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The relationship between dairy intake, body composition, physical activity, and bone health among pre-pubertal children

A thesis presented in partial fulfilment of the requirements for the degree of Master of Science in Human Nutrition

at Massey University, Palmerston North, New Zealand.

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ABSTRACT

Objective: To examine the effects of dairy intake, body composition parameters, and physical activity on the bone mineral status of pre-pubertal children.

Study design: This was a cross-sectional study of 45 healthy pre-pubertal children aged 5-10 years. Total headless bone mineral content (tBMC), total headless bone mineral density (tBMD), lumbar spine bone mineral content (LS-BMC), and lumbar spine bone mineral density (LS-BMD) were measured with dual energy x-ray absorptiometry (DEXA). Dietary calcium intake was assessed using a food frequency questionnaire (FFQ) and a 3-day estimated food record. Anthropometric data was collected and a previous day physical activity recall (PDPAR) was used to measure the physical activity as metabolic equivalents of task (METs) and energy expenditure (EE). The FFQ was also validated against the 3-day estimated food record.

Results: The average daily serves of dairy consumed by children were above the recommended levels. Similarly the FFQ analysis also showed mean calcium intake of the sample to be higher than the recommended dietary intake (RDI) level. Boys had significantly higher lean body mass (LBM) than girls ($p < 0.02$). Girls on the other hand had significantly higher percent body fat (%BF) compared to boys ($p<0.0005$). Multiple linear regression analyses for the population sample showed no significant association was present between calcium or dairy intake and any bone parameters. Furthermore, calcium/dairy intake was also not significantly related to body composition, physical activity, and anthropometric variables. tBMC and tBMD did show a positive significant relationship with LBM and TFM but an inverse significant association with %BF. Average EE showed a significantly positive relationship with tBMD only. Whereas, METs were a negative significant predictor of only %BF. Validation of the FFQ showed that it overestimated daily calcium intake.

Conclusions: Calcium or dairy intakes were not significantly associated with bone health status. LBM, TFM, and %BF are important significant predictors of children's bone health. And finally, physical activity has beneficial effects upon the bone health and body fat.

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List of Abbreviations used:

%BF	Percent body fat
$1,25(OH)_2D_3$	1α,25- dihydroxyvitamin D_3
AI	Adequate intake
BMC	Bone mineral content
BMD	Bone mineral density
BMDC	Bone mineral density childhood study
BMI	Body mass index
BMP	Bone morphogenic protein
BMU	Bone multicellular unit
BRC	Bone remodelling cycle
cAMP	cyclic adenosine monophosphate
CB	Calbindin
CDC	Centres for Disease Control and Prevention
CLA	Conjugated linoleic acid
cm	Centimetre
CMPA	Cow's milk protein allergy
CT	Calcitonin
CTR	Calcitonin receptor
DEXA	Dual energy x-ray absorptiometry
DLX5	Distal-less homeobox 5
EAR	Estimated average requirement
EE	Energy expenditure
EGF	Epidermal growth factor
EPG	Epiphyseal growth plate
F	Females
FAO	Food and agriculture organization
FFQ	Food frequency questionnaire
FFSS	Fluid flow sheer stress
FGF	Fibroblast growth factor
FGFR-3	Fibroblast growth factor receptor-3
FSANZ	Food standards Australia & New Zealand
GC	Glucocorticoid
GF	Growth Factor
GH	Growth hormone
GHD	Growth hormone deficiency
GHRH	Growth hormone releasing hormone
HCl	Hydrochloric acid
IFN	Interferon
Ig-E	Immunoglobulin E
IGF-1	Insulin like growth factor-1
IL	Interleukin
IQR	Interquartile range
IU	International unit
kg	Kilogram
LBM	Lean body mass

LI	Lactose intolerance
LS-BMC	Lumbar spine bone mineral content
LS-BMD	Lumbar spine bone mineral density
M	Males
M-CSF	Macrophage colony stimulating factor
MET	Metabolic equivalent of task
mg	Milligram
MOH	Ministry of health
MRI	Magnetic resonance imaging
MSC	Mesenchymal stem cell
MSX2	msh homeobox homologue 2
MT1-MMP	molecule membrane type-1 matrix metalloproteinase
N	Number
NCN survey	National children's nutrition survey
NCP	Non collagenous protein
NCX	Na^{+}-Ca^{2+} pump
NHMRC	National health and medical research council
NO	Nitric oxide
NRV	Nutrient reference value
NZEO	New Zealand European origin
OPG	Osteoprotegerin
OSx	Osterix
PAL	Physical activity level
PAQ	Physical activity questionnaire
PBM	Peak bone mass
PDGF	Platelet derived growth factor
PDPAR	Previous day physical activity recall
PEM	Protein energy malnutrition
PGE2	Prostaglandin E-2
PMCA	Ca-ATPase pump
pQCT	Peripheral computed tomography
PTH	Parathyroid hormone
PTHrP	Parathyroid hormone receptor protein
QUS	Quantitative ultrasound
RANK	Receptor activator of nuclear factor-kB
RANK-L	Receptor activator of nuclear factor-kB ligand
RCT	Randomised controlled trial
RDA	Recommended dietary allowance
RDI	Recommended dietary intake
Runx2	Runt-related transcription factor 2
S1P	sphingolipid 1-phosphate
SAS	Statistical analysis system
SD	Standard deviation
SOST	Seclerostin
Spns2	Spinster Homologue 2
SPSS	Statistical package for social sciences
T_3	3,5,3'-L-triiodothyronine
T_4	Thyroxine

TFM	Total fat mass
TGF-β	Transforming growth factor β
TH	Thyroid hormone
TNF-α	Tumour necrosis factor α
TNF-β	Tumour necrosis factor β
TSH	Thyroid stimulating hormone
ug	Microgram
UK	United kingdom
UL	Upper level of intake
US	United states
VDR	Vitamin D receptor
VDRE	Vitamin D responsive element
VIF	Variation inflation factor
WBPA	Weight bearing physical activity
WC	Waist circumference
WHO	World health organization

CHAPTER 1: INTRODUCTION

With an increase in life expectancy over the past century, the rate of prevalence of chronic diseases has also increased. Osteoporosis is a bone disorder that is one of the most commonly encountered diseases among developed countries (Cashman, 2007) resulting in 8.9 million fractures every year across the globe (World Health Organization, 2007). A high incidence rate of bone diseases not only affects health but also imposes an enormous financial burden upon the economy of a nation. In New Zealand, the estimated incidence of osteoporotic fractures is expected to increase by 37% from 2007 to 2020, with the cost of osteoporosis treatment and management also increasing from $330 million (2007) to $458 million (2020) (Brown et al, 2011).

Maintaining good bone health in childhood and adolescence can optimise peak bone mass and reduce the risk of fractures, which then could decrease the likelihood of osteoporosis in later life (McDevitt and Ahmed, 2014). Calcium intake is a very important determinant for normal bone development during childhood and adolescence. Hence, optimal calcium levels within the body are required to achieve the maximum peak bone mass during young adulthood (Zhu and Prince, 2012). Numerous studies in children have shown the beneficial effects of calcium or dairy food supplementation on bone mass (Johnston et al, 1992; Bonjour et al, 1997; Cadogan et al, 1997; Dibba et al, 2000; Zhu et al, 2008; Ward et al, 2014). In addition to calcium, some other dietary and lifestyle factors can also influence bone mineral accretion during growth. Several nutrients (such as vitamin D, phosphorous, magnesium, protein, and dietary fibre) (Bonjour et al, 2009a; Gat-Yablonski et al, 2009), genetics (Nguyen et al, 1998), body fat (Casazza et al, 2010), and physical activity (Fuchs et al, 2001; Lofgren et al, 2012) can have an impact on the development of bone in children. In short, the bone mineral content (BMC) and bone mineral density (BMD) are influenced by the complex relationships between dietary, lifestyle, and genetic factors.

Puberty is the period of accelerated growth and maturation which includes peak height velocity (Hoppe et al, 2006). Due to this reason many bone health studies

have been conducted in pubertal children. The pre-pubertal phase of life is also a very important period to study bone accretion since at this stage the transition towards puberty starts. Therefore, it is important to consider whether supplementation is more beneficial prior to or during rapid growth. A few calcium supplementation studies only focusing on pre-pubertal children have shown significant improvements in bone parameters (Chan et al, 1995; Bonjour et al, 1997; Chevalley et al, 2005) and overall health (Lien et al, 2009).

The New Zealand National Children's Nutrition (NCN) survey found that 15.1% of children had insufficient calcium intakes (MOH, 2003). Contrary to the survey's findings, other studies conducted in New Zealand children found the baseline calcium intake to be above the recommended dietary intake (RDI) level (Gibbons et al, 2004; Houghton et al, 2010). Considering these inconsistent findings, it is important to reflect upon why and where the inadequacy persists in the children's population.

To determine the bone parameters and dietary intake correctly, it is essential to choose assessment techniques of adequate sensitivity and specificity. The choice of the dietary assessment method to be used in a study depends on; the objectives being measured, the study design, and the target population (Thompson and Subar, 2008). Since dietary assessment techniques are self-reported or proxy reported in the case of children, there is a possibility of inherent errors (Illner et al, 2012). In comparison, the dual energy x-ray absorptiometry (DEXA) which measures bone variables and body composition is a precise and fast machine with a low co-efficient of variation (Crabtree et al, 2014). Physical activity can be assessed using recording devices like activity monitors or physical activity questionnaires (Bonomi and Westerterp, 2012).

This cross-sectional 'bone health study' aims at examining the effects of dairy intake, body composition, and physical activity upon the bone health status of pre-pubertal children. Also the dietary assessment method used in this study i.e. a food frequency questionnaire (FFQ) will be validated. The second chapter (Chapter 2) includes a critical review of the literature relevant to bone health. Chapter 3 presents the aims, hypotheses, and primary and secondary objectives of this study. An overview of the study design is presented in chapter 4. Chapter 5

includes the results found and finally chapter 6 provides a detailed discussion of the key findings in association with available data. Chapter 6 also encompasses the conclusions, limitations, and recommendations.

CHAPTER 2: LITERATURE REVIEW

SECTION 1: Bone Structure, Growth & Metabolism

2.1 Bone:

The bone is a complex multifunctional organ which is only present in vertebrates. It is capable of carrying out various functions within a human body system ranging from providing stature support to maintaining mineral homeostasis (Watkins et al, 2001). Optimal bone health is not only essential for ossification of growing bones in children and adolescents but good bone health status in early years of life can also lower the risk of developing osteoporosis in later life (Gibbons et al, 2004).

2.2 Classification of Skeletal Bones:

A total of 206 bones are present in the human skeleton that can be classified into two general categories of "Axial skeleton" which comprises bones of long axis including the skull, rib cage and vertebral column, and "Appendicular skeleton" which consists of bones of the upper and lower limbs (Marieb, 2009).

Histologists have various classifications for bones such as woven vs lamellar, cellular vs acellular, and coarse fibre vs fine fibre (Hall, 2005). The bone classification system (Marieb, 2009) most commonly used is based on the shape and is described below:

Long bones: A long bone is made up of a central shaft and heads at bone ends. Examples are most of the limb bones, even the small bones in fingers are categorised as long bones.

Short bones: Short bones are small and cubical in shape. The ankle and wrist bones are examples of short bones. "Sesamoid bones", which resemble a sesame seed are also a type of short bone. A good example of the sesamoid bone is patella.

Flat bones: These bones are flat, levelled, thin, and a little curved. Mostly axial skeleton bones are flat, such as the sternum and skull bones.

Irregular bones: This category of bones does not fit into any of the above groups. These bones are asymmetrical and some examples are the vertebrae and hip bones.

2.3 Structure of Bone:

A bone is an organ composed of osseous tissues, nervous tissues, fibrous connective tissues, cartilage, and muscle as well as epithelial tissues which make up the blood vessels in bone (Marieb and Hoehn, 2010). Bone is a very complex network of interconnected structures and hence to better understand its structural anatomy, various hierarchical levels are used (Ritchie et al, 2009). The hierarchical levels of bone structure are generally categorised as "Macrostructure or Macroscopic anatomy", and "Microstructure or Microscopic anatomy" (Rho et al, 1998). Structure of a long bone is shown in figure 2. 1.

2.3.1 Macrostructure of bone:

2.3.1.1 Textures of bone:

At a macroscopic level, bone can be structurally differentiated into "Cortical or Compact bone" and "Trabecullar or Spongy or Cancellous bone". Compact bone is the dense bone present on the outer surface. It is mostly mineralised with only small spaces (lacunae) available to accommodate bone cells and vascular arrangement (Ritchie et al, 2009). Outer layer compact bone cortically wraps around the internal spongy bone which is more porous and less dense. The spongy bone has a honeycomb like structure filled with small open spaces called trabeculae. The living trabeculae are filled with either red or yellow bone marrow (Marieb and Hoehn, 2010).

Due to the unique structure of both types of bones; compact bone is regarded as stronger and spongy bone on the other hand offers higher flexibility. Under normal circumstances, spongy bone is metabolically more active than compact bone and hence is considered as the younger bone (Rho et al, 1998).

2.3.1.2 Gross anatomy of bone:

Complex cascades of events within the long bone are responsible for linear growth of the human body (Gat-Yablonski et al, 2009) and thus in this section the structure of a typical long bone will be examined in detail. A long bone can be distinguished into three structural sections: Diaphysis, Epiphysis, and Metaphysis (Figure 2.1).

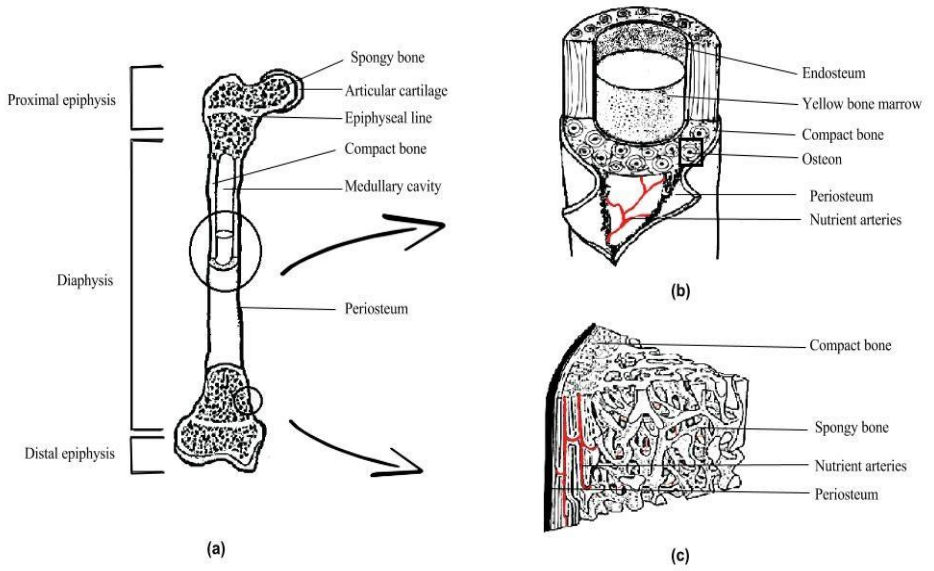

Figure 2.1: Structure of a typical long bone. (a) the anterior view of macroscopic bone (b) microscopic structure of the bone shaft showing compact bone (c) cross sectional view of compact and spongy bone (modified from Marieb and Hoehn, 2010).

The *Diaphysis* or the shaft forms vertical axis of a long bone. The outer surface is made up of compact bone which surrounds a central marrow or medullary cavity (Marieb and Hoehn, 2010). The *Epiphysis* is divided into a proximal and distal epiphysis present at extremities of the diaphysis. The outer surface of the epiphysis is made up of compact bone, however, unlike the diaphysis the anterior is composed of spongy bone. During childhood, a hyaline cartilage epiphyseal plate is present between each epiphysis and diaphysis. The function of this plate is to lengthen bone during growth. In adults this plate is replaced by an epiphyseal line which is a remnant of the epiphyseal plate. The area of epiphysis and

diaphysis unification whether it is epiphyseal line or plate is called the *Metaphysis* (Marieb and Hoehn, 2010). The outer layer of bone is covered by a fibrous double layered membrane called the Periosteum. The internal layer of bone is covered by a connective tissue membrane called the Endosteum. Both of these layers contain bone forming and resorbing cells (Marieb and Hoehn, 2010).

2.3.2 Microstructure of bone:

2.3.2.1 Bone composition:

A mature bone consists of 70% inorganic mineral phase (hydroxyapatite), 22% organic collagenous matrix, and 8% water by weight (Augat and Schorlammer, 2006). Figure 2.2 represents the bone composition distribution in percentage.

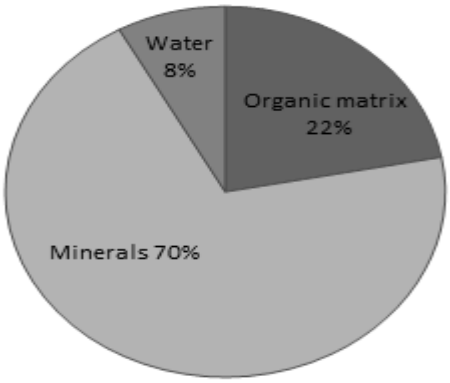

Figure 2.2: Composition of bone-around 70% is hydroxyapatite, 22% is organic matrix (90% is collagen and 10% NCPs), and the remaining 8% is water.

Inorganic phase or minerals: The inorganic matrix of bone mainly comprises of spindle shaped hydroxyapatite crystals and the organisation of these crystals is influenced by collagen fibre organisation. *Intrafibrillar* crystals form when minerals are embedded between the organic fibrils and *Interfibrillar* crystals form when minerals are present between or on the surface of collagen fibres (Olszta et al, 2007). The main function of minerals in bone is to provide rigidity and strength to the skeleton.

Organic matrix: The organic matrix comprises of approximately 90% collagen protein (Knott and Bailey, 1998) and the remaining 10% is made up of non-collagenous proteins (NCP) (Weiner and Wagner, 1998). Type 1 collagen is the main type of collagen protein present in the human bone extracellular matrix and is made up of two α1 chains and one α2 chain (Viguet-Carrin et al, 2006). The collagen fibres are organised in intricate interlinked patterns to increase the toughness and flexibility of bone (Knott and Bailey, 1998; Viguet-Carrin et al,

2006). NCPs such as osteocalcin, osteonectin, sialoprotein, proteoglycan, and osteopontin make up a small fraction of organic matrix (Hall, 2005). In an animal study, NCPs have shown to play an important role in stabilising the mechanical structure of bone by binding the mineralised collagen fibrils (Hang et al, 2014). However, no solid evidence for direct mechanical evaluation of NCPs in human studies is present and thus, more research needs to be conducted (Hang et al, 2014).

2.3.2.2 Microstructure of compact bone:

An osteon or Haversian system is the basic lamellar structural unit of compact bone which runs parallel to the vertical axis of bone. Osteon is made up of osteoblasts and mineralised collagen fibres known as lamellae which wrap around a core or cavity called the central or Haversian canal. Within these canals lie nerve fibres, blood vessels, and lymph vessels which nourish and help the osteons function properly. Volkmann's or perforating canals are another type of passages which run along the horizontal axis of bone. The function of these canals is to connect the periosteum blood and nerve supply with medullary cavity and Haversian canals (Rho et al, 1998; Marieb and Hoehn, 2010). In hard bone matrix at lamellar junctions the osteocytes occupy fluid cavities called lacunae. These lacunae are linked to one another and the Haversian canal via hair-like structures called canaliculi. Other than forming connections, the canaliculi also provide osteocytes with nourishment (Marieb and Hoehn, 2010).

Not all collagen fibres form parallel lamellar fibres, different fibre arrangements and alignments can also give rise to random and irregular parallel, tilted, or woven bundle structures in bone (Ritchie et al, 2009). These distinguished fibre shapes provide the strength to bone and prevent it from twisting.

2.3.2.3 Microstructure of spongy bone:

Spongy bone cellular structure is less organised in comparison to compact bone. Spongy bone is very porous and consists of small interconnecting structures called trabeculae (Marieb and Hoehn, 2010). In appearance the trabeculae look irregular; however, they are very intricate structures which allow nerves and blood vessels to carefully pass through them. The trabeculae also help bones resist stress by

absorbing the shock. Trabeculae can be structurally classified as rod-rod, rod-plate, and plate-plate interactions (Rho et al, 1998).

2.3.2.4 Bone cells:

Three main types of bone cells are osteoblasts, osteocytes and osteoclasts.

2.3.2.4.1 Osteoblasts:

Osteoblasts are cuboidal mononucleated cells which originate from the multipotent mesenchymal stromal cells (MSC) that also induce myoblast, adipocyte, fibroblast, and chondrocyte formation (Nombela-Arrieta et al, 2011). Osteoblasts are the bone forming cells which are located beneath the endosteum and periosteum, and within the internal medullary cavity (Harada and Rodan, 2003).

Osteoblasts are a type of fibroblast, the main function of which is to synthesise and mineralise bone matrix or osteoid. Osteoblasts are responsible for producing extracellular proteins like osteocalcin, alkaline phosphate, and type 1 collagen (Long, 2011). Osteoblasts are also responsible for regulating bone formation or bone resorption by producing prostaglandins (PGs), growth factors, and cytokines. (Watkins et al, 2001). Pre-osteoblasts are converted into mature osteoblasts once they reach the site of bone formation and start to produce bone matrix proteins. After completion of the required function osteoblasts can undergo one of three fates: (1) become embedded in mineralised collagen matrix as osteocytes (Shapiro, 2008), (2) they can become inactive and form bone lining cells (Kim et al, 2012) or (3) they can undergo cytokine derived apoptosis and die (Jilka et al, 1998).

Osteoblast differentiation is regulated by local growth factors (GFs) especially in the embryonic stages. Some of the widely studied GFs are insulin like growth factor-1 (IGF-1), bone morphogenic proteins (BMPs), transforming growth factor-β (TGF-β), and members of fibroblast growth factor (FGF) family (Ducy et al, 2000). Wnt signalling pathways are made up of a set of Wnt proteins which also play a complex role in regulating osteoblast differentiation and suppression (Boland et al, 2004). Figure 2.3 shows the effect of Wnt signalling on osteoblast differentiation. An inhibitor of the Wnt signalling pathway or bone formation is known as sclerostin that is expressed by mature osteocytes. Sclerostin, which is a

glycoprotein belonging to the DAN family is encoded by the *SOST* gene (ten Dijke et al, 2008). Sclerostin is responsible for the suppression of osteoblastic differentiation which in turn results in the inhibition of bone formation (Matsuo and Irie, 2008). Just like Wnt pathways, sclerostin indirectly acts as an antagonist of bone forming BMPs as well.

In addition to the above stated regulators, transcriptional factors also play a vital role in bone formation. One of the earliest and influential determinant of osteoblastic differentiation is the runt-related transcription factor 2 (Runx2 gene) also known as *Cbfa*1. It is a member of the Runt domain family and this particular gene is highly expressed in osteoblasts. Administration of Runx2 to non-osteoblastic cells can lead to expression of osteoblastic genes like osteocalcin (Karsenty, 2008). Runx2 gene is also responsible for regulating another osteoblast specific transcriptional factor called osterix (Osx). In mice it is shown that Osx activity is necessary for proper osteoblast functioning as Osx knockout mice do not have a normal mineralised bone matrix (Nakashima et al, 2002). Some other transcriptional factors influencing osteoblast differentiation are Distal-less homeobox 5 (*DLX5*) and msh homeobox homologue 2 (*MSX2*) (Harada and Rodan, 2003).

2.3.2.4.2 Osteocytes:

In the adult human skeleton, osteocytes are the most abundant type of bone cell making up about 90% of the bone cell population. Unlike osteoblasts and osteoclasts which have a very short life span, osteocytes can remain viable for decades. Osteocytes form the canaliculi which connect adjacent lacunae and also allow communication between other osteocytes (Hall, 2005; Bonewald, 2007).

For over a century it has been known that osteocytes are derived from mature osteoblasts once mineralisation of bone has occurred (Klein-Nulend et al, 2005). It is believed that on the bone surface a selected population of osteoblasts reduce the bone matrix production which then results in these osteoblasts becoming embedded within the matrix. The exact mechanism responsible for osteoblast to osteocyte conversion still remains to be fully elucidated (Dallas and Bonewald, 2010). Since the osteoblasts are passively embedded within the osteoid, osteocytogenesis is thought to be a passive process (Franz-Odendaal et al, 2006).

A few arguments, however, propose that osteocytogenesis might be an active process instead. Formation of dendritic processes is one of the primary changes taking place within the buried cells where the dendrites start to extend towards bone surface and mineral formation begins. The embedded osteocytes develop a polarised state in regards to both the dendrites and mineralisation because the mineral deposition occurs at one side rather than throughout the cell surface. The cell within which all the above mentioned transitions take place is called an osteoid osteocyte and it is responsible for the regulation of mineralisation (Bonewald, 2011). Another argument that also suggests osteocyte formation may be an active process is that osteocytogenesis requires an invasive active mechanism for the cleavage of matrix molecules as well as collagen fibrils. As shown by Holmbeck and colleagues (2005) that mice lacking the cleaving molecule membrane type-1 matrix metalloproteinase (MT1-MMP) have significantly decreased number of dendrites due to disruption of osteocytogenesis (Holmbeck et al, 2005).

Several functions have been associated with osteocytes such as calcium sensors, regulators of matrix maturation in osteoid, mineralisation, and mechanosensors (Kamioka et al, 2001). The dendritic processes of osteocytes are important for transferring nutrients and converting mechanical stress into a biological response (Miyauchi et al, 2000). One of the main objectives of the bone is to bear functional load and the canalicular system which acts as a conduit for bodily fluids helps perform this very action. Canaliculi are small fluid filled channels between lacunae where osteocytes reside, hence osteocytes are the bone cells which are most likely to sense and respond to strain because of fluid flow sheer stress (FFSS) (Klein-Nulend et al, 2005). Fluxes in mineral (especially calcium) levels within the body can have a strong influence upon the mechanical strain endured by osteocytes as changes in fluid pressure occur. The FFSS also impacts the bone remodelling cycle by adjusting changes in the mineral gradient (Kaiser et al, 2012). A major beneficial effect of FFSS is that mechanical stress produces nitric oxide (NO) and in turn prostaglandin E2 (PGE2). These changes have partly been shown to reduce the TNF-α induced osteocyte apoptosis (Klein-Nulend et al, 1995; Tan et al, 2006). The connectivity between osteocytes and osteons decreases with increasing age as the canalicular network begins to degrade

resulting in lesser supply of nutrients to osteocytes, reduced mechanosensitivity, and increased bone fragility (Milovanovic et al, 2013).

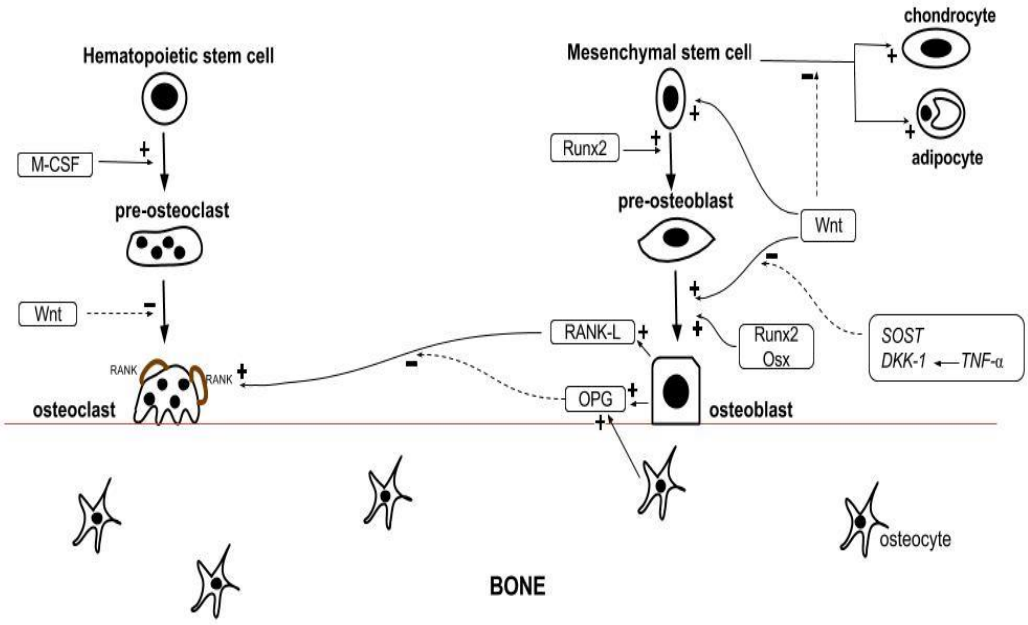

Figure 2.3: Diagrammatic presentation of osteoblast and osteoclast differentiation along with their stimulatory (+) and inhibitory factors (-). Bold line represents stimulation and dotted line is used to show an inhibitory effect. (modified from Kruger et al, 2010). M-CSF=macrophage colony stimulating factor, RANK= receptor activator of nuclear factor-kB, RANK-L= receptor activator of nuclear factor-kB ligand, Runx2= runt-related transcription factor 2, Osx=osterix, SOST=seclerostin, DKK-1=dickkopf-related protein-1, TNF-α=tumour necrosis factor α, OPG=osteoprotegerin.

2.3.2.4.3 Osteoclasts:

Osteoclasts are large multinucleated cells, the main function of which is resorption of skeletal bone. Osteoclasts arise from hematopoietic precursors of the monocyte-macrophage lineage after several cellular fusions and thus share common origins with dendritic cells and macrophagic liver Kupfer cells (Vaananen and Laitala-Leinonen, 2008). Osteoclastogenesis takes place at or near the surface of bone from where osteoclasts break down bone tissue by releasing acidic and lytic enzymes within an extracellular compartment (Boyle et al, 2003). Osteoclastic cells that assist in efficient and rapid bone resorption normally have five to eight nuclei, however, the mononucleated cells also are capable of resorbing bone (Vaananen and Laitala-Leinonen, 2008).

When bone resorption occurs the mature osteoclasts become polarised due to synthesis and mobilisation of various electrolytes and degenerative enzymes (Teitelbaum, 2007). After the cell becomes polarised three distinct zones arise in the cytoskeleton; a ruffled border, a sealing zone, and a secretory domain. At the sealing zone the actin rings formed by the cytoskeleton attach plasma membranes of the osteoclasts to the bone matrix. Evidence strongly shows that integrin receptors, especially the vitronectin receptor (αvβ3 integrin) plays a vital role in this attachment (Nakamura et al, 2012). It has also been suggested that at lower levels a few other integrins; αvβ1 and α2β1 play an important role in osteoclast attachment to the matrix (Nakamura et al, 2012). After the attachment is complete a ruffled border forms, in the membrane of which a vacuolar type H^+-ATPase pump is located. This pump releases hydrochloric acid (HCl) to dissolute minerals and proteases which then dissolves the organic matrix forming lacunae (Nakamura, 2007). The degraded material is then endocytosed via the secretory domain. Finally after resorption occurs, the osteoclasts undergo apoptosis (Vaananen et al, 2000).

Osteoclastogenesis is up-regulated by two cytokines; receptor activator of nuclear factor-kB ligand (RANK-L) and macrophage colony stimulating factor (M-CSF) (Teitelbaum, 2007). See figure 2.3 for osteoclast differentiation along with factors affecting it. RANK-L belongs to the TNF family and can be expressed by osteoblastic cells. The ligation of RANK-L to its receptor RANK activates osteoclastogenesis (Boyce and Xing, 2008) and in some cases can promote proliferation of tumor cells (Jones et al, 2002). The proliferation of osteoclast is further enhanced by several other cytokines such as TNF-α, interleukin-1 (IL-1), and interleukin-6 (IL-6), and hormones like thyroid hormone (TH), parathyroid hormone (PTH), glucocorticoids (GCs), and 1α,25- dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) (Boyle et al, 2003; Kanatani et al, 2004; Rao et al, 2006).

Osteoblasts express osteoprotegerin (OPG) that is a member of the tumor necrosis factor-α (TNF-α) family. OPG plays an osteo-protective role in humans by acting as a decoy receptor for the RANK-L (Boyce and Xing, 2008). OPG plays a reverse role to RANK-L and inhibits the differentiation of osteoclasts by preventing the binding and stimulation of RANK-L with its receptor RANK (Simonet et al, 1997; Schoppet et al, 2002). OPG, hence, down regulates

osteoclastogenesis by inhibiting the binding of RANK-L to receptor activator of nuclear factor-kB (RANK) along with the promotion of osteoblast differentiation. Since OPG competes for the same receptor as RANK-L/RANK, the ratio of RANK-L to OPG or in other words the RANK/RANK-L/OPG pathway is very important in determining whether bone resorption or formation will take place (Gori et al, 2000; Theoleyre et al, 2004).

Osteocyte apoptosis also impacts osteoclast functioning because osteoclasts do not resorb bone in the presence of live osteocytes, and they are also unable to produce actin filaments when osteocytes are still viable (Gu et al, 2005).

2.4 Bone Modelling and Remodelling:

2.4.1 Bone modelling:

In a lifetime the bones undergo modelling and then remodelling. Growth of the skeleton or bone modelling is described as the sequence of events that alter the size, shape, and position of skeletal bones (Raisz, 2004). At any particular site, bone modelling requires activation of either osteoclasts followed by the resorption of bone or it involves the activation of osteoblasts which then leads to bone formation. In other words during bone modelling, the formation and resorption of bone are independent and uncoupled which means that osteoclast and osteoblast activities occur at different locations at different time intervals (Brandi, 2009).

Both bone forming and bone resorbing cells function throughout life. During childhood and adolescence, both bone modelling and remodelling occur, however, to support the increased growth velocity bone modelling rate is higher than bone remodelling (Roberts et al, 2004). On the other hand in adults, when the skeleton has fully matured, only bone remodelling occurs to maintain the integrity of bone matrix (Ruimerman, 2005; Gafni and Baron, 2007). Another distinction between these two mechanisms is that bone modelling is versatile in contrast to remodelling as it can produce bones of various shapes and sizes depending on the intensity of the mechanical strain (Brandi, 2009).

A theory very strongly linked to bone modelling is that the development of a bone is influenced by mechanical strain and physiological stimuli. This is known as Wolff's law, according to which a growing skeleton adapts to functional loads

(Clarke, 2008). The exact mechanism by which physiological stress influences bone modelling is unclear but chemicals released in response to mechanical stimulation like prostaglandins are known to enhance bone growth (Roberts et al, 2004). In addition to mechanical stress, genes also play an important role in shaping the architecture and determining mass of bones (Roberts et al, 2004; Clarke, 2008). Bone modelling rate can also be enhanced during hypoparathyroidism, renal osteodystrophy, and when anabolic drugs are ingested (Kini and Nandesh, 2012).

2.4.2 Bone remodelling:

Bone is a metabolically active organ which needs to be remodelled on a regular basis to replace old bone tissues with new ones (Raisz, 1999). The bone remodelling cycle (Figure 2.4) is a highly regulated sequence of events that involves a coordinated action of osteoclasts and osteoblasts within the bone multicellular unit (BMU). Unlike bone modelling, bone resorption and formation are tightly coupled in bone remodelling (Crockett et al, 2011). Cellular activity within BMU is coupled meaning that the bone resorption by osteoclasts is equal to bone formation by osteoblasts (Sims and Gooi, 2008). Bone remodelling cycle (BRC) can be divided into categories of activation, resorption, reversal, formation, and finally termination. Each of these stages has a different duration of completion; resorption takes about 2 weeks, reversal requires approximately a month, and formation lasts up to 3 months or until the bone formation process has completed (Hadjidakis and Androulakis, 2006).

The first step of BRC is ***activation*** where recruitment and activation of osteoclasts takes place for bone to be resorbed. RANK/RANKL system along with M-CSF is a very important regulator of the BRC as it is responsible for the differentiation, fusion, activation, and subsistence of multicellular osteoclastic cells (Hadjidakis and Androulakis, 2006). On the other hand, OPG which is mainly produced by osteoblasts inhibits bone resorption by blocking the RANK/RANKL system altogether (Raisz, 2004). Therefore, osteoclastogenesis is believed to be controlled by the OPG/RANK-L signalling axis as it acts as a mediator of the resorption effecting factors like PTH, vitamin D_3, prostaglandins, interleukins, growth factors, and cytokines (Robling et al, 2006). Signals which

supposedly stimulate osteoclast formation are apoptosis of osteocytes and bone strain (Sims and Gooi, 2008).

After activation, ***resorption*** of bone begins. Resorption is triggered after osteoclasts attach to the bone matrix where they begin to degrade the osteoid matrix. As a result of osteoclastic resorption irregular Howship lacunae form in trabecular bone and cylindrical Haversian canals in compact bone (Raisz, 1999).

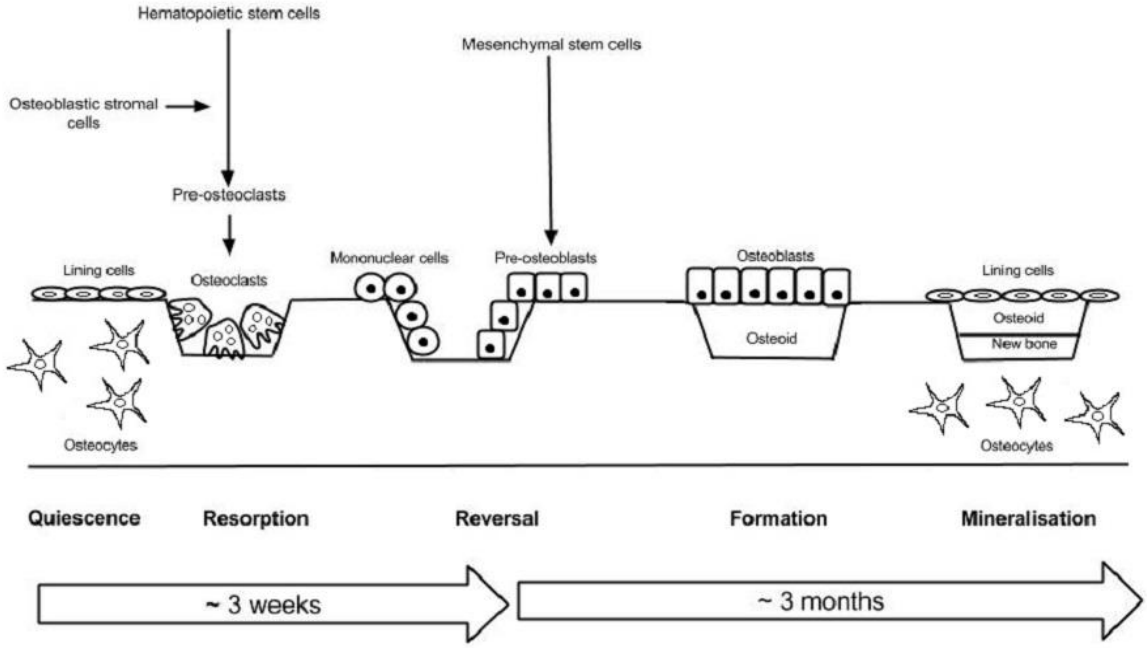

Figure 2.4: Bone remodelling process demonstrated in a trabecular bone which starts with activation of osteoclasts and terminated when bone mineral deposition completes (modified from Raisz, 2004).

Once bone resorption is completed mononuclear cells degrade collagen, deposit proteoglycans and release growth factors which lead to bone formation (Matsuo and Irie, 2008). This is the ***reversal*** phase and it is believed that changes which occur here attract osteoblasts to bone at junction of old and new bone cells called the cement line (Raisz, 2004).

Osteoblasts now begin to replicate, migrate, and differentiate in the ***formation*** phase (the longest phase of BRC). Numerous factors affect osteoblast differentiation such as osteoclasts, growth factors like IGF-1, hormones like PTH, bone morphogenic proteins (BMPs), and angiogenic factors like endothelin-1 (Eriksen, 2010). Once osteoblasts have secreted the osteoid matrix they either

undergo apoptosis or become bone lining cells or osteocytes (Hernandez-Gil et al, 2006).

After bone formation, osteocytes release sclerostin (ten Dijke et al, 2008) which leads to the suppression of osteoblastic differentiation which then results in the inhibition of bone formation (Matsuo and Irie, 2008). Research has shown that sclerostin suppresses bone formation by acting as an antagonist of canonical Wnt signalling pathway (inducer of osteoblast formation) and bone formation promoting BMPs (ten Dijke et al, 2008). So after bone formation is completed and osteoblastic activity is suppressed, the bone remodelling cycle is finally ***terminated*.**

In bone remodelling, bone formation and resorption are coupled thus remodelling can be either in balance (bone formed is equal to bone resorbed) or imbalanced (bone gain or bone loss) depending upon various systemic and local regulators (Table 2.1) (Raisz, 2004). The process of bone remodelling occurs in both cortical and trabecular bone, however, the mechanisms for each differ. In cortical bone remodelling the osteoclasts dig holes through solid mineralised bone which is closely followed by the formation of an osteon by osteoblasts. In trabecular bone the osteoclasts form channels across the trabecular surface which when filled by osteoblasts creates a hemi-osteon (van Oers et al, 2008). Even though cortical bone is present in greater amounts in body, the trabecular bone has a higher metabolic rate due to its greater surface area (Clarke, 2008). Bone remodelling can be both targeted and non-targeted. In targeted bone remodelling there is a predetermined removal of damaged bone which will be later replaced by new healthy bone and the main factor responsible for triggering such a response is believed to be micro-damage sensed by mature osteocytes. In contrast, non-targeted bone remodelling is not predetermined and can be affected by hormones like PTH, GH, thyroxine and oestrogen or antiresorptive drugs such as bisphosphonates (Eriksen, 2010). Approximately 5-10% of total bone is renewed per year (Hernandez-Gil et al, 2006).

Table 2.1: Systemic and local regulatory factors affecting bone remodelling.

	Stimulate bone resorption	Stimulate bone formation
Systemic regulation:		
PTH	↑	↓
1,25$(OH)_2$ Vitamin D_3	↑	↑
Oestrogen	↓	↑
GH	↑	↑
TH	↑	↑
Glucocorticoids	↑	↓
Calcitonin	↓	?
Local regulatory factors:		
Cytokines	IL-1, IL-6, IL-8, IL-11, TNF-α, PGE_2	IL-4, IL-3, IL-18, IFN, OPG, TNF-β, adiponectin
Growth factors	M-CSF, PDGF, FGF, EGF	BMP, IGF-1, IGF-2, PDGF, FGF

PTH: parathyroid hormone, GH: growth hormone, TH: thyroid hormone, IL: interleukin, TNF-α: tumour necrosis factor-α, PGE: prostaglandins, M-CSF: macrophage colony stimulating factor, PDGF: platelet derived growth factor, FGF: fibroblast growth factor, EGF: epidermal growth factor, OPG: osteoprotegerin, IFN: interferon, BMP: bone morphogenic protein.

2.5 Bone Mass Accrual and Peak Bone Mass:

Bone mass accrual is the accumulation of minerals in the bone during growth. At puberty and post-puberty the storage of minerals takes place until the highest level of bone mass is achieved (Bonjour et al, 1991; McDevitt and Ahmed, 2009). Peak bone mass (PBM) is defined as the attainment of highest bone mass during normal growth and is believed to be achieved towards the end of second or early third decade of life (Matkovic et al, 2003). The exact age at which PBM is attained is ambiguous due to lack of definitive data, differential methodology in trials used, and different skeletal sites measured (Baxter-Jones et al, 2011) but a longitudinal study showed that the estimated mean age at which PBM is achieved was 18-20 years in females and 18-23 years in males (Boot et al, 2010). This gender difference possibly exists because of the late puberty attainment, later growth spurt, and greater bone size in males which results in higher periosteal deposition, hence taking longer to achieve PBM along with providing better strength and resistance to mechanical strain (Bonjour et al, 2009b; Boot et al, 2010).

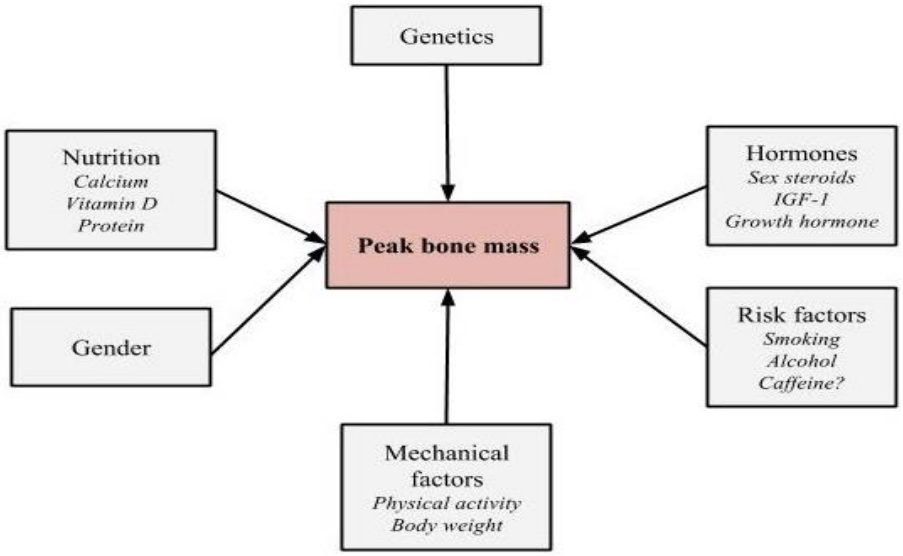

Figure 2.5: Physiological determinants of PBM during normal growth.

PBM determines resistance to bone fractures and has been shown to be a significant risk predictor of osteoporosis (Matkovic et al, 2003). An increase in PBM of 1 standard deviation is associated with a 50% reduction in fracture risk (Bonjour et al, 2009b). Maintaining high bone mass during childhood may lower the risk of bone fractures in later years of life (Rizzoli et al, 2010). Evidence suggests that females with optimal milk intake or higher bone mass during childhood and adolescence will have greater BMD and a lower risk of developing osteoporosis in older age (Rubin et al, 1999; Kalkwarf et al, 2003).

PBM is influenced by both genes and environmental factors (Figure 2.5). Genes have been shown to contribute around 70-80% of the variance in bone mineral status (Nguyen et al, 1998) and the remaining 20-30% is contributed by environmental and lifestyle factors like diet, endocrine hormones and mechanical forces (Figure 2.5) (Matkovic et al, 2003; Bonjour et al, 2009b). To maintain optimal bone mass during growth, the calcium balance needs to be positive in order to meet the extra skeletal needs. Weight bearing exercise of moderate to vigorous intensity along with a healthy diet that is rich in both calcium and vitamin D is believed to be the most potent strategy to establish good bone health (McDevitt et al, 2014). Fractures during growth, lack of exercise, poor nutrition and in some cases hormonal imbalance are important influential factors of low PBM (McDevitt and Ahmed, 2009). Some other lifestyle factors which might

influence bone mineral status can be smoking, alcohol consumption, caffeine intake, and oral contraceptive usage (Valimaki et al, 1994; Heaney et al, 2000; Lacerda et al, 2010).

2.6 Functions of the skeletal system:

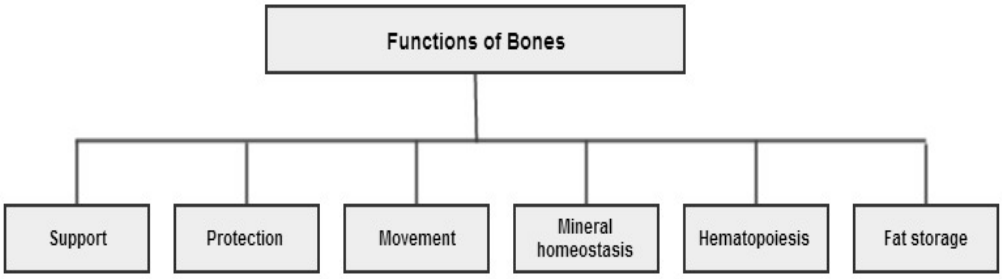

Figure 2.6: Functions performed by bones of the skeletal system.

The two main types of bones have differing compositions as the compact bone is 80-90% calcified and mainly used for structural purposes, and the spongy bone is only 15-25% calcified and predominantly considered as metabolic (Flynn, 2003). Bones are capable of carrying out a wide range of functions (Figure 2.6) within the body but the main role of bones is believed to be (1) the provision of *Support and stature* (Humphrey, 1998). The bones of vertebrae act as shock absorbers providing flexibility which allows them to bend without breaking (Seeman, 2008). Other important functions performed by bones are (2) *Protection* of vital organs e.g. the cranium protects the brain and ribcage the thoracic organs (Marieb and Hoehn, 2010), (3) *Movement* where tubular bones or long bones function as levers and provide stiffness so the body is held upright. Axial bones also provide flexibility to aid the bone in absorbing shock during movement (Seeman and Delmas, 2006), (4) Bone is also a good *Reservoir of minerals* (especially calcium, phosphorus and magnesium), growth factors and helps maintain the acid-base balance by controlling alkaline salts. Two more functions of bones are (5) *Red blood cell formation* within the medullary cavity of certain bones and (6) *Storage of extra lipids or* triglycerides within cavities in the form of yellow marrow (Marieb and Hoehn, 2010).

2.7 Regulation of Bone Metabolism:

2.7.1 Nutrition and bone metabolism:

2.7.1.1 Calcium:

Based on composition, calcium is the fifth most abundant element present within the human body making up to 1.9% of body weight (FAO, 2002). Approximately 99% of this calcium is located in the skeletal system as hydroxyapatite, which is a calcium-phosphate complex, that provides strength and rigidity to the bones (Peacock, 2010). The remaining amount is distributed across extracellular fluid and soft tissues as complexes of calcium phosphate, calcium oxalate, and calcium carbonate (Greer and Krebs, 2006). Calcium plays an important role in development, growth, and sustenance of the skeleton and teeth. In addition to this, some other critical roles involve cellular metabolism, blood pressure regulation, blood clotting, muscle contraction, and nerve impulse transmission (Chung et al, 2009; Peacock, 2010).

Calcium can be obtained from the diet as well as supplements, however, in both cases calcium is bound to other dietary components or micronutrients. Therefore, within the gut, these complexes must be broken down into a soluble and ionised form before calcium is absorbed and utilised by the body (Allen, 1982). The majority (90%) of calcium absorption occurs in the small intestine with the order of absorption being duodenum>jejunum>ileum. Remaining absorption occurs in the stomach and large intestine (Wasserman, 2004).

Intestinal calcium absorption takes place using two mechanisms. *Paracellular transport* is the mechanism where calcium ions move through the tight junctions which are present between the adjacent intestinal epithelial cells (Khanal and Nemere, 2008). It is a passive movement of calcium ions that is dependent upon the concentration and electrochemical gradient across the epithelium as well as the integrity and permeability of tight junctions within the epithelium (Perez et al, 2008). Tight junctions are located in the apical epithelial membrane to form a barrier to certain solutes hence allowing only selective permeability (Hoenderop et al, 2005). *Active transport* on the other hand is saturable, taking place mainly in the upper small intestinal regions. Active transport is believed to involve three steps; (1) *Epithelial calcium channels* TRPV5 and TRPV6 both of which are

homologues of vanilloid subfamily of Transient Receptor Potential (TRPV) allow calcium entry into cell by facilitated diffusion (van Abel et al, 2005). (2) *Intracellular calbindin* i.e., Calbindin$_{9k}$ (CB_{9k}) facilitates the movement of calcium ions intracellularly (Bronner, 2003; Perez et al, 2008). (3) *Ca-ATPase (PMCA) and* Na^{+}*-*Ca^{2+} *(NCX) pumps* allow efflux of calcium ions from the enterocytes into the blood plasma (Perez et al, 2008).

Low intake of calcium results in increased efficiency of calcium absorption by the intestine. It is believed that this increase in calcium absorption is mainly mediated by 1,25-dihydroxyvitamin D_3 ($1,25(OH_2)_3$) (Fleet and Schoch, 2010). The presence of adequate Vitamin D levels is essential for optimal calcium absorption as low calcium intake increases vitamin D turnover and production whereas high calcium status is vitamin D sparing (Lips, 2012). Further details on vitamin D's effect upon calcium metabolism are discussed in detail under the 'Vitamin D' heading.

Calcium requirements change throughout the life of human beings with the requirements increasing from early childhood until the peak bone mass (PBM) is achieved in late puberty, with a decline afterwards (Abrams, 2001). During childhood and adolescence sufficient calcium intake should be maintained as it is a period of rapid growth and development of the skeletal system (Gibbons et al, 2004). Furthermore, adequate calcium consumption during early years of life leads to large peak bone mass accrual which in turn reduces the risk of bone disorders such as osteoporosis in later life (Wosje et al, 2000).

Different countries have different recommendations for calcium. The recommended dietary intake (RDA) for US children is 800-1200mg/d (Baker et al, 1999), in UK it ranges from 450-550mg/d (More, 2007), and in NZ/Australia it is 700mg/d for 4-8year olds and 100mg/d for 9-13 year old children (NHMRC, 2006). No exact single value has been adopted globally because evidence from calcium balance studies has shown inconclusive results as to what value should be set to achieve maximum benefit and minimum adverse outcomes (Winzenberg and Jones, 2008).

In New Zealand, the MOH suggests that 2-3 servings of milk or dairy should be consumed daily as they are a good source of not only calcium but other nutrients

as well, which help maintain good bone health (MOH, 2012a). The National Children's Nutrition (NCN) survey which included children aged from 5-14 years concluded that inadequate calcium intake prevalence in NZ children was 15.1% (12.2% in males and 18.2% in females) (MOH, 2002). Detailed analysis of the survey also showed that Pacific children (both genders) had higher inadequacy of calcium than NZ European origin (NZEO) and Maori children. And when Maori and NZEO children's calcium intake was compared it was seen that Maori females had higher calcium intake compared to NZEO females but Maori males had lower calcium intake then NZEO males. Milk provided about 1/3 of calcium in NZ children's diet, followed by other dairy products, bread, vegetables/fruits and grains (MOH, 2012b).

The best dietary sources of calcium are milk and other milk products such as cheese, cream, curd, and yoghurt (National Institute of Health, 2013). Some non-dairy sources include green vegetables, legumes and nuts, tofu, fish with edible bones and fortified food products such as soy milk and breakfast cereal (MOH, 2012b). Table 2.2 below shows the calcium content of some foods obtained from the International Osteoporosis Foundation's website (International Osteoporosis Foundation, 2015).

Table 2.2: Calcium content of various foods.

Food	Serving Size	Calcium content (mg)
Milk (skimmed)	200ml	244
Milk (whole)	200ml	236
Yoghurt (natural)	150g	207
Cheese (cheddar)	30g	240
Kale	50g	32
Broccoli	120g	112
Tofu	120g	126
Sardines	60g	240
Almonds	30g	75

2.7.1.2 Vitamin D:

Vitamin D is a prohormone that can be obtained from two sources: the main source (around 90%) of vitamin D is made in skin as vitamin D_3 or cholecalciferol from sunlight (UV-B radiation) and the remaining 10% comes from diet (Neer, 1975; Holick, 1996) or sometimes from supplements (Houghton and Vieth, 2006)

in the form of vitamin D_2 or ergocalciferol Both of these are inactive forms, thus for activation to occur vitamin D_3 is converted to 25-hydroxyvitamin D_3 (25(OH)D) in the liver and finally into active 1α,25-dihydroxyvitamin D_3 (1,25-$(OH_2)D_3$) mainly in the kidneys (Lips, 2006). This final conversion is catalysed by 25-(OH)-D-1α-hydroxylase which is a cyp27b1 encoded enzyme (Peterlik et al, 2009). The cyp27b1 enzyme can also be found in other non-renal sites like the gut, bone, parathyroid glands, immune cells, and epithelia of numerous other tissues (Hewison et al, 2007). The regulation of cyp27b1 by these non-renal tissues is different and could be more substrate dependent (Bikle, 2012). The synthesis, absorption and metabolism of Vitamin D through two sources is illustrated in figure 2.7.

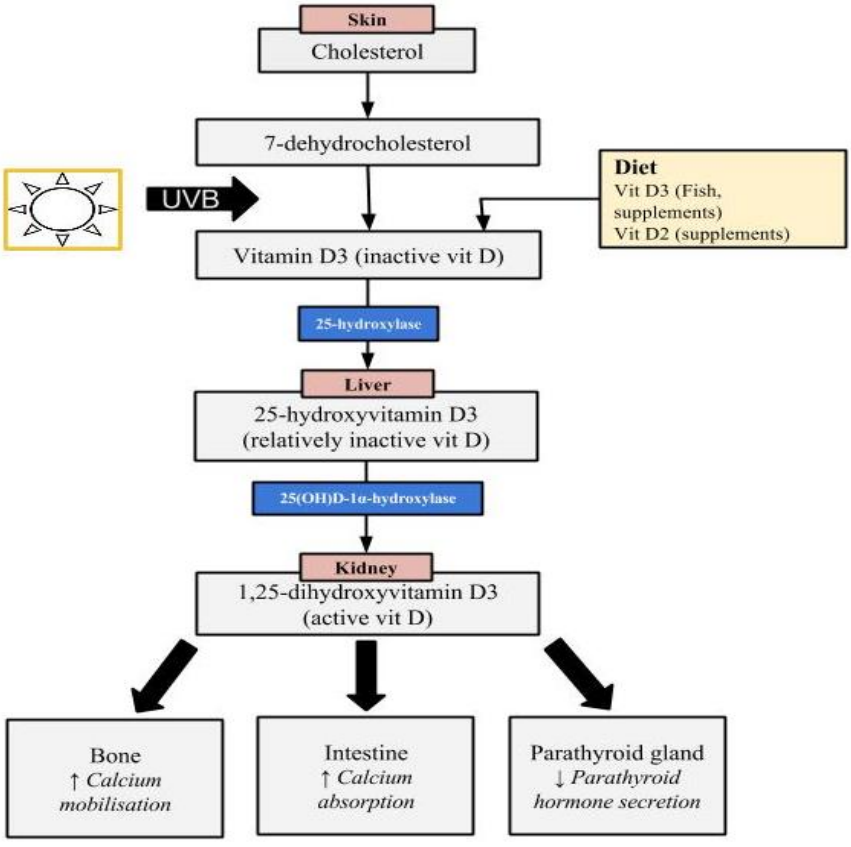

Figure 2.7: Absorption and metabolism of vitamin D via both sunlight and diet (modified from Peterlik, 2009).

All three bone cell types have Vitamin D receptors (VDR) but the action of vitamin D on osteoblasts is best understood (St-Arnaud, 2008). Vitamin D's effect

on osteoblasts leads to the production of non-collagenous proteins; osteopontin, osteocalcin, and osteonectin and also results in up regulation of RANKL hence increasing bone resorption (Holick, 1996; Turner et al, 2012). Two functions exerted by vitamin D on bone health are; firstly maintenance of serum calcium and phosphate homeostasis, and secondly mineralisation of bone matrix (Turner et al, 2012).

Optimal vitamin D intake is important for the maintenance of normal calcium homeostasis. 1,25-$(OH_2)D_3$ or calcitriol is the metabolite which facilitates calcium absorption in the gut by both genomic and non-genomic mechanisms (Heaney, 2008; Girgis et al, 2012). The mechanisms mainly studied on vitamin D's effect on calcium are genomic and are believed to affect the individual transcellular transport steps in the intestine. In this particular process 1,25$(OH_2)D_3$ binds with the VDR promoting heterodimerisation with retinoid X receptor (RXR) which is a nuclear receptor and is a common binding partner of VDR (Christakos et al, 2011). The VDR-1,25-$(OH_2)D_3$-RXR complex regulates gene transcription of vitamin D target genes by binding 1,25-$(OH_2)D_3$ to vitamin D responsive elements (VDREs) (St-Arnaud, 2008). Within the small intestine, 1,25-$(OH_2)D_3$ enhances some or all of the calcium transport proteins; transient receptor potential 5 and 6 (TRPV5, TRPV6), calbindins (CB_{9K} in mammals, CB_{28K} in avian species), Ca-ATPase (PMCA), Na^+-Ca^- (NCX1). All of these transporters respond differently to 1,25-$(OH_2)D_3$ possibly due to affinity and sensitivity of their respective genes (Perez et al, 2008). Animal studies have shown that the regulation of epithelial calcium transporters is controlled by 1,25-$(OH_2)D_3$ with differing effects observed upon the duodenal and kidney calcium transport mechanisms (Song et al, 2003). Other trials on VDR knockout mice also concluded the importance of vitamin D, as knockout mice showed impaired duodenal calcium absorption compared to the wild type mice (van Cromphaut et al, 2001; Bouillon et al, 2003; Okano et al, 2004). Extrusion of calcium at the basolateral membrane is carried out by the calcium transport proteins PMCA1b and PMCA2c1 and research has shown that 1,25-$(OH_2)D_3$ could amplify this effect (Goltzman et al, 2014).

Vitamin D assisted calcium absorption via paracellular diffusion is not as comprehensively studied as the transcellular transport (Wasserman, 2004; Fleet and Schoch, 2010) but a research trial conducted by Chirayath and colleagues in

1998 showed that tight junction conductance as well as paracellular transport of calcium ions across intestinal Caco-2 cells increased in the presence of vitamin D due to genomic mechanisms. 1,25-$(OH_2)D_3$ also enhances paracellular diffusion of calcium by activating the protein kinase C (signalling pathway) which increases permeability by altering the cytoskeleton activity (Perez et al, 2008).

The non-genomic effects of vitamin D consist of complex intracellular signal transduction pathways which begin after 1,25$(OH_2)D_3$ has bound to non-nuclear receptors (Girgis et al, 2012).

During vitamin D deficiency, calcium absorption is not altered until the serum 25(OH)D levels fall below 10nM (Need et al, 2008). Below this threshold, calcium malabsorption occurs, which consequently results in increased PTH secretion from the parathyroid glands that may lead to mobilisation of calcium from the skeleton—a condition known as hyperparathyroidism (Bonjour et al, 2009a). Therefore, both inadequate calcium and raised PTH levels enhance bone mineral loss resulting in low bone mass and increased risk of osteoporosis. If vitamin D deficiency occurs before the epiphyseal plates close it leads to defective mineralisation which is the cause of rickets in children. If the deficiency arises after closure of the epiphyseal plates it can result in osteomalacia in adults (Cashman, 2007). Hypervitaminosis D (serum 25(OH)D>250nmol/litre) is uncommon but can occur due to excessive use of vitamin D supplements or vitamin D fortified foods (Jones, 2008). In this state bone resorption increases rapidly with high levels of plasma calcium present which might cause hypercalcemia. Formation of kidney stones, conjunctivitis, fever, and weight loss are some of the symptoms of hypervitaminosis D (Jones, 2008; Bonjour et al, 2009a).

Dietary sources of vitamin D are fatty fish (salmon, sardines), and egg yolk. Other sources include fortified food products such as bread, cereals, and margarines. Vitamin D supplementation is essential, especially in the most vulnerable population groups of young children, pregnant and lactating mothers, dark skinned people, vegetarians, and people living in the northern latitudes (Misra et a, 2008). Vitamin D deficiency can be caused by a number of factors like skin pigmentation, as the risk of vitamin D deficiency is higher in darker skinned

people. In addition sunscreen, coverage of skin, air pollution, and latitude also affect vitamin D synthesis (Misra et al, 2008; Munns et al, 2006).

New Zealand is a country located at 35-46˚S latitude with a multi-ethnic population. Due to this factor a high prevalence of vitamin D deficiency exists in this country with 1 in 25 NZ children having vitamin D levels below the normal threshold (Rockell et al, 2005). Vitamin D deficiency is defined as having serum 25(OH)D <50nmol/litre (Holick et al, 2011) and to maintain adequate vitamin D intake by NZ children the recommendations for adequate intake (AI) are set at 5ug of vitamin D per day (NHMRC, 2006). The international recommended dietary allowance (RDA) and upper intake level (UL) of vitamin D for children is set at 600IU/day and 3000IU/day respectively (Institute Of Medicine, 2011). Furthermore, the vitamin D estimated average requirement (EAR) and RDA for children and adolescents are specified on basis of the serum 25(OH)D concentrations of 40nmol/litre and 50nmol/litre respectively (Institute Of Medicine, 2011).

2.7.1.3 Phosphorous:

Similar to calcium, phosphorus also plays an important role in bone mineralisation with the majority constituted within the bones and teeth (Peacock, 2010). Phosphorous deficiency is uncommon in humans unless hydroxide medication is taken or suffering from a chronic kidney disease (Allen, 1982).

Phosphorus is considered to be an important micronutrient required for bone deposition, however, the “acid-ash hypothesis” raises some concerns regarding its intake (Fenton et al, 2009). The acid-ash hypothesis is described as an increase in demineralisation of bone and a decrease in urinary calcium retention due to high acid load from the diet where dietary phosphorus is considered to be a major contributor towards acid production. High phosphorus levels might also form insoluble calcium-phosphate complexes which lower calcium availability for absorption (Heaney, 2000).

Studies however have shown that high phosphorous intake does not negatively interfere with calcium metabolism in healthy subjects as no increase in urinary calcium excretion is observed (Spencer et al, 1978; Heaney, 2000; Fenton et al, 2009) and also there is no firm evidence that increased phosphate level decreases

bone mineralisation and increases the risk of osteoporosis (Fenton et al, 2009). In older people who consume calcium carbonate or calcium citrate supplements, low serum phosphorus levels are found due to high calcium intake. Despite high calcium consumption, this phosphorus deficiency can lead to hyperphosphatemia and thus weakened bone (Heaney and Nordin, 2002).

2.7.1.4 Magnesium:

Magnesium is the second most abundant intracellular cation, two-thirds of which is located in the skeleton. Magnesium plays an important role in maintaining the integrity of the skeleton by influencing both bone matrix and minerals (Jahnen-Dechent and Ketteler, 2012). In bones, magnesium is not an important part of the hydroxyapatite crystals but is located on the outer lattice surface (Sojka, 1995).

Magnesium absorption mainly takes place in the small intestine where the amount absorbed is linear to the amount consumed and when dietary intake is low, only slight increases in absorption occur. Magnesium absorption through the intestine occurs via two pathways; non saturable passive transport and saturable active transport with the majority of absorption occurring via the former process (Vormann, 2003). Intracellular magnesium accounts for the majority of total body magnesium and the extracellular magnesium only contributes 1%. Thus, under normal circumstances intracellular magnesium concentration is tightly regulated but the extracellular concentration can often fluctuate (Jahnen-Dechent and Ketteler, 2012).

Magnesium deficiency is not very common in people who consume a western diet, but none the less hypomagnesemia can still occur. A trial in rats showed that 50% reduction of magnesium from RDI resulted in (1) a decrease in osteoblasts, (2) increase in osteoclast production, and (3) bone loss. Furthermore, these effects were exacerbated by calcium deficiency (Rude et al, 2009). People suffering from bone disorders are also likely to suffer from magnesium deficiency. Some other risk factors for magnesium deficiency are increasing age, alcohol consumption, malabsorption, and diabetes (Rude et al, 2009). During magnesium depletion the bone quality is altered and perfect crystals start to form rather than irregular ones. Due to this change in structure, the bones (trabecular more than compact bone) become brittle and fragile (Tucker et al, 1999). Magnesium homeostasis is also

essential for optimal calcium metabolism as hypomagnesemia causes increased PTH secretion which in turn leads to hypocalcemia and low BMD. Furthermore, magnesium deficiency might also alter the normal metabolism of vitamin D by either a direct action on the kidneys by decreasing 1,25-$(OH_2)D_3$ synthesis or by decreasing PTH release (Velazguez et al, 1999).

2.7.1.5 Zinc:

Zinc is an important trace element that is essential for growth and maintenance of the skeleton. Zinc is required by almost all body tissues but bone tissues have the highest concentrations and in case of zinc depletion bone homeostasis is strongly altered (Eberle et al, 1999).

The main functions that zinc plays in bone metabolism are: stimulation of osteoblast formation and inhibition of osteoclastogenesis. In cell culture studies, bone growth stimulating factors IGF-1 and TGF-β1 were significantly increased by the addition of zinc (Yamaguchi, 2010). Zinc promotes bone growth and it also plays an important role in inhibiting the production of bone resorbing components. Zinc has been shown to reduce the effects of PTH and pro-inflammatory cytokines like IL-1 and it also helps in inhibiting the early stage osteoclast-like cell formation from marrow (Yamaguchi, 1998).

Due to the anabolic properties, zinc is believed to be essential for normal growing bones, it also helps in repairing fractures, and can also be considered as a therapeutic drug for treatment of bone disorders (Eberle et al, 1999).

2.7.1.6 Sodium:

In 1961 it was shown that urinary calcium and urinary sodium excretion mechanisms are related as sodium intake reduces calcium retention by the kidneys (Walser, 1961). Both of these micronutrients compete with one another for reabsorption at the renal tubule (Lanou et al, 2005). Urinary sodium is believed to be one of the determinants that effects urinary calcium excretion as shown in a study of pre-adolescent girls (Matkovic et al, 1995). This study also concluded that a high sodium and low calcium diet can lead to increased urinary calcium loss which can then have a detrimental impact on the skeletal health. Another study performed on children and adolescents also showed that sodium significantly increased calcium excretion and an increase of 0.9mmol urinary calcium excretion

occurred per 100mmol of sodium excreted (Shi et al, 2012). With a high sodium load the loss of urinary calcium increases leading to a drop in the calcium ion concentration within the extracellular fluid, which then increases the secretion of PTH and subsequently 1,25-$(OH_2)D_3$. These changes then result in an increase in bone turnover rate and if the calcium consumed is less than the calcium amount required, bone mass is negatively affected (Heaney, 2006). In short, high sodium intake could impair calcium re-absorption, making the body susceptible to a risk of low BMD and high fracture rate.

2.7.1.7 Proteins:

The association of protein and calcium functioning is complex, with protein showing both a positive and negative effect. Both high and low protein diets are believed to alter calcium homeostasis leading to hypercalciuria and hypocalciuria respectively. (Allen, 1982). Diets high in protein (mainly animal source) increase acidosis and the bone is then used to buffer this acid load (Jesudason and Clifton, 2011). The constant balancing of pH homeostasis results in increased urinary calcium excretion and demineralisation of bones. Also diet induced acidosis can result in hypercalciuria, and the suggested reasons for this mechanism to occur are direct dissolution of bone by acid, reduced renal reabsorption of calcium, and enhanced cell mediated resorption of bones (Dawson-Hughes, 2003).

This notion, however, is surrounded by controversy as numerous studies have shown no significant negative impact of a high protein diet upon calcium balance. A review by Calvez and colleagues (2012) established a relationship between calcium and protein metabolism. The review concluded that in healthy subjects, a high protein diet did not significantly increase calcium excretion and kidney stone formation especially when calcium intake was adequate. Furthermore, it was concluded that protein consumption had an impact on calcium balance only when the calcium intake was inadequate. A study in healthy postmenopausal women showed a positive effect, as dietary protein increase of 10-20% resulted in enhanced calcium absorption rather than increased calcium excretion (Hunt et al, 2009).

Not only high but low protein levels can affect calcium metabolism as well. Therefore, optimal protein intake should be maintained through diet because

protein deficiency can cause secondary hyperparathyroidism in humans (Conigrave et al, 2008). In protein deficiency, parathyroid hormone (PTH) levels increase resulting in elevated 1,25-$(OH_2)D_3$ concentrations as well. These hormonal levels remain elevated for approximately 2-4 weeks which ultimately results in reduced intestinal calcium absorption (Kerstetter et al, 2003).

2.7.1.8 Fibre:

Dietary fibre is the indigestible component of plant derived foods which aids in maintenance of healthy gastrointestinal tract movement and cardiovascular health (Bosscher et al, 2001). Despite the advantageous effects, some adverse consequences are also observed such as reduction in the bioavailability of essential micronutrients e.g. calcium, iron and zinc which then leads to poor bone mineral status. Extensive research has shown that in fact it is not dietary fibre that is the main culprit but phytates and oxalates which are commonly associated with it. Two research trials concluded that wheat fibre extracts significantly reduced calcium absorption due to high phytate content and not the fibre per se (Kennefick and Cashman, 2000; Harrington et al, 2001).

Phytates and oxalates form insoluble phytate-calcium, phytate-iron, phytate-zinc complexes (Heaney et al, 1991) and calcium-oxalate salts (Weaver et al, 1997) which prevent the minerals from being absorbed by the body. In nations like China where the majority of calcium intake is via vegetables rather than milk, calcium insufficiency is a big problem to address. The incidence of fractures is high in Chinese people most probably because of reduced dairy consumption and low bioavailability of calcium from plant sources (Lau et al, 1991).

2.7.2 Hormones and bone metabolism:

2.7.2.1 Parathyroid hormone (PTH):

Extracellular calcium concentration is constantly sensed by calcium sensing receptors (CaSR) which are present in PTH producing chief cells of parathyroid gland (Hoenderop et al, 2005). PTH, therefore, plays an important role in maintaining extracellular calcium and phosphate concentrations while acting on the intestinal, renal and bone tissues (Khanal and Nemere, 2008). PTH levels are strongly correlated with calcium homeostasis as during low calcium levels, PTH is released into blood and from there it travels to target receptors where it is bound

to the PTH/PTH receptor protein (PTHrP) receptor to stimulate calcium reabsorption from bone and kidneys (Perez et al, 2008). The effect of PTH on bone and kidney can be either direct or indirect via the production of 1α-hydroxylase which in turn increases $1,25(OH)_2$ D_3 synthesis (Nemere and Larsson, 2002; Khanal and Nemere, 2008).

PTH regulation of bone can be either anabolic or catabolic but the mechanisms by which PTH stimulates bone resorption (catabolic) are better understood than processes of bone formation (anabolic) (Silva et al, 2011).

The effect of PTH released in an intermittent pattern is anabolic as an increase in collagen synthesis is observed afterwards. An in vitro study has shown that PTH stimulates osteoblast-collagen synthesis by increasing osteoblast proliferation rate in a manner that is similar to the effect of IGF-1 (Canalis et al, 1989). In addition to affecting gene regulation and cell proliferation, PTH has also been shown to increase the absorption of calcium by enterocytes in the intestine (Nemere and Larsson, 2002).

When PTH administration exists for longer continuous periods bone resorption exceeds bone formation rate. PTH along with Vitamin D stimulates RANKL activity and suppress OPG leading to osteoclast proliferation. Furthermore, the abnormally high levels of PTH in the blood and calcium deficiency can likely lead to primary hyperparathyroidism (Silva et al, 2011).

2.7.2.2 Calcitonin:

Calcitonin (CT) is a polypeptide hormone that is secreted by the parafollicular cells of the thyroid gland and it acts directly on the osteoclasts via calcitonin receptors (CTRs) (Pondel, 2000; Hirsch and Baruch, 2003). CT was first discovered over 50 years ago but its role in mammalian skeletal physiology is still not fully known (Martin and Sims, 2015). CT is secreted when plasma calcium concentrations are high and it is considered a regulator of calcium metabolism as it inhibits bone resorption particularly at the cortical sites (Nieves et al, 1998). It was also suggested that CT increases levels of cyclic adenosine monophosphate (cAMP) which then enhances the expression of active vitamin D encoding enzyme, the cyp27b1 (Yoshida et al, 1999). Another study also showed similar results and stated that CT maintains calcium balance indirectly via a pathway that

is different from what PTH would use (Murayama et al, 1999). Therefore, CT can promote calcium homeostasis by regulating the production of active vitamin D.

Over the past decades, it was mainly thought that CT regulated the bone resorptive process, however, a surprising finding came forth when a study showed that knockout mice lacking the CT encoding gene had a higher bone formation rate than that of the wild type mice (Hoff et al, 2002). A recent study performed in mice showed that the osteoclast expression of Spinster Homologue 2 *(Spns2)*, which mediates export of a transporter for the sphingolipid called sphingolipid 1-phosphate (S1P), was suppressed by CT. Mice that lacked CTRs had increased levels of S1P which resulted in higher osteoclast activity. Another interesting finding was that bone formation increase in CTR deficient mice was also due to locally produced S1P. When S1P antagonists were administered to the CTR deficient mice, not only a decrease in osteoclast activity was observed but an osteoanabolic action was also seen (Keller et al, 2014). These findings, thus, provide new areas of research regarding calcitonin's role in human physiology.

2.7.2.3 Thyroid hormone (TH):

Thyroid hormones, Thyroxine (T_4) and 3,5,3'-L-triiodothyronine (T_3), play an important role in bone metabolism. In children T_3 which is the active hormone, is required for bone development and linear growth as it affects both endochondral and intramembranous bone (Gogakos, 2010). T_3 also enhances the expression of the fibroblast growth factor receptor-3 (FGFR-3) gene. FGFR-3 is a FGF pathway protein that plays an important role in the stimulation of cell growth and any mutations in the *FGFR-3* gene may cause achondroplasia, that is the main cause of dwarfism in humans (Shao et al, 2006).

Hypothyroidism occurs when serum thyroid stimulating hormone (TSH) levels are increased but T_3 and T_4 serum levels remain normal (Cooper and Biondi, 2012). Children with hypothyroidism can have delayed bone maturation and the bone-tendon reflexes might be slow (Setian, 2007). Hyperthyroidism is a condition where the serum TSH levels are very low but the levels of T_4 and T_3 remain normal. It can occur due to iodine deficiency and can increase the risk of fractures and osteoporosis by increasing the bone turnover rate (Cooper and Biondi, 2012). During thyrotoxicosis i.e. hyperthyroidism, children show

accelerated growth and bone development which can result in the premature closure of growth plates (Reddy et al, 2012). In adults, the risk of fractures is increased in both TH deficiency and excess (Duncan-Bassett and Williams, 2003).

At a cellular levels TH affects bone metabolism by interfering with calcium balance. Evidence has shown that long term hyperthyroidism alters calcium balance by increasing bone resorption. Two studies comparing hyperthyroid rats and hypothyroid rats showed that the latter group had a reduced rate of calcium influx at the brush border membrane vesicles and also a decreased rate of calcium efflux from the basolateral membrane of the enterocytes probably due to changes in membrane fluidity (Kumar and Prasad, 2002; Kumar and Prasad, 2003). Another finding was that the calcium transporting Na^{+}-Ca^{2+} (NCX) pump within enterocytes was also activated by TH. Moreover, a synergistic association has been shown between 1,25-dihydroxyvitamin D_3 and thyroid hormone as these two hormones enhance each other's effects (Cross et al, 1990).

2.7.2.4 Growth hormone (GH):

Growth hormone (GH) plays a vital role in regulating linear growth and bone homeostasis in childhood and adolescence. It stimulates the proliferation of cartilage, bone, and other tissues by increasing the number but not the size of cells (Ohlsson et al, 1998). The production of GH involves a complex pathway which is controlled by peripheral and central signalling mechanisms (Giustina et al, 2008). The anterior pituitary gland is responsible for synthesising and secreting GH, and within the hypothalamus, the growth hormone releasing hormone (GHRH) stimulates, and somatostatin suppresses, GH secretion (Gat-Yablonski et al, 2009). Figure 2.8 shows the pathway of GH production.

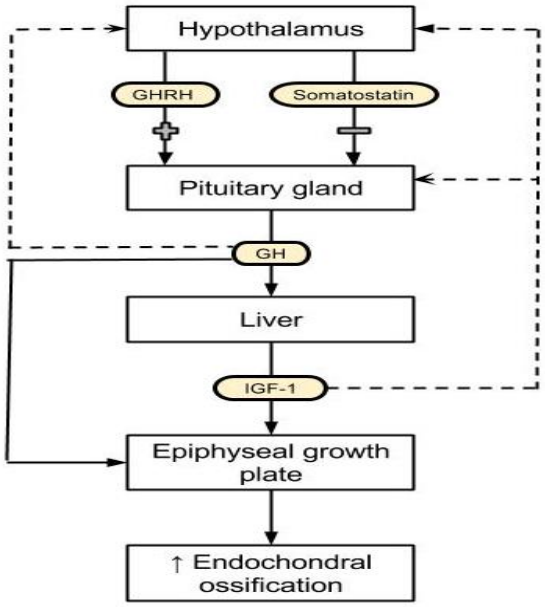

Figure 2.8: GH and IGF-1 effects upon linear bone growth. (Solid line shows a positive effect and dotted line shows a negative feedback effect).

GH can affect linear growth directly by binding to the growth hormone receptors (GHRs) in the epiphyseal growth plate (EPG) or indirectly via insulin like growth factor-1 (IGF-1) (Figure 2.8). IGF-1 is a single polypeptide chain the production of which is stimulated by growth hormone (GH) in not only the liver but also within bone and other non-skeletal tissues. IGF-1 binds with the insulin like growth factor-1 receptor (IGF-1R) resulting in stimulation of bone cell proliferation and enhancing growth (Gat-Yablonski et al, 2009). Chondrocytes are the cells found in healthy cartilage and their differentiation in the EGP determines long bone growth. GH acts directly upon prechondrocytes in the EGP, leading to IGF-1 production (Mackie et al, 2011). GH-IGF-1 axis, thus, is responsible for determining the size of skeleton and also for promoting longitudinal bone growth (Isaksson et al, 1982). Growth defects and low bone mass can arise due to suppression of the GH/IGF-1 axis by inflammatory cytokines or because of growth hormone deficiency (GHD) (Ohlsson et al, 1998; Gahlot et al, 2012).

In addition to the stimulation of growth, the GH/IGF-1 axis exerts anabolic effects on bone modelling and the remodelling cycle (Giustina et al, 2008). IGFs have been shown to have an anabolic effect on osteoblasts similar to chondrocytes. IGF-1 stimulates bone formation mainly when in low concentrations. At higher

concentrations it still promotes the formation of bone but increases bone resorption as well (Ohlsson et al, 1998).

2.7.2.5 Glucocorticoids (GCs):

Due to their immunosuppressive and anti-inflammatory properties, glucocorticoids are used as a medicine to treat numerous diseases such as asthma (Christakos et al, 2011). Usage of GCs has been shown to reduce bone mineral density as they inhibit functioning of mature osteoblasts and insulin like growth factor 1 (IGF-1) (Canalis, 2005). In addition to bone resorption, GCs also reduce intestinal calcium absorption efficiency. Trials conducted on mice showed that calcium absorption decreases in the duodenum due diminished activity of transport proteins TRPV6 and CB_{9k} (Lee et al, 2006; Huybers et al, 2007). These studies have also concluded that the effect of GCs upon calcium absorption is direct rather than via the vitamin D assisted pathway (Lee et al, 2006; Huybers et al, 2007; Scholz-Ahrens et al, 2007).

2.7.2.6 Oestrogen:

Sex hormones play an important role in regulating and maintaining bone growth and development. Oestrogen is the main mediator of growth not only in adolescent girls but boys as well and its presence is especially important during the pubertal growth spurt. The human growth spurt is a two way process as on one side it accelerates the growth and on the other side it leads to epiphyseal plate fusion (Cutler Jr, 1997). Girls have an early pubertal maturation compared to boys because of higher levels of oestradiol and faster oestrogen induced epiphyseal closure (Cutler Jr, 1997).

Oestrogen can influence bone growth directly and indirectly. In direct mechanisms oestrogen stimulates osteoblastogenesis and inhibits osteoclastogenesis via three oestrogen receptors (ER); ERα, ERβ, and ER GPR30 that are expressed in the EGP (Chagin and Savendahl, 2007). Oestrogen prevents bone resorption by suppressing the production of inflammatory cytokines like IL-6, IL-1, M-CSF, and TNF-α and is also used as a therapeutic drug for treating low bone mineral density levels (Syed et al, 2010). During early stages of bone cell proliferation, oestrogen indirectly affects the hematopoietic cell lineage and its deficiency is one of the main causes of osteoclast hyperactivity (Redlich and

Smolen, 2012). Oestrogen deficiency can result in the increased production of osteoclasts by increasing expression of RANK and decreasing OPG activity (Redlich and Smolen, 2012).

During menopause the ovarian function ceases and production of oestrogen declines. This oestrogen deficiency can then lead to an increase (1) in the loss of bone mass and (2) incidence of fractures (Eastell and Hannon, 2008). In post-menopausal women, oestrogen treatment along with calcium supplementation showed beneficial effects upon bone health (Bone et al, 2000). Oestrogen treatment has shown to also increase the risk of diseases such as breast cancer, CVD, and stroke (Writing Group for the Women's Health Initiative Investigators, 2002). Schierbeck et al (2012) studied these effects further and their 10 yearlong RCT concluded that early initiation of oestrogen therapy in menopausal women does not impose any harmful influence on the heart health. For cancer risk the outcome was inconclusive, due to not being able to follow up for longer.

2.7.3 Other determinants of bone mass:

Bone mineral content (BMC) in peripubertal children is dependent upon numerous determinants such as genetics, physical activity level, body fat content, hormones, and diet (Casazza et al, 2010).

2.7.3.1 Genetics:

Genetics are the main determinant of bone mass (Davies et al, 2005). Decades ago Smith and colleagues (1973) showed that genetics was a significant determinant of bone mass at maturity, and it alone or along with environmental factors can effect osteoporosis development. The strong influence of genes on bone status is shown in a study where daughters of osteoporotic women had significantly reduced BMD compared to those women who had healthy mothers (Seeman et al, 1989). Similarly another study also showed that daughters of women with hip fractures are at a higher risk of suffering from hip fractures as well due to low BMD (Seeman et al, 1994). The role of genetics on bone mass can also be seen in a twin study including both monozygotic and dizygotic twins (Hunter et al, 2001). The results of this study concluded that genes influenced variance in bone formation by 74% and bone resorption by 52%, however, this variance significantly changed after menopause. Individuals belonging to

different racial backgrounds have different genetic makeup and hence would have different bone structures. A study examining BMD in different racial groups concluded that black individuals had significantly higher areal and volumetric bone density in comparison to non-black (Horlick et al, 2000). Another study also showed similar outcomes along with Asian females having the lowest BMD followed by white and then Hispanic females. In men, however, Hispanic males had the lowest BMD followed by Asian and then white males (Bachrach et al, 1999).

The exact and main genes which are associated with bone mass are yet not fully known explaining only a fraction of this phenomenon (Delgado-Calle et al, 2012). One of the main reasons being that genes are not the only single determinant of bone mass and numerous environmental factors interact with the genes. In addition, the intrauterine environment of a foetus can affect the skeletal development from the start of life (Delgado-Calle et al, 2012). Therefore, the epigenetic mechanisms from embryonic development should be carefully studied because factors like maternal stress, gestation time, and nutritional levels can immensely alter the gene functionality for life (Holroyd et al, 2012).

2.7.3.2 Physical activity:

Childhood is a period of rapid bone growth and is the optimal period of life when long term skeletal benefits can be attained. Along with a diet that is rich in calcium and vitamin D, physical activity or exercise is also required to attain optimal PBM and a strong bone structure (Specker and Vukovich, 2007). In young children, physical activity has a very strong relationship with bone strength as mechanical loading exercises, especially, during the first two decades of life stimulate greater bone mass deposition (Anderson, 2000). A prospective study in children showed that physical activity was associated with gain in skeletal mass especially in the radius and hip region regardless of the age or gender (Slemenda et al, 1991). Weight bearing physical activities like running and dancing increase bone mass in children. About 20-30 minutes of moderately intense exercise is recommended to attain the maximum benefits (Heaney et al, 2000). A summary of studies which describe the relationship between bone health and physical activity is present in section 3.

2.7.3.3 Body fat:

The relationship between adiposity and bone health is debatable as research studies have shown inconclusive results. Fat mass has been proposed to exert a beneficial effect on bone mineral status via the phenomenon of mechanical loading (Cao et al, 2011). The total body fat can apply a load on the skeletal bones which could then stimulate the proliferation of osteoblasts and osteocytes (Ehrlich and Lanyon, 2002). Several human studies have supported this notion by showing body fat to be a positive predictor of bone status (Pietrobelli et al, 2002; Ackerman et al, 2006; Clark et al, 2006; Ka et al, 2013).

A recent review of research trials, showed the opposite and concluded that body fat mass has a negative and detrimental influence upon bone density (Fazeli et al, 2013). Similarly, other bone health trials have also found adiposity to 'not' have a protective role on bone health as analyses have shown obese children to have lower bone mass and density and also have a higher risk of bone fractures (Goulding et al, 2001; Goulding et al, 2005; Rocher et al, 2008). The exact mechanism of how this complex association occurs is not yet fully known. It is suggested that pro-inflammatory cytokines like TNF-α, IL-1, and IL-6, which are released at an abnormal rate in obese patients are also shown to be key regulators of bone resorption and osteoporosis (Mundy, 2007). Thus, these cytokines may contribute to the complex bone-fat phenomenon. Osteoblasts and adipocytes are derived from the same multipotent mesenchymal stem cell and therefore, could interfere with the functioning of one another (Cao et al, 2011). A study in rat models showed that the activation of osteoblast formation occurred at the expense of inhibition of adipogenesis (David et al, 2007). Hormones like leptin, oestrogen, insulin, and resistin might also affect the bone-adipocyte association (Magni et al, 2010; Cao et al, 2011).

In addition to achieving a high PBM and maintaining good bone mineral status, keeping an optimal and healthy weight is necessary for the prevention of many other diseases like diabetes mellitus, cardiovascular disease, and metabolic syndrome (McDevitt et al, 2014).

SECTION 2: Importance of Milk/Dairy Products

Dairy products are high energy yielding nutrient rich foods which support growth and development especially during the period of childhood and adolescence (Fiorito et al, 2006). Milk is the most commonly consumed dairy product and is a source of both macronutrients (carbohydrates, lipids and proteins) and numerous micronutrients such as calcium, iodine, phosphorous, potassium, magnesium, zinc, vitamin D, vitamin A, vitamin B12, and vitamin B2 (Dror and Allen, 2013). In comparison to other beverages milk provides the best nourishment as it contains numerous essential nutrients especially calcium (Miller et al, 2007). Some frequently consumed dairy products across the globe include cheese, yoghurt, cream, and fermented milk.

2.8 Milk composition:

In general, milk is referred to as cow milk, however, human beings also consume milk of other mammalian species. In Asian countries, particularly buffalo, goat, and sheep milk are widely used (FAO, 2013). A few other mammals which are less commonly used for their milk across different regions of the world include yak milk, camel milk, and reindeer and moose milk (FAO, 2013). As cow milk is the most frequently used type of milk in a western society its composition will be further discussed below. Table 2.3 provides the nutritional information of cow milk that is most commonly consumed by the NZ population. And table 2.4 shows the nutrient composition of whole milk.

Table 2.3: Composition of Anchor whole milk (blue top) and skimmed milk (light blue) per 100ml (Anchor, 2014).

Nutrition	Whole milk	Skimmed milk
Energy (kJ)	260	194
Protein (g)	3.3	3.3
Fat-total (g) **-Fat-saturated (g)**	3.3 2	1.5 0.9
Carbohydrate-total (g) **-Carbohydrate-sugars (g)**	4.8 4.8	4.9 4.9
Sodium (mg)	40	41
Calcium (mg)	120	120

Table 2.4: Nutrient composition of whole milk (The Dairy Council, 2012).

Constituent	Per 100ml
Energy (KJ)	282
Protein (g)	3.4
Carbohydrate (g)	4.7
Fat (g)	4.0
Dietary fibre (g)	0
Thiamin (mg)	0.03
Riboflavin (mg)	0.24
Niacin (mg)	0.2
Vitamin B6 (mg)	0.06
Vitamin B12 (ug)	0.9
Folate (ug)	8
Biotin (ug)	2.6
Vitamin C (mg)	2
Retinol (ug)	31
Vitamin D (ug)	Trace
Vitamin E (mg)	0.08
Sodium (mg)	44
Potassium (mg)	160
Calcium (mg)	122
Magnesium (mg)	11
Phosphorous (mg)	96
Iron (mg)	0.03
Zinc (mg)	0.4
Selenium (ug)	1
Iodine (ug)	32

2.8.1 Macronutrients:

The major constituent of any kind of milk is water. Lactose is the chief carbohydrate present in cow milk (bovine milk) along with oligosaccharides and glycoconjugates. Colostrum has the highest concentrations of oligosaccharides and glycoconjugates. These molecules are present as important bioactive compounds, with their main function being immunological protection rather than nutrition in the new-born (Gopal and Gill, 2000). Cow milk contains high quality protein providing essential amino acids. Two main forms of protein found in milk are the soluble whey proteins and the insoluble caseins (Pereira, 2013). Whey proteins make up 20% of total milk proteins which mainly consist of branched chain amino acids that play an important role in human metabolism e.g. whey proteins undergo rapid digestion and absorption providing high concentration of plasma amino acids following a meal (Haug et al, 2007). A few important whey

components are β-lactoglobulin, α-lactalbumin, immunoglobulins, albumin, and lactoferrin (Pereira, 2013). Caseins make up around 80% of the total milk protein fraction and its main function is to provide various amino acids. Casein also helps carry calcium and phosphorus, then bind them forming a coagulum which in turn increases their digestibility (Pereira, 2013). The three types of caseins found in milk are α, β, and κ-caseins.

The amount of fat present in milk varies and in bovine milk triglycerides make up about 95% of the lipid fraction. Other lipids present in milk are diacylglycerol, cholesterol, phospholipids, and free fatty acids. Oleic acid is an unsaturated fatty acid also found in milk along with trans fatty acid (vaccenic acid) and conjugated linoleic acid (CLA) (Pereira, 2013). Although these lipids are believed to improve the blood cholesterol levels by decreasing the total cholesterol/high density lipoprotein ratio, more research needs to be undertaken to better understand them (Pereira, 2013).

2.8.2 Minerals:

Milk is also a good source of many micronutrients. Several important minerals found in milk are calcium, phosphorus, magnesium, zinc, and selenium (Hoppe et al, 2006). Section 2 contains details of the interaction of these nutrients with calcium metabolism.

2.8.3 Vitamins:

Milk contains both fat soluble vitamins (A, D (trace amounts), and E) and water soluble vitamins (B_{12} and Riboflavin). Vitamin A helps in maintaining good vision, skin and immunity whereas vitamin D most importantly is involved in calcium and phosphorus homeostasis as well as immunity (Dror and Allen, 2013). Both of these vitamins can be found in cow milk, but only in very small amounts. In many countries including NZ, milk is fortified with vitamin D, however, vitamin A fortification is not common. In New Zealand, only calci-trim (Meadowfresh) and calci+ (Anchor) milk is fortified with vitamin D providing approximately 0.5ug of vitamin D per 100ml (Food Standards Australia and New Zealand-FSANZ, 2013). Vitamin E is also present in bovine milk as α-tocopherol which is a lipid soluble antioxidant capable of scavenging free radicals (Lindmark-Mansson and Akesson, 2000).

2.9 Milk and growth:

An overview of the available evidence suggests that an inverse association exists between dairy intake and bone fractures (Prentice, 2014). Numerous hypotheses have been proposed to describe the beneficial effects of milk on growth (especially skeletal growth) including the effects of the individual nutrients like calcium, phosphorus, vitamin D, and potassium found in milk (Dror and Allen, 2013). A widely accepted concept put forth by Bogin is the "milk hypothesis" according to which optimal milk consumption during early years of life leads to taller height or stature in adulthood. The increment in height is believed to be due to a growth enhancing nutrient or group of nutrients rather than the energy provided by milk (Bogin, 1998). A review by de Beer (2012) summarised various studies looking at the effect of consumption of dairy on physical growth and concluded that on average 0.4cm of additional growth per annum occurs with the daily consumption of 245ml of milk.

Studies have shown that children who avoid cow milk consumption for long term have poorer bone health, shorter stature, higher weight gain prevalence, and increased fracture risks compared to their milk drinking counterparts (Black et al, 2002; Goulding et al, 2004). Children avoid drinking milk for numerous reasons; which can be intolerance, dislike of flavour, or concern of weight gain due to milk fats (Fayet et al, 2013). A rapidly increasing and concerning factor held accountable for low milk intake of children within the Western society is the displacement of milk with high sugar containing soft drinks and caffeinated beverages (Ma and Jones, 2004; Libuda et al, 2008).

2.10 Factors affecting milk intake:

A complex interplay of race, familial/peer influence and environmental factors are involved in the development of a child's dietary habits (Scaglioni et al, 2008).

2.10.1 Ethnicity:

Research has shown that ethnicity plays a vital role in selection of calcium rich or dairy foods. The National Children's Nutrition (NCN) Survey conducted in 2002 showed that the proportion of New Zealand European (NZEO) children drinking milk was higher than Maori and Pacific children (MOH, 2003).

A study conducted in America concluded that white children had a higher dairy consumption rate compared to Hispanic and Asian children (Novotny et al, 2003). The most significant barrier towards calcium rich food consumption in the Hispanic population was displacement of milk with soda drinks mainly due to a dislike of milk's taste, whereas in Asian population the constraints were high mal-digestion rates, mismatch of milk with Asian diet, and very low exposure to milk (Auld et al, 2002; Novotny et al, 2003; Vue and Reicks, 2007).

2.10.2 Sugary drinks:

Soft drink consumption especially by children has rapidly increased over the past few decades and is linked to the rising obesity epidemic (Sturm et al, 2012). Soft drinks not only include the carbonated soda drinks but also involve non-carbonated sugary fruit juices and fruit punch (Fisher et al, 2001). Amongst children sugary beverages are now commonly substituted in place of milk and other nutritious beverages, however, the exact reasons behind it are not known. 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 et al, 2001; Grimm et al, 2004). Some other reasons for increased sugary beverage consumption can be peer influence, television advertisements, increasing age and independency, and availability at school (Grimm et al, 2004; Fiorito et al, 2006).

A cross sectional analyses conducted in New Zealand children and adolescents showed that TV viewing duration (Utter et al, 2006) and holidays (Rockell et al, 2011) were positively related to the consumption of high energy and sugar yielding snacks and beverages. These food products were the most widely advertised commercials on TV, and therefore, children were more likely to prefer these over healthier options like milk.

2.10.3 Parental influence:

Parents especially the mothers are the role models for their children (Scaglioni et al, 2008). A healthy food environment in the home is created by the parents and it is them who shape a child's dietary preferences and eating patterns (Scaglioni et al, 2008). The reasons for why children (especially young children) follow their parents could be either the availability of healthy foods e.g. health conscious

parents would provide their children with healthy foods or it could simply be that the children imitate the parents. Some other socioeconomical and sociocultural barriers faced by parents in providing nutritious food to the children are time constraints for working parents which leads them to buying convenience energy dense foods, parental education level (as a well-educated parent will possibly be more health conscious), and low income of a family can influence the food choices and eating patterns (Patrick and Nicklas, 2005).

Without doubt it is believed that the parents influence a child's eating habits and patterns, but to what extent, it is not yet fully known. An extensive meta-analysis looking at the diet similarity of a parent and child (of any age) showed that only a weak association was present. It was concluded that a strong relationship was not able to be detected because of various confounders present. The confounders identified by the researchers were peer influence, milk intolerance, and breakfast consumption (Wang et al, 2011). Another study stated that in developed countries children eat meals at schools and care centres as well so this factor should not be ignored when observing overall dairy consumption of children (Ortega et al, 1998).

2.11 Adverse reactions to milk:

Two commonly observed adverse reactions to milk due to malabsorption are discussed below:

2.11.1 Lactose intolerance:

Lactose is the primary sugar found only in mammalian milk and is composed of two monosaccharides; glucose and galactose (Heyman, 2006). A β-galactosidase enzyme known as lactase is responsible for the breakdown of lactose into its constituent monosaccharides which are than absorbed by the enterocytes and transported into the blood stream (Pereira, 2013).

For human beings, main sources of lactose are human milk (for mainly young child) and cow milk constituting about 7% and 5% lactose respectively (Kwak et al, 2012). Lactase is the enzyme which breaks down and helps intestinal lactose metabolism. Certain susceptible people are lactose intolerant due to either lactase enzyme deficiency, congenital lactase deficiency, or developmental lactase

deficiency (Prentice, 2014). Lactose intolerance (LI) can be either primary or secondary. Primary or congenital lactase deficiency remains throughout life and cannot be completely cured leading to complete avoidance of lactose. Secondary LI occurs due to gastrointestinal injury and is less severe and is treatable (Lomer et al, 2008).

Around 70% of the world's population has lactase non-persistence but not all suffer from lactose intolerance due to involvement of genetic and other nutritional factors like pattern of dairy food consumption (Matthews et al, 2005). Genes are a very important determinant of lactase deficiency as different racial groups show differing prevalence rates. For instance, Asian population has the highest rate of lactase non-persistence with almost 100% being affected (Lomer et al, 2008). People with lactose intolerance generally have lower bone mass thought to be due to absence of lactase, but recent studies have shown that consumption of lactose containing milk improves calcium absorption more efficiently in lactase deficient rather than lactase tolerant subjects (Tremaine et al, 1986; Griessen et al, 1989), thus the negative effects most probably would be a result of low dairy intake rather than absence of the enzyme itself. Even after the presence of evidence, lactose's effect on calcium bioavailability is still debatable because studies conducted on animal models are not the perfect predictor for humans and also due to various inconclusive results which show no effect of lactose enriched milk or dairy upon calcium absorption (Zitterman et al, 2000).

As lactose is absorbed in the small intestine, the majority of lactose intolerance symptoms are related to the distal gastrointestinal tract. Some typical symptoms are abdominal pain, bloating, flatulence, diarrhoea, nausea, and vomiting (Lomer et al, 2008; Pereira, 2013). LI individuals usually avoid any dairy products which can result in calcium deficient diet. Numerous techniques have been devised to enable lactase deficient people to consume dairy without showing any severe adverse effects. Dairy products like fermented milk, cheese, and yoghurt are considered to be good sources of lactose and calcium in LI individuals. In addition to this, adverse symptoms also may not persist when milk is consumed with a varied diet in smaller portions (Shaukat et al, 2010).

2.11.2 Milk protein allergy:

Around 2-6% of children are affected by cow milk protein allergy (CMPA) with the highest prevalence at one year of age (Caffarelli et al, 2010). The prevalence then starts to decrease with increasing age and is very rare but severe in adults. More than 20 different proteins are found in cow milk and all are believed to cause allergic reactions. Casein and whey make up the bulk of milk proteins and are the most prevalent milk allergies (Kattan et al, 2011).

CMPA can be divided into *immediate* immunoglobulin-E (Ig-E) mediated reactions or the *delayed* non Ig-E mediated reactions (El-Agamy, 2007). Immediate reactions occur within minutes or hours and the symptoms are a variety of skin reactions, gastrointestinal symptoms, respiratory symptoms, and sometimes anaphylaxis. Delayed reactions are T cell dependent and take place over several days or sometimes hours. Symptoms are somewhat similar to the immediate response mainly concentrated around the intestinal and cutaneous regions (El-Agamy, 2007).

CMPA can be managed by numerous methods which include complete avoidance of milk, substituting cow milk with other mammals' milk or milk extracted from plants, and heating and enzymatically treating milk to hydrolyse proteins (El-Agamy, 2007; Kattan et al, 2011).

2.12 NZ milk consumption:

The usage of cattle's milk by human beings is dated back to the seventh millennium BC, showing that milk has always been a major component of their diet (Evershed et al, 2008). Milk is the first food that is consumed by humans after birth. Cow milk is the most common form of milk consumed worldwide, however, sheep and goat milk consumption is also frequent in some countries (Pereira, 2013).

To meet the requirements for calcium in children, NZ Ministry of Health (MOH) recommends consumption of 2-3 servings of dairy foods per day (MOH, 2012a). The 2002 NCN survey findings showed that only 38% of children consumed non-flavoured milk once a day (MOH, 2003). Figure 2.9 shows the proportion of milk consumption by NZ children.

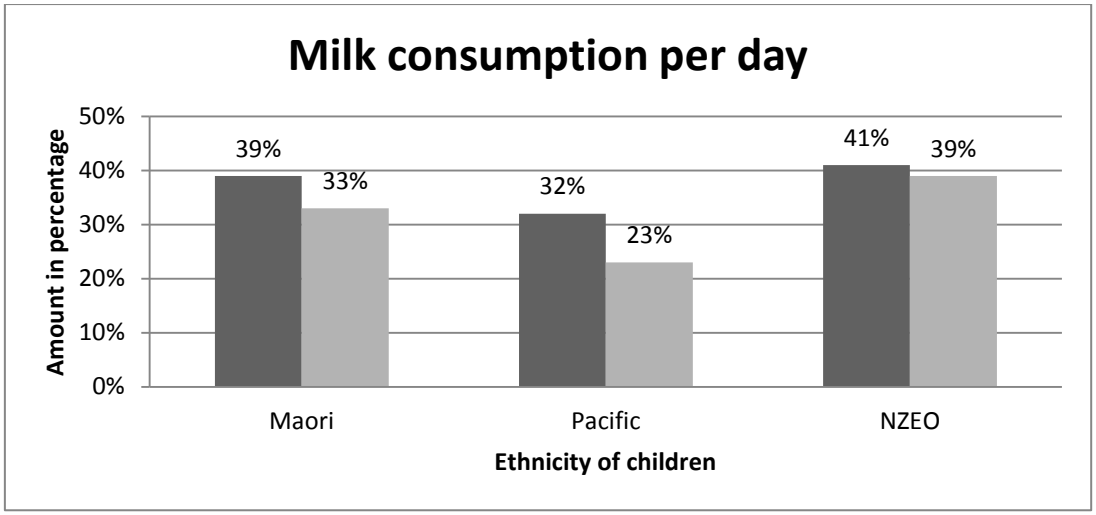

Figure 2.9: Milk consumption of NZ children per day. NZEO = New Zealand European and Other. Modified from MOH (2003).

Over the recent years, production of milk has increased worldwide including New Zealand (DairyCo, 2014), and the Canadian Dairy Information Centre (2014) reported that in New Zealand milk consumption rate has also increased from 73.3 litres per capita in 2008 to 96.8 litres per capita in 2013.

History of New Zealand school milk programme (Bulford and Alexander, 2001):

The New Zealand school milk programme was started at a time when milk production was surplus and it was thought that New Zealand children were malnourished after the great economic depression. The programme ran for 30 years, starting in 1937 and terminating in 1967. The main objective of the school milk programme was to tackle nutritional deficiency by providing half a pint (approximately 230ml) bottle of milk to both primary and secondary school children each school day.

This programme was fully subsidised by the government and pasteurised milk was delivered to schools every morning. However, in winter months dried milk was used as an alternative due to low milk supply. During this programme, 68% of primary and 38% of secondary schools were receiving milk. The beneficial outcomes of the milk supply were reported as increased alertness and attendance among children. Other positive outcomes were increased average height, better dental health, and lower rates of goitre. It should be kept in mind that milk might not have been the only contributing factor to improve health.

The school milk programme was terminated in 1967 due to a number of factors. Bulford and Alexander (2001) stated that the main reason for this was that the annual cost of the programme had risen above what was originally planned. There was also a problem with the distribution of milk as deterioration occurred when temperatures increased and light reduced the vitamin levels. No doubt the school milk programme improved the nutrition of children but it was believed that by the end, this programme had outlived its usefulness and malnutrition actually was never a concern amongst New Zealand children.

SECTION 3: Summary of Studies on Calcium/Milk Supplementation, & Physical Activity

Pre-pubertal children despite having a slower growth velocity in comparison to adolescents still require adequate dairy intake to maintain normal skeletal growth and development (Hoppe et al, 2006). Optimal peak bone mass attainment in childhood or adolescence is strongly related to bone disorders like osteoporosis in later life (McDevitt and Ahmed, 2014). Below are summarised a few studies which examine the effect of dairy or elemental calcium upon bone health and growth of children.

2.13 Intervention trials:

2.13.1 Milk/dairy supplementation:

Main characteristics of trials which showed a significant improvement following milk supplementation are summarised in table 2.5.

In early 20th century one of the first school milk based intervention studies was conducted in Scotland by Boyd Orr (Orr, 1928). It was a relatively simple and well-designed trial where school children were supplied with whole milk, skim milk, biscuits, or no supplementation at all. Both kinds of milk supplementation increased the weight and height by 20% in comparison to the other two groups (Orr, 1928). In addition to growth, at the end of the trial Orr also found that milk

consuming children showed better growth outcomes, with children who were undernourished at baseline attaining the most benefit.

In 1980, socioeconomically disadvantaged British children who were receiving free milk (190ml) in schools were studied. Observations on these children showed that a slight but significant increment was found in height and weight gain within the milk supplemented group. The reason for such a small increase was most probably due to greater baseline heights of children in the control group which then diminished the difference of height in between the study groups (Baker et al, 1980).

A school milk intervention was performed in both rural and urban areas of Nigeria in early 1980s where all children were provided with powdered milk daily on school days (Nnanyelugo, 1984). The results concluded that both rural and urban children had increased calcium intake following the intervention, however, the positive effects of milk were more apparent in rural children. Only rural boys showed significant increases in height and weight probably because they had lower baseline characteristics. This study shows that children who are disadvantaged will benefit the most.

The impact of school milk feeding programme in children of various ethnicities was studied by Chen (Chen, 1989). The results of this study showed that after provision of milk the rate of prevalence of protein energy malnutrition (PEM) significantly reduced. Other than this, the attendance rates of children also improved over the span of this trial most likely due to improved general health as a result of consumption of milk as no other important developmental changes took place at that time period.

Cadogan et al (1997) conducted a randomised controlled trial (RCT) with adolescent secondary school girls. The intervention group was provided with either whole or skim milk based on preference. The controls received no milk. At the end of the trial, the milk supplementation group had significantly greater bone mineral density (BMD) and bone mineral content (BMC) in comparison to controls. Girls who received milk also had significantly higher serum IGF-1 levels and micronutrient intakes. The height, weight, body composition, and bone

markers did not significantly differ between the girls who consumed milk and those who did not.

In China, a school milk based intervention was conducted to study the effects of milk supplementation on pre-pubertal girls who had low calcium and vitamin D intakes (Du et al, 2004). Participants were divided into three groups; (1) received 330ml calcium (560mg) fortified milk, (2) received 330ml calcium (560mg) and vitamin D (8ug) fortified milk, or (3) controls which received no milk. Both milk supplemented groups had significantly higher BMC, BMD, height, and weight compared to controls. When groups 1 and 2 were compared, group 2 had the highest increments in BMC and BMD. Serum PTH concentration was significantly lower in groups 1 and 2 in comparison to group 3. Furthermore, group 2 had significantly lower prevalence of vitamin D deficiency than group 1 and 3. A study with similar characteristics was performed to observe the effects of milk supplementation upon size adjusted total body BMD (Zhu et al, 2008). After implementation of the intervention it was observed that calcium supplementation positively affected BMD with the majority of benefit seen in the lower limbs. Another RCT was conducted in Asian girls where the first group received a high dose of calcium (1300mg) in the form of powdered milk, the second group received a low dose (650mg), and the third group acted as controls (Lau et a, 2004). The results showed that no significant changes in anthropometry were present but both intervention groups had significantly higher BMD in comparison to controls. When the high and low calcium dose groups were compared, no significant difference was present.

Lien et al (2009) provided stunted and underweight pre-pubertal children with fortified and normal milk. Bone parameters were not studied in this trial and height and weight also did not show a significant improvement. However, the results showed that overall health related quality of life and mental health significantly improved along with a significant reduction in stunting rates.

Even though many trials have shown the benefit and efficacy of milk supplementation in children, there are a few studies that have shown no beneficial effects at all. A simple study by Cook et al (1979) concluded that children aged 6-7 years did not show any significant improvements in health and growth outcomes

after consuming milk from 1972 to 1976. Similarly a RCT conducted in pre-pubertal New Zealand children showed that 18 months of milk powder supplementation (1200mg/d) neither improved the bone status or the anthropometric variables (Gibbons et al, 2004). The authors concluded that children included in the trial already had high calcium intakes, so calcium supplementation would be more effective when targeted at children who have low habitual dairy consumption.

2.13.2 Calcium enriched food intervention trials:

In addition to using dairy products or elemental calcium, foods enriched with calcium can also be used as supplements in intervention trials. Table 2.6 summarises main characteristics of studies which use calcium enriched food products as an intervention supplement.

A well-known RCT was performed by Bonjour and colleagues where the effects of calcium enriched foods were studied upon the bone mass at various skeletal sites (Bonjour et al, 1997). Pre-pubertal girls were divided into either the intervention or control group. The intervention group consumed different food products which were fortified with milk powder containing calcium and the controls received similar foods but without any fortification. After 12 months of intervention, high calcium intakes led to an increase of 3.5-5% of bone mass accretion per year with greatest benefits in the appendicular skeleton. BMD at all skeletal sites and BMC was significantly higher in the intervention group compared to controls. Girls who had lower baseline calcium intakes were found to be the ones most benefited from supplementation in terms of height gain when examined after 1 year follow up. Furthermore, the higher growth rate of intervention group was attributed to increased stimulation of bone modelling possibly due to calcium.

Another RCT where food products enriched with milk minerals were administered to the intervention group was performed by Iuliano-Burns and colleagues (2003). In this trial along with supplemented food, exercise intervention was also introduced to extensively study the combined effects of calcium and physical activity upon bone health (Iuliano-Burns et al, 2003). This study concluded that bone mass was most efficiently improved by calcium supplementation

accompanied by exercise rather than any one of these interventions alone. In addition to the combined effect, moderate exercise but not calcium supplementation led to increased bone mass at tibia and fibula (loaded sites) and calcium supplementation but not exercise improved bone mass status at the humerus and ulna-radius regions (non-loaded sites).

In pre-pubertal boys bone mass gain was studied by supplementing food products with milk based calcium-phosphate salt (Chevalley et al, 2005). The intervention group consumed calcium enriched food products daily with the controls receiving similar food products but without added calcium. After 12 months of intervention the areal BMD significantly increased at appendicular skeletal sites in the intervention group compared. No changes in BMD were observed at the lumbar spine in both groups. After a further 12 months of follow up, the improvements in BMD were still observed in pre-pubertal boys but whether this effect will still be present after several years needs to be further studied. A positive but not significant association was found between physical activity and calcium supplementation mainly due to smaller sample size. Similarly no significant anthropometric differences were found between both groups.

2.13.3 Elemental calcium supplementation trials:

Table 2.7 summarises the main characteristics of intervention trials studying elemental calcium supplementation and its effects upon bone status and growth of children.

A co-twin model study conducted by Johnston and colleagues in the early 1990s placed one twin of each pair in the intervention group where they received calcium tablets and the other twin in the control group (Johnston et al, 1992). After 3 years of supplementation, calcium supplemented group showed a significant increase in BMD at some skeletal sites in comparison to controls. The intervention group also had higher serum osteocalcin concentrations then controls. One more interesting finding of this trial was that younger children benefited the most from this supplementation. Similar results were seen in Chinese children who were supplemented with a much lower dose of calcium per day (Lee et al, 1994). The calcium supplemented children had significantly greater BMC and BMC/BW in comparison to the placebo group but height increment remained

unchanged (Lee et al, 1994). Another RCT conducted in Chinese children studied the effect of calcium supplementation upon the bone mineral acquisition of more specific skeletal sites (Lee et al, 1995). The results of this trial showed that children who received calcium had significantly higher lumbar spine and radius bone mass but no changes were observed in the bone mass of femoral neck.

Gambian children who had lower dietary calcium intake were selected to take part in a RCT where the intervention group received 1000mg of calcium per day and the controls received a placebo tablet (Dibba et al, 2000). At the end of the trial period calcium supplemented children had significantly higher BMC, size corrected BMC, and BMD but no changes in anthropometric measures were seen. An interesting finding of this study was that despite the increase in bone mass, serum osteocalcin levels decreased within the intervention group. This phenomenon is believed to have occurred due to bone remodelling transient where high calcium level causes osteoclasts to inactivate at the start of remodelling which in turn decreases bone resorption as well as bone formation via the bone remodelling cycle (Dibba et al, 2000). Another RCT conducted in pre-pubertal Gambian boys who had low baseline calcium intake showed that calcium supplementation improved bone health only during mid-adolescence and not later on (Ward et al, 2014). This trial was also followed up for 12 years which showed that as calcium supplementation stopped, the beneficial effects seen on bone health started to decrease as well.

Physical activity also is believed to play a vital role in maintaining good bone health as shown in a RCT that exercise plus calcium supplementation was the most beneficial with highest BMD. Calcium without exercise showed no significant improvement in BMD acquisition (Courteix et al, 2005). Cameron et al (2004) found a significant increase in areal BMD at different skeletal sites after 12 to 18 months of intervention but unfortunately similar results were not perceived after 24 months. The height and weight did not differ at all throughout the trial period of this study. Somewhat similar findings were presented by Matkovic et al (2005) where BMD increased after 4 years of intervention but after 7 years of follow up these effects diminished. Ward et al (2014) also presented similar results where an increase in BMC and BMD of whole body, lumbar spine, and

total hip was seen but only during the period of supplementation. After the supplementation stopped the effects started to diminish.

In conclusion, increasing calcium intake either via diet or supplementation generally improves growth and bone outcomes in children who have low baseline calcium intake. Vitamin D and physical activity might further enhance the beneficial effects of calcium supplementation towards bone strength. However, in children who already have an adequate calcium intake, calcium supplementation as a public health intervention has not been proven very useful so far.

Limitations of the data available so far would be that majority of these trials did not determine fracture risks. Also these results cannot be used to represent children who are suffering from any medical conditions which affect bone metabolism because these trials used a healthy population of children.

Further work needs to be done to fill the gaps in research such as more long term calcium supplementation trials should be conducted in 'pre-pubertal' children who have low baseline calcium intake as not enough data are present for this age group. Many nutrients and other confounders like ethnicity, physical activity level, and gender should also be explored when carrying out these studies.

Table 2.5: Summarisation of milk and dairy supplemented intervention trials in children.

References	Study Population	Study Design	Study Duration	Treatment	Main Findings
Orr (1928)	-n=40-50 children per group -age = 5-14 years (M & F)	-RCT -Height and weight measured at 3 consecutive days.	7 months	-Group 1: received whole milk. -Group 2: received separated milk. -Group 3: received biscuits with similar energy value as separated milk. -Group 4: acted as controls and received nothing. *Volume:* *5-6 years age = 3 quarters of a pint per school day* *8-9 = 1 pint per school day* *13-14 = 1 and a quarter pint per school day*	Milk supplemented groups had a significantly greater increase in height compared to the other groups however the difference between groups 1 and 2 is insignificant. Similar to height, weight was also significantly higher in milk supplementation groups. Altogether addition of milk increases weight and height by 20%.
Baker et al (1980)	-n = 581 -age = 7-8 years (M & F)	-Double blind RCT -Height and weight recorded at baseline and 6 subsequent visits. -General questionnaire evaluating lifestyle administered to parents.	24 months	-Intervention group: received 190ml of free milk daily. -Control group: no milk provided.	A slight significant increase was found in the height and weight of milk supplemented group when compared to control group. The gain in height was 3% and gain in weight was 130g at the end of trial.
Nnanyelugo (1984)	-n = 246 -age = 5-10 years (M & F)	-Intervention trial -Height & weight measured.	12 months	Intervention: consumption of full cream milk powder which provides 135.50mg of calcium per 100ml.	Children in both rural and urban environments who drank milk daily on weekdays had significantly higher calcium intake then those who did not.

Table 2.5 continued: Summarisation of milk and dairy supplemented intervention trials in children.

References	Study Population	Study Design	Study Duration	Treatment	Main Findings
		-Questionnaire used to assess household food consumption pattern. -In urban areas dietary intake was measured by 7 day weighed food records. -In rural areas dietary intake was measured by 4 day weighed food records. -All questionnaires were administered every 3 months.		A dose of 250ml milk was supplied to all children on weekdays. Gradually the amount of milk consumed increased to 300ml only in older children aged 8-10 years.	Only rural school boys had significantly higher weights and heights compared to non-drinkers. Beneficial effects of milk supplementation were more apparent in rural rather than urban children.
Chen (1989)	-n = 2766 -age = 6-9 years (M & F)	-Intervention trial -Weight & height measured at baseline, mid and end of trial. -Parent's occupation, school attendance, and nutritional status were assessed.	24 months	Intervention: 250ml of cow milk twice a week.	During the study period a significant decline was observed in the prevalence of stunting, underweight, and wasting. Following the school milk feeding programme the attendance rates also increased.
Cadogan et al (1997)	-n = 82 -age = 12.2 years on average (only F)	-RCT -Primary outcomes were determination of bone mass and body composition using DEXA. And biochemical analysis of blood and urine samples. -Secondary outcomes involve height and weight measurements,	18 months	-Intervention group: received 568ml of whole or skimmed milk per day. -Control group: no milk provided.	A significant increase in total BMD and BMC was present in the intervention group however no significant benefits in weight, height and body mass were found.

Table 2.5 continued: Summarisation of milk and dairy supplemented intervention trials in children.

References	Study Population	Study Design	Study Duration	Treatment	Main Findings
		pubertal assessment via Tanner staging and -PAQ to measure physical activity. -Dietary intake was determined by a 7day diet record at baseline and end of study. -Except for dietary intake all other measurements were made every 6 months.			The intervention group had higher calcium, magnesium, phosphorus and other micronutrient levels along with higher IGF-1 and protein concentrations most probably due to milk consumption.
Du et al (2004)	-n = 757 -age = 10-12 years (only F)	-Double blind RCT -BMC, bone area and BMD at distal & proximal forearm was assessed using DEXA. -Biochemical analyses of blood & urine samples were collected. -Anthropometric measurements of weight, height and dosimeters. -Physical activity measured via a PAQ. -All the measurements were performed at baseline, mid and end of trial. -Dietary intake was assessed using a 7-day food record at baseline & 3-day food records for remaining intervals.	24 months	-Group 1: 330ml of milk fortified with 560mg of calcium per day. -Group 2: 330ml of milk fortified with 560mg of Ca and 8µg of Vitamin D per day. -Group 3: control group was provided no supplementary milk.	Groups 1 and 2 had significantly higher height, body mass, BMD and BMC levels compared to group 3. Group 2 who received an additional vitamin D dose had the greatest increments in total body BMC and BMD. The levels of PTH also significantly decreased in both supplementation groups. The plasma concentration of Ca was significantly lower in groups 1 and 2 probably due to accelerated creatinine excretion.

Table 2.5 continued: Summarisation of milk and dairy supplemented intervention trials in children.

References	Study Population	Study Design	Study Duration	Treatment	Main Findings
Lau et al (2004)	-n = 344 -age = 9-10 years (only F)	-RCT -BMD at lumbar spine and proximal femur measured every 6 months via DEXA. Body composition was also assessed by DEXA. -Height and weight measured. -Pubertal status determined by Tanner staging. -PA determined by one on one interviews. -Dietary intake evaluated by 3 day diet records every year.	24 months(18 months intervention and 6 months follow up)	-Group 1: milk powder provided 1300mg Ca per day. -Group 2: 650mg of Ca obtained from milk powder. -Group 3: control group continued with normal diet without placebos.	Group 1 had an increased BMD of 7.4% at total hip, 6.5% at femoral neck, 8.4% at spine and 2.9% at total body. Group 2 did not show significant increases in BMD regions when compared to controls except for the total body BMD. There were no significant differences in anthropometry between all three groups.
Zhu et al, (2008)	-n = 345 -age = 10 years (only F)	-RCT -BMD at total body, head, chest, midriff, pelvis, arms and legs along with BMC and bone area measured by DEXA at baseline and end of trial. -Height (sitting and standing) and weight measured. -Pubertal status assessed by Tanner staging. -Dietary intake evaluated	24 months	-Group 1: 330ml of milk providing 560mg Ca per day. -Group 2: 330ml of milk providing 560mg of Ca and 5-8µg of vitamin D. -Group 3: control group consumed habitual diet with no supplement.	Groups 1 and 2 had significantly increased total and lower limb BMD after adjustment for size.

Table 2.5 continued: Summarisation of milk and dairy supplemented intervention trials in children.

References	Study Population	Study Design	Study Duration	Treatment	Main Findings
		by using un-weighed records at baseline followed by 3 day diet recalls every 24 months.			
Lien et al (2009)	-n = 454 -age = 7-8 years (M & F)	-Double blind RCT -Height and weight measured at baseline and every 3 months. -Health and mental performance questionnaire. -Biochemical analysis of both blood and urine samples were performed.	6 months	-Group 1: regular milk providing 111mg of Ca per school day. -Group 2: fortified milk providing 156mg of Ca per school day. -Group 3: control group did not receive any compensation for energy and protein.	Height and weight were not significantly different between all three groups. The general health and performance of supplemented children was better than controls. Milk supplementation increased micronutrient levels and reduced stunting and underweight by 10%.

Table 2.6: Summarisation of studies where calcium enriched food products were used as supplemental intervention.

References	Study Population	Study Design	Study Duration	Treatment	Main Findings
Bonjour et al (1997)	-n = 149 -age = 7.9 on average (Only F)	-Double blind RCT -BMC and BMD were assessed by DEXA at baseline and end of study. -Six skeletal sites examined were distal metaphysis of radius, diaphysis of radius, femoral neck, femoral trochanter, femoral diaphysis and L2-L4 vertebrae. -Height and weight were also measured. -FFQ used to estimate dietary intakes at 0, 24 and 48 weeks.	12 months intervention and 8 years of follow up.	-Intervention group: Milk calcium extract used to fortify cakes, biscuits, fruit juices, powdered drinking chocolate, chocolate bars and yoghurts. Two products on average provided 850mg of Ca per day. -Control group: Nutritionally similar products excluding calcium fortification.	Calcium supplementation led to an increase of 3.5-5% of bone mass accrual per year. BMD at all sites, BMC and height all significantly increased in the intervention group. The gains in BMD were greatest at the appendicular skeleton regions in girls who had lower baseline calcium intakes. No significant difference was found between the weights of two groups.
Iuliano-Burns et al (2003)	-n = 66 -age = 7-11 years (M & F)	-Double blind RCT -Body composition and BMC of total body and lumbar spine determined by DEXA. -Sitting and standing height, weight, and limb length were measured. -Tanner staging was used to assess pubertal status. -PA determined by a PAQ.	8.5 months	Foods like muesli bars, muffin and cookies were fortified with 2g of milk minerals containing around 20% of calcium. Placebo had similar foods without calcium fortification. -Group 1: consumption of calcium fortified food + moderate exercise.	BMC increased 3% more in the exercise plus calcium group then non exercise and only calcium groups. At the humerus and radius-ulna BMC increased 2-4% in calcium supplemented groups with more benefit observed when exercise was included.

Table 2.6 continued: Summarisation of studies where calcium enriched food products were used as supplemental intervention.

References	Study Population	Study Design	Study Duration	Treatment	Main Findings
		-Dietary intake was evaluated using 3 day diet records at start, mid and end of trial.		-Group 2: consumption of placebo + moderate exercise. -Group 3: consumption of calcium fortified foods +low impact exercise. -Group 4: consumption of placebo diet + low impact exercise.	Loaded bone mass increased by exercise but not calcium. On contrary calcium and not exercise increased bone mass at non loaded sites.
Chevalley et al (2005)	-n = 235 -age = 7.4 years on average (Only M)	-Double blind RCT -DEXA was used to measure areal BMD at six skeletal sites: radius, hip, femoral diaphysis and L2-L4 vertebrae. -Height, weight and BMI measured at baseline, 12 and 24 months. -PA was assessed via recordings. -Dietary calcium intake was assessed by FFQ at baseline, 12 and 24 months.	24 months (12 months intervention and 12 months follow up)	-Intervention group: calcium phosphate enriched foods (chocolate or caramel cakes, biscuits, and fruit juices, powdered drinking chocolate and chocolate bars) were consumed as 2 servings per day providing 850mg Ca/day. -Control group: similar foods were consumed but without fortified calcium present.	Areal BMD was significantly higher at six skeletal sites when compared with controls. PA showed no significant benefit on bone health. At the end of intervention bone size did not change and anthropometric measurements were also not significant.

Table 2.7: Main characteristics of studies where elemental calcium was used as an intervention.

References	Study Population	Study Design	Measurements	Study Duration	Treatment
Johnston et al (1992)	n = 70 pairs of identical twins age = 6-14 years (M & F)	Double blind RCT	-Radius, hip & lumbar spine BMD -PA -Blood & urine samples -FFQ & 3 day food records -Height	36 months	One twin received 1000mg of calcium a day from calcium citrate malate tablet. Second twin received placebo tablet.
Lee et al (1994)	n = 162 age = 7 years (M & F)	Double blind RCT	-Radius BMC & bone width -Blood samples -PA -5 day food record -Height & weight	18 months	Intervention group: received 300mg Ca/day as calcium carbonate tablets. Control group: sucrose tablet.
Lee et al (1995)	n = 84 age = 7 years (M & F)	Double blind RCT	-Radius, hip & lumbar spine BMC & bone width -Blood samples -PA -FFQ & 24-hr diet recall -Weight & height	18 months	Intervention group: 300mg of calcium per day as calcium carbonate. Control group: sucrose tablet.
Dibba et al (2000)	n = 160 age = 8.3-11.9 years (M & F)	Double blind RCT	-Radius BMC, bone width & BMD -Blood samples -Weighed food records -Weight, height & Tanner stage.	12 months	Intervention group: calcium carbonate tablets providing 1000mg Ca/d. Control group: no calcium tablets.

Table 2.7 continued: Main characteristics of studies where elemental calcium was used as an intervention.

References	Study Population	Study Design	Measurements	Study Duration	Treatment
Cameron et al (2004)	n = 102 (51 pairs of twins) age = 8-13 years (only F)	Single blind RCT	-Lumbar spine, hip, femoral neck, forearm & total body BMC -Body composition -PA -FFQ & 4 day food record -Height & weight	48 months (24 months intervention and 24 months follow up)	Intervention group: 1200mg calcium carbonate tablet per day. Control group: no calcium carbonate tablet.
Matkovic et al (2005)	n = 354 age = 8-13 years (only F)	RCT	-Radius & total body BMD. -Blood, stool & urine samples -Body composition -3 day diet records -Height, weight & pubertal stage.	7 years (4 years supplementation and 3 years follow up)	Intervention group: 1000mg of calcium per day via calcium citrate malate tablet. Control group: no calcium tablet consumed.
Courteix et al (2005)	n = 113 age = 8-13 years (only F)	RCT	-Radius, hip, lumbar spine & total body BMC & BMD -Body composition -PA -FFQ & 3 day diet record -Height, weight & pubertal status.	24 months (12 months intervention and 12 months follow up)	Intervention group: 800mg of calcium per day consumed as calcium phosphate. Control group: placebo tablets.
Ward et al (2014)	N = 80 Age = 8-11.9 years (only M)	Double blind RCT	-Whole body, lumbar spine & total hip BMC, BMD and BA (bone area). -Body composition (lean mass) - Height & weight	13 years (12 months intervention and 12 years of follow up)	Intervention group: 100mg of calcium carbonate tablet 5days/week. Control group: placebo tablets.

2.14 Observational studies:

Dairy products especially milk in general play a very important part in meeting the RDI of calcium for children. Adequate calcium intake maintains optimal bone mass and reduces the risk of fractures or bone loss in later life (Spence, 2013). A cross sectional analysis conducted in Australian children and adolescents showed that bovine or cow milk is a good source of various nutrients and can help achieve the recommended intake levels of calcium (Fayet et al, 2013). Japanese children who drank large amounts of bovine milk over a prolonged time period had significantly greater height increments in comparison to those who did not (Okada, 2004). An observational study of short stature children and adolescents showed that these children had low calcium and vitamin D intakes and biochemical analysis further revealed that their PTH levels were high. All of these factors can lead to an alteration in normal bone growth, hence increasing the risk of future bone disorders (Bueno et al, 2010). Physical activity has been considered to improve bone health in children and adolescents with the best outcome observed when the mean calcium intake is adequate or high (Harvey et al, 2012). Slightly similar results were found by Boot et al (1997), but only boys showed positive correlations between BMD, calcium intake, and physical activity.

The avoidance of milk by New Zealand children aged 3-10 years for a long period of time was associated with lower calcium levels and poorer bone health in comparison to the community control counterparts(Black et al, 2002). These children also had shorter statures and low BMD. Another observational study in New Zealand showed that in addition to shorter stature and poor bone status, children who avoided milk also had higher rates of fractures (Goulding et al, 2004). Goulding and colleagues (2005) further demonstrated that milk-avoiding children had higher body mass or adiposity, which is also believed to be a risk factor for increased fracture rate. Low dairy intake in children can lead to poor bone health which can then further increase the health care costs. In New Zealand, not enough studies have been conducted in children, where bone health status is studied in association with dairy consumption. Hence, there is a substantial need to conduct more research trials in this population group which will not only provide information about children's bone health but also give a reference data for researchers to use in the future.

2.15 Physical activity and bone mass accrual:

Two strategies during childhood which are deemed to be effective in increasing peak bone mass (PBM) are calcium consumption and weight bearing physical activity (WBPA) (French et al, 2000). Weight bearing exercise can influence bone mass deposition especially at the growth phase of a skeleton because during this time period the impact of external stimuli is most profound (Boreham and McKay, 2011). Therefore, during childhood an active and healthy lifestyle should be maintained to achieve maximum PBM and reduce risk of low bone mass loss in older years.

A substantial amount of evidence has been published which shows the positive influences of mechanical loading upon bone mineral accrual. A general example of WBPA is gymnastics which impacts loading at both upper and lower limbs. A study conducted in gymnast boys concluded that musculoskeletal loading as a result of training had positive influences upon skeletal health (Daly et al, 1997). Several RCTs have also shown that indeed WBPA positively influences the bone mass loading in the skeleton of growing children. A trial investigating the effects of jumping (3 times a week for ten minutes) on hip and lumbar spine of pre-pubertal girls and boys concluded that the jumpers had significantly higher BMC at femoral and lumbar spine regions than controls (Fuchs et al, 2001). In addition, BMD was greater only at the lumbar spine region of jumpers. Van Langendonck et al (2003) studied the effects of WBPA on bone mass loading of monozygotic pre-pubertal female twins. Intervention of 9 months led to an increase of areal BMD and BMC of proximal femur only. The beneficial osteogenic effect of load bearing exercise was more pronounced in girls who generally did not take part in physical activities. Mackelvie et al (2004) also reported that the boys in the intervention group had statistically higher BMC at the femoral neck area after 20 months of intervention. Other than that no changes in BMD at femoral neck and BMD and BMC at other regions were observed. The intervention in this trial was high impact circuit training 3 times a week. A RCT which included children of both genders implemented an intervention of jumping for strength training for 10 minutes 3 times a week (Meyer et al, 2011). The results of this study showed that the intervention group had significantly increased BMC at the femoral neck, lumbar spine, and total body regions whereas the BMD was significantly higher

only at the lumbar spine and total body areas. An increase of 10% in PBM can lead to the reduction of osteoporotic risk by 50% and the intervention programme in this trial helped achieve an increase of 8.4% in bone development within one year which proves that mechanical loading exercise can lower the risk of future bone disorders. Lofgren and colleagues (2012) performed the longest intervention trial regarding the relationship between bone mass accretion and physical activity where intervention group performed moderately intense exercise for 40 minutes every school day for 4 years. At the end of this trial both pre-pubertal gender populations within the intervention group showed a significant improvement in not only bone mass but bone size as well without increasing the risk of fracture.

In summary, overall results suggest that high impact WBPA does impose positive effects upon bone mass accrual in pre-pubertal children with the most consistent finding being the improved bone status of the femoral neck. In addition to bone health, physical activity is also important for maintaining optimal weight, lean mass and overall fitness.

SECTION 4: DEXA and Nutritional assessment

2.16 Dual energy X-ray absorptiometry (DEXA):

Bone densitometry assessment is the gold standard in determining the bone health status. In children, the bone mineral content (BMC) or bone mineral density (BMD) of growing bones should be closely examined due to two reasons; first is to observe the normal growth of bone and to diagnose if any disorders like osteopenia cause bone mineral loss, and the second reason is to study bone growth patterns in young life and its relation to osteoporosis risk in later adult life (Gordon et al, 2008). Numerous techniques have been developed over the recent years to determine bone densitometry but before applying any of these processes a few points should be taken in to consideration. Such as the technique used should be practical and easy to use, different types of bones can be easily differentiated and assessed, and finally measurements should not be significantly influenced by the height or weight of individual subjects (Gilsanz, 1998; Binkovitz and Henwood, 2007).

The most commonly used non-invasive bone density measurement techniques include quantitative computed tomography (QCT), magnetic resonance imaging (MRI), quantitative ultrasound (QUS), and dual energy x-ray absorptiometry (DEXA) (Petit et al, 2005). In this review, however, only DEXA is discussed further. DEXA scans are the most commonly used tools to determine bone densitometry in children.

DEXA was first developed in the late 1980s and today is considered to be the gold standard for bone density measurement (Fewtrell, 2003) because of its precision, speed, low radiation exposure, and global availability (Crabtree et al, 2014). DEXA works by differential absorption of x rays at two different energies which then determine the amount of mineral present at that particular region. In adults frequent DEXA measurement sites are the total body with head, lumbar spine, and hip but when required other specific sites can be measured as well (Choplin et al, 2014). In children, the ideal sites are the lumbar spine and total body without head, as at these regions bony sites are easier to detect and are highly reproducible (Gordon et al, 2008). Paediatric total body scans are highly precise and provide information about the whole body bone status. In addition, the whole body scan estimates the body composition as well (Crabtree et al, 2014). Even though the skull constitutes a major portion of the skeleton, there is a slight controversy surrounding the inclusion of head in total body scans. In growing children bone mass of the skull highly varies and also it is not influenced by stimuli such as physical activity (Crabtree et al, 2014). Thus, including a child's head in total body scans can lead to under or over reporting especially when suffering from bone disorders (Taylor et al, 1997). Lumbar spine measurement in children is favoured due to the speed and precision of scans and easy identification of any bony landmarks (Crabtree et al, 2014). Furthermore, the lumbar spine is considered a common outcome variable when the influence of physical activity is studied on bone accrual (Fuchs et al, 2001; Gunter et al, 2012).

DEXA results of bone mass can be presented as BMC (g) or BMD (g/cm^2) which are then compared with standards of similar characteristics like age and gender (Bachrach and Sills, 2011). In adults the comparison is made using a T score which represents the BMD of a healthy adult. An error often made is the usage of T scores to interpret the results of paediatric DEXA scans. T scores should not be

used for children because they are set scores for fully developed skeletons which have achieved peak bone mass (PBM) and children's bones are constantly growing and undergoing bone mass accrual (Gafni and Baron, 2004). Instead of T scores, Z scores are used for children where a comparison with the same aged population is made. Nevertheless, even this method can generate errors as not all children have similar growth patterns and weight gain (Fewtrell, 2003), therefore, Z-scores should be adjusted in children who have delayed growth or shorter statures (Gordon et al, 2014). The lumbar spine z-scores can be corrected by using areal-BMD or height adjusted z-scores and for total body (without head) the height adjusted z-scores should be used (Crabtree et al, 2014).

A significant factor affecting bone densitometry measurements is fat mass. It is believed that the higher the level of fat that exist on a subject the greater the chances of error to occur. The studies looking at this interaction have, however, provided inconclusive results and more research needs to be carried out (Manzoni et al, 1996; Yu et al, 2012). There are various other factors which can affect growth such as different physical characteristics, genes, geographics, and lifestyle. In addition to these natural characteristics there are numerous disorders (see table 2.8) which can also affect the normal skeletal growth (Bishop et al, 2008).

Table 2.8: Disorders which could affect normal skeletal growth.

Primary bone disorders
Idiopathic juvenile osteoporosis
Osteogenesis imperfecta
Potential secondary bone diseases
-Chronic inflammatory disorders
Inflammatory bowel disease
Juvenile idiopathic arthritis
Cystic fibrosis
-Chronic immobilization
Cerebral palsy
Myopathic disease
Epidermolysis bullosa
-Endocrine disturbance
Turner syndrome
Anorexia nervosa
-Cancer and therapies with adverse effects on bone health
Acute lymphoblastic leukemia
Status post chemotherapy for childhood cancer
Status post transplantation (nonrenal)
Hematologic disorders
Thalassemia

The correct collection and interpretation of DEXA results is very important for the validity and reliability of data. Problems can arise at any stage starting from the positioning of a subject up to statistical calculations. Most commonly encountered errors as reported by Gafni and Baron (2004) in order of impact were incorrect use of T scores, lack of gender and ethnic differentiation, incorrect bone map, no correction of short stature, and some statistical errors. Due to these errors under or over estimation of bone mass can lead to incorrect diagnosis of low bone mass or fracture risk especially in children. Paediatric DEXA reports should not contain the terms 'osteoporosis' and 'osteopenia'; instead 'low bone mass or low bone mineral density' should be used when the BMC or BMD is equal to less than -2 standard deviation (Gordon et al, 2014).

In addition to measuring bone density, DEXA is a very efficient tool also used to measure body composition i.e., fat mass and bone free lean mass (Toombs et al, 2012). In a paediatric setting it is very often difficult to assess the body composition. A study in young children showed that most of the body composition assessment techniques in children provided inaccurate estimates except for DEXA, which gave a comparatively supportive outcome (Djafarian et al, 2014). However, still DEXA or any other body composition assessment method should be used with caution in children.

A few drawbacks of using DEXA are that it is very expensive, requires trained personnel for use, and the derived values vary according to the manufacturers (Lockner et al, 2000; Ittenbach et al, 2006). In addition, DEXA results can be misinterpreted when factors such as skeletal maturity, ethnicity, puberty, and body fatness are not considered (Djafarian et al, 2014). These variables should be adjusted by comparison to the reference data available. Unfortunately there is no single technique that that can adjust these variables altogether and a child's unique medical profile is important to determine how and what techniques (bone size, height, pubertal stage, and body composition adjustments to name a few) need to be applied. (Crabtree et al, 2014).

2.17 Nutritional assessment:

The dietary or nutritional assessment at an individual or group level is used to evaluate the food intake over a certain time period. Three most commonly used

methods to assess diet intake are weighed food records, 24 hour diet recalls, and FFQs with each process having both its advantages and disadvantages (Kolodziejczyk et al, 2012). Table 2.9 summarises the strengths and limitations of all three methods.

Table 2.9: Advantages and disadvantages of dietary assessment methods (information obtained from Thompson and Subar, 2008 & Illner et al, 2012).

Dietary assessment method	Advantages	Disadvantages
24 hour diet recall	-Intake quantified -Applicable by various means like paper, telephone, computer. -Low cost of administration. -Low respondent burden. -No changes in eating habits as it is recorded afterwards.	-Completely relies on memory of respondent. -One day is not enough to capture usual eating habits. -Intake can be misreported.
Food record	-Intake quantified. -No recall of food is required as reporting of food is mostly suggested right after consuming it. -Food can be weighed, photographed or estimated as well. -More than one day provides good enough idea of diet patterns. -Detailed information regarding food consumed and also includes less commonly consumed foods.	-High respondent burden. -High investigator cost. -Requires many days to record food e.g., 3, 5, 7 or even more days. -The respondent is extensively trained on how to fill in the records. -Respondent must be literate. -Can affect eating habits. -Intake can be misreported.
Food frequency questionnaire	-Low cost. -A well designed FFQ can provide good estimation of the nutrient(s) for which it was created. -Does not affect eating habits. -Low respondent burden. -Distinguishes high and low consumers in a population. -Can be administered in larger populations.	-Not all questionnaires can be used for different groups e.g ethnicities, age, gender therefore must be kept in mind. -Intake is not precisely quantified. -Respondents must be literate. -Memory of respondent should be good. -difficult estimating portion sizes. -Intake is misreported often.

2.17.1 24-hour diet recall:

The 24 hour diet recall provides an estimate of the individual's eating habits in detail which can then be used to calculate total dietary intake for that day. The respondents are asked probing questions by the interviewer making this method time consuming and it does not provide population means as well (McPherson et al, 2000). Since a 24-hour diet recall is completely based upon the memory of the previous day, it is not very feasible to use in young children (Ontiz-Andrellucchi et al, 2009).

2.17.2 Food record:

The Food record provides an actual intake of a person over a certain period of time, usually 3, 5 or 7 days. It can be recorded by weighing as well as estimating food but the weighed food record method is considered gold standard (McPherson et al, 2000). In estimated food records the portion size is estimated by mainly using pictures, models or household items like cup, spoon, bowl whereas in weighed food records the food ingredients or food as a whole is weighed. Weighing food is time consuming and requires co-operation of respondents but is considered to be the most accurate dietary intake reporting tool (Rutishauser, 2005).

Diet histories (24-hr recall and food records) consist of lengthy interviews that contain open ended unstandardised questions which need to be administered and assessed by qualified nutritionists (Kristal et al, 2005) and therefore are not ideal to use in large population based epidemiological studies to investigate a relationship between diet and disease (Molag et al, 2007). Also these dietary assessment methods are not reliable when used directly on young children (Ontiz-Andrellucchi et al, 2009). A proxy such as parent or guardian would be better at providing an idea of the child's eating habits.

2.17.3 Food Frequency Questionnaire (FFQ):

A FFQ is composed of a set of questions which asks the respondents about how often and how much of a food they consumed over a certain period of time (Cade et al, 2002). It is very effective in ranking and distinguishing low nutrient vs high nutrient intakes no matter the gender, race, or age of the population (Magkos et al, 2006). Often FFQs are also used to assess the total dietary intake such as

determining what percentage of a population meets the RDI standards (Molag et al, 2007). In comparison to other dietary assessment methods, FFQs can estimate the diet of large populations over a longer period of time i.e., weeks or months and are most frequently used for a specific nutrient(s) rather than the total diet (Rockett and Colditz, 1997). Hence, a FFQ can be quite convenient and efficient in assessing calcium intake of a desired population group.

A FFQ can be quantitative, semi-quantitative, or non-quantitative which can be administered by an interviewer or the respondent him/herself (McPherson et al, 2000). The questionnaire may either be made by the researchers from basic principles regarding the nutrient(s) of interest or in case of time and money constraints a modified version of pre-existing questionnaires can also be used (Cade et al, 2002). Advantages of using a FFQ are that it is cheap, less time consuming and easily administered. Qualified interviewers are not required and often the FFQ can be self-administered. During data collection the normative dietary intake of individuals does not alter. And finally, literacy is not a restricting factor and the respondent burden is also low (McPherson et al, 2000).

Even with all the benefits, a FFQ is not easy to administer in children since the recalling of dietary intake is not simple for children (as compared to adults) due to restricted cognitive abilities. A study showed that children (approximately less than 9 years of age) have difficulty in recalling their long term dietary intake with the greatest struggle with estimation of portion sizes (Ontiz-Andrellucchi et al, 2009). To overcome this problem it is often suggested that the parents should either report (act as proxy) or simply just aid in reporting their children's estimated intake. Another FFQ which was specially designed to assess calcium intake in children and adolescents showed that 6-10 year olds had a more precise estimated intake probably due to a more stable and parent controlled diet but this approximation only helped to distinguish low and high calcium consumers without providing any specific details such as what type of food contributed the most to calcium intake (Bertoli et al, 2005). For younger children, parents are better at providing reliable information as shown in a study in Belgian preschool children where parental estimation of calcium intake was fairly good and helped distinguish low dairy consumers from high dairy consumers but underreporting was observed as well (Huybrechts et al, 2006). One main concern surrounding

parental involvement is that they are no doubt reliable reporters of a child's dietary intake at home but out of home the recall is not very reliable, for instance, at school, church, or afterschool activity centres (Livingstone and Robson, 2000). To overcome such a problem, dietary intake out of home should ideally be managed by the care taker present at that particular place. A study has shown that school aged children (7-10 year olds) who were assisted by the teacher or interviewer in completing their FFQs displayed good reproducibility and moderate validity (Barros et al, 2007).

The validity and reproducibility of a FFQ is very important and should always be pre-tested before administration is carried out. It helps the researchers know whether the FFQ is properly assessing the desired nutrient intake. Along with the validation, reproducibility should also be tested by administering the FFQ more than once to a population group with similar characteristics (Cade et al, 2002; Magkos et al, 2006). The main purpose of carrying out validation is to determine if similar outcomes are attained by different dietary assessment methods. There is no perfect technique present to determine an FFQ's validity, however, a weighed food record is the most frequently used tool for the validation of a FFQ and has shown the least correlated errors (Cade et al, 2002). Since a weighed food record is considered to be the gold standard it should be the first choice for validation, however, due to the number of days involved it can impose a respondent burden. A 24-hour recall, thus, can also be used to validate a FFQ but it is recommended that it should be implemented several times to obtain a good average estimate of diet (Carroll et al, 2012). In addition to these, several isotopic and biochemical techniques like urea content (for protein) and blood plasma concentration can be analysed to assess specific nutrients and therefore used to validate an FFQ (Cade et al, 2002). The doubly labelled water technique provides an estimate of the total energy expended and hence can be used to validate an FFQ when energy intake is being measured (Dutman et al, 2011).

When using a FFQ a few factors should be considered and corrected for. The reporting of single foods consumed is easy but combined meals are difficult to report, especially in children, leading to over or underestimation. Related food items should be grouped together for the ease of both the respondent and researcher (Cade et al, 2002). It should be noted that different FFQs have different

designs and the number of items, portion sizes and methods of administration also differ. A questionnaire should be long enough to encompass all the relevant questions but not too long that it increases pressure on the respondent which then further leads to errors (Kolodziejczyk et al, 2012). A review has shown that longer food lists provided better ranking results in comparison to short questionnaires and that portion size did not affect the validity of outcome (Molag et al, 2007). The questions of a FFQ should be close ended to reduce the time of interpretation as well as lower transcription errors (Cade et al, 2002). Administration of FFQs should be carefully carried out as systematic and random errors can arise very easily affecting the reliability of results by producing bias in disease-diet association (Carroll et al, 2012; Illner et al, 2012).

No doubt FFQs have numerous benefits however a few disadvantages are also present. FFQs are not very efficient in identifying unique details in diet, for example, less commonly consumed foods can be misreported (Rockett and Colditz, 1997). To counter this problem FFQs can be often used in association with 24 hour diet recalls or food records to help the researcher in obtaining information about foods that would only be consumed periodically (Carroll et al, 2012).

CHAPTER 3: AIMS AND OBJECTIVES

Objectives:

The main purpose of this study was to investigate the relationship of bone health with dairy intake/calcium intake and body composition in pre-pubertal New Zealand school children living within the Manawatu district, New Zealand.

The secondary aim was to determine whether there is an association between bone health status and physical activity. Another secondary objective was to study the effects of other nutrients on bone health. One more secondary aim of this study was to validate the FFQ against an estimated 3-food record.

Hypotheses:

Children with better-quality bone status would have higher dairy and calcium intake.

A negative relationship would be present between bone mineral status and body fat.

Children who have an adequate or high dairy intake will have increased height and weight.

Children with higher levels of physical activity will have a higher BMD and BMC and lower body fat measures.

Outcomes:

Primary outcomes:

Weight (kg), height (cm), waist circumference (cm), BMI, total fat mass-TFM (kg), lean body mass-LBM (kg), and % body fat.

Total headless bone mineral density (tBMD), total headless bone mineral content (tBMC), lumbar spine BMD (LS-BMD), and lumbar spine BMC (LS-BMC).

Calcium intake and diary serves consumed per day.

Secondary outcomes:

Measure physical activity level.

To determine vitamin D, protein, phosphorous, magnesium, zinc, sodium, and dietary fibre intake.

CHAPTER 4: METHODOLOGY

Sponsorship for the 'bone health study' was provided partly by Fonterra Co-Operative Ltd, Palmerston North, New Zealand and partly by Massey University School of Food and Nutrition, Palmerston North, New Zealand. This study was reviewed and ethically approved by the Massey University Human Ethics Committee: Southern A, Application 15/03 (appendix 1). The signed parental consent forms were obtained before any type of data were collected (appendix 3). Participation in the research study was voluntary and the confidentiality of each participant was assured by referring an identification code to each child. Only the researchers at Massey University had access to the data and consent forms.

4.1 Subjects:

4.1.1 Recruitment process:

Pre-pubertal children aged 5-9 years living within the Manawatu region were recruited to take part in the current study. The number of children to be included was determined by a power calculation (appendix 2). 33 children were recruited under the ethics application named Southern A, Application 14/02 from local schools. 12 more children were further recruited by the researcher, through advertisement of the study through Massey University internal staff e-mailing system and kids activity clubs (Southern A, Application 15/03). A total of 45 children participated in this study. Once the parent/guardian's permission was attained via a signed consent form (appendix 3), a health screening questionnaire (appendix 4) was filled out and a visit to the human nutrition research unit was arranged. On arrival to the human nutrition research unit, the children also had to sign their informed assent forms which were also the information sheets (appendix 5) before commencement of the measurements. The parental information sheet is present in appendix 6.

4.1.2 Participants involved:

45 children ranging from 5 to 10 years of age were included in this study. All children were either brought or they came with the parent themselves to the human nutrition research unit at Massey University, Palmerston North where the

DEXA scans and anthropometric measurements were taken. The FFQ and physical activity questionnaire was also completed by each child at the human nutrition research unit but the food records were provided to be taken home.

All measurements were undertaken by the same researcher except for DEXA scans which were performed by another qualified technician. Standard procedures were carried out where ever necessary and all measurements were taken in the metric form (cm and kg). The children wore very light clothing with no shoes or artefact on when being measured.

4.1.3 Inclusion criteria:

The inclusion criteria were that the children were healthy and pre-pubertal. They were fluent in speaking and understanding English and the caregiver of the child would also understand English and must be over the age 18 years to provide informed consent.

4.1.4 Exclusion criteria:

A health screening questionnaire (appendix 4) was completed by the guardian of each child and children suffering from any medical condition that would alter bone metabolism were excluded from this study. These conditions could be; diagnosed bone, gastrointestinal or renal disorders as well as diabetes. Furthermore children consuming any medication that interferes with bone homeostasis would also have been excluded.

4.2 Study Design and Data Collection:

This was a cross sectional study. All measurements were performed at one point in time only within the human nutrition research unit, School of Food and Nutrition, Massey University, Palmerston North.

4.2.1 Anthropometry:

Height: The height of each child was measured using a calibrated wall mounted stadiometer. Two measurements were taken per child and the average was used as the final value. In case of a difference >1cm between the two readings a third measurement was taken and used.

Weight: A mechanical spring weighing scale was used to measure the weight of every child. Similar to height, two measurements were taken for weight and the average was used, however, if the difference between the recorded values was >0.1kg, the third measurement was used.

Waist circumference: The waist circumference was measured by placing a measuring tape slightly above the hip bone at the abdomen. At the time of measurement the abdomen was bare and children were asked to breathe normally. Two measurements were taken and an average was used, but in case of a difference of >1cm, a third value was recorded.

BMI: Body mass index (BMI) was calculated to determine overweight and obesity. The formula used to calculate BMI was $BMI = mass(kg)/height(m)^2$.

4.2.2 Dual energy x ray absorptiometry (DEXA):

DEXA was used to determine the total headless bone mineral density (tBMD) and bone mineral content (tBMC). The sites selected for BMD and BMC analysis were total body and lumbar spine (L1-L4). The reasons for choosing these two sites in paediatrics are explained in the literature review section 4 (Chapter 2). Body composition including the LBM, TFM, and percent body fat (%BF) were also determined from the total body DEXA scan.

The scans were analysed using DEXA Hologic Discovery A, (Wisconin, MA, USA) by a qualified technician who followed the quality control protocol. Based on the manufacturer's instructions, quality control scans were performed everyday using a certified calibration block (calibration values accepted when co-efficient of variation was below 0.05%). Z scores for children aged 8 years or over were analysed by the software itself and for children aged 7 years and below the z scores were calculated manually using reference populations (Kalkwarf et al, 2007, Navachakara et al, 2014, Zemel et al, 2011). A z score of 0 was equivalent to the mean and children with z scores equal to or less than -2 standard deviation were categorised as having low bone mineral density or low bone mass (Gordon et al, 2014).

4.2.3 Measurement of nutrient intake:

FFQ: The calcium intake was estimated using a pre-designed baseline FFQ (appendix 7) devised by researchers at the University of Auckland. The reason for using this questionnaire was that the researchers at University of Auckland used this FFQ in a study quite similar to this current study. The FFQ inquired about dairy intake of children at school, dairy intake on weekdays at home, and dairy intake on the weekends. Questions about the child's personal perception and liking for dairy were also included. In addition to these, basic demographic questions were also present at the start of the FFQ. To aid the understanding of portion sizes and help identify common foods, children were shown different food models. Some models used were; drinks (diet coke vs regular coke, flavoured water, sports drink), a pottle of yoghurt, and different milk bottle tops (dark blue, yellow, green, and light blue). The children were also helped by the researcher to identify portion sizes by showing them different cup sizes, the size of a slice of cheese, and the size of a regular bowl or plate. Children filled the FFQs themselves with assistance mainly provided by the researcher but in some instances when the parent/caregiver was present a few helpful inputs were made by them as well.

The calcium intake of children from the FFQs were analysed by using Foodworks 7 (2012, Xyris software, Australia, Pty Ltd).

3 day estimated diet record: An estimated food record was used to assess the dietary intake of the child over a period of 3 days. On visiting the human nutrition research unit, the child was provided with the diet record that had to be filled in by the parent or guardian with consultation with the child. The parent was asked to record everything their child ate or drank for three days (including two week days and one weekend day). An instructional manual with examples on how to fill in the food record was provided to the parents (see appendix 8). On completion, the 3 day estimated diet records were mailed back to the researcher via a provided pre-paid envelope. The amount of desired macronutrients and micronutrients were assessed using Foodworks 7 (Xyris software). Furthermore, Goldberg cut-off was used to test for reporting bias in energy intake (Black, 2000). It was calculated by dividing the energy intake (obtained from food records) by the basal metabolic rate (obtained from foodworks). Under reporters and over reporters were

identified by the energy intake:basal metabolic rate ratio with under reporters having a value below the minimum (<95% confidence limit) and over reporters having a value above the maximum (>95% confidence limit) range (Black et al, 2000). More information about the calculation of Goldberg cut-offs can be obtained from Black et al, 2000.

4.2.4 Physical activity:

To assess the physical activity level of the children after school, a previous day physical activity recall (PDPAR) was used (appendix 9). PDPAR is a self-reporting tool specially designed for the younger population in which children recall their past day behaviour as accurately as possible (Trost et al, 1999). For the child's feasibility, the PDPAR is divided into 30-minute time intervals which are in turn grouped into afternoon, supper, evening, and night. In each time block the child enters the activity code along with the intensity of the activity as well. Metabolic equivalent of task (MET) values were used to score each activity and a mean was then used to estimate the average physical activity level after school. 1 MET* represents the resting metabolic rate when a person is sitting quietly and the higher the MET value the more intense the activity is. MET levels can be divided into rest (1-1.5 METs), light (1.5-3.0 METs), moderate (3.0-6.0 METs), and vigorous (>6.0 METs) activity. The mean MET value of 3 or greater was considered as moderate to vigorous physical activity and MET of 6 or greater was referred to as vigorous physical activity. (Trost et al, 1999; Welk et al, 2004). To make the understanding of physical activity easier, METs were further categorised in relation to physical activity levels (PAL). Low PAL = <1.45 METs per day, moderate PAL = 1.45-1.60 METs per day, and high PAL = >1.60METs per day (Di Pietro et al, 2004). The METs were also used to calculate kilocalorie energy expenditure (EE) by using this equation: "EE = METs x sessions per week x hours per session x body weight(kg)" (Ainsworth et al, 2000). Additional in depth details on the PDPAR assessment and scoring can be found in the validation study of Weston et al, 1997.

*For an adult 1 MET is approximately 1 kcal/kg/hr or 3.5 ml oxygen/kg/min

4.3 Statistical Analyses:

Normality of all variables was tested before beginning any type of analysis using the Shapiro-Wilk test in IBM SPSS statistics 22. The normally distributed data

were presented as mean ± SD and not normal or skewed data were presented as median ± IQR. SAS version 9.4 (SAS Institute Inc. Cary, NC 27513-2414, USA) was used to perform all the other tests such as independent t-test to find a significant difference between normally distributed data. Wilcoxon-Mann-Whitney test was performed as a substitute of t-test where data were not normal. Multiple linear regression analysis was used to determine the association of various factors with bone mineral status. Finally, a Pearson's correlation was used to evaluate a correlation between the calcium intake from the FFQ and the 3-day estimated food record.

CHAPTER 5: RESULTS

5.1. Basic demographic and anthropometric data:

Children (n=45) included in this study were residents of the Manawatu region, North Island, New Zealand. All participants were pre-pubertal with ages ranging from 5 to 10 years. The total sample consisted of 62% males and 38% females. Children belonging to various ethnic backgrounds represented the study population with the New Zealand European origin (NZEO) or Pakeha making up the majority (n=28), followed by Others (n=10), Maori (n=5), and Tongan (n=2). At the time of measurements, the study participants were in school years 1 to 6.

Of the total sample, one child reported being lactose intolerant, one child suffered from attention deficit hyperactivity disorder (ADHD), and one had a cast on a broken arm. None of these children, however, were taking any calcium supplements and medication which would alter bone metabolism.

Firstly to check for any abnormality in growth, the anthropometric data of participants were compared to reference growth charts. The body mass index (BMI)-for-age of the participants on comparison with the World Health Organization (WHO) child growth standards showed that 67% of the population sample was classified as normal (5^{th} to $<85^{th}$ percentile), 5% as underweight ($<5^{th}$ percentile), 11% as overweight (85^{th} to $<95^{th}$ percentile), and 18% obese ($>95^{th}$ percentile). The BMI, however, is not a good indicator of body mass as DEXA analysis showed that children classified as overweight/obese by BMI actually had optimal fat mass. The growth charts used as a reference for comparison with the growth parameters i.e. height-for-age, weight-for-age, BMI-for-age (WHO standards) and waist circumference (WC)-for-age (Centres for Disease Control and Prevention[CDC] growth charts) are available in appendix 10.

Table 5.1 summarises the characteristics of the study population. Mean ± SD was used for variables which were normal, however, for those which were not normally distributed the median ± interquartile range (IQR) was used. Two sample independent t tests (for parametric data) and Wilcoxon-Mann-Whitney test (for non-parametric data) showed that in our population group, a statistically

significant difference between the two genders was only present for percent body fat (% BF) and lean mass. The boys had a significantly higher LBM and a significantly lower % BF compared to girls. All other characteristics were not significantly different between boys and girls.

Table 5.1: Characteristics of the study population.

Characteristics of the children	**Total** (n=45) Mean ± SD or Median ± IQR	**Boys** (n=26) Mean ± SD or Median ± IQR	**Girls** (n=19) Mean ± SD or Median ± IQR	***t value/ z value*****	***p***
Age (years)^	7.5 ± 1.0	7.6 ± 1.0	7.3 ± 1.1	0.98	0.33
Height (cm)	130.6 ± 12.6	131.2 ± 9.9	126.4 ± 9.1	-1.68	0.09
Weight (kg)	28 ± 9	28.7 ± 10.2	27.5 ± 8.5	-1.02	0.30
BMI (Kg/m^2)	16.4 ± 2.8	16.5 ± 2.9	16.4 ± 2.7	-0.16	0.43
Mean WC (cm)	57.5 ± 8.75	57.8 ± 7.8	56.5 ± 8.5	-0.28	0.77
tBMD (g/cm^2)^	0.631 ± 0.06	0.64 ± 0.06	0.61 ± 0.05	1.79	0.08
tBMC (g)	673.6 ± 150.4	685.8 ± 160.2	651.4 ± 159.8	-1.2	0.22
LS-BMD (g/cm^2)^	0.66 ± 0.04	0.66 ± 0.04	0.67 ± 0.04	-0.35	0.72
LS-BMC (g)^	20.4 ± 2.8	20.8 ± 2.8	19.9 ± 2.8	1.05	0.30
BF (%)	29.2 ± 10.6	25.9 ± 4.9	32.3 ± 7.3	3.5	0.0005*
TFM (kg)	7.6 ± 3.7	6.9 ± 3.0	9.03 ± 4.1	1.73	0.08
LBM (kg)	17.9 ± 5.0	19.7 ± 4.8	16.9 ± 4.2	-2.2	0.02*
MET	1.69 ± 0.75	1.79 ± 0.8	1.58 ± 0.5	-1.10	0.31

(BMI=body mass index, WC=waist circumference, tBMD=total bone mineral density minus head, tBMC=total bone mineral content minus head, LS-BMD=lumbar spine bone mineral density, LS-BMC=lumbar spine bone mineral content, BF=body fat, MET=metabolic equivalent of task).

^only these variables were normal (mean ± SD)

*there was a significant difference present between the % body fat and lean mass of boys vs girls ($p<0.05$).

**t value was obtained from two sample independent t-test (performed when data were normal) and z value from Wilcoxon-Mann-Whitney test (performed where data were not-normal).

5.2. Dietary intake:

5.2.1. Food Frequency Questionnaire:

All participants completed the food frequency questionnaire (FFQ) and table 5.2 shows the frequency of both daily and weekly consumption of dairy foods by the study population.

Table 5.2: An average estimation of dairy servings* consumed using the FFQ.

Dairy food	Servings per day Mean ± SD	Servings per week Mean ± SD
Plain milk	1.0 ± 0.7	7.1 ± 5.0
Plain milk with cereal	0.8 ± 0.5	5.9 ± 3.5
Flavoured milk	0.5 ± 0.6	3.7 ± 4.5
Cheese	1.2 ± 0.6	8.4 ± 4.7
Yoghurt	1.2 ± 0.6	8.4 ± 4.7

*1 serving of milk = 1cup, 1 serving of milk on cereal = 1cup, 1 serving of flavoured milk = 1cup, 1 serving of cheese = 40g, 1 serving of yoghurt = 150g

On average, children consumed approximately 5 serves of dairy per day and met the recommendations of 2-3 serves per day (MOH, 2012a). In addition to estimating the servings, the FFQ also elucidated what type of milk children consumed (Figure 5.1). Whole milk was the most commonly consumed option, followed by skimmed milk.

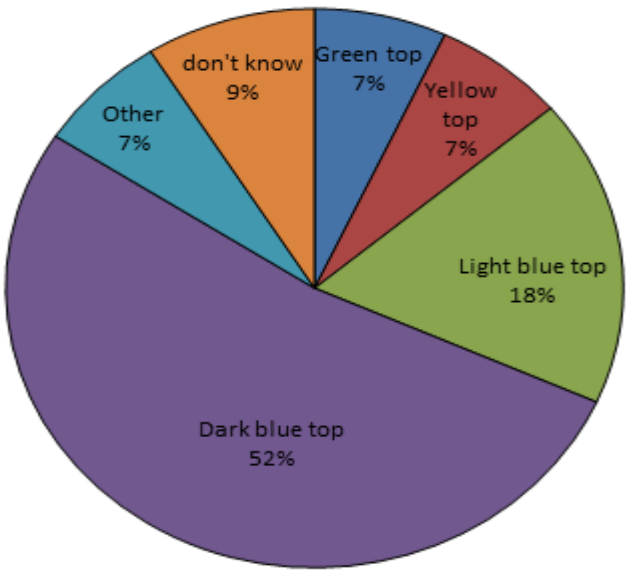

Figure 5.1: A variety of different milk types consumed by children (Dark blue top=whole milk, light blue top=reduced-fat milk, green top=trim milk, yellow top=low-fat calcium enriched milk, other included raw milk).

Another interesting finding from the FFQ was the frequency of consumption of beverages other than milk by the children during school hours, before and after school, and on weekends (Figure 5.2). Water was the most frequently consumed beverage overall followed by fruit juice. When asked about the nutritional value of these beverages, children rated water as the healthiest option and soft drinks as the unhealthiest choice of all (refer to Figure 5.3).

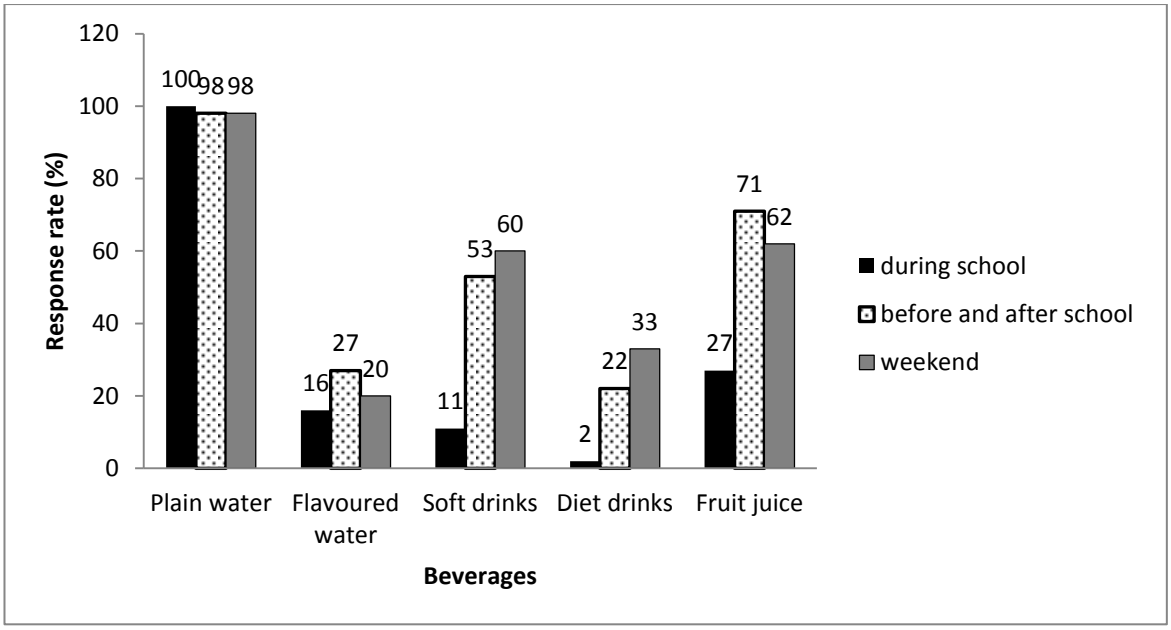

Figure 5.2: Frequency of the consumption of various beverages during school, before and after school, and on weekends.

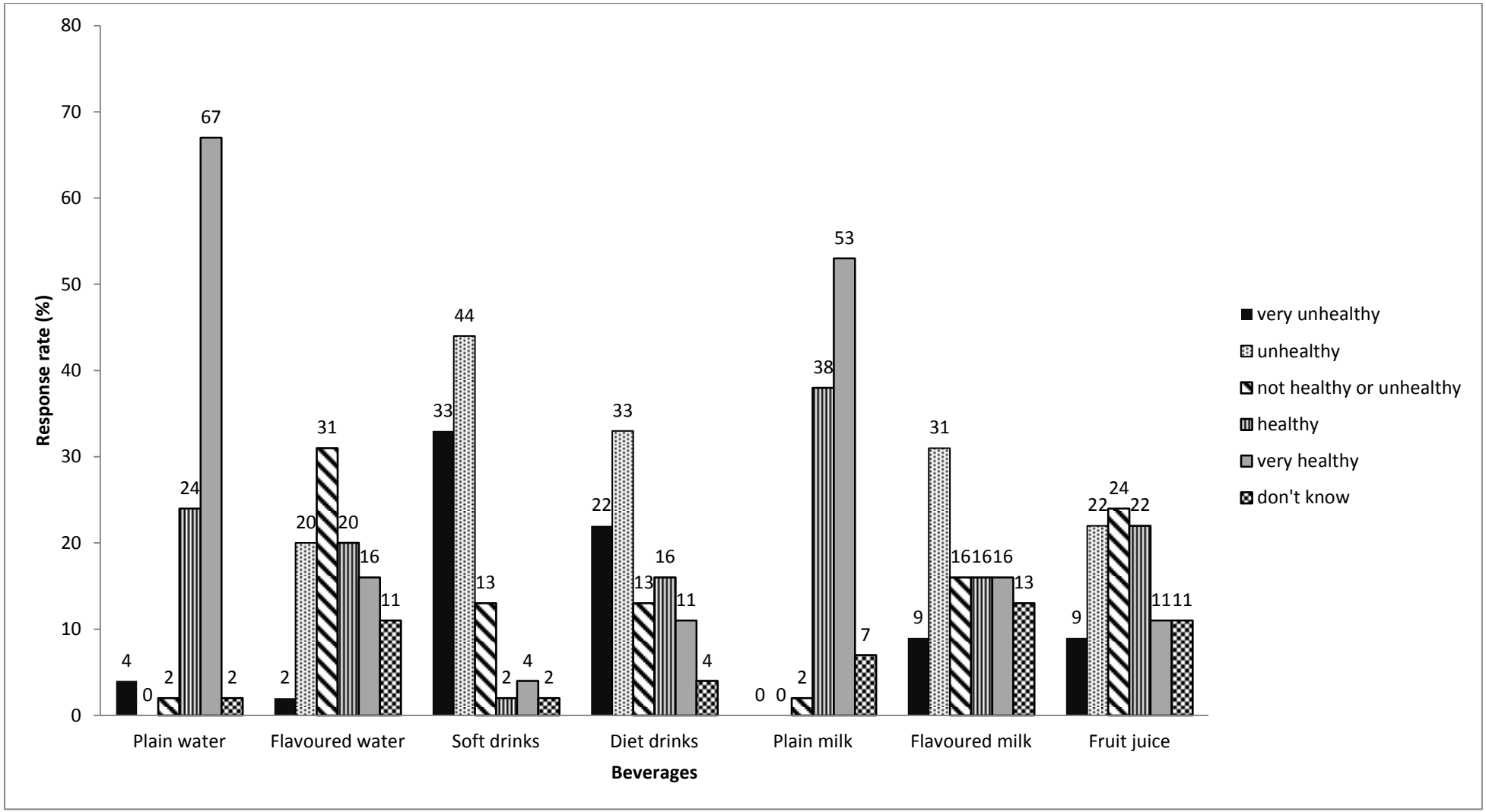

Figure 5.3: Children's perception about the healthiness of different beverages.

The FFQ also asked the children about their taste preference for dairy products like plain milk, flavoured milk, cheese, and yoghurt. Children rated yoghurt as the most liked dairy food (60%), flavoured milk (51%) was second, plain milk (49%) was third and cheese (44%) came fourth (Figure 5.4).

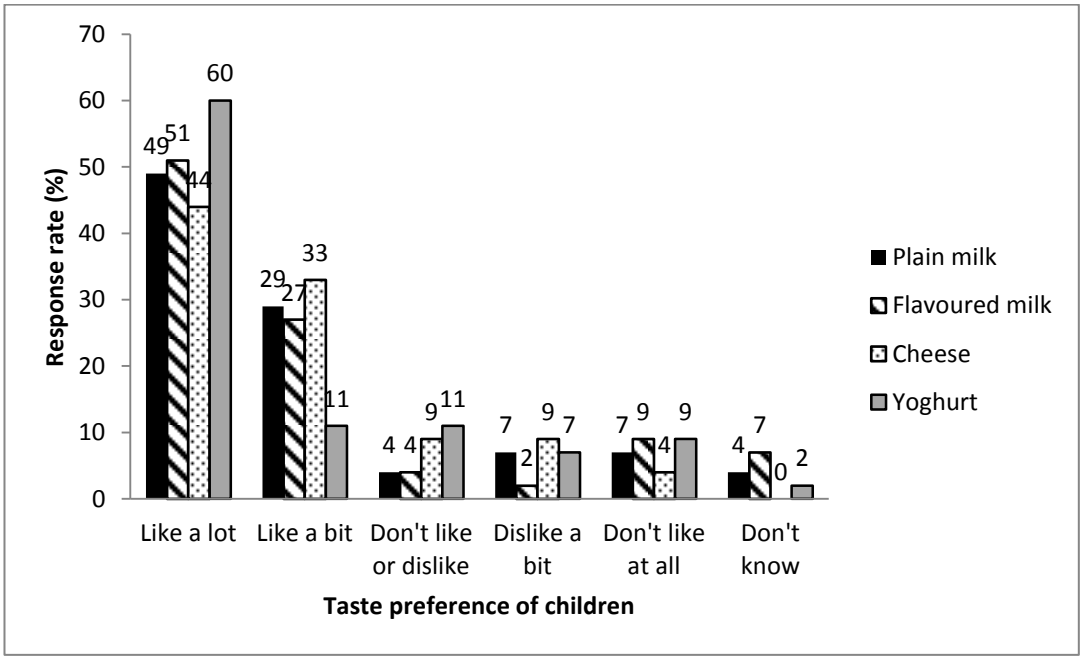

Figure 5.4: Children's taste preference regarding various dairy products.

The FFQ used in this study was designed to estimate the average serves of dairy consumed per day, allowing the calcium content from these serves to be estimated. The participants ranged in age from 5 to 10 years, thus the calcium intake was examined separately for children aged ≤ 8 years (n=38) and ≥ 9 years (n=7) due to different reference intake values (Table 5.3). Both age groups had mean calcium intakes higher than the RDI but still below the upper limit (UL) levels.

Table 5.3: FFQ mean calcium intake per day of the population divided in groups based on the RDI.

Age	Calcium intake/d (mg) ± SD	RDI (mg)	UL (mg)
≤ 8 years	1423 ± 533	700	2500
≥ 9 years	1305 ± 545	1000-1300	2500

When the whole population sample was examined together, the mean intake of calcium was still above the RDI (NHMRC, 2006) and so were the average dairy serves consumed per day (MOH, 2012a). There was no significant difference between the daily calcium or dairy intake of boys vs girls. Two sample independent t tests showed no significant difference in the calcium or dairy intake between boys vs girls (Table 5.4).

Table 5.4: Total daily calcium intake of the population.

Dietary variable	Total sample (n=45) Mean ± SD	Boys (n=26) Mean ± SD	Girls (n=19) Mean ± SD	t value	p
Calcium (mg)*	1474 ± 547	1492 ± 546	1285 ± 541	1.26	0.21
Dairy serves/d	4.8 ± 1.7	4.7 ± 1.8	4.9 ± 1.8	-0.12	0.90

*for children aged 4-8 years the RDI for calcium is 700mg/d and for 9-13 year olds it is 1000-1300mg/d. The upper level (UL) for calcium is 2500mg/d for children aged 4-13 years.

Regression was performed to find a relationship of calcium or dairy intake with the anthropometric (Table 5.5a) and bone characteristics (Table 5.5b) but no significant association was found.

Table 5.5a: Regression outcomes of calcium intake and dairy serves per day (parameter estimate ± SE and p value).

Variables	WC (cm)	BMI (g/cm^2)	TFM (kg)	LBM (kg)	% BF
Calcium (mg/d)	6.15 ± 11.5 *0.59*	-8.7 ± 4.8 *0.07*	-0.6 ± 1.5 *0.65*	-4.8 ± 5.3 *0.38*	3.5 ± 4.4 *0.43*
Dairy serves/d	67.6 ± 110 *0.54*	-65 ± 46.2 *0.17*	-5.3 ± 14.2 *0.70*	-64.2 ± 51.2 *0.22*	51.4 ± 42 *0.22*

Table 5.5b: Regression outcomes of calcium intake and dairy serves per day (parameter estimate ± SE and p value).

Variables	tBMC (g)	tBMD (g/cm^2)	LS-BMC (g)	LS-BMD (g/cm^2)
Calcium (mg/d)	0.2 ± 0.4 *0.69*	0.3 ± 0.7 *0.63*	-5.3 ± 5.2 *0.31*	-0.2 ± 0.3 *0.59*
Dairy serves/d	2.1 ± 3.8 *0.57*	3.0 ± 6.7 *0.65*	-30.4 ± 50 *0.54*	-0.7 ± 3.6 *0.84*

$r^2 = 0.75$ (for calcium intake mg/d)

$r^2 = 0.19$ (for dairy serves /day)

Subjects were divided into two groups based on their calcium intake i.e. below or above median. A comparison of means of all variables was then tested by ANOVA based upon the below and above median calcium intakes, but no statistically significant difference was found for any variable when divided in to high and low intake groups.

5.2.2. Food Record:

An estimated 3 day food record was provided to each child who took part in this study but unfortunately only 25 completed food records were returned. The intakes of several nutrients with roles in bone metabolism are shown in table 5.6.

The data for calcium, vitamin D, and protein intake were not normally distributed hence they are represented as median and IQR. All the other nutrients had normally distributed data so mean and SD was used. Since the NRVs are separately set for younger children (4-8 years old) and older children (9-13 years old), the sample was divided into two groups depending on the age. From the 25 food record received back, 20 were of children below 9 years of age and 5 were for children who were all 9 years old.

The median usual per day intake of calcium for younger children was above the RDI but for older children it was below the RDI. Similar to calcium, the mean daily intake of phosphorous in younger children was above the RDI but for older children it was below the RDI. The median daily vitamin D intake was below the AI for both age groups. Protein median intake for the younger children was very high, approximately three times above the RDI. Older children also had median protein intake above the RDI but not as high as the younger children. Mean intakes of magnesium and zinc, for both age groups were above RDIs. Also daily mean intake of dietary fibre was higher than the AI in both groups. Average sodium intake was very high, approximately four times the AI in younger children and three times the AI in older children. These values even exceeded the UL of 1400mg/d (4-8 y) and 2000mg/d (9-13 y).

When nutrient intakes were examined further we found that 15% of the younger children and 40% of older children had calcium intake <EAR respectively. In a few older children, the intakes for phosphorous, magnesium, and zinc were also below the EAR (Table 5.6). On comparison to the RDIs, 20% of the younger and

all of the older children had median intake values below RDI. Vitamin D usual median intake was <AI for 80% of younger and all of the older children. Only 1 young child (5%) and 4 older children (80%) had mean phosphorous intakes below the RDI. The dietary fibre intakes were below recommended AI in 35% of young and 20% of older children. Finally, average magnesium and zinc intakes were below the RDIs in 40% of 9-13 year olds (Table 5.6). Sodium and protein intakes were above the RDI/AI and EAR for both age groups.

Table 5.6: Comparison of the nutrient intakes of participants with NRVs. Participants divided into two groups; 4-8 year olds (n=20) and 9-13 year olds (n=5).

Nutrient	Mean ± SD Median ± IQR		<EAR % (n)		<RDI /AI % (n)		NRV					
							EAR		RDI		AI	
	4-8 y	9-13y	4-8 y	9-13y	4-8 y	9-13y	4-8 y	9-13y	4-8 y	9-13y	4-8y	9-13y
Calcium (mg)*	922 ± 440	809.7 ± 259	15 (3)	40 (2)	20 (4)	100 (5)	520	800-1050	700	1000-1300	-	-
Vitamin D (ug)*	2.5 ± 2.75	2.4 ± 0.4	-	-	80 (16)	100 (5)	-	-	-	-	5	5
Phosphorous(mg)	1240.5 ± 413	704.5 ± 393	0	60 (3)	5 (1)	80 (4)	405	1055	500	1250	-	-
Magnesium (mg)	247 ± 83.4	245 ± 78.4	0	20 (1)	0	40 (2)	110	200	130	240	-	-
Zinc (mg)	8.2 ± 2.2	7.1 ± 2.5	0	20 (1)	0	40 (2)	3	5	4	6	-	-
Sodium (mg)	2509 ± 729.2	2839 ± 1347	-	-	0	0	-	-	-	-	300-600	400-800
Protein (g)*	73.2 ± 23.8	67.8 ± 10.2	0	0	0	0	16	24-31	20	35-40	-	-
Dietary fibre (g)	20.6 ± 7.4	24.5 ± 6.4	-	-	35 (7)	20 (1)	-	-	-	-	18	20-24

*Median ± IQR

NRV=nutrient reference value ("the levels of intakes of nutrients considered to be essential for optimal functioning of the human body"), EAR=estimate average requirement ("a daily nutrient level estimated to meet the requirements of half the healthy individuals in a particular life stage and gender group"), RDI=recommended dietary intake ("the average daily dietary intake level that is sufficient to meet the nutrient requirements of nearly (97-98%) healthy individuals in a particular life stage and gender group"), AI=adequate intake (" the average daily nutrient intake level based on observed or experimentally-determined approximations or estimates of nutrient intake by a group/s of apparently healthy people that are assumed to be adequate"), UL=upper level of intake ("The highest average daily nutrient intake level likely to pose no adverse health effects to almost all individuals in the general population. As intake increases above the UL, the potential risk of adverse effects increases"). (Sourced from NHMRC, 2006).

5.2.3. Comparison of calcium intakes from the FFQ and 3-day estimated food record:

The data obtained from FFQs were normal but calcium intake data from food records had to be normalised using log10 transformation. After normalisation, a Pearson's correlation was applied to test the validation of the FFQ. Table 5.7 below shows the mean and median calcium intakes from FFQ and food record respectively.

Table 5.7: Correlation between calcium intake assessed via a FFQ and a 3-day estimated food record.

	Mean ± SD/Median ± IQR (mg)	Range (mg)	Pearson's correlation	p-value
FFQ	1474 ± 547	320-2645	0.387	0.056
3-day estimated food record	884 ± 373	155-2227		

A non-significant ($p>0.05$) positive Pearson's correlation coefficient of 0.387 was obtained on comparing the two dietary assessment methods. To test how much variability in FFQ is shared by food record, a coefficient of determination (r^2) was calculated. These two variables had a correlation of 0.387, so r^2 would be $(0.387)^2 = 0.15$ and when converted into percentage it becomes 15%. This shows that FFQ calcium estimation shares only 15% of variability in food record calcium estimated intake. Therefore, 85% of the variability is accounted for by other unknown variables.

5.3. Bone status:

Bone mineral data for 44 children were used because one child had a cast on a broken arm and her data were excluded. A comparison of the total bone mineral density without head (tBMD) with reference population showed that for all children except one the z scores fell within the normal range. BMD height adjusted z scores for all children are present in appendix 11.

There was no significant difference present between any of the bone characteristics between boys and girls (Table 5.1). All variables except otherwise specifically mentioned were transformed by reciprocal transformation to

normalise the data. Multivariate regression with dependent variables tBMD, tBMC, LS-BMD, and LS-BMC was performed. Variance inflation factor (VIF) was used to detect any effects due to multi-collinearity. After the removal of WC and calcium intake (because the VIF >10 and both WC and calcium intake were not significantly related to any bone parameters) the standard error visibly reduced for all other variables. BMI was also excluded from the model because it was not a very good predictor of body mass. Five participants (four boys and one girl) who had a BMI value categorised as overweight or obese on further inspection showed optimal % BF. Another finding is that out of these five children, four were non-white.

Table 5.8 shows that both tBMC and tBMD were significantly and positively associated with TFM and LBM but a negative significant association was found with %BF. Weight was only significantly related to tBMD and not tBMC. LS-BMC and LS-BMD showed no significant associations with any of the variables.

Table 5.8: Associations between bone mineral status and anthropometric measurements (parameter estimate ± SE and p value).

Variables	Height (feet)	Weight (kg)	TFM (kg)	LBM (kg)	% BF	Dairy serves/d
tBMC (g)	0.12 ± 1.1	8.50 ± 4.2	2.10 ± 0.7	13.8 ± 2.0	-11.3 ± 1.6	0.02 ± 0.01
	0.917	*0.053*	*0.0035**	*<0.0001**	*<0.0001**	*0.185*
tBMD (g/cm^2)	-0.17 ± 0.1	4.70 ± 2.1	1.38 ± 0.3	8.14 ± 1.05	-6.92 ± 0.8	0.006 ± 0.005
	0.764	*0.037**	*0.0003**	*<0.0001**	*0.020**	*0.270*
LS-BMC (g)	0.05 ± 0.06	0.32 ± 0.2	-0.03 ± 0.04	0.15 ± 0.1	-0.070 ± 0.09	0.0001 ± 0.0007
	0.450	*0.217*	*0.337*	*0.206*	*0.430*	*0.21*
LS-BMD (g/cm^2)	1.38 ± 0.8	4.16 ± 3.0	-0.51 ± 0.4	-0.65 ± 1.5	0.16 ± 1.17	-0.0008 ± 0.008
	0.105	*0.182*	*0.293*	*0.662*	*0.14*	*0.922*

*statistically significant p value ($p<0.05$).

$r^2 = 0.86$ (tBMC)

$r^2 = 0.95$ (tBMD)

$r^2 = 0.93$ (LS-BMC)

$r^2 = 0.86$ (LS-BMD)

Calcium intake of children (from FFQ) had no statistically significant associations with the bone variables. Similarly dairy serves consumed per day also had no significant associations with any of the bone variables as well.

5.4. Physical activity and bone status:

A physical activity questionnaire was completed by all children (n=45). A metabolic equivalent of task (MET) which represents how much oxygen the body is taking in per minute was used as the primary indicator of physical activity, however, energy expenditure (EE) was also calculated using the average MET levels. The median METs for the sample were 1.69 and no significant difference was found between the METs of boys when compared to girls (Table 5.1). The average METs of the children were also categorised as low (<1.45 METs/d), medium (1.45-1.60 METs/d), or high (>1.60 METs/d) physical activity levels (Figure 5.5).

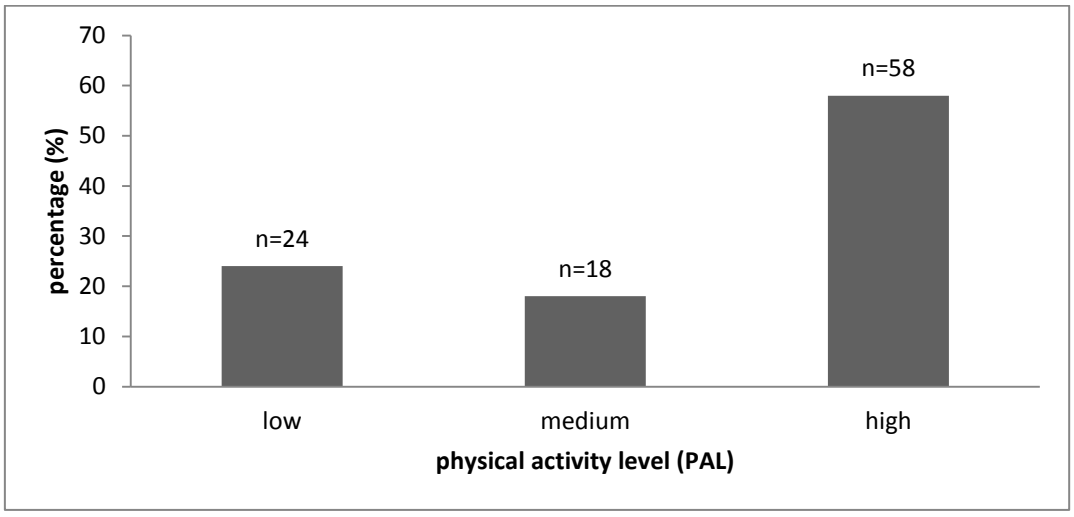

Figure 5.5: Physical activity level of the children.

Regression analyses were also performed to test the relationship between bone mineral status and physical activity variables i.e. METs and EE. Before the analysis was performed, all data were normalised by applying the transformation of log10. Table 5.9 shows the outcome of multiple linear regression.

Table 5.9: Multiple linear regression outcomes for physical activity variables (parameter estimate ± SE and p value).

Variables	tBMD (g/cm^2)	% BF
METs	-1.06 ± 0.7 *NS*	-0.8 ± 0.2 *0.003**
Energy expended (kJ)	3.73 ± 1.4 *0.013**	-0.3 ± 0.3 *NS*

*statistically significant p value ($p<0.05$).

The METs showed no significant associations with bone variables, however, the EE calculated from METs did show a significant positive association with tBMD. When other variables were inserted into the regression model, a negative significant association was only found between METs and % BF, which meant an increase in METs resulted in a decrease in total % BF.

CHAPTER 6: DISCUSSION, CONCLUSIONS, AND RECOMMENDATIONS

This cross-sectional study was designed to investigate the relationship of bone health status with calcium intake and body composition in pre-pubertal children. The secondary aim was to determine whether bone mineral status was affected by physical activity and other influential nutrients.

The results of this study suggest that neither calcium intake nor dairy serves haa a significant association with any of the bone mineral characteristics (Table 5.5a, 5.5b). Both tBMD and tBMC showed a positive relationship with lean body mass (LBM) and total fat mass (TFM) (Table 5.8). In addition to this, an increase in physical activity was associated with an increase in tBMD and a decrease in %BF (Table 5.8).

6.1 Anthropometric Indicators:

The rate of obesity has increased over the past decade with one in nine New Zealand children being classified as obese (MOH, 2013). The BMI of this study's sample also showed a small proportion of obese children, but since BMI can be misleading (as it does not take into account the muscle mass, bone weight, and other factors), a body composition analysis was performed using DEXA.

Body composition generally is not found to differ between younger boys and girls (Djafarian et al, 2015), but as age increases and sexual maturation approaches the difference in body composition based on gender becomes more apparent. Numerous studies have shown that a gender based difference in body composition exists in pre-pubertal and pubertal children (Park et al, 2011; Rajeswari et al, 2012; Zhang et al, 2015). In these studies fat mass and % BF was found to be higher among girls than boys and LBM or fat free mass was significantly higher in boys. This current study also showed similar results with girls having a higher % BF and boys having greater LBM (Table 5.1). Numerous factors are believed to play a role in this disparity of fat distribution between genders; hormonal or endocrinal factors near or during puberty can affect the body composition in children. Girls gain more fat during puberty due to oestrogen whereas boys have

greater lean and skeletal mass possibly due to androgen (Loomba-Albrecht et al, 2009). Genetic variation (Comuzzie et al, 2009), rate of the growth spurt (Malina et al, 1999), stress or emotional strain (Donoho et al, 2011), physical activity level, and lifestyle are a few other factors associated with fat mass in children (Staiano and Katzmarzyk, 2012).

6.2 Calcium and dairy intake:

6.2.1 Analysis of the FFQ:

The population sample in this study had both mean calcium and dairy intakes above the recommended levels and no significant difference was found between the intakes of boys and girls (Table 5.2, 5.3 and 5.4). Previous studies in New Zealand children have also shown the baseline calcium intakes to be above the RDI levels (Gibbons et al, 2004; Houghton et al, 2010). In accordance with the National Children's Nutrition (NCN) survey's findings younger children in this study's sample also had higher calcium intake compared to the older ones. However, in contradiction to the NCN survey where calcium insufficiency of 15.1% was found (MOH, 2003), FFQ analyses of this study showed that the mean calcium intake of participants were above the RDI (Table 5.3).

The New Zealand NCN survey reported that the most commonly consumed type of milk was whole milk and likewise the majority of participants in this study also consumed whole milk or dark blue top milk (MOH, 2003). Other than milk, the most frequently consumed beverage by children was plain water. The average daily consumption of fruit juice by the sample was low at school (27%) but on weekdays and weekends it increased to 71% and 62% respectively. Soda drinks were most commonly consumed on the weekends (60%). When asked about the healthiness of these beverages, children believed that plain water was the healthiest (67%) followed by plain milk (53%). The unhealthiest beverage was chosen to be soda drinks (33%) and diet drinks (22%). Flavoured water and fruit juice were the drinks that these children were unsure about, as they rated them as neither healthy nor unhealthy. Participants were also asked about the taste of different dairy products; the sample responded with yoghurt (60%) as the most liked dairy food, followed by flavoured milk (51%), plain milk (49%), and cheese (44%).

A single factor or cause explaining why children responded this way cannot be precisely explained. Participants in this study were ≤10 years of age and hence their food choices and taste perceptions can be influenced by numerous innate and environmental factors. A child inherits many traits through genetic transmission from the parents including food preference and taste perception (Kral and Rauh, 2010). Several other important factors which influence the food choices are the family environment, peer influence, and media exposure such as advertisements on television (Patrick and Nicklas, 2005; Ferguson et al, 2012). Holsten et al (2012) explained that the most influential factors affecting children's food choices are the food preference and the role of the parent. The parent(s) can influence a child's dietary habits in many ways; food purchasing and preparation, setting rules, and most importantly acting as a role model for the child (Scaglioni et al, 2008). A study by Forshee and Storey (2003) showed that children's beverage consumption and food choices were strongly related to age, race, and gender. Younger children are more likely to consume plain milk and older children prefer high fat dairy foods (Kranz et al, 2007). In this bone healthy study, however, a comparison based on these characteristics could not be made due to a small sample size.

The FFQ used in this study was not detailed enough to find out what particular type or brand of dairy product was being consumed, and hence a general brand of that product had to be used. When putting data into the foodworks database, 'colby' cheese was used as it along with cheddar is the most commonly consumed type of cheese by New Zealand children (MOH, 2006). Also 'sweetened fruit flavoured yoghurt' was used, because majority of the children mentioned eating it. The serving size for each dairy food (as reported in the results) was adapted from the food and nutrition guidelines for children (MOH, 2012b).

6.2.2 Food record:

First of all Goldberg cut-off was used to test for reporting bias in energy intake (Black, 2000). The EE (measured form physical activity questionnaires) and basal metabolic rate (obtained from foodworks) for each child was used to calculate the Goldberg cut-off and the results showed that all food records fell within the acceptable range and thus none were excluded from analyses.

The main function of the food record was to obtain information regarding calcium and other nutrients that can affect bone development. The total median daily intake of calcium was above the recommended levels for the younger group but below RDI for children ≥ 9 years of age (Table 5.6). The New Zealand NCN survey also concluded that children had a 15.1% prevalence of inadequate calcium intake (MOH, 2003). Previous studies in New Zealand children (Gibbons et al, 2004; Houghton et al, 2010) as well as studies in children of other developed countries have also shown the calcium intake to be higher than the set recommended allowance (Emmett et al, 2002; Paeratakul et al, 2003; Royo-Bordonada et al, 2003).

Vitamin D intake in this study's sample regardless of age was below the AI. Vitamin D deficiency in New Zealand children is a serious emerging issue as the incidence of rickets has increased (Munns et al, 2006). Rockell et al (2005) have shown that 1 in 25 children and adolescents in New Zealand are vitamin D deficient with a serum concentration <17.5nmol/L. A recent study in European children also found paediatric vitamin D deficiency to be prevalent (Dobrescu et al, 2014). Vitamin D deficiency is a rapidly increasing problem in children living within both developed and developing countries (Ashraf et al, 2014). Vitamin D deficiency is common across the world but special consideration should be given to high risk groups like young children, pregnant women, elderly, and dark skinned individuals (Mithal et al, 2009). Very few natural foods contain vitamin D and thus endogenous skin synthesis of active vitamin D from 7-dehydrocholesterol via sunlight is essentially important (Brannon et al, 2008). Also the endogenously produced vitamin D is more efficient than that consumed from diet due to its gradual and continuous plasma availability and more bioactive compound, 25-hydroxyvitamin D_3 (Haddad et al, 1993).

Median daily intake of protein for the participants was higher compared to the RDI. The NCN survey also found similar findings where the daily intake of protein for children was twice the recommended levels (MOH, 2003). A cross sectional survey in New Zealand babies and toddlers also showed that the median intake of protein was much higher than the RDI value (Soh et al, 2002). Protein inadequacy as well as over consumption could impact calcium balance in situations of calcium insufficiency (Calvez et al, 2012). Therefore, for the

maintenance of optimal bone health adequate protein consumption should be maintained.

Other nutrients which can affect growth and bone metabolism are phosphorous, magnesium, zinc, sodium, and dietary fibre (Ilich and Kerstetter, 2000; Bonjour et al, 2009a). Phosphorous intake was adequate for the younger population in this study but for older children the intake was insufficient. Magnesium, zinc, and dietary fibre, all had mean daily population intakes above the recommended values. The NCN survey similar to this study found that New Zealand children had adequate phosphorous, magnesium, and dietary fibre intakes but in contrast to this study zinc consumption was low with an inadequate prevalence of 7.3% (MOH, 2003). Sodium intake of this study's population was very high; for younger children it was four times and in older children three times greater than the AI and also above the UL. Other studies in children and adolescents also showed sodium intake was very high, sometimes as high as that of the adults (Magriplis et al, 2011; Yang et al, 2012). High sodium intake is harmful to health as it is shown to be one of the main risk factor for cardiovascular disease especially in overweight and obese individuals (Strazzullo et al, 2009). Also high sodium levels increase calcium excretion and thus could contribute towards bone loss (Shi et al, 2012).

A secondary aim of this study was to examine the relationship between the above stated nutrients and bone health, but unfortunately low numbers of food records were received back (25 out of 45) and a whole picture of the population sample could not be obtained.

6.2.3 Validation of dietary calcium intake:

Studies have shown that even though a FFQ is good at distinguishing subjects into extremes of high or low nutrient intake, it can often overestimate that nutrient (Taylor and Goulding, 1998; Bertoli et al, 2005; Del Pinno and Friedman, 2011). Therefore, to test the reliability and validity of a FFQ it should be validated against another dietary assessment method such as several 24 hour recalls or weighed/estimated food records (Thompson and Subar, 2008). One of the objectives of this study was to validate the FFQ against a reference method. Thus, an estimated 3 day diet record was used. Only 25 food records were received and

due to these low numbers an evaluation rather than a validation was performed with what food records were available.

The FFQ used in this study helped in ranking low and high calcium intake groups. However, it was not useful in estimating the dietary calcium intake accurately due to overestimation. Previous studies have also shown FFQs to overestimate or underestimate calcium intake in comparison to reference methods (Taylor and Goulding, 1998; Bertoli et al, 2005; Magkos et al, 2006) but unlike this study's results they did find significant correlations, probably due to the usage of better FFQs. In this bone health study, the correlation of dietary calcium intake between the FFQ and 3-day estimated food record was non-significant with a low correlation coefficient (r=0.387, *p=0.056*). Calcium intake by the FFQ was overestimated (Table 5.7) and this could have been due to children being young as some were as young as 5 years old. Another inaccuracy that could have led to incorrect estimation of calcium intake by FFQ could be error in portion size estimation.

6.2.4 Calcium/dairy intake and body composition:

No significant associations were found between calcium or dairy intake and body composition in this study's sample. Neither TFM nor %BF was significantly related to calcium or dairy intake (Table 5.5a). Unlike this study previous observational studies in children have shown a significant inverse relationship of adiposity with calcium as well as dairy intake (Davies et al, 2000; Carruth and Skinner, 2001; Moreira et al, 2005; Barr et al, 2007). Several mechanisms have been postulated as to why calcium affects adiposity. During high calcium intake, circulating 1,25-dihydroxyvitamin D can increase intracellular calcium which can then lead to the suppression of lipogenesis and the stimulation of lipolysis. This in turn leads to an inhibition of lipid filling in adipocyte i.e. decreased adiposity (Zemel 2005). Increasing dairy intake can also result in calcium and lipids forming insoluble salts within the intestine. These salts would then decrease fat absorption and increase faecal fat excretion and if no compensation follows, the weight loss can be accentuated (Christensen et al, 2009). Many studies have shown a negative impact of calcium on body fat but this phenomenon is not always true as a meta-analysis of RCTs performed to study the effect of dairy

foods on body fat concluded that dairy products do facilitate weight loss in short term studies but in long term studies (> 1 year) and in trials where no energy restriction occurs, a beneficial effect does not exist (Chen et al, 2012).

6.3 Bone Mineral Density and Bone Mineral Content:

6.3.1 Lean body mass and body fat:

In this study of pre-pubertal children lean body mass (LBM) and fat mass (FM) showed significant positive associations with tBMC and tBMD. Only tBMD was positively related to weight. A negative but significant association of BF % with tBMC and tBMD was also present (Table 5.8). Similar to these findings, a recent study has also shown that LBM and TFM are positively correlated to tBMC and tBMD but negative associations were found between BF % and femur and lumbar spine in girls and BF % and tBMC and tBMD in boys (Mosca et al, 2014). Another study also found that LBM and FM were positively related to tBMC and tBMD but unlike this bone health study the BF % was positively associated with tBMC and tBMD (El-Soud et al, 2006).

Numerous bone health studies have been conducted in children but the results are varied; some studies have found that both LBM and TFM are positive predictors of bone mineral status (Pietrobelli et al, 2002; Ackerman et al, 2006; Clark et al, 2006; Ka et al, 2013) while others show a positive relation with LBM but an inverse association with FM (El-Hage et al, 2008; Hrafnkelsson et al, 2010). Some other trials where bone health was studied in association to obesity concluded that children with high adiposity had lower bone mass and bone area suggesting that obesity does not have a protective effect on the skeleton and could be a risk factor for fractures (Goulding et al, 2001; Rocher et al, 2008).

As discussed above, studies have shown inconclusive results with some suggesting a protective role of body fat on bones and others showing that adiposity is detrimental to bone health (Cao et al, 2011). An explanation for why different studies in children present different findings regarding the effect of body fat on the skeleton could be the use of DEXA. The LBM or fat free mass hydration can alter the %BF calculated especially in overweight and obese subjects. Hence, errors in a DEXA scan can result when a child has high fat mass (Wildman and Henwood-Finley, 2012). Also DEXA does not provide a true

volumetric measure of the bone density because it releases only two dimensional x-rays. A study in pre-pubertal children showed that fat mass was positively related to bone area and BMC in DEXA scans but when true volumetric bone density was measured using peripheral quantitative computed tomography (pQCT) a negative association was found between fat mass and bone density (Cole et al, 2012). The mechanisms by which body fat could interfere with bone mineral are previously discussed in Chapter 2.

This study similar to many other trials also confirmed that LBM is a better determinant of bone mineral status than FM (Pietrobelli et al, 2002; Ackerman et al, 2006; Gjesdal et al, 2008). Table 5.7 shows a stronger association of LBM with tBMC and tBMD than FM. Gender can play an important role in determining bone mass because of differing hormonal levels between boys and girls. Bone studies have shown a gender based effect on bone; in girls FM is considered a better determinant of bone mineral status and in boys the LBM is more strongly related to bone status (El-Hage et al, 2008). These effects, however, are believed to be exaggerated after puberty (Ackerman et al, 2006). Gender difference and its effect on the relationship between body composition and bone mass could not be observed in this study due to a small sample size and also no significant difference was found between any of the bone variables based on gender. In this study, LS-BMC and LS-BMD showed no significant associations with any of the body composition variables.

6.3.2 Calcium and dairy intake:

Dairy products which are a rich source of calcium and protein play an important role in improving bone health and reducing the risk of fractures throughout life (Rizzoli, 2014). Meta-analyses have confirmed that dairy and calcium supplementation exerts a beneficial effect upon the bones and growth of children (Winzenberg et al, 2006; Huncharek et al, 2008). Studies in New Zealand children have also shown this association as children with low calcium intakes have poor bone health, shorter statures, and increased susceptibility to fractures (Black et al, 2002; Goulding et al, 2004). This bone health study did not show a significant association of any bone parameters with calcium or dairy products. Neither multiple regression nor simple linear regression for each parameter produced a significant effect. In this study, children had high baseline calcium intakes (Table

5.3) and this could be a reason why no significant relationship was found between bone parameters and dairy or calcium intake. A Cochrane review of children has also shown that calcium supplementation does not significantly improve bone health when the baseline calcium consumption is already high (Winzenberg et al, 2006). Another reason for not finding a significant effect of dairy/calcium intake on bone health could well be the already adequate or high bone mass of the participants. As mentioned in the results all children in this study (except one) had tBMD z-scores which fell within the normal healthy range.

The beneficial effects of calcium supplementation on bone health have been extensively studied in observational as well as intervention trials. Elemental calcium supplementation trials (Johnston et al, 1992; Lee et al, 1995; Dibba et al, 2000; Cameron et al, 2004; Matkovic et al 2005, Courteix et al, Ward et al, 2014), calcium enriched food supplementation trials (Bonjour et al, 1997; Iuliano-Burns et al, 2003; Chevalley et al, 2005), and finally milk/dairy products supplementation studies (Cadogan et al, 1997; Du et al, 2004; Lau et al, 2004; Zhu et al, 2008) all showed a significant positive association of calcium/dairy with bone mineral status. The characteristics of these trials are summarised in Tables 2.5 to 2.7.

Unlike majority of the observational cross-sectional studies (Boot et al, 1997; Bueno et al, 2010; Harvey et al, 2012) which show a significant association between bone health and calcium intake, this study was not able to find a statistically significant relationship between calcium intake and bone mineral status. A few previous studies were also not able to show an effect of calcium on bone mass in children (Kroger et al, 1992; Kroger et al, 1993). The reason for not being able to find a significant relationship between calcium and bone could be a flaw in the study design, sampling error, or inaccuracies in statistical analyses.

6.4 Physical activity:

6.4.1 Bone mineral accrual:

Physical activity in this study was expressed as METs and EE. The EE but not METs were significantly and positively related to tBMD (Table 5.8). Similar to this study, Meyer et al, (2011) also showed that in children physical activity significantly increased tBMD. Other improvements seen by them were in LS-

BMD, tBMC, LS-BMC, and femoral neck BMC. Several other intervention trials have also shown that moderate to vigorous physical activity in both pre-pubertal and pubertal children improves bone mineral accretion (Fuchs et al 2001; Mackelvie et al, 2003; Stear et al, 2003; Janz et al, 2006; Heidemann et al, 2013). Though all of these studies show a positive effect, the specific type of exercise, intensity, and duration vary between them. In addition to that, the bone site and bone parameters which are affected are also different. Hence, it is difficult to propose what the optimal stimulus for peak bone mineral accrual will be and at which site.

Regular physical activity in children is essential for the maintenance of a healthy skeleton, movement control, mental wellbeing, and weight management assistance (MOH, 2012b). High impact physical activity in children can positively impact bone mineral accrual by inducing osteogenic effects (Van Langendonck et al, 2003) which are believed to be heightened in pubertal children (Hind and Borrows, 2007). It is often believed that vigorous physical activity would lead to fractures but this notion was disproved by intervention trials where children who had received physical activity education of greater than 30minutes/day. The intervention group showed an improvement in bone mass and size without an increase in fracture rate (Lofgren et al, 2012; Detter et al, 2013).

It has been confirmed by many trials that calcium has beneficial effects upon bone health (Winzenberg et al, 2006; Huncharek et al, 2008). However, this effect can be further enhanced as moderate to vigorous physical activity or exercise augments the positive effects of calcium consumption on bone mass accrual in children (Iuliano-Burns et al, 2003; Harvey et al, 2012; Ma et al, 2014).

6.4.2 Body fat:

Only the METs showed a negative significant relationship with %BF (Table 5.8). These results have been reported in pre-pubertal children previously as well (Barr, 2007). An active lifestyle starting from a young age is essential in controlling body fat levels (Vale et al, 2010) and even modest amounts of physical activity can have remarkable health benefits especially in overweight or obese children (Janssen and LeBlanc, 2010). Physical activity is not only an influential factor in maintaining a healthy body weight but it could also help control or reduce blood

lipids, decrease the incidence of metabolic syndrome, help achieve maximum bone mineral accretion, and improve mental health (Janssen and LeBlanc, 2010).

The MOH in New Zealand suggests that children should perform at least 60 minutes of moderate to vigorous physical activity every day (MOH, 2012b). This amount of exercise could be suitable to avoid excess adiposity in young children (Martinez-Gomez et al, 2010). The physical activity questionnaire used in this study was not detailed enough to give an overview of the whole day's activities but it did help us estimate the activity levels of children after school. We found that boys were overall more active than girls but when tested the difference was not significant. Furthermore, playing a game outside was the most frequently reported type of moderate to vigorous physical activity and 73% of the population sample watched television for at least 30 minutes in the time between 3pm to 11pm (as per the PDPAR). In this study, when METs were categorised based on PAL, majority of the participants had high PAL (Figure 5.5).

6.5 Limitations of the study:

There were several limitations of this study. The first group of children (n=29) were recruited for the baseline 'milk in schools project' and hence had not received any milk at school whereas the second group (n=16) which was recruited for this 'bone health study' was receiving milk in schools. This disparity could mean that the first group would have low calcium intake, but overall FFQ analyses of the population showed that mean dairy serves consumed per day were above the recommended guidelines. The FFQ analysis also estimated a high calcium intake for the whole sample. This could be because many children in the first group came from farms and therefore have high milk consumption. Furthermore, another limitation is that no information was collected on the socioeconomic status of the children as it can provide vital information of dietary habits.

Another limitation could be a human error in anthropometric measurements because of the researcher's skills and knowledge, as well as equipment can affect the measurement techniques. The BMI of this study's participants varied from underweight to normal and obese but this would not have a major impact on

statistical analysis as it was not used due to BMI being misleading. Instead TFM and LBM were used.

The FFQ used in this study was basically designed to estimate average dairy serves consumed on a weekday and weekend. It was not designed to specifically estimate total calcium intake, however, it was still done and this could be the reason why calcium intake levels reported were very high. A further limitation of the FFQ was that it only contained a few dairy foods and did not include other dietary sources of calcium such as green vegetables, legumes, fish with edible bones e.g. sardines, and calcium fortified foods. It also excluded a few commonly consumed foods made from milk like butter, cream, and ice cream and dietary supplements of calcium. In addition to calcium, many other nutrients can also affect bone development but the FFQ used in this study did not collect information about these. A FFQ is good at differentiating high and low nutrient intake groups but is not good at estimating an average habitual intake (Thompson and Subar, 2008). A main limitation of almost all FFQs is the measurement error caused due to self-reporting. Errors can arise when all relevant food items are not listed and due to misreporting of the portion sizes (Thompon and Subar, 2008). The food database used can also contribute towards inaccuracy as only the portion sizes and foods already present in it are often used, however, in this study many new food items were added to the database and used. Also the portion sizes used were based on the MOH guidelines (MOH, 2012a) and different models were shown to the children to obtain an estimate as reliable as possible. The researcher also helped the children fill out their FFQs and in some instances the parent was present to guide the child. The involvement of the researcher and parent could lead to either better or mis-reporting of foods (depending on their skills and knowledge).

A FFQ should be validated to test its reliability and sensitivity as many validation studies have shown that FFQs can often over or under report a nutrient intake (Taylor and Goulding, 1998; Montomoli et al, 2002; Del Pino and Friedman, 2011). To test the reliability and cogency of the FFQ used in this study, the FFQ used was validated against the estimated 3-day food record but unfortunately due to small numbers of food records available the statistical outcome was not

considered very reliable. Thus, the secondary objective of this study instead became an evaluation rather than a validation of the FFQ.

The participants in this study were thought to be too young to fill in their estimated 3-day food diaries themselves, thus the parent/s acted as a proxy for their child/children. Parents are considered to be reliable reporters of their children's food intake at home, but out of home the reliability becomes limited (Livingstone and Robson, 2000), and hence this could be a limiting factor in recording children's food intakes. Food records can also have high respondent burden and could affect the eating pattern leading to over or under reporting. All dietary assessment methods have some sort of errors and it is very hard to obtain a completely perfect profile of an individual's dietary habits.

PDPAR has been used numerous times and has been previously validated as well (Trost, 1999). It is a very good tool in terms of reporting all levels of activities; even the sedentary behaviours. Some limitations of this physical activity questionnaire were that it was based on the child's memory of the previous day hence there is a chance that physical activities would be misreported. The reporting of activities started after 3.pm, hence a big part of the day (morning and noon) had been missed out. The researcher tried to extend the PDPAR for the whole day but it did not work as children found it very difficult to remember the whole previous day. One more limitation is that in this study PDPAR was administered only on one day, but it has been suggested that it would be best to administer the PDPAR over a few days with both weekdays and weekends included to get an idea of the individual's typical activity behaviours (Welk, 2008; Hart et al, 2011). Thus the limitation of using a one day physical activity record and a 3-day diet record is that these tools only provide a snapshot of the diet/physical activity at one point in time and there is a possibility that typical diet/physical activity routine could be completely different.

Several errors can also arise in DEXA measurements. The body fat assessment by DEXA might be affected by the hydration of LBM but a study comparing DEXA with the four compartment model which included total body water assessment as well showed no correlation of LBM hydration with body fat (LaForgia et al, 2009). Other errors can arise due to presence of artefacts and movement during

the scans and when using different software versions (Fosbel and Zerahn, 2014). In this study the same software was used every time. There was slight movement reported by the technician in several children. Also scan for one child could not be used due to a cast on the arm. Another limitation was the usage of two dimensional x-rays by DEXA on a three dimensional structure like bone. Thus, true volumetric density of bone could not be obtained by DEXA (Wildman and Henwood-Finley et al, 2012). Children are growing and hence their bones are still developing but in this study changes in bone remodelling were not studied as no blood or urine bone biomarkers were assessed. Also in young children errors can arise due to their low absolute BMC. A major limitation of this study is that any appendicular skeleton sites were not measured. Previously it has been confirmed by many trials that in pre-pubertal children appendicular skeleton sites are more responsive to calcium supplementation than axial skeleton (Johnston et al, 1992; Lee et al, 1995; Bonjour et al, 1997; Cadogan et al, 1997; Dibba et al, 2000; Zhu et al, 2008).

This is an observational cross-sectional study where data were collected on one occasion only, hence reverse causality could not be used to interpret any results. Another limitation of the cross sectional study is that it only captures a snapshot of that particular time and there is a possibility that different outcomes will be obtained when the same study is performed at a different time period (Man, 2003). Also, bias can occur when the response rate is low and significant information can be missed out (similar to low retrieval of food records in this study). Finally, a cross sectional study cannot determine the cause and effect relationship.

6.6 Conclusions:

FFQ analyses concluded that the mean calcium as well as dairy serves consumed per day by the participants were above the recommended guideline values. Despite numerous studies showing a beneficial effect of calcium or dairy intake on bone health and body composition of pre-pubertal children (Johnston et al, 1992; Bonjour et al, 1997; Cadogan et al, 1997; Black et al, 2002; Barr et al, 2007; Zhu et al, 2008; Bueno et al, 2010), the results from this study found no such significant relationships, most probably due to a small sample size or flaws in the assessment methods. Only 25 out of a total 45 children returned the

completed 3 day estimated food records and hence a reliable validation of the FFQ could not be performed and it could hence be considered as a pilot study for validation. The available food records showed that the average calcium intake per day for younger children (4-8 years old) was above RDI, but for older children (≥ 9 years old) it was below it. As shown previously in New Zealand children (Rockell et al, 2005), this study also concluded that vitamin D intake in children was below the RDI. Sodium intake was very high as the mean of the population even exceeded the UL intake recommendations.

tBMC and tBMD both increased with an increase in LBM and TFM. However, the tBMC and tBMD were negatively associated with %BF showing that total bone mineral status decreases as the %BF increases. Furthermore, only tBMD increased with an increase in the body weight. Lumbar spine bone mineral status was not significantly affected by calcium intake, body composition parameters, or physical activity.

Habitual physical activity is believed to have a positive impact on bone mineral accrual in children (Harvey et al, 2012) thus in this study the relationship of physical activity and bone health was studied. The physical activity was measured as MET and EE and the results showed that the tBMD increased with an increase in EE but not MET. Also the effects of physical activity were studied in relation to %BF. The MET and not EE was negatively associated with %BF, concluding that the higher the levels of physical activity the lower the %BF in pre-pubertal children.

Finally, we did not find an association of bone parameters with calcium or dairy intake, but other variables such as LBM, TFM, %BF, physical activity did affect the tBMD and tBMC status in pre-pubertal children.

6.7 Recommendations:

Below are listed some recommendations based on the literature cited and the results of this study.

1) Adequate calcium intake is the most extensively studied factor in association with bone health. It is not only required for the attainment of PBM in adolescence but also for the prevention of osteoporosis in later years of life

(Zhu and Prince, 2012). Therefore, public health messages should promote the importance of calcium and dairy foods intake for children in maintenance of stronger bones. Despite adolescence being the period of rapid growth and development, considerable attention should also be given to the nutrition promotion in pre-pubertal children as at this stage the transition into puberty starts to begin. The NCN survey showed that NZ children were consuming insufficient calcium, hence there is a need to identify which children in particular are at risk of low dairy/calcium intake. Interventions should be carried out targeting these children.

2) More intervention studies of calcium or dairy supplementation should be focussed towards pre-pubertal children as there are not enough data present for this particular age group. Furthermore, the trials should be focused towards children with low calcium intake because children who already have a high or optimal calcium intake showed no effects of intervention (Gibbons et al, 2004).
3) Dairy is a very good source of calcium, but other non-dairy food sources such as green vegetables, legumes, fish that can be eaten with bone (e.g. sardines), and tofu should also be used to promote growth and good bone health. In addition to calcium, there are some other nutrients such as vitamin D, phosphorous, and protein (to name a few) which affect bone metabolism and thus should be examined in studies as well.
4) If a study of similar nature was to be conducted again, the following few points should be taken into consideration:
    - In this study a mechanical spring weighing scale was used, and it might lead to unknown errors during reporting of decimal places. In future, it would be preferable to use a good quality electronic scale so that the machine could itself provide an accurate result excluding any human measurement error.
    - The FFQ that was used only enquired about the dairy intake. More details need to be added to the FFQ; such as milk based food products like ice-cream, cream and butter/margarine. Also supplements should also be included in the FFQ to find out if any calcium is being consumed through supplementation.

- The food record retrieval rate was not very good in this study, but food diaries especially the gold standard 'weighed food records' should still be used in the future to assess dietary habits, as this method is the most comprehensive and detailed method of knowing about an individual's diet. Rewarding of a prize or vouchers can be used a strategy to improve the retrieval rate.
- Using a pQCT scan to assess bone mass could overcome the problems faced with DEXA such as not being able to obtain a true volumetric density of bone. However, QCT is expensive with a higher exposure to radiation and would not be very feasible to use in a study of children with limited funding.
- In the current study only axial skeleton bone parameters were used, in the future appendicular skeleton should also be analysed and examined as studies in children have shown calcium supplementation to have a more beneficial effect on the limbs.
- To examine bone development in children in more detail, the blood and urinary bone biomarkers should also be studied.
- PDPAR for one day does not provide a picture of an individual' s typical activities per day. Hence, the PDPAR should be administered for several days including both weekdays and weekends. Also it would be even better to use accelerometers because young children are not very reliable reporters of previous day's activities.
- In future, a larger population sample should be recruited. A long term longitudinal study with a large sample size would establish a better sequence of events and more reliable cause-effect relationships.

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Appendices

Appendix 1 – Ethics approval of the study

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA

23 February 2015

Tahibia Awan
31b Worcester Street
PALMERSTON NORTH

Dear Tahibia

Re: HEC: Southern A Application – 15/03
The relationship of bone status and body composition with calcium intake in pre-pubertal children

Thank you for your letter dated 23 February 2015.

On behalf of the Massey University Human Ethics Committee: Southern A I am pleased to advise you that the ethics of your application are now approved. Approval is for three years. If this project has not been completed within three years from the date of this letter, reapproval must be requested.

If the nature, content, location, procedures or personnel of your approved application change, please advise the Secretary of the Committee.

Yours sincerely

Mr Jeremy Hubbard, Chair
Massey University Human Ethics Committee: Southern A

cc Prof Marlena Kruger
School of Food and Nutrition
PN452

A/Prof Jane Coad
School of Food and Nutrition
PN452

Dr Raewyn Poulsen
School of Food and Nutrition
PN452

Distinguished Prof Harjinder Singh, HoS
School of Food and Nutrition
PN452

Appendix 2 – Sample size calculation for this study

The power calculation for sample size estimation is as follows:

-Prevalence of inadequate calcium intake in NZ children (p) = 15.1% (National children's nutrition survey, 2002).

-confidence interval = 90%

-z-score for 90% confidence interval =1.645

$$\text{Standard error (SE)} = \frac{10}{1.645} = 6.07$$

Sample size calculation:

$$N = \frac{p(100-p)}{(SE)^2} \qquad N = \frac{15.1(100-15.1)}{(6.07)^2} \qquad N = 1282/36.8$$

$$N = 35\ (\pm\ 10)$$

Appendix 3 – Parental consent form

MASSEY UNIVERSITY
COLLEGE OF HEALTH
TE KURA HAUORA TANGATA

Bone Health Research Project

PARENT 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 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 .....

Te Kunenga ki Pūrehuroa

Institute of Food, Nutrition and Human Health

Private Bag 11222, Palmerston North 4442, New Zealand **T** 64 6 350 4336 **F** 64 6 350 5657
http//:ifnhh.massey.ac.nz

Appendix 4 – Health screening questionnaire

Massey University
COLLEGE OF SCIENCES
School of Food and Nutrition
Te Kura Hangarau o Kai-orangā-ā-tāngata
Human Nutrition & Health

HUMAN NUTRITIONAL STUDIES: Laboratory Procedure

Health Screening Questionnaire

Name of the child……

Child's date of birth……

Telephone #……

Email address…….

Has the child ever had any of the following:	**√** if yes or X if no
Any type of bone disease	
A history of gastrointestinal disease other than appendicitis	
Diabetes or persistent sugar in the urine	
Endocrine disease (hormone trouble)	
Kidney problems or a renal disease	
Disorders of the liver	
A recent gut infection	
Any chronic or recent constipation	
Diarrhoea in the last 1 month	
Cancer, tumour or growth of any type	

- Is the child allergic or sensitive to any dairy products? ……
- Is the child allergic to plasters or antiseptic wipes? ……

- Does the child have clotting problems? ……

Does the child take any of the following **√** if yes or X if no

Vitamin supplements	
Mineral supplements	
Medication prescribed by your Doctor	
Extra calcium	
Pills for diabetes	
Water pills (diuretics)	

Please list any medication of the child below:

……
……
……
……
……
……

Appendix 5 – Information sheet and assent form for child

Bone Health Study

Information Sheet for Children

We would like it invite you to take part in a study to find out about how much milk you drink and how this affects your bones. Nutrients found in milk can help your bones grow and stay strong. We want to find out if drinking milk makes your bones healthier.

About 20 children are going to take part in this study. If you want to take part you will be asked to fill in a form to tell us how much milk, cheese, yoghurt and ice cream you eat. You will need to fill in the form once so we can see what you eat. You will also be asked to fill in another form which asks about the sorts of activities you do. You will only need to fill in this form once as well.

All children will also be asked to visit us at Massey University once where we would take pictures of your bones using our special x-ray machine. You will be asked to wear scrubs (similar to pyjamas) on top of your underwear and would need to lie still on a bed for 15 minutes so the picture can be taken. It does not hurt and you can keep the picture. Here is a photo showing someone having their 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 Bone health study.

My name is ……

This project has been reviewed and approved by the Massey University Human Ethics Committee: Southern A, Application 15/03. If you have any concerns about the conduct of this research, please contact Mr Jeremy Hubbard, Acting Chair, Massey University Human Ethics Committee: Southern A, telephone 04 801 5799 x 63487,email humanethicsoutha@massey.ac.nz.

Appendix 6 – Information sheet for parent

MASSEY UNIVERSITY

Bone Health Study
Does milk/dairy improve bone health in pre-pubertal children?
INFORMATION SHEET FOR PARENTS

Researcher's introduction
This study is being conducted by MSc student Tahibia Awan under the supervision of Prof Marlena Kruger, Dr Jane Coad and Dr Raewyn Poulsen from the School of Food and Nutrition, Massey University.

Why are we doing this research?

Drinking milk or eating products made from milk is linked with better bone health. We want to find out whether adequate milk intake results in improved bone health in children.

We are inviting around 20 children aged 7-9 years who do not have any gastrointestinal disorder or bone disease and who are enrolled in Year 3 or 4 at a Manawatu 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 involved?
All participating children will be asked to fill out a simple questionnaire to find out how often they eat milk products or drink milk.The questionnaire will take approximately 15 minutes to complete. Children will also be asked to complete a second questionnaire which asks about their level of physical activity. Children will only need to complete this questionnaire once. This questionnaire will also take approximately 15 minutes to complete.

The children will have measurements made to determine their bone density and their body composition (level of muscle and fat). For these measurements, children will come to the School of Food and Nutrition at Massey University in Palmerston North. We will arrange for transportation of all children if necessary. **Caregivers or family/whanau members are welcome to accompany children and can be present for all measurements.**

<u>Anthropometry</u>

Standing height using a stadiometer, weight using a weighing scale and waist circumference using a measuring tape will be measured for each child.

DEXA scan

We will provide the child with surgical scrubs (similar to pyjamas) to wear on top of their underwear. Your child will then lie on a bed under our DEXA scanner as shown in the picture on the next page. DEXA scanning is a form of x-ray so does involve exposure to a very low dose of radiation. The radiation exposure from DEXA 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 DEXA to take home.The room is private and your child can enter the DEXA room in complete privacy. Two female staff members who are certificated to operate the DEXA 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 DEXA machine.

All participants will have the opportunity to receive a copy of their own data.

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 informationthat 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 at least until your child is 26 years of age. 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 cupboard 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 willbe accessible by nominated staff only, who require pin numbers for ID. 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.

What if my child cannot or will not drink milk?
Should your child be unable to drink cow's milk or simply not like milk they can still take part in this study. If your child is lactose intolerant or allergic to cow's milk or dairy products we ask that you please indicate this on the consent form. If your child does not like milk but is not allergic or intolerant to cow's milk you do not need to do anything.

What I have to do?

After your child has attended the human nutrition laboratory at Massey University, he/she will be provided with an estimated 3-day diet record to take home which is then to be completed by you. A 3 day estimated food record is similar to a food diary which provides detailed data on the food and beverages consumed. In the present study, this food record is used to assess the diet of a child over a period of three days (two weekdays and one weekend). The parent or guardian of the child has to fill out the food record and while doing so it is very important that the child is consulted and thoroughly asked about what he/she ate or drank the whole day. The rest of the instructions on how to complete the food record are thoroughly explained within the food record provided. *You have also been provided with a pre-paid Massey University envelope so that on completing the food record you can mail it back to us.*

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 provided consent form.

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

- refuse to answer any particular question, and to withdraw from the study at any time
- refuse to drink any milk provided by the Milk in Schools programme
- 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 accordance with the Compensation Act 2001, in an unlikely event where 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. 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 questions please contact Tahibia Awan or Raewyn Poulsen or Marlena Kruger who will be happy to discuss the project in more detail.

Contact details:

Dr Raewyn Poulsen
School of Food and Nutrition
Massey University
Private Bag 11222
Palmerston North
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This project has been reviewed and approved by the Massey University Human Ethics Committee: Southern A, Application 15/03. If you have any concerns about the conduct of this research, please contact Mr Jeremy Hubbard, Acting Chair, Massey University Human Ethics Committee: Southern A, telephone 04 801 5799 x 63487,email humanethicsoutha@massey.ac.nz.

Appendix 7 – Food frequency questionnaire (FFQ)

Office use only

Participant initials

Participant date of birth

day month 2 0 year

Study ID

Baseline Questionnaire

Administrative Section: Data entered into database

signature

printed name

day month year

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DW03

1.01	What is today's date? (Example: 23 April 2012)	
		Day
		Month
		Year

Section A: Tell us about yourself

2.01	When is your birthday? (Example: 15 May 2002)	
		Day
		Month
		Year

2.02	How old are you? (Example: 8)	

2.03	Are you a (tick one)
	○ Boy
	○ Girl

2.04	What school year are you in? (tick one)
	○ Year 3
	○ Year 4

2.05 Would you call yourself

- ○ New Zealand European
- ○ Māori
- ○ Samoan
- ○ Cook Islands Maori
- ○ Tongan
- ○ Niuean
- ○ Chinese
- ○ Indian
- ○ Other

- ○ Don't know

Section B: School Breakfast Club

3.01 Does your school have a breakfast club? (tick one)

- ○ Yes → please go to **Q3.02**
- ○ No → please go to **Section C**

3.02 Do you usually have breakfast at the school breakfast club?

- ○ Never
- ○ One day a week
- ○ Two days a week
- ○ Three days a week
- ○ Four days a week
- ○ Five days a week

Section C: Tell us about what you eat and drink at school

4.01 When you are at school, how much plain milk do you drink? (tick one)

- ○ None
- ○ ½ a serving
- ○ 1 serving
- ○ 2 servings
- ○ 3 servings
- ○ 4 servings or more

4.02 When you are at school, how much plain milk do you have on your cereal? (tick one)

- ○ None
- ○ ½ a serving
- ○ 1 serving
- ○ 2 servings
- ○ 3 servings
- ○ 4 servings or more

4.03 When you are at school, how much flavoured milk do you drink? (tick one)

- ○ None
- ○ ½ a serving
- ○ 1 serving
- ○ 2 servings
- ○ 3 servings
- ○ 4 servings or more

4.04 When you are at school, how much cheese do you eat? (tick one)

- ○ None
- ○ ½ a serving
- ○ 1 serving
- ○ 2 servings
- ○ 3 servings
- ○ 4 servings or more

4.05 When you are at school, how much yoghurt do you eat? (tick one)

- ○ None
- ○ ½ a serving
- ○ 1 serving
- ○ 2 servings
- ○ 3 servings
- ○ 4 servings or more

4.06 When you are at school do you drink these drinks? (tick one per line)

		Yes	No
a)	Plain water e.g. from a tap, bottle, cooler or water fountain	○	○
b)	Bottled water with fruit flavour	○	○
c)	Regular or full sugar fizzy or soft drinks, sports drinks, or energy drinks	○	○
d)	Diet or sugar free fizzy or soft drinks, including zero	○	○
f)	Fruit juice or fruit drinks, e.g. Raro	○	○

Section D: Tell us about what you eat and drink before and after school

5.01 How much plain milk do you drink before and after school? (tick one)

- ○ None
- ○ ½ a serving
- ○ 1 serving
- ○ 2 servings
- ○ 3 servings
- ○ 4 servings or more

5.02 How much plain milk do you have on your cereal before and after school? (tick one)

- ○ None
- ○ ½ a serving
- ○ 1 serving
- ○ 2 servings
- ○ 3 servings
- ○ 4 servings or more

5.03 How much flavoured milk do you drink before and after school? (tick one)

- ○ None
- ○ ½ a serving
- ○ 1 serving
- ○ 2 servings
- ○ 3 servings
- ○ 4 servings or more

5.04 How much cheese do you eat before and after school? (tick one)

- ○ None
- ○ ½ a serving
- ○ 1 serving
- ○ 2 servings
- ○ 3 servings
- ○ 4 servings or more

5.05 How much yoghurt do you eat before and after school? (tick one)

- ○ None
- ○ ½ a serving
- ○ 1 serving
- ○ 2 servings
- ○ 3 servings
- ○ 4 servings or more

5.06 Do you drink these drinks before and after school? (tick one per line)

		Yes	No
f)	Plain water e.g. from a tap, bottle, cooler or water fountain	○	○
g)	Bottled water with fruit flavour	○	○
h)	Regular or full sugar fizzy or soft drinks, sports drinks, or energy drinks	○	○
i)	Diet or sugar free fizzy or soft drinks, including zero	○	○
j)	Fruit juice or fruit drinks, e.g. Raro	○	○

Section E: Tell us about what you eat and drink on Saturdays and Sundays

6.01 On Saturdays and Sundays, how much plain milk do you drink? (tick one)

- ○ None
- ○ ½ a serving
- ○ 1 serving
- ○ 2 servings
- ○ 3 servings
- ○ 4 servings or more

6.02 On Saturdays and Sundays, how much plain milk do you have on your cereal? (tick one)

- ○ None
- ○ ½ a serving
- ○ 1 serving
- ○ 2 servings
- ○ 3 servings
- ○ 4 servings or more

6.03 On Saturdays and Sundays, how much flavoured milk do you drink? (tick one)

- ○ None
- ○ ½ a serving
- ○ 1 serving
- ○ 2 servings
- ○ 3 servings
- ○ 4 servings or more

6.04 On Saturdays and Sundays, how much cheese do you eat? (tick one)

○	None
○	½ a serving
○	1 serving
○	2 servings
○	3 servings
○	4 servings or more

6.05 On Saturdays and Sundays, how much yoghurt do you eat? (tick one)

○	None
○	½ a serving
○	1 serving
○	2 servings
○	3 servings
○	4 servings or more

6.06 On Saturdays and Sundays do you drink these drinks? (tick one per line)

		Yes	No
k)	Plain water e.g. from a tap, bottle, cooler or water fountain	○	○
l)	Bottled water with fruit flavour	○	○
m)	Regular or full sugar fizzy or soft drinks, sports drinks, or energy drinks	○	○
n)	Diet or sugar free fizzy or soft drinks, including zero	○	○
o)	Fruit juice or fruit drinks, e.g. Raro	○	○

Section F: Tell us what you think

7.01 What type of plain milk do you mostly drink? (tick one)

- ○ Green top
- ○ Yellow top
- ○ Light blue top
- ○ Dark blue top
- ○ Other type of plain milk (please list)

- ○ Don't know

7.02 How healthy are the following drinks? (please tick one circle for each line)

		Very unhealthy	Unhealthy	Not healthy and not unhealthy	Healthy	Very healthy	Don't know
a)	Plain water e.g. from a tap, bottle, or cooler	○	○	○	○	○	○
b)	Bottled water with fruit flavour	○	○	○	○	○	○
c)	Regular or full sugar fizzy or soft drinks, sports drinks, or energy drinks	○	○	○	○	○	○
d)	Diet or sugar free fizzy or soft drinks, including zero	○	○	○	○	○	○
e)	Plain milk	○	○	○	○	○	○
f)	Chocolate milk or other flavoured milk, including milo	○	○	○	○	○	○
g)	Fruit juice or fruit drinks, e.g. Raro	○	○	○	○	○	○

7.03 How much do you like the taste of the following? (tick one)

		Like a lot	Like a bit	Don't like or dislike	Dislike a bit	Don't like at all	Don't know
a)	Plain milk	○	○	○	○	○	○
b)	Flavoured milk	○	○	○	○	○	○
c)	Cheese	○	○	○	○	○	○
d)	Yoghurt	○	○	○	○	○	○

Section G: Physiological Measures

8.01 ___.___ **cm** Standing Height 1

8.02 ___.___ **cm** Standing Height 2

If difference between Height 1 and Height 2 is > 1cm, take a third measure below.

8.03 ___.___ **cm** Standing Height 3

8.04 ___.___ **cm** Sitting Height 1

8.05 ___.___ **cm** Sitting Height 2

If difference between Height 1 and Height 2 is > 1cm, take a third measure below.

8.06 ___.___ **cm** Sitting Height 3

8.07 ___.___ **kg** Weight 1

8.08 ___.___ **kg** Weight 2

If difference between weight 1 and weight 2 is >0.1kg, take a third measure:

8.09 ___.___ **kg** Weight 3

8.10 ___.___ **cm** Waist circumference 1

8.11 ___.___ **cm** Waist circumference 2

If difference between Waist Circumference 1 and Waist Circumference 2 is > 1cm, take a third measure below.

8.12 ___.___ **cm** Waist circumference 3

---

9. _____ *signature* _____ *printed name* ___ ___ *day* ___ ___ *month* ___ ___ ___ ___ *year*

Appendix 8 – 3-day estimated food record

MASSEY UNIVERSITY
COLLEGE OF HEALTH
TE KURA HAUORA TANGATA

Dear Parent,

Thank you for agreeing to allow your child to participate in a research study entitled "**Bone health study**" conducted by the Massey University's School of Food and Nutrition. The main purpose of this study is to comprehend nutritional intakes and physical activity of young children, and whether the consumption of milk has any kind of effect/s upon the bone status and body composition of pre-adolescent children aged 7-9 years living within the Manawatu region.

Your child has now attended the human nutrition laboratory at Massey and as part of the research we ask you to please help us understand your child's dietary intakes. A 3-day estimated food record is enclosed for you to complete. A 3 day estimated food record is similar to a food diary which provides detailed data on the food and beverages consumed. In the present study, this food record is used to assess the diet of a child over a period of three days (two weekdays and one weekend). The parent or guardian of the child has to fill out the food record and while doing so it is very important that the child is consulted and thoroughly asked about what he/she ate or drank the whole day. Rest of the instructions on how to complete the food record are thoroughly explained within the food record provided. *You have also been provided with a pre-paid Massey University envelope so that on completing the food record you can mail it back to us.*

To ensure confidentiality, the information obtained will only be used for research purposes and the responses obtained will be reported in aggregated form to protect the identity of the respondents.

Thank you for taking the time to assist me in my educational endeavours. The data collected will provide useful information towards the completion of this project. Please feel free to contact me (researcher) or the degree supervisor using the contact details provided below if you have any further questions.

Contact details:

Tahibia Awan (Researcher)
MSc Student
School of Food and Nutrition
Massey University, Palmerston North
Telephone: 021-02533309
e-mail: t.awan@massey.ac.nz

Supervisor:
Prof Marlena Kruger
School of Food and Nutrition
Massey University, Palmerston North
Telephone: 063505905
m.c.kruger@massey.ac.nz

Bone Health Study

To determine the effect/s of milk and dairy products upon bone health and body composition of pre-pubertal children aged 7-9 years

3 Day Estimated Food Record

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA

If you have any questions regarding this food record please contact me either via:

e-mail: t.awan@massey.ac.nz

mobile: 02102533309

Information

- This study is aimed at determining the effect of "Milk in Schools Programme" upon the bone mass and body composition of New Zealand school children between the ages of 6 to 9 years.
- It is ***very important that you sit and interact with your child*** while you are filling in the particular day's food intake.
- Please do your best to record everything your child eats and drinks as accurately and with as much detail as possible.
- As we are interested in learning about your child's usual diet patterns ***please do not change their diet*** while writing it down in the food record.
- An example of a pre-filled food record for 1 day has been attached for your convenience and also to give you an idea of what we are looking for.
- After you have completed the 3 day food record please put it in the prepaid envelope and mail back to the address provided.

General Instructions

- Ask your child to eat as they normally do; but try to remember everything about what they eat and drink.
- Remember to record all drinks ***even water***. If your child drinks from a water bottle throughout the day record it as such i.e. 1x750 mL water. You do not need to record every sip.
- Please do not start a 3 day record if your child is sick or if it is not a normal day (e.g. attending a party) as it can alter normal diet habits.
- Please record everything consumed by your child for 3 days. It should be ***2 weekdays and 1 weekend day***. An example is: Wednesday, Friday & Saturday.
- It is best if you record everything the child eats as soon as possible as it can be forgotten later on. If you are not with your child e.g. school, ask the provider or the child him/herself to explain what they ate.
- If you pack school lunches, after school ask the child if he/she has ***swapped food*** with a friend or ***did not finish*** all their packed lunch or all of an item.

- Please also record any vitamin and mineral supplements, or other health products the child is consuming.
- Write down the type of meat used (lean, with skin etc), type of bread (white, brown etc), thinness of bread (toast or sandwich), type of milk (blue top, yellow top, soy milk etc), fruit type (small or medium apple), brand names (Kellogg's, Hubbard's etc), and the cooking method (stir fry, shallow fry, boil etc).
- Please write down the amount of the items consumed e.g. when the child eats a sandwich, do they eat the crusts or are these removed, when the child eats a feijoa or persimmon do they eat the skin or is this removed. Don't worry about noting obvious discards such as apple core or banana skin.
- If a homemade mixed dish was consumed please attach the recipe if possible and note the amount the child consumed.
- It is ok to cross out something if you have recorded something wrong. The food record below shows one example.

Helpful tips:

- Record food as soon as eaten.
- Purchased foods and drinks provide the weight and serving size of that food which can be recorded directly.
- For liquid foods use cups as a standard measure.

Instructions for recording food:

- Record the ***time*** at which your child ate or drank food.
- Record ***where*** the food was consumed.
- Record the ***brand*** of the food as well.
- If a home cooked food is consumed please write down the details of how and with what ingredients it was prepared.
- Please be as accurate as possible about the amount of food consumed.
- Please list any ***condiments*** e.g., sauces, dressings used.

- Record all ***supplements, mineral, and vitamins*** consumed with the brand name and quantity.
- If the pack of food says something like; low in fat, 30% reduced sodium, fortified with Vitamin D please note it.

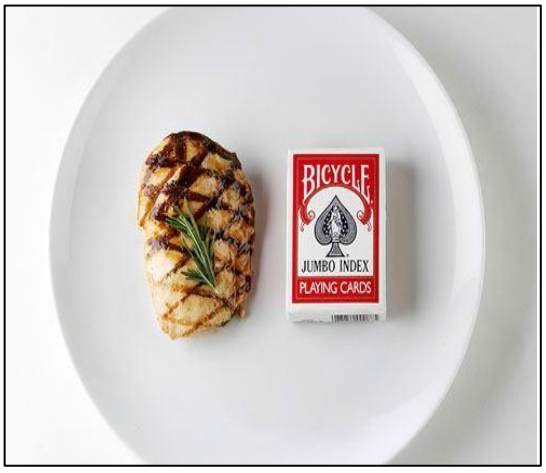
Common measures:

Wherever possible please use measuring instruments like cups or spoons.

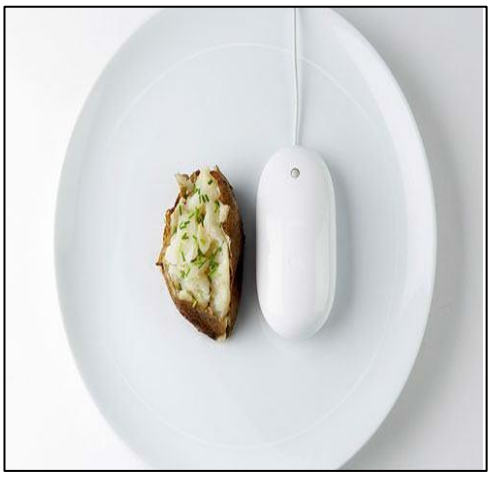
1 cup = 250ml 1 tablespoon =15ml 1 teaspoon = 5ml

Or you could estimate food portions based on common items, see following illustrations.

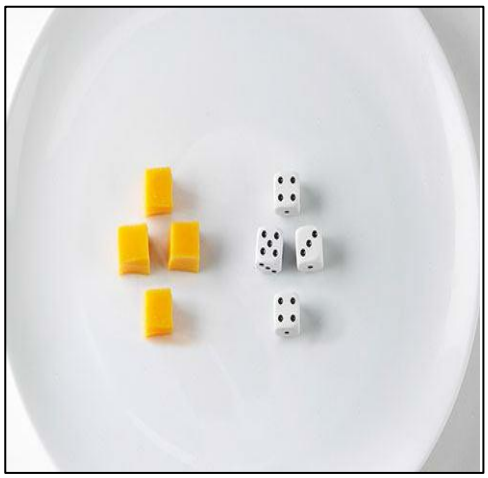
1 cup = the size of softball or tennis ball.



85 grams of meat = the size of deck of cards

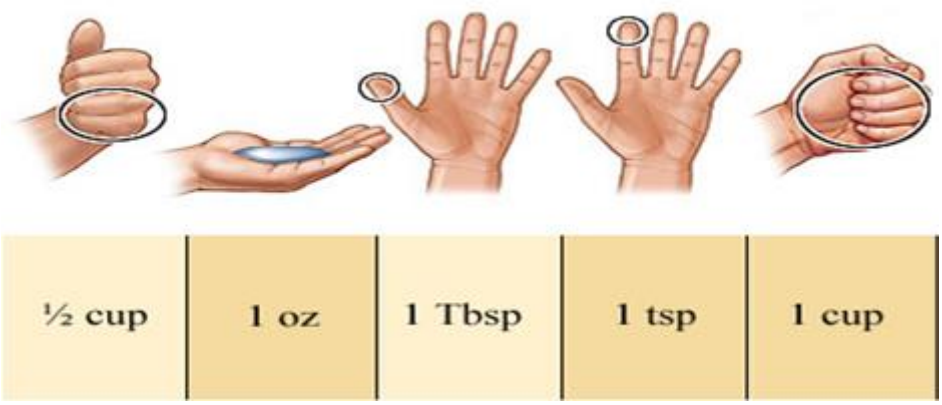


1 potato = the size of a computer mouse.



57 grams of cheese = the size of 4 dice

Another method is to estimate food portion size the Handy way. Trace around the main caregivers hand, and the child's hand. The child can then describe how much they ate compared to the caregivers hand and their own hand. For example compared to an adults' hand:



For better understanding of this picture please see the table listed on the next page.

Part of hand	**Approximate portion size**	**Examples of foods**
Whole fist	1 cup	2 servings of vegetables 1 serving of rice or pasta
Half fist	Half a cup	Half a serving of 1 cup rice
Palm of hand	1 oz.	1 serving of snack size crisps 1 serving of meat
Thumb tip	1 Tablespoon	2 servings of Nutella or peanut butter
Finger tip	1 teaspoon	1 serving of margarine

FOOD RECORD (EXAMPLE)

Date & Day ____01-08-2014___(Friday)_____

Time food was eaten	**Place eaten**	**Complete description of food** (preparation, variety, brand) If possible attach the recipe or the nutrition label (fortified foods).	**Amount consumed (units, measures, weight)**
8.15 am	Home	Anchor blue top milk	1 cup
" "	" "	Kellogg's Nutrigrain cereal	1 serving (30g) or 1 cup
" "	" "	Keri premium orange juice	1 serving (250ml) or 1 cup
10.30 am	School	Grain waves (golden cheddar)	1 serving of small snack pack or 18g
" "	" "	Apple (green skinned and medium size)	Half apple
" "	" "	Raisins (Sunmaid)	1 small pack (28g)
12.30 pm	School	Sandwich (Tip Top white sandwich bread, Hellers champagne leg ham, Flora original margarine, fresh lettuce, Mainland edam cheese slice) Note: did not eat crusts	2 slices of bread, 2 slices of ham, 1 tablespoon, 1 oz., 1 slice (21g)
" "	" "	Watties fruit squirtz (Apple and Mango)	1 serving or 120g
" "	" "	Mother Earth natural cashews	0.5 cup
3.30 pm	Home	Instant noodles chicken flavoured	1 pack (75g)
" "	" "	Mandarin (small)*	1
" "	" "	Water (tap)	1 cup
4.00 pm	Home	Tip Top traffic light popsicle	1 serving (78g)
6.30 pm	Home	Lasagna (Beef mice, diamond pasta lasagna sheets, Leggos pasta bake pasta sauce, watties mixed frozen vegetables, Mainland colby cheese, salt, cracked pepper, canola oil)	1 serving
" "	" "	Water (tap)	2 cups
8.00 pm	Home	Fun size snickers bar	1 serving (15g)

" "	" "	Anchor blue top milk	1 cup
" "	" "	Cadbury drinking chocolate	1 tablespoon (15g)
8.30 pm	Home	Water (tap)	1 cup

*mandarin has been crossed out because it was put in by mistake.

Beef lasagna recipe:

Serves 6 people.

1.5 cup ground beef

2.5 cups Leggos pasta bake sauce

6 diamond pasta lasagne sheets

1.5 cup colby cheese

2 cups watties frozen mixed vegetables

1 tablespoon salt

2 tablespoons cracked pepper

2 tablespoon canola oil

Heat the oil in a large skillet and when hot add mince along with pasta sauce plus salt and pepper. When cooked add mixed vegetables and stir for a while. Remove the pan from heat and separately layer the lasagna sheets in a dish two at a time. Add the mince mixture on top and then add shredded cheese. Keep on repeating the process until all pasta sheets have been used. Cover the fish with a foil and place in a hot oven for 15 minutes.

FOOD RECORD (day 1)

*Name of child:*_____

Date & Day _____

Time food was eaten	**Place eaten**	**Complete description of food** (preparation, variety, brand) If possible attach the recipe or the nutrition label (fortified foods).	**Amount consumed**

FOOD RECORD (day 1)

*Name of child:*_____

Date & Day_____

Time food was eaten	**Place eaten**	**Complete description of food** (preparation, variety, brand) If possible attach the recipe or the nutrition label (fortified foods).	**Amount consumed**

FOOD RECORD (day 2)

*Name of child:*_____

Date & Day_____

Time food was eaten	**Place eaten**	**Complete description of food** (preparation, variety, brand) If possible attach the recipe or the nutrition label (fortified foods).	**Amount consumed**

FOOD RECORD (day 2)

*Name of child:*_____

Date & Day_____

Time food was eaten	**Place eaten**	**Complete description of food** (preparation, variety, brand) If possible attach the recipe or the nutrition label (fortified foods).	**Amount consumed**

FOOD RECORD (day 3)

*Name of child:*_____

Date & Day_____

Time food was eaten	**Place eaten**	**Complete description of food** (preparation, variety, brand) If possible attach the recipe or the nutrition label (fortified foods).	**Amount consumed**

FOOD RECORD (day 3)

*Name of child:*_____

Date & Day_____

Time food was eaten	**Place eaten**	**Complete description of food** (preparation, variety, brand) If possible attach the recipe or the nutrition label (fortified foods).	**Amount consumed**

Recipes:

Appendix 9 – Previous day physical activity recall (PDPAR)

Activities scale

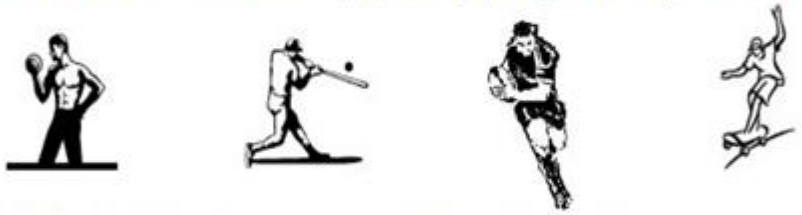
On the next page is a scale which records the **main** activities you did yesterday. Please be certain to write on the scale the **day of the week** that 'yesterday' was.

1. For each time period write in the **number(s)** of the main activities you actually did in the boxes on the time scale.
2. Then rate how physically **hard** these activities were. Place an 'X' on the rating scale to indicate if the activities for each time period were very light, light, medium, or hard.
3. Below are few examples of different types of activities children perform at a daily basis:

- **Very Light** = Slow breathing, little or no movement.

- **Light** = Normal breathing, regular movement.

- **Medium** = Increased breathing, moving quickly for short periods of time.



- **Hard** = Hard breathing, moving quickly for 20 minutes or more.

Please be as accurate as possible but fill out the scale quickly.

Activity Numbers

Eating

1. Meal
2. Snack
3. Cooking

Sleep/Bathing

4. Sleeping
5. Resting
6. Shower/bath

Transportation

7. Ride in car, bus
8. Travel by walking
9. Travel by bike

Work/School

10. Job (list):_____
11. Housework/paperwork
12. House chores (list):_____

Spare Time

13. Watch TV
14. Go to movies/concert
15. Listen to music
16. Talk on the phone
17. Hang around
18. Shopping
19. Play video games
20. Other (list):_____

Physical Activities

21. Walk
22. Jog/run
23. Dance (for fun)
24. Aerobic dance
25. Swim (for fun)
26. Swim laps
27. Ride bicycle
28. Lift weights
29. Use skateboard
30. Play organized sport
31. Did individual exercise
32. Did active game outside
33. Other (list):_____

1. Put Activity Numbers in this column

2. Put an "X" to rate how hard these activities were

	Time	Activity Numbers	Very Light 	Light 	Medium 	Hard 
Afternoon	3:00					
	3:30					
	4:00					
	4:30					
	5:00					
Supper	5:30					
	6:00					
	6:30					
	7:00					
Evening	7:30					
	8:00					
	8:30					
	9:00					
	9:30					
Night	10:00					
	10:30					
	11:00					

Circle the day of the week that you did these activities:

M T W Th F Sa Su

Date_____ ID Code_____

Questionnaire

Your Name_____

Your Birth Date_____

First Name of Your Mother (or other adult who takes care of you)

_____

Researcher use only

Weight of the child _____kg

Appendix 10 – WHO growth charts; BMI-for-age, Height –for-age, Weight-for-age.

CDC-growth charts for waist circumference-for-age

BMI-for-age BOYS

5 to 19 years (percentiles)

World Health Organization

Year: Month	Month	L	M	S	Percentiles (BMI in kg/m^2)										
					1st	3rd	5th	15th	25th	50th	75th	85th	95th	97th	99th
5: 1	61	-0.7387	15.2641	0.08390	12.7	13.1	13.4	14.0	14.4	15.3	16.2	16.7	17.7	18.1	18.8
5: 2	62	-0.7621	15.2616	0.08414	12.7	13.1	13.4	14.0	14.4	15.3	16.2	16.7	17.7	18.1	18.9
5: 3	63	-0.7856	15.2604	0.08439	12.7	13.1	13.4	14.0	14.4	15.3	16.2	16.7	17.7	18.1	18.9
5: 4	64	-0.8089	15.2605	0.08464	12.7	13.1	13.4	14.0	14.4	15.3	16.2	16.7	17.7	18.1	18.9
5: 5	65	-0.8322	15.2619	0.08490	12.7	13.1	13.4	14.0	14.4	15.3	16.2	16.7	17.7	18.1	18.9
5: 6	66	-0.8554	15.2645	0.08516	12.7	13.1	13.4	14.0	14.4	15.3	16.2	16.7	17.7	18.1	19.0
5: 7	67	-0.8785	15.2684	0.08543	12.7	13.1	13.4	14.0	14.4	15.3	16.2	16.7	17.7	18.2	19.0
5: 8	68	-0.9015	15.2737	0.08570	12.7	13.1	13.4	14.0	14.4	15.3	16.2	16.8	17.8	18.2	19.0
5: 9	69	-0.9243	15.2801	0.08597	12.7	13.1	13.4	14.0	14.4	15.3	16.2	16.8	17.8	18.2	19.1
5:10	70	-0.9471	15.2877	0.08625	12.7	13.1	13.4	14.0	14.4	15.3	16.2	16.8	17.8	18.2	19.1
5:11	71	-0.9697	15.2965	0.08653	12.7	13.2	13.4	14.0	14.5	15.3	16.2	16.8	17.8	18.3	19.1
6: 0	72	-0.9921	15.3062	0.08682	12.7	13.2	13.4	14.0	14.5	15.3	16.3	16.8	17.9	18.3	19.2
6: 1	73	-1.0144	15.3169	0.08711	12.7	13.2	13.4	14.0	14.5	15.3	16.3	16.8	17.9	18.3	19.2
6: 2	74	-1.0365	15.3285	0.08741	12.7	13.2	13.4	14.1	14.5	15.3	16.3	16.9	17.9	18.4	19.3
6: 3	75	-1.0584	15.3408	0.08771	12.8	13.2	13.4	14.1	14.5	15.3	16.3	16.9	17.9	18.4	19.3
6: 4	76	-1.0801	15.3540	0.08802	12.8	13.2	13.4	14.1	14.5	15.4	16.3	16.9	18.0	18.4	19.4
6: 5	77	-1.1017	15.3679	0.08833	12.8	13.2	13.4	14.1	14.5	15.4	16.3	16.9	18.0	18.5	19.4
6: 6	78	-1.1230	15.3825	0.08865	12.8	13.2	13.4	14.1	14.5	15.4	16.4	16.9	18.0	18.5	19.4
6: 7	79	-1.1441	15.3978	0.08898	12.8	13.2	13.4	14.1	14.5	15.4	16.4	17.0	18.1	18.5	19.5
6: 8	80	-1.1649	15.4137	0.08931	12.8	13.2	13.5	14.1	14.5	15.4	16.4	17.0	18.1	18.6	19.6
6: 9	81	-1.1856	15.4302	0.08964	12.8	13.2	13.5	14.1	14.6	15.4	16.4	17.0	18.1	18.6	19.6
6:10	82	-1.2060	15.4473	0.08998	12.8	13.2	13.5	14.1	14.6	15.4	16.5	17.1	18.2	18.7	19.7
6:11	83	-1.2261	15.4650	0.09033	12.8	13.3	13.5	14.2	14.6	15.5	16.5	17.1	18.2	18.7	19.7
7: 0	84	-1.2460	15.4832	0.09068	12.8	13.3	13.5	14.2	14.6	15.5	16.5	17.1	18.3	18.8	19.8
7: 1	85	-1.2656	15.5019	0.09103	12.9	13.3	13.5	14.2	14.6	15.5	16.5	17.1	18.3	18.8	19.8
7: 2	86	-1.2849	15.5210	0.09139	12.9	13.3	13.5	14.2	14.6	15.5	16.6	17.2	18.3	18.8	19.9

2007 WHO Reference

BMI-for-age BOYS

5 to 19 years (percentiles)

World Health Organization

Year: Month	Month	L	M	S	Percentiles (BMI in kg/m^2)										
					1st	3rd	5th	15th	25th	50th	75th	85th	95th	97th	99th
7: 3	87	-1.3040	15.5407	0.09176	12.9	13.3	13.5	14.2	14.6	15.5	16.6	17.2	18.4	18.9	20.0
7: 4	88	-1.3228	15.5608	0.09213	12.9	13.3	13.6	14.2	14.7	15.6	16.6	17.2	18.4	18.9	20.0
7: 5	89	-1.3414	15.5814	0.09251	12.9	13.3	13.6	14.2	14.7	15.6	16.6	17.3	18.5	19.0	20.1
7: 6	90	-1.3596	15.6023	0.09289	12.9	13.3	13.6	14.3	14.7	15.6	16.7	17.3	18.5	19.0	20.2
7: 7	91	-1.3776	15.6237	0.09327	12.9	13.4	13.6	14.3	14.7	15.6	16.7	17.3	18.6	19.1	20.2
7: 8	92	-1.3953	15.6455	0.09366	12.9	13.4	13.6	14.3	14.7	15.6	16.7	17.4	18.6	19.2	20.3
7: 9	93	-1.4126	15.6677	0.09406	12.9	13.4	13.6	14.3	14.7	15.7	16.7	17.4	18.7	19.2	20.4
7:10	94	-1.4297	15.6903	0.09445	13.0	13.4	13.6	14.3	14.8	15.7	16.8	17.4	18.7	19.3	20.4
7:11	95	-1.4464	15.7133	0.09486	13.0	13.4	13.7	14.3	14.8	15.7	16.8	17.5	18.8	19.3	20.5
8: 0	96	-1.4629	15.7368	0.09526	13.0	13.4	13.7	14.4	14.8	15.7	16.8	17.5	18.8	19.4	20.6
8: 1	97	-1.4790	15.7606	0.09567	13.0	13.4	13.7	14.4	14.8	15.8	16.9	17.5	18.9	19.4	20.6
8: 2	98	-1.4947	15.7848	0.09609	13.0	13.5	13.7	14.4	14.8	15.8	16.9	17.6	18.9	19.5	20.7
8: 3	99	-1.5101	15.8094	0.09651	13.0	13.5	13.7	14.4	14.9	15.8	16.9	17.6	19.0	19.5	20.8
8: 4	100	-1.5252	15.8344	0.09693	13.0	13.5	13.7	14.4	14.9	15.8	17.0	17.7	19.0	19.6	20.9
8: 5	101	-1.5399	15.8597	0.09735	13.1	13.5	13.7	14.4	14.9	15.9	17.0	17.7	19.1	19.7	21.0
8: 6	102	-1.5542	15.8855	0.09778	13.1	13.5	13.8	14.5	14.9	15.9	17.0	17.7	19.1	19.7	21.0
8: 7	103	-1.5681	15.9116	0.09821	13.1	13.5	13.8	14.5	14.9	15.9	17.1	17.8	19.2	19.8	21.1
8: 8	104	-1.5817	15.9381	0.09864	13.1	13.5	13.8	14.5	15.0	15.9	17.1	17.8	19.2	19.9	21.2
8: 9	105	-1.5948	15.9651	0.09907	13.1	13.6	13.8	14.5	15.0	16.0	17.1	17.9	19.3	19.9	21.3
8:10	106	-1.6076	15.9925	0.09951	13.1	13.6	13.8	14.5	15.0	16.0	17.2	17.9	19.3	20.0	21.4
8:11	107	-1.6199	16.0205	0.09994	13.2	13.6	13.8	14.6	15.0	16.0	17.2	17.9	19.4	20.0	21.4
9: 0	108	-1.6318	16.0490	0.10038	13.2	13.6	13.9	14.6	15.1	16.0	17.2	18.0	19.5	20.1	21.5
9: 1	109	-1.6433	16.0781	0.10082	13.2	13.6	13.9	14.6	15.1	16.1	17.3	18.0	19.5	20.2	21.6
9: 2	110	-1.6544	16.1078	0.10126	13.2	13.7	13.9	14.6	15.1	16.1	17.3	18.1	19.6	20.2	21.7
9: 3	111	-1.6651	16.1381	0.10170	13.2	13.7	13.9	14.6	15.1	16.1	17.4	18.1	19.6	20.3	21.8

2007 WHO Reference

BMI-for-age BOYS

5 to 19 years (percentiles)

World Health Organization

Year: Month	Month	L	M	S	Percentiles (BMI in kg/m^2)										
					1st	3rd	5th	15th	25th	50th	75th	85th	95th	97th	99th
9: 4	112	-1.6753	16.1692	0.10214	13.2	13.7	13.9	14.7	15.1	16.2	17.4	18.2	19.7	20.4	21.9
9: 5	113	-1.6851	16.2009	0.10259	13.3	13.7	14.0	14.7	15.2	16.2	17.4	18.2	19.8	20.5	22.0
9: 6	114	-1.6944	16.2333	0.10303	13.3	13.7	14.0	14.7	15.2	16.2	17.5	18.3	19.8	20.5	22.1
9: 7	115	-1.7032	16.2665	0.10347	13.3	13.8	14.0	14.7	15.2	16.3	17.5	18.3	19.9	20.6	22.2
9: 8	116	-1.7116	16.3004	0.10391	13.3	13.8	14.0	14.8	15.3	16.3	17.6	18.4	20.0	20.7	22.3
9: 9	117	-1.7196	16.3351	0.10435	13.3	13.8	14.1	14.8	15.3	16.3	17.6	18.4	20.0	20.8	22.4
9:10	118	-1.7271	16.3704	0.10478	13.4	13.8	14.1	14.8	15.3	16.4	17.7	18.5	20.1	20.8	22.5
9:11	119	-1.7341	16.4065	0.10522	13.4	13.8	14.1	14.8	15.3	16.4	17.7	18.5	20.2	20.9	22.6
10: 0	120	-1.7407	16.4433	0.10566	13.4	13.9	14.1	14.9	15.4	16.4	17.7	18.6	20.2	21.0	22.7
10: 1	121	-1.7468	16.4807	0.10609	13.4	13.9	14.2	14.9	15.4	16.5	17.8	18.6	20.3	21.1	22.8
10: 2	122	-1.7525	16.5189	0.10652	13.4	13.9	14.2	14.9	15.4	16.5	17.8	18.7	20.4	21.1	22.9
10: 3	123	-1.7578	16.5578	0.10695	13.5	13.9	14.2	15.0	15.5	16.6	17.9	18.7	20.4	21.2	23.0
10: 4	124	-1.7626	16.5974	0.10738	13.5	14.0	14.2	15.0	15.5	16.6	17.9	18.8	20.5	21.3	23.1
10: 5	125	-1.7670	16.6376	0.10780	13.5	14.0	14.3	15.0	15.5	16.6	18.0	18.8	20.6	21.4	23.2
10: 6	126	-1.7710	16.6786	0.10823	13.5	14.0	14.3	15.1	15.6	16.7	18.0	18.9	20.7	21.5	23.3
10: 7	127	-1.7745	16.7203	0.10865	13.6	14.0	14.3	15.1	15.6	16.7	18.1	19.0	20.7	21.6	23.4
10: 8	128	-1.7777	16.7628	0.10906	13.6	14.1	14.3	15.1	15.6	16.8	18.1	19.0	20.8	21.6	23.5
10: 9	129	-1.7804	16.8059	0.10948	13.6	14.1	14.4	15.2	15.7	16.8	18.2	19.1	20.9	21.7	23.6
10:10	130	-1.7828	16.8497	0.10989	13.6	14.1	14.4	15.2	15.7	16.9	18.2	19.1	21.0	21.8	23.7
10:11	131	-1.7847	16.8941	0.11030	13.7	14.2	14.4	15.2	15.8	16.9	18.3	19.2	21.0	21.9	23.8
11: 0	132	-1.7862	16.9392	0.11070	13.7	14.2	14.5	15.3	15.8	16.9	18.4	19.3	21.1	22.0	23.9
11: 1	133	-1.7873	16.9850	0.11110	13.7	14.2	14.5	15.3	15.8	17.0	18.4	19.3	21.2	22.1	24.0
11: 2	134	-1.7881	17.0314	0.11150	13.8	14.3	14.5	15.3	15.9	17.0	18.5	19.4	21.3	22.2	24.1
11: 3	135	-1.7884	17.0784	0.11189	13.8	14.3	14.6	15.4	15.9	17.1	18.5	19.4	21.4	22.2	24.2

2007 WHO Reference

BMI-for-age GIRLS

5 to 19 years (percentiles)

World Health Organization

Year: Month	Month	L	M	S	Percentiles (BMI in kg/m^2)										
					1st	3rd	5th	15th	25th	50th	75th	85th	95th	97th	99th
5: 1	61	-0.8886	15.2441	0.09692	12.4	12.9	13.1	13.8	14.3	15.2	16.3	16.9	18.1	18.6	19.6
5: 2	62	-0.9068	15.2434	0.09738	12.4	12.9	13.1	13.8	14.3	15.2	16.3	16.9	18.1	18.6	19.6
5: 3	63	-0.9248	15.2433	0.09783	12.4	12.9	13.1	13.8	14.3	15.2	16.3	17.0	18.1	18.7	19.7
5: 4	64	-0.9427	15.2438	0.09829	12.4	12.9	13.1	13.8	14.3	15.2	16.3	17.0	18.2	18.7	19.7
5: 5	65	-0.9605	15.2448	0.09875	12.4	12.9	13.1	13.8	14.3	15.2	16.3	17.0	18.2	18.7	19.8
5: 6	66	-0.9780	15.2464	0.09920	12.4	12.8	13.1	13.8	14.3	15.2	16.3	17.0	18.2	18.7	19.8
5: 7	67	-0.9954	15.2487	0.09966	12.4	12.8	13.1	13.8	14.3	15.2	16.3	17.0	18.2	18.8	19.8
5: 8	68	-1.0126	15.2516	0.10012	12.4	12.8	13.1	13.8	14.3	15.3	16.4	17.0	18.3	18.8	19.9
5: 9	69	-1.0296	15.2551	0.10058	12.4	12.8	13.1	13.8	14.3	15.3	16.4	17.0	18.3	18.8	19.9
5:10	70	-1.0464	15.2592	0.10104	12.4	12.8	13.1	13.8	14.3	15.3	16.4	17.0	18.3	18.9	20.0
5:11	71	-1.0630	15.2641	0.10149	12.4	12.8	13.1	13.8	14.3	15.3	16.4	17.1	18.3	18.9	20.0
6: 0	72	-1.0794	15.2697	0.10195	12.4	12.8	13.1	13.8	14.3	15.3	16.4	17.1	18.4	18.9	20.1
6: 1	73	-1.0956	15.2760	0.10241	12.4	12.8	13.1	13.8	14.3	15.3	16.4	17.1	18.4	19.0	20.1
6: 2	74	-1.1115	15.2831	0.10287	12.4	12.8	13.1	13.8	14.3	15.3	16.4	17.1	18.4	19.0	20.2
6: 3	75	-1.1272	15.2911	0.10333	12.4	12.8	13.1	13.8	14.3	15.3	16.4	17.1	18.5	19.0	20.2
6: 4	76	-1.1427	15.2998	0.10379	12.4	12.8	13.1	13.8	14.3	15.3	16.5	17.2	18.5	19.1	20.3
6: 5	77	-1.1579	15.3095	0.10425	12.4	12.8	13.1	13.8	14.3	15.3	16.5	17.2	18.5	19.1	20.4
6: 6	78	-1.1728	15.3200	0.10471	12.4	12.8	13.1	13.8	14.3	15.3	16.5	17.2	18.6	19.2	20.4
6: 7	79	-1.1875	15.3314	0.10517	12.4	12.8	13.1	13.8	14.3	15.3	16.5	17.2	18.6	19.2	20.5
6: 8	80	-1.2019	15.3439	0.10562	12.4	12.8	13.1	13.8	14.3	15.3	16.5	17.3	18.6	19.3	20.5
6: 9	81	-1.2160	15.3572	0.10608	12.4	12.8	13.1	13.9	14.3	15.4	16.6	17.3	18.7	19.3	20.6
6:10	82	-1.2298	15.3717	0.10654	12.4	12.9	13.1	13.9	14.3	15.4	16.6	17.3	18.7	19.3	20.7
6:11	83	-1.2433	15.3871	0.10700	12.4	12.9	13.1	13.9	14.4	15.4	16.6	17.3	18.8	19.4	20.7
7: 0	84	-1.2565	15.4036	0.10746	12.4	12.9	13.1	13.9	14.4	15.4	16.6	17.4	18.8	19.4	20.8
7: 1	85	-1.2693	15.4211	0.10792	12.4	12.9	13.1	13.9	14.4	15.4	16.6	17.4	18.9	19.5	20.9
7: 2	86	-1.2819	15.4397	0.10837	12.4	12.9	13.2	13.9	14.4	15.4	16.7	17.4	18.9	19.6	20.9

2007 WHO Reference

BMI-for-age GIRLS

5 to 19 years (percentiles)

World Health Organization

Year: Month	Month	L	M	S	Percentiles (BMI in kg/m^2)										
					1st	3rd	5th	15th	25th	50th	75th	85th	95th	97th	99th
7: 3	87	-1.2941	15.4593	0.10883	12.4	12.9	13.2	13.9	14.4	15.5	16.7	17.5	19.0	19.6	21.0
7: 4	88	-1.3060	15.4798	0.10929	12.4	12.9	13.2	13.9	14.4	15.5	16.7	17.5	19.0	19.7	21.1
7: 5	89	-1.3175	15.5014	0.10974	12.4	12.9	13.2	13.9	14.4	15.5	16.8	17.5	19.1	19.7	21.2
7: 6	90	-1.3287	15.5240	0.11020	12.5	12.9	13.2	14.0	14.5	15.5	16.8	17.6	19.1	19.8	21.2
7: 7	91	-1.3395	15.5476	0.11065	12.5	12.9	13.2	14.0	14.5	15.5	16.8	17.6	19.2	19.8	21.3
7: 8	92	-1.3499	15.5723	0.11110	12.5	13.0	13.2	14.0	14.5	15.6	16.9	17.6	19.2	19.9	21.4
7: 9	93	-1.3600	15.5979	0.11156	12.5	13.0	13.2	14.0	14.5	15.6	16.9	17.7	19.3	20.0	21.5
7:10	94	-1.3697	15.6246	0.11201	12.5	13.0	13.3	14.0	14.5	15.6	16.9	17.7	19.3	20.0	21.6
7:11	95	-1.3790	15.6523	0.11246	12.5	13.0	13.3	14.0	14.6	15.7	17.0	17.8	19.4	20.1	21.7
8: 0	96	-1.3880	15.6810	0.11291	12.5	13.0	13.3	14.1	14.6	15.7	17.0	17.8	19.4	20.2	21.7
8: 1	97	-1.3966	15.7107	0.11335	12.6	13.0	13.3	14.1	14.6	15.7	17.0	17.9	19.5	20.2	21.8
8: 2	98	-1.4047	15.7415	0.11380	12.6	13.1	13.3	14.1	14.6	15.7	17.1	17.9	19.6	20.3	21.9
8: 3	99	-1.4125	15.7732	0.11424	12.6	13.1	13.4	14.1	14.7	15.8	17.1	18.0	19.6	20.4	22.0
8: 4	100	-1.4199	15.8058	0.11469	12.6	13.1	13.4	14.2	14.7	15.8	17.2	18.0	19.7	20.4	22.1
8: 5	101	-1.4270	15.8394	0.11513	12.6	13.1	13.4	14.2	14.7	15.8	17.2	18.1	19.8	20.5	22.2
8: 6	102	-1.4336	15.8738	0.11557	12.6	13.1	13.4	14.2	14.7	15.9	17.2	18.1	19.8	20.6	22.3
8: 7	103	-1.4398	15.9090	0.11601	12.7	13.2	13.4	14.2	14.8	15.9	17.3	18.2	19.9	20.7	22.4
8: 8	104	-1.4456	15.9451	0.11644	12.7	13.2	13.5	14.3	14.8	15.9	17.3	18.2	20.0	20.7	22.5
8: 9	105	-1.4511	15.9818	0.11688	12.7	13.2	13.5	14.3	14.8	16.0	17.4	18.3	20.0	20.8	22.6
8:10	106	-1.4561	16.0194	0.11731	12.7	13.2	13.5	14.3	14.9	16.0	17.4	18.3	20.1	20.9	22.7
8:11	107	-1.4607	16.0575	0.11774	12.8	13.3	13.5	14.4	14.9	16.1	17.5	18.4	20.2	21.0	22.8
9: 0	108	-1.4650	16.0964	0.11816	12.8	13.3	13.6	14.4	14.9	16.1	17.5	18.4	20.2	21.1	22.9
9: 1	109	-1.4688	16.1358	0.11859	12.8	13.3	13.6	14.4	15.0	16.1	17.6	18.5	20.3	21.1	23.0
9: 2	110	-1.4723	16.1759	0.11901	12.8	13.3	13.6	14.4	15.0	16.2	17.6	18.5	20.4	21.2	23.1
9: 3	111	-1.4753	16.2166	0.11943	12.8	13.4	13.6	14.5	15.0	16.2	17.7	18.6	20.5	21.3	23.2

2007 WHO Reference

BMI-for-age GIRLS

5 to 19 years (percentiles)

World Health Organization

Year: Month	Month	L	M	S	Percentiles (BMI in kg/m^2)										
					1st	3rd	5th	15th	25th	50th	75th	85th	95th	97th	99th
9: 4	112	-1.4780	16.2580	0.11985	12.9	13.4	13.7	14.5	15.1	16.3	17.7	18.7	20.5	21.4	23.3
9: 5	113	-1.4803	16.2999	0.12026	12.9	13.4	13.7	14.5	15.1	16.3	17.8	18.7	20.6	21.5	23.4
9: 6	114	-1.4823	16.3425	0.12067	12.9	13.4	13.7	14.6	15.1	16.3	17.8	18.8	20.7	21.6	23.5
9: 7	115	-1.4838	16.3858	0.12108	13.0	13.5	13.8	14.6	15.2	16.4	17.9	18.8	20.7	21.6	23.6
9: 8	116	-1.4850	16.4298	0.12148	13.0	13.5	13.8	14.6	15.2	16.4	17.9	18.9	20.8	21.7	23.7
9: 9	117	-1.4859	16.4746	0.12188	13.0	13.5	13.8	14.7	15.2	16.5	18.0	18.9	20.9	21.8	23.8
9:10	118	-1.4864	16.5200	0.12228	13.0	13.6	13.9	14.7	15.3	16.5	18.0	19.0	21.0	21.9	23.9
9:11	119	-1.4866	16.5663	0.12268	13.1	13.6	13.9	14.7	15.3	16.6	18.1	19.1	21.1	22.0	24.0
10: 0	120	-1.4864	16.6133	0.12307	13.1	13.6	13.9	14.8	15.4	16.6	18.2	19.1	21.1	22.1	24.1
10: 1	121	-1.4859	16.6612	0.12346	13.1	13.6	14.0	14.8	15.4	16.7	18.2	19.2	21.2	22.2	24.2
10: 2	122	-1.4851	16.7100	0.12384	13.1	13.7	14.0	14.9	15.4	16.7	18.3	19.3	21.3	22.2	24.3
10: 3	123	-1.4839	16.7595	0.12422	13.2	13.7	14.0	14.9	15.5	16.8	18.3	19.3	21.4	22.3	24.4
10: 4	124	-1.4825	16.8100	0.12460	13.2	13.7	14.1	14.9	15.5	16.8	18.4	19.4	21.5	22.4	24.6
10: 5	125	-1.4807	16.8614	0.12497	13.2	13.8	14.1	15.0	15.6	16.9	18.5	19.5	21.5	22.5	24.7
10: 6	126	-1.4787	16.9136	0.12534	13.3	13.8	14.1	15.0	15.6	16.9	18.5	19.5	21.6	22.6	24.8
10: 7	127	-1.4763	16.9667	0.12571	13.3	13.9	14.2	15.1	15.7	17.0	18.6	19.6	21.7	22.7	24.9
10: 8	128	-1.4737	17.0208	0.12607	13.3	13.9	14.2	15.1	15.7	17.0	18.6	19.7	21.8	22.8	25.0
10: 9	129	-1.4708	17.0757	0.12643	13.4	13.9	14.2	15.1	15.8	17.1	18.7	19.8	21.9	22.9	25.1
10:10	130	-1.4677	17.1316	0.12678	13.4	14.0	14.3	15.2	15.8	17.1	18.8	19.8	22.0	23.0	25.2
10:11	131	-1.4642	17.1883	0.12713	13.4	14.0	14.3	15.2	15.9	17.2	18.8	19.9	22.1	23.1	25.3
11: 0	132	-1.4606	17.2459	0.12748	13.5	14.0	14.4	15.3	15.9	17.2	18.9	20.0	22.2	23.2	25.4
11: 1	133	-1.4567	17.3044	0.12782	13.5	14.1	14.4	15.3	16.0	17.3	19.0	20.0	22.2	23.3	25.6
11: 2	134	-1.4526	17.3637	0.12816	13.6	14.1	14.4	15.4	16.0	17.4	19.0	20.1	22.3	23.4	25.7
11: 3	135	-1.4482	17.4238	0.12849	13.6	14.2	14.5	15.4	16.1	17.4	19.1	20.2	22.4	23.5	25.8

2007 WHO Reference

Height-for-age BOYS

5 to 19 years (percentiles)

World Health Organization

Year: Month	Month	L	M	S	SD	Percentiles (height in cm)										
						1st	3rd	5th	15th	25th	50th	75th	85th	95th	97th	99th
5: 1	61	1	110.2647	0.04164	4.5914	99.6	101.6	102.7	105.5	107.2	110.3	113.4	115.0	117.8	118.9	120.9
5: 2	62	1	110.8006	0.04172	4.6226	100.0	102.1	103.2	106.0	107.7	110.8	113.9	115.6	118.4	119.5	121.6
5: 3	63	1	111.3338	0.04180	4.6538	100.5	102.6	103.7	106.5	108.2	111.3	114.5	116.2	119.0	120.1	122.2
5: 4	64	1	111.8636	0.04187	4.6837	101.0	103.1	104.2	107.0	108.7	111.9	115.0	116.7	119.6	120.7	122.8
5: 5	65	1	112.3895	0.04195	4.7147	101.4	103.5	104.6	107.5	109.2	112.4	115.6	117.3	120.1	121.3	123.4
5: 6	66	1	112.9110	0.04203	4.7456	101.9	104.0	105.1	108.0	109.7	112.9	116.1	117.8	120.7	121.8	124.0
5: 7	67	1	113.4280	0.04211	4.7765	102.3	104.4	105.6	108.5	110.2	113.4	116.7	118.4	121.3	122.4	124.5
5: 8	68	1	113.9410	0.04218	4.8060	102.8	104.9	106.0	109.0	110.7	113.9	117.2	118.9	121.8	123.0	125.1
5: 9	69	1	114.4500	0.04226	4.8367	103.2	105.4	106.5	109.4	111.2	114.5	117.7	119.5	122.4	123.5	125.7
5:10	70	1	114.9547	0.04234	4.8672	103.6	105.8	106.9	109.9	111.7	115.0	118.2	120.0	123.0	124.1	126.3
5:11	71	1	115.4549	0.04241	4.8964	104.1	106.2	107.4	110.4	112.2	115.5	118.8	120.5	123.5	124.7	126.8
6: 0	72	1	115.9509	0.04249	4.9268	104.5	106.7	107.8	110.8	112.6	116.0	119.3	121.1	124.1	125.2	127.4
6: 1	73	1	116.4432	0.04257	4.9570	104.9	107.1	108.3	111.3	113.1	116.4	119.8	121.6	124.6	125.8	128.0
6: 2	74	1	116.9325	0.04264	4.9860	105.3	107.6	108.7	111.8	113.6	116.9	120.3	122.1	125.1	126.3	128.5
6: 3	75	1	117.4196	0.04272	5.0162	105.8	108.0	109.2	112.2	114.0	117.4	120.8	122.6	125.7	126.9	129.1
6: 4	76	1	117.9046	0.04280	5.0463	106.2	108.4	109.6	112.7	114.5	117.9	121.3	123.1	126.2	127.4	129.6
6: 5	77	1	118.3880	0.04287	5.0753	106.6	108.8	110.0	113.1	115.0	118.4	121.8	123.6	126.7	127.9	130.2
6: 6	78	1	118.8700	0.04295	5.1055	107.0	109.3	110.5	113.6	115.4	118.9	122.3	124.2	127.3	128.5	130.7
6: 7	79	1	119.3508	0.04303	5.1357	107.4	109.7	110.9	114.0	115.9	119.4	122.8	124.7	127.8	129.0	131.3
6: 8	80	1	119.8303	0.04311	5.1659	107.8	110.1	111.3	114.5	116.3	119.8	123.3	125.2	128.3	129.5	131.8
6: 9	81	1	120.3085	0.04318	5.1949	108.2	110.5	111.8	114.9	116.8	120.3	123.8	125.7	128.9	130.1	132.4
6:10	82	1	120.7853	0.04326	5.2252	108.6	111.0	112.2	115.4	117.3	120.8	124.3	126.2	129.4	130.6	132.9
6:11	83	1	121.2604	0.04334	5.2554	109.0	111.4	112.6	115.8	117.7	121.3	124.8	126.7	129.9	131.1	133.5
7: 0	84	1	121.7338	0.04342	5.2857	109.4	111.8	113.0	116.3	118.2	121.7	125.3	127.2	130.4	131.7	134.0
7: 1	85	1	122.2053	0.04350	5.3159	109.8	112.2	113.5	116.7	118.6	122.2	125.8	127.7	130.9	132.2	134.6
7: 2	86	1	122.6750	0.04358	5.3462	110.2	112.6	113.9	117.1	119.1	122.7	126.3	128.2	131.5	132.7	135.1

2007 WHO Reference

Height-for-age BOYS

5 to 19 years (percentiles)

World Health Organization

Year: Month	Month	L	M	S	SD	Percentiles (height in cm)										
						1st	3rd	5th	15th	25th	50th	75th	85th	95th	97th	99th
7: 3	87	1	123.1429	0.04366	5.3764	110.6	113.0	114.3	117.6	119.5	123.1	126.8	128.7	132.0	133.3	135.7
7: 4	88	1	123.6092	0.04374	5.4067	111.0	113.4	114.7	118.0	120.0	123.6	127.3	129.2	132.5	133.8	136.2
7: 5	89	1	124.0736	0.04382	5.4369	111.4	113.8	115.1	118.4	120.4	124.1	127.7	129.7	133.0	134.3	136.7
7: 6	90	1	124.5361	0.04390	5.4671	111.8	114.3	115.5	118.9	120.8	124.5	128.2	130.2	133.5	134.8	137.3
7: 7	91	1	124.9964	0.04398	5.4973	112.2	114.7	116.0	119.3	121.3	125.0	128.7	130.7	134.0	135.3	137.8
7: 8	92	1	125.4545	0.04406	5.5275	112.6	115.1	116.4	119.7	121.7	125.5	129.2	131.2	134.5	135.9	138.3
7: 9	93	1	125.9104	0.04414	5.5577	113.0	115.5	116.8	120.2	122.2	125.9	129.7	131.7	135.1	136.4	138.8
7:10	94	1	126.3640	0.04422	5.5878	113.4	115.9	117.2	120.6	122.6	126.4	130.1	132.2	135.6	136.9	139.4
7:11	95	1	126.8156	0.04430	5.6179	113.7	116.2	117.6	121.0	123.0	126.8	130.6	132.6	136.1	137.4	139.9
8: 0	96	1	127.2651	0.04438	5.6480	114.1	116.6	118.0	121.4	123.5	127.3	131.1	133.1	136.6	137.9	140.4
8: 1	97	1	127.7129	0.04446	5.6781	114.5	117.0	118.4	121.8	123.9	127.7	131.5	133.6	137.1	138.4	140.9
8: 2	98	1	128.1590	0.04454	5.7082	114.9	117.4	118.8	122.2	124.3	128.2	132.0	134.1	137.5	138.9	141.4
8: 3	99	1	128.6034	0.04462	5.7383	115.3	117.8	119.2	122.7	124.7	128.6	132.5	134.6	138.0	139.4	142.0
8: 4	100	1	129.0466	0.04470	5.7684	115.6	118.2	119.6	123.1	125.2	129.0	132.9	135.0	138.5	139.9	142.5
8: 5	101	1	129.4887	0.04478	5.7985	116.0	118.6	120.0	123.5	125.6	129.5	133.4	135.5	139.0	140.4	143.0
8: 6	102	1	129.9300	0.04487	5.8300	116.4	119.0	120.3	123.9	126.0	129.9	133.9	136.0	139.5	140.9	143.5
8: 7	103	1	130.3705	0.04495	5.8602	116.7	119.3	120.7	124.3	126.4	130.4	134.3	136.4	140.0	141.4	144.0
8: 8	104	1	130.8103	0.04503	5.8904	117.1	119.7	121.1	124.7	126.8	130.8	134.8	136.9	140.5	141.9	144.5
8: 9	105	1	131.2495	0.04511	5.9207	117.5	120.1	121.5	125.1	127.3	131.3	135.2	137.4	141.0	142.4	145.0
8:10	106	1	131.6884	0.04519	5.9510	117.8	120.5	121.9	125.5	127.7	131.7	135.7	137.9	141.5	142.9	145.5
8:11	107	1	132.1269	0.04527	5.9814	118.2	120.9	122.3	125.9	128.1	132.1	136.2	138.3	142.0	143.4	146.0
9: 0	108	1	132.5652	0.04535	6.0118	118.6	121.3	122.7	126.3	128.5	132.6	136.6	138.8	142.5	143.9	146.6
9: 1	109	1	133.0031	0.04543	6.0423	118.9	121.6	123.1	126.7	128.9	133.0	137.1	139.3	142.9	144.4	147.1
9: 2	110	1	133.4404	0.04551	6.0729	119.3	122.0	123.5	127.1	129.3	133.4	137.5	139.7	143.4	144.9	147.6
9: 3	111	1	133.8770	0.04559	6.1035	119.7	122.4	123.8	127.6	129.8	133.9	138.0	140.2	143.9	145.4	148.1

2007 WHO Reference

Height-for-age BOYS

5 to 19 years (percentiles)

World Health Organization

Year: Month	Month	L	M	S	SD	Percentiles (height in cm)										
						1st	3rd	5th	15th	25th	50th	75th	85th	95th	97th	99th
9: 4	112	1	134.3130	0.04566	6.1327	120.0	122.8	124.2	128.0	130.2	134.3	138.4	140.7	144.4	145.8	148.6
9: 5	113	1	134.7483	0.04574	6.1634	120.4	123.2	124.6	128.4	130.6	134.7	138.9	141.1	144.9	146.3	149.1
9: 6	114	1	135.1829	0.04582	6.1941	120.8	123.5	125.0	128.8	131.0	135.2	139.4	141.6	145.4	146.8	149.6
9: 7	115	1	135.6168	0.04589	6.2235	121.1	123.9	125.4	129.2	131.4	135.6	139.8	142.1	145.9	147.3	150.1
9: 8	116	1	136.0501	0.04597	6.2542	121.5	124.3	125.8	129.6	131.8	136.1	140.3	142.5	146.3	147.8	150.6
9: 9	117	1	136.4829	0.04604	6.2837	121.9	124.7	126.1	130.0	132.2	136.5	140.7	143.0	146.8	148.3	151.1
9:10	118	1	136.9153	0.04612	6.3145	122.2	125.0	126.5	130.4	132.7	136.9	141.2	143.5	147.3	148.8	151.6
9:11	119	1	137.3474	0.04619	6.3441	122.6	125.4	126.9	130.8	133.1	137.3	141.6	143.9	147.8	149.3	152.1
10: 0	120	1	137.7795	0.04626	6.3737	123.0	125.8	127.3	131.2	133.5	137.8	142.1	144.4	148.3	149.8	152.6
10: 1	121	1	138.2119	0.04633	6.4034	123.3	126.2	127.7	131.6	133.9	138.2	142.5	144.8	148.7	150.3	153.1
10: 2	122	1	138.6452	0.04640	6.4331	123.7	126.5	128.1	132.0	134.3	138.6	143.0	145.3	149.2	150.7	153.6
10: 3	123	1	139.0797	0.04647	6.4630	124.0	126.9	128.4	132.4	134.7	139.1	143.4	145.8	149.7	151.2	154.1
10: 4	124	1	139.5158	0.04654	6.4931	124.4	127.3	128.8	132.8	135.1	139.5	143.9	146.2	150.2	151.7	154.6
10: 5	125	1	139.9540	0.04661	6.5233	124.8	127.7	129.2	133.2	135.6	140.0	144.4	146.7	150.7	152.2	155.1
10: 6	126	1	140.3948	0.04667	6.5522	125.2	128.1	129.6	133.6	136.0	140.4	144.8	147.2	151.2	152.7	155.6
10: 7	127	1	140.8387	0.04674	6.5828	125.5	128.5	130.0	134.0	136.4	140.8	145.3	147.7	151.7	153.2	156.2
10: 8	128	1	141.2859	0.04680	6.6122	125.9	128.9	130.4	134.4	136.8	141.3	145.7	148.1	152.2	153.7	156.7
10: 9	129	1	141.7368	0.04686	6.6418	126.3	129.2	130.8	134.9	137.3	141.7	146.2	148.6	152.7	154.2	157.2
10:10	130	1	142.1916	0.04692	6.6716	126.7	129.6	131.2	135.3	137.7	142.2	146.7	149.1	153.2	154.7	157.7
10:11	131	1	142.6501	0.04698	6.7017	127.1	130.0	131.6	135.7	138.1	142.7	147.2	149.6	153.7	155.3	158.2
11: 0	132	1	143.1126	0.04703	6.7306	127.5	130.5	132.0	136.1	138.6	143.1	147.7	150.1	154.2	155.8	158.8
11: 1	133	1	143.5795	0.04709	6.7612	127.9	130.9	132.5	136.6	139.0	143.6	148.1	150.6	154.7	156.3	159.3
11: 2	134	1	144.0511	0.04714	6.7906	128.3	131.3	132.9	137.0	139.5	144.1	148.6	151.1	155.2	156.8	159.8
11: 3	135	1	144.5276	0.04719	6.8203	128.7	131.7	133.3	137.5	139.9	144.5	149.1	151.6	155.7	157.4	160.4

2007 WHO Reference

Height-for-age GIRLS

5 to 19 years (percentiles)

World Health Organization

						Percentiles (height in cm)										
Year: Month	Month	L	M	S	SD	1st	3rd	5th	15th	25th	50th	75th	85th	95th	97th	99th
5: 1	61	1	109.6016	0.04355	4.7731	98.5	100.6	101.8	104.7	106.4	109.6	112.8	114.5	117.5	118.6	120.7
5: 2	62	1	110.1258	0.04364	4.8059	98.9	101.1	102.2	105.1	106.9	110.1	113.4	115.1	118.0	119.2	121.3
5: 3	63	1	110.6451	0.04373	4.8385	99.4	101.5	102.7	105.6	107.4	110.6	113.9	115.7	118.6	119.7	121.9
5: 4	64	1	111.1596	0.04382	4.8710	99.8	102.0	103.1	106.1	107.9	111.2	114.4	116.2	119.2	120.3	122.5
5: 5	65	1	111.6696	0.04390	4.9023	100.3	102.4	103.6	106.6	108.4	111.7	115.0	116.8	119.7	120.9	123.1
5: 6	66	1	112.1753	0.04399	4.9346	100.7	102.9	104.1	107.1	108.8	112.2	115.5	117.3	120.3	121.5	123.7
5: 7	67	1	112.6767	0.04407	4.9657	101.1	103.3	104.5	107.5	109.3	112.7	116.0	117.8	120.8	122.0	124.2
5: 8	68	1	113.1740	0.04415	4.9966	101.6	103.8	105.0	108.0	109.8	113.2	116.5	118.4	121.4	122.6	124.8
5: 9	69	1	113.6672	0.04423	5.0275	102.0	104.2	105.4	108.5	110.3	113.7	117.1	118.9	121.9	123.1	125.4
5:10	70	1	114.1565	0.04431	5.0583	102.4	104.6	105.8	108.9	110.7	114.2	117.6	119.4	122.5	123.7	125.9
5:11	71	1	114.6421	0.04439	5.0890	102.8	105.1	106.3	109.4	111.2	114.6	118.1	119.9	123.0	124.2	126.5
6: 0	72	1	115.1244	0.04447	5.1196	103.2	105.5	106.7	109.8	111.7	115.1	118.6	120.4	123.5	124.8	127.0
6: 1	73	1	115.6039	0.04454	5.1490	103.6	105.9	107.1	110.3	112.1	115.6	119.1	120.9	124.1	125.3	127.6
6: 2	74	1	116.0812	0.04461	5.1784	104.0	106.3	107.6	110.7	112.6	116.1	119.6	121.4	124.6	125.8	128.1
6: 3	75	1	116.5568	0.04469	5.2089	104.4	106.8	108.0	111.2	113.0	116.6	120.1	122.0	125.1	126.4	128.7
6: 4	76	1	117.0311	0.04475	5.2371	104.8	107.2	108.4	111.6	113.5	117.0	120.6	122.5	125.6	126.9	129.2
6: 5	77	1	117.5044	0.04482	5.2665	105.3	107.6	108.8	112.0	114.0	117.5	121.1	123.0	126.2	127.4	129.8
6: 6	78	1	117.9769	0.04489	5.2960	105.7	108.0	109.3	112.5	114.4	118.0	121.5	123.5	126.7	127.9	130.3
6: 7	79	1	118.4489	0.04495	5.3243	106.1	108.4	109.7	112.9	114.9	118.4	122.0	124.0	127.2	128.5	130.8
6: 8	80	1	118.9208	0.04502	5.3538	106.5	108.9	110.1	113.4	115.3	118.9	122.5	124.5	127.7	129.0	131.4
6: 9	81	1	119.3926	0.04508	5.3822	106.9	109.3	110.5	113.8	115.8	119.4	123.0	125.0	128.2	129.5	131.9
6:10	82	1	119.8648	0.04514	5.4107	107.3	109.7	111.0	114.3	116.2	119.9	123.5	125.5	128.8	130.0	132.5
6:11	83	1	120.3374	0.04520	5.4393	107.7	110.1	111.4	114.7	116.7	120.3	124.0	126.0	129.3	130.6	133.0
7: 0	84	1	120.8105	0.04525	5.4667	108.1	110.5	111.8	115.1	117.1	120.8	124.5	126.5	129.8	131.1	133.5
7: 1	85	1	121.2843	0.04531	5.4954	108.5	110.9	112.2	115.6	117.6	121.3	125.0	127.0	130.3	131.6	134.1
7: 2	86	1	121.7587	0.04536	5.5230	108.9	111.4	112.7	116.0	118.0	121.8	125.5	127.5	130.8	132.1	134.6

2007 WHO Reference

Height-for-age GIRLS

5 to 19 years (percentiles)

World Health Organization

Year: Month	Month	L	M	S	SD	Percentiles (height in cm)										
						1st	3rd	5th	15th	25th	50th	75th	85th	95th	97th	99th
7: 3	87	1	122.2338	0.04542	5.5519	109.3	111.8	113.1	116.5	118.5	122.2	126.0	128.0	131.4	132.7	135.1
7: 4	88	1	122.7098	0.04547	5.5796	109.7	112.2	113.5	116.9	118.9	122.7	126.5	128.5	131.9	133.2	135.7
7: 5	89	1	123.1868	0.04551	5.6062	110.1	112.6	114.0	117.4	119.4	123.2	127.0	129.0	132.4	133.7	136.2
7: 6	90	1	123.6646	0.04556	5.6342	110.6	113.1	114.4	117.8	119.9	123.7	127.5	129.5	132.9	134.3	136.8
7: 7	91	1	124.1435	0.04561	5.6622	111.0	113.5	114.8	118.3	120.3	124.1	128.0	130.0	133.5	134.8	137.3
7: 8	92	1	124.6234	0.04565	5.6891	111.4	113.9	115.3	118.7	120.8	124.6	128.5	130.5	134.0	135.3	137.9
7: 9	93	1	125.1045	0.04569	5.7160	111.8	114.4	115.7	119.2	121.2	125.1	129.0	131.0	134.5	135.9	138.4
7:10	94	1	125.5869	0.04573	5.7431	112.2	114.8	116.1	119.6	121.7	125.6	129.5	131.5	135.0	136.4	138.9
7:11	95	1	126.0706	0.04577	5.7703	112.6	115.2	116.6	120.1	122.2	126.1	130.0	132.1	135.6	136.9	139.5
8: 0	96	1	126.5558	0.04581	5.7975	113.1	115.7	117.0	120.5	122.6	126.6	130.5	132.6	136.1	137.5	140.0
8: 1	97	1	127.0424	0.04585	5.8249	113.5	116.1	117.5	121.0	123.1	127.0	131.0	133.1	136.6	138.0	140.6
8: 2	98	1	127.5304	0.04588	5.8511	113.9	116.5	117.9	121.5	123.6	127.5	131.5	133.6	137.2	138.5	141.1
8: 3	99	1	128.0199	0.04591	5.8774	114.3	117.0	118.4	121.9	124.1	128.0	132.0	134.1	137.7	139.1	141.7
8: 4	100	1	128.5109	0.04594	5.9038	114.8	117.4	118.8	122.4	124.5	128.5	132.5	134.6	138.2	139.6	142.2
8: 5	101	1	129.0035	0.04597	5.9303	115.2	117.9	119.2	122.9	125.0	129.0	133.0	135.2	138.8	140.2	142.8
8: 6	102	1	129.4975	0.04600	5.9569	115.6	118.3	119.7	123.3	125.5	129.5	133.5	135.7	139.3	140.7	143.4
8: 7	103	1	129.9932	0.04602	5.9823	116.1	118.7	120.2	123.8	126.0	130.0	134.0	136.2	139.8	141.2	143.9
8: 8	104	1	130.4904	0.04604	6.0078	116.5	119.2	120.6	124.3	126.4	130.5	134.5	136.7	140.4	141.8	144.5
8: 9	105	1	130.9891	0.04607	6.0347	117.0	119.6	121.1	124.7	126.9	131.0	135.1	137.2	140.9	142.3	145.0
8:10	106	1	131.4895	0.04608	6.0590	117.4	120.1	121.5	125.2	127.4	131.5	135.6	137.8	141.5	142.9	145.6
8:11	107	1	131.9912	0.04610	6.0848	117.8	120.5	122.0	125.7	127.9	132.0	136.1	138.3	142.0	143.4	146.1
9: 0	108	1	132.4944	0.04612	6.1106	118.3	121.0	122.4	126.2	128.4	132.5	136.6	138.8	142.5	144.0	146.7
9: 1	109	1	132.9989	0.04613	6.1352	118.7	121.5	122.9	126.6	128.9	133.0	137.1	139.4	143.1	144.5	147.3
9: 2	110	1	133.5046	0.04614	6.1599	119.2	121.9	123.4	127.1	129.4	133.5	137.7	139.9	143.6	145.1	147.8
9: 3	111	1	134.0118	0.04615	6.1846	119.6	122.4	123.8	127.6	129.8	134.0	138.2	140.4	144.2	145.6	148.4

2007 WHO Reference

Height-for-age GIRLS

5 to 19 years (percentiles)

World Health Organization

						Percentiles (height in cm)										
Year: Month	Month	L	M	S	SD	1st	3rd	5th	15th	25th	50th	75th	85th	95th	97th	99th
9: 4	112	1	134.5202	0.04616	6.2095	120.1	122.8	124.3	128.1	130.3	134.5	138.7	141.0	144.7	146.2	149.0
9: 5	113	1	135.0299	0.04616	6.2330	120.5	123.3	124.8	128.6	130.8	135.0	139.2	141.5	145.3	146.8	149.5
9: 6	114	1	135.5410	0.04617	6.2579	121.0	123.8	125.2	129.1	131.3	135.5	139.8	142.0	145.8	147.3	150.1
9: 7	115	1	136.0533	0.04617	6.2816	121.4	124.2	125.7	129.5	131.8	136.1	140.3	142.6	146.4	147.9	150.7
9: 8	116	1	136.5670	0.04616	6.3039	121.9	124.7	126.2	130.0	132.3	136.6	140.8	143.1	146.9	148.4	151.2
9: 9	117	1	137.0821	0.04616	6.3277	122.4	125.2	126.7	130.5	132.8	137.1	141.4	143.6	147.5	149.0	151.8
9:10	118	1	137.5987	0.04616	6.3516	122.8	125.7	127.2	131.0	133.3	137.6	141.9	144.2	148.0	149.5	152.4
9:11	119	1	138.1167	0.04615	6.3741	123.3	126.1	127.6	131.5	133.8	138.1	142.4	144.7	148.6	150.1	152.9
10: 0	120	1	138.6363	0.04614	6.3967	123.8	126.6	128.1	132.0	134.3	138.6	143.0	145.3	149.2	150.7	153.5
10: 1	121	1	139.1575	0.04612	6.4179	124.2	127.1	128.6	132.5	134.8	139.2	143.5	145.8	149.7	151.2	154.1
10: 2	122	1	139.6803	0.04611	6.4407	124.7	127.6	129.1	133.0	135.3	139.7	144.0	146.4	150.3	151.8	154.7
10: 3	123	1	140.2049	0.04609	6.4620	125.2	128.1	129.6	133.5	135.8	140.2	144.6	146.9	150.8	152.4	155.2
10: 4	124	1	140.7313	0.04607	6.4835	125.6	128.5	130.1	134.0	136.4	140.7	145.1	147.5	151.4	152.9	155.8
10: 5	125	1	141.2594	0.04605	6.5050	126.1	129.0	130.6	134.5	136.9	141.3	145.6	148.0	152.0	153.5	156.4
10: 6	126	1	141.7892	0.04603	6.5266	126.6	129.5	131.1	135.0	137.4	141.8	146.2	148.6	152.5	154.1	157.0
10: 7	127	1	142.3206	0.04600	6.5467	127.1	130.0	131.6	135.5	137.9	142.3	146.7	149.1	153.1	154.6	157.6
10: 8	128	1	142.8534	0.04597	6.5670	127.6	130.5	132.1	136.0	138.4	142.9	147.3	149.7	153.7	155.2	158.1
10: 9	129	1	143.3874	0.04594	6.5872	128.1	131.0	132.6	136.6	138.9	143.4	147.8	150.2	154.2	155.8	158.7
10:10	130	1	143.9222	0.04591	6.6075	128.6	131.5	133.1	137.1	139.5	143.9	148.4	150.8	154.8	156.3	159.3
10:11	131	1	144.4575	0.04588	6.6277	129.0	132.0	133.6	137.6	140.0	144.5	148.9	151.3	155.4	156.9	159.9
11: 0	132	1	144.9929	0.04584	6.6465	129.5	132.5	134.1	138.1	140.5	145.0	149.5	151.9	155.9	157.5	160.5
11: 1	133	1	145.5280	0.04580	6.6652	130.0	133.0	134.6	138.6	141.0	145.5	150.0	152.4	156.5	158.1	161.0
11: 2	134	1	146.0622	0.04576	6.6838	130.5	133.5	135.1	139.1	141.6	146.1	150.6	153.0	157.1	158.6	161.6
11: 3	135	1	146.5951	0.04571	6.7009	131.0	134.0	135.6	139.7	142.1	146.6	151.1	153.5	157.6	159.2	162.2

2007 WHO Reference

Weight-for-age BOYS

5 to 10 years (percentiles)

World Health Organization

Year: Month	Month	L	M	S	Percentiles (weight in kg)										
					1st	3rd	5th	15th	25th	50th	75th	85th	95th	97th	99th
5: 1	61	-0.2026	18.5057	0.12988	13.8	14.6	15.0	16.2	17.0	18.5	20.2	21.2	23.0	23.8	25.3
5: 2	62	-0.2130	18.6802	0.13028	13.9	14.7	15.1	16.4	17.1	18.7	20.4	21.4	23.3	24.0	25.6
5: 3	63	-0.2234	18.8563	0.13067	14.1	14.8	15.3	16.5	17.3	18.9	20.6	21.6	23.5	24.3	25.8
5: 4	64	-0.2338	19.0340	0.13105	14.2	15.0	15.4	16.7	17.4	19.0	20.8	21.9	23.7	24.5	26.1
5: 5	65	-0.2443	19.2132	0.13142	14.3	15.1	15.6	16.8	17.6	19.2	21.0	22.1	24.0	24.8	26.4
5: 6	66	-0.2548	19.3940	0.13178	14.4	15.3	15.7	17.0	17.8	19.4	21.2	22.3	24.2	25.1	26.7
5: 7	67	-0.2653	19.5765	0.13213	14.6	15.4	15.8	17.1	17.9	19.6	21.4	22.5	24.5	25.3	27.0
5: 8	68	-0.2758	19.7607	0.13246	14.7	15.5	16.0	17.3	18.1	19.8	21.6	22.7	24.7	25.6	27.3
5: 9	69	-0.2864	19.9468	0.13279	14.8	15.7	16.1	17.4	18.3	19.9	21.8	23.0	25.0	25.8	27.6
5:10	70	-0.2969	20.1344	0.13311	15.0	15.8	16.3	17.6	18.4	20.1	22.1	23.2	25.3	26.1	27.9
5:11	71	-0.3075	20.3235	0.13342	15.1	16.0	16.4	17.7	18.6	20.3	22.3	23.4	25.5	26.4	28.2
6: 0	72	-0.3180	20.5137	0.13372	15.2	16.1	16.6	17.9	18.8	20.5	22.5	23.6	25.8	26.7	28.5
6: 1	73	-0.3285	20.7052	0.13402	15.4	16.3	16.7	18.1	18.9	20.7	22.7	23.9	26.0	26.9	28.8
6: 2	74	-0.3390	20.8979	0.13432	15.5	16.4	16.9	18.2	19.1	20.9	22.9	24.1	26.3	27.2	29.1
6: 3	75	-0.3494	21.0918	0.13462	15.7	16.5	17.0	18.4	19.3	21.1	23.1	24.3	26.6	27.5	29.4
6: 4	76	-0.3598	21.2870	0.13493	15.8	16.7	17.2	18.6	19.5	21.3	23.4	24.6	26.8	27.8	29.7
6: 5	77	-0.3701	21.4833	0.13523	16.0	16.8	17.3	18.7	19.6	21.5	23.6	24.8	27.1	28.1	30.0
6: 6	78	-0.3804	21.6810	0.13554	16.1	17.0	17.5	18.9	19.8	21.7	23.8	25.0	27.4	28.3	30.3
6: 7	79	-0.3906	21.8799	0.13586	16.2	17.2	17.7	19.1	20.0	21.9	24.0	25.3	27.6	28.6	30.7
6: 8	80	-0.4007	22.0800	0.13618	16.4	17.3	17.8	19.2	20.2	22.1	24.2	25.5	27.9	28.9	31.0
6: 9	81	-0.4107	22.2813	0.13652	16.5	17.5	18.0	19.4	20.4	22.3	24.5	25.8	28.2	29.2	31.3
6:10	82	-0.4207	22.4837	0.13686	16.7	17.6	18.1	19.6	20.5	22.5	24.7	26.0	28.5	29.5	31.6
6:11	83	-0.4305	22.6872	0.13722	16.8	17.8	18.3	19.8	20.7	22.7	24.9	26.3	28.8	29.8	32.0
7: 0	84	-0.4402	22.8915	0.13759	17.0	17.9	18.4	19.9	20.9	22.9	25.2	26.5	29.1	30.1	32.3
7: 1	85	-0.4499	23.0968	0.13797	17.1	18.1	18.6	20.1	21.1	23.1	25.4	26.8	29.3	30.4	32.7
7: 2	86	-0.4594	23.3029	0.13838	17.3	18.2	18.8	20.3	21.3	23.3	25.6	27.0	29.6	30.7	33.0

2007 WHO Reference

Weight-for-age BOYS

5 to 10 years (percentiles)

World Health Organization

Year: Month	Month	L	M	S	Percentiles (weight in kg)										
					1st	3rd	5th	15th	25th	50th	75th	85th	95th	97th	99th
7: 3	87	-0.4688	23.5101	0.13880	17.4	18.4	18.9	20.5	21.5	23.5	25.9	27.3	29.9	31.1	33.4
7: 4	88	-0.4781	23.7182	0.13923	17.6	18.5	19.1	20.6	21.6	23.7	26.1	27.5	30.2	31.4	33.7
7: 5	89	-0.4873	23.9272	0.13969	17.7	18.7	19.2	20.8	21.8	23.9	26.4	27.8	30.5	31.7	34.1
7: 6	90	-0.4964	24.1371	0.14016	17.8	18.8	19.4	21.0	22.0	24.1	26.6	28.1	30.8	32.0	34.4
7: 7	91	-0.5053	24.3479	0.14065	18.0	19.0	19.6	21.2	22.2	24.3	26.8	28.3	31.1	32.3	34.8
7: 8	92	-0.5142	24.5595	0.14117	18.1	19.1	19.7	21.3	22.4	24.6	27.1	28.6	31.4	32.7	35.2
7: 9	93	-0.5229	24.7722	0.14170	18.3	19.3	19.9	21.5	22.6	24.8	27.3	28.9	31.8	33.0	35.6
7:10	94	-0.5315	24.9858	0.14226	18.4	19.5	20.0	21.7	22.8	25.0	27.6	29.1	32.1	33.3	36.0
7:11	95	-0.5399	25.2005	0.14284	18.6	19.6	20.2	21.9	22.9	25.2	27.8	29.4	32.4	33.7	36.3
8: 0	96	-0.5482	25.4163	0.14344	18.7	19.8	20.4	22.0	23.1	25.4	28.1	29.7	32.7	34.0	36.7
8: 1	97	-0.5564	25.6332	0.14407	18.9	19.9	20.5	22.2	23.3	25.6	28.3	30.0	33.1	34.4	37.1
8: 2	98	-0.5644	25.8513	0.14472	19.0	20.1	20.7	22.4	23.5	25.9	28.6	30.2	33.4	34.7	37.6
8: 3	99	-0.5722	26.0706	0.14539	19.1	20.2	20.8	22.6	23.7	26.1	28.8	30.5	33.7	35.1	38.0
8: 4	100	-0.5799	26.2911	0.14608	19.3	20.4	21.0	22.7	23.9	26.3	29.1	30.8	34.1	35.5	38.4
8: 5	101	-0.5873	26.5128	0.14679	19.4	20.5	21.2	22.9	24.1	26.5	29.4	31.1	34.4	35.8	38.8
8: 6	102	-0.5946	26.7358	0.14752	19.6	20.7	21.3	23.1	24.3	26.7	29.6	31.4	34.7	36.2	39.2
8: 7	103	-0.6017	26.9602	0.14828	19.7	20.8	21.5	23.3	24.5	27.0	29.9	31.7	35.1	36.6	39.7
8: 8	104	-0.6085	27.1861	0.14905	19.8	21.0	21.6	23.5	24.7	27.2	30.2	32.0	35.5	37.0	40.1
8: 9	105	-0.6152	27.4137	0.14984	20.0	21.1	21.8	23.6	24.9	27.4	30.4	32.3	35.8	37.4	40.6
8:10	106	-0.6216	27.6432	0.15066	20.1	21.3	22.0	23.8	25.0	27.6	30.7	32.6	36.2	37.8	41.0
8:11	107	-0.6278	27.8750	0.15149	20.3	21.4	22.1	24.0	25.2	27.9	31.0	32.9	36.6	38.2	41.5
9: 0	108	-0.6337	28.1092	0.15233	20.4	21.6	22.3	24.2	25.4	28.1	31.3	33.2	36.9	38.6	42.0
9: 1	109	-0.6393	28.3459	0.15319	20.6	21.8	22.4	24.4	25.6	28.3	31.5	33.5	37.3	39.0	42.5
9: 2	110	-0.6446	28.5854	0.15406	20.7	21.9	22.6	24.6	25.9	28.6	31.8	33.8	37.7	39.4	43.0
9: 3	111	-0.6496	28.8277	0.15493	20.9	22.1	22.8	24.7	26.1	28.8	32.1	34.2	38.1	39.8	43.5

2007 WHO Reference

Weight-for-age BOYS

5 to 10 years (percentiles)

World Health Organization

					Percentiles (weight in kg)										
Year: Month	Month	L	M	S	1st	3rd	5th	15th	25th	50th	75th	85th	95th	97th	99th
9: 4	112	-0.6543	29.0731	0.15581	21.0	22.2	22.9	24.9	26.3	29.1	32.4	34.5	38.5	40.3	44.0
9: 5	113	-0.6585	29.3217	0.15670	21.1	22.4	23.1	25.1	26.5	29.3	32.7	34.8	38.9	40.7	44.5
9: 6	114	-0.6624	29.5736	0.15760	21.3	22.6	23.3	25.3	26.7	29.6	33.0	35.2	39.3	41.1	45.0
9: 7	115	-0.6659	29.8289	0.15850	21.5	22.7	23.5	25.5	26.9	29.8	33.3	35.5	39.7	41.6	45.5
9: 8	116	-0.6689	30.0877	0.15940	21.6	22.9	23.6	25.7	27.1	30.1	33.6	35.8	40.1	42.0	46.1
9: 9	117	-0.6714	30.3501	0.16031	21.8	23.1	23.8	25.9	27.3	30.4	34.0	36.2	40.6	42.5	46.6
9:10	118	-0.6735	30.6160	0.16122	21.9	23.2	24.0	26.1	27.6	30.6	34.3	36.6	41.0	43.0	47.2
9:11	119	-0.6752	30.8854	0.16213	22.1	23.4	24.2	26.3	27.8	30.9	34.6	36.9	41.4	43.5	47.7
10: 0	120	-0.6764	31.1586	0.16305	22.2	23.6	24.4	26.6	28.0	31.2	34.9	37.3	41.9	43.9	48.3

2007 WHO Reference

Weight-for-age GIRLS

5 to 10 years (percentiles)

World Health Organization

Year: Month	Month	L	M	S	Percentiles (weight in kg)										
					1st	3rd	5th	15th	25th	50th	75th	85th	95th	97th	99th
5: 1	61	-0.4681	18.2579	0.14295	13.4	14.2	14.6	15.8	16.6	18.3	20.2	21.3	23.4	24.3	26.2
5: 2	62	-0.4711	18.4329	0.14350	13.5	14.3	14.7	16.0	16.8	18.4	20.4	21.5	23.7	24.6	26.5
5: 3	63	-0.4742	18.6073	0.14404	13.6	14.4	14.9	16.1	16.9	18.6	20.6	21.7	23.9	24.9	26.8
5: 4	64	-0.4773	18.7811	0.14459	13.7	14.5	15.0	16.3	17.1	18.8	20.8	21.9	24.2	25.1	27.1
5: 5	65	-0.4803	18.9545	0.14514	13.9	14.7	15.1	16.4	17.2	19.0	21.0	22.2	24.4	25.4	27.4
5: 6	66	-0.4834	19.1276	0.14569	14.0	14.8	15.2	16.5	17.4	19.1	21.2	22.4	24.7	25.7	27.7
5: 7	67	-0.4864	19.3004	0.14624	14.1	14.9	15.4	16.7	17.5	19.3	21.4	22.6	24.9	25.9	28.0
5: 8	68	-0.4894	19.4730	0.14679	14.2	15.0	15.5	16.8	17.7	19.5	21.6	22.8	25.2	26.2	28.3
5: 9	69	-0.4924	19.6455	0.14735	14.3	15.2	15.6	17.0	17.8	19.6	21.8	23.0	25.4	26.5	28.6
5:10	70	-0.4954	19.8180	0.14790	14.4	15.3	15.8	17.1	18.0	19.8	22.0	23.2	25.7	26.7	28.9
5:11	71	-0.4984	19.9908	0.14845	14.5	15.4	15.9	17.2	18.1	20.0	22.2	23.5	25.9	27.0	29.2
6: 0	72	-0.5013	20.1639	0.14900	14.6	15.5	16.0	17.4	18.3	20.2	22.4	23.7	26.2	27.3	29.5
6: 1	73	-0.5043	20.3377	0.14955	14.8	15.6	16.1	17.5	18.4	20.3	22.6	23.9	26.4	27.5	29.8
6: 2	74	-0.5072	20.5124	0.15010	14.9	15.8	16.3	17.7	18.6	20.5	22.8	24.1	26.7	27.8	30.1
6: 3	75	-0.5100	20.6885	0.15065	15.0	15.9	16.4	17.8	18.7	20.7	23.0	24.3	27.0	28.1	30.4
6: 4	76	-0.5129	20.8661	0.15120	15.1	16.0	16.5	17.9	18.9	20.9	23.2	24.6	27.2	28.4	30.8
6: 5	77	-0.5157	21.0457	0.15175	15.2	16.1	16.6	18.1	19.0	21.0	23.4	24.8	27.5	28.7	31.1
6: 6	78	-0.5185	21.2274	0.15230	15.3	16.3	16.8	18.2	19.2	21.2	23.6	25.0	27.8	28.9	31.4
6: 7	79	-0.5213	21.4113	0.15284	15.5	16.4	16.9	18.4	19.4	21.4	23.8	25.3	28.0	29.2	31.7
6: 8	80	-0.5240	21.5979	0.15339	15.6	16.5	17.0	18.5	19.5	21.6	24.0	25.5	28.3	29.5	32.1
6: 9	81	-0.5268	21.7872	0.15393	15.7	16.6	17.2	18.7	19.7	21.8	24.2	25.7	28.6	29.8	32.4
6:10	82	-0.5294	21.9795	0.15448	15.8	16.8	17.3	18.8	19.9	22.0	24.5	26.0	28.9	30.1	32.7
6:11	83	-0.5321	22.1751	0.15502	15.9	16.9	17.5	19.0	20.0	22.2	24.7	26.2	29.2	30.4	33.1
7: 0	84	-0.5347	22.3740	0.15556	16.1	17.0	17.6	19.2	20.2	22.4	24.9	26.5	29.5	30.8	33.5
7: 1	85	-0.5372	22.5762	0.15610	16.2	17.2	17.8	19.3	20.4	22.6	25.2	26.7	29.8	31.1	33.8
7: 2	86	-0.5398	22.7816	0.15663	16.3	17.3	17.9	19.5	20.6	22.8	25.4	27.0	30.1	31.4	34.2

2007 WHO Reference

Weight-for-age GIRLS

5 to 10 years (percentiles)

World Health Organization

					Percentiles (weight in kg)										
Year: Month	Month	L	M	S	1st	3rd	5th	15th	25th	50th	75th	85th	95th	97th	99th
7: 3	87	-0.5423	22.9904	0.15717	16.5	17.5	18.1	19.7	20.7	23.0	25.6	27.3	30.4	31.7	34.6
7: 4	88	-0.5447	23.2025	0.15770	16.6	17.6	18.2	19.8	20.9	23.2	25.9	27.5	30.7	32.1	34.9
7: 5	89	-0.5471	23.4180	0.15823	16.7	17.8	18.4	20.0	21.1	23.4	26.1	27.8	31.0	32.4	35.3
7: 6	90	-0.5495	23.6369	0.15876	16.9	17.9	18.5	20.2	21.3	23.6	26.4	28.1	31.3	32.8	35.7
7: 7	91	-0.5518	23.8593	0.15928	17.0	18.1	18.7	20.4	21.5	23.9	26.7	28.4	31.7	33.1	36.1
7: 8	92	-0.5541	24.0853	0.15980	17.2	18.2	18.8	20.6	21.7	24.1	26.9	28.7	32.0	33.5	36.5
7: 9	93	-0.5563	24.3149	0.16032	17.3	18.4	19.0	20.7	21.9	24.3	27.2	28.9	32.3	33.8	36.9
7:10	94	-0.5585	24.5482	0.16084	17.5	18.6	19.2	20.9	22.1	24.5	27.5	29.2	32.7	34.2	37.4
7:11	95	-0.5606	24.7853	0.16135	17.6	18.7	19.4	21.1	22.3	24.8	27.7	29.5	33.0	34.6	37.8
8: 0	96	-0.5627	25.0262	0.16186	17.8	18.9	19.5	21.3	22.5	25.0	28.0	29.8	33.4	34.9	38.2
8: 1	97	-0.5647	25.2710	0.16237	17.9	19.1	19.7	21.5	22.7	25.3	28.3	30.2	33.8	35.3	38.6
8: 2	98	-0.5667	25.5197	0.16287	18.1	19.2	19.9	21.7	22.9	25.5	28.6	30.5	34.1	35.7	39.1
8: 3	99	-0.5686	25.7721	0.16337	18.3	19.4	20.1	21.9	23.2	25.8	28.9	30.8	34.5	36.1	39.5
8: 4	100	-0.5704	26.0284	0.16386	18.4	19.6	20.3	22.1	23.4	26.0	29.2	31.1	34.9	36.5	40.0
8: 5	101	-0.5722	26.2883	0.16435	18.6	19.8	20.4	22.3	23.6	26.3	29.5	31.4	35.3	36.9	40.5
8: 6	102	-0.5740	26.5519	0.16483	18.8	20.0	20.6	22.6	23.8	26.6	29.8	31.8	35.7	37.4	40.9
8: 7	103	-0.5757	26.8190	0.16532	18.9	20.1	20.8	22.8	24.1	26.8	30.1	32.1	36.0	37.8	41.4
8: 8	104	-0.5773	27.0896	0.16579	19.1	20.3	21.0	23.0	24.3	27.1	30.4	32.5	36.4	38.2	41.9
8: 9	105	-0.5789	27.3635	0.16626	19.3	20.5	21.2	23.2	24.5	27.4	30.7	32.8	36.9	38.6	42.4
8:10	106	-0.5804	27.6406	0.16673	19.5	20.7	21.4	23.4	24.8	27.6	31.0	33.2	37.3	39.1	42.9
8:11	107	-0.5819	27.9208	0.16719	19.7	20.9	21.6	23.7	25.0	27.9	31.4	33.5	37.7	39.5	43.4
9: 0	108	-0.5833	28.2040	0.16764	19.8	21.1	21.8	23.9	25.3	28.2	31.7	33.9	38.1	40.0	43.9
9: 1	109	-0.5847	28.4901	0.16809	20.0	21.3	22.0	24.1	25.5	28.5	32.0	34.2	38.5	40.4	44.4
9: 2	110	-0.5859	28.7791	0.16854	20.2	21.5	22.3	24.4	25.8	28.8	32.4	34.6	38.9	40.9	44.9
9: 3	111	-0.5872	29.0711	0.16897	20.4	21.7	22.5	24.6	26.0	29.1	32.7	35.0	39.4	41.3	45.5

2007 WHO Reference

Weight-for-age GIRLS

5 to 10 years (percentiles)

World Health Organization

Year: Month	Month	L	M	S	Percentiles (weight in kg)										
					1st	3rd	5th	15th	25th	50th	75th	85th	95th	97th	99th
9: 4	112	-0.5883	29.3663	0.16941	20.6	21.9	22.7	24.8	26.3	29.4	33.1	35.3	39.8	41.8	46.0
9: 5	113	-0.5895	29.6646	0.16983	20.8	22.1	22.9	25.1	26.6	29.7	33.4	35.7	40.3	42.3	46.5
9: 6	114	-0.5905	29.9663	0.17025	21.0	22.3	23.1	25.3	26.8	30.0	33.8	36.1	40.7	42.7	47.1
9: 7	115	-0.5915	30.2715	0.17066	21.2	22.6	23.3	25.6	27.1	30.3	34.1	36.5	41.1	43.2	47.6
9: 8	116	-0.5925	30.5805	0.17107	21.4	22.8	23.6	25.8	27.4	30.6	34.5	36.9	41.6	43.7	48.1
9: 9	117	-0.5934	30.8934	0.17146	21.6	23.0	23.8	26.1	27.6	30.9	34.8	37.3	42.1	44.2	48.7
9:10	118	-0.5942	31.2105	0.17186	21.8	23.2	24.0	26.3	27.9	31.2	35.2	37.7	42.5	44.7	49.3
9:11	119	-0.5950	31.5319	0.17224	22.0	23.4	24.3	26.6	28.2	31.5	35.6	38.1	43.0	45.2	49.8
10: 0	120	-0.5958	31.8578	0.17262	22.2	23.7	24.5	26.9	28.5	31.9	35.9	38.5	43.5	45.7	50.4

2007 WHO Reference

Table 18. Waist circumference in centimeters for children and adolescents aged 2–19 years and number of examined persons, mean, standard error of the mean, and selected percentiles, by sex and age: United States, 2007–2010

Sex and age[1]	Number of examined persons	Mean	Standard error of the mean	Percentile								
				5th	10th	15th	25th	50th	75th	85th	90th	95th
Male				Centimeters								
2 years	270	48.5	0.29	43.4	44.8	45.6	46.5	48.2	50.3	51.2	52.0	53.4
3 years	182	50.5	0.35	44.5	46.0	46.9	47.9	50.1	52.3	53.3	54.6	56.9
4 years	231	52.7	0.31	46.8	47.9	48.9	50.1	51.5	54.2	56.7	57.5	62.7
5 years	194	54.8	0.42	47.7	49.0	50.2	51.3	53.6	56.0	58.3	60.8	66.0
6 years	188	57.4	0.74	48.4	49.5	50.6	52.1	54.6	59.1	65.3	69.6	78.7
7 years	208	59.0	0.56	50.4	50.9	51.9	53.4	56.7	62.8	68.1	71.3	74.6
8 years	205	62.7	0.79	51.2	53.3	54.1	56.3	59.5	66.7	73.0	78.1	81.0
9 years	183	66.0	1.13	53.4	54.6	55.8	57.2	61.0	72.9	79.5	85.0	91.2
10 years	194	69.2	1.09	55.4	57.1	57.9	59.8	66.5	76.5	81.1	85.6	89.9
11 years	205	71.9	0.98	57.4	58.9	60.3	62.4	67.2	78.6	87.2	90.4	97.0
12 years	156	74.6	0.98	56.7	59.9	61.3	64.0	71.5	83.1	91.6	93.7	98.5
13 years	141	76.8	1.34	62.4	64.6	65.5	68.2	72.7	81.2	90.8	96.7	104.7
14 years	173	78.8	1.48	61.7	64.4	65.8	68.1	74.2	84.5	93.9	101.3	107.4
15 years	157	80.9	1.41	65.9	67.1	67.6	71.1	76.3	87.6	94.8	99.9	113.1
16 years	170	83.9	1.21	67.8	68.3	70.4	73.4	80.0	92.3	99.3	106.1	110.5
17 years	184	85.6	1.82	68.5	70.0	73.0	74.8	79.5	92.2	101.6	108.0	118.6
18 years	137	88.1	1.62	70.6	72.2	73.7	77.5	85.3	95.5	101.6	105.9	†
19 years	176	85.9	1.17	71.7	73.1	74.1	76.8	82.8	91.1	93.9	101.3	†
Female												
2 years	235	48.2	0.36	43.4	43.9	44.5	45.4	47.5	50.3	51.6	52.9	54.7
3 years	179	50.2	0.36	43.1	45.4	46.4	47.4	49.9	52.3	54.3	55.0	57.3
4 years	191	52.1	0.27	46.2	46.9	47.7	48.9	51.1	54.2	56.2	58.3	60.2
5 years	168	55.6	0.84	47.9	49.2	50.4	51.3	54.0	57.4	60.4	63.3	†
6 years	173	56.5	0.60	48.5	49.8	50.8	52.3	56.0	58.8	63.5	64.3	67.9
7 years	202	59.4	0.67	49.1	51.0	51.4	53.7	57.0	61.9	67.3	72.9	78.1
8 years	199	63.8	1.08	49.7	52.3	53.2	55.2	61.8	71.3	75.2	78.7	82.7
9 years	198	66.6	1.08	53.1	54.5	56.0	57.6	63.3	72.3	79.6	83.0	88.1
10 years	174	69.8	0.97	57.1	58.3	59.1	61.2	68.6	75.1	82.0	84.1	88.8
11 years	212	73.2	1.27	56.2	58.9	60.8	62.7	69.2	81.5	87.2	93.6	103.0
12 years	162	74.7	1.22	†	60.6	63.6	65.7	73.9	82.0	87.3	95.1	98.8
13 years	130	77.6	1.40	63.3	64.8	65.7	68.4	73.7	85.6	92.1	96.8	100.6
14 years	164	80.4	0.95	65.5	67.5	68.3	71.2	77.3	85.5	91.2	94.4	108.0
15 years	134	81.7	1.20	64.7	68.5	70.6	72.6	78.4	87.0	95.3	101.4	106.0
16 years	152	81.0	1.03	66.9	67.9	69.7	71.6	78.2	85.4	92.2	102.2	106.6
17 years	141	82.2	1.50	66.6	67.1	69.0	72.0	79.6	86.9	95.5	99.5	114.1
18 years	131	83.1	1.35	67.9	70.0	70.8	72.3	77.2	90.1	99.6	107.1	112.6
19 years	115	85.4	1.72	65.2	68.4	70.5	72.8	80.8	93.4	103.2	109.6	118.4

† Standard error not calculated by SUDAAN.
[1]Refers to age at time of examination.

NOTE: Pregnant females were excluded.

SOURCE: CDC/NCHS, National Health and Nutrition Examination Survey.

Appendix 11 – z-scores of tBMD

Subject id	Age	Total headless bone mineral density (g/cm^2)	z-score	Source of z-scores
1	7	0.623	-0.4	BMDCS
2	8	0.576	-0.7	DEXA
3	9	0.765	1.1	DEXA
4	8	0.669	0.7	DEXA
5	6	0.503	-1.2	BMDCS
6	8	0.633	0.7	DEXA
7	5	0.55	0.6	BMDCS
8	6	0.559	0.4	BMDCS
9	10	0.78	0.5	DEXA
10	8	0.592	-1	DEXA
11	9	0.573	-0.6	DEXA
12	5	0.558	0.4	BMDCS
13	7	0.578	0.3	BMDCS
14	8	0.594	0.2	DEXA
15	8	0.646	0.3	DEXA
16	7	0.608	-0.6	BMDCS
17	8	0.674	1	DEXA
18	7	0.686	0.02	BMDCS
19	7	0.652	-0.09	BMDCS
20	8	0.635	-0.5	DEXA
21	8	0.688	1	DEXA
22	8	0.638	0.3	DEXA
23	7	0.64	0.3	BMDCS
24	8	0.597	0	DEXA
25	8	0.611	0.2	DEXA
26	8	0.706	-1.1	DEXA
27	9	0.682	-0.1	Nakavachara et al 2014
28	7	0.592	-1.6	Nakavachara et al 2014
29	6	0.528	-2.3	Nakavachara et al 2014
30	8	0.609	-0.3	Nakavachara et al 2014
31	7	0.547	-0.8	BMDCS
32*	8	0.622	0	DEXA
33	8	0.703	1	DEXA
34	7	0.594	-0.84	BMDCS
35	6	0.562	-0.1	BMDCS
36	6	0.578	-0.1	BMDCS
37	8	0.566	-0.2	DEXA
38	8	0.703	0.6	DEXA
39	6	0.674	1.45	BMDCS
40	8	0.741	2	DEXA
41	8	0.748	1.9	DEXA
42	9	0.663	1.1	DEXA
43	9	0.678	1.5	DEXA
44	7	0.67	0.61	BMDCS
45	9	0.631	0	DEXA

*participant had a cast on the arm, hence data not used. BMDC=bone mineral density childhood study.