et al. 2012, Sethuraman, Sreenivas et al. 2015, Sahin, Serdar et al. 2018). In a retrospective study of 90,042 children, Sahin et al. (2018) reported that the maximum suppression of PHT was at a 25(OH)D concentration of 75 nmol/L. Based on the Endocrine Society 25(OH)D below 50 nmol/L is considered as vitamin D deficiency, 25(OH)D between 50 to 75 nmol/L as vitamin D insufficient and 25(OH)D ≥ 75.0 nmol/L as vitamin D sufficient (Holick et al. 2011).

It is well-established vitamin D promotes intestinal calcium absorption. A cohort study, conducted by Abrams et al. (2009) in 251 healthy children (4.9-16.7 years old) found a significant relationship between total calcium absorption and 25(OH)D concentration and this correlation was closer in early puberty (higher 25(OH)D = higher calcium absorption). They also found that compared to children

with serum 25(OH)D concentration of 50-80 nmol/L, calcium absorption was higher than in children with 25(OH)D of 28-50 nmol/L. The authors concluded that the reason for higher calcium absorption is likely related to compensatory increases in PTH with decreases in serum 25(OH)D concentration. Serum 25(OH)D concentration has been shown to correlate positively with BMD and negatively with the incidence of fractures. In 100 healthy Indian school-age children (5-10 years old) Sharawat and Dawman (2019) found a positive correlation between lumbar spine BMD and serum concentration of 25(OH)D.

Serum 25(OH)D concentrations of at least 50 nmol/L are necessary for a better health outcome, however, the amount of 25(OH)D ≥ 75 nmol/L is optimal, since it has the maximum suppression of PHT (Sahin, Serdar et al. 2018). However, there is evidence that some New Zealand children and young people have sub-optimal vitamin D status. Results of the 2002 National Children's Nutrition Survey showed that 31 per cent of children aged 5–14 years had a 25(OH)D level below 37.5 nmol/L and four per cent of them had 25(OH)D concentrations less than 17.5 nmol/L (Rockell et al. 2005). Cairncross (2015) collected capillary blood spots late winter to early spring and measured 25(OH)D in 1329 preschool children aged 2-4 years old. Results showed 86 (7%) and 642 (48%) of children had vitamin D < 25 nmol/L and < 50 nmol/L, respectively.

2.11 Daily Requirement of Vitamin D

The Australia/New Zealand Nutrient Reference Values recommended adequate intake (AI) and upper level of intake (UL) of vitamin D for children and adolescents (aged 2-18 years old) who have little or no exposure to sunlight is 5 and 80 µg per day (200-3200 IU), respectively (Ministry of Health 2012).

Vitamin D Sources

In New Zealand, exposure to natural sunshine is the main source of vitamin D since there are very few foods fortified with vitamin D or naturally containing a high amount of vitamin D (Ministry of Health and Cancer Society of New Zealand 2012). A small quantity of vitamin D_3 can be obtained

naturally from oily fish (salmon, herring, tuna, and mackerel). There is a limited range of foods including milk and yoghurt, which are sometimes fortified with vitamin D (Ministry of Health and Cancer Society of New Zealand 2012). There is no mandatory fortification in New Zealand (Ministry of Health and Cancer Society of New Zealand 2012), so it would be hard to reach an acceptable blood concentration of vitamin D through diet alone.

Supplementation of Vitamin D in New Zealand

Children and young people who are at risk of vitamin D deficiency may benefit from vitamin D supplementation (Ministry of Health 2012). According to the Ministry of Health (2012), consumption of 10 µg (400 IU) per day may be necessary for children and young people with the following risk factors:

- Individuals with naturally very dark skin (e.g. African, Indian) or Middle Eastern populations with coverage (veils and full-body coverage clothing).
- Individuals with skin cancer or skin-related conditions, where avoidance of sun exposure is necessary.
- People with some health conditions such as liver or renal disease.
- People who consume some type of medications that have contraindications with vitamin D metabolism.

2.12. Factors Affecting Vitamin D Absorption

Bioavailability can be defined as the amount of a nutrient that is digested, absorbed by intestinal cells, transported to body cells, and finally available for physiological functions (Etcheverry, Grusak et al. 2012). Bio accessibility refers to the proportion of an ingested nutrient that is extractable from a food matrix and potentially available for absorption in the gut (Etcheverry, Grusak et al. 2012). The bioavailability or bioaccessibility of vitamin D may be enhanced or inhibited by some factors. These factors are discussed below.

2.12.1 Chemical Forms of Vitamin D

The chemical form of a nutrient may influence its bioavailability. A large body of evidence suggests that vitamin D_3 is more effective than vitamin D_2 in raising circulating 25(OH)D concentration, though the evidence is inconsistent. For instance, Armas et al. (2004) and Heaney et al. (2011) found that the potency of vitamin D_2 is less than D_3 in increasing serum 25(OH)D concentration. Contrary to these results, Holick et al. (2008) conducted a study to investigate the difference between D_2 and D_3 in maintaining 25(OH)D concentration. Their participants randomly were divided into four groups: placebo, 25 µg (1000 IU) vitamin D_3, 25 µg (1000 IU) vitamin D_2, or 12.5 µg (500 IU) vitamin D_2 plus 12.5 µg (500 IU) vitamin D_3 daily for 11 weeks. They concluded that all forms of supplementation had similar effects and vitamin D_2 was equally as effective as vitamin D_3.

2.12.2 Amount Consumed in a Meal

It is generally assumed that like other vitamins, there is an inverse linear relationship between the amount of vitamin consumption and the absorption efficiency of vitamin D (Borel, Caillaud et al. 2015). However, previous studies have shown vitamin D absorption efficiency does not significantly decrease with increasing dose (DeLuca 2004).

2.12.3 Dietary Factors Affecting Vitamin D Absorption

Lipids have an important role for the absorption of fat-soluble vitamins such as vitamin D. However, it has been shown that the amount of dietary lipids ingested with vitamin D does not affect absorption while the type of lipids (e.g. medium vs. long-chain fatty acids) incorporated with vitamin D can affect vitamin D bioavailability (more bioavailability after consumption of long-chain fatty acids compared to medium-chain fatty acids) (Borel, Caillaud et al. 2015). Also, it has been proven in previous studies that the food matrix has little or no noticeable effect on the bioavailability of vitamin D (Borel, Caillaud et al. 2015).

2.12.4 Host-related Factors in a Paediatric Population

There is a relationship between obesity and vitamin D insufficiency since vitamin D can be trapped in fat cells (Borel, Caillaud et al. 2015). Turer et al. (2000) investigated the prevalence of vitamin D deficiency (25(OH)D < 50 nmol/L) among children aged 6-18 years old and found that there is a significantly greater odds of vitamin D deficiency in overweight, obese, and severely obese children than normal-weight children. This data was supported by Mazahery et al. (2015) who also reported a better response to vitamin D supplementation in women with lower body fat percentage. Also findings revealed that vitamin D deficient and insufficient obese adolescents needed higher doses of vitamin D for treatment compared to their non-obese peers (Castaneda et al. 2012). Results from another study conducted by Reinehr et al. (2007) showed that vitamin D status was normalised after a weight-loss intervention programme without increasing dietary vitamin D intake. Some diseases (e.g. cystic fibrosis, gastrointestinal disorders) or surgeries (e.g. gastric bypass surgery) can affect vitamin D status by decreasing its absorption, and reducing its intake and consequently causing vitamin D deficiency.

Ethnicity is one of the main determinants of 25(OH)D status. In a review paper, Akhtar (2016) reported that vitamin D deficiency (20-50 nmol/L) is highly prevalent among the South Asian population. Rockell et al (2005) found the highest 25(OH)D concentrations in European/Other children (53 nmol/L), then in Māori children (44 nmol/L) and the lowest 25(OH)D concentrations were in Pacific children (37 nmol/L). A possible reason for these ethnic differences can be related to differences in skin colour as documented in previous studies (Weng, Shults et al. 2007, Poopedi, Norris et al. 2011). Individuals with naturally dark skin colour have high levels of pigmentation in their skin, which can decrease the amount of cutaneous vitamin D through reduced absorption of ultraviolet radiation and result in up to a six-fold slower production rate (Ministry of Health and Cancer Society of New Zealand 2012). This, therefore, puts them at higher risk of vitamin D deficiency and this is important for our population because of our ethnic diversity.

2.12.5 Environmental and Geographical Factors

A certain amount of ultraviolet (UV) radiation is necessary for 7-dehydrocholesterol (pro-vitamin D) to convert to pre-vitamin D_3. The angle that the sun hits the earth is called the solar zenith angle. Latitude, seasons, and time of the day can influence the zenith angle. By decreasing the solar zenith angle, the UV radiation is decreased therefore vitamin D synthesis is decreased as well. In wintertime and some places with a latitude above 70°, the zenith angle reduces and reaches the point that UV radiation is not enough for vitamin D synthesis (Engelsen, Brustad et al. 2005). Rockell et al. (2005) showed that the prevalence of vitamin D deficiency was significantly higher in winter than in summer. New Zealand has an average of 40° latitude. The New Zealand Ministry of Health recommends a daily walk or some other form of outdoor physical activity in the early morning or late afternoon during the summer months between September and April (Ministry of Health and Cancer Society of New Zealand 2012). Between May and August (winter in New Zealand), except at high altitudes or near highly reflective surfaces (e.g. snow or water), sun protection is generally not recommended. During this time exposure to direct sunlight, especially in the hours around noon when ultraviolet radiation beta (UVβ) levels are highest, will be enough for sufficient cutaneous production of vitamin D if sufficient skin is exposed. For people with light skin colour approximately 20% skin exposure is adequate (e.g. arms and legs) (Ministry of Health and Cancer Society of New Zealand 2012). However, using sun protection such as shade, sun-protective clothing and hat, sunscreen, sunglasses is recommended for those who have a special condition (e.g. history of skin cancer, skin damage from the sun) (Ministry of Health and Cancer Society of New Zealand 2012).

Importance of Milk/Dairy Products

Generally, 'milk' refers to cow's milk, since it is the most frequently consumed variety. Milk and dairy products are nutrient-rich foods, which are important throughout the life cycle, to support growth and development particularly during childhood and adolescence (Fiorito, Mitchell et al. 2006). Milk and other dairy products are good sources of energy, protein, and important macronutrients (e.g. carbohydrates, lipids, and proteins) and various micronutrients (e.g. calcium, phosphorus, iodine, magnesium, zinc, potassium, and vitamins A, B_{12}, B_2) (Dror and Allen 2014). Children who consume plenty of milk have been shown to have better BMD in adulthood than adults who did not (Sandler, Slemenda et al. 1985, Teegarden, Lyle et al. 1999, Movassagh, kontulanien et al. 2017, Movassagh, Baxter-Jones et al. 2018). Kruger et al. (2017) conducted a case-control study and examined 118 children (aged 5-10 years) over one year. Their study aimed to investigate the bone mineral status of pre-pubertal children participating in the 'milk for school programme' in New Zealand. Children whole body headless BMC and whole body headless BMD, lumbar spine BMC, and BMD were measured by DXA. They found greater changes in whole-body headless BMC in the milk consumption group. Also, they found that children in the drinking milk group were taller than the control group. Their results showed that most of their population (85% of the controls and 94-98% of the milk-drinking groups) met the New Zealand Ministry of Health recommendations to consume 2-3 servings of milk and dairy products per day (Ministry of Health 2012).

2.13 Milk Composition

2.13.1 Macronutrients

Any type of milk is mostly composed of water (87%). The main type of carbohydrate in cow's milk is lactose. The other crucial bioactive components in milk are oligosaccharides and glycoconjugates, which have the highest concentration in early post parturition milk (colostrum) (Gopal and Gill 2000). The chief function of these molecules is immunological protection (Gopal and Gill 2000). There are two main forms of proteins available in milk, whey and caseins. Whey is a soluble protein that makes

up 20% of total milk proteins (Pereira 2014). Casein is an insoluble protein and contributes 80% of the total milk protein. Casein has an important role in carrying calcium and phosphorous (Pereira 2014). There is an abundant glycoprotein in milk called lactoferrin, which is a potent regulator of bone cell activity. Lactoferrin increases bone formation (e.g. proliferation and differentiation of osteoblast cells) and inhibits osteoclast-mediated bone resorption (Cornish 2004, Cornish, Callon et al. 2004). The amount and composition of cow's milk fatty acids vary. The milk fat fraction is usually composed of triacylglycerol 98%, diacylglycerol 2%, cholesterol < 0.5%, phospholipids about 1%, and free fatty acids 0.1%. Milk also includes unsaturated fatty acids (oleic acid), trans-fatty acids (vaccenic acid), and conjugated linoleic acid (CLA) (Pereira 2014).

However, the current evidence suggested that dairy products should be considered as a whole when looking at their nutritional and physiological health effects instead of looking at specific nutrients (Thorning and Bertram 2017, Turgeon and Brisson 2020). This fact is based on people eat food and usually in conjunction with meal not nutrients in isolation. Therefore, the health effects of a food are much more complex than a single nutrient, which is known as a "food matrix" effect. In fact, food matrix can be effected by food's structure, its nutrient composition, and the interaction between these nutrients (Fardet, Dupont et al. 2019, Thorning and Bertram 2017). For instance, the bone health benefits of milk and dairy products consumption can be referred to the complex interactions between calcium, protein and phosphorus with each other and with lactose and bioactive peptides in the dairy matrices (Heaney 2009). Further research needs to investigate in depth the bioavailability of protein, lipid and calcium fractions and the benefits effect of dairy food as a matrix on our health (Fardet, Dupont et al. 2019).

2.13.2 Micronutrients

Minerals

The beneficial effects of milk and dairy products on bone health are mainly related to the presence of calcium and phosphorus; two nutrients necessary for the inorganic bone matrix and potassium, and

regulating bone turnover (Dror and Allen 2014). Milk also contains other bone minerals including zinc, selenium, and magnesium which are important for bone health as well.

Vitamins

Milk and dairy products are composed of both water-soluble (e.g. thiamin, riboflavin, and vitamin B_{12}) and fat-soluble vitamins (e.g. A, E, and trace amount D). Vitamin A plays a vital role in our body such as skin health, vision, immunity, fertility, and gene regulation (Gaucheron 2011) while vitamin D helps in calcium and phosphorous balance, bone formation, immune system, and cell differentiation (Dror and Allen 2014). Fortification of food with vitamin D is voluntary in New Zealand. Some margarine, milk and yoghurts are fortified with vitamin D, however, vitamin A fortification is not common (Ministry of Health and Cancer Society of New Zealand 2012). Vitamin E is an antioxidant, which can scavenge free radicals and is found in bovine milk as α-tocopherol (Lindmark-Mansson and Akesson 2000).

2.14 Milk and Growth

Leg length has been identified as a marker of growth before puberty (Schooling, Jiang et al. 2010). Milk and dairy products intake showed a relationship with stimulation of bone formation and leg length through increasing expression of IGF-1 (Hoppe, Udam et al. 2004, Rogers, Emmett et al. 2006). Insulin-like growth factor-1 is stimulated by GH and consequently increases bone cell differentiation and proliferation and enhances bone growth (Gat-Yablonski, Yackobovitch-Gavan et al. 2009). Bovine milk also contains the osteoclastogenesis inhibitory factor (osteoprotegerin), which is a negative regulator of osteoclast differentiation and activation (Kanezler, Bodamyali et al. 2001). Moreover, milk whey protein especially its basic protein (MBP), consists of different components, which can promote bone formation and suppress bone resorption (Takada, Aoe et al. 1996, Yamamura, Aoe et al. 2002, Uenishi, Ishida et al. 2007).

Results from a meta-analysis by de Beer (2012) illustrated that consumption of 245 ml milk daily can cause additional growth of about 0.4 cm per annum in a group of children and adolescents 2-18 years

old. Therefore, prolonged avoidance of milk and dairy product consumption during growth (e.g. allergic reaction to cow milk, dislike of the taste or a lifestyle choice) could jeopardise general bone health and development (Goulding, Rockell et al. 2004). Black et al. (2002) investigated 50 milk avoiders (aged 3-10 years old) and found an association between long-term milk avoidance and small stature and poor bone health. Kalkwarf et al. (2003) demonstrated that low milk intake during childhood can increase the risk of bone fractures by 2-fold. Yeh et al. (2006) investigated the rate of fractures among 313 New Zealand children under 13 years old. They found those children who consumed less than the recommended 2 to 3 servings milk daily had more fractures.

2.15 Factors Affecting Milk Consumption

Children avoid drinking milk for numerous reasons, which are briefly discussed below.

2.15.1 Age

Dairy product consumption decreases significantly as children move from childhood to adolescence (Mensink, Kleiser et al. 2007, Keller, Kirzner et al. 2009). Fiorito et al. (2006) followed up 151 girls aged 5 to 11 years to investigate their dairy intake patterns and found that there was a significant decrease in total milk consumption over time, mainly due to a decline in consumption of milk as a beverage ($P < 0.001$). It is suggested that as children become older the consumption of milk decreases and it is replaced with other beverages such as sweetened beverages (Lo, Coles et al. 2008) or other dairy products like cheese and dairy desserts (Fiorito, Mitchell et al. 2006).

2.15.2 Gender

Mensink et al. (2007) found that more boys than girls consume milk and dairy products. It has been shown that female adolescents limit milk and dairy products intake to lose or maintain their weight (Larson, Story et al. 2006).

2.15.3 Ethnicity

Based on previous research findings, ethnicity has an important role in milk and dairy products consumption. For instance, the New Zealand Ministry of Health (2003) conducted a nation-wide

children's nutrition survey in 2002 which found that the proportion consuming milk daily was higher in New Zealand European children than Māori and Pacific children. Veghari (2013) investigated the milk consumption of 7430 primary school children among Fars-native, Turkman, and Sisstanish ethnic groups, and found significantly more milk intake in Turkman children (66.0%) compared to Fars-native (61.4%) and Sisstani (58.2%) children. He suggested that a lower nutritional situation of Fars-native and Sisstani children than Turkman children may be a barrier to milk consumption.

2.15.4 Sugary Drinks

Children and adolescents are likely to change their beverage intake patterns by decreasing milk consumption and increasing sweetened beverages as they get older (Lo, Coles et al. 2008) and the exact reasons for this behaviour are not known. However, it is postulated that parental influence, television advertisements, increasing age and independency, and milk and dairy product availability at school can be some reasons for these changing patterns. This shift has harmed children and adolescents' bone health by decreasing calcium intake due to replacement of milk in the diet with sweetened beverages, which are high in caffeine, phosphoric acid and/or sugar (Kinney 2002, Fitzpatrick and Heaney 2003). Results from a systematic and meta-analysis revealed that consumption of noncarbonated or carbonated beverages could contribute to increased risk of bone fractures (Handel and Heitmann, 2015). Ma and Jones (2004) ran a case-control study among children aged 9–16 years to explore the relationship between the consumption of soft drink and milk with upper limb fracture risk. The authors showed a positive correlation between cola beverage consumption and wrist and forearm fracture risk.

2.15.5 Parental Influence

Parental influence is a plausible factor as studies showed that children of parents who have a high intake of sugary drinks also drank sugary beverages more often (Fisher, Mitchell et al. 2001). Milk consumption patterns of children and adolescents were negatively influenced by the availability of healthy foods and provision of nutritious foods by parents (Auld, Boushey et al. 2002, Vue and Reicks 2007), and occasionally parental choices and eating patterns (Fisher, Mitchell et al. 2001, Vue and

Reicks 2007). The role of beverage knowledge of parents and their practice on beverage intake of children have been investigated in a few studies. For example, Cluskey et al. (2008) interviewed 201 parents of children aged 10-13 years old and found limited knowledge of parents regarding calcium food sources, function, and requirements. Zahid et al. (2017) aimed to investigate the association between sugar-sweetened beverages (SSB) and dairy beverages and home availability and parental factors (beverages knowledge) among 194 children aged 9–12 years. They found that there was a relationship between parent dairy intake and child dairy intake. Their results also showed that knowledge of parents about sugar in beverages had an association with the children's dairy intake. Although, their sample size was small and they used a convenience sample, one of the strengths of the study was that they recruited parents and their children, and therefore had better information regarding relationships between parent knowledge, practise of the parent, and child behaviours. Therefore, parents play a crucial role in the development of their child's dietary patterns, eating preferences, and expectations (Scaglioni, Salvioni et al. 2008).

2.15.6 Dietary Habits

Some studies supported that children and adolescents who eat breakfast have a higher consumption of milk and calcium intakes (Nicklas, O'Neil et al. 1998, Ortega, Requejo et al. 1998, Bowman 2002). Based on the Ministry of Health (2003) findings approximately 10% of New Zealand children and young people skipped breakfast, with the highest prevalence in Pacific children, followed by Māori children. Data suggests that the most important factors for the consumption of breakfast regularly could be the availability of breakfast and parental modelling (Pearson, Biddle et al. 2009).

2.16 Adverse Reactions to Milk

There are two reasons for adverse reactions to milk, including lactose intolerance and milk protein allergy, which will be discussed briefly in the next sections.

2.16.1 Lactose Intolerance

Lactose is a disaccharide and the main carbohydrate of mammalian milk and dairy products (Heyman 2006). In the small intestine, a β-galactosidase enzyme termed lactase breaks down lactose into its two primary molecules, glucose and galactose. Then, these two monosaccharaides are absorbed through the enterocytes and transported into the blood (Pereira 2014). Certain susceptible individuals experience abdominal symptoms during lactose ingestion (Heyman 2006). Either congenital, primary or secondary lactase deficiency (hypolactasia) can cause lactose intolerance (Prentice 2014). The lactase enzyme activity is at the lowest level of activity in congenital lactose deficiency. This type of intolerance is a lifelong disorder and cannot be completely cured and results in complete avoidance of lactose from birth. It is suggested that primary lactase deficiency or lactase non-persistence individuals may tolerate up to 12 g lactose if consumption is spread throughout the day (e.g. with breakfast and in tea or coffee) (Lomer, Parkes et al. 2008). Some illnesses such as gastrointestinal illness can lead to secondary lactase intolerance or acquired lactase deficiency. This type of lactose intolerance is less severe and is usually reversible (Lomer, Parkes et al. 2008).

When lactose is not absorbed in the small intestine, it passes into the gastrointestinal tract, ferments in the colon and produces lactose intolerance symptoms such as abdominal cramps, bloating, flatus, diarrhoea, nausea, and vomiting (Lomer, Parkes et al. 2008, Pereira 2014). Usually, individuals with lactose intolerance avoid intake of dairy products and without compensatory dietary adjustments, they may receive inadequate calcium in their diet and consequently increase fracture risk (Goulding 2007). Suggestions regarding consumption of dairy products by lactase deficient people without showing any severe adverse effects include dividing milk consumption into small portions or consuming fermented milk, cheese, and yoghurt as a source of lactose and calcium (Shaukat, Levitt et al. 2010).

2.16.2 Milk Protein Allergy

About 2 to 6% of children suffer from cow's milk protein allergy (CMPA), with the highest prevalence during the first year of their life (Caffarelli, Baldi et al. 2010). With increasing age, the prevalence starts to decrease and becomes an uncommon problem in adulthood. There are more than

20 different proteins available in cow's milk, which can lead to allergic reactions (El-Agamy 2007). Since casein and whey are the most abundant milk proteins they are the most prevalent reason for milk allergies (Kattan, Cocco et al. 2011). Cow's milk protein allergy by avoiding cow's milk intake, replacing cow's milk with other mammals' milk, or plant-based milk (e.g. soy milk, almond milk) (El-Agamy 2007, Kattan, Cocco et al. 2011).

2.17 New Zealand Milk Consumption

The Ministry of Health (2012) recommends consuming 2-3 servings of milk and dairy products per day for 2-12 years old children to help achieve total calcium recommendations. However, according to the 2002 survey results, only 38% of New Zealand children drink milk (not flavoured) once a day, 34% once a week, 10% once a month, and 17% less than once a month or never (Ministry of Health 2003). Figure 2.5 shows the proportion of milk intake by ethnic groups in New Zealand. Children in the New Zealand European and other group had the highest proportion of milk consumption (Ministry of Health 2003).

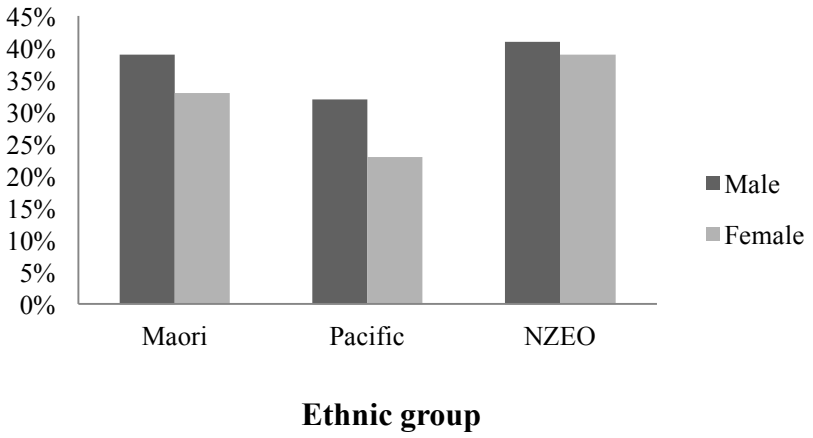


Figure 2.5. Milk consumption of New Zealand children per day.
Ministry of Health (2003). *NZEO*, New Zealand European and Other.

History of the New Zealand School Milk Programme

The New Zealand government started to provide free milk for schools between 1937 and 1967 (when milk production was surplus) intending to improve the health of young New Zealanders. By 1940, over 80% of school-age children received milk. The New Zealand school milk programme was terminated in 1967 due to the annual cost and some questions around the health benefits of milk (Bulford and Alexander 2001). However, the scheme was started again in 2013 by Fonterra Co-operative Ltd and milk has been provided for more than 1100 New Zealand schools nationwide since then.

Conclusion

Previous studies have attempted to investigate the risk factors of childhood fractures (e.g. age, obesity, physical activity, nutrition, vitamin D status) (Ma and Jones 2002, Ma and Jones 2004, Manias, McCabe et al. 2006, Clark 2014). Goulding (2007) suggested a checklist for every child with their first fracture and recommended that these children must be carefully reviewed for any skeletal health problem by health care professionals (Table 2.4). However, there is limited data available regarding paediatric fractures and known risk factors (e.g. milk consumption, calcium, and SSB intake, vitamin D status, PA, ethnicity, body composition, and family history of fractures) in the paediatric population living in Auckland, New Zealand. Early identification of risk factors during childhood will allow changes their lifestyle, thus enhancing bone mineralisation, preventing fractures, and improving adult bone health.

Table 2.4. Checklist for every child with a fracture

How did the fracture happen?
Has the child had any previous fractures?
Is there any family history of fracture?
Has the child any health condition affecting bone?
Are there any signs of endocrine disorders?
Are any medications or treatments being given that can affect bone density?
What is the child weight and BMI?
What is the pubertal status of the child?
Is the child be involved in regular weight-bearing physical activity
Does the child have appropriate safety clothing for sport?
Does the child have adequate sunshine exposure?
Does the child consume adequate milk and dairy products?
Does the child have a balanced diet (meet the recommended levels of calcium, proteins, calories, beverages, salt, caffeine)?

Goulding (2007)

Methods for Measurement of Paediatric Body Composition

Childhood overweight and obesity can lead to developing musculoskeletal problems, injuries, and fractures (Adams, Kessler et al. 2013, Paulis, Silva et al. 2014). There are some charts such as weight-for-age and body mass index (BMI)-for-age, which are used for measuring children's physical growth. They can give a general clinical overview of the health and nutritional status and they only provide proxy measures for changes in body composition of children (Lee, Liao et al. 2017). Therefore, they are not suitable for measuring body composition, which undergoes dynamic changes throughout growth and development (Lee, Liao et al. 2017).

Also, there are a variety of other methods available for measuring body composition, including skinfold thickness (Himes, Roche et al. 1979, Lohman 1981), ultrasound (Haymes, Lundegren et al. 1976), hydro-densitometry (or underwater weighing) (Wilmore 1969), electrical conductivity (Presta, Casullo et al. 1987), deuterium distribution (Loeppky, Myhre et al. 1977), computed tomography (CT) (Borkan, Gerzof et al. 1982), magnetic resonance imaging (MRI), dual-energy X-ray absorptiometry (DXA), measurement of potassium (Forbes and Hursh 1963), nitrogen (Vartsky, Ellis et al. 1979), and calcium (Cohn, Shukla et al. 1972). The accuracy, feasibility, cost, and accessibility of these methods are varied and important to use methods that are as accurate and precise as possible when investigating body composition in children.

Among these body composition measurement methods, DXA is one of the most applicable techniques and becoming increasingly available for both clinical and research purposes. Dual-energy X-ray absorptiometry measures body composition using a three-compartment model: BMC, fat-free mass, and fat mass (Pietrobelli, Formica et al. 1996). The advantages of this technique are its precision and moderate requirement for subjects' co-operation (Okasora, Takaya et al. 1999). However, this method has some drawbacks: 1) it is not cost-effective, 2) exposes subjects to radiation, and 3) requires specific laboratory facilities and trained operators. Accordingly, it is not suitable for routine paediatric clinical practice (Fors, Gelander et al. 2002).

Recently, bioelectrical impedance analysis (BIA) has received much attention as an alternative method for measuring body composition. Compared to laboratory-based techniques, it is a rapid, safe, relatively simple, child-friendly method both in the field and in clinical settings for tracking of body composition changes in children (Noradilah, Ang et al. 2016). Other advantages of this technique are low cost, equipment portability, and needing minimal operator training (Houtkooper, Lohman et al. 1989). The BIA can measure the volume of total body water (TBW), and since there is an assumption that TBW is a fixed proportion of fat free mass (FFM), values for FFM can be calculated. Finally, body fat mass (FM) is computed from TBW and FFM (Kushner 1992). The BIA is based on the conductibility of an electrical current in the body (Okasora, Takaya et al. 1999). Since FFM has greater electrolyte content compared to FM, it causes less electrical resistance (Okasora, Takaya et al. 1999). In BIA, measurements are according to the correlation between the conductor volume (i.e. the child's body), the length of the conductor (i.e. the child's height), the components of the conductor (i.e. FM or FFM), and its impedance (Brodie, Moscrip et al. 1998).

In segmental models, subjects are in the supine position and a current passes from hand-to-foot, foot-to-foot, or hand-to-hand (Khalil, Mohktar et al. 2014). The whole-body BIA technique measures the electrical resistance of the body through four electrodes attached to different sites of the body (Kushner 1992). The amount of FFM (kg), FM (kg), and body fat percentage (%BF) are computed through specific equations (Kushner 1992). A vertical 8-point model of BIA has footpads containing electrodes on the base platform and similar sensors on the handles, which extend from the up-stand and control panel (von Hurst, Walsh et al. 2015). Both footpads and handles each contain two electrodes, providing eight points of contact. A small electrical current is passed through the body, resistance is measured and the inbuilt software calculates TBW and the corresponding %BF (von Hurst, Walsh et al. 2015). Body composition parameters such as FFM (kg), FM (kg), and %BF are calculated for each participant based on recorded impedance, height, age, gender, anthropometric index, and other factors. Although BIA has some advantages for measuring body composition, it has a fundamental problem. It is a predictive method based on algorithms so its results are not as accurate

as may be desired (Ward 2019).

There are only a few studies in healthy children available which compare the validity of BIA measurements with DXA (Gutin, Litaker et al. 1996, Okasora, Takaya et al. 1999, Fors, Gelander et al. 2002, Noradilah, Ang et al. 2016). Results have varied because of the wide range of BIA equipment available. The validity of BIA devices considered in other similar studies included ankle-to-wrist electrode systems (Eisenmann, Heelan et al. 2004), hand-to-foot electrode systems (Elberg, McDuffie et al. 2004) as well as 8-point models (Kriemler, Puder et al. 2009, Lee, Liao et al. 2017). Although BIA devices have been used in the assessment of body composition, their validity against conventional reference methods (e.g. DXA) as a measure of body composition in children remains uncertain and needs to be investigated.

Methods for Measurement of Paediatric Bone Health

The demand for evaluating of paediatric bone mineral status has increased with the development of more bone measurement methods (Baroncelli 2008). There are two main reasons for measuring paediatric bone mineral status. The first is to find children who might be at increased risk of bone problems (e.g. osteopenia, osteoporosis). Since the amount of bone gained during growth and its subsequent rate of loss is an important determinant of osteoporosis in adulthood (Specker and Schoenau 2005). Some several disorders and factors can have an adverse effect on bone mineral status (Baroncelli, Bertelloni et al. 2005) and diagnosis and assessing their association with bone loss can be helpful (Kovanlikaya, Loro et al. 1996). The second is to start timely intervention programmes (e.g. adequate nutritional intake and physical activity) to optimise bone accrual and consequently bone strength and quality for children who are at risk of osteopenia and/or osteoporosis later in life (Specker and Schoenau 2005).

There are several conventional methods to assess children's bone density such as dual-energy X-ray absorptiometry (DXA) and quantitative ultrasound (QUS). It is highly important to choose easy to apply, accurate, valid and reliable, non-invasive, and harmless methods in research and clinical

settings. Moreover, these methods should provide information on bone mass, geometry, and bone density and ideally not be influenced by the bone size, body size or soft tissue (Specker and Schoenau 2005). These conventional methods for assessment of paediatric bone status will be briefly discussed below.

2.18 Dual-energy X-ray Absorptiometry (DXA)

The most widely employed technique for assessing bone mineral status is DXA. In the late 1980s, DXA was primarily used for diagnosing osteoporosis in postmenopausal women and has since become a commonly available densitometry technique to evaluate and manage adult bone disease (Binkovitz and Henwood 2007). In the early 1990s, after demonstrating improvements in detecting bone in children with low bone density, the door for using DXA software in a paediatric population had been opened (Specker and Schoenau 2005, Binkovitz and Henwood 2007).

Dual-energy X-ray is a radiological device which depends on the differential absorption of photons to compare tissues of different radiographic density (Binkovitz and Henwood 2007). The source of photons in DXA is an X-ray tube which causes a higher photon flux, and therefore better edge detection and more precise information (Gilsanz 1998). A computer then detects and measures X-rays' photons exiting from various body sites (Gilsanz 1998) and the attenuation values determined using calibration materials are converted into BMC and quantified (in grams) (Gilsanz 1998). Bone mineral density is calculated by dividing BMC values by bone area (BA) (BMD = BMC/BA (g/cm^2) (Gilsanz 1998). Since there are two dimensions available for DXA bone images and they are not a true volumetric density, the DXA-derived BMD is referred to as areal BMD (aBMD) (Binkley, Berry et al. 2008). Bone size can affect aBMD measurements and it is an important problem in paediatric bone assessment due to large differences in body size and bone size between different age groups (larger bones having artificially inflated aBMD measurements) (Specker and Schoenau 2005). According to the International Society for Clinical Densitometry (ISCD) recommendation, children who are considered for the treatment of osteoporosis due to chronic inflammatory conditions,

prolonged immobilisation, and/or idiopathic juvenile osteoporosis, should undergo a DXA examination (The Writing Group for the ISCD 2004).

It is important to consider that there are different DXA manufacturers, one manufacturer produces different DXA models, and even results from one DXA device can differ as different software analysis packages are available (Specker and Schoenau 2005). In paediatric studies, DXA scans have usually been used for bone measurements in the total body and regional sites (Specker and Schoenau 2005). Regional sites include the lumbar spine, the proximal femur, the forearm and the hand (Gilsanz 1998) and it is important to realise which bone site is measured since the proportion of cortical and trabecular bone is different (Specker and Schoenau 2005). For instance, trabecular bone has the greatest proportion in the spine while the long bones are predominantly formed from cortical bone. However, any site which is chosen for bone measurement should provide a robust evaluation of the paediatric bone density status (Binkovitz and Henwood 2007).

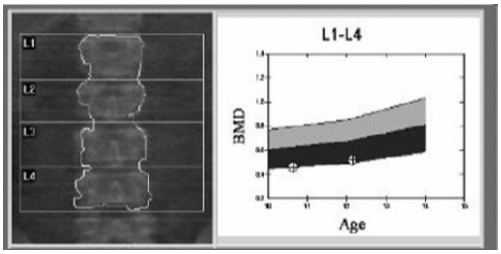
Depending on the DXA machine used, the measurement can take between 2 and 15 minutes. There is DXA manufacturers' normative data for aBMD at some bone sites and they are specific for age, however, using these data may not be appropriate for diagnosing low aBMD and/or BMC, especially when the child is smaller than his/her age (Specker and Schoenau 2005).

The bone density (aBMD) test results are usually interpreted as 'T-score' and 'Z-score'. According to the WHO criteria T-scores are used for diagnosing osteoporosis in adults (World Health Organization 2004). T-scores are based on comparing aBMD from normal young adults with the observed aBMD standard deviation scores. The normal range of the average bone density for young healthy adults is when T-scores are -1.0, 0, +1.0, and above. Bone density less than -1 in adults indicates osteopenia and less than −2.5 indicates osteoporosis (World Health Organization 2004). However, the T-score is used in adult DXA reports and compares the observed aBMD results with normal range so it is not appropriate to use in growing children (The Writing Group for the ISCD, 2004). Therefore, Z-score is a more appropriate method for evaluation and comparing a child's aBMD results with other normal children ("Z-score defined as the standard deviation (SD) score based on

age-specific and gender-specific norms") (Specker and Schoenau 2005, Binkley, Berry et al. 2008). The ISCD suggests measuring whole body and lumbar spine as the skeletal sites for DXA (Figure 2.6) (Leib, Lewiecki et al. 2004). In paediatric DXA reports, the phrase low bone density is recommended when the Z-score is below -1 (aBMD normal range = −1 and +1.5) (The Writing Group for the ISCD, 2004). However, the terms osteopenia (Z-score < 1-) and osteoporosis (Z-score < -2.5) are used in children by some clinicians and researchers (Binkovitz and Henwood 2007). Nevertheless, it should be noted that not only the DXA reports should be used as osteoporosis diagnosing criteria but other participant factors should also be taken into account (Binkovitz and Henwood 2007).

A

B



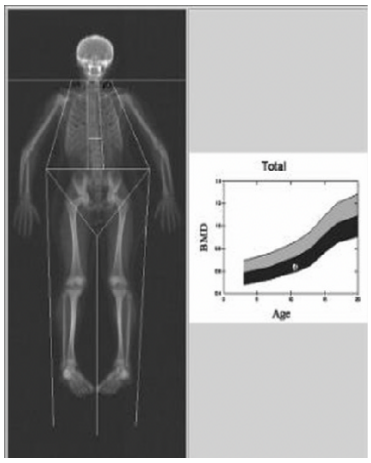


Figure 2.6. Dual-energy X-ray absorptiometry (DXA) measurements results
Dual-energy X-ray absorptiometry (DXA) measurements performed on a Hologic QDR Discovery A (Hologic Inc, Bedford, MA, USA) with APEX V. 3.2 software. Areal BMD results are transformed into Z-scores and presented with age- and gender-specific reference curves. Measured sites (A) whole body and (B) lumbar spine.

The DXA can assess bone mass accurately and precisely in a short period (Gilsanz 1998). However, an important point to remember is that DXA measures bone in two dimensions and provides only an estimation of BMD (Fewtrell 2003, van Rijn, van der Sluis et al. 2003, Specker and Schoenau 2005). The inability of DXA to measure cortical and trabecular bone separately, and account for body

changes and bone size during growth, limits its utility in children (Gilsanz 1998, Binkley, Berry et al. 2008) particularly when study populations are children with eating disorders (e.g. obesity or anorexia nervosa) (Gilsanz 1998). Another disadvantage of DXA is the unknown composition of soft tissues around the region of interest (ROI) that can affect DXA measurements thus longitudinal DXA values in children may not reflect true changes in BMC (Gilsanz 1998). Lastly, this method is expensive, requires trained operators, and exposes children to ionising radiation (Binkovitz and Henwood 2007). It is therefore not suitable for routine paediatric clinical and research practice and is impractical for use in the field.

2.19 Quantitative Ultrasound (QUS)

In both clinical and research settings, the technique must provide information on paediatric bone mass and bone density (Specker and Schoenau 2005). In recent years, the use of radiation-free techniques has increased for the assessment of skeletal health status. In 1984, Langton et al. (1984) developed the first QUS as a device for assessing bone status in adults (Langton, Palmer et al. 1984) and since then QUS has been suggested as an alternative method of assessing bone density at the peripheral skeleton (Genant, Engelke et al. 1996). The QUS devices are radiation-free portable scanners and only take a few minutes for measurements (Baroncelli 2008). QUS is also able to provide some additional structural information beside bone mineral status, which may be important in determining fracture risk (Genant, Engelke et al. 1996, Gregg, Kriska et al. 1997, Njeh, Boivin et al. 1997). There are some other benefits in using the QUS method for evaluating bone status in children and adolescents. This technique is low cost, easy to apply, and non-invasive (Baroncelli 2008) (Figure 2.7).

Quantitative ultrasound measures the velocity of transmitting sound through a body tissue (e.g. bone) and the amplitude of ultrasound wavelengths are absorbed through transducers placed on the opposite side of the tissue, reflecting its density, architecture, and elasticity (Kaufman and Einhorn 1993, Genant, Engelke et al. 1996, Gregg, Kriska et al. 1997, Njeh, Boivin et al. 1997). In the case of bone,

ultrasound velocity is dependent on the specific biomechanical, structural properties, resistance to deformation (elasticity modulus), and compressive strength of bone (bone's load-carrying capacity) (Kaufman and Einhorn 1993, Genant, Engelke et al. 1996, Gregg, Kriska et al. 1997, Njeh, Boivin et al. 1997), which are also related to bone density (Njeh, Fuerst et al. 2001). There is a reduction in beam amplitude; when ultrasound waves travel through the bone, they are absorbed by the medium, therefore they lose their acoustic energy and this phenomenon is known as attenuation (Baroncelli 2008).

Figure 2.7. Sahara Clinical Bone Sonometer Hologic Inc, USA

Two main variables can be measured by QUS devices, derived from the velocity or attenuation of the ultrasound waves, which travel through the bone tissue (Baroncelli 2008). Unfortunately, there is not a universal term for ultrasound transmission velocity and it is known by the speed of sound (SOS), the velocity of sound, and apparent velocity of ultrasound (Specker and Schoenau 2005) which do not rely on ultrasound wave attenuation (Lum, Wang et al. 1999). The SOS reflects the velocity of ultrasound waves inside of the bone (e.g. heel, radius, tibia, and patella) (Baroncelli 2008) and is defined by the ratio of the width of the ROI by the travel time (m/s) (Gilsanz 1998). In biological soft tissue, the total ultrasound attenuation is linearly proportional to frequency over the range of 200–600 kHz (Gilsanz 1998). In clinical practice, the broadband ultrasound attenuation (BUA) represents the slope of attenuation of mechanical waves travelling through the bone and is expressed as dB/MHz

(Baroncelli 2008). Some calcaneal QUS devices can combine both SOS and BUA measurements mathematically and provide an additional variable known as the stiffness index (SI) through [(0.67 × BUA) + (0.28 × SOS) - 420] (Jaworski, Lebiedowski et al. 1995, Sundberg, Gardsell et al. 1998) and a quantitative ultrasound index through [0.41 × (BUA + SOS) - 571] calculations, which is expressed as a percentage (Magkos, Manios et al. 2005).

2.19.1 Main Technical Characteristics of QUS Methods

There are various QUS devices based on their technical characteristics, such as the device precision, frequency of released ultrasound beam, ROI, the number of skeletal sites, the number of bone components and variables, and acoustic pathways of transmitting beam inside of body tissue (Guglielmi, Adams et al. 2009). The frequency range of acoustic generated waves through QUS devices are between 500 kHz and 1.25 MHz (Guglielmi, Adams et al. 2009). The ultrasound beam is absorbed by transducers, which are placed on either side of the ROI. The position of the transducers and the bone components at the ROI are two important factors that can influence the pathways of ultrasound broadcast inside the bone tissue (Baroncelli 2008). Unfortunately, the majority of available QUS devices are designed for adults and have relatively large transducers (Gilsanz 1998) therefore, they are not appropriate equipment for bone measurements in paediatric populations because of unsuitable transducer sizes. Thus to avoid measurement error, smaller transducers with special foot-pads or appropriately sized callipers are recommended when children are the target population (Schonau, Radermacher et al. 1994, Jaworski, Lebiedowski et al. 1995). Also similar to adults, the transmission of the ultrasound waves in paediatric QUS devices requires a coupling medium (e.g. a water bath or gel) between the transducers and the skin, covering the ROI (Gilsanz 1998). The coupling medium must have a constant temperature to avoid inaccurate velocity and attenuation values (Gilsanz 1998). In calcaneal QUS devices, the temperature of the water bath or coupling gel (water- or oil-based) should be controlled to transmit the ultrasound beam, whereas other dry QUS devices (gel-free) work by using isopropyl or ethylic alcohol (70%) and do not require thermal control (Baroncelli 2008).

The amount and composition of bone marrow and other soft tissue in the acoustic pathway's beam can affect ultrasound measurements (Genant, Engelke et al. 1996). Most of the QUS devices measure only one skeletal site (e.g. the heel, the tibia, or the proximal phalanges of the hand), however, some other devices are available, which have different probes and therefore can measure more than one site (multisite QUS device) (e.g. the tibia, radius and third phalanx of the hand) (Guglielmi, Adams et al. 2009). Multisite QUS devices have more than one ultrasound signal transmitter and more than one ultrasound receiver concerning the examined bone (Baroncelli 2008). Among all body sites for bone measurement, the calcaneus is the most common site, and less frequently selected are the patella, tibia, and phalanges (Gilsanz 1998).

The ISCD suggests the calcaneus as the best skeletal site of measurement compared to the other peripheral sites (e.g. radius, tibia) since it shows an acceptable level of scientific validation results in osteoporosis management (Krieg, Barkmann et al. 2008). In healthy children, there are not any differences between left and right sides of proximal phalanges of the hand (Baroncelli, Federico et al. 2001) or mid-shaft tibia (Lequin, van Rijn et al. 1999) in QUS variables. However, there are some discrepancies in the heel results likely due to some anatomical differences in the heterogeneity of the calcaneus structure (Bayer and Kutilek 1997, Magkos, Manios et al. 2005). Also, the bone thickness, the amount of bone mineral, and the three-dimensional arrangement of the trabecular bone in the calcaneus can influence BUA and SOS measurements (Gilsanz 1998).

Based on the anthropometric variables, and the ISCD suggestions, a Z-score less than −2.0 is considered as a low bone mineral status (Lewiecki, Watts et al. 2004). It has been suggested that it is more appropriate to report the Z-score for evaluating the bone mineral status and estimating the degree of bone mineral reduction in children (Fewtrell 2003, Lewiecki, Watts et al. 2004, Baroncelli, Bertelloni et al. 2005).

2.19.2 Children QUS Validation Studies

A major issue with the use of QUS is the lack of information regarding the validity of ultrasonic measurement of bone in a paediatric population. Therefore, a review of published studies from 1995 to 2020 was conducted focusing on QUS with these criteria: 1) examined validity of QUS 2) using DXA as a gold standard method (reference method) 3) in a group of healthy children and/or adolescents and young adults (4 to 25 years old), and 4) published in English.

There are only a few studies that have validated QUS against DXA in children and adolescents (Mughal, Langton et al. 1996, Sundberg, Gardsell et al. 1998, Lum, Wang et al. 1999, Sioen, Goemare et al. 2011, Xu, Guo et al. 2014, Chong, Poh et al. 2015, Srichan, Thasanasuwan et al. 2016, Weeks, Hirsch et al. 2016). However, to the best of our knowledge, there is no published validation of QUS in children living in New Zealand as revealed in Table 2.5.

For instance, Chong et al. (2015) and Srichan et al. (2016) both conducted studies and investigated the validity of radial QUS against DXA measurements. In both studies, SOS (m/s) was measured at the non-dominant arm at a distal one-third-radius point. Then by using the manufacturer's reference values (data derived from a gender- and age-matched Asian population) SOS Z-score values were calculated for each participant. Chong et al. (2015) concluded that radial QUS and DXA are not comparable and interchangeable in assessing the bone status of children. Srichan et al. (2016) also found a low level of agreement between radial QUS variables and DXA measurements. However, five other studies examined the validity of calcaneal QUS against DXA measurements (Mughal, Langton et al. 1996, Sundberg, Gardsell et al. 1998, Lum, Wang et al. 1999, Sioen, Goemare et al. 2011, Xu, Guo et al. 2014). In these studies, calcaneal QUS measured BUA (dB/MHz) and/or SOS (m/s) and/or SI parameters. Total body (TB) BMD was obtained in all studies. Their findings showed correlation coefficients (ranging from 0.44 to 0.88) between calcaneal QUS and DXA-derived BMD or BMC (Mughal, Langton et al. 1996, Sundberg, Gardsell et al. 1998, Xu, Guo et al. 2014). For instance, Xu et al. (2014) measured 329 healthy boys and girls aged 5-17 years and found positive correlations between calcaneal QUS SI and DXA TB BMD ($r = 0.69$, $P < 0.001$) and BMC ($r = 0.69$,

$P < 0.001$). However, Sioen et al. (2011) did not find significant correlation results between SI measured by QUS and BMD values measured by DXA. Another study investigated the validity of QUS against DXA in a paediatric population in Australia. Weeks et al. (2016) examined 389 boys and girls (4-18 years old). They used calcaneal QUS and they measured BUA (dB/MHz) values. Bone mineral content and BMD of total body, lumbar spine, and non-dominant femoral neck were obtained. They concluded that there was a weak to moderate correlation between calcaneal BUA and DXA-derived bone mass measurements in children. This lack of agreement may be associated with some discrepancies in using different types of QUS machines (different brands), different sites of measurements (radius or calcaneal), and considering different QUS variables (BUA and/or SOS and/or SI).

Conclusion

To date, the use of QUS in paediatric populations has been limited. Many ultrasound devices are not appropriate for use in paediatric populations due to inappropriate transducer sizes. Also, inadequate reference values for a large number of existing ultrasound devices are problematic. Moreover, even if the density, microstructure, and elasticity remain constant within the trabecular and cortical compartments, the distribution of cortical bone (i.e., varying cortical thickness in particular) may influence the results. Significant changes in cortical bone distribution during growth interpret QUS results to differ. However, since almost the entirety of the calcaneus consists of trabecular bone, measurements at this site may be less problematic (Binkley, Berry et al. 2008).

Another major issue with the use of QUS is the lack of information regarding the validity of ultrasonic measurement of bone in paediatric populations. However, QUS is a low cost, easy to use, transportable, non-invasive and radiation-free method, and only takes a few minutes for measurements (Baroncelli 2008).

Table 2.5. Overview of studies, which have been validated QUS against DXA among children and adolescents (n = 8 studies)

Author (year) country	Sample size and age	Type of QUS	QUS variable	DXA body part measurements	QUS Z-score reference population	DXA Z-score reference population	Statistical analysis for validity	Main results
Chong et al. (2015) Malaysia	n = 134 7-11 years	Radial QUS	SOS (m/s)	Total body BMD (g/cm^2)	Manufacturer data base	Manufacturer data base	• Cross-classification (quartile) • Bland-Atman plot	• Observed poor agreement; 70.1% of subjects were correctly classified and 29.9% were grossly misclassified. • Mean difference between two techniques was relatively big (0.6 ± 0.9; $P < 0.001$), ranging from -1.4 to 2.6. • Suggested radial QUS and DXA are not comparable and interchangeable in evaluating the bone status of children.
Srichan et al. (2016) Thailand	n = 181 6-12 years	Radial QUS	SOS (m/s)	Total body BMD (g/cm^2)	Manufacturer data base	ISCD paediatric official position	• Pearson's correlation • κ-statistic (tertile) • Bland-Altman plot	• SOS measurements at the radius are not appropriate for assessing bone quality status. • No correlation between the two different techniques. • The mean difference of the Z-scores of BMD and SOS was −0.61 (1.27) that was different from zero ($P < 0.05$). • Tertiles of Z-scores of BMD and QUS showed low agreement ($\kappa = 0.022$, $P = 0.677$) and the limits of agreement in Bland and Altman statistics were wide.
Lum et al. (1999) America	n = 125 9-25 years	Calcaneal QUS	SOS (m/s) BUA (dB/MHz), and Vb (m/s)	Total body, femoral neck, and lumbar spine BMD (g/cm^2)	NA	NA	• Spearman's correlation	• QUS measurements correlated moderately well with DXA measurements of the spine, femoral, and whole-body BMD (r = 0.71-0.88).
Mughal et al. (1996) UK	n = 58 7-17 years	Calcaneal QUS	BUA (dB/MHz)	Total body BMD (g/cm^2)	NA	NA	• Correlations	• Calcaneal BUA was significantly correlated with total body BMD (r = 0.74, $P < 0.001$).

Table 2.5. Continue

Author (year) country	Sample size and age	Type of QUS	QUS variable	DXA body part measurements	QUS Z-score reference population	DXA Z-score reference population	Statistical analysis for validity	Main results
Sioen et al. (2011) Belgium	n = 37 4-8 years	Calcaneal QUS	SOS (m/s) BUA (dB/MHz), and SI	Total body and lumbar spine BMD (g/cm^2)	NA	NA	• Spearman's correlation	• QUS SI was significantly negatively correlated with BMD of the total body (r = -0.370, $P = 0.02$). • SI measured by QUS does not correlate significantly with BMD values measured by DXA.
Xu et al. (2014) China	n = 329 5-19 years	Calcaneal QUS	SOS (m/s) BUA (dB/MHz), and SI	Total body and lumbar spine BMD (g/cm^2)	NA	NA	• Pearson's correlation	• Significantly positive correlations between SI and total body BMD (r = 0.693, $P < 0.001$) and BMC (r = 0.690, $P < 0.001$). • There were significant positive correlations between the calcaneal SI and total body BMD (r = 0.693, $P < 0.001$).
Sundberg et al. (1998) Sweden	n = 280 11-16 years	Calcaneal QUS	SOS (m/s) BUA (dB/MHz), and SI	Total body, lumbar spine, and femoral neck BMD (g/cm^2)	NA	NA	• Pearson's correlation	• There were significant positive correlations between QUS measurements of the calcaneus in children and DXA (r = 0.44–0.70).
Weeks et al. (2016) Australia	n = 389 4-18 years	Calcaneal QUS	BUA (dB/MHz)	Total body, lumbar spine, and non-dominant femoral neck BMC (g) BMD (g/cm^2)	NA	NA	• Pearson's correlation • Quartile classifications and Fisher's exact test	• Agreement in quartile rankings between QUS and DXA measures of bone mass was generally poor (27.3% to 38.2%). • Calcaneal BUA has a weak to the moderate relationship with DXA measurements of bone mass and tends to misclassify children based on quartile rankings.

QUS, quantitative ultrasound; *DXA*, dual-energy X-ray absorptiometry; *SOS*, speed of sound; *BMD*, bone mineral density; *ISCD*, International Society for Clinical Densitometry; *BUA*, broadband ultrasound attenuation; *Vb*, velocity bone; *SI*, stiffness index; *BMC*, bone mineral content; *BMI*, body mass index.

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Chapter Three

Fracture Risk Factors among Children Living in New Zealand (paper I)

While fractures are common in childhood, bone research is predominantly focused around peri- and post-menopausal women, and those with diagnosed osteoporosis, that is, when the problems manifest. The identification of factors that can affect bone health enables preventative strategies and solutions for fractures to be put in place. Therefore, the objective of this study is to identify related risk factors for fracture history (e.g. intake of milk, calcium, and sugar-sweetened beverages, vitamin D status, physical activity, ethnicity, body composition, bone stiffness index, siblings' history of fractures, and family history of osteoporosis) in children.

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To align with the formatting and referencing style of this thesis, there are some changes in formatting and referencing style of the published paper

Abstract

Aim: This study aimed to investigate fracture history and associated risk factors in New Zealand children.

Methods: Children aged 8-12 years were recruited. Capillary blood spots collected from a finger prick were analysed for 25(OH)D concentrations. Bioelectrical impedance analysis (InBody720, Seoul, Korea) was used to measure body fat percentage (%BF). Information about fracture history, siblings' history of fractures, family osteoporosis history, physical activity (PA), ethnicity, and intake of calcium-containing foods, and sugar-sweetened beverages (SSB) were collected using questionnaires.

Results: Children (n = 647, 354 girls), mean ± SD age 9.8 ± 0.7 years were recruited from six Auckland primary schools. New Zealand European (n = 252) and South Asian (n = 68) children reported the lowest (20.2%) and highest (44.1%) fracture proportion, respectively. New Zealand European compared to South Asian children, had higher 25(OH)D concentrations (74.6 ± 19.8 vs. 48.4 ± 19.3 nmol/L, $P < 0.001$), higher total calcium intake (764.0 ± 394.4 vs. 592.7 ± 266.3 mg/day, $P < 0.018$), and lower %BF (19.5 ± 6.6 vs. 23.4 ± 8.4, $P < 0.003$). Māori children had the next highest fracture rate (32.5%). This group had adequate 25(OH)D (64.2 ± 18.9 nmol/L), but high %BF (23.9%) and most participated in vigorous PA. After stratifying by gender, binary logistic regression analysis revealed the main determinants of fracture history for boys were high %BF, low 25(OH)D, low calcium intake, high SSB consumption, siblings' fracture history, family osteoporosis history, and being South Asian; and in girls, high SSB consumption, siblings' fracture history, and family osteoporosis history.

Conclusion: Our results showed that South Asian ethnicity was a significant risk factor for boys. Some children were at high risk of vitamin D deficiency and for whom supplementation may be necessary for winter. Good nutrition (especially good sources of calcium and reducing SSB intakes) should be recommended to children during growth and development to reduce their risk of fractures.

Introduction

Fractures are one of the most common injuries in healthy children (Landin 1983) but are a neglected public health issue. Fractures impact on daily activity and cause pain, lack of mobility, and dependency (Manias, McCabe et al. 2006). Falls (Lyons, Sellstrom et al. 2000, Rennie, Court-Brown et al. 2007), slips, and trips during activities like walking, running, climbing, and playing (Lyons, Sellstrom et al. 2000) are the most common type of fractures. It has been estimated that 7500 fractures occur annually in New Zealand children aged 3-15 years, an incidence of approximately 1% in this age group (Goulding, Cannan et al. 1998). A study of 313 children aged under 13 years living in Dunedin (New Zealand) revealed 468 fracture events over 6 months, with 212 (67.7%) sustaining a single fracture and 101 children more than one fracture (Yeh, Grant et al. 2006). A cohort study in New Zealand involving 601 children of up to 18 years old found 172 cases of a single fracture, with 119 children having had more than one fracture (Jones, Williams et al. 2002). There is an increasing concern that children who have multiple fractures become adults with poor bone quality and increased risk of further fractures (Cooper, Walker-Bone et al. 2000).

A complex interplay of non-modifiable (e.g. genetics) (Dequeker, Nijs et al. 1987, Clark 2014) and modifiable risk factors (e.g. obesity, physical activity, nutrition, vitamin D status) have been associated with childhood fractures (Ma and Jones 2002, Ma and Jones 2004, Manias, McCabe et al. 2006, Clark 2014). Early identification of risk factors during childhood could enable lifestyle changes to be instigated, thus enhancing bone mineralisation, preventing fractures, and improving adult bone health.

Therefore, this study aims to investigate the relationship between fracture history and known risk factors such as bone stiffness index (SI), intake of milk, calcium, and sugar-sweetened beverages (SSB), vitamin D status, physical activity (PA), ethnicity, body composition, siblings' history of fractures, and family osteoporosis history in children living in Auckland, New Zealand.

Methods

This was an observational and cross-sectional study in a sample of school-age children (aged 8 – 12 years old) living in Auckland (during August 2016 and 2017 – late winter in the southern hemisphere). Six local primary schools across Auckland were selected to include a range of socio-demographic and ethnic groups. We originally approached schools through a collaboration with primary school science teachers and asked for expressions of interest. We then endeavoured to recruit schools specifically to include a range of socio-demographic levels and ethnicities. All children within the age group specified, and attending the school were invited to participate. The study protocol was approved by the Human Ethics Committee of Massey University (Southern A; approval no. MUHECN 16/42) and had an agreement with the Declaration of Helsinki (World Medical Association Declaration of Helsinki 2013).

Children and their parents received an envelope containing a study information sheet (see Appendix 1 and 2), consent form (see Appendix 3), demographic questionnaire (see Appendix 6), and a food frequency questionnaire (FFQ) focusing on foods and beverages containing calcium (see Appendix 7). The selected participants involve children (and their parents) who gave written consent and returned the completed questionnaires. Also, children were eligible if they are healthy and fully mobile. Children were excluded if they had a history of any disease affecting calcium, and vitamin D metabolism (e.g. cardiac, kidney or liver disease), gastrointestinal disorders, a history of any long-term medication (e.g. corticosteroids, anticonvulsants and immune-suppressants) or had any surgical implants, metal screws or similar, or had a cast.

Data Collection

Anthropometric Measurements

Information about participants' weight and height were collected. Children were asked to remove their shoes to measure their heights (to an accuracy of 0.1 cm) using a Seca 213 portable stadiometer with operational standard based on the International Society for the Advancement of Kinanthropometry (ISAK) protocol and standards (International Society for the Advancement of

Kinanthropometry 2001). Body mass index (BMI) was calculated based on BMI adjusted-for-age as described by Cole et al. (2000) and Cole et al. (2007). Body fat percentage (%BF) was measured by bioelectrical impedance analysis (BIA), using the InBody 230 (Biospace Co. Ltd., Seoul, Korea) according to the standard procedures provided by the manufacturer. Body weights were measured in minimal clothing to the nearest 0.1 kg also on the InBody 230. The Inbody 230 demonstrated good validity for measuring fat-free mass, fat mass, and %BF when compared to dual-energy X-ray absorptiometry (DXA) in a sub-group of the same population of healthy children (Delshad, Beck et al. 2020). Body fat percentage is classified as 1) low %BF, 2) normal %BF, and 3) high %BF (McCarthy, Cole et al. 2006).

Health and Demographic Questionnaires

Fracture history, defined as any fracture occurring before taking part in the present study, were declared by participants including information such as skeletal site, age of event, and type of event. The severity of the trauma was classified based on Landin (1983) categories and includes;

1. Slight trauma: falling to the ground from standing height on the same level, falling from less than 0.5 metre (e.g. stools, chairs or beds), and low energy sport type (e.g. ball sports, skiing, wrestling, judo, skateboard, and roller skating),
2. Moderate trauma: falling from less than 0.5-3 metres (e.g. bunk-bed, stairs, bicycle, horse-back, swings, or similar playing equipment),
3. Severe trauma: falling from more than 3 metres (e.g. from window or roofs) and all traffic accidents. Information about children's consumption of dietary supplements, siblings' history of fracture, and family history of osteoporosis were also obtained.

Parents were asked to identify the ethnic group of their children. The ethnic groups were categorised as 1) New Zealand European, 2) Māori, 3) Pacific, 4) South Asian, 5) Chinese/Korean/Southeast Asian, and 6) Other ethnicities (Ministry of Health 2004, Statistics New Zealand 2009). Physical activity was evaluated using the short version of the international physical activity questionnaire (IPAQ) (Patterson 2005). Metabolic equivalent (MET) minutes/week of physical activity was

calculated as the MET intensity (3.3 or 4.0 or 8.0 METs) multiplied by the minutes of each physical activity multiplied by the number of days each physical activity occurred (Patterson 2005).

Current servings of milk, calcium intake, and SSB (e.g. fruit juice, cola, fizzy drinks, sports drinks) intake were determined using a non-validated FFQ. Available frequency of food and beverages consumption ranged from 'never/once a month' to 'once or more than once a day'. The calcium intake was assessed from food (e.g. yoghurt, broccoli, tofu) and beverage items rich in calcium (e.g. milk). The cumulative daily calcium intakes were calculated from the weekly consumption of calcium-containing food/beverages per day multiplying by the amount of calcium in one serving size. The amount of calcium for each food/beverage was extracted from the Concise New Zealand Food Composition Tables (Plant and Food Research Limited and Ministry of Health 2016).

Based on the estimated average requirement (EAR), total calcium intake was divided into < 800 mg/day and ≥ 800 mg/day (Ministry of Health 2006). Sugar-sweetened beverages were classified into <1 serving/day and ≥ 1 serving/day (Ministry of Health 2012).

Dried Blood Spots Sampling and Vitamin D Assay

The dried blood spots (DBS) method was used, as it is a minimally invasive and a convenient technique for children. A trained researcher used a single-use safety lancet to collect sufficient capillary blood from the child's finger. Blood spots were collected onto a Whatman filter paper card within a pre-marked area. The sample cards were air-dried at room temperature before being placed into a sealed plastic bag. All the DBS cards were refrigerated and stored at 4°C until they were sent to Canterbury Health Laboratories for further analysis.

Measurement of 25(OH)D in DBS was performed by a newly developed method utilising two-dimensional liquid chromatography tandem mass spectrometry (2D LC-MS/MS) following methanol and hexane extraction of the DBS. Capillary calculated serum concentrations of 25(OH)D were determined by assuming a haematocrit of 0.39. The method has been thoroughly validated for research applications (Jensen, Saraf et al. 2018). Serum 25(OH)D were categorised into 25(OH)D < 75 nmol/L and ≥ 75 nmol/L (Holick, Binkley et al. 2011).

Quantitative Ultrasound (QUS)

The International Society for Clinical Densitometry (ISCD) suggests calcaneus as the best skeletal site of measurement compared to the other peripheral sites (e.g. radius, tibia) as it demonstrates an acceptable level of scientifically validated results in osteoporosis management (Krieg, Barkmann et al. 2008). In this study, the calcaneal bone mineral density (BMD) was measured using a commercial QUS device (Sahara Clinical Bone Sonometre Hologic Inc, USA). Previous studies showed that QUS is a valid tool for measuring BMD in children (Sundberg, Gardsell et al. 1998, Lum, Wang et al. 1999, Xu, Guo et al. 2014). Furthermore, positive correlations between calcaneal QUS stiffness index (SI) and DXA BMD were observed in a sub-group of the same population (r = 0.4) ($P < 0.01$) (Delshad, Beck et al. 2020). The QUS can assess both speed of sound (SOS) (m/s) and broadband ultrasound attenuation (BUA) (decibels/mega-hertz). From these a third parameter is calculated known as the stiffness index (SI). The QUS device was calibrated before each data collection period using a verification phantom (an external bone mineral reference), supplied by the manufacturer. The coefficient of variance for stiffness was 2.6%. Children positioned their bare right foot into the machine and the analysis took less than one minute.

Statistical Analysis

The computer software statistical package program SPSS version 24 (IBM Corporation, New York, NY, USA) was used to analyse the data. The variables were tested for normality using the Kolmogorov-Smirnov test and Shapiro-Wilk tests and for homogeneity using the Levene's test.

The data were normally distributed so parametric tests were used. For baseline characteristics and measurements, continuous variables were expressed as mean ± standard deviation (SD) and categorical data, as number and percentages (n (%)). To compare with- and without-fracture history groups, the independent T-test (for continuous variables) and Chi-squared test (for categorical variables) were used. An ANOVA (Turkey Post Hoc test) was used to determine differences for the continuous variable (e.g. %BF, calcium intake, 25(OH)D) between ethnic groups. Pearson's correlation coefficient was used to investigate the relationship between dairy/milk consumption and

SSB.

Binary logistic regression (univariate and multivariate) was performed to determine which factors were independent predictors for fracture history. The following independent variables were included in the model: age, %BF, 25(OH)D, total calcium intake, SSB consumption, siblings' history of fractures, history of osteoporosis in family, and ethnicity. Body mass index (BMI) was not included in the regression analyses to avoid the violation of multicollinearity. All variables were treated as categorical variables. Reference categories were younger (8-9 years), having normal %BF, 25(OH)D ≥ 75nmol/L, calcium intake ≥ 800 mg/day, SSB intake < 1 serving/day, no siblings' history of fractures, no family history of osteoporosis, and from a New Zealand European ethnic group. Also, interaction terms were added into the models to investigate the interaction effects between variables but no significant results were observed. Associations were described using adjusted odds ratios (OR) and 95% confidence intervals (CI). Predictors were considered significant if the *P*-value was less than 0.05.

Results

Seven hundred and forty-one healthy children were invited to participate in this study. 665 children agreed to participate but 18 children were absent from school on the day of data collection. Of the 647 children, only 513 children agreed to participate in the finger prick test.

One hundred and sixty-five children reported at least one fracture, 33 reported two fractures, and 7 reported three fractures during their lifetime. Fractures of the upper limb were more common (n = 161, 78.5%) than those of the lower limb (n = 39, 19.0%), and few fractures occurred elsewhere (n = 5, 2.5%). Trauma resulting in fracture showed 110 (53.6%) mild, 88 (42.9%) moderate, and 7 (3.5%) severe cases (Landin 1983). No skull fracture but one neck vertebral and one nose fracture were reported. New Zealand European and South Asian ethnic groups had the lowest (20.2%) and highest (44.1%) fracture rate, respectively. Māori children had the next highest fracture rate (32.5%).

Table 3.1 shows characteristics and a comparison of risk factors between those with and without fracture history in boys and girls. Boys with fractures had higher BMI (18.6 ± 4.7 kg/m^2 vs. 18.2 ±

3.5 kg/m^2, $P = 0.037$) and %BF (21.0 ± 10.2 vs. 20.4 ± 7.5, $P < 0.004$). The percentage of fracture was lower in boys with 25(OH)D ≥ 75 nmol/L (16.2% vs. 34.3%), total calcium ≥ 800 mg/day (18.7% vs. 33.9%), and SSB < 1 serving/day (24.5% vs. 35.2%), no history of siblings' fracture (19.3% vs. 57.1%), and no family history of osteoporosis (21.8% vs. 51.6%). Lower percentage of fracture was found in girls with SSB < 1 serving/day (16.5% vs. 37.5%), no siblings' fracture of history (15.2% vs. 58.4%), and no family history of osteoporosis (19.5% vs. 40.3%).

Table 3.1. Characteristics of population group (n = 647)

		Boys			Girls		
	Total population (n = 647)	**Fracture history (n = 83)**	**No Fracture history (n = 210)**	***P*-value[1]**	**Fracture history (n = 82)**	**No Fracture history (n = 272)**	***P*-value[1]**
	Mean ± SD	**Mean ± SD**	**Mean ± SD**		**Mean ± SD**	**Mean ± SD**	
Age (years)	9.8 ± 0.7	9.8 ± 0.7	9.8 ± 0.6	0.148	9.8 ± 0.7	9.8 ± 0.7	0.838
Weight (kg)	38.6 ± 11.0	38.7 ± 13.1	37.6 ± 10.3	0.140	39.9 ± 11.2	39.0 ± 10.7	0.347
Height (cm)	143.4 ± 8.3	143.1 ± 7.9	143.2 ±9.2	0.916	144.8 ± 8.2	143.2 ± 7.7	0.240
Body mass index (kg/m^2)	18.5 ± 3.8	18.6 ± 4.7	18.2 ± 3.5	**0.037**	18.8 ± 3.9	18.7 ± 3.7	0.656
Body fat percentage	21.9 ± 7.9	21.0 ± 10.2	20.4 ± 7.5	**0.004**	23.4 ± 8.0	22.8 ± 7.3	0.601
25(OH)D (nmol/L)[2]	63.5 ± 20.6	60.7 ± 18.7	68.0 ± 20.4	0.238	61.4 ± 18.4	61.4 ± 21.6	0.082
Physical activity (MET-minutes/week)	1627.2 ± 1089.1	1762.1 ± 1011.7	1749.7 ± 1110.2	0.298	1634.7 ± 1186.5	1488.6 ± 1053	0.541
Total calcium intake (mg/day)	683.2 ± 341.0	621.9 ± 301.8	757.5 ± 382.0	0.055	635.1 ± 278.8	659.1 ± 327.4	0.233
Dairy calcium intake (mg/day)	623.4 ± 333.2	576.4 ± 311.5	686.9 ± 372.2	0.286	567.1 ± 257.5	605.8 ± 321.8	0.158
Dairy consumption (servings/day)	2.5 ± 1.3	2.3 ± 1.3	2.7 ± 1.5	0.234	2.3 ± 1.0	2.4 ± 1.2	0.199
Milk consumption (servings/day)	1.6 ± 1.0	1.5 ± 0.9	1.8 ± 1.1	0.239	1.3 ± 0.8	1.5 ± 1.0	0.283
SSB (servings/day)	0.7 ± 0.8	0.8 ± 0.6	0.8 ± 1.0	**0.017**	0.9 ± 1.0	0.5 ± 0.6	**0.006**
QUS SI	99.4 ± 34.6	98.7 ± 19.1	99.8 ± 16.5	0.642	97.9 ± 15.7	100.3 ± 49.7	0.737
	n (%)	**n (%)**	**n (%)**		**n (%)**	**n (%)**	
Body fat percentage[3]				**0.002**			0.818
Low	151 (23.3)	26 (40.6)	38 (59.4)		20 (23.0)	67 (77.0)	
Normal	315 (48.6)	28 (19.8)	113 (80.2)		38 (22.0)	136 (77.0)	
High	181 (28.0)	29 (33.0)	59 (67.0)		24 (25.8)	69 (74.2)	
25(OH)D (nmol/L)[2]				**0.004**			0.238
< 75	367 (71.3)	58 (34.3)	111 (65.7)		54 (27.3)	144 (72.7)	
≥ 75	148 (28.7)	12 (16.2)	62 (83.8)		15 (20.3)	59 (79.7)	

Total calcium intake (mg/day)				**0.005**			0.624
< 800	443 (68.5)	63 (33.9)	123 (66.1)		61 (23.8)	196 (76.2)	
≥ 800	204 (31.5)	20 (18.7)	87 (81.3)		21 (21.6)	76 (78.4)	
SSB (servings/day)				**0.050**			**< 0.0001**
< 1	430 (66.5)	46 (24.5)	142 (75.5)		40 (16.5)	202 (83.5)	
≥ 1	217 (33.5)	37 (35.2)	68 (64.8)		42 (37.5)	70 (62.5)	
Siblings' history of fracture (%)				**< 0.0001**			**< 0.0001**
Yes	135 (20.9)	40 (57.1)	30 (42.9)		38 (58.4)	27 (41.6)	
No	512 (79.1)	43 (19.3)	180 (80.7)		44 (15.2)	245 (84.8)	
Osteoporosis family history (%)				**< 0.0001**			**< 0.001**
Yes	126 (19.5)	33 (51.6)	31 (48.4)		25 (40.3)	37 (59.7)	
No	521 (80.5)	50 (21.8)	179 (78.2)		57 (19.5)	235 (80.5)	
Ethnic group (%)				**< 0.0001**			
New Zealand European	252 (38.9)	24 (21.1)	90 (78.9)		27 (19.9)	111 (80.4)	0.219
Māori	77 (11.9)	8 (24.2)	25 (75.8)		17 (38.6)	27 (61.4)	
Pacific	133 (20.6)	14 (23.3)	46 (76.7)		17 (23.3)	56 (76.7)	
South Asian	68 (10.5)	22 (62.9)	13 (37.1)		8 (24.2)	25 (75.8)	
Chinese/Korean/Southeast Asians	89 (13.8)	10 (26.3)	28 (73.7)		10 (19.6)	41 (80.4)	
Other	28 (4.3)	5 (38.5)	8 (61.5)		3 (20.0)	12 (80.0)	

SSB, sugar-sweetened beverages; *QUS SI*, quantitative ultrasound stiffness index
[1]Independent T-test (for continuous variables) and Chi-squared test (for categorical variables) between with and without fracture history
[2]Data present for 513 participants
[3]Based on McCarthy (2006) categories

Current milk consumption was generally low in both groups and 35.3% of children who never drank milk had a fracture history. A weak but significant inverse correlation was observed between dairy and SSB consumption (r = -0.111, $P < 0.01$) and milk and SSB consumption (r = -0.078, $P < 0.05$). No participants used calcium and/or vitamin D supplements.

Binary logistic regression analysis revealed the main determinants of fracture history for boys were high %BF (OR = 3.1, 95%CI: 1.2-7.5, $P = 0.011$), low 25(OH)D (OR = 2.7, 95%CI: 1.1-6.5, $P = 0.021$), low calcium intake (OR = 2.8, 95%CI: 1.2-6.1, $P = 0.009$), high SSB consumption (OR = 2.0, 95%CI: 1.0-4.3, $P = 0.044$), siblings' fracture history (OR = 4.1, 95%CI: 1.8-9.2, $P = 0.001$), family history of osteoporosis (OR = 2.4, 95%CI: 1.0-6.0, $P = 0.043$), and being of South Asian ethnicity (OR = 3.2, 95%CI: 1.0-9.6, $P = 0.038$) (Table 3.2). Girls who consumed higher SSB (OR = 4.6, 95%CI: 2.3-9.1, $P < 0.0001$), had siblings' history of fracture (OR = 7.4, 95%CI: 3.4-16.3, $P < 0.0001$), and family history of osteoporosis (OR = 2.6, 95%CI: 1.1-6.2, $P = 0.028$) had higher odds of having a history of fractures (Table 3.2).

Table 3.2. Factors predicting fracture history

Variables[1]	Association with fracture in boys (n = 293)		Association with fracture in girls (n = 354)	
	Univariate OR (95%CI)	Multivariate OR (95%CI)	Univariate OR (95%CI)	Multivariate OR (95%CI)
Age (8-9 vs. 10-12)	1.2 (0.7, 2.2)	1.1 (0.5, 2.3)	1.0 (0.6, 1.7)	1.4 (0.7, 2.9)
Body fat percentage[2]				
(Normal vs. low)	**2.9 (1.5, 5.6)**	**5.0 (2.1, 11.9)**	1.0 (0.5, 1.9)	1.2 (0.5, 2.8)
(Normal vs. high)	**2.0 (1.1, 3.7)**	**3.1 (1.2, 7.5)**	1.2 (0.6, 2.1)	1.0 (0.4, 2.2)
25(OH)D (nmol/L) (≥ 75 vs. <75)[3]	**2.7 (1.3, 5.4)**	**2.7 (1.1, 6.5)**	0.6 (0.3, 1.2)	0.9 (0.3, 2.1)
Total calcium intake (mg/day) (≥ 800 vs. < 800)	**2.2 (1.2, 3.9)**	**2.8 (1.2, 6.1)**	0.8 (0.4, 1.5)	1.0 (0.5, 2.2)
SSB (servings/day) (< 1 vs. ≥ 1)	1.6 (0.9, 2.8)	**2.0 (1.0, 4.3)**	**2.9 (1.7, 4.8)**	**4.6 (2.3, 9.1)**
Siblings' history of fracture (No vs. yes)	**5.5 (3.1, 9.9)**	**4.1 (1.8, 9.2)**	**8.3 (4.6, 15.0)**	**7.4 (3.4, 16.3)**
Osteoporosis family history (No vs. yes)	**3.8 (2.1, 6.8)**	**2.4 (1.0, 6.0)**	**2.9 (1.6, 5.3)**	**2.6 (1.1, 6.2)**
Ethnicity group				
(NZ European vs. Māori)	1.2 (0.4, 2.9)	1.3 (0.3, 3.1)	**2.5 (1.2, 5.4)**	1.4 (0.5, 3.8)
(NZ European vs. Pacific)	1.1 (0.5, 2.4)	1.2 (0.2, 2.1)	1.2 (0.6, 2.4)	1.1 (0.4, 3.5)
(NZ European vs. South Asian)	**6.3 (2.7, 14.4)**	**3.2 (1.0, 9.6)**	1.3 (0.5, 3.2)	1.8 (0.4, 7.1)
(NZ European vs. Chinese/Korean/Southeast Asian)	1.3 (0.5, 3.1)	1.5 (0.4, 5.1)	1.1 (0.5, 2.4)	2.0 (0.6, 6.3)
(NZ European vs. other)	2.3 (0.7, 7.8)	1.1 (0.2, 6.2)	1.0 (0.2, 3.8)	1.2 (0.1, 3.8)

CI, confidence intervals; *OR,* odds ratio; *NZ,* New Zealand; *SSB,* sugar-sweetened beverages

[1]Variables included in the multivariable model were: age, body fat percentage, 25(OH)D, total calcium consumption, SSB, siblings' history of fracture, history of osteoporosis in family, and ethnicity.

Boys (Cox and Snell = 0.26, Nagelkerke = 0.37. Model X^2=73.565), girls (Cox and Snell = 0.21, Nagelkerke = 0.31. Model X^2=64.845).

[2]Based on McCarthy (2006) categories

[3]Data present for 513 participants

Table 3.3 shows the comparison of different variables between the ethnic groups. South Asian children had the lowest 25(OH)D (48.4 ± 19.3 nmol/L), total calcium intake (592.7 ± 266.3 mg/day), and dairy calcium intake (541.2 ± 271.2 mg/day). Māori children had 25(OH)D concentration 64.2 ± 18.9 nmol/L, but high %BF (23.9%) and a large number of them participated in vigorous PA (42.6%). Pacific children had the highest %BF (25.2 ± 9.4) as well as the highest consumption of SSB among the other ethnic groups (1.1 ± 1.2 servings/day).

Table 3.3. Comparison of factors in ethnic groups

Ethnic group	New Zealand European (reference group)	Māori	Pacific	South Asian	Chinese/Korean/ Southeast Asian	Other ethnicity	*P*-value[1]
Body fat percentage	19.5 ± 6.6	**23.9 ± 7.6**	**25.2 ± 9.4**	**23.4 ± 8.4**	21.0 ± 6.7	20.9 ± 7.7	< 0.003
25(OH)D (nmol/L)[2]	74.6 ± 19.8	**64.2 ± 18.9**	**54.9 ± 14.1**	**48.4 ± 19.3**	**57.7 ± 15.7**	**52.7 ± 23.1**	< 0.001
Physical activity (MET-minutes/week)	1925.1 ± 1037.1	1691.6 ± 1126.2	**1483.5 ± 1144.8**	**1346.1 ± 1108.8**	**1297.3 ± 956.7**	**1182.6 ± 816.5**	< 0.006
Total calcium intake (mg/day)	764.0 ± 394.4	**601.4 ± 221.1**	**649.0 ± 342.0**	**592.7 ± 266.3**	666.8 ± 293.4	615.7 ± 240.4	< 0.018
Dairy calcium intake (mg/day)	712.2 ±391.3	**549.4 ± 215.1**	**596.4 ± 320.9**	**541.2 ± 271.2**	**555.4 ± 265.8**	571.4 ± 240.7	< 0.013
Dairy consumption (servings/day)	2.8 ±1.5	**2.2 ± 0.8**	2.4 ± 1.3	**2.2 ± 1.0**	**2.2 ± 1.1**	2.3 ± 1.0	< 0.016
Milk consumption (servings/day)	1.8 ± 1.2	**1.4 ± 0.7**	1.5 ± 0.9	1.4 ± 0.8	**1.4 ± 0.8**	1.4 ± 0.7	< 0.047
SSB (servings/day)	0.5 ± 0.5	**0.9 ± 1.0**	**1.1 ± 1.2**	0.8 ± 0.8	0.6 ± 0.7	0.8 ± 0.6	< 0.003

SSB, sugar-sweetened beverages
Values are shown as mean ± standard deviation
[1]ANOVA test (Bold number shows significant results in Turkey Post Hoc test compared to New Zealand European ethnic group)
[2]Data present for 513 participants

Discussion

There are limited sources of data available regarding fracture determinants in New Zealand children. Approximately one quarter (25.5%) of the study population reported one or more fractures in their lifetime. The strongest predictors of fracture in boys were high %BF, low 25(OH)D concentration, low total calcium intake, high SSB consumption, having siblings' history of fractures, having a family history of osteoporosis, and being South Asian, while in girls high SSB consumption, having siblings' history of fractures and having a history of osteoporosis in the family were predictors of fractures.

In keeping with other studies (Maasalu, Raukas et al. 2009, Jenkins, Nimphius et al. 2018), in the current study, the upper limb was found to be the most common site of fractures (78.5%) in children compared to the lower limb.

Boys with fracture history compared with those without a fracture history have a higher BMI and %BF. Boys with high %BF compared with boys with normal %BF have a higher prevalence of fracture (33.0% vs. 19.8%). Also, the odds of fracture for them was 3.1 times more compared to the children who had normal %BF. Goulding et al. (2001) found that high adiposity was associated with an increased risk of fracture in boys. The same author in another study found that high body weight is a risk factor for fracture in children and adolescents with repeated forearm fractures (Goulding, Grant et al. 2005). Kim et al. (2013) found a greater risk of pelvic bone fracture in children with obesity compared with their non-obese counterparts. Different theories have been postulated regarding a greater risk of bone fractures in children with obesity. Childhood obesity is associated with a higher propensity for falls with injuries or fractures due to poor musculoskeletal control and decline in motor skills (Cheng, East et al. 2016). In overweight and children with obesity, there is a structural adaptation in response to muscle forces and local mechanical strain, not to static loads represented by body weight (Petit, Beck et al. 2005). Plausible explanations for a greater risk of bone fractures may be lower at physical activity levels, inadequate intake of dietary calcium, and low vitamin D concentration (Wortsman, Matsuoka et al. 2000, Farr and

Dimitri 2017).

It is suggested that higher fracture risk in a group of people could be associated with genetic factors, either inherent skeletal weakness or lower bone mineralisation (Goulding, Jones et al. 2005). The literature suggests that genetic factors are responsible for up to 80% of inter-subject variance in bone mass (Pocock, Eisman et al. 1987). In the current study, the group with a fracture history compared to the group without a fracture history reported a higher proportion of fractures in their siblings (boys = 57.1% vs. 19.3% and girls = 58.4% vs. 15.2%) and also a stronger history of osteoporosis in their family (boys = 51.6% vs. 21.8% and girls = 40.3% vs. 19.5%). The odds of fracture for boys and girls with siblings' history of fractures was four and seven times more compared to the children who did not report siblings' history of fractures, respectively. Boys and girls with a family history of osteoporosis had 2.4 and 2.6 times greater odds for fracture, respectively.

There was no significant difference in SI between children with or without fracture history. This is consistent with a previous study (Cvijetic, Baric et al. 2003) but in contrast to other studies (Landin and Nilsson 1983, Chan, Hess et al. 1984, Goulding, Cannan et al. 1998, Goulding, Jones et al. 2001, Ma and Jones 2003, Schalamon, Singer et al. 2004, Clark, Ness et al. 2006), which found children with fractures had significantly lower bone mineral density. A lack of association in our population may relate to a moderate degree of correlation between QUS SI measurements and DXA results ($r = 0.4$). Alternatively, a bone mass may not be an adequate proxy measure of bone strength in this age group. Indeed, Cook et al. (1987) and Ma and Jones (2002) used DXA to evaluate bone mass and did not find a significant difference between a fracture and non-fracture groups. Skaggs et al. (2001) ran a case-control study in 100 healthy girls aged 4-15 years old. Using computed tomography, they found only a smaller cross-sectional area is a risk factor for forearm fracture but not cancellous, cortical, or integral bone density.

In this study, our results showed that the consumption of SSB $\geq$ 1 serving/day can increase the risk of fracture in boys 2.0 times and in girls 4.6 times more than children who consumed < 1

serving/day. Ma and Jones (2004) confirmed that there is a deleterious correlation between consumption of cola beverages and wrist and forearm fracture risk. Furthermore, a small but significant inverse correlation between dairy consumption and SSB ($r = -0.111$, $P < 0.01$) and milk consumption and SSB ($r = -0.078$, $P < 0.05$) were found. Intake of high sugar foods and drinks needs to be limited (less than once a week) (Ministry of Health 2012). In line with the Ministry of Health (2003) data, Pacific children had the highest consumption of SSB compared with other ethnic groups (1.1 ± 1.2 servings/day). In the United States, milk consumption has been shown to decrease and intake of sweetened beverages increase with age in 3-7 years old children over a period of three years (Keller, Kirzner et al. 2009). The exact reasons for this behaviour are not known. However, it is postulated that parental influence, television advertisements, increasing age and independency, and availability at school are some reasons for this substitution. This shift had a negative impact on children's and adolescents' bone health and may contribute to increased risk of bone fractures (Wyshak and Frisch 1994, Petridou, Karpathios et al. 1997, Wyshak 2000) by decreasing calcium intake due to replacement of milk in the diet with sweetened beverages, which are high in caffeine, phosphoric acid and/or sugar (Kinney 2002, Fitzpatrick and Heaney 2003).

The present findings showed that 35.3% of children who avoid milk intake had a fracture history. As shown by Goulding et al. (2004), pre-pubertal children who avoid drinking milk had more fractures than their control pair. Black et al. (2002) studied 50 milk avoiders (aged 3-10 years old) and found that children who habitually avoid consumption of cow's milk had sustained a high number of bone fractures. The beneficial effects of milk and dairy products regarding bone health are related to the presence of calcium (Dror and Allen 2014). Calcium is an essential nutrient, which is long known for its important role in the acquisition of strong and healthy bones (Stagi, Cavalli et al. 2013). During skeletal maturity, calcium deficiency can have an adverse effect on gaining PBM in children and increase the risk of fractures later in life (Heaney, Abrams et al. 2000). Other nutrients in milk may also be important for optimal bone development. Milk and other dairy products are an important source of energy, protein, vitamins, minerals (e.g.

phosphorus, zinc, selenium, and magnesium), and bone anabolic growth factors (e.g. osteoprotegerin) (Kanezler, Bodamyali et al. 2001). The milk basic protein consists of different components, which can promote bone formation and increasingly suppress bone resorption (Yamamura, Aoe et al. 2002, Uenishi, Ishida et al. 2007). Milk and dairy products intake are also associated with stimulating bone formation and leg length growth through increasing expression of insulin-like growth factor-1 (Rogers, Emmett et al. 2006). Therefore, without appropriate dietary substitutions, young children who consume very little milk for long periods of their life, when their bone demands for calcium are high, may be more prone to fractures.

The main sources of dietary calcium in New Zealand are milk and dairy products (e.g. cheese, ice cream, and yoghurt) (Ministry of Health 2012). Because milk and yoghurt contain about 300 mg calcium per serving, consuming 2-3 servings of milk and dairy products per day are recommended to help achieve the required daily intake (Ministry of Health 2012). Children in this study had a total dairy consumption of 2.5 ± 1.3 servings/day and mean milk consumption of 1.6 ± 1.0 servings/day. South Asian children had the lowest total calcium (592.7 ± 266.3 mg/day) and dairy calcium intakes (541.2 ± 271.2 mg/day). Based on the New Zealand Ministry of Health report (2003), the median intake of calcium in boys and girls, 7-10 years old was 788 mg and 628 mg, respectively compared with the recommended intake of 800-1000 mg/day for 8-11 year-olds. In line with these findings, many of our study children consumed less dietary calcium than the recommendation (total calcium intake < 800 mg/day = 68.5%) and current calcium intake were generally low (683.2 ± 341.0 mg/day). The prevalence of fracture was higher in boys who consumed < 800 mg/day calcium than those who consumed ≥ 800 mg/day (33.9% vs. 18.7%). Furthermore, logistic regression analysis showed that boys who had lower calcium intake had higher odds for fracture. Just before the growth spurt, young individuals must be in positive calcium balance since bone skeletal calcium requirements for bone modelling and remodelling are high (Matkovic 1992). During rapid growth, cortical porosity increases as a consequence of an increase in bone turnover in order to supply adequate calcium needed by the growth plate (Parfitt

1994) therefore, dietary calcium insufficiency can accentuate this process. In the present study, the majority of our population did not meet the current calcium recommended dietary intake (RDA) for age. Thus, in some children, low dietary calcium intakes could cause increased cortical bone resorption to provide an increased demand for calcium and consequently lead to bone fragility (Parfitt 1994).

Vitamin D plays an important role in the absorption of calcium from the intestine, regulation, and maintenance of calcium levels, and bone metabolism (Lieben, Carmeliet et al. 2011). Optimal vitamin D status is necessary for maximising bone mineralisation and minimising of fracture risk (Laird, Ward et al. 2010). It is shown that 25(OH)D concentration less than 50 nmol/L may cause hypo-calcemia and secondary hyperparathyroidism in children (Holick 2007) and according to the Endocrine Society (Holick, Binkley et al. 2011) and the Society for Adolescent Health and Medicine (Society for Adolescent Health and Medicine 2013), 25(OH)D less than 75 nmol/L is insufficient. Most of our population had 25(OH)D less than 75 nmol/L (71.3%) and the percentage of fractures was higher in boys with 25(OH)D < 75 nmol/L (34.3%), and 25(OH)D < 75 nmol/L was one of the predictors of fractures in boys. In our study, South Asian children have the lowest 25(OH)D (48.4 ± 19.3 nmol/L). South Asians are often reported to have low 25(OH)D concentration (Akhtar 2016, Poh, Rojroongwasinkul et al. 2016). The cutaneous synthesis of vitamin D is lower in individuals with a darker skin colour due to the ultraviolet radiation beta (UVβ) photons being poorly absorbed through melanin pigmentation (Ministry of Health and Cancer Society of New Zealand 2012).

In our study, Māori children had the second-highest fracture rate (32.5%) and a large percentage of them participated in vigorous physical activity, presumably leading to increased risk of injuries. Previous studies showed that 36% (Lyons, Delahunty et al. 1999) and 50% (Houshian, Mehdi et al. 2001) of fractures in children are due to sporting activities, especially in children involved in vigorous physical activity, suggesting exposure to injuries is an important risk factor of childhood fracture. Field et al. (2011) suggested that there is an association between physical activity and

fracture risk. They found that girls who were involved in eight or more hours of activity such as running, basketball, and cheerleading/gymnastics per week were more likely to develop a stress fracture compared to their less active peers. Clark et al. (2008) found that vigorous physical activity was an independent risk factor of fracture during childhood. They concluded that higher bone mass in children who are involved in more physical activity can only partially compensate for the fracture risk caused by increased exposure to injuries. The pre- and early peri-pubertal years are ideal for being involved in light and weight-bearing physical activity since it can reduce fracture risk, probably through increased osteogenic effects of exercise on bone density (Ma and Jones 2003) and increase bone strength in response to biomechanical forces (Ma and Gordon 2012). However, participation in vigorous physical activity probably not only increases bone mass but also the number of injuries and consequently the fracture risk.

Strengths and Limitations

Our results are not necessarily transferable to other populations since only healthy children living in Auckland, New Zealand were recruited for the study. Information related to fracture risk factors were collected (e.g. ethnicity, siblings' history of fracture, history of osteoporosis in family, calcium intake, SSB, and PA) through self-reported questionnaires so recall bias could have influenced our results. This study was conducted in children aged 8-12 years old therefore, the age range presumably encompassed subjects in different pubertal status. At the bone growth spurt during puberty, areal bone density can decrease since bones enlarge more quickly then they mineralise (Magarey, Boulton et al. 1999). Therefore, they are more vulnerable to fractures and it can be an issue since some of the girls in our study could have been undergoing a growth spurt. However, the ages that fracture happened were asked and none of the girls ≥ 10 years old had a fracture at 10 years old or after. The DBS method was used for measuring 25(OH)D concentrations since it is minimally invasive, convenient to use for children and shown to be an alternative method to measuring 25(OH)D serum concentrations (Avagyan, Neupane et al. 2016, Cairncross, Stonehouse et al. 2017, Jensen, Saraf et al. 2018). However, although validated in adults and

neonates, it has not been specifically validated in children. Another limitation is using a short questionnaire for the estimation of dietary calcium intakes, which may not be valid in a New Zealand population. Despite these limitations, this study has some notable strengths and illustrates a general pattern of fracture status and its related risk factors among school-age children living in Auckland, New Zealand. A large sample of healthy children from a broad range of socio-demographic and ethnic backgrounds was recruited.

Conclusion

In summary, our study identified an association between fracture history and its related risk factors in children living in Auckland, New Zealand. Approximately one-quarter of our participants reported one or more fractures during their short lifetime. Our findings showed that South Asian ethnicity was a significant risk factor for boys. Some children were at high risk of vitamin D deficiency and for whom supplementation may be necessary during the winter season. Good nutrition (especially good sources of calcium and reducing SSB intakes) should be recommended to children during growth and development to reduce their risk of fractures.

Conflict of interest: The authors declare that they have no conflict of interest.

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Chapter Four

Wintertime Vitamin D Status and its Related Risk Factors (Paper II)

While vitamin D deficiency is associated with several skeletal health consequences (e.g. rickets, metabolic bone disease), information regarding vitamin D status and risk factors for vitamin D deficiency in New Zealand children are limited. Also, some children are likely to be vulnerable to vitamin D deficiency. This study aims to investigate wintertime vitamin D status in New Zealand children living in Auckland and to identify related risk factors for deficiency.

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To align with the formatting and referencing style of this thesis, there are some changes in formatting and referencing style of the published paper.

Abstract

Aim: To investigate vitamin D status and its determinants in school-aged children living in Auckland, New Zealand (NZ).

Methods: Healthy children (n = 507) aged 8-11 years, were recruited from six primary schools to include a range of ethnicities and socio-demographic characteristics. Finger-prick blood spots were collected and analysed for capillary 25-hydroxyvitamin D (25(OH)D). Weight and body fat percentage (%BF) were measured using the InBody 230 (Biospace Co. Ltd., Seoul, Korea). Information related to ethnicity, skin colour, physical activity, and sun exposure were sought from parents through a questionnaire.

Results: Mean ± standard deviation (SD) 25(OH)D concentration were 64.0 ± 20.8 nmol/L, with 31% of the population presenting with 25(OH)D ≥ 75 nmol/L, 41% 50-75 nmol/L, and 28% < 50 nmol/L. Capillary 25(OH)D was significantly higher in NZ European compared to all other ethnic groups (75.0 ± 20.1 nmol/L, $P < 0.001$). As expected, children with dark/brown skin colour had lower 25(OH)D concentration compared to other skin colour categories (51.7 ± 18.0 nmol/L, $P < 0.001$). Using multiple logistic regression analysis, determinants of 25(OH)D were %BF and ethnicity.

Conclusion: Approximately one-third of this population had 25(OH)D < 50 nmol/L. Determinants of a 25(OH)D < 50 nmol/L included %BF and ethnicity. Wintertime serum 25(OH)D was highly variable. There are some children at high-risk of 25(OH)D < 50 nmol/L for whom supplementation may be considered.

Introduction

Vitamin D is a secosteroid and an essential nutrient, which has a crucial role in the absorption of calcium from the intestine, regulation of serum calcium, and bone health (Lieben, Carmeliet et al. 2011). Observational and clinical studies suggest there is a relationship between vitamin D deficiency and insufficiency and skeletal health problems (e.g. rickets, metabolic bone disease, and hypocalcaemia) during childhood (Wagner and Greer 2008, Moon, Harvey et al. 2014). Therefore, an adequate 25(OH)D concentration is considered important for ensuring good bone health during childhood and later in life.

In New Zealand, a small quantity of vitamin D comes from a limited number of foods that naturally contain vitamin D (salmon, herring, tuna, and mackerel). There is a limited range of foods including milk and yoghurt, which are sometimes fortified with vitamin D (Ministry of Health and Cancer Society of New Zealand 2012), although there is no mandatory fortification in New Zealand (Ministry of Health and Cancer Society of New Zealand 2012). Therefore, it would be hard to reach an acceptable blood concentration of vitamin D through diet alone. However, endogenous synthesis through sunlight exposure is the major source of vitamin D for most people living in New Zealand since diet alone is not adequate to meet recommended 25(OH)D concentrations (Ministry of Health and Cancer Society of New Zealand 2012). Nevertheless, the efficacy of cutaneous synthesis of vitamin D can be influenced by various factors including geographic latitude, season, time of day (Kimlin 2008), ethnicity (Rockell, Green et al. 2005), obesity (Vierucci, Del Pistoia et al. 2013), waist circumference (Elizondo-Montemayor, Ugalde-Casas et al. 2010), age (Tolppanen, Fraser et al. 2012), and gender (Kolokotroni, Papadopoulou et al. 2015). Furthermore, some other conditions such as skin pigmentation, use of sunscreens, exposed body surface, and exposure duration (Godar, Pope et al. 2012), and restricted sunlight exposure habits (e.g. clothing) (Wharton and Bishop 2003) can affect the cutaneous vitamin D synthesis.

Unfortunately, there is limited availability of data regarding the vitamin D status and risk factors for vitamin D deficiency in New Zealand children (Rockell, Green et al. 2005, Cairncross 2015,

Cairncross, Stonehouse et al. 2017). Therefore, this study aimed to assess wintertime vitamin D status in New Zealand children living in Auckland and to identify related risk factors for deficiency.

Methods

Study Participants

In this cross-sectional study, children aged 8-11 years old were recruited from six primary schools in Auckland, New Zealand (1 from the north, 2 from the east, 2 from the south, and 1 from central) in August 2016 and 2017 – late winter in the southern hemisphere. We approached schools through a collaboration with primary school science teachers and asked for expressions of interest. We then endeavoured to recruit schools specifically to include a range of socio-demographic levels and ethnicities. The study protocol was approved by the Human Ethics Committee of Massey University (Southern A; approval no. MUHECN 16/42). All children and their parents provided written informed consent before participating in the study (see Appendix 3). The study was conducted in agreement with the Declaration of Helsinki (World Medical Association Declaration of Helsinki 2013). Healthy children were sought for this study and considered ineligible when they met the following exclusion criteria 1) a history of any disease affecting vitamin D metabolism (e.g. cardiac, kidney or liver disease) or 2) a history of any long-term medication use (e.g. steroids) 3) having had any surgical implants, metal screws or similar, or 4) having a cast. All data collected from the children took place at their schools on one occasion.

Data Collection

Sample Size

Children were stratified by gender (2 groups), ethnicity (6 categories), and skin colour (4 groups) and logistic regression used to determine the contribution of risk factors for vitamin D deficiency. A sample of 10-15 per factor per group is the standard requirement for regression analysis. Therefore: 2*6*4*10-15 = 480-720 participants (Field 2009).

Anthropometric Measurements

Information about participants' weight and height were collected. Children were asked to remove their shoes to measure their heights (to an accuracy of 0.1 cm) using a Seca 213 portable stadiometer. Body weights were recorded in minimal clothing to the nearest 0.1 kg through InBody 230 (Biospace Co. Ltd., Seoul, Korea). Body mass index (BMI) was derived by dividing weight (kg) by the square of height (m^2). Body mass index was adjusted-for-age as described by Cole et al. (2000) and Cole et al. (2007). Body fat percentage (%BF) was measured by bioelectrical impedance analysis (BIA), the InBody 230 (Biospace Co. Ltd., Seoul, Korea) according to the standard procedure provided by the manufacturer. The Inbody 230 has been validated in the same population (Delshad, Beck et al. 2020).

Health and Demographic Questionnaires

Parents were asked to identify the ethnic group of their children (see Appendix 6). Ethnicity was categorised as 1) European New Zealanders, 2) Māori, 3) Pacific, 4) South Asian, 5) Chinese/Korean/Southeast Asian, and 6) Other ethnicities. If participants reported more than one ethnic group, then based on the priority system (Ministry of Health 2004, Statistics New Zealand 2009), the child was assigned to one ethnic group. The following ethnicity prioritisation was used for analytical purposes: Māori > Pacific > South Asian > Chinese/Korean/Southeast Asian > European New Zealanders > Other. For instance, if Māori was one of the reported groups, then the child was allocated to the Māori group. Parents were asked to classify their children's skin colour. Skin colour was categorised as 'fair', 'medium', 'olive', or 'dark/brown'.

Information on sunlight exposure, applying sunscreen, and the frequency of using sunscreen were obtained from a self-reported parents' questionnaire (see Appendix 6). Parents were asked to identify which parts of the body were usually exposed to sunlight, and what the usual sunscreen usage was.

Physical activity was evaluated with the short version of the international physical activity questionnaire (IPAQ) (Patterson 2005). Three types of activity were assessed: walking, moderate-intensity, and vigorous-intensity activities. Data collected using the IPAQ were categorised into three levels: low, moderate, and high (Patterson 2005) (see Appendix 6).

Dried Blood Spots Sampling and Vitamin D Assay

The dried blood spots (DBS) method was used, as it is a minimally invasive and convenient technique for children. A trained researcher used a single-use safety lancet to collect sufficient capillary blood from the child's finger. Blood spots were collected onto a Whatman filter paper card within a pre-marked area. The sample cards were air-dried at room temperature before being placed into a sealed plastic bag. All the DBS cards were refrigerated and stored at 4°C until they were sent to the Canterbury Health Laboratories for further analysis.

Measurement of 25(OH)D in DBS was performed by a newly developed method utilising two-dimensional liquid chromatography tandem mass spectrometry (2D LC-MS/MS) following methanol and hexane extraction of the DBS. Capillary calculated serum concentrations of 25(OH)D were determined by assuming a haematocrit of 0.39. The method has been thoroughly validated for research applications (Jensen, Saraf et al. 2018).

Statistical Analysis

The computer software statistical package program SPSS version 24 (IBM Corporation, New York, NY, USA) and R statistical package, version 2.15.1 (R Foundation for Statistical Computing, Vienna, Austria, http://www.R- project.org/) were used to analyse the data. The variables were tested for normality using the Kolmogorov-Smirnov test and Shapiro-Wilk tests and for homogeneity using the Levene's test.

The data were normally distributed so parametric tests were used. For baseline characteristics and measurements, continuous variables were expressed as mean ± standard deviation (SD) (Table 4.1) and categorical as number and percentages (n (%)) (Table 4.2). Pearson's correlation coefficients were used to test relationships between continuous variables. The independent T-test, ANOVA test and Chi-squared test were used to compare 25(OH)D concentration between groups.

The association between 25(OH)D status (< 50 nmol/L) and potential determinants were tested using univariate analysis. The following independent variables (all with a *P*-value of < 0.20) were included in the model: age, %BF, gender, physical activity, and ethnicity. Age and %BF were entered into the

model as continuous variables. All other variables were treated as categorical variables. Reference categories were male, having normal physical activity, and of New Zealand European ethnicity. Body mass index, body part sun exposure and sunscreen user did not meet the screening criteria (*P*-value ≥ 0.20), so they were not included as they were unlikely to contribute to a model contacting other potential determinants of 25(OH)D < 50 nmol/L. Interaction between ethnicity and skin colour did not pass the initial screen, therefore only ethnicity was entered to the model. There was no collinearity between variables. Forward stepwise multiple logistic regression analysis with the entry criterion set at *P*-value < 0.05 was used to determine which variables to include in the final model.

Results

A total of 507 children (237 (46.7%) boys) participated. The main characteristics of the study population group are presented in Table 4.1.

Table 4.1. Characteristics of population group (n = 507) and Pearson correlation between 25(OH)D and continuous variables.

	Mean ± SD	Correlation coefficient (*r*)	*P*-value
Age (years)	10.0 ± 0.7	-0.123	0.006
Weight (kg)	39.1 ± 11.0	-0.178	< 0.0001
Height (cm)	144.0 ± 8.3	-0.020	0.656
Body mass index (kg/m^2)	18.6 ± 4.0	-0.201	< 0.0001
Body fat percentage	22.1 ± 9.0	-0.233	< 0.0001
25(OH)D (nmol/L)	64.0 ± 20.8	-	-

SD, standard deviation.

The distribution of ethnic groups was: 191 (37.7%) New Zealand European, 64 (12.6%) Māori, 108 (21.3%) Pacific, 47 (9.3%) South Asian, 65 (12.8%) Chinese/Korean/Southeast Asian, and 32 (6.3%) of others not specified. The mean age was 10.0 ± 0.7 years (range 8-11 years). The concentrations of 25(OH)D were negatively associated with age (*P* < 0.05), weight, BMI, and %BF (*P* < 0.01). Most participants were normal (85.6%) with a mean BMI of 18.6 kg/m^2. High physical activity levels were reported in 36.5% of participants. The most common skin colour was ‘medium’ (39.3%) with ‘olive’

(28.8%) ranking second. Our results showed 89.5% of children exposed only legs; arms and legs; face and arms and legs to the sunlight. The majority used sunscreen only in summer time (87.0%) and 75.3% applied sunscreen on only their legs; arms and legs; face and arms and legs.

Capillary (calculated) serum concentrations of 25(OH)D ranged from 9.0-123.0 nmol/L. Mean ± SD 25(OH)D concentration was 64.0 ± 20.8 nmol/L. The prevalence of 25(OH)D < 50 nmol/L, 50-75 nmol/L, and ≥ 75 nmol/L was 27.8, 41.4, and 30.8%, respectively (Figure 4.1).

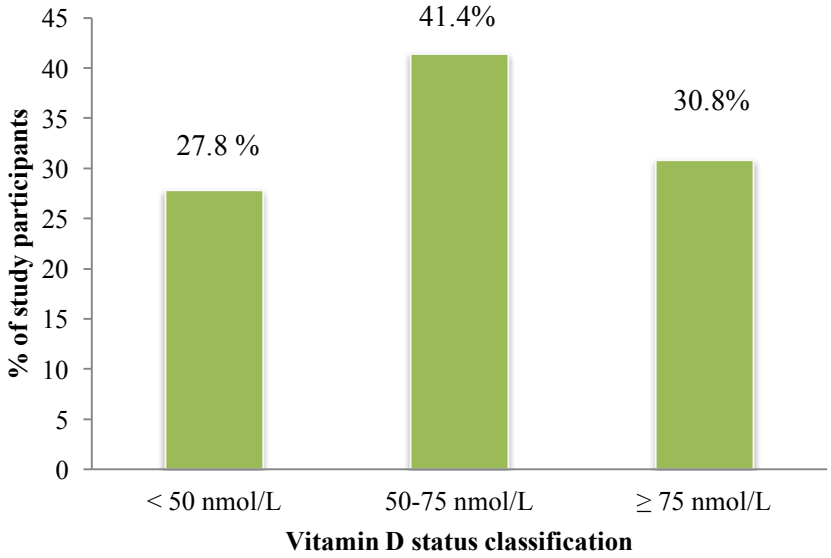

Figure 4.1. 25(OH)D status among the study population

The relationship between 25(OH)D concentration and their potential determinants are summarised in Table 4.2. Mean 25(OH)D did not differ significantly between boys and girls (66.0 ± 20.2 vs. 62.4 ± 21.3 nmol/L; $P > 0.05$), or by levels of physical activity. However, mean 25(OH)D was significantly higher in normal compared with overweight/obese children (65.0 ± 21.1 vs. 55.4 ± 18.1 nmol/L; $P < 0.005$). New Zealand European children had the highest mean 25(OH)D concentrations of 75.0 ± 20.1 nmol/L and South Asian children had the lowest 49.2 ± 20.3 nmol/L. Participants reporting 'dark/brown' skin colour had lower mean 25(OH)D concentration (51.7 ± 18.0 nmol/L) compared to the other skin colours. Body parts exposed to sunlight, use of sunscreen, frequency of use of sunscreen and body parts where sunscreen applied were significantly associated with 25(OH)D (Table 4.2).

Table 4.2. 25(OH)D concentration and its related determinants

	n (%)	25(OH)D nmol/L Mean ± SD	*P*-value[1]	25(OH)D < 50 nmol/L n (%)	25(OH)D 50-75 nmol/L n (%)	25(OH)D ≥ 75 nmol/L n (%)	*P*-value[2]
Gender			0.190				0.140
Boys	237 (46.7)	66.0 ± 20.2		56 (23.6)	105 (44.3)	76 (32.1)	
Girls	270 (53.3)	62.4 ± 21.3		85 (31.5)	105 (38.9)	80 (29.6)	
BMI status[3]			< 0.005				< 0.004
Underweight	26 (5.1)	65.3 ± 23.3		6 (23.1)	9 (34.6)	11 (42.3)	
Normal weight	434 (85.6)	65.0 ± 21.1		114 (26.3)	179 (41.2)	141 (32.5)	
Overweight and obese	47 (9.3)	55.4 ± 18.1		21 (44.7)	22 (46.8)	4 (8.5)	
Physical activity levels			0.627				0.129
Low level	176 (34.7)	65.1 ± 21.9		45 (25.6)	70 (39.8)	61 (34.7)	
Moderate level	146 (28.8)	63.8 ± 22.5		49 (33.6)	52 (35.6)	45 (30.8)	
High level	185 (36.5)	64.0 ± 19.3		47 (25.4)	88 (47.6)	50 (27.0)	
Ethnic group			< 0.0001				
New Zealand European	191 (37.7)	75.0 ± 20.1		22 (11.5)	73 (38.2)	96 (50.3)	< 0.0001
Māori	64 (12.6)	66.0 ± 19.0		20 (31.3)	23 (35.9)	21 (32.8)	
Pacific	108 (21.3)	570 ± 17.0		37 (34.3)	56 (51.9)	15 (13.9)	
South Asian	47 (9.3)	49.2 ± 20.3		29 (61.7)	13 (27.7)	5 (10.6)	
Chinese/Korean/Southeast Asian	65 (12.8)	57.4 ± 15.2		21 (32.3)	33 (50.8)	11 (16.9)	
Other	32 (6.3)	57.9 ± 23.6		12 (27.8)	12 (37.5)	8 (25.0)	
Skin colour group			< 0.0001				
Fair	58 (11.4)	66.5 ± 22.6		14 (24.1)	24 (41.4)	20 (34.5)	< 0.0001
Medium	199 (39.3)	69.2 ± 20.4		37 (18.9)	83 (41.7)	79 (39.7)	
Olive	146 (28.8)	65.5 ± 19.0		36 (24.7)	64 (43.8)	46 (31.5)	
Dark/Brown	104 (20.5)	51.7 ± 18.0		54 (51.9)	39 (37.5)	11 (10.6)	
Body part exposed to sun			0.001				< 0.001
Only face; only arm; face and arm	53 (10.5)	54.3 ± 21.8		28 (52.8)	15 (28.3)	10 (18.9)	
Only legs; arms and legs; face, arms, and legs	454 (89.5)	65.4 ± 20.0		113 (24.9)	195 (43.0)	146 (32.2)	
Sunscreen user			0.003				0.099
Yes, all year	31 (6.1)	63.0 ± 24.7		9 (29.0)	12 (38.7)	10 (32.3)	
Yes, only summer	441 (87.0)	65.0 ± 21.6		116 (26.3)	184 (41.7)	141 (32.0)	
No	35 (6.9)	53.6 ± 18.9		16 (45.7)	14 (40.0)	5 (14.3)	
Frequency sunscreen use			< 0.0001				0.001
Always	205 (40.4)	69.5 ± 22.8		46 (22.4)	83 (40.5)	76 (37.1)	
Sometimes	241 (47.5)	62.5 ± 19.5		67 (27.8)	102 (42.3)	72 (29.9)	
Rarely and never	61 (12.0)	54.6 ± 17.0		28 (45.8)	25 (41.0)	8 (13.1)	
Body part apply sunscreen			<0.0001				0.060
Only face; only arm; face and arm	88 (17.3)	65.1 ± 22.6		25 (28.4)	31 (35.2)	32 (36.4)	
Only legs; arms and legs; face, arms, and legs	382 (75.3)	65.0 ± 21.2		100 (26.2)	163 (42.7)	119 (31.2)	

[1]Independent T-test (for two categories) and ANOVA test (more than two categories), [2]Chi-squared test, [3]Cole et al. (2000) and Cole et al. (2007)
SD, standard deviation.

Logistic regression analysis results on 25(OH)D and its determinants are shown in Table 4.3. There was a significant inverse association between %BF and increased odds ratio (OR) of 25(OH)D < 50 nmol/L (OR = 0.96, 95%CI: 0.94-0.99, $P < 0.001$). All other ethnic groups compared to reference group (New Zealand European) were at higher risk of 25(OH)D < 50 nmol/L (OR from 0.07 to 0.28, $P < 0.0001$).

Table 4.3. Results of stepwise linear regression identifying determinants of 25(OH)D < 50 nmol/L

Variable	B (SE)	25(OH)D < 50 nmol/L vs. 25(OH)D ≥ 50 nmol/L)		
		OR[1]	95%CI for OR	*P*-value
Body fat percentage	-0.03 (0.01)	0.96	0.94, 0.99	0.008
Ethnicity group				
(NZ European vs. Maori)	-1.26 (0.39)	0.28	0.13, 0.60	0.001
(NZ European vs. Pacific)	-1.39 (0.35)	0.24	0.12, 0.49	< 0.0001
(NZ European vs. South Asian)	-2.54 (0.40)	0.07	0.03, 0.17	< 0.0001
(NZ European vs. Chinese/Korean/ Southeast Asian)	-1.58 (0.37)	0.20	0.09, 0.42	< 0.0001
(NZ European vs. other)	-1.80 (0.45)	0.16	0.06, 0.40	< 0.0001

Following independent variables were included in the model: age, body fat percentage, gender, physical activity, and ethnicity.
[1]Change in odds of 25(OH)D < 50 nmol/L occurring for each unit change in determinant variable. If >1.0, as determinant variable increases, odds of 25(OH)D < 50 nmol/L increase. If < 1.0, as determinant variable increases, odds of 25(OH)D < 50 nmol/L decrease.
$R^2 = 0.22$ (Hosmer and Lemeshow), 0.12 (Cox and Snell), and 0.17 (Nagelkerke). Model $X^2 = 65.49$.
CI, confidence intervals; *OR,* odds ratio; *NZ,* New Zealand.

Discussion

There is limited data available regarding wintertime vitamin D status and its related determinants in New Zealand children. Approximately, one-third of the study population had 25(OH)D ≥ 75 nmol/L and about one-third had 25(OH)D < 50 nmol/L. Body fat percentage and ethnicity were the strongest predictors of 25(OH)D < 50 nmol/L, while many established contributors to vitamin D status (e.g. gender, physical activity) were not associated with 25(OH)D < 50 nmol/L in this population.

There is not a worldwide consensus about acceptable vitamin D concentrations. Different cut-off points have been set for populations based on the relationship between vitamin D status and various criteria such as parathyroid hormone levels, intestinal calcium absorption, and bone mineralisation. It has been shown that 25(OH)D concentration of less than 50 nmol/L may cause hypo-calcemia and

secondary hyperparathyroidism in children (Holick 2007). Based on New Zealand consensus, aiming for 25(OH)D ≥ 50 nmol/L seems prudent (Ministry of Health and Cancer Society of New Zealand 2012). In our study, nearly one third (30.8%) of participants had 25(OH)D ≥ 75 nmol/L, 41.4% had 25(OH)D = 50-75 nmol/L, and 25(OH)D < 50 nmol/L was detected in approximately one third (27.8%) of children. Our data are comparable to previous studies in children and adolescents (Rockell, Green et al. 2005, Weng, Shults et al. 2007, Alemzadeh, Kichler et al. 2008, Elizondo-Montemayor, Ugalde-Casas et al. 2010, Poopedi, Norris et al. 2011, Vierucci, Del Pistoia et al. 2013, Avagyan, Neupane et al. 2016). For instance, Alemzadeh et al. (2008) using the same cut-off points for vitamin D categories, showed 32% and 41.7% of 6 to 18 year olds (49 Caucasian, 39 Hispanic, and 39 African American) had 25(OH)D < 50 nmol/L and between 50 and 75 nmol/L, respectively. There are a few studies that have investigated the status of 25(OH)D among the New Zealand paediatric population. Rockell et al. (2005) used 37.5 nmol/L 25(OH)D as the cut-off value and found a high prevalence of 25(OH)D insufficiency 41%, 59%, and 25% among Māori, Pacific, and New Zealand European school-age children aged 5-14 years, respectively. Cairncross (2015) collected capillary blood spots late winter to early spring and measured 25(OH)D in 1329 preschool children (2 to 4 years old). Results showed that 86 (7%) and 642 (48%) of children had vitamin D deficiency (< 25 nmol/L) and insufficiency (< 50 nmol/L), respectively. Therefore, further demonstrating that wintertime 25(OH)D concentrations are of concern for some New Zealand children.

Previous studies have suggested a significant inverse association between vitamin D status and fat mass, due to vitamin D being a fat-soluble vitamin (Wortsman, Matsuoka et al. 2000, Arunabh, Pollack et al. 2003). Our results showed an inverse correlation between weight, BMI, and %BF and vitamin D status. Children with normal BMI showed significantly higher 25(OH)D compared to overweight/obese children (65.0 nmol/L vs. 55.4 nmol/L). Wortsman et al. (2000) suggested obesity is associated with vitamin D insufficiency since vitamin D can be deposited in adipocytes, and therefore its bioavailability decreases. Also, in our logistic regression analysis, having a higher %BF increased the chance of having 25(OH)D < 50 nmol/L after accounting for age, gender, and physical

activity.

In our study, New Zealand European children had higher 25(OH)D concentration (75.0 nmol/L) compared with all other ethnic groups. Rockell et al. (2005) demonstrated the prevalence of vitamin D insufficiency (< 37.5 nmol/L) was 38, 58 and 23% in Māori, Pacific, and New Zealand European children aged 5-14 years, respectively. Cairncross (2015) found that the prevalence of vitamin D deficiency (< 25 nmol/L) was 9 and 23% in Māori and Pacific children, respectively compared with 3% in New Zealand European children. Our findings showed that 11.5% of New Zealand European children, 31.3% of Māori children and 34.3% of Pacific children had 25(OH)D concentration less than 50 nmol/L. The highest prevalence of 25(OH)D < 50 nmol/L was in South Asian children (61.7%) with a mean of 49.2 nmol/L. Results from the logistic regression analysis demonstrated ethnicity is a predictor of 25(OH)D < 50 nmol/L and South Asian children had the highest odds of 25(OH)D < 50 nmol/L compared to the New Zealand European group. In a review paper, Akhtar (2016) reported that vitamin D deficiency (20-50 nmol/L) is highly prevalent among the South Asian population. A possible reason for these results can be related to differences in skin colour among different ethnic groups. In the present study, most of the New Zealand European children (56.5%) reported 'medium' skin colour while 35.9% of Māori children reported having 'olive' skin colour. About half (47.2%) of Pacific children and more than half (57.4%) of South Asian children had 'dark/brown' skin colour. The negative association of vitamin D status with skin colour is well documented in previous studies (Weng, Shults et al. 2007, Poopedi, Norris et al. 2011). Our study results revealed that children who reported a 'dark/brown' skin colour had significantly lower 25(OH)D concentrations than children who reported a 'fair' skin colour (51.7 nmol/L vs. 66.5 nmol/L). It is suggested that the cutaneous synthesis of vitamin D is lower in individuals with a darker skin colour due to the ultraviolet radiation beta (UVβ) photons being less absorbed through melanin pigmentation (Ministry of Health and Cancer Society of New Zealand 2012).

Contrary to previous studies (Al-Ghamdi, Lanham-New et al. 2012, Al-Musharaf, Al-Othman et al. 2012, Flores, Macias et al. 2013, Kolokotroni, Papadopoulou et al. 2015), and in line with Vierucci

et al. (2013) and Avagyan et al. (2016), a significant difference in 25(OH)D concentration between boys (66.0 nmol/L) and girls (62.4 nmol/L) was not found. It has been proposed that gender differences in vitamin D are related to BMI status (Wortsman, Matsuoka et al. 2000, Arunabh, Pollack et al. 2003). In the current study, there was not a significant difference in BMI between boys (mean 18.2 kg/m^2) and girls (mean 19.0 kg/m^2).

It has been established that sedentary lifestyle and less physically active people have lower 25(OH)D values (Valtuena, Gonzalez-Gross et al. 2013). Less physically active people usually spend less time outdoors and therefore have limited opportunity for sun exposure, with an increased risk of obesity. In this study, a significant relationship between vitamin D status and physical activity was not found. Also, the mean of BMI across all three physical activity levels did not differ significantly.

In the literature, several factors have been postulated that could affect the cutaneous synthesis of vitamin D through sun exposure, such as body surface area, time of the day, season, latitude, degree of skin pigmentation, and sunscreen use (Hossein-nezhad and Holick 2013, Saggese, Vierucci et al. 2015). In this study, parents were asked to identify which parts of the body were usually exposed to sunlight, frequency of use and where sunscreen was applied. Our data confirm the prevalence of vitamin D deficiency is lower in children who exposed more surface of their body (only legs; arms and legs; face and arms and legs) to the sunlight.

It is suggested that properly applying sunscreen with a sun protection factor (SPF) of 30 can decrease the cutaneous synthesis of vitamin D by as much as 95 to 99 % (Hossein-nezhad and Holick 2013). In our study, similar to Cairncross et al. (2017), our participants who reported that they applied sunscreen and were more frequent users, had higher 25(OH)D concentrations compared to the other groups. An explanation for these findings may be because of the lack of parents' knowledge about how to apply sunscreen. Furthermore, parents or children were not asked how often they renewed application of sunscreen each day and the SPF of the sunscreen used. The amount of sunscreen applied (Kim, Oh et al. 2010) and how often it is re-applied influences its effectiveness. It was also speculated that the children who were using sunscreen were going out in the sun, but those who were

not applying sunscreen were not exposing themselves.

In this study, 27.8% of the population presented with 25(OH)D values < 50 nmol/L. An effective strategy is needed to prevent vitamin D status less than 50 nmol/L and its related health consequences among children. Increasing sun exposure, especially in high-risk children (overweight/obese, darker skin colour, specific ethnic groups) should be the first-line treatment. For most people living in New Zealand, exposure to the sun is the major source of vitamin D (Ministry of Health and Cancer Society of New Zealand 2012). The body is able to synthesise sufficient vitamin D with adequate skin sunlight exposure (Holick 2012). However, there is no evidence regarding the safe threshold level of ultraviolet (UV) radiation exposure from the sun without increasing risk of skin cancer (Ministry of Health and Cancer Society of New Zealand 2012). A balance between avoiding excessive sun exposure to prevent skin cancer, and enough sun exposure to achieve adequate vitamin D concentration, is required (Ministry of Health and Cancer Society of New Zealand 2012). The New Zealand Ministry of Health recommends a daily walk or some other form of outdoor physical activity in the early morning or late afternoon between the summer months of September and April (Ministry of Health and Cancer Society of New Zealand 2012). Between May and August (winter in New Zealand), except at high altitudes or near highly reflective surfaces (e.g. snow or water), sun protection is generally not recommended. During this time exposure to direct sunlight, especially in the hours around noon when ultraviolet radiation beta (UVβ) levels are highest, will be enough for adequate cutaneous production of vitamin D (Ministry of Health and Cancer Society of New Zealand 2012). However, paediatricians should monitor children's sun exposure and vitamin D concentration in high-risk subjects including those who are overweight/obese, have dark skin colour or who are of South Asian ethnicity. These groups may benefit from vitamin D supplementation (Ministry of Health and Cancer Society of New Zealand 2012).

Strengths and Limitations

One of the limitations of this study is that our results are not necessarily transferable to other populations since only healthy children living in Auckland, New Zealand were recruited. Also,

children based on their schools' collaboration were recruited. So we cannot claim that our participants are representative of all children living in Auckland. For instance, the proportion of Pacific and Asian groups seem high while Māori are on the low side. However, we tried to include a range of socio-demographic levels and ethnicities. This study was a cross-sectional study, at the end of winter, and lacked a longitudinal assessment of vitamin D status, therefore, we could not consider the effect of seasonal variation on vitamin D status. In addition, we collected some information regarding vitamin D risk factors (e.g. body exposure area, use of sunscreen, physical activity) through self-reported questionnaires so recall bias could have influenced our results. Although the DBS method is minimally invasive for measuring vitamin D and previous studies have used it (Cairncross, Stonehouse et al. 2017), it has not been specifically validated in children. Despite these limitations, this study had some notable strengths and suggested a general pattern of vitamin D status and its related risk factors among school-age children living in Auckland, New Zealand. We recruited a large sample of healthy children from a broad range of socio-demographic and ethnic backgrounds. Also, we used %BF, which is a better indicator of fatness in an individual than BMI.

Conclusion

Our data indicate an association between winter vitamin D status and its related factors in children living in Auckland, New Zealand. About one-third of our participants had 25(OH)D < 50 nmol/L. Ethnicity and %BF were the only significant predictors of 25(OH)D < 50 nmol/L, with no association shown between gender, physical activity and 25(OH)D in our population. Being overweight and obese, being from South Asian ethnicity, having a darker skin colour, and exposing less surface of the body to sunlight significantly affected vitamin D status. Poor vitamin D status during childhood can affect long-term health, so opportunities to intervene during childhood should be pursued. A strong consideration should be given to the high-risk children and production of cutaneous vitamin D through sunlight exposure.

Conflict of interest: The authors declare that they have no conflict of interest.

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Chapter Five

Validity of Quantitative Ultrasound and Bioelectrical Impedance Analysis for Measuring Bone Density and Body Composition in Children

(paper III)

In order to determine bone density and body composition in children, quantitative ultrasound (QUS) and bioelectrical impedance analysis (BIA) were examined against dual-energy X-ray absorptiometry (DXA). It is important that the relative validity of newly developed tools ('test' QUS and BIA) are tested with a standard and independent assessment method ('reference' DXA) prior to being used. This study aimed to investigate the validity of QUS against DXA for measuring bone density and the validity of an in-built algorithm of BIA for measuring body composition in children (8-13 years) living in New Zealand.

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To align with the formatting and referencing style of this thesis, there are some changes in formatting and referencing style of the published paper.

Abstract

Aim: This study aimed to examine: 1) validity of quantitative ultrasound (QUS) against dual-energy X-ray absorptiometry (DXA) for measuring bone density and 2) the validity of in-built algorithm of bioelectrical impedance analysis (BIA) for measuring body composition in children (8-13 years) living in New Zealand.

Methods: Total body less head (TBLH), bone mineral content (BMC), bone mineral density (BMD), and body composition were measured with DXA (QDR Discovery A, Hologic, USA); calcaneal BMD and stiffness index (SI) with QUS (Sahara QUS, Hologic, USA), and BIA on the InBody 230 (Biospace Ltd., Seoul, Korea). Relative validity was assessed using Pearson's and Lin's concordance correlation coefficients (CCC), and Bland-Altman plots.

Results: In 124 healthy children, positive correlations between QUS SI and DXA (BMC and BMD) were observed (range = 0.30-0.45, $P < 0.01$). Results from Lin's CCC test showed almost perfect correlations between BIA and DXA fat-free mass (0.96), fat mass (0.92), and substantial correlation for body fat percentage (0.75) ($P < 0.05$).

Conclusion: Although BIA results were not as accurate as DXA and DXA remains the gold standard method for clinical assessment, BIA can be an alternative method for investigating body composition among children in large cohort field studies. Calcaneal QUS and DXA are not interchangeable methods for measuring bone density in children similar to our study population.

Introduction

One of the important osteoporosis risk factors in adulthood is the amount of bone gained during childhood and adolescence and the subsequent rate of loss with ageing (Specker and Schoenau 2005). Growth monitoring and investigation of body composition in children is crucial for early evaluation of nutritional status and detecting health and nutritional problems during child development. Dual-energy X-ray absorptiometry (DXA) is a radiological technique, which is designed primarily for measuring bone mineral density (BMD) (Binkley, Berry et al. 2008). It can also provide information on bone mineral content (BMC) (Specker and Schoenau 2005), and other compartments of body composition including fat mass (FM), fat-free mass (FFM), and body fat percentage (%BF) (Ellis, Shypailo et al. 1994). Although DXA measurements are accurate and valid, the method is expensive, requires trained operators, time-consuming, and exposes children to ionising radiation (Binkovitz and Henwood 2007). It is therefore not suitable for routine paediatric research practice and is impractical for use in the field.

In recent years, there has been an increasing demand for the assessment of skeletal health status using radiation-free techniques. Quantitative ultrasound (QUS) measures the velocity of sound through a body tissue (e.g. bone) and the amplitude of ultrasound wavelengths are absorbed through a transducer placed on opposite sides of tissue, reflecting its density, architecture, and elasticity (Kaufman and Einhorn 1993, Genant, Engelke et al. 1996). It is a low cost, easy to use, transportable, non-invasive, radiation-free method, and only takes a few minutes for measurements (Baroncelli 2008). The attractiveness of QUS lies in additional structural information beside bone mineral status, which may be important in determining fracture risk (Genant, Engelke et al. 1996). However, a major issue with the use of QUS is the lack of information regarding the validity of ultrasonic measurement of bone in a paediatric population.

On the other hand, a number of methods are available to estimate body composition of paediatric populations with various accuracy, feasibility, cost, and accessibility. Compared to other methods (e.g., underwater weighing, isotope dilution), bioelectrical impedance analysis (BIA) is a relatively simple non-invasive field method and has distinct advantages including low cost (Houtkooper, Lohman et al. 1989). Bioelectrical impedance analysis can estimate the volume of

total body water (TBW), FFM, and FM (cited by Houtkooper et al. 1989). There are several studies available which compare the validity of BIA measurements with DXA in adults (Huang, Chen et al. 2015, von Hurst, Walsh et al. 2015), however, there are only a few in healthy children (Gutin, Litaker et al. 1996, Okasora, Takaya et al. 1999, Fors, Gelander et al. 2002, Eisenmann, Heelan et al. 2004, Elberg, McDuffie et al. 2004, Kriemler, Puder et al. 2009, Noradilah, Ang et al. 2016, Lee, Liao et al. 2017). Results have varied due to a wide range of BIA equipment available (e.g. ankle-to-wrist electrode systems, hand-to-foot electrode systems, 4-point and 8-point models).

Although both QUS and BIA devices have been used in the assessment of BMD and body composition respectively, their validity against conventional reference methods (e.g. DXA) as a measure of BMD and body composition in children remains uncertain. To the best of our knowledge, there is no published validation of QUS and BIA in children living in New Zealand. Therefore, the proposed study aimed to investigate 1) the validity of QUS against DXA values for measuring bone density and 2) the validity of in-built algorithm BIA against DXA values for measuring body composition in children living in Auckland, New Zealand.

Methods

The current study was a cross-sectional and observational study in children aged 8-13 years living in Auckland, New Zealand. The study protocol was approved by the Human Ethics Committee of Massey University (Southern A; approval no. MUHECN16/42). Prior to the study, children and their parents received an envelope containing a study information sheet (see Appendix 4 and 5) and consent form (see Appendix 3).

Study Participants

A sample of 128 children was calculated based on G*Power program [version 3.1 software (Faul, Erdfelder et al. 2009): medium effect size: 0.6; power: 95%; level of significance: 5%]. Healthy children were recruited from primary schools. Children were ineligible if they have 1) a history of any disease affecting bone health or calcium and vitamin D metabolism 2) a history of long-term medication use (e.g. corticosteroids) affecting bone growth and metabolism 3) any surgical implants, metal screws or similar or 4) having a cast. All data collection took place in the Human

Nutrition Research Unit at Massey University, Albany campus, in Auckland, New Zealand on one occasion.

Data Collection

Anthropometric Measurements

Height was measured using a stadiometer to the nearest 0.1 cm. Body weights were recorded in minimal clothing to the nearest 0.1 kg through InBody 230 (Biospace Co. Ltd., Seoul, Korea). Body mass index (BMI) was calculated based on BMI adjusted-for-age as described by Cole et al. (2000) and Cole et al. (2007).

DXA Scan

The DXA measurements were performed on a Hologic QDR Discovery A (Hologic Inc, Bedford, MA, USA) with APEX V. 3.2 software. It depends on the differential absorption of X-ray photons to compare tissues of different radiographic density (Binkovitz and Henwood 2007). A computer detects and measures photons exiting from various body sites and the attenuation values determined using calibration materials are converted into BMC and quantified (in grams). The BMD is calculated by dividing BMC values by bone area (BA) (BMD = BMC/BA (g/cm^2)) (Binkley, Berry et al. 2008). The total body less head (TBLH) and lumbar spine (LS) were chosen as the skeletal sites for DXA measurements based on the International Society for Clinical Densitometry (ISCD) recommendations and the highly reproducible nature of these measures in paediatric measurements (Crabtree, Arabi et al. 2014). Final measurements from the DXA were BMC (g), BMD (g/cm^2), FFM (kg), FM (kg), and %BF.

Quantitative Ultrasound (QUS)

The ISCD suggests calcaneus as the best skeletal site of measurement compared to the other peripheral sites (e.g. radius, tibia) as it demonstrates an acceptable level of scientifically validated results in osteoporosis management (Krieg, Barkmann et al. 2008). In this study, the calcaneal BMD measurements were evaluated using a commercial QUS device (Sahara Clinical Bone Sonometre Hologic Inc, USA). The device can assess both speed of sound (SOS) (m/s) and broadband ultrasound attenuation (BUA) (decibels/mega-hertz). The SOS reflects the velocity of ultrasound waves inside of the bone (Baroncelli 2008) and the BUA represents the slope of

attenuation of mechanical waves travelling through the bone (Baroncelli 2008). From these a third parameter is calculated (stiffness index (SI)). The coefficient of variance (CV) for stiffness was 2.6%.

BIA

Body composition was measured using InBody 230 (Biospace Co. Ltd., Seoul, Korea). The Inbody 230 is a vertical 8-point model of BIA with footpads containing electrodes on the base platform and similar sensors on the handles, which extend from the up-stand and control panel. Children were asked to take off their shoes and socks and stand upright, positioning their bare feet on the footpads and their hands on the handles. Footpads and handles each contain two electrodes, providing eight points of contact. A small electrical current is passed through the body, resistance is measured and the inbuilt software calculates TBW and the corresponding percentage of fat mass. An inbuilt equation was used to convert the input impedance to body composition estimates. The amount of FFM (kg), FM (kg), and %BF were calculated for each participant.

Statistical Analysis

The computer software statistical package program SPSS version 24 was used to analyse the data (IBM Corporation, New York, NY, USA). The variables were tested for normality using the Kolmogorov-Smirnov and Shapiro-Wilk tests and for homogeneity using the Levene's test. The data were normally distributed so parametric tests were used and data expressed as mean and standard deviation (SD).

Pearson correlation coefficients were used to quantify the strength of association between QUS parameters (calcaneal SI and BMD) versus DXA measurements (LS BMC and BMD; TBLH BMC and BMD). Lin's concordance correlation coefficient (CCC) was used for comparing BIA measurements (FM, FFM, and %BF) with the reference method (DXA) measurements (FM, FFM, and %BF) (Lin 1989, Lin 2000). A P-value <0.05 was considered significant.

Agreement between the FFM by DXA and BIA was assessed using a Bland-Altman plot. Mean differences were calculated and the limits of agreement (LOA) (mean difference ± 1.96 standard deviations) were determined (Bland and Altman 1986). Linear regression analysis was undertaken to investigate bias.

Results

One hundred and thirty healthy children volunteered to participate in this study however, only 124 (58 boys) met the inclusion criteria and completed the DXA, QUS, and BIA measurements. The mean ± SD age in both boys and girls was 10.4 ± 1.1 years. Most of our participants were classified as normal weight (75.4%). The baseline characteristics of the participants are summarised in Table 5.1.

Table 5.1. Baseline characteristics of participants (mean ± SD)[1]

Variable	Total (n = 124)	Boys (n = 58)	Girls (n = 66)
Age (years)	10.4 ± 1.1	10.4 ± 1.1	10.4 ± 1.2
Height (cm)	148.0 ± 9.1	147.8 ± 9.5	148.1 ± 8.8
Weight (kg)	41.6 ± 10.1	41.0 ± 10.3	42.1 ± 9.9
BMI (kg/m^2) [2, 3]	18.7 ± 3.2	18.5 ± 2.9	19.0 ± 3.4
Under weight	3.1 ± 2.4	-	3.3 ± 4.5
Normal weight	93.2 ± 75.0	48.0 ± 82.8	45.0 ± 68.2
Over weight	22.0 ± 17.7	7.5 ± 12.1	15.2 ± 22.7
Obese	6.2 ± 4.8	3.1 ± 5.2	3.6 ± 4.5

[1]*SD,* standard deviation; [2]*BMI,* body mass index; [3]Cole et al. (2000) and Cole et al. (2007)

The mean ± SD of QUS SI was 84.2 ± 8.6. The mean ± SD of BMD measured by QUS was 0.5 ± 0.1 g/cm^2 and by DXA TBLH 0.7 ± 0.1 g/cm^2. Positive associations were observed between QUS SI and DXA BMC and BMD in LS and TBLH, ranging from 0.30 (LS BMC) to 0.45 (TBLH BMD) ($P < 0.01$) as indicated in Table 5.2. Correlation coefficients between QUS SI and DXA BMC and BMD in LS and TBLH were slightly higher in girls (r = 0.32-0.50; $P < 0.01$) compared to boys (r = 0.26-0.43; $P < 0.05$). Calcaneal BMD had a significant association with DXA LS BMD (r = 0.25) and DXA TBLH BMD (r = 0.30) ($P < 0.05$) (Table 5.2).

Table 5.2. Bone quality measurements from DXA[1] and correlations with QUS[2] variables

Variable	DXA Mean ± SD[3]	QUS SI[4] correlations	QUS BMD (g/m^2) [5] correlations
Total (n = 124)			
LS BMC (g) [6]	28.1 ± 7.3	0.30**	0.18*
LS BMD (g/cm^2) [7]	0.7 ± 0.1	0.33**	0.25*
TBLH BMC (g) [8]	931.3 ± 228.6	0.43**	0.08
TBLH BMD (g/cm^2) [9]	0.7 ± 0.1	0.45**	0.30*
Boys (n = 58)			
LS BMC (g)	27.5 ± 6.5	0.30*	0.17
LS BMD (g/cm^2)	0.7 ± 0.1	0.26*	0.24*
TBLH BMC (g)	922.8 ± 231.3	0.32*	0.06
TBLH BMD (g/cm^2)	0.7 ± 0.1	0.43**	0.30*
Girls (n = 66)			
LS BMC (g)	28.6 ± 8.0	0.32**	0.18*
LS BMD (g/cm^2)	0.7 ± 0.1	0.36**	0.35*
TBLH BMC (g)	938.7 ± 227.6	0.50**	0.22
TBLH BMD (g/cm^2)	0.7 ± 0.1	0.46**	0.36*

*Correlation is significant at the 0.05 level (two-tailed), ** Correlation is significant at the 0.01 level (two-tailed)
[1]*DXA,* dual-energy X-ray absorptiometry; [2]*QUS,* quantitative ultrasound; [3]*SD,* standard deviation; [4]*SI,* stiffness index; [5]*BMD,* bone mineral density; [6]*LS BMC,* lumbar spine bone mineral content; [7]*LS BMD,* lumbar spine bone mineral density; [8]*TBLH BMC*, total body less head bone mineral content; [9]*TBLH BMD,* total body less head bone mineral density.

The mean ± SD of FM measured by BIA was 9.5 ± 5.1 kg and by DXA 10.8 ± 4.3 kg as indicated in Table 5.3 Results from Lin's CCC test showed almost perfect correlations between BIA and DXA FFM (0.96), FM (0.92), and substantial correlation for %BF (0.75) ($P < 0.05$). Similar results were found for boys and girls as indicated in Table 5.3.

Table 5.3. Body composition measurements and correlations between BIA[1] and DXA[2]

Variable	BIA Mean ± SD[3]	DXA Mean ± SD	Lin's concordance correlations
Total (n = 124)			
Total FFM[4] (kg)	32.0 ± 6.4	30.7 ± 6.8	0.96
Total FM[5] (kg)	9.5 ± 5.1	10.8 ± 4.3	0.92
%BF[6] (kg)	22.0 ± 7.6	25.6 ± 5.8	0.75
Boys (n = 58)			
Total FFM (kg)	32.4 ± 6.6	31.1 ± 7.0	0.97
Total FM (kg)	8.6 ± 4.8	9.8 ± 4.1	0.92
%BF (kg)	19.9 ± 6.5	23.4 ± 4.9	0.74
Girls (n = 66)			
Total FFM (kg)	31.7 ± 6.3	30.3 ± 6.7	0.95
Total FM (kg)	10.4 ± 5.2	11.7 ± 4.3	0.92
%BF (kg)	23.9 ± 8.0	27.5 ± 5.8	0.73

*Correlation is significant at the 0.05 level (two-tailed), ** Correlation is significant at the 0.01 level (two-tailed)
[1]*BIA,* bioelectrical impedance analysis; [2]*DXA,* dual-energy X-ray absorptiometry; [3]*SD,* standard deviation; [4]*FFM,* fat-free mass; [5]*FM,* fat mass; [6]*%BF,* body fat percentage.

The Bland-Altman plot showed the agreement between FFM measured by DXA and BIA (difference in DXA and BIA FFM the dependent variable, and mean DXA and BIA FFM the independent variable) as indicated in Figure 5.1. Linear regression analysis demonstrated differences were significantly dependent on mean FFM, indicating variation in the agreement between methods across the mean FFM. Compared with DXA, BIA overestimated FFM and underestimated FM by 1.3 kg. The slope of bias was 0.06 and the LOA analysis was around -3.8 to 1.2 (+/- ~5).

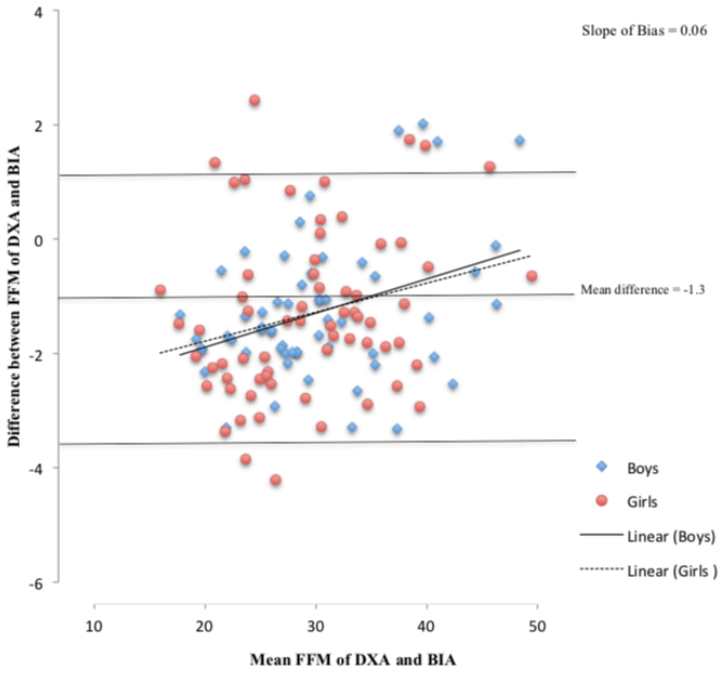

Figure 5.1. Bland-Altman plots demonstrating agreement between DXA and BIA FFM measurements (total population). A solid line represents the mean difference between two devices measurements and dashed lines representing the limits of agreement (mean difference ± 1.96 standard deviations).

Discussion

The main findings of the study in a population of healthy children were as follows: 1) a fair to moderate agreement between QUS SI and DXA parameters (LS BMC and BMD, TBLH BMC and BMD); 2) an 'almost perfect agreement' was found between BIA (FFM and FM) and the same DXA parameters and 'substantial agreement' was found for %BF measuring by BIA and DXA.

Typically, in order to investigate how well a newly developed method ('test' QUS and BIA) agrees with a standard and independent assessment method ('reference' DXA), relative validity is used (Waern, Cumming et al. 2015). In this study, relative validity was examined by using a range of statistical tests to ensure the robustness of the validity process. The QUS calcaneal stiffness index showed a moderate correlation with the DXA parameters ($r = 0.30$-0.45; $P < 0.01$). There are only a few studies that have validated QUS against DXA in healthy children and adolescents (Mughal, Langton et al. 1996, Sundberg, Gardsell et al. 1998, Lum, Wang et al. 1999,

Sioen, Goemare et al. 2011, Xu, Guo et al. 2014, Chong, Poh et al. 2015, Srichan, Thasanasuwan et al. 2016, Weeks, Hirsch et al. 2016). Our findings for QUS validity are similar to those of Xu et al. (2014), who measured 329 healthy boys and girls aged 5-17 years and found significant positive correlations between calcaneal QUS SI and DXA TB BMD (r = 0.69; $P < 0.001$) and BMC (r = 0.69; $P < 0.001$). In line with our results, others found correlation coefficients (ranging from 0.40-0.80) between calcaneal QUS and DXA-derived BMD or BMC (Mughal, Langton et al. 1996, Sundberg, Gardsell et al. 1998, Lum, Wang et al. 1999). Correlations between calcaneal BMD and DXA BMD for TBLH and LS were small but significant (r = 0.30 and 0.25 respectively; $P < 0.05$). Our findings suggested that calcaneal QUS is a reasonably valid method for assessing BMD in a paediatric population.

A recent systematic review investigated the diagnostic accuracy of QUS for the assessment of osteoporosis in children and adolescents and the authors concluded that although this technique provides reliable measurements, there is insufficient evidence to support the diagnostic value of QUS (Wang, Wang et al. 2014). In the current study, a fair to moderate agreement between the QUS and DXA in our study further supports this conclusion. While other studies observed stronger correlations (0.71-0.88) (Lum, Wang et al. 1999) and another systematic review in women over 45 years concluded that calcaneal QUS has potential to be used as a pre-screening tool for assessment of osteoporosis (Thomsen, Jepsen et al. 2015), it seems that further investigation is required to establish QUS clinical use in children.

Compared to the other anthropometric indexes (e.g. body mass index), BIA is more sensitive and specific for estimating adiposity in individuals (Houtkooper, Lohman et al. 1996). In this study, the precision of the BIA was investigated by comparing FFM, FM, and %BF values between BIA and DXA measurements. Almost perfect to substantial agreement was found between BIA parameters and DXA values (r = 0.96-0.75; $P < 0.05$). Our data shows there is a close agreement between FFM of BIA and DXA measurements, however, BIA overestimates FFM compared with DXA. Our findings were supported by prior studies. For instance, a recent study conducted by Noradilah et al. (2016) found good agreement between BIA and DXA values and similar to our data, they found BIA underestimated %BF in compared with DXA measurements. Okasora et al.

(1999) investigated the validity of BIA against DXA in a sample of children aged 6 to 16 years old. They showed a high correlation (r = 0.9) between DXA and BIA derived variables (%BF, FFM, and FM).

The good levels of correlation seen in this study may relate to the BIA machine. In this study, InBody 230 was used, which has fixed footpads and handles and is a convenient way to measure whole body segments with the participant standing. Although this can be equally achieved with a tetra-electrode device (e.g. hand-to-foot or foot-foot electrode systems) by moving the connections around the body, it can increase susceptibility to error. Our results extend those by Kriemler et al. (2009) that an octopolar-BIA device (8-BIA), similar to that used in our study, is an accurate predictor of body composition.

Our data confirm BIA is a valid and useful tool to assess body composition in children in epidemiologic and field studies. The simplicity of use of the BIA device, portability, lack of radiation exposure for the child, and low cost represents the clear advantage of the BIA method compared to DXA.

Strengths and Limitations

Participants in this study were healthy children and therefore, the results cannot be generalised to other population such as the elderly or those with specific health conditions and/or disease. In this study, the pubertal status of our participants was not investigated. Bone density and fat mass content and distribution considerably depend on the maturational status and the pattern of bone mineralisation and %BF is different in boys and girls. All the analyses of the agreement/validity between DXA and QUS, and DXA and BIA were performed separately for boys and girls.

This study had some notable strengths regarding the three different devices, which were used. First, DXA was used as the criterion method. It is considered the gold standard method for measuring bone and body composition. Second, the QUS software provided SOS and BUA, and consequently, the combined parameter called ‘stiffness index’ was available. It is known that SOS and BUA measure different properties of bone tissue and it has been shown that stiffness is a better predictor of bone fractures than SOS or BUA measurements alone (Sakata, Kushida et al. 1997). Finally, the validity of the QUS and BIA with the DXA was assessed using a range of

statistical tests. The rationale for using more than one approach to assess the same concept helps demonstrate the robustness of the validity process.

Conclusion

The current study is the first to evaluate the validity of calcaneal QUS and BIA against DXA in a New Zealand paediatric population and for measuring bone density and body composition, respectively. Although QUS is easy and convenient to use, moderate correlations were found between QUS and DXA parameters, suggesting the calcaneal QUS and DXA are not interchangeable methods. DXA is the benchmark method for diagnostic purposes in children such as our study population.

The BIA demonstrated good validity and provided similar estimates to DXA for measurements of FFM, FM, and %BF, however, BIA results were not as accurate as DXA measurements. Although BIA overestimates FFM as compared with DXA, BIA is an effective, portable, safe, and economically feasible alternative method for investigating body composition among children especially in large cohort field studies with a population that is similar to this study sample. However, the DXA method remains the gold standard and is not an interchangeable method for clinical assessment.

Conflict of interest: The authors declare that they have no conflict of interest.

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Chapter Six

Discussion and Conclusions

6.1 Introduction

The primary aim of this cross-sectional study is to explore fracture history and related risk factors in children living in Auckland, New Zealand. The secondary aims were to determine the wintertime vitamin D status of children living in Auckland and its determinants and to validate QUS and BIA measurements against DXA values.

The discussion brings together the different studies focusing on key findings, the main results, methodological issues, the significance of the results, and recommendation for future studies. The final conclusions will also be drawn.

6.2 Summary of Findings

The results of the study showed that high %BF, low 25(OH)D concentration, low total dietary calcium intake, high SSB consumption, siblings' history of fractures, family history of osteoporosis, and South Asian ethnicity were the strongest predictors of fracture in boys. In girls, high SSB consumption, siblings' history of fractures, and family history of osteoporosis were predictors of fractures **(hypothesis 1 accepted)**. Good nutrition (especially good sources of calcium and reducing SSB intake) should be recommended to children and their caregivers during growth and development to reduce their risk of fractures (chapter three).

The vitamin D status in wintertime and its determinants were investigated in children living in Auckland. Approximately one-third of our population had 25(OH)D less than 50 nmol/L. Percentage body fat and ethnicity were observed to be significant determinants of 25(OH)D < 50 nmol/L **(hypothesis 2 accepted)**. Wintertime serum 25(OH)D was highly variable and New Zealand European children had the highest mean 25(OH)D concentrations of 75 ± 20 nmol/L and South Asian children had the lowest at 49 ± 20 nmol/L as revealed in chapter four of the study.

The use of QUS provides a novel approach to investigating BMD in children. This approach considers a device, which is a low cost, easy to use, transportable, non-invasive, and radiation-free method, and only takes a few minutes for measurements. This study is the first to evaluate the validity of calcaneal QUS against DXA, for measuring bone density in a New Zealand paediatric population. Only a fair to a moderate agreement was found between QUS and DXA

parameters, suggesting that the calcaneal QUS and DXA are not interchangeable methods **(hypothesis 3 rejected)** and DXA remains the benchmark method for diagnostic purposes in children such as our study population as highlighted in chapter five of the study.

The study also investigated the validity of BIA to measure body composition in children. This approach considers a device, which is a relatively simple non-invasive field method and has distinct advantages including low cost. An almost perfect to a substantial agreement was found between BIA parameters (FFM, FM, %BF) and the same DXA parameters **(hypothesis 4 accepted)**. As far as we are aware, and as highlighted in chapter five, this is the first study to determine the validity of BIA against DXA among New Zealand children.

6.3 Methodological Considerations

6.3.1 Study Design

The current study is a cross-sectional study. Although, cross-sectional studies have some limitations such as they do not analyse behaviour over a period of time, and therefore cause and effect cannot be determined, and they are not able to measure incidence (Hennekens and Buring 1987). However, there are some advantages of using this type of study. Cross-sectional studies are relatively quick and are usually conducted to measure the prevalence of all health-related problem factors under investigation. In this type of study, all variables are collected only once and multiple outcomes and exposures can be studied (Sedgwick 2014).

6.3.2 Study Population

We originally approached schools through a collaboration of primary school science teachers and asked for expressions of interest. We then endeavoured to recruit schools specifically to include a range of socio-demographic levels and ethnicities. All children within the age group specified, and attending the school were invited to participate. Therefore, children were recruited based on their schools' collaboration. To decrease the potential risk of using a convenience sample of volunteer subjects, we mostly recruited all the children in a selected age group.

The proportion of children who identified as Pacific and Asian were higher than those children who identified as Maori. Although we tried to include a wide range of socio-demographic levels

and ethnicities, we cannot claim that our participants are representative of all children living in Auckland.

We used a separate study population for the validation study. Some children in the validation study were older (aged 8-13 years old) compared to the fracture risk factors study (aged 8-12 years old) and wintertime vitamin D study (aged 8-11 years old).

In planning the research, our studies were adequately powered to observe clinically significant results. The validation study involves a sample of 128 children, 720 children for fracture risk factors and 480 participants in the wintertime vitamin D studies.

6.3.3 Measurements

This study was conducted in children aged 8-13 years old, therefore, the age range presumably encompasses subjects in different pubertal status which we did not investigate because of ethical considerations. Also, it is not appropriate in the school setting where children can be exposed to peer pressure. At the bone growth spurt during puberty, areal bone density can decrease since bones enlarge more quickly than they mineralise (Magarey, Boulton et al. 1999). Therefore, children of this age are more vulnerable to fractures and this may have been an issue in the fracture risk factors study since some of the girls in our study could have been undergoing a growth spurt. However, the ages that fracture happened were asked and none of the girls ≥ 10 years old had a fracture at 10 years of age or older. Moreover, bone density and fat mass content and distribution considerably depend on the maturational status and the pattern of bone mineralisation and %BF are different in boys and girls. Therefore, all the analyses of the agreement/validity between DXA and QUS, and DXA and BIA were performed separately for boys and girls.

Information related to fracture risk factors (e.g. ethnicity, siblings' history of fracture, history of osteoporosis in family, calcium intake, SSB, and PA) were collected through self-reported questionnaires, so recall bias could have influenced our results. In addition to recall bias, the use of a calcium FFQ may be particularly challenging when dealing with children, or when an adult is answering on behalf of the children and who may not be paying attention to portion size, frequency of intake, or left-overs. Also, the short questionnaire for estimation of dietary calcium intakes had not been validated in a New Zealand population.

Information regarding vitamin D risk factors (e.g. body area exposure to sunlight, use of sunscreen, physical activity) was also collected through self-reported questionnaires so again recall bias could have influenced our results. The DBS method was used for measuring 25(OH)D concentrations since it is minimally invasive, convenient to use for children, and is an alternative method to measuring 25(OH)D serum concentrations (Avagyan, Neupane et al. 2016, Cairncross, Stonehouse et al. 2017, Jensen, Saraf et al. 2018). However, although validated in adults and neonates, it has not been specifically validated in children.

6.4 Overview of Main Findings and Discussion

6.4.1 Causes and Consequences of Fractures in Children

The major objective of this research is to determine the related risk factors for fractures in children. The identification of factors that can affect bone health enables preventative strategies and solutions for fractures to be put in place.

Fractures impact on daily activity, and cause pain, lack of mobility, and dependency (Manias, McCabe et al. 2006). During childhood and adolescence, fracture events can happen simply because of accidents or a transient reduction in bone density, which soon normalises (Goulding, Jones et al. 2005). Achieving maximal genetic PBM throughout the first two decades of life can maintain good bone mass and consequently decrease the risk of osteoporotic fractures later in life. Having a balanced diet and body weight, doing daily weight-bearing physical activity, and healthy endocrine development are necessary during childhood growth (Goulding 2007).

Similar to adults (Marshall, Johnell et al. 1996, Johnell, Kanis et al. 2005), there is an association between low bone density and fracture risk in growing children. In adults, low BMD, and the site of bone fracture (e.g. vertebral, hip) has been documented (Marshall, Johnell et al. 1996). In some but not all studies among children and adolescents (boys and girls), upper limb fractures are considered as a site for fracture prediction (Goulding, Jones et al. 2000, Clark, Tobias et al. 2006, Ferrari, Chevalley et al. 2006, Jones and Boon 2008, Kalkwarf, Laor et al. 2011). Our findings showed the upper limb was the most common site of fractures (n = 161, 78.1%) in children compared to the lower limb (n = 39, 18.9%), with few fractures occurring elsewhere (n = 6, 3.0%).

In the current study, the proportion of fractures was higher in the group of children who reported fracture history in their siblings (boys = 57.1% vs. 19.3% and girls = 60.0% vs. 15.2%) and history of osteoporosis in their family (boys = 51.6% vs. 21.8% and girls = 41.9% vs. 19.5%). Also, siblings' history of fractures and a family history of osteoporosis were predictors of fractures in our population, suggesting genetic factors (either inherent skeletal weakness or lower bone mineralisation), similar lifestyle, or food choices may contribute to their vulnerability to fractures (Goulding, Jones et al. 2005).

In line with other studies (Cook, Harding et al. 1987, Ma and Jones 2002, Cvijetic, Baric et al. 2003) a significant difference in SI between children with and without a fracture history was not found, which is in contrast with previous studies (Landin and Nilsson 1983, Chan, Hess et al. 1984, Goulding, Cannan et al. 1998, Goulding, Jones et al. 2001, Ma and Jones 2003, Schalamon, Singer et al. 2004, Clark, Ness et al. 2006). These contradictory results may be because of different instruments measuring bone density. Also, we found a moderate ($r = 0.4$) degree of correlation between QUS SI measurements and DXA results so a lack of association in our population may relate to these results as revealed in chapter five.

The prevalence of fracture was higher in boys with high %BF compared to boys with normal %BF (33.0% vs. 19.8%) and 3.1 times greater odds of having had a fracture. Our findings are in agreement with previous studies showing that high body mass index is a risk factor for fracture in children and adolescents (Goulding, Jones et al. 2000, Goulding, Jones et al. 2001, Goulding, Grant et al. 2005, Kim, Hsieh et al. 2013). Different theories have been suggested by other authors for the increased risk of fracture in children with obesity. Cheng et al. (2016) concluded that children with obesity have poor musculoskeletal control, less efficient postural stability, and obesity preceded decreases in motor skills, therefore they have a higher tendency for falls with injuries or fractures. In overweight and children with obesity, the strength of bone adjusts primarily to local mechanical strain from muscle forces rather than static loads represented by body weight (Petit, Beck et al. 2005). However, this bone strength is inadequate to overcome the greater forces from high body weight when an obese child falls (Petit, Beck et al. 2005). Moreover, it has been shown that overweight and children with obesity have lower bone mass

and bone area relative to their body weight (Goulding, Taylor et al. 2000). At the growth spurt, bone density decreases because bone size increases more quickly than its mineralisation (Magarey, Boulton et al. 1999). In overweight and children with obesity, this mismatch between weight and bone mineral deposition can lead to fragile bones and increase their susceptibility for repeated fractures (Goulding, Taylor et al. 2000). This raises concern as obesity rates are increasing among NZ children and adolescents, especially in some ethnic groups. The New Zealand Health Survey 2018/19 (Ministry of Health 2019) found that around one in nine (11.3%) children aged 2-14 years old were obese and following their findings our results showed that Pacific children had the highest %BF (25.2 ± 9.4), followed by Māori (23.9 ± 7.6), South Asian (23.4 ± 8.4), and NZ European children who had the lowest %BF (19.5 ± 6.6).

If children want to achieve their genetically potential PBM, adequate dietary calcium is crucial to satisfy the needs of the skeleton (Matkovic and Heaney 1992). Children should be encouraged to consume a diet with adequate protein, energy, and essential nutrients (e.g. calcium, vitamin D) to maintain their muscle mass and bone health. However, some children chronically avoid drinking milk either because they show adverse reactions to milk (lactose intolerance or milk protein allergy) (Caffarelli, Baldi et al. 2010, Prentice 2014) or they simply do not like the taste or it is a lifestyle choice (Black, Williams et al. 2002). Usually, individuals who avoid intake of milk without compensatory nutritional modification receive inadequate important nutrients in their diet and consequently have an increased fracture risk (Goulding 2007). In line with previous studies (Black, Williams et al. 2002, Goulding, Rockell et al. 2004), the present findings showed that 35.3% of children who avoided milk reported a fracture compared with children who did drink milk (25.4%). The importance of milk and dairy products on bone health and bone mass acquisition during childhood is related to their containing minerals especially calcium and phosphorus, which are essential for forming the inorganic bone matrix, and potassium which indirectly regulates bone turnover (Ministry of Health 2012), and bone anabolic growth factors (e.g. osteoprotegerin) (Kanezler, Bodamyali et al. 2001). The basic protein fraction of whey has been found to increase bone formation and decrease markers of bone resorption (Jesudason and Clifton 2011). Milk also contains an abundant glycoprotein called lactoferrin, which can mediate

bone resorption through the promotion of osteoblast proliferation and differentiation and osteoclast inhibition (Cornish 2004, Cornish, Callon et al. 2004, Amini and Nair 2011). Therefore, monitoring children who avoid drinking milk without compensatory nutritional changes (e.g. increasing intake of other calcium-rich foods and/or consuming calcium supplements) is important to ensure they are not jeopardising their growth and bone health.

In New Zealand, most of the dietary calcium intake is derived from the consumption of milk and dairy products (e.g. cheese, ice cream, and yoghurt) (Ministry of Health 2012). Therefore, consuming 2 to 3 servings of milk and dairy products per day is recommended to help achieve the required daily intake (Ministry of Health 2012). However, similar to the New Zealand Ministry of Health results (Ministry of Health 2012), many of our study children consumed less dietary calcium than the recommendation (total calcium intake < 800 mg/day = 68.5%), and current calcium intake was generally low (683.2 ± 341.0 mg/day). Alshamrani et al. (2019) conducted a cross-sectional study of children 6-15 years old. Their results showed that 98% of their population did not meet the daily dietary recommendation for calcium intake and low levels of calcium consumption were significantly associated with an increase in fracture risk. In the current study, low calcium intake was a predictor of fracture in boys and the prevalence of fracture was higher in boys who consumed < 800 mg/day calcium compared with those who consumed ≥ 800 mg/day (33.9% vs. 18.7%). Just before the growth spurt, bone skeletal calcium requirements for bone modelling and remodelling are high thus it is suggested children must be in positive calcium balance (Matkovic 1992). Low dietary calcium intakes could cause increased bone resorption to fulfil an increased demand for calcium during rapid growth and consequently leading to bone fragility (Parfitt 1994).

Studies in children and adolescents suggest that consumption of SSB has an adverse effect on bone growth and density (McGartland, Robson et al. 2003, Libuda, Alexy et al. 2008), specifically cola due to its phosphoric acid content (Wyshak 2000, Whiting, Healey et al. 2001). In the current study, and in line with a previous study (Ma and Jones 2004) our results showed the consumption of SSB ≥ 1 serving/day increased the odds of fracture in boys 2.0 times and in girls 4.6 times more than children who consumed < 1 serving/day. Some mechanisms have been postulated to

explain this relationship. Sugar-sweetened beverages contain phosphoric acid, which generates acid load and consequently increase calcium excretion (Doumid Borges Pretto, Correa Kaufmann et al. 2014). Also, SSB phosphoric acid decreases 25-hydroxyvitamin D conversion to 1,25-hydroxyvitamin D through inhibition of 1α-hydroxylase and finally may increase bone resorption (Calvo and Tucker 2013). An animal study showed that cola beverages can cause renal acid load and consequently osteoclast stimulation and therefore bone resorption, which leads to lower BMD (Ogur, Uysal et al. 2007). Moreover, caffeine in cola drinks seems to affect the mechanical properties of bone (Ohta, Ide et al. 2002).

Also, SSB consumption can result in the displacement of milk in a diet. A small but significant inverse correlation was found between dairy consumption and SSB ($r = -0.111$, $P < 0.01$) and milk consumption and SSB ($r = -0.078$, $P < 0.05$). Pacific children have the highest consumption of SSB compared with other ethnic groups (1.1 ± 1.2 servings/day), similar to the Ministry of Health data (Ministry of Health 2003). Based on the New Zealand Ministry of Health recommendations, consumption of high sugar foods and drinks needs to be limited to less than once a week (Ministry of Health 2012). In the United States, milk consumption has been shown to decrease and intake of sweetened beverages increases with age in 3-7 years old children for three years (Keller, Kirzner et al. 2009). The exact reasons for this behaviour are not known. However, it is postulated that parental influence, television advertisements, increasing age and independence, and availability at school are some reasons for this substitution. This shift had a negative impact on children's and adolescents' bone health and may contribute to increased risk of bone fractures (Wyshak and Frisch 1994, Petridou, Karpathios et al. 1997, Wyshak 2000) by decreasing calcium intake due to replacement of milk in the diet with sweetened beverages, which are high in caffeine, phosphoric acid and/or sugar (Kinney 2002, Fitzpatrick and Heaney 2003).

In our study, Māori children have the second-highest fracture rate (32.5%) and a large percentage of them participated in vigorous physical activity, presumably leading to an increased risk of injuries. During different stages of life, physical activity has been recommended since it is a preventive and curative factor of bone density loss and consequently osteoporosis later in life (Doumid Borges Pretto, Correa Kaufmann et al. 2014). Randomised controlled trials results

suggested that bone density increases in children who are involved in physical activity (Nichols, Sanborn et al. 2001, Johannsen, Binkley et al. 2003, MacKelvie, Khan et al. 2003, Caulton, Ward et al. 2004), and it is in line with previous study results that light physical activity acts as a protective and decreases fracture risk in children (Ma and Jones 2003). However, the type and intensity of physical activity are important. Weight-bearing physical activity with a minimum degree of trauma stimulates bone remodelling and thus increases bone density and reduces fracture risk (Ma and Jones 2003), while participation in strenuous physical activity or high-risk sports probably not only increases bone density but also the number of injuries and consequently the fracture risk (Ma and Jones 2003, Clark, Ness et al. 2008). Children need to be encouraged to undertake regular physical activity to develop their motor skills and coordination and improve their balance and prevent falls, which may predispose them to fractures (Goulding 2007).

6.4.2 Causes and Consequences of Vitamin D Deficiency in Children

Little is known about the vitamin D status of the New Zealand children, a country with minimal vitamin D fortification in food. In this work, late-winter 25(OH)D concentrations were lower than 50 nmol/L in approximately one-third of our participants, and one-third of the study population had 25(OH)D concentrations ≥ 75 nmol/L. Our results showed boys with 25(OH)D < 75 nmol/L had a higher percentage of fracture (34.3%) compared to boys with 25(OH)D ≥ 75 nmol/L, and 25(OH)D < 75 nmol/L was one of the predictors of fractures in this group as highlighted in chapter three. Also, our results revealed that 25(OH)D was significantly correlated with being overweight and obese, being from South Asian ethnicity, having darker skin colour, and exposing less surface of the body to sunlight. None of our participants used vitamin D supplementation. The strongest predictors of 25(OH)D < 50 nmol/L were %BF and ethnicity as highlighted in chapter four.

Based on the Endocrine Society (Holick, Binkley et al. 2011) and the Society for Adolescent Health and Medicine (Society for Adolescent Health and Medicine 2013), 25(OH)D less than 75 nmol/L is insufficient. Vitamin D regulates serum calcium and phosphate homeostasis (Lieben, Carmeliet et al. 2011). It also has important roles in bone metabolism through osteoclast formation, and mobilisation of calcium from the bone stores into the blood circulation (DeLuca 2004). In vitamin D insufficiency, the ability to absorb calcium from the intestine decreases,

which can lead to impaired bone mineralisation (Lips 2001). Therefore, optimal vitamin D status is necessary for maximising bone mineralisation and minimising fracture risk (Laird, Ward et al. 2010). For instance, Thompson et al. (2017) recruited 120 children, 2-14 years old to investigate vitamin D's role in children's fracture risk. They found an association between lower concentration of vitamin D and higher fracture risk. Saglam et al. (2017) confirmed that the prevalence of vitamin D deficiency or insufficiency was higher in children with distal radius fractures compared with healthy children. Ryan et al. (2012) supported the association between vitamin D deficiency and an increased odds of forearm fracture in African American children. Therefore, optimal vitamin D status is necessary for maximising bone mineralisation and minimising of fracture risk (Laird, Ward et al. 2010).

There is evidence that suggests that obesity in children is associated with lower 25(OH)D concentrations (Rockell, Green et al. 2005, Alemzadeh, Kichler et al. 2008, Mark, Gray-Donald et al. 2008, Kumar, Muntner et al. 2009, Turer et al. 2013) but not in all studies (Stein, Laing et al. 2006, Weng, Shults et al. 2007). Our results showed an inverse correlation between weight, BMI, and %BF and vitamin D status, and %BF was one of the predictors of 25(OH)D < 50 nmol/L. This association could be explained by some potential pathways. Vitamin D is a fat-soluble vitamin and can be sequestered in adipose tissue and therefore its bioavailability decreases (Wortsman, Matsuoka et al. 2000, Arunabh, Pollack et al. 2003). In addition, children with obesity may have a more sedentary and indoor lifestyle, and consequently less sun exposure.

Results from the National Nutrition Survey of New Zealand children (aged 5-14 years) in 2002 showed the mean concentration of 50 nmol/L, with a high prevalence of 25(OH)D insufficiency (defined as < 37.5 nmol/L) at 41%, 59%, and 25% among Māori, Pacific, and New Zealand European school-age children, respectively (Rockell, Green et al. 2005). Our findings showed that 11.5% of New Zealand European, 31.3% of Māori, and 34.3% of Pacific children had 25(OH)D less than 50 nmol/L. In our population, New Zealand European and South Asian children had the highest (75.0 nmol/L) and lowest (49.2 nmol/L) 25(OH)D concentrations, respectively. Also, South Asian children had the highest odds of 25(OH)D less than 50 nmol/L compared to the New Zealand European group. A review by Akhtar (2016) reported that vitamin

D deficiency (20-50 nmol/L) is highly prevalent among the South Asian population. A possible explanation is skin colour. More than half (57.4%) of South Asian children and about half (47.2%) of Pacific children in our study selected 'dark/brown' skin colour. One-third (35.9%) of Māori children reported having 'olive' skin colour while most New Zealand European children (56.5%) reported 'medium' skin colour. The role of skin pigmentation in the cutaneous synthesis of vitamin D has been demonstrated in previous studies conducted in New Zealand (Rockell, Green et al. 2005, Rockell, Skeaff et al. 2008, von Hurst, Stonehouse et al. 2010). Skin pigmentation hinders the ability of the skin to synthesise vitamin D. The melanin in the skin acts as a sunscreen, therefore the ultraviolet radiation beta (UVβ) photons are less absorbed through the skin (Ministry of Health and Cancer Society of New Zealand 2012). Thus children with darker skin colour require longer exposure to sunlight (5-10 times longer) compared to those with lighter skin to produce adequate amounts of vitamin D through their skin.

An effective strategy is necessary to prevent 25(OH)D concentrations less than 50 nmol/L and the related health consequences among children especially in high-risk children (overweight/obese, darker skin colour, specific ethnic groups). In New Zealand, increasing sun exposure should be the first-line treatment since only a small quantity of vitamin D comes from a limited number of foods that naturally contain vitamin D (salmon, herring, tuna, and mackerel) and there is a limited range of foods including milk and yoghurt, which are sometimes fortified with vitamin D (Ministry of Health and Cancer Society of New Zealand 2012). Therefore, it would be hard to reach acceptable blood concentrations of vitamin D through diet alone. Through adequate skin sunlight exposure, our body can synthesise sufficient vitamin D (Holick 2012). Unfortunately, there is no evidence regarding the safe threshold level of ultraviolet (UV) radiation exposure from the sun without increasing the risk of skin cancer (Ministry of Health and Cancer Society of New Zealand 2012). Therefore, children should be advised to have a balance between avoiding excessive sun exposure and preventing skin cancer, and having enough sun exposure to achieve adequate vitamin D concentration (Ministry of Health and Cancer Society of New Zealand 2012). For instance, a daily walk or some other form of outdoor physical activity in the early morning or late afternoon during summer months (September and April) and direct exposure

to sunlight between May and August (winter in New Zealand), especially in the hours around noon when ultraviolet radiation beta (UVβ) levels are the highest, is recommended by the New Zealand Ministry of Health for adequate cutaneous production of vitamin D (Ministry of Health and Cancer Society of New Zealand 2012).

6.4.3 Measuring Bone Density and Body Composition in Children

In adults, DXA is a widely accepted method for detecting osteoporosis (Gonnelli, Caffarelli et al. 2008). The advantages of DXA in adults include accuracy, reproducibility, validity, and low radiation dose (Binkovitz, Henwood et al. 2007). Nevertheless, in a paediatric population, the effect of puberty on bone density makes DXA data interpretation difficult in children (Leonard, Propert et al. 1999) therefore, it is not suitable for routine paediatric research practice and is impractical for use in the field. Also, there are concerns about exposing children unnecessarily to radiation. Quantitative ultrasound has been developed as a proxy for DXA since it is a low cost, easy to use, transportable, non-invasive, and a radiation-free method (Baroncelli 2008). Moreover, the parameters of QUS (SOS, BUA, and SI) can assess bone properties other than BMD like structural information, elasticity, and thickness, which may be important in determining fracture risk (Genant, Engelke et al. 1996, Fielding, Nix et al. 2003). In adults, QUS has been approved as a technique for screening osteoporosis since it can predict fracture risk in postmenopausal women (Fielding, Nix et al. 2003, Hartman, Brik et al. 2004). However, data regarding the diagnostic accuracy of QUS in the paediatric population is sparse. A recent systematic review investigated the diagnostic accuracy of QUS for the assessment of osteoporosis in children and adolescents and the authors concluded that although this technique provides reliable measurements, there is not enough evidence to support the diagnostic value of QUS (Wang, Wang et al. 2014). In the current study, the fair to moderate agreement between the QUS and DXA further supports this conclusion. While other studies observed stronger correlations (0.71-0.88) (Lum, Wang et al. 1999) and another systematic review in women over 45 years concluded that calcaneal QUS has the potential to be used as a pre-screening tool for assessment of osteoporosis (Thomsen, Jepsen et al. 2015), it seems that further investigation is required to establish QUS clinical use in children.

Body composition can be measured by various methods, which differ in their accuracy, feasibility, cost, and accessibility. Some of these methods are not appropriate for young children (e.g., underwater weighing, isotope dilution), whereas others are limited due to cost and accessibility (e.g. DXA). Compared to these methods BIA is a relatively simple non-invasive field method (Houtkooper, Lohman et al. 1989). Our data confirm BIA is a valid and useful tool to assess body composition in children in epidemiologic and field studies. The simplicity of use of the BIA device, portability, lack of radiation exposure for the child, and low cost represents the clear advantage of the BIA method compared to DXA.

6.5 Limitations

Fracture information and vitamin D risk factors were collected through self-reported questionnaires, so recall bias could have influenced our results. Previous studies have shown there is a relationship between preterm birth (Michaud, Luu et al. 2020, Xie, Alos et al. 2019) or being breastfed (Blanco, Burrows et al 2017, Handel and Heitmann 2015, Muniz, Menezes et al 2015, Jones, Riley et al. 2000) and fracture during childhood. However, we did not ask children's weight at their birth or the methods that they fed when they were infants. Using children's medical history through hospitals such as X-ray information is suggested. Also in our study, a short questionnaire for estimation of dietary calcium intakes was used, which may not be valid in a New Zealand population. A validated calcium FFQ with more details is needed. The DBS method was used for measuring vitamin D. Although this method was used in previous studies, it has not been specifically validated in children. A study for the validation DBS technique in this age group is recommended. Moreover, information about vitamin D status was collected at the end of winter, therefore, we lacked a longitudinal assessment of vitamin D status and could not consider the effect of seasonal variation on vitamin D status.

6.6 Final Conclusions

In general, normal activities should not easily cause fracture of healthy and strong bones. It is impossible to prevent all accidents and therefore some fractures will be unavoidable, however, we should do our best to decrease childhood fracture rates. Approximately one-quarter of our

participants reported one or more fractures during their short lifetime. Being South Asian was a significant risk factor for fractures in boys. Supplementation may be necessary for some children who had vitamin D deficiency, especially in wintertime. During growth and development, children should be encouraged to consume a good diet (especially good sources of calcium and reducing SSB intakes) to reduce their risk of fractures and maintain a healthy %BF.

Every child in New Zealand is susceptible to vitamin D insufficiency and skeletal health problems unless they have adequate sunlight exposure or consume vitamin D supplements. This condition is more pronounced in some groups such as for overweight and obese children, South Asian ethnicity, darker skin colour, and less exposure of the body to sunlight. Poor vitamin D status during childhood can cause undesirable skeletal and non-skeletal health outcomes, so opportunities to intervene during childhood should be pursued. A strong consideration should be given to the high-risk children encouraging activities in the sun to support the production of cutaneous vitamin D through sunlight exposure.

The current study was the first to evaluate the validity of calcaneal QUS and BIA against DXA in a paediatric New Zealand population for measuring bone density and body composition, respectively. Based on our findings, the calcaneal QUS and DXA are not interchangeable methods and DXA is the best method for diagnostic purposes in children. Moreover, although BIA results were not as accurate as DXA and DXA remains the gold standard method for clinical assessment, BIA can be an alternative method for investigating body composition among children in large cohort field studies.

6.7 Research Recommendations

1. Further research should consider the pubertal status of children, to allow an improved description of bone health and body composition of New Zealand children.
2. In the future, a study should be designed to include more samples from a high-risk population (e.g. South Asian).
3. Investigate the effect of seasonal variation on vitamin D status in primary school children.
4. A well-designed long-term intervention trial examining whether improved nutrition (e.g.

increasing milk and calcium intake, and 25(OH)D concentrations, decreasing SSB intake) and PA (e.g. having regular moderate PA) can lower future fracture rates in paediatric population.

6.8 References

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Appendices

Appendix 1. Information Sheet for Parents (no DXA information)

MASSEY UNIVERSITY

The children's bone study

Information sheet for parents

Thank you for your interest in the children's bone study. This sheet gives information on the conduct and organisation of this study, including confidentiality and data protection. It is important that you read this and are happy with the information given before agreeing to take part in the study.

Why is this research important?
Anecdotal evidence suggests that a greater number of primary school children are incurring broken bone than did their grandparents generation. We want to find out the relationships between broken bone history, bone mineral density, dietary intake of nutrients related to bone health, beverage choices and preferences, physical activity, sun exposure behaviours, and body composition of children living in Auckland.

Who are we looking for?
We are inviting around 600+ children aged 8-13 years who do not have any gastrointestinal disorder or bone disease and who are enrolled in Year 5 or 6 in Auckland primary school to take part in this study. Each child and at least one of the child's parents/guardians need to be able to read and comprehend English to a sufficient level that they can understand the information provided about the study and make an informed decision about whether or not they wish to participate.

What is going to happen?
Initially, the children will have a science lesson, facilitated by our specialist science teacher, on the topic of bone health. Classroom sessions will be arranged at times to suit teachers and teaching schedules, and will be designed to link with curriculum. All data collection from the children will take place in school. Further information will be sought from parents through 3 questionnaires, which your child will bring home, along with a consent form.

The questionnaires will have questions such as 1) ethnicity, 2) fracture history, 3) skin exposure to sunlight, 4) physical activity and type of sports your child plays, 5) your child's milk/dairy product and sweetened beverages consumption.
These questionnaires will take approximately twenty minutes to complete. You will only need to complete these questionnaires once.

All data collection from the children will take place in school. The children will have measurements made to determine their body composition (level of muscle and fat), and a finger prick blood spot to measure vitamin D concentration. We will also measure the bone density of their heel bone using the quantitative ultrasound (QUS).

Height measurement

We will ask your child to remove his/her shoes due to measuring his/her height using a stadiometer.

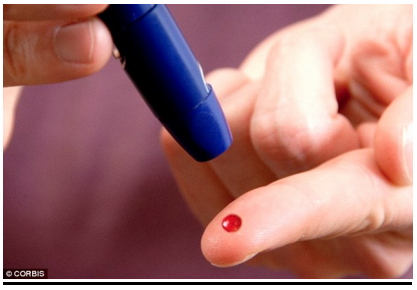
BIA (see picture)

BIA is a method for measuring body composition. This machine is used to tell us how much fat and muscle mass tissue your child has on his/her body. This test will take only a few minutes and won't hurt at all. Your child will need to take off his/her shoes and socks and then stands on the machine's scale and holds two handles for few minutes (like the picture). These numbers will be used in an equation to estimate the amount of fat and lean tissue on your body

Machine Show

Finger prick blood spot

We will ask children to wash their hands with warm water. Then they will ask to shake their hand and choose a finger for finger prick. Then we will prick the finger with a lancet and collect a full drop of blood.



Quantitative Ultrasound (QUS)

Quantitative ultrasound is radiation-free technique for providing a proxy for bone mineral density by determining how rapidly sound travels through the tissue and how different sonic wavelengths are absorbed. QUS is used to diagnose osteopenia and osteoporosis. We will ask your child to bare one of his/her legs (no shoes or socks) and then put his/her heel into the machine for less than one minute.

Who will see the information about your child?
All information about your child will be stored in a locked filing cabinet accessed by the research team only. No names or any other information that could be used to identify your child will be used in any publication.

We are required to keep any data that may be medically relevant for your child in the future for ten years. All electronic data will be stored password-protected on the University's secure server. For the first 5 years we will store any paper copies of data in a locked filing cabinet within a locked office. For the remainder of the time, data will be stored in a secure archive in boxes labelled by barcode only. This data will be accessible by nominated staff only. After the mandatory storage time has passed, all data filed on paper will be shredded and electronic data will be deleted from our computer records and databases.

Would your child like to take part?
If "YES"
If your child would like to take part in this study and you are happy for them to do so, please sign the attached consent form and ask your child to return it to their teacher.

If "YES" to your child taking part, but NO to the blood spot, do not tick the box on the consent form giving permission for the blood spot.

If "NO"
If you do not want your child to participate or your child does not want to take part in this study then you do not need to do anything.

Who is funding the research?
This is funded by a grant from the Massey University Research Fund.

What are my rights and the rights of my child?
We respect your rights and your child's rights to:

- refuse to answer any particular question or take part in any testing (finger prick blood spot, QUS or BIA)
- withdraw from the study at any time
- ask further questions about the study that occur to you during your participation
- provide information on the understanding that it is completely confidential to the researchers. All information is collected confidentially, and it will not be possible to identify you or your child in any reports that are prepared from the study
- be given access to a summary of the findings from the study when it is concluded.

Compensation for Injury
In the unlikely event that physical injury results from your child's participation in this study, you

should visit a treatment provider to make a claim to ACC as soon as possible. ACC cover and entitlements are not automatic and your claim will be assessed by ACC in accordance with the Compensation Act 2001. If your claim is accepted, ACC must inform you of your entitlements, and must help you access those entitlements. Entitlements may include, but not be limited to, treatment costs, travel costs for rehabilitation, loss of earnings, and/or lump sum for permanent impairment. Compensation for mental trauma may also be included, but only if this is incurred as a result of physical injury.

If your ACC claim is not accepted, you should immediately contact the researcher. The researcher will initiate processes to ensure you receive compensation equivalent to that to which you would have been entitled had ACC accepted your claim from Massey University.

If you have any question, please contact Dr Pamela von Hurst who will be happy to discuss the project in more detail.

Contact details:

Dr Pamela von Hurst
Institute of Food Nutrition and Human Health, Massey University
Email P.R.vonHurst@massey.ac.nz
Phone (09) 4140800

This project has been reviewed and approved by the Massey University Human Ethics Committee: Southern A, Application 16/42. If you have any concerns about the conduct of this research, please contact Mr Jeremy Hubbard, Chair, Massey University Human Ethics Committee: Southern A, telephone 04 801 5799 x 63487,

Appendix 2. Information Sheet for Children (no DXA information)

MASSEY UNIVERSITY

Bone Health Study

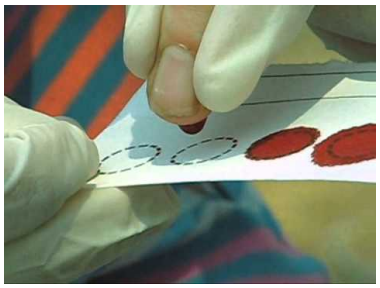
Information sheet for children

We would like to invite you to take part in a study to find out the relationships between fracture history, bone mineral density, dietary intake of nutrients related to bone health, beverage choices and preferences, physical activity, sun exposure behaviours and body composition of children living in Auckland. We want to figure out how much bones of primary children are healthy? About 600+ children are going to take part in this study. In this study we will measure your height weight, body composition, vitamin D concentration, and bone density.

Height measurement
We will ask you to remove your shoes due to measuring your height using a stadiometer.

BIA (see picture)
For measuring your body composition, we will ask you to remove your shoes and socks, and stand on the machine's scale. Then grab two handles.

Finger prick blood spot
Also we need a drop of your blood in order to determine your 25(OH)D concentration. We will ask you to wash your hands with warm water. Then you will ask to shake your hand and choose a finger for finger prick. Then we will prick the finger with a lancet and collect a full drop of blood.



Quantitative Ultra Sound (QUS)

Moreover, we will ask you to bear one of your legs (no shoe no sucks) and then put your calcaneal into the machine for less than one minute.

You do not have to take part in this study. You should only say yes if you want to. If you say yes now but change your mind later, you don't have to keep doing the study. No one will be cross with you if you don't want to do the study. You should talk to your family/whanau to help you decide. You can also ask us anything you want at any time.
If you want to take part in this study, please fill in the bottom of this form. (You do not have to decide now. You should think about it and talk to your family/whanau). If you do not want to take part, you do not need to do anything.

I want to take part in the children's bone study.
My name is ……

Appendix 3. Parent and Children Consent Form

MASSEY UNIVERSITY

The children's bone study

Parents and children consent form

I have read the Information Sheet and have had the details of the study explained to me. My questions have been answered to my satisfaction, and I understand that I may ask further questions at any time.

☐ I agree for my child to participate in this study including the finger prick blood spot under the conditions set out in the Information Sheet.

☐ I agree for my child to participate in this study but not the finger prick blood spot under the conditions set out in the Information Sheet.

Signature: ..... **Date:** .....

Full Name (printed) .....

Child's Full Name .....

Child's date of birth .....

Any allergies and/or medication .....

☐ I agree to participate in this study including the finger prick blood spot under the conditions set out in the Information Sheet.

☐ I agree to participate in this study but not the finger prick blood spot under the conditions set out in the Information Sheet.

Signature: ..... **Date:** .....

Child's Full Name

Child's date of birth

Any allergies and/or medication

Appendix 4. Information Sheet for Parents (with DXA information)

MASSEY UNIVERSITY

The children's bone study

Information sheet for parents

Thank you for your interest in the children's bone study. This sheet gives information on the conduct and organisation of this study, including confidentiality and data protection. It is important that you read this and are happy with the information given before agreeing to take part in the study.

Why is this research important?
Anecdotal evidence suggests that a greater number of primary school children are incurring broken bone than did their grandparents generation. We want to find out the relationships between broken bone history, bone mineral density, dietary intake of nutrients related to bone health, beverage choices and preferences, physical activity, sun exposure behaviours and body composition of children living in Auckland.

Who are we looking for?
We are inviting around 128 children who do not have any gastrointestinal disorder or bone disease and who are enrolled in Auckland primary school to take part in this study. Each child and at least one of the child's parents/guardians need to be able to read and comprehend English to a sufficient level that they can understand the information provided about the study and make an informed decision about whether or not they wish to participate.

What is going to happen?
Initially, the children will have a science lesson, facilitated by our specialist science teacher, on the topic of bone health. Classroom sessions will be arranged at times to suit teachers and teaching schedules, and will be designed to link with curriculum. Children will come to Massey, Albany for a DXA scan and the other data collection.

The children will have measurements made to determine their body composition (level of muscle and fat). We will also measure the bone density of their heel bone using the quantitative ultrasound (QUS).

Your child will come to the Massey University campus in Albany as part of a school trip. As well as participating in the study, they will also visit some of our research laboratories and have the opportunity to do some science experiments.

Height measurement
We will ask your child to remove his/her shoes due to measuring his/her height using a stadiometer.

BIA

BIA is a method for measuring body composition. This machine is used to tell us how much fat and muscle mass tissue your child has on his/her body. This test will take only a few minutes and won't hurt at all. Your child will need to take off his/her shoes and socks and then stands on the machine's scale and holds two handles for few minutes (like the picture). These numbers will be used in an equation to estimate the amount of fat and lean tissue on your body

DXA scan

We will ask your child to bring his/her sport gear (T-shirt and elastic short, without any metal), and wear them during DXA examination. Your child will then lie on a bed under our DXA scanner as shown in the picture. DXA scanning is a form of X-ray so does involve exposure to a very low dose of radiation. The radiation exposure from DXA is much lower than that received from other medical devices. It is about five times lower than the level a person is exposed to during a dental X-ray. The scanning procedure is completely painless. Your child will be given a picture of their own skeleton which is produced by the DXA to take home. The room is private and your child can enter the DXA room in complete privacy. Staff members who are certificated to operate the DXA will perform the scans. The scans will be assessed and approved by our consultant Radiologist. Should any abnormalities be found in your child's scans, the Radiologist would advise you and refer your child for treatment if required.

This is a picture of a person having their bone density measured by a DXA machine.

Quantitative Ultrasound (QUS)

Quantitative ultrasound is radiation-free technique for providing a proxy for bone mineral density by determining how rapidly sound travels through the tissue and how different sonic wavelengths are absorbed. QUS is used to diagnose osteopenia and osteoporosis. We will ask your child to bare one of his/her legs (no shoes or socks) and then put his/her heel into the machine for less than one minute.

Who will see the information about your child?
All information about your child will be stored in a locked filing cabinet accessed by the research team only. No names or any other information that could be used to identify your child will be used in any publication.

We are required to keep any data that may be medically relevant for your child in the future for ten years. All electronic data will be stored password-protected on the University's secure server. For the first 5 years we will store any paper copies of data in a locked filing cabinet within a locked office. For the remainder of the time, data will be stored in a secure archive in boxes labelled by barcode only. This data will be accessible by nominated staff only. After the mandatory storage time has passed, all data filed on paper will be shredded and electronic data will be deleted from our computer records and databases.

Would your child like to take part?
If "YES"
If your child would like to take part in this study and you are happy for them to do so, please sign the attached consent form and ask your child to return it to their teacher.

If "YES" to your child taking part, but NO to the blood spot, do not tick the box on the consent form giving permission for the blood spot.

If "NO"
If you do not want your child to participate or your child does not want to take part in this study then you do not need to do anything.

What are the benefits and risks of taking part in this study?

- You will receive a brief report summarising the main findings of the project via mail or email.
- The principal benefit of taking part in this study is that you will contribute to a study and our understanding of bone mineral density and body composition of children.
- It is not envisaged that there will be any discomforts or risks to the participants as a result of participation.

- If you have any specific requirements including cultural requirements or concerns about the project, or about being a participant, please contact a member of the research team to discuss.

Who is funding the research?
This is funded by a grant from the Massey University Research Fund.

What are my rights and the rights of my child?
We respect your rights and your child's rights to:

- refuse to answer any particular question or take part in any testing (DXA, QUS or BIA)
- withdraw from the study at any time
- ask further questions about the study that occur to you during your participation
- provide information on the understanding that it is completely confidential to the researchers. All information is collected confidentially, and it will not be possible to identify you or your child in any reports that are prepared from the study
- be given access to a summary of the findings from the study when it is concluded.

Compensation for Injury
In the unlikely event that physical injury results from your child's participation in this study, you should visit a treatment provider to make a claim to ACC as soon as possible. ACC cover and entitlements are not automatic and your claim will be assessed by ACC in accordance with the Compensation Act 2001. If your claim is accepted, ACC must inform you of your entitlements, and must help you access those entitlements. Entitlements may include, but not be limited to, treatment costs, travel costs for rehabilitation, loss of earnings, and/or lump sum for permanent impairment. Compensation for mental trauma may also be included, but only if this is incurred as a result of physical injury.

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If you have any questions please contact Dr Pamela von Hurst who will be happy to discuss the project in more detail.

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Appendix 5. Information Sheet for Children (with DXA information)

MASSEY UNIVERSITY

Bone health study
Information sheet for children

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About 128 children are going to take part in this study. You will come to the Massey University campus in Albany as part of a school trip. As well as participating in the study, you will also visit some of our research laboratories and have the opportunity to do some science experiments. In this study we will measure your height, body composition, and bone density.

Height measurement
We will ask you to remove your shoes due to measuring your height using a stadiometer.

BIA (see picture)
For measuring your body composition, we will ask you to remove your shoes and socks, and stand on the machine's scale. Then grab two handles.

Quantitative Ultrasound (QUS)

Moreover, we will ask you to bear one of your legs (no shoe no sucks) and then put your calcaneal into the machine for less than one minute.

DXA scan

You need to bring your own sport gear (T-shirt and elastic short, without any metal), and wear them during DXA examination. You will lie on a bed under our DXA scanner as shown in the picture on the next page. It does not hurt and you can keep the picture. The room is private and you can enter the DXA room in complete privacy. Staff members who are certificated to operate the DXA will perform the scans. Here is a photo showing someone having her bones photographed.

You do not have to take part in this study. You should only say yes if you want to. If you say yes now but change your mind later, you don't have to keep doing the study. No one will be cross with you if you don't want to do the study. You should talk to your family/whanau to help you decide. You can also ask us anything you want at any time.

If you want to take part in this study, please fill in the bottom of this form. (You do not have to decide now. You should think about it and talk to your family/whanau). If you do not want to take part, you do not need to do anything.

I want to take part in the children's bone study.

My name is ……

Appendix 6. Demographic Questionnaire

Date…..

The Children's Bone Study

To be completed by parent or guardian

Thank you for participating in this study, if you have any questions please feel free to discuss them with the researcher.

Principal Investigator:
Dr Pamela von Hurst, School of Food and Nutrition, Massey University
Email: p.r.vonhurst@massey.ac.nz

All information you provide will remain strictly confidential

Participant Demographics

First Name of your child

.....

Family Name of your child

.....

Date of birth of your child

.....

Address

.....

.....

Phone (home)

.....

Phone (mobile)

.....

Email

.....

Which ethnic group or groups does your child belong to? (Please √ all that apply)

New Zealand European	□	
Maori	□	
Pacific	□	Please specify _____
South Asian	□	
Chinese	□	
Korean	□	
Southeast Asian	□	Please specify _____
Other ethnicity	□	Please specify _____

How would you describe your child skin colour? (Please √ one)

☐	Fair	Easily burns in the sun, doesn't tan
☐	Medium	Can burn, but tans after some sun exposure
☐	Olive	Rarely gets sunburnt, becomes quite tanned in summer
☐	Brown	Light to medium brown, very rarely gets sunburnt
☐	Dark	Very dark brown, never gets sunburnt

Is your child taking any medication or supplements? Please list

.....

.....

.....

Does your child have any chronic illness (for example, asthma) or food allergy? Please list

.....

.....

.....

Participant fracture history

Has your child ever been diagnosed with any bone fracture (broken bone)?
□ Yes (Please put the details in the table)

□ No (go to next page)

Which bone For instance: upper right arm, lower left leg	Age when it happened	How did it happen For instance: Fell out of a tree, fell off skateboard, was doing a cartwheel
1.		
2.		
3.		
4.		
5.		
6.		

Does your child have brothers and/or sisters who have also had a bone fracture?
□ Yes □ No or □ No siblings

A large part of how strong our bones are is determined by our genes. Therefore, family history provides important information about the health of your child's bones.
Do any other family members have a history of broken bones or osteoporosis?

Sun light exposure

How many hours each day does your child usually spend outside in summer?
During school time.....
During weekends and holidays .....

Which part of his/her body is usually exposed to the sunlight?
□ Only face

□ Only arms

□ Face and arms

□ Only legs

□ Arms and legs

□ Face, arms, and legs

Does he/she use sunscreen cream?
□ Yes - all year round □ Yes - only in summer

□ No (go to next page)

If "Yes" how often does he/she use it?
□ Always

□ Some times

□ Rarely

□ Never

To which part of his/her body does he/she apply sunscreen?
□ Only face

□ Only arms

□ Face and arms

□ Only legs

□ Arms and legs

□ Face, arms, and leg

Physical activity levels

Does your child walk to school?
□ Yes - approximately how far, or how long does the walk take?
……
□ No

Does your child play sport or some other kind of physical activity like dance?
□ Yes
□ No

What kind of activity?
.....
.....
How many times each week does he/she do this activity?
.....
.....
How many hours is he/she active for each time?
.....
.....

Anything else you would like to tell us about your child?

……
……
……

……
……
……

Appendix 7. Food Frequency Questionnaire

Children's Bone Study
Food frequency questionnaire

Complete this questionnaire with help from your Mum, Dad or other family member.
Put a tick in the box, which best tells HOW OFTEN you eat the food or drinks listed below

1. Milk (not flavoured) – one glass serving size

Never or less than once a month	1-3 times a month	1-2 times a week	3-4 times a week	5-6 times a week	Once a day	2 or more times a day

2. Flavoured milk – one glass serving size

Never or less than once a month	1-3 times a month	1-2 times a week	3-4 times a week	5-6 times a week	Once a day	2 or more times a day

3. Milk shake or milk drink e.g. Up and Go

Never or less than once a month	1-3 times a month	1-2 times a week	3-4 times a week	5-6 times a week	Once a day	2 or more times a day

4. Yoghurt or dairy food dessert – one pot or one cup serving size

Never or less than once a month	1-3 times a month	1-2 times a week	3-4 times a week	5-6 times a week	Once a day	2 or more times a day

5. Ice cream – one cup serving size

Never or less than once a month	1-3 times a month	1-2 times a week	3-4 times a week	5-6 times a week	Once a day	2 or more times a day

6. Fruit Smoothie e.g. Simply Squeezed, Meadow Fresh Yoghurt Smoothie

Never or less than once a month	1-3 times a month	1-2 times a week	3-4 times a week	5-6 times a week	Once a day	2 or more times a day

7. Cottage cheese – half a cup serving size

Never or less than once a month	1-3 times a month	1-2 times a week	3-4 times a week	5-6 times a week	Once a day	2 or more times a day

8. Broccoli – half a cup serving size

Never or less than once a month	1-3 times a month	1-2 times a week	3-4 times a week	5-6 times a week	Once a day	2 or more times a day

9. Tofu – half a cup serving size

Never or less than once a month	1-3 times a month	1-2 times a week	3-4 times a week	5-6 times a week	Once a day	2 or more times a day

10. Bread – two slices serving size

Never or less than once a month	1-3 times a month	1-2 times a week	3-4 times a week	5-6 times a week	Once a day	2 or more times a day

11. Juice e.g. fresh orange juice, juices such as McCoys, Robinsons, Keri

Never or less than once a month	1-3 times a month	1-2 times a week	3-4 times a week	5-6 times a week	Once a day	2 or more times a day

12. Powdered fruit drink e.g. Refresh, Raro

Never or less than once a month	1-3 times a month	1-2 times a week	3-4 times a week	5-6 times a week	Once a day	2 or more times a day

13. Fruit drink from concentrate or cordial e.g. Just Juice, Ribena

Never or less than once a month	1-3 times a month	1-2 times a week	3-4 times a week	5-6 times a week	Once a day	2 or more times a day

14. Standard soft drinks or other fizzy drinks e.g. Coke, Lemonade, Fanta, Mountain Dew

Never or less than once a month	1-3 times a month	1-2 times a week	3-4 times a week	5-6 times a week	Once a day	2 or more times a day

15. Soda stream

Never or less than once a month	1-3 times a month	1-2 times a week	3-4 times a week	5-6 times a week	Once a day	2 or more times a day

16. Diet drinks / Artificially sweetened drinks e.g. Diet Coke, Coke Zero

Never or less than once a month	1-3 times a month	1-2 times a week	3-4 times a week	5-6 times a week	Once a day	2 or more times a day

17. "Other drinks" group. If you often have another drink that is not listed, give the name and tick how often you have it.
Drink _____

Never or less than once a month	1-3 times a month	1-2 times a week	3-4 times a week	5-6 times a week	Once a day	2 or more times a day

Appendix 8. Statement of Contribution Doctorate with Publications

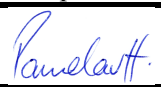
MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION DOCTORATE WITH

PUBLICATIONS/MANUSCRIPTS

We, the candidate and the candidate's Primary Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of candidate:	Maryam Delshad Siyahkaly
Name/title of Primary Supervisor:	A/P Pamela von Hurst
In which chapter is the manuscript /published work: Chapter Three	
Please select one of the following three options: The manuscript/published work is published • Please provide the full reference of the Research Output: Maryam Delshad, Kathryn L Beck, Cathryn A Conlon, Owen Mugridge, Marlena C Kruger, Pamela R von Hurst (2020). Fracture risk factors among children living in Auckland, New Zealand. Journal of Steroid Biochemistry and Molecular Biology 200: 105655. Describe the contribution that the candidate has made to the manuscript/published work: Responsible for all aspects of the manuscript including: conceptualisation and design of manuscript, searching the literature, data extraction, data analysis, drafting manuscript, and manuscript submission	
Candidate's Signature:	 Maryam Delshad
Date:	2 September 2020
Primary Supervisor's Signature:	
Date:	2 September 2020

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PUBLICATIONS/MANUSCRIPTS

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Name of candidate:	Maryam Delshad Siyahkaly
Name/title of Primary Supervisor:	A/P Pamela von Hurst
In which chapter is the manuscript /published work: Chapter Four	
Please select one of the following three options: The manuscript/published work is published • Please provide the full reference of the Research Output: Maryam Delshad, Kathryn L Beck, Cathryn A Conlon, Owen Mugridge, Marlena C Kruger, Berit P Jensen, Jing Ma, Pamela R von Hurst (2019). Wintertime Vitamin D status and its related risk factors among children living in Auckland, New Zealand. New Zealand Medical Journal 132(1504): 67-76. Describe the contribution that the candidate has made to the manuscript/published work: Responsible for all aspects of the manuscript including: conceptualisation and design of manuscript, searching the literature, data extraction, data analysis, drafting manuscript, and manuscript submission	
Candidate's Signature:	 Maryam Delshad
Date:	2 September 2020
Primary Supervisor's Signature:	
Date:	2 September 2020

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Name of candidate:	Maryam Delshad Siyahkaly
Name/title of Primary Supervisor:	A/P Pamela von Hurst
In which chapter is the manuscript /published work: Chapter Five	
Please select one of the following three options: The manuscript/published work is published • Please provide the full reference of the Research Output: Maryam Delshad, Kathryn L Beck, Cathryn A Conlon, Owen Mugridge, Marlena C Kruger, Pamela R von Hurst (2020). Validity of quantitative ultrasound and bioelectrical impedance analysis for measuring bone density and body composition in children. European Journal of Clinical Nutrition (online access). Describe the contribution that the candidate has made to the manuscript/published work: Responsible for all aspects of the manuscript including: conceptualisation and design of manuscript, searching the literature, data extraction, data analysis, drafting manuscript, and manuscript submission	
Candidate's Signature:	 Maryam Delshad
Date:	2 September 2020
Primary Supervisor's Signature:	
Date:	2 September 2020

This form should appear at the end of each thesis chapter/section/appendix submitted as a manuscript/ publication or collected as an appendix at the end of the thesis.

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