Elmantaser, Dr Musab Elmabrouk M. (2013) *Bone health in children with acute lymphoblastic leukaemia.*

PhD thesis

http://theses.gla.ac.uk/4447/

Copyright and moral rights for this thesis are retained by the author

A copy can be downloaded for personal non-commercial research or study, without prior permission or charge

This thesis cannot be reproduced or quoted extensively from without first obtaining permission in writing from the Author

The content must not be changed in any way or sold commercially in any format or medium without the formal permission of the Author

When referring to this work, full bibliographic details including the author, title, awarding institution and date of the thesis must be given

Glasgow Theses Service
http://theses.gla.ac.uk/
theses@gla.ac.uk

Bone Health in Children with Acute Lymphoblastic Leukaemia

Dr Musab Elmabrouk M Elmantaser

MBChB & MSc (Medical Genetics)

A Thesis Submitted in Fulfilment of the Requirements of the University of Glasgow for the Degree of Doctor of Philosophy

Bone and Endocrine Research Group

Department of Child Health

Royal Hospital for Sick Children

Faculty of Medicine

University of Glasgow

March 2013

AUTHOR'S DECLARATION

I hereby declare that this thesis has been composed by myself and is a record of work performed by myself under the title 'Bone Health in Children with Acute Lymphoblastic Leukaemia'. This work has not been submitted previously for a higher degree and was carried out under the supervision of Professor Syed Faisal Ahmed

I conducted all research at Royal Hospital for Sick Children and the University of Glasgow, Glasgow, UK.

Dr Musab Elmabrouk M Elmantaser

I certify that the work reported in this thesis has been performed by Dr Elmantaser and that during the period of study he has fulfilled the conditions of the ordinances and regulations governing the Degree of Doctor of Philosophy, University of Glasgow.

Prof Syed Faisal Ahmed

Abstract

In chapter 1, bone structure, bone growth and development, osteoporosis in children and skeletal morbidities in children with acute lymphoblastic leukaemia (ALL) are discussed. After that, the mechanostat and the effect of whole body vibration (WBV) on bone health are considered. Finally, I examine diagnostic approaches to assess the musculoskeletal system.

In chapter 2, the incidence and risk factors for skeletal morbidity in ALL children are determined. The medical records of all (n,186, male,110) children presenting with ALL between 1997 and 2007 and treated on UKALL97, UKALL97/01 or UKALL2003 were studied. Skeletal morbidity included musculoskeletal pain (MSP), fractures and osteonecrosis (ON). MSP was classified as any event of limb pain, muscle pain, joint symptoms or back pain that required radiological examination. Fractures and ON were confirmed by X-rays and MRI respectively. We found that skeletal morbidity, presenting as MSP, fractures or ON were reported in 88(47%) children of whom 56(63%) were boys. Of 88 children, 49(55%), 27(30%) and 18(20%) had MSP, fracture(s) or ON, respectively. Six (7%) had both fractures and ON. The median(10^{th},90^{th}centiles) age at diagnosis of ALL children without skeletal morbidity was 3.9years(1.4,12), which was lower than in those with skeletal morbidity at 8.2years(2.2,14.3) ($p<0.00001$,95%CI:1.7,4.4). Children with ALL diagnosed over 8years of age were at increased risk of developing fracture(s) ($p=0.01$,odds ratio(OR)=2.9,95%CI:1.3,6.5), whereas the risk of ON was higher in those who were diagnosed after 9years of age ($p<0.0001$,OR=15,95%CI:4.1,54.4). There was no gender-difference in the incidence of skeletal morbidity. Children who received dexamethasone had a higher incidence of skeletal morbidity than those who were treated with prednisolone ($p=0.027$,OR=2.6,95%CI:1.1,5.9). We concluded that the occurrence of skeletal morbidity in ALL children may be influenced by age and the type of glucocorticoids (GCs). These findings may facilitate the development of effective bone protective intervention.

In chapter 3, the aim is to investigate the influence of physical activity, age and mineral homeostasis over the first 12months of chemotherapy on subsequent skeletal morbidity. We reviewed 56 children who presented with ALL between 2003 and 2007 and treated only on

UKALL2003. The number of in-patient days over the first 12months of chemotherapy was collected and used as a surrogate marker of inactivity and lack of well-being. Data for serum calcium (Ca), phosphate (Pho), magnesium (Mg) and albumin were also collected over this period. Skeletal morbidity was defined as any episode MSP or fractures. We found that the median duration of in-patient days over the first 12months of treatment in children with no skeletal morbidity was 58days(40,100), whereas the median number of in-patient days during the first 12months in those children with any skeletal morbidity, MSP only or fractures only was 83days(54,131), 81days(52,119) and 91days(59,158), respectively (p=0.003). Children with skeletal morbidity and fractures particularly had lower levels of serum Ca, Mg and Pho compared with those without skeletal morbidity over the first 12 months of chemotherapy. There was a higher risk of skeletal morbidity in those who were diagnosed after the age of 8years (p=0.001,OR=16,CI:3,80). Multiple regression analysis showed that the incidence of skeletal morbidity only had a significant independent association with age at diagnosis (p=0.001) and the number of inpatient days (p=0.03) over the first 12months (r=23). All children who were diagnosed after the age of 8years with an inpatient stay of greater than 75 days in the first 12 months of the chemotherapy (n,14) had some form of skeletal morbidity (OR=64). The conclusion was that the incidence of skeletal morbidity in children receiving chemotherapy for UKALL2003 is associated with a higher likelihood of being older and having longer periods of inpatient stay. The close link between age and changes in bone mineral status may be one explanation for the increased bone morbidity in ALL children

In chapter 4, the effects of two WBV regimens on endocrine status, muscle function and markers of bone turnover are compared. We recruited 10adult men with a median age of 33years(29,49), who were randomly assigned to stand on the Galileo platform (GP) (frequency (f)=18-22Hz, peak to peak displacement (D)=4mm, peak acceleration (a_{peak}) =2.6-3.8g) or Juvent1000 (f=32-37Hz, 0.085mm,0.3g) platform (JP) three times/week for a period of eight weeks. The measurements were performed at five time points (T0, T1, T2, T3, T4) and performed in a four week period of run-in (No WBV), eight weeks of WBV and a four-week period of washout (No WBV). The measurements included anthropometries, body composition measured by Tanita, muscle function measured by Leonardo mechanography and biochemical markers of endocrine status and bone turnover. The immediate term effect of WBV at 22Hz was associated with an increase in serum growth hormone (GH), increasing

from 0.07µg/l(0.04,0.69) to 0.52µg/l(0.06,2.4) (p=0.06),0.63µg/l(0.1,1.18)(p=0.03) ,0.21µg/l (0.07,0.65) (p=0.2) at 5minutes, 20minutes and 60minutes after WBV, respectively in the GP group. The immediate term effect of GP at 18Hz was associated with a reduction in serum cortisol from 316nmol/l (247,442) at 60minutes pre-WBV to 173nmol/l(123,245)(p=0.01), 165nmol/l(139,276)(p=0.02) and 198nmol/l(106,294)(p=0.04) at 5minutes, 20minutes and 60minutes post-WBV, respectively. At 22 Hz, GP was associated with a reduction in serum cortisol from 269nmol/l(115,323) at 60minutes before WBV to 214nmol/l(139,394)(p=0.5), 200nmol/l(125,337)(p=0.08) and 181nmol/l(104,306)(p=0.04) at 5minutes, 20minutes and 60minutes post-WBV, respectively. Median serum cortisol decreased after eight weeks of WBV from 333nmol/l(242,445) to 270nmol/l(115,323)(p=0.04). Median serum of the carboxy-terminal telopeptide (CTX, bore resorption marker) reduced significantly after eight weeks of WBV from 0.42ng/ml(0.29,0.90) to 0.29ng/ml(0.18,0.44)(p=0.03). None of these changes were observed in the JP group. Therefore, WBV at a certain magnitude can stimulate GH secretion, reduce circulating cortisol and reduce bone resorption. These effects are independent of clear changes in muscle function and depend on the type of WBV that is administered.

In chapter 5, the effect of WBV using GP on the bone health of children receiving chemotherapy for ALL was assessed. We recruited 16children with ALL with a median age of 7.8years(5-13.8; 9males), who were randomized either to receive side-alternating WBV (f=16-20Hz,D=2mm, a_{peak} =1-1.6g)(n,9) or to stand on a still platform as a control group (n,7) for 9minutes, once/week for four months. Measurements were performed at baseline, two-month and four-month assessing bone health (DXA and p.QCT), body composition and muscle function by imaging and biochemical assessment. DXA BMC data were corrected for bone area and presented as BMC z-score. We found that the median compliance rate measured as a ratio of actual completed minutes and expected minutes of WBV was 55%(17,100). The median percentage change of total body BMC z score in the WBV group from baseline to four months dropped by 10%(-25,10)(p=0.1), whereas it was 87%(-203,4)(p=0.07) in the control group. The median lumbar spine BMC z-score (L2-L4) in the WBV group was -0.4(-1.3,0.3) and -0.3(-1.4,1.5) at baseline and four months, whereas the respective data in the control group were 0.04(-0.6,2.4) and -0.1(-1.1,1), respectively. The median percentage change in LS-BMC z-score declined from baseline to four-month by19%(-349,365)(p=0.1)

and 75%(-1016,178)(p=0.1) in the WBV and control groups, respectively. We concluded that WBV is tolerated by children receiving chemotherapy. WBV might improve bone health in ALL children receiving chemotherapy

Chapter 6 summarises the findings of this thesis, discussing recommendations for improving bone health in ALL children and exploring weaknesses inherent in registry data and limitation. To sum up exercise in ALL children may be most effective if started at the time of diagnosis in parallel with chemotherapy but user acceptability of WBV may not be high at this point. Also, where sufficient data are available, there is a need to compare outcomes between WBV and conventional exercise for improvement in children' bone health in order to find the optimal dose. Whereas in this thesis, the effect of WBV on the musculoskeletal and endocrine systems was assessed, for any further work, also it may be useful to consider the interactive effect of nutritional optimisation and Mg supplementation on bone health during chemotherapy.

Acknowledgements

The one who is not grateful to people is not grateful to Allah''

''Muhammad peace be upon him''

First and foremost I offer my sincerest gratitude to my principal supervisor, Professor Syed Faisal Ahmed, who has supported me throughout my thesis with his patience and knowledge whilst allowing me the room to work in my own way. He is a wonderful role model and has provided me with many exciting opportunities over the last four years. Not to forget my former co-supervisor, Dr Brenda Gibson for their helpful comments and generously providing me a space in the haematological department

Most of the results described in this thesis would not have been obtained without a close collaboration with few people. I owe a great deal of appreciation and gratitude to Dr Sheila Khanna for measuring bone scanning in children and Martin McMillan for analysing blood samples. I would like to thank all participants who took part in this study. I also gratefully acknowledge the help of Ward 1C staff for providing a space and material for this study.

I am indebted to my many colleagues for providing a stimulating and fun filled environment. My thanks go in particular to Dr Salma Malik and Dr Mabrouka AL.Towati with whom I started this work and many rounds of discussions on my project with them helped me a lot. I wish to express my sincere gratitude to all those who influenced and motivated me to pursue a career at the Bone and Endocrine Research Group (BERG), Department of Child Health, Royal Hospital for Sick and special thanks to Karyn Cooper for her continued support at various stages of the PhD, her co-operation and for assisting in several administrative matters.

I would also like to thank my committee members, Dr Christina Halsey Dr Justin Davies and Professor Charlotte Wright for serving as my committee members even at hardship. I also want to thank you for letting my defense be an enjoyable moment, and for your brilliant comments and suggestions, thanks to you.

The financial support from Libyan Government is gratefully acknowledged.

DEDICATIONS

This thesis is dedicated to my wife Wafa and my children Muhammad, Amina and Luqman for they gave me values; enjoyment and love. Without their encouragement and understanding it would have been impossible for me to finish this work. I dedicate this work to them.

This thesis is also dedicated to my mother Ayada and my father Elmabrouk who have all supported me and given me the strength to complete this thesis.

Publications

1. Ahmed S, Elmantaser M. Secondary osteoporosis. *Endocrine Development* 2009;16:170-190.

2. Elmantaser M, Stewart G, Young D, Duncan R, Gibson B, Ahmed SF. Skeletal morbidity in children receiving chemotherapy for acute lymphoblastic leukaemia. *Archives of Disease in Childhood* 2010;95(10):805-809.

3. Elmantaser M, Young D, Gibson B, Ahmed SF. Skeletal morbidity in children receiving chemotherapy for acute lymphoblastic leukemia and its association with mineral homeostasis and duration of inpatient stay. *Journal of Pediatric Hematology Oncology* 2011 Oct;33(7):516-520.

4. Elmantaser M , McMillan M, Smith K, Khanna S, Chantler D, Panarelli M , Ahmed SF. A Comparison Of The effect of two types of vibration exercise on the endocrine and musculoskeletal system. *Journal of Musculoskeletal and Neuronal Interactions*. 2012 (in press)

5. Elmantaser M, Shaikh GM. Bone health in childhood cancer survivors. *SIGN Guidelines*. 2012 (in press)

Table of Contents

Abbreviations

11β-HSD	11β-hydroxysteroid dehydrogenases
A	Amplitude
aBMD	Areal bone mineral density
ALL	Acute lymphoblastic leukaemia
AN	Anorexia nervosa
a_{peak}	Peak acceleration
BA	Bone area
BAP	Bone-specific alkaline phosphatase
BMAD	Bone mineral apparent density
BMD	Bone mineral density
BMP	Bone morphogenetic proteins
BMU	Basic Multicellular Unit
BSP	Sialoprotein
BTT	Bone transmission time
BUA	Broadband attenuation
Ca	Calcium
c-AMP	Cyclic adenosine monophosphate
CD	Crohn's disease
CK	Creatine kinase
COX-2	Cyclooxygenase-2
CP	Cerebral palsy
CRT	Chair rising test
CSF-1	Colony-stimulating factor-1
CV	Coefficient variance
D	Peak to peak displacement
DMD	Duchenne muscular dystrophy
DXA	Dual energy X-ray absorptiometry
ECM	Extracellular matrix

EMG	Electromyography
ER	Estrogen receptors
f	Frequency
FFM	Fat free mass
FGF	Fibroblast growth factors
FM	Fat mass
FM%	Fat mass percent
FN-BMC	Femoral neck BMC
FN-BMD	Femoral neck BMD
FSS	Fluid shear stress
g	Gravity force
GCs	Glucocorticoids
GFR	Ground force reaction
GH	Growth hormone
GHBP	Growth hormone binding protein
GHD	Growth hormone deficiency
GHR	Growth hormone receptors
GHRH	Growth hormone releasing hormone
GIO	Glucocorticoid-induced osteoporosis
GP	Galileo platform
GRFP	Ground reaction force platform
HAZ	Height for age z-score
HPV	Puberty height velocity
HRT	Heal rise test
Hz	Hertz
IBD	Inflammatory bowel disease
ICTP	Pyridinoline cross-linked telopeptide domain of type I collagen
IGF-1	Insulin like growth factor-1
IGFBP	Insulin like growth factor binding proteins
IL	Interleukin
IP3	Inositol triphosphate3
ISCD	The International Society for Clinical Densitometry
ISO	International Safety Organisation
JAK2	Tyrosine kinase Janus kinase 2

JIA	Juvenile idiopathic arthritis
JP	Juvent1000 platform
LM	Lean mass
LRP	Low density lipoprotein related receptors
LS-BMC	Lumbar spine BMC
LS-BMD	Lumbar spine BMD
m1LJ	Multiple one leg jump
M-CSF	Macrophage colony-stimulating factor
MDT	Micro-damage threshold
Mg	Magnesium
MIGF	Maximal isometric grip force
MSC	Mesenchymal stem cells
MSP	Musculoskeletal pain
NCP	Non-collagenous proteins
NO	Nitric oxide
NTX	Cross-linked N-terminal telopeptide of type I collagen
OCN	Osteocalcin
OHP	Hydroxyproline
OI	Osteogenesis imperfect
ON	Osteonecrosis
ONN	Osteonectin
OPG	Osteoprotegerin
OR	Odds ratio
p.QCT	Peripheral quantitative computed tomography
PDGF	Platelet-derived growth factor
PG	Prostaglandin
Pho	Phosphate
PICP	Carboxy-terminal propeptide of type I procollagen
PINP	Amino-terminal propeptide of type I procollagen
PPBA	Percentage predicted bone area
PTH	Parathyroid hormone
PTHrP	Parathyroid hormone related peptide
QUS	Quantitative ultrasound
RANK	Receptor activator of nuclear factor κβ

RANKL	Receptor activator of nuclear factor κβ ligand
RCT	Randomised controlled trial
rhGH	Recombinant human growth hormone
ROI	Region of interest
s2LJ	Single two leg jump
Scl	Sclersotin
SCOS2	Suppressor of cytokine signalling-2
SD	Standard deviation
SDS	Standard deviation score
SLE	Systemic lupus erythematosis
SNP	Single nucleotide polymorphisms
SOS	Speed of sound
SSI	Stress-strain index
STAT	Signal transducers and activators of transcription
TB-BMC	Total Body BMC
TB-BMD	Total Body BMD
TBW	Total body water
TGF-β	Transforming growth factor-β
THR	Thyroid hormone receptors
TNF-α	Tumour necrosis factors-α
TRAP	Tartrate-resistant acid phosphates
TSH	Thyroid-stimulating hormone
vBMD	Volumetric BMD
VDR	Vitamin-D receptor
WBC	White blood cells
WBV	Whole body vibration

List of Figures

List of Tables

Chapter 1

Introduction

1.1 Bone Structure

1.1.1 Introduction

Bone is a dynamic connective tissue and has several physiological functions. It provides mechanical support of muscle for locomotion and load bearing, protects vital organs such as brain, heart and lungs, plays a central role in controlling mineral homeostasis and supports the haemopoiesis in the bone marrow. Recently, it has been revealed that bone is an endocrine organ that may regulate glucose homeostasis, energy expenditure and testosterone production. This chapter gathers together some recent studies in bone physiology and bone cell biology and discusses the biochemical bone markers and bone growth and development. Skeletal complications in children with leukaemia will also be considered in this review. Furthermore, this chapter will explore bone biomechanics and whole body vibration (WBV) training and their effects on enhancing bone mass and strength. Finally, diagnostic approaches to musculoskeletal system and body composition assessments will be summarised.

1.1.2 Anatomical Structure

Bone has a remarkable variation in shape and size. Therefore, this variety allows human bones to be classified anatomically into three main categories: flat bones such as skull, scapula, mandible and ileum; short bones like foot and hand bones; and long bones (humours, femurs, tibia and fibula). The long bones (Fig.1.1) are composed of three physiologic sections: epiphysis, metaphysis and diaphysis (midshaft). The epiphyses are located at the peripheries, the diaphysis is found in the middle of bones and the metaphysis (developmental growth plate) is situated between the epiphysis and the diaphysis. The epiphysis and the metaphysis are derived from two different ossification centres and also separated from each other by an epiphysial cartilage plate, which is known as a growth plate. This layer plays a pivotal role in the longitudinal or linear growth during the puberty period. This cartilage matrix in the growth plate becomes completely calcified and converted into bone at the end of the growth time. The external layer of the bones is composed of a thick and dense calcified tissue which is known as the cortex (cortical or compact bone) and this type of bone accounts for 80% of adult human skeleton. This layer is mainly found in the centre of the mid-shaft and becomes progressively thinner towards the direction of the

metaphysis. The internal layer of bone is called trabeculae (also named cancellous or spongy bone) and comprises of 20% of total bone. Both trabecular and cortical bone (Tab.1.1) are made from the same bone cells and matrix, but they have different structure and organisation. There are two bone surfaces: periosteum, which covers both the external surface of the compact and cancellous bone, and endosteum, which lines medullary cavity and covers the trabeculae (1).

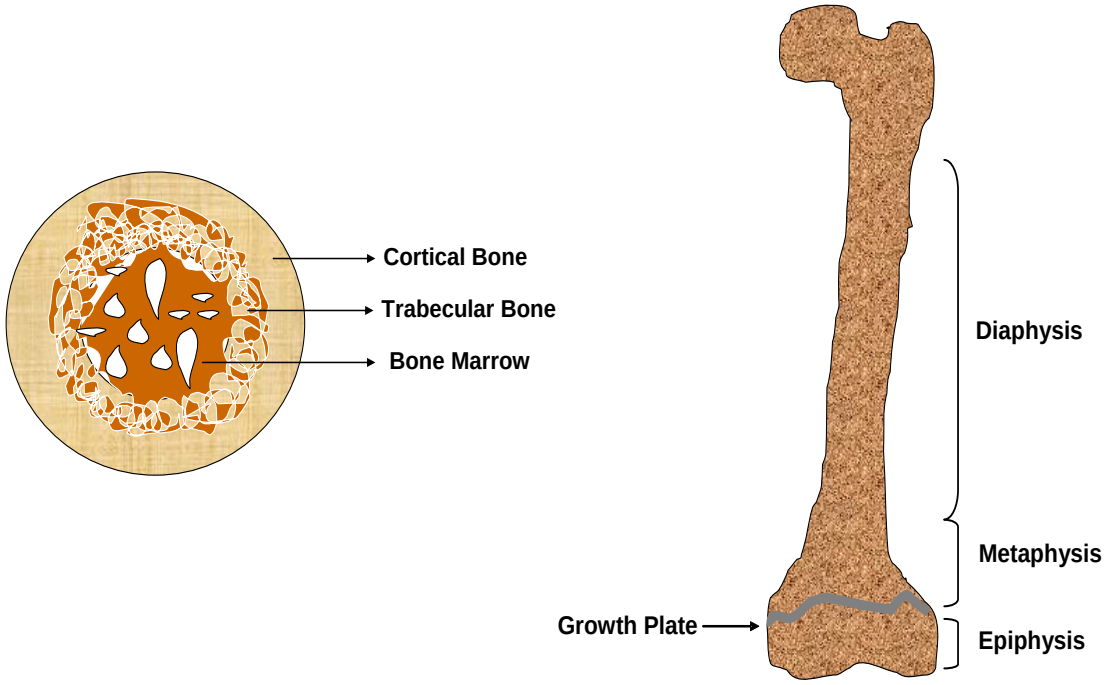


Fig 1.1: Schematic structure of long bone (transverse and longitudinal section), viewing different structures: epiphysis, metaphysic, diaphysis and growth plate.

Characteristic	Cortical bone	Trabecular bone
Calcified Bone	80-90%	15-25%
Porous	10%	50%-90%
Function	Mainly protection	Mainly metabolic

Tab 1.1: The structural and functional differences between cortical and trabecular bones.

1.1.3 Extracellular Matrix

Extracellular matrix (ECM) is very abundant and is made from collagen fibres and non-collagenous proteins (NCP). The organic matrix of bone comprises of about 85% to 90% collagen proteins (90% type I collagen) and the remaining is NCP. Collagens and minerals together play an important function in the biomechanical properties and functional integrity of bone (2). Collagen proteins are organised in a preferential way in order to increase bone toughness and reduce the risk of fracture. The mineral composition is formed mainly by spindle crystals of hydroxyapatite [$Ca_3(PO_4)_2(OH)_2$], which resist compression (Fig.1.2). The latter composition is present in the ground substance, on the collagen fibres and between them. Similarly, the hydroxyapatite is aligned along the fibrils of type 1 collagen. The ground substance is formed from NCPs and consists of glycoproteins and proteoglycans and are characterised by highly anionic complexes which might be responsible for bone mineralisation. As a result of a high ion-binding capacity, the ground substance might have a role in the calcification mechanism and increasing the affinity of hydroxyapatite crystals to the collagen fibre. Some NCPs may play a role in binding the collagen and minerals together (3).

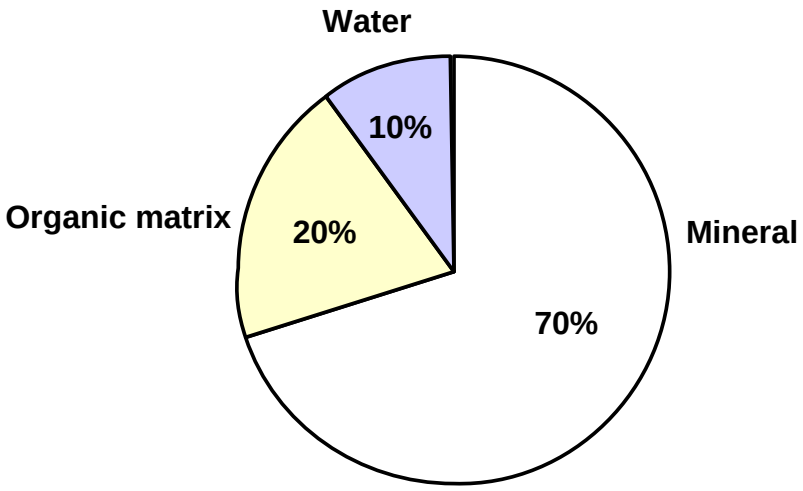


Fig 1.2: Bone composition – around 70% is hydroxyapatite, 20% is organic matrixes, which are comprised of collagen protein (90% type1collagen) and non-collagenous proteins, and the remaining 10% is water.

1.1.4 Collagen Fibres

Collagen proteins are found mainly in skin, tendon and bone. They have the same protein structure and responsible for the structural integrity of the tissues. In bone, type I collagen is the most abundant fibrillar collagen in the ECM. Type I collagen accounts for about 90% of the organic mass of bone; this protein is released from fibroblasts, osteoblasts and odontoblasts in large quantities. Moreover, other collagen proteins such as types III, V and VI are found in loose connective tissues and bone matrix together with type I collagen. Type V collagen comprises about 5% of the organic bone matrix. In bone, type I collagen is incorporated into heterofibrils containing type V collagen. The bone matrix especially type I collagen provides flexibility of bone and is also responsible for its structural orientation. The molecular structure of type I collagen consists of three polypeptide chains, which are twisted around each other and organised in the form of a triple helix composed of two identical α1 chains and one α2 chain. Each polypeptide chain has repetitive Gly-X-Y- repeating triplet (amino acids sequences), with proline and hydroxyproline residues in the X and Y position, respectively (Fig.1.3). A homozygous mutation in a gene that encodes for α1chains is fatal during a prenatal period, whereas, a heterozygous genetic abnormality of one α1 results in osteogenesis imperfecta (OI) type 1A phenotype. The chain α1 and α2 (Tab.1.2) are expressed by COL1A1 and COL1A2 genes respectively (4).

The organisation of type I collagen in parallel array and cross linked telopeptides is formed after removing the procollagens. For instance, the carboxy-terminal propeptide of type I procollagen (PICP) and the amino-terminal propeptide of type I procollagen (PINP) of the procollagen molecule are released during collagen synthesis by specific propeptidases. The indices of type I collagen fibril such as PICP and PINP can be measured in blood and urine and can be involved as bone formation markers. However, because of the peptides are also released from other different tissues such as skin and non-specificity for type I collagen, it reduces the sensitivity of these markers in bone formation (4). The pyridinoline cross-linked telopeptide domain of type I collagen (ICTP) and the cross-linked N-terminal telopeptide of type I collagen (NTX) represent cross linking structures of the collagen type 1 and are released during degradation of the mature type I collagen primarily in bone. Hence, it appears to be a potential marker of bone resorption (5). In metabolic bone diseases, the serum level of these markers increases owing to high levels of bone resorption (6).

The minor fibrillar type V collagen occurs predominantly in tissues as an $\alpha1(V)_2$ α2(V) heterotrimers that is widely expressed in type I collagen fibrils and may regulate the collagen diameter. Type V collagen can also be found in other molecular structures such as α1(V) α2(V) α3(V) heteromers that have been isolated from the placenta and $\alpha1(V)_3$ homomeres (7). The other trace amount of collagen fibres are summarised in the Tab.1.3 below.

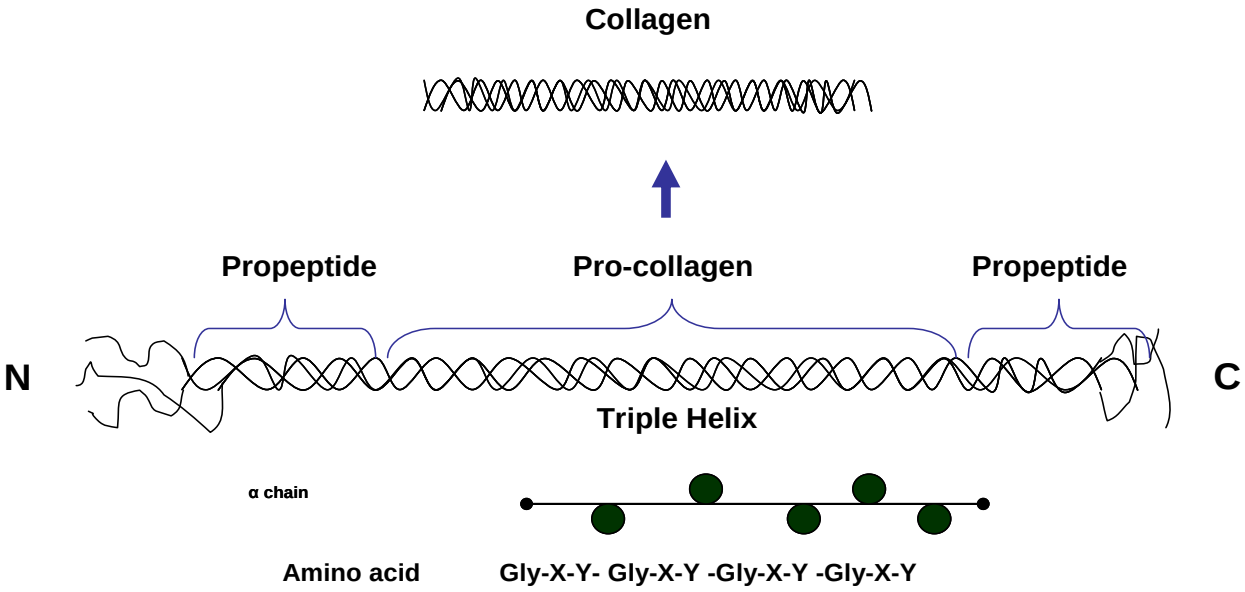


Fig 1.3: The metabolism of type I collagen, procollagen is converted into collagen by removing propeptides. N represents amino-terminal end of the propeptide, C is carboxy-terminal end of the propeptide. It also shows α-chain composition of Gly-X-Y amino.

COL1A1	COL1A2
Chromosome 7	Chromosome 17
α1 chains	α2 chains
51 exons	51 exons

Tab 1.2: The differences between COL1A1 and COL1A2 genes in terms of locations, expression and the numbers of exons.

Protein	Gene	Function
Type III collagen $[\alpha1(III)_3]$	COL3A1, 2q24.3-31	Found in trace amount in bone, May regulate the collagen diameter
Type V collagen $[\alpha1(V)_2\ \alpha2(V)]$ $[\alpha1(V)\ \alpha2(V)\ \alpha3(V)]$	COL5A1, 19q34.2-34.3	It may regulate the collagen diameter
Type X collagen $[\alpha1(X)_3]$	COL10A1, 6q21-22.3	Produced specifically by hypertrophic chondrocytes of the growth plate, but may also be involved in bone mineralisation.

Tab 1.3: The characteristic features of the minor fibrillar collagen protein in bone in terms of the gene location and the function.

1.1.5 Non-Collagenous Fibres

The NCPs account for 10–15% of the total bone proteins. A large amount of NCPs are produced by osteoblasts (endogenous sources), of which the major ones are osteocalcin (OCN), osteonectin (ONN), osteopontin and bone sialoprotein (BSP). Both OPN and BSP are important in the initiation of bone mineralisation process. OCN and ONN may have a role in controlling the size and speed of bone mineralisation process (8). Ninomiya et al. (9) reported that cortical and trabecular bones have different ratios of NCP. For example, OCN is more predominant in cortical bone, whereas ONN is higher in trabecular bone. Approximately, 25% of these proteins are derived from non-bone cells (exogenous sources) and arise from serum-derived proteins and are predominately formed from albumin and α2-HS-glycoprotein. Some of these proteins are not specific to the bone tissue in human beings. NCPs are acidic in nature with high affinity to bone matrix and hydroxyapatite. The remainder of the exogenous sources contain potent growth factors such as transforming growth factor-β (TGF-β), platelet-derived growth factor (PDGF), insulin like growth factor-1 (IGF-1), fibroblast growth factors (FGF) and interleukin-1(IL-1) in trace amounts, which may have a role in bone mineralisation. The local sources (endogenous) are derived from bone cells and can be categorised into three main groups: proteoglycans, glycoslyated proteins and gamma carboxylated proteins (4). Chondroitin sulphate proteoglycans like decorin and biglycan are found in small amount in the ECM of bone. The physiological role of these fractions in the bone tissue is not well defined; however, decorin has high affinity for the bone matrix, particularly type I collagen,

and might have a role in the mineralisation, whereas biglycan tends to be found in the osteoid and may have a function in the early stages of osteogenic processes and bone formation (10).

1.1.6 Bone Minerals

Bone mineral composition, hydroxyapatite [$Ca_3(PO_4)_2(OH)_2$] has a unique mechanical, protective and homeostataic function. This composition can be influenced by age, growth, hormones, diet and health condition. Generally, approximately 70% of adult human bone is formed from minerals. The mechanical rigidity and load bearing strength are dependent on calcium (Ca), hydroxyl-deficient analog of the geologic mineral and hydroxyapatite. These form large geologic crystals, while small crystals are formed from the carbonate, magnesium (Mg) and acid phosphate and no hydroxyl group, enabling them to be more soluble than the large crystals. Moreover, these small crystals act as reservoir for Ca, Mg and phosphate (Pho) ions. Bone mineralisation is initially performed by deposition of minerals in the collagen matrix. As bone tissue matures, the crystals increase in their size and become more organised. Crystal growth (addition of ions to the crystals) and crystal aggregations lead to enlargement in the crystal dimension (4). The chemical mechanism of crystal formation occurs in events, including nucleation, crystal growth and crystal proliferation. The nucleation process results from collision of ions or cluster of ions (Ca, Pho and hydroxide). This step consumes the highest energy for bone crystal formation and then organises ions clusters or the colliding ions in the final structure of crystal lattice, forming a crystal nucleus. After that, crystal growth is formed by adding more ions on the existing stable nuclei. This process requires less energy compared to the nucleation. Finally, the crystal proliferation is established by increasing the number of these nuclei on the surface (11).

Mineral deposition occurs at discrete sites in the collagen matrix and these crystals increase in their size. This process is provoked by ECM vesicles, which are produced by chondrocytes and osteoblasts. These vesicles release Ca and Pho ions and several other enzymes that block degradation of bone mineralisation. ECM vesicles also contain proteins, acidic phospholipids and inorganic phosphate and all of these products play a vital role in apatite formation. The nucleation process may be influenced by macro-molecules such as collagen, osteopontin, BSP and bone acetic glycoprotein-75. These products can bind to Ca in solution

and on the apatite crystal surface. Moreover, bone mineralisation is regulated by phosphorylation and dephosphorylation. For instance, alkaline phosphatase induces phosphorylation and patients with deficiency of this enzyme (hypophosphatasia) have abnormal bone mineralisation mechanism. The crystal growth is also dependent on collagenous and NCP. Mg and strontium are dietary cations and can bind directly to the bone mineral and replacing Ca. This provides bone crystals that are less perfect, smaller and more soluble. Nonetheless, the solubility of crystals can be reduced by fluoride. Bisphosphonate is another compound, reducing bone solubility without affecting the size of crystals. Tetracycline antibiotics chelate Ca and bind to the newly formed bone with high affinity. Therefore, this property can be used as a histological quantitative marker of new bone formation. Several studies have related bone strength to bone architecture and bone mineral density (BMD). Some studies have also reported that the mechanical property of bone is dependent on the distribution and size of mineral crystals. Therefore, if there is a large amount of crystals such as in skeletal flourosis, the bone may become more brittle and more susceptible to fracture (12).

In summary, bone material properties are greatly dependent on the amount of collagen proteins and the degree of mineralisation. This designation results in a proper combination of bone stiffness and toughness.

1.1.7 Normal mineral homeostasis

Normal mineral homeostasis is regulated to maintains serum levels (Ca, Pho, Mg), intracellular levels and also to optimize the mineral content in bone. This regulation is controlled mainly by two hormones, parathyroid hormone (PTH) and vitamin D. This complex mechanism principally occurs at three major target organs, the intestine, kidney and bone.

Ca is the most abundant mineral element in the body. The total adult store of Ca is about 1200gm in the form of hydroxyapatite [(Ca)10(PO4)6(OH)2] in bone which represents approximately 98%. Therefore, the serum Ca level reflects poorly the total body Ca. Serum Ca can be classified into two main types; ionized Ca (physiologically active) which is found in the form of the free ionic fraction and the non-ionized Ca is mainly bound to albumin (90%) or anions such as citrate, bicarbonate and phosphorus. The Ca homeostatic system depends on

several important factors: PTH, vitamin D, Pho, and Mg. A small fall in ionized calcium will quickly lead to a rise in PTH secretion. PTH stimulates osteoclasts to induce bone resorption, increases renal Ca reabsorption and activates vitamin D-25-hydroxylation in the kidney. Ultimately, vitamin D increases the intestinal absorption of calcium and at the kidney increases tubular reabsorption of calcium.

Approximately 85% of the 700gm of Pho in the adult is in the form of hydroxyapatite in the skeleton. Of the remaining 15%, 14% is intracellular, and only 1% is extracellular. The serum Pho level plays a vital role in mineral homeostasis therefore, it is important to maintain the serum levels of Pho between (0.81-1.45mmol/L). When serum Pho levels decrease, there is an increase in the conversion of 25(OH)D to 1,25(OH)2D in the kidney, thereby increasing gastrointestinal Pho absorption. Furthermore, it reduces the urinary excretion of Pho. Generally the relationship between Ca and Pho shows that a rise in serum Pho usually leads to a fall in serum Ca whereas a drop in serum Pho will conversely lead to an increase in the serum Ca.

Mg is the second must abundant intracellular cation, with 67% of total body stores found in bone, 31% intracellular, and only 2% in the extracellular. Alterations of serum Mg within the normal range (0.7-0.85mmol/L) do not appear to affect the concentration of serum Ca. However, a rise in serum Mg may lead to suppression of PTH secretion which in turn may reduce serum Ca. The gastrointestinal absorption of Mg is independent on vitamin D. Mg reabsorption occurs along with Ca by specific Mg transport channels in the distal renal tubule (13).

1.2 Bone Cell Biology

Bone tissue is maintained by cells which are involved continuously in the process of remodelling and modelling to adapt mechanical and physiological demands. These cells include osteoblasts, osteoclasts and osteocytes.

1.2.1 Osteoblasts

Osteoblasts are cuboidal, mononuclear and basophilic cells which are derived from undifferentiated local mesenchymal stem cells (MSC) (bone marrow stem cells or connective tissue stem cells) under stimulation of local growth factors such as FGFs, bone morphogenetic proteins (BMPs) and Wnt proteins. In addition, some transcriptional factors like Runx2, Sox9 and Osterix are also important in the differentiation of osteoblasts. The stem cells differentiate into pro-osteoblasts and then into mature osteoblasts (14). These cells produce bone matrix and are involved in bone mineralisation by releasing collagen, NCPs and alkaline phosphatase. Once they complete matrix production, some of them undergo programmed cell death (apoptosis) and others become lining cells and osteocytes (Fig.1.4), which are embedded in calcified bone and connected with each other by dendritic processes in the canaliculi (15).

The cytoplasmic membranes of the osteoblasts are rich in alkaline phosphatase and have receptors for prostaglandin (PG) and PTH. They also express receptor activator of nuclear factor κβ ligand (RANKL), receptors for oestrogen, vitamin D, integrins and cytokines. Furthermore, they down regulate osteoclastogenesis by secreting cytokines in their membranes, particularly colony-stimulating factor-1 (CSF-1). Osteoblasts (Fig.1.4) also express osteoprotegerin (OPG), which can inhibit osteoclast differentiation by interrupting the RANKL/RANK interaction (16). OPG is a tumour necrosis factors-α (TNF-α) receptor family member and inhibits the final differentiation and activation of osteoclasts by blocking RANKL and by inducing their apoptosis (17). The Wnt signalling pathway in osteoblasts acts through receptors composed of Frizzled and low density lipoprotein related receptors5/6 (LRP5/LRP6). The interaction between these components results in the intracellular accumulation of β-catenin, which in turn stimulates osteoblasts gene expression (18). The Wnt signalling pathway can be inhibited either by Dickopf family members through binding

with LRP5 or by sclersotin (Scl) through competing with LRP5/Frizzled protein complex. Bone alkaline phosphatase and type I collagen products are categorised as earlier markers for bone formation. On the other hand, OCN is released at the terminal stages of bone formation. In conclusion, the main functions of the osteoblasts are; firstly, formation of the ECM, secondly, expression of genes that are involved in bone calcification and stimulation and inhibition of osteoclasts through the interaction between RANK /RANKL and expression of OPG.

Among the many local and systemic factors that control osteoblats, IGF-1 has an anabolic effect on bone formation. IGF releases collagen proteins from osteoblasts and reduce bone matrix degradation by inhibiting collagenase (19). They have a key role in bone turnover and bone growth (20). IGFs combine with a group of six secreted IGFBPs (1-6) with the IGFBP-4 and IGFBP-5 being most abundant in bone. IGFBP4 has the ability to block the action of IGFs and inhibit bone formation. IGFBP-5 is a polypeptide chain and the amino terminal is attached with IGFs. This protein is released during endochondral formation and global knockout of IGFBP-5 in mice is associated with osteoporosis. On the other hand, a recent study has shown that IGFBP-5 inhibits BMP-2 induced osteoblast differentiation and function andblocks bone growth. According to this finding, IGFBP-5 inhibits IGF actions in bone cells (21). Vanderschueren et al. (22;23) demonstrated that androgens increase the rate of periosteal bone formation in males at puberty, whereas oestrogens decrease this rate, but stimulate endosteal bone apposition. Furthermore, oestrogens increase expression of Fas ligand in osteoblasts. Fas ligand pathways induce the apoptosis of pre-osteoclasts (24). There are many factors that promote osteoblast differentiation including BMPs, FGF, PDGF, PTH, PTH, PTHrP, vascular endothelial growth factor and peptides such as activin, inhibin and amylin (25).

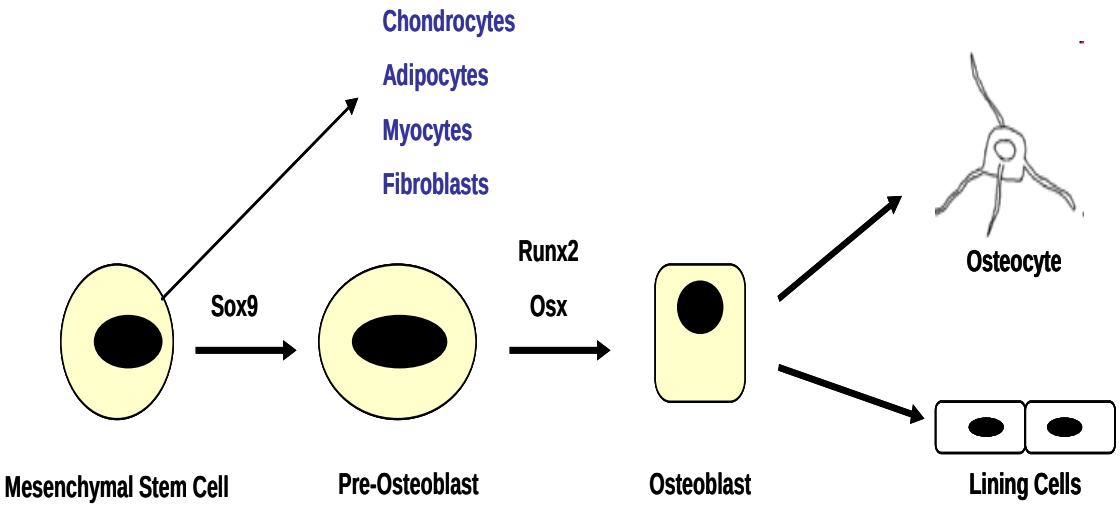


Fig 1.4: Osteoblasts arise from the same pluripotent mesenchymal stem cell with chondroblasts adipocytes, myoblasts and fibroblasts. Under influence of several factors, mesenchymal stem cell will differentiate into the pre-osteoblast, osteoblast, osteocyte and bone-lining cell.

1.2.2 Osteoclasts

Osteoclasts are multinucleated giant cells (4-20 nuclei) present only in bone and they are responsible for bone resorption. Osteoclasts are originated from hematopoietic precursors of the monocyte-macrophage lineage as a result of an interaction with cells of the osteoblastic lineage (26). These cells usually found in bone surfaces and within lacuna. One or two cells only usually appear in the resorptive site. Under light microscope, the nuclei are heterogeneous in shape and size; this may be due to the asynchronous fusion of mononuclear cells. The cytoplasm appears foamy and characterised by a large number of molecules and many stacks of Golgi membranes. The most interesting features are the presence of ruffled borders and clear zones the (sealing zone) (15). The cells are attached to bone matrix by integrin receptors (αv B3, αv B5, α2 B1). These receptors bind to a specific sequence in the matrix proteins and also require a specific molecule in order to provide a proper adhesion to the matrix and cell motility. Differentiation of the osteoclasts requires several transcription factors at different stages. PU-1 and MiTf are required at the early stage. After that, macrophage colony-stimulating factor (M-CSF) proliferates monocytes and ensures expression of the RANK receptor. Under the right stimuli, monocytes and macrophages fuse together to form a pre-fusion osteoclast, an immature osteoclast and eventually a mature osteoclast, so that they begin the process of resorption of bone matrix

(Fig.1.5) (4). Moreover, there are several factors that are involved in osteoclastogenesis at different stages of development, including 1,25-dihydroxyvitamin D3, RANKL/RANK, M-CSF, IL-1 and TNF-α and PTH. Osteoclasts also express many autocrine and paracrine factors that regulate their own activity. A study shows that α9 B1 integrin is produced by osteoclasts at the very low levels and this integrin is a receptor for ADAM8 that play an important role in the later stage of osteoclastogenesis (27). Osteoclasts can express the receptors for PTH, oestrogen and vitamin D (4). Osteoclasts have several specific marker enzymes like tartrate-resistant acid phosphates (TRAP). They have a large number of proton pumps (V-H+ ATPase) and proteolytic enzyme such as matrix metalloproteinase-9 (MMP-9) and cathepsin K in lysosomes. In addition; there are calcitonin receptors in these cells, which are located on the basolateral membranes (15). Osteoclasts release 1-2 protons for each Ca ions in order to stimulate decalcification and degradation of bone matrix. The electrogenic H-ATPase, a highly conductive chloride channel, chloride bicarbonate exchangers, carbonic anhydrase and accessory pumps have a physiological function in the secretion of protons across the ruffled border membrane to dissolve ECM (27).

A balance between the activation and inhibitory factors that control osteoclastogenesis is important in dictating the level of bone resorption. Osteoclastogenesis (Fig.1.5) and osteoclast survival are promoted by RANKL and M-CSF, which are released from osteoblasts or stromal cells. RANK-RANKL interaction can be blocked by the decoy receptor OPG and RANKL expression can be upregulated by cytokines such as TNF-α and IL-1. PTH and GCs increase RANKL and decrease OPG. IL-1, IL6 and PGE2 have a positive impact on the release of RANKL (28). RANKL can also be promoted by 1,25-dihydroxyvitamin D; on the other hand, osteoclastogenesis can be inhibited by TGF-ß and estrogen. Oestrogen induces bone loss by downregulating IL-1, IL-6, M-CSF, RANKL and TNF-α (29;30), thus inhibiting the activation of osteoclast production. Besides proinflammatory cytokines, activated T cells can also secrete a number of the above factors (OPG, RANKL and M-CSF) that are involved in regulating osteoclast activity (31). Thyroid hormone (T3) is essential for osteoclast differentiation but T3-induced osteoclastogenesis is not regulated by RANKL/OPG interaction. T3 can induce osteoclast formation in the absence of osteoblasts and its effects on osteoclasts are likely to be mediated by other mechanisms such as increased expression of c-Fos and Fra-1 (32). The effect of thyroid-stimulating hormone (TSH) on bone is

independent of the circulating T3. The TSH receptor is expressed on the surface of both osteoclasts and osteoblasts. TSH inhibits bone cell differentiation and reduces bone turnover by suppressing LRP-5 and FLK-1 in osteoblast and by down-regulating RANKL in osteoclasts. An absence of TSH receptor signalling results in increased bone turnover in favour of bone resorption rather than bone formation (33).

In conclusion, osteoclasts are involved in bone remodelling and induce bone resorption. A Large number of these steps require cell-cell and cell-matrix interaction (27). Tab.1.4 summarises the differences between osteoblasts and osteoclasts. It is known that osteoclasts are catabolic and destroying bone and osteoblasts are anabolic and forming a new bone, but both together have very important function in bone health. Therefore, maintaining a balance between them has a positive impact on the musculoskeletal system (34).

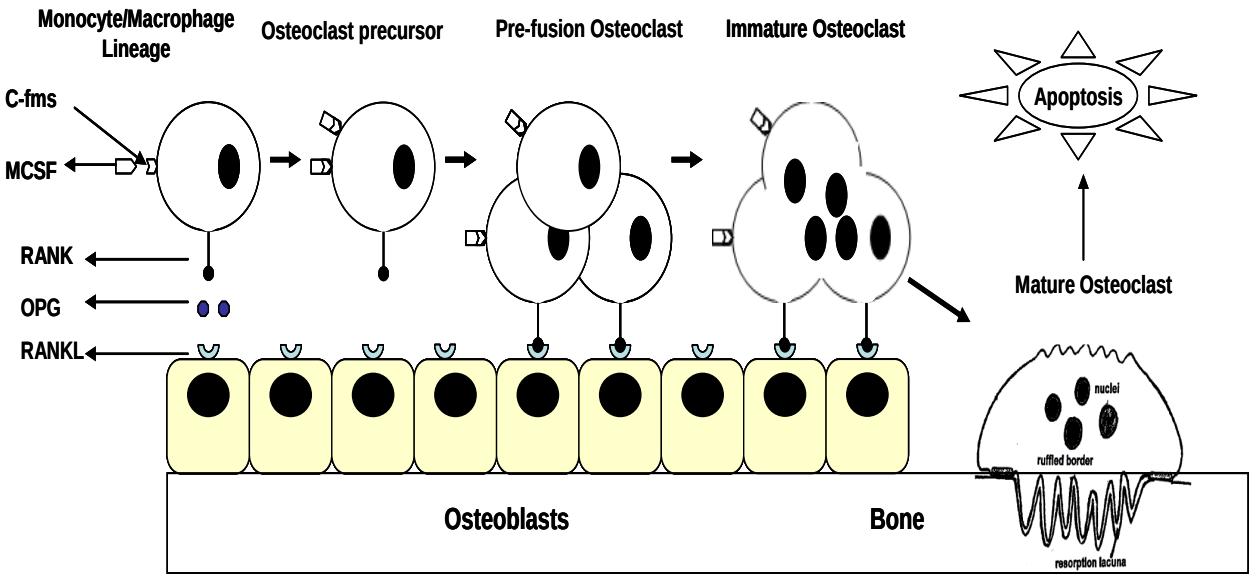


Fig 1.5: Osteoclasts are originated from monocyte/macrophage lineage. Osteoclast precursors are activated by binding MCSF (osteoblast products) and C-fms receptors. RANKL binds to RANK and stimulate the[i] fusion of osteoclasts precursors resulting in the multinucleated immature osteoclast. This finally forms the mature osteoclasts with ruffled membrane borders against the bone surface from which they secrete acid and proteolytic enzymes forming a resorption lacuna.

Characteristics	Osteoblasts	Osteoclasts
Origin	Mesenchymal stem cells (MSC)	Monocyte/macrophage lineage
Structure	Mononuclear, basophilic	Multinucleated, acidophilic
Function	Bone formation	Bone resorption
Production	Bone matrix, collagen and nocollagen proteins	Proteolytic enzymes, MMP-9, cathepsin K, collagenase
Receptors	PTH, PG, vitamin D3, oestrogen, CSF1, RANKL	calcitonin, CSF1, TNFα, PTH, RANK
Fate	Osteocytes, lining cells, Apoptosis	Apoptosis

Tab 1.4: The differences between osteoblasts and osteoclasts cells.

1.2.3 Osteocytes

Osteocytes are the most abundant cells in bone tissue (90%). They are the terminally differentiated cells of the osteoblast lineage (Fig.1.4). They are located within the lacunae and the canaliculi of the lacuno-canalicular network surrounded by bone matrix. The osteocyte cell bodies reside within lacunae from which long actin-rich slender cytoplasmic processes radiate through the canaliculi to connect with the surrounding osteocytes. Osteocytes have several functions including that of Ca sensor, a regulatory function in matrix maturation and mineralisation and mechanosensor (35). Mechanical loading induces fluid flow through the canalicular network leading to a fluid shear stress (FSS) at the cell membrane of osteocytes. FSS stimulates osteocytes to produce osteogenic factors such PGE2, COX and NO. These products play an important role in initiation of bone remodelling process, which depends on osteoclast and osteoblast activity. Recently, van Hove et al. (36) reported that the size of osteocytes is negatively correlated with BMD. The osteocyte size is relatively smaller in a condition associated with high BMD such as osteopetrosis, whereas these cells were relatively large and round in osteopenia and osteoarthritis (low BMD). Therefore, the osteocyte alignment and morphology are likely an important parameter in adaptation of bone to mechanical loading. The smaller cells are more sensitive to mechanical loading than the larger cells. According to the mechanostat theory (37) (Fig.1.6), the mechanical stimulus of bone is divided into four zones depending on different loading environments. Firstly, trivial loading zone, which is characterised by strain magnitude, is below 200μ-strains and the rate

bone resorption exceeds bone formation. If mechanical strain stays below this threshold, then bone remodelling by BMU will be activated in favour of bone resorption (disuse mode) resulting in net bone loss. Therefore, physical inactivity, reduced muscle strength and lack of weight bearing activities may result in osteoporosis (38). Secondly, normal physiological zone (bone remodelling) (200-2000μ-strains) where there is a balance between the rate of bone formation and bone resorption and this zone maintain bone strength. The third zone is overload zone (bone modelling) (2000-3000μ-strains), which induces new bone formation. Lastly, in the pathological overload zone (>4000 μ-strains) bone is subjected to higher risk of fracture. With the recent reports of increased bone fragility and osteoblastic dysfunction in osteocyte-ablated mice, the role of osteocytes in maintaining bone health has become increasingly important. It is found that osteocyte less mice are resistant to bone loss due to unloading, supporting the importance of these cells in mechanotransduction (39). Mechanical stimuli can be translated into intracellular signals by osteocytes through extracellular transmembrane receptors such as integrins and CD44, which stimulate bone remodelling through the production of secondary intracellular messengers including PGE2, cyclooxygenase-2 (COX-2) and NO (40). Proinflammatory cytokines such as TNFα can induce osteocyte apoptosis and in vitro studies suggest that mechanical loading can reduce TNF-α-induced apoptosis in osteocytes (41). On the other hand, mechanical unloading which occurs under conditions of microgravity and a long bed rest result in canaliculi fluid stasis and induce osteocyte apoptosis (41;42). Recently, it was reported that Scl secreted by osteocytes can inhibit bone formation by blocking a Wnt/β-catenin pathway antagonist through binding to LRP5/6. Furthermore, it has been found that hypersclerostinemia associated with immobilised patients may lead to reduced bone formation (43).

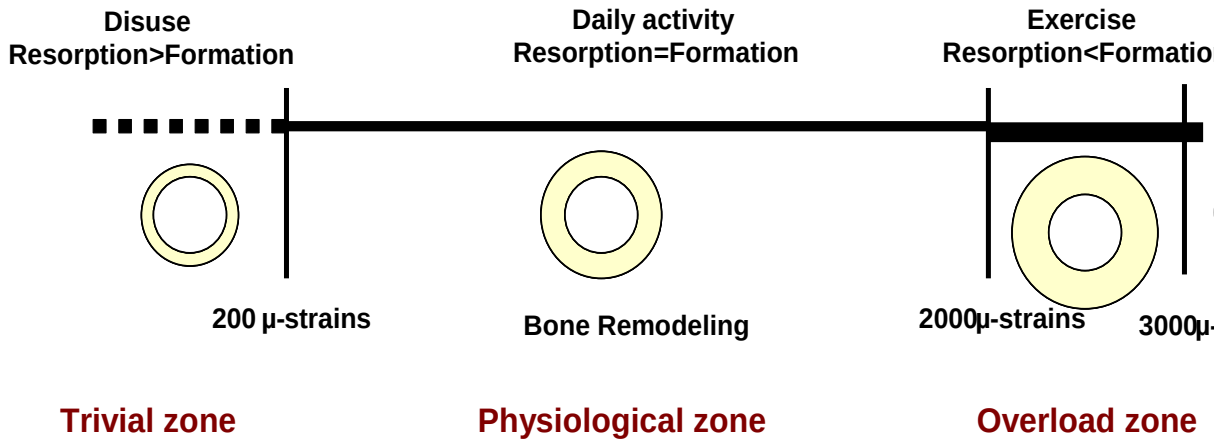


Fig 1.6:Mechanostat theory. The mechanical stimulus of bone to strain magnitudes resulting from different loading environments into four distinct zones: trivial load zone (<200 micro(µ)-strains), physiological loading zone (200-2000µ-strains), overload zone (2000-3000µ-strains) and pathological overload zone (4000µ-strains).

1.2.4 Bone Remodelling

Bone is a dynamic tissue, in which bone formation and bone resorption continue throughout life in response to mechanical and metabolic influences. This process is described as bone remodelling through which bone mass can be regulated. This process is coordinated by bone cells. The interaction of these cells is indicated as the 'Basic Multicellular Unit' (BMU). This indicates that a coupling mechanism must keep a balance between bone formation and bone resorption and no adding bone (44). Bone remodelling is characterised by a balance between the amount of bone resorption and bone formation (coupled) and also plays a vital role in changing material properties (bone renovation) (45). The rate of BMU mechanism occurs at different levels in cortical and trabecular bone. Although cortical bone represents 80% of total volume, the metabolic rate is 10times as high as in trabecular bone because the surface volume ratio is much larger (trabecular bone area represents 60% of the total bone surface). Therefore, approximately 5–10% of total bone is renewed per year. In the third decade of life, bone reaches to the peak bone mass and it is maintained with small variations, up to 50years of life. After that, bone resorption predominates and bone mass begins to decline (46). Bone remodelling is categorised into five phases: resting, activation, resorption, reversal and formation (Fig.1.7). These phases are conducted by complex interaction between osteoblast,

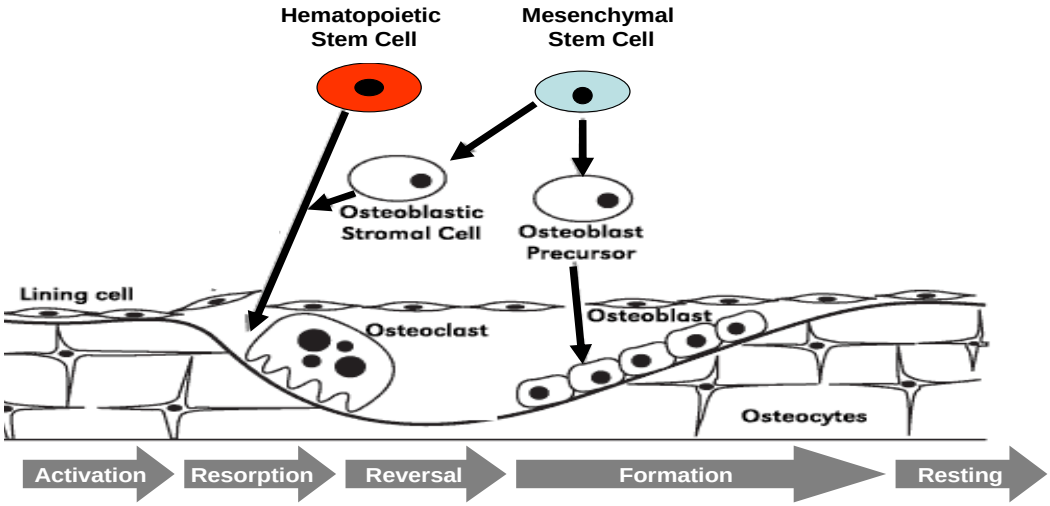
osteocytes and osteoclasts. Bone remodelling begins with activation (activation phase) of osteoclasts at a quiescent bone surface. Signals, which stimulate osteoclasts in these processes, are likely produced by bone deformation (strain) and apoptosis of osteocytes (47). Once osteoclasts are formed, a resorption phase is initiated by acidification and destruction of bone matrices. Subsequently, the osteoblasts appear at the same resorptive site and cover the bone surface (reversal phase). In a formation phase (the longest phase), osteoblasts secrete bone matrix and some of these cells differentiate into osteocyte and bone lining cells and the remainder undergo apoptosis and then transference to resting phase (48). In bone remodelling, osteoblasts and osteoclasts are working very closely in a cooperative manner and forming coupling mechanism (49;50).

1.2.4.1 . Genetic Factors

Genetic factors play an important role in determining the maximum bone mass, since up to 80% of bone mass is genetically determined, whereas the remaining 20% is controlled by environmental factors and sex hormone levels during puberty (51). Several genetic polymorphisms are modulating bone mass in human beings such as vitamin D receptor gene, LRP5 and COLIA1 (51). It has been found that black women have greater mean levels of BMD at all skeletal sites compared with the other ethnic groups such as Asian, black, Hispanic and white females (52)

1.2.4.2 Mechanical Factors

Mechanical loading is essential for the correct development of bone. It is generally agreed that mechanical stimuli are detected by the osteocytes within lacunae and through this mechanism these cells produce ontogenetic factor such as PGs, nitric oxide (NO) and IGF-1, all of which stimulate bone formation (53). Bone remodelling in favour of bone resorption may occur in children with conditions that are associated with impaired mobility, such as cerebral palsy (CP), spinal cord injury, head injury, muscular dystrophy and spinal muscular atrophy (54). Many children will suffer from additional skeletal morbidity (SM) including scoliosis, fixed flexion deformities as well as joint subluxation and dislocation. In children with CP, pathological fractures are commoner at the femoral shaft and supracondylar region and this may be due to abnormalities of growth and presence of contractures in the major joints,



Stimulate Bone Formation	Stimulate Bone Resorption
Genetic Factors (70%)	PTH, GCs
Mechanical Factors	TNF-Alpha
1,25(OH)$_2$ vitamin D3	IL1,6,8,11,7
Endocrine (GH, IGF,	M-CSF
Androgen, Estrogens)	GM-CSF
Local Factors (IGFs, TGF-β, BMPs, FGF, PGE2)	T3

Fig 1.7: Bone remodelling is divided into the following phases: activation, resorption, reversal, formation and resting. In activation phase, osteoclasts are attracted to the resorption site. Then, the osteoclasts start to dissolve bone matrix in the resorption phase. In the reversal phase, osteoblast precursors differentiate into mature osteoblasts and migrate into the resorption area. In the formation phase, osteoblasts start depositing un-mineralised bone matrix in the resorption lacunae and, finally, in the resting phase; osteoblasts terminally differentiate into bone lying cells and osteocytes and then bone at rest. This bone remodelling process is controlled by several factors either in favour of bone formation or bone resorption.

particularly knees and hips (55). In addition, there are several contributory factors including muscle weakness, malnutrition and use of anticonvulsants. In Duchenne muscular dystrophy (DMD), the use of GC as therapy for slowing down the progression of the muscular dystrophy may also contribute to the pathogenesis of secondary osteoporosis in DMD boys. In a longitudinal study of the use of deflazocort in 79 children with DMD who had regular spine X-rays, the incidence of limb fractures was similar in the treated and untreated group (56).

Moreover, vertebral fractures only occurred in 20% of the treated group, no vertebral fractures were observed in the untreated group. Abnormalities in markers of bone turnover and may have lower BMD (57;58). However, lumbar spine BMD (LS-BMD) is often preserved and should not be the sole means of assessing bone fragility (54).

1.2.4.3 Neurovascular Factors

Bone is like any other organ that is innervated by the nervous system and the nerve fibres are either primary afferent sensory or sympathetic fibres and often associated with blood vessels. Nerve fibres are found in the periosteum, bone marrow and mineralised bone, the periosteum achieve close sensory innervations. In cortical bone, nerve fibres run in Haversian and Volkmann canals (59). There are a number of neuropeptides containing receptors on bone cells such as vasoactive intestinal polypeptide, calcitonin gene-related peptide, pituitary adenylate cyclase activating peptides , neuropeptide Y, substance P, as well as classical neuromediators such as noradrenaline, serotonin and glutamate. These substances are important in the bone remodelling process (60). Moreover, vascularisation is essential for bone development, not only supplying blood cells, oxygen, minerals, ions, glucose, hormones and growth factors, but also playing an active role in bone formation and remodelling by mediating the interaction between osteoblasts, osteocytes, osteoclasts and vascular cells at a variety of levels. Studies show that over-expression of hypoxia inducible factor alpha in mouse osteoblasts results in profound increases in angiogenesis and osteogenesis, which are important in endochondral bone formation and bone repair following fracture (61). In the hypertrophic zone of growth plate, vascular invasion is down-regulated by Sox9 and, therefore, inhibition of Sox9 in the hypertrophic zone of the normal growth plate is essential for allowing vascular invasion, bone marrow formation and endochondral ossification (62).

1.2.4.4 Nutritional Factors

Factors believed to influence bone accretion and peak bone mass include maintaining nutritional requirement such as Ca and vitamin D. Adequate Ca intake during childhood and adolescence is necessary to attain peak bone mass, which may play an important role in reducing the risk of bone fractures and osteoporosis later in life. The optimisation of Ca and Pho intake are especially important in adolescence. Peak bone accretion is achieved with an

average of 12.5years and 14years for girls and boys respectively. Approximately 40% of total lifetime bone mass is accumulated during adolescence in 3–4 years of increased bone mass acquisition (63).

1.2.4.5 Glucocorticoids

Glucocorticoids (GCs) have inhibitory effect on osteoblast number, increasing osteoblast and osteocyte apoptosis. These effects lead to suppression in the rate of bone formation (64). GCs have also stimulatory effect on osteoclasts by increasing cell differentiation and recruitment (65). Therefore, GCs induce rapid bone loss and increase the risk for osteoporotic fractures. GCs are commonly prescribed as anti-inflammatory and immunosuppressive agents in the treatment of several diseases such as chronic inflammatory diseases, asthma, cancer therapy, post-transplant and rheumatoid arthritis (66). Nevertheless, the long-term detrimental effects result in glucocorticoid-induced osteoporosis (GIO), which is considered the commonest cause of secondary osteoporosis. The catabolic effect of GCs on bone cells has various mechanisms. Generally, GCs decrease the rate of bone formation by inhibiting osteoblastogenesis and increase bone resorption by stimulating osteoclastogenesis. It has been found that MSC can be shifted away from osteoblasts into adipocytes by induction of GCs. They also inhibit bone matrix produced by osteoblasts as well as induce apoptosis of osteoblasts. Furthermore, the sensitivity of IGF-1 can be suppressed by steroids. Osteoclastogenesis can be promoted indirectly by increasing the expression of RANKL and decreasing the production of OPG, resulting in increased bone resorption (67). In addition, GCs reduce intestinal Ca absorption and increase renal execration rate of Ca (54). According to Wolff's law, bone development is dependent on the muscle forces. It is well known that muscle weakness can also be caused by GCs (67). Therefore, patients treated with GCs particularly during growth show a decline in the rate of bone formation, increase in vertebral compression fractures and suppression in linear growth (54;66).

Approximately 5–10% of children may use GCs at some time during childhood (68). In adults, a rapid loss of BMD is observed in GC therapy particularly in the first year of treatment. Whilst this loss may persist throughout duration of treatment, it may reverse partially on cessation of GCs. A study in children receiving chemotherapy for leukaemia shows an imbalance between

markers of bone formation and bone resorption and reversibility (69). This is particularly marked during periods of high dose GC therapy. However, markers of bone formation remain low even during maintenance chemotherapy when children received relatively low doses of GC (69). Trabecular bone seems to be more sensitive than cortical bone to the catabolic effects of GC (70), but this is not universally reflected in fractures in children; in some conditions such as ALL children receiving GC therapy, appendicular fractures are commoner than fractures of the axial skeleton (71;72). However, a recent report shows that 16% of ALL children had vertebral fractures during the first month of chemotherapy (73). The largest study to evaluate the incidence of fractures among paediatric GC users was a case-control study involving over 37,000 children treated with four or more courses of oral GCs for a mean duration of 6.4 days (74). Compared with controls, GC-treated children had an adjusted odds ratio for fracture of 1.32 (95% confidence interval, 1.03-1.69). Moreover, the risk of fracture may depend on the dose of GC with an incidence of about 2.6% versus 1.6% in the low dose group (75). For the treatment of some conditions such as ALL, dexamethasone is preferred over prednisolone, and our group's preliminary data confirm previous observation of higher bone morbidity in those children receiving dexamethasone compared with prednisolone (71). In comparison with prednisolone, dexamethasone may be almost 10 times more potent at suppressing bone turnover (68). Besides GC, other chemotherapy, abnormal mineral homeostasis and even the disease process itself might affect bony morbidity (72;75). For example, methotrexate induces bone resorption and decreases bone formation, which may be treated with administration of antidote folinic acid (76).

1.2.4.6 Adipocytokines

Bone mass can be controlled by adipocytokines such as leptin and adiponectin. Leptin and adiponectin are polypeptide hormones produced primarily by adipocytes. Leptin is positively correlated with fat mass (FM) and controls body weight, whereas adiponectin is negatively correlated with FM. Leptin controls weight through specific receptors located in the hypothalamus. Adiponectin modulates energy storage and communicates primarily with skeletal muscle and the liver (77). A high bone mass is observed in leptin-deficient and leptin receptor-deficient mice, although they have hypogonadism and hypercortisolism. It shows also that intracerebroventricular infusion of leptin in leptin deficient and wild-type mice increases the rate of bone loss. This study suggests that leptin is a strong inhibitor of bone

formation (78). In contrast, adiponectin increases bone formation by inhibition of osteoclastogenesis – decreasing osteoclast numbers and blocking M-CSF and RANKL molecules – and stimulation of osteoblastogenesis – increasing mRNA expression of alkaline phosphatase and mineralisation activity of osteoblasts (79).

1.2.4.7 Local Factors

Bone remodelling is also regulated by local factors which are divided into two main groups: growth factors and cytokines. Generally, growth factors stimulate bone formation and cytokines stimulate bone resorption (Tab.1.5).

	Growth Factors	**Cytokines**
Bone Formation	BMP-2(+) BMP-4(+) BMP-6(+) BMP-7(+) IGF-I(+) IGF-II(+) TGF-β(+) FGF(+) PDGF(+)	Adiponectin(+)
Bone Resorption	EGF(+) M-CSF(+) GM-CSF(+) PDGF(+) FGF(+)	TNF-α(+) IL-1(+) IL-6(+) IL-8(+) IL-11(+) PGE1(+) PGG2(+) PGI2(+) PGH2(+) Leptin(+)

Tab 1.5: Regulatory local factor in bone remodelling (+) stimulates bone formation and bone resorption. The stimulatory local factors are bone morphogenic protein (BMP), Insulin-like growth factor1 -2 (IGF-1, IGF-2), transforming growth factor (TGF), fibroblast growth factors (FGF) and platelet-derived growth factor (PDGF). The inhibitory local factors are epidermal growth factor (EGF), macrophage colony-stimulating factor (M-CSF), Granulocyte macrophage colony-stimulating factor (GM-CSF), tumour necrosis factors-α (TNF-α), Interleukin (IL-1,6, 8,11) and prostaglandins (PG).

1.2.5 Bone Modelling

The cellular activity of bone modelling is similar to bone remodelling (48). In bone modelling, osteoclasts and osteoblasts are working at the same time, but at different sites or surfaces (Fig.1.5). For example, the osteoclast cells dissolve bone matrices at the endosteal bone surface and the cells osteoblasts form a new bone at the periosteal bone surfaces. Bone formation and bone resorption are uncoupling and the rate of bone formation is higher than resorption. Consequently, bone modelling causes changes in bone shape and size (80). Bone modelling can be described as building up the skeleton.

1.3 Biochemical Markers of Bone Metabolism

Different bone markers reflect different steps in bone formation or bone resorption and enzymatic activity of bone cells. Biochemical markers of bone metabolism are divided into two groups; bone formation markers and bone resorption markers. These makers can be measured either in blood or urine.

1.3.1 Bone Formation Makers

Bone formation markers reflect osteoblast activity; bone-specific alkaline phosphatase (BAP) and OCN are produced by osteoblast. The N-terminal and C-terminal extension peptides of procollagen are released during collagen synthesis.

1.3.1.1 Bone Alkaline Phosphatase

BAP is the most frequently used as a biochemical marker of osteoblastic bone formation due to the wide availability of inexpensive and simple detection methods. The serum level of BAP is highly specific and increases significantly in osteoporotic post-menopausal women. In leukemic children BAP is low throughout treatment, which suggests impaired osteoblast differentiation resulting from a direct effect of chemotherapy on bone (69). BAP is expressed in the early period of osteoblastic differentiation (81) and is produced in high amounts during the cycle of bone formation. Therefore, BAP represents a good indicator of osteoblast activity (82). BAP is a glycoprotein and functions as an ectoenzyme attached to the cell membrane by a hydrophobic glycosyl-phosphatidylinositol anchor. In humans, there are four gene loci encode alkaline phosphatase: placental alkaline phosphatase, germ cell alkaline phosphatase, intestinal alkaline phosphatase and tissue non-specific alkaline phosphatase (liver and bone). These four different genes produce four alkaline phosphatase isoenzymes. Both BAP and liver alkaline phosphatase are glycoproteins encoded by the same tissue nonspecific gene locus and they have the same amino acid sequences and are referred to as isoforms rather than isoenzymes (83). Approximately 95% of the circulating total alkaline phosphatase in humans is derived from bone and liver sources found in serum with a ratio of ~1:1 (84). The four isoforms of BAP can be measured in human serum. These are two major isoforms (B1 and B2) and two minor isoforms (B/I and B1x) present in serum of 60% of patients with severe chronic kidney disease. The B/1 isoforms is mainly found in bone (70%)

and in 30% of cases in intestine. The major isoforms B1 and B2 may provide a clue as to where bone metabolism is active; cortical bone is richer in B1, whereas trabecular bone is richer in B2 (85).

1.3.1.2 Osteocalcin

OCN has low molecular mass (58KDa), 49amino acid peptides synthesised by osteoblasts and is vitamin K and D dependent (86). OCN is expressed in the later period of bone formation (81). Vitamin K stimulates the carboxylation process of OCN at positions 17, 21 and 24 by adding glutamyl residues forming alpha-carboxylglutamyl residues, which in turn leads to increasing the affinity binding to the hydroxyapatite (87). Vitamin D has a direct stimulation on the OCN gene transcription (88). OCN can be found in two forms; the most abundant protein is present as NCP in bone matrix and the newly synthesised form of OCN is found in the circulation. Approximately 10–40% of OCN is released into the circulation and can be used as a reliable marker of bone formation (89). However, OCN is easily subject to degradation in circulation at residues 19–20 and 43–44 producing various sizes of OCN in the circulation (1–19 N-terminal, 20–43-mid-OCN, 43–49 C-terminal, 1–43 mid-N-terminal OCN, 20–49 mid-C-terminal OCN). Some studies have reported that N-terminal OCN (1–19) may be released even during bone turnover (90). The instability of OCN might produce some problems in the specificity and sensitivity of the OCN assays. To overcome this problem, measuring of the N-terminal/mid molecule (1/3 of OCN) is more reliable to assess bone formation (91). Recently, it was shown that in addition to simply acting as a marker of bone formation, OCN plays an important role in regulating blood glucose homeostasis and energy metabolism. The activation of circulating OCN can result from the conversion of carboxylated form into decarboxylated form of OCN in the resorption lacuna in acidic media. The decarboxylated form of OCN stimulates B-cell proliferation and adiponectin in adipocytes. The decarboxylated OCN acts as a hormone favouring β-cell proliferation, insulin secretion, insulin sensitivity and energy expenditure (92;93). Another hormonal action of bone is mediated by an osteoblast-specific secreted molecule. OCN is able to induce testosterone production by stimulating the Leydig cells of testes but does not have an effect on estrogen secretion (94).

1.3.1.3 Procollagen Type I Propeptides

Procollagen type I propeptides are resulted from the degradation of procollagen in the process of type I collagen production. Type I collagen can also be found in other tissues such as skin, dentin, cornea, vessels, fibrocartilage and tendons. In bone, osteoblasts produce primarily the collagen in the structure of pro-procollagen which then will be subject to cleavage at the amino and carboxy terminal releasing these two molecule PINP and PICP, respectively (95). These propeptides circulate in blood, where they are used as markers of bone formation and can be measured by specific immunoassays (96). PICP is a single protein (115kDa), while PINP (70kDa) circulates as three different forms, including an intact trimer, a monomer and several fragments (97). However, the measurements of PINP show more validity in a clinical practice than PICP (98).

1.3.2 Bone Resorption Markers

The majority of bone resorption makers result from degradation products of bone collagen except for tartrate-resistant acid phosphatase. More recently, non-collagenous proteins such as BSP and osteoclast-derived enzymes such as cathepsin K and L have been applied as a marker of bone turnover. Bone resorption markers are divided into three groups: collagen-related markers, NCP and osteoclastic enzymes.

1.3.2.1 Hydroxyproline

Hydroxyproline (OHP) is one index of total collagen degradation, and is formed around 12–14% of the total amino acid content of collagen and produced intracellularly by post-translational hydroxylation of peptide chain. Approximately 90% of OHP is metabolised in the liver, so that only 10–15% appears in urine (99). The urinary OHP is usually considered as an index of bone resorption marker. However, it is noticeable that a major part of urinary OHP is derived from the newly synthesised collagen. Moreover, OHP can be found in other tissues such as skin (100) and derived from other sources such as diet and C1q, which has collagen-like sequences (101). The urinary OHP is, therefore, considered to be poorly correlated with bone resorption rate and has now been largely replaced by more specific and sensitive assays.

1.3.2.2 Urinary Hydroxylysine Glycosides

Hydroxylysine is also an amino-acid used as a marker of bone resorption. Hydroxylysine results from collagen degradation. It is present in two forms: glycosyl-galactosyl-hydroxylysine and galactosyl-hydroxylysine. The glycosyl-galactosyl-hydroxylysine form can be found in skin and C1q component, whereas galactosyl-hydroxylysine is present primarily in bone. It is not recycled and significantly metabolised during collagen turnover, and the levels are not influenced by diet (102). The measurements of urinary hydroxylysine provide more sensitive reflection of collagen breakdown than OHP (103).

1.3.2.3 Urinary Pyridinoline

The urinary excretion of pyridinium cross-links of collagen, pyridinoline and deoxypyridinoline can be used as a marker of bone resorption. Pyridinium, pyridinoline and deoxypyridinoline are non-reducible cross-links that bridge several collagen peptides and mechanically stabilise the collage chain within ECM (104;105). The cross-link components are released into the circulation and the urine due to breakdown of collagen stabilisers during bone resorption (106). These two components have several advantages. Firstly, their measurements are mainly influenced by degradation of mature cross-linked collagen and independent of the degradation of the newly synthesised collagens. Secondly, the urinary execration of pyridinium cross-links is intrinsic as their components are not taken up from food (107). Finally, they are also highly specific for skeletal tissue since pyridinoline is present in cartilage, bone, ligaments and vessels, while deoxypyridinoline is highly specific for bone and dentin. As tissue turnover is much higher in bone than the other tissues containing these proteins, the measurements of pyridinoline and deoxypyridinoline in serum or urine mostly reflect bone resorption (108). Gineyts et al. (75) have published a highly sensitive and specific method for measuring pyridinoline and deoxypyridinoline (109).

1.3.2.4 Cross-linked Telopeptides of Type I Collagen

The cross-linked telopeptides of type I collagen are derived from degradation of specific regions of the type I collagen, called the amino-terminal and the carboxy-terminal telopeptide. ICTP was the first collagen marker measured in serum by a radio-immunoassay (RIA) and can be assessed in serum and urine. In the past the ICTP assay was relatively insensitive for

measuring changes in physiological bone resorption (6). On the other hand, this assay has potentially a clinical application in the conditions that include local destruction of bone tissue such as multiple myeloma (110), metastatic bone disease (111) and rheumatoid arthritis (112). The carboxy terminal and the amino terminal telopeptide can also be assessed by another group of immunoassay, called CTX and NTX assays, respectively. Recently, a new assay was developed to measure serum CTX (ββ-CTX and αα-CTX) using sandwich ELISA. Furthermore, Rosen et al. (113) reported that the serum CTX assay was more sensitive for assessing efficacy of pamidronate treatment than NTX and free deoxypyridinoline (113). NTX has two α (α1,α2) chains in the N-terminal. The NTX assay is detecting the epitope on the α2 chain by using a monoclonal antibody. However, this antibody has several cross reactions with other components such as skin collagen (114).

1.3.2.5 Bone Sialoprotein

BSP is an acidic phosphorylated glycoprotein (MW 70-80KDa) and is produced from different cells – primarily osteoblasts (115) and odontoblasts – and also found in osteoclast-like and malignant cell lines. BSP composes of 5–10% of NCP. The main function of BSP is stimulating the attachment of osteoclasts and osteoblasts as it has a Arg-Gly-Asp integrin recognition sequence (116). This protein has an affinity to α2 chain of collagen and stimulates the nucleation process of hydroxyaptite (117). Therefore, BSP plays an important role in bone cell matrix adhesion processes and mineralisation of ECM. In serum, the large proportion of BSP is bound to factor H, a major regulator of the alternate complement pathway (118). There are several immunoassays developed based on polyclonal antisera to measure the serum BSP. Based on clinical data, it is suggested that serum BSP can be used as an index for assessing bone resorption (119). It is found that serum BSP decreases after administration of bisphosphonate in post-menopausal women (120).

1.3.2.6 Tartrate-Resistant Acid Phosphatase

TRAP has five different isoforms are expressed by different tissues and cells such as prostate, bone, spleen, platelets, RBC and macrophages. However, the two main isoforms of TRAP are known as TRAP5a and TRAP5b with optimal pH of 5.0 and 5.6, respectively. Recently, it was shown that TRAP5b is secreted by osteoclasts, whereas the origin of

TRAP5a is still unknown. Therefore, the measurement of serum TRAP5b by specific immunoassays can be used as a marker of bone resorption. Moreover, Halleen et al. (121) reported that in osteoporotic patients, there was a negative correlation between BMD and TRAP5b, but not with TRAP5a.

1.3.2.7 Cathepsin K

Cathepsin K is the most abundant cysteine protease expressed and secreted by osteoclasts and during active bone resorption. In clinical practice, a mutation in this gene can lead to a condition known as pycnodysostosis (autosomal recessive), which is characterised by high BMD (osteopetrosis) and multiple fractures (122). Serum cathepsin K can be detected by immunoassays and may be a useful and specific biochemical marker of osteoclasts. Recently, a cathepsin K inhibitor (odanacatib) was tried as a new treatment for osteoporosis as it has a sustained suppression of bone resorption (123).

1.3.2.8 Sclerostin

Scl is produced by osteocytes under regulation of mechanical loading. Scl is a Wnt signalling antagonist. Recent data showed that non-ambulatory women have a higher level of serum Scl than control. Moreover, there is a negative correlation between BAP and Scl positive correlation with CTX. Therefore, the evaluation of serum Scl by ELISA can be a useful marker of bone resorption particularly in those who have mechanical unloading (124).

1.4 Bone Development and Growth

Skeletal development is the development of the human skeletal system from the early days of gestational life until the bones have reached their peak of development in late puberty. There are two processes involved in bone growth and development: endochondral ossification (deposition of bone matrix on a pre-existing cartilage matrix, which occurs mainly in short and long bones) and intramembranous ossification (direct mineralisation of bone matrix produced by osteoblasts, which occurs mainly in flat bones).

1.4.1 Endochondral Ossification

Endochondral (Greek word means Endo=within and Chondral=cartilage) ossification takes place within a small piece of cartilage model (125). Bone development is started by formation of mesenchymal condensation, which then results in the initial cartilage model under the direction of local growth factors such as homeobox (hox) genes (126) TGF-β , FGF, BMP (127), Wnt protein, and also requires transcription factors Runx2 and osterix. These MSC condense and then differentiate into chondrocytes, which secrete collagen proteins such as II, IX, XI and aggrecan under the influence of transcription factors including Sox9. Then, the cartilaginous model grows (Fig.1.7) in its size through proliferation of the chondrocytes and through formation of specific cartilage matrix. In the centre of the cartilage model, there will be some changes including that the chondrocytes halt proliferating. Instead of these cells enlarging in their size (chondrocyte hypertrophy) and secrete matrix which are subsequently invaded by capillaries. Additionally, the composition of cartilage matrix is replaced by collagen X. After chondrocyte hypertrophy and cartilage matrix mineralisation, pre-osteoblasts, osteoclasts and blood vessels migrate into this region. The hypertrophic chondrocytes subsequently undergo apoptosis and pre-osteoblasts differentiate into mature osteoblasts, which predominately produce type I collagen. The collagen fibres at this stage are not tightly oriented and form a woven bone. This region is remodelled by invasion more osteoblasts and osteoclasts in order to form mature trabecular bone. All of these processes result in the formation of a primary ossification centre. The primary ossification begins in the shaft, and then proceeds outward from the medullary cavity and inward from the periosteum. Eventually, all cartilages are replaced by bone. The same mechanism occurs at the periphery of bones and forms secondary ossification centres. Once the shaft and epiphysis are ossified, cartilage

growth located between the primary and the secondary ossification centres is known as the growth plate (128).

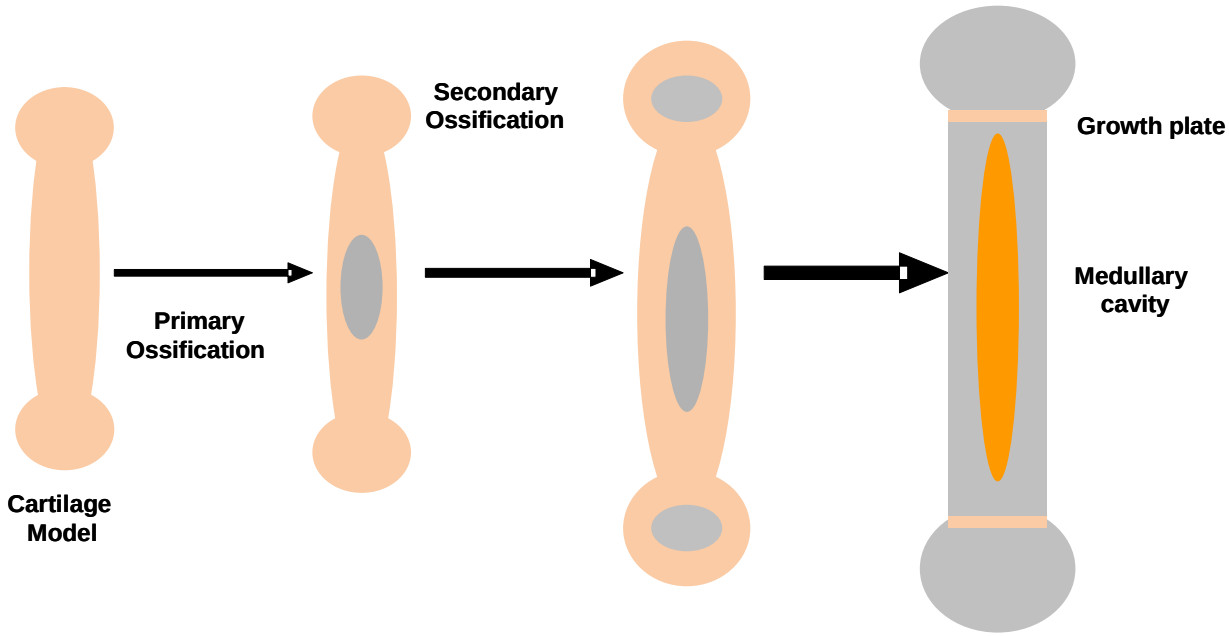


Fig 1.8: Bone development. The schematic diagram shows the serial steps of endocondral bone formation.

1.4.2 Intramembranous Ossification

Intramembranous ossification utilises a direct bone deposition by osteoblasts. Perichondrial cells develop around the chondrocytes and both of these cells regulate each other. The genetic expression of the perichondrial cells is different from the chondrocytes by lacking of Sox9 gene. However, they can produce BMP and parathyroid hormone related peptide (PTHrP) that are of vital importance in the regulation and differentiation of the chondrocytes. They also have a role in differentiation of chondrocytes into pre-osteoblasts and then into mature osteoblasts under the influence of Indian Hedgehogs and other signalling molecules. These osteoblasts synthesise bone matrix (type I collagen) and form intramembranous bone surrounding the cartilage model. The remaining intramembranous bone undergoes remodelling processes by endosteal osteoclastic activity and periosteal osteoblastic activity forming the marrow space and periosteal new bone formation (periosteal calcification)

respectively. Haversian systems are formed in the areas surrounding the infiltrating blood vessels (129).

In conclusion, we can see clearly that bone growth and development utilise both mechanisms. For instance, long bones such as the femur initially form and grow longitudinally by an endochondral ossification, but grow in diameter by the intramembranous ossification.

1.4.3 Longitudinal Bone Growth

Longitudinal bone growth takes place at the growth plate by endochondral ossification. Longitudinal growth depends on both proliferation and hypertrophy of chondrocytes in the growth plate. The rate of longitudinal bone growth is controlled by biomechanical factors and numerous systemic and local growth mediators that interact to regulate the activities of the growth plate chondrocytes. Lengthening of the appendicular bones is dependent on chondrocyte proliferation and differentiation in the epiphyseal growth plate of long bones, forming endochondral bone formation (20). The growth plate is an avascular tissue and is found near the ends of long bones and vertebrae. The chondrocytes in the growth plate are arranged into columns that parallel the longitudinal axis of the bone (130). ECM comprising collagenous and proteoglycan make longitudinal and transverse septae, which separate each column and each chondrocyte respectively (131). The growth plate has four stages of differentiation during growth period: resting zone (zone I), proliferative zone (zone II), hypertrophic zone (zone III) and terminal zone (zone IV) (Fig.1.8). Undifferentiated stem cells (resting zone) differentiate into chondrocytes. Then these chondrocytes in the centre of the growth plate enlarge proliferate (proliferative zone) and differentiate into further hypertrophic chondrocytes (from 6-10 times). The hypertrophic cells (hypertrophic zone) grow in columns in the direction of the long bone axis. However, the chondrocyte proliferation rate becomes very slow on the top of the growth plate and these cells are known as resting zone chondrocytes. The latter may serve as stem cells for the remaining chondrocytes (66;132).

The hypertrophic cells are eliminated by apoptosis and this zone will be invaded by blood vessels and become completely calcified bone tissue. Eventually, the growth plate will be replaced by calcified tissue at the end of the growth period. This process is complex and controlled by a number of systemic and local autocrine/paracrine mechanisms (20).

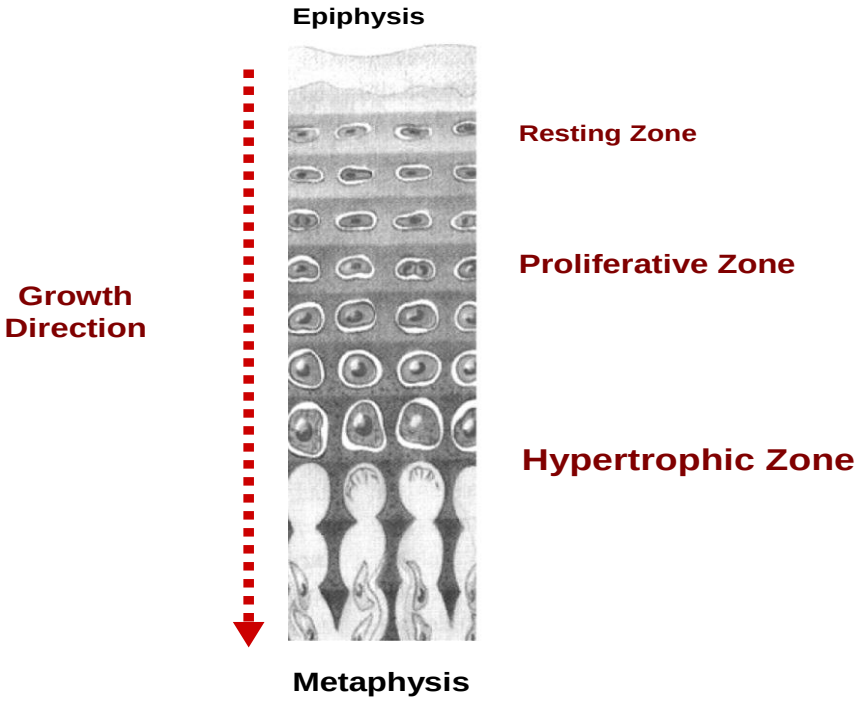


Fig 1.9: The histological structure of the growth plate. It is categorised into three zones: resting, proliferative and hypertrophic (131).

1.4.4 Systemic Control of Growth

1.4.4.1 Growth Hormone

GH is a single chain 191-amino acid polypeptide, secreted in pulsatile and intermittent manner from anterior pituitary gland under the control growth hormone releasing hormone (GHRH) and somatstatin, which are positive and negative regulators respectively. Although several hormones are important for the control of longitudinal growth, GH is considered to be the most important hormone regulating postnatal growth. Therefore, recombinant human GH (rhGH) is widely used to treat conditions that are associated with short stature such as Turner's syndrome and Prader Willi syndrome (133). Abnormal GH response is also prescribed in chronic inflammatory condition that has a negative impact on linear growth (134). A randomised controlled trial (RCT) shows that daily subcutaneous injections rhGH in children (n,22) with Crohn's disease (CD) can improve short-term linear growth (135). GH can also be induced by Ghrelin which has an important role in proliferation and differentiation of osteoblasts (136). GH stimulates growth at the growth plate by increasing cell size rather than

increasing cell number (137). GH receptors (GHR) can be expressed by chondrocytes and osteoblasts. The GHR is a type of the class 1 cytokine receptor super-family and composed of three components (Fig.1.9): an extracellular and a transmembrane and an intracellular domain. GH activates GHR through dimerisation of the extracellular domain. This leads to phosphorylation of the intracellular domain with the tyrosine kinase Janus kinase 2 (JAK2) and, subsequently, induces intracellular the signal transducers and activators of transcription (STAT1, STAT3, STAT5). STAT is the main pathway in the function of GH. STAT5 proteins often have two isoforms such as STAT5a and STAT5b. STAT1 null mice are associated with normal size, while STAT3 null mice are incompatible with life in the early embryonic stages (20). Kofoed et al. (138) reported that human mutation in STAT5b is associated with GH insensitivity and leads to a severe short stature. Interestingly, growth retardation and reduced circulating IGF-1 are found in STAT5b null mice, but not in STAT5a null mice. This might suggest that GH signalling is more effected by the STAT5b isoform (139). The GH signalling can be inactivated by suppressor of cytokine signalling-2 (SCOS2) through blocking STAT5 or inhibiting phosphorylation in JAK2 (140). SCOS2 null mice display an increased longitudinal skeletal growth and wilder growth plates with wilder proliferative and hypertrophic zone. It also shows increased total cross sectional bone area, bone volume and trabecular thickness. The SOCS2 protein expression can be stimulated by TNF-α in the growth plate (141).

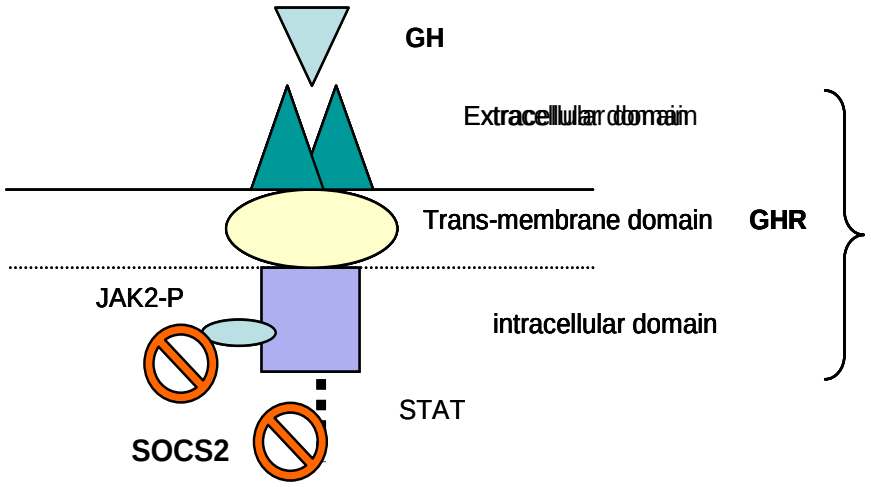


Fig 1.10: This diagram shows the mechanism of growth hormone (GH). GH activates the GH receptor (GHR)-associated tyrosine kinase JAK2 by phosphorylation (JAK2-P). This results in auto-phosphorylation and to phosphorylation of the intracellular domain and stimulates the signal transducers and activators of transcription (STAT). Suppressor of cytokine signalling-2 (SCOS2) is also able to bind the GHR and may, therefore, block STAT5 and JAK2.

1.4.4.2 Insulin-Like Growth Factors

IGF-1 stimulates growth both prenatally and postnatally. IGF-1 is a single polypeptide chain produced mainly from liver under stimulation of GH. IGF-1 promotes longitudinal bone growth by increasing both size of the hypertrophic zone and the chondrocyte proliferation rate during the early stage of bone growth (142). The effect of IGF-1 at the proliferative phase is induced by shortening the time cycle rather than by clonal expansion (143). An in vitro study shows that the IGF-1 signalling pathway plays a vital role in regulating endochondral bone growth independently through the p44/42 mitogen activated protein kinase (Erk1/2) and phosphor-inositide 3-kinase (PI3K) pathways (144). The cellular actions of IGF-1 are activated by a receptor tyrosine kinase (IGF-1R) and this receptor is expressed in the chondrocytes of growth plate. Binding IGF-1 to its receptors in the chondrocytes initiates a number of autophosphorylation reactions. The IGF-1 signalling pathway can be interrupted by different expressions such as TNF-α and IL-1 (145). IGF-1R null mice die shortly after birth and show disorganisation in chondrocyte pattern and abnormalities in vascularisation and mineralisation (146). Furthermore, the GHR/IGF-1R knocked out mice show a greater reduction in bone growth compared with individual gene mutation only in the IGF-1R and missing the GHR. In fact, the functional correlation between the GH and IGF-1 is still unclear. Nevertheless, IGF-1

can stimulate bone growth in the lack of GH. For example, the short stature in Laron syndrome can be improved by IGF-1 only (4).

IGF-1 influences on chondrocyte functions is regulated, in part, by IGF-binding proteins (IGFBP) (147). IGF-1 circulates bound to one of the six IGFBPs (148) and IGFBP-3 predominates in the circulation (90-95%) (149). Mukherjee and Rotwein. (21) demonstrated that IGFBP-4 and IGFBP-5 predominate in bone. IGFBP-4 has ability to block the action of IGFs and inhibits bone formation. On the other hand, the exact function of IGFBP-5 remains controversial. IGFBP-5 is a polypeptide chain and the amino terminal is attached with IGF1. This protein is released during endochondral formation and it deposits in adult bone. Global knockout of IGFBP-5 in mice causes low changes in bone minerals or whole animal physiology. However, its overexpression has a significant detrimental effect on mineralisation. In contrast, other studies show that IGFBP-5 in combination with IGF-1 has both stimulatory and inhibitory effects on bone. However, it has been found that the BMP-2- induced osteoblasts differentiation can be blocked by IGFBP-5 and also suppress longitudinal growth and bone mineralisation in mice. Therefore, this study supports the inhibitory effect of IGFBP-5 (21).

The secretion of GH and IGF-1 decreases with age; the declining rate is much higher in men than women. The underlying mechanism could be related to either central or peripheral causes. The central causes might be due to decrease of the secretion of GHRH and over expression of somatostadin. The peripheral causes might be due to decline of the sex steroid activity and decreasing the physical activity. Therefore, the muscular performance and bone density decrease with age (20).

In conclusion, GH and IGF-1 are considered as major regulators and stimulators of longitudinal bone growth. The effect of GH on growth plates are likely to be mediated predominately by locally produced IGF-1. Therefore, both of these compounds are found in each stage in chondrocyte differentiation, but at a varying degree. They, moreover, have a key role in bone remodelling and modelling processes.

1.4.4.3 Insulin

Overweight and obese children grow faster, have accelerated bone maturation and start puberty earlier compared with normal weight peers. It has been suggested that elevated serum insulin levels in overweight children is responsible for stimulation longitudinal bone growth as they are often insulin-resistant. A recent study in mice has shown insulin increased growth of cultural metatarsal bones and cultured chondrocyte. Furthermore, after normalisation of insulin by pioglitazone, the increased longitudinal bone growth had been abolished, but weight did not change. This finding suggests that insulin level, not obesity, has a direct effect through receptors on skeletal maturation (150).

1.4.4.4 Pro-inflammatory Cytokines

Pro-inflammatory cytokines (IL-1, IL-6 and TNF-α) have an adverse effect on longitudinal bone growth. It has been shown that TNF-α induces an apoptosis in chondrocytes by increasing caspase-3 activity, whereas the cytokine (IL-1) inhibits proteoglycan synthesis by these cells (131). A recent report has shown that there is an improvement in linear growth in children with CD treated with Adalimumab (Anti-TNF-α) (151). A direct effect of cytokines at the level of growth plate might provide an explanation for growth disorders in chronic inflammatory conditions (152). An indirect effect of pro-inflammatory cytokines on growth plate can be meditated through inhibition of anabolic hormones (GH, IGF-1 and sex steroid) (148). Furthermore, there are other multiple factors contributing to poor growth in patients with inflammatory diseases such as poor nutrition and GCs (148;152). IL-6 inhibits the formation of IGF-1/IGFBP-3 and increases the proteolysis of IGFBP-3 and this results in enhanced clearance of IGF-1(148).

1.4.4.5 Glucocorticoids

GCs may impair growth through a negative influence on the GH/IGH-1 axis by a reduction of GH secretion or altering IGF-1 sensitivity (148). At the level of the growth plate, GCs inhibit chondrocyte proliferation (153) and increase in apoptosis of terminal hypertrophic through down regulation of anti-apoptotic proteins (154). IGF-I can also prevent growth impairment resulting from dexamethasone by stimulating chondrocyte proliferation (153). A clear association of growth retardation has been reported with prolonged dose duration (155). In

children treated with GCs, particularly during growth, catch-up growth often follows completion of GCs treatment, but those patients might have reduced final height (54;66).

1.4.4.6 Sex Steroids

Sex steroids are important for the growth and maintenance of both the female and the male skeleton. It is found that estrogen plays an important role in the initiation of the pubertal growth spurt. Therefore, blocking the effect of oestrogen through a gene mutation such as ERα;hERKO man and aromatase P450 (CYP19; converts testosterone to oestrogen in boys) by a drug such as letrozole (aromatase inhibitor) can lead to immature bones, open growth plates and lack of pubertal growth spurt and continuation of linear growth (156). Estrogen hormone has direct and indirect effects on the bone longitudinal growth. The direct effect is influenced by stimulation of the estrogen receptors (ER) in the growth plate. Two different types of ER (ER-ß and ER-α) can be expressed in the growth plate and both of them release gene expression through stimulation of activating protein-1. It is found that ER-α can be expressed in all zones in the growth plate, whereas ER-ß are localised only in the hypertrophic zone. Each receptor is responding differently to estradiol concentration and has a dual effect on growth depending on the level of concentration. A higher concentration of estradiol is required to activate ER-ß compared with ER-α. The highest level of estradiol will occur at the end of puberty, when the growth plate fuses. The presence of ER-ß in the hypertrophic zone and their stimulation under high concentration of estrogens can speculate the significance of ER-ß in the growth plate fusion (156). This concentration of estrogens has also an inhibitory effect on bone growth mediated by preventing gene clonal expansion and halting the proliferating stage in the growth plate. Moreover, high doses of estrogen have an apoptotic effect on the hypertrophic zone and also facilitate osteoblast invasion in the growth plate. Subsequently, these can lead to an epiphyseal fusion. On the other hand, at a lower concentration, estrogen stimulates linear growth through increased secretion of GH and can also proliferate chondrocytes at the proliferative zone. It is reported that males with estrogen deficiency due to either ER gene mutation or a mutation in the aromatase gene result in tall stature syndrome. The growth can be influenced indirectly by estrogen. It is found that ER-ß and ER-α receptors are also expressed in pituitary gland and hypothalamus. Several studies demonstrated that GH levels are higher in prepubertal girls in comparison with prepubertal boys. This higher level could be related to the estrogen hormone (157). In girls, the estrogen

level is positively correlated with tibial length before menarche, but negatively after menarche (158). A recent study has shown that ER-α knockout mice have normal growth and normal sexual maturation during early life. This growth continues beyond the age when growth normally stops. Therefore, ER-α signalling in growth plate plays a vital role in growth cessation (159).

Androgens have direct effects on linear growth via stimulation of all stages in the growth plate through androgen receptors. These receptors have been detected in all zones of the growth plate. The indirect effects can be induced by their aromatisation into oestrogens and interaction with ER-α. Androgens have biphasic action on growth plate similar to oestrogens. At the beginning of puberty, androgens increase endochondral bone formation, whilst at the end of puberty; they stimulate to close growth plate by conversion of testosterone into oestrogen. Osteoblasts-derived OCN can stimulate production of testosterone from testes (94). Moreover, androgens increase tibial length, periosteal bone formation in males at puberty, whereas estrogens decrease this rate, but they stimulate endocortical bone apposition (158;160).

1.4.4.7 Thyroid Hormone

Thyroid hormone is a critical regulator of bone development in humans. It plays a positive role in chondrocytes differentiation from proliferative zone into hypertrophic zone of the growth plate. Animal models found that thyroid hormone receptors (THR) are expressed in the proliferative zone, but not in the hypertrophic zone in the growth plate. The molecular mechanism of thyroid hormone is mediated by activation of Wnt-4/β-catenin signalling pathway. β-catenin promotes terminal differentiation of growth plate chondrocytes (161) (Fig.1.10).

Children with hypothyroidism, thyroid hormone deficiency presented with bone age delay, disorganisation of the normal cartilage columns of the growth plates, impaired differentiation of growth plate chondrocytes into terminal stages and reduced thickness of the growth plates (161). In vitro studies suggested that the mechanism of growth arrest in hypothyroidism caused by disorganisation of chondrocytes in epiphyseal growth plate and also prevent them from differentiating into the hypertrophic zone. Two THR (THR-ß and THR-α1 and α2) have

been detected in the growth plate. However, THR isoforms expressed in all layers of the growth plate chondrocytes except in the hypertrophic zone. THR-α null mice cause complete growth arrest, disorganisation of epiphyseal growth plate chondrocytes and delayed skeletal maturation. On the other hand, THR-ß knockout mice result in no evidence of growth arrest, abnormalities in endochondral bone formation, or disorganisation of growth plate chondrocytes. Robson et al. (162) also reported that the effect of T3 on primary monolayer cultures of rat tibial growth plate increases the rate of cell proliferation rather than the number of chondrocytes. Many investigators have also noted that T3 organises chondrocyte proliferation in columns and plays a pivotal role in terminal differentiation of growth plate chondrocytes into the hypertrophic zone (162). Recently, the expression of THR-α can be activated by leptin and thyroid hormone also increases leptin signalling activity in growth plate cells (163). Thyroid hormone increases production of collagen type X, BAP and proteoglycans in the growth plate cartilage (132).

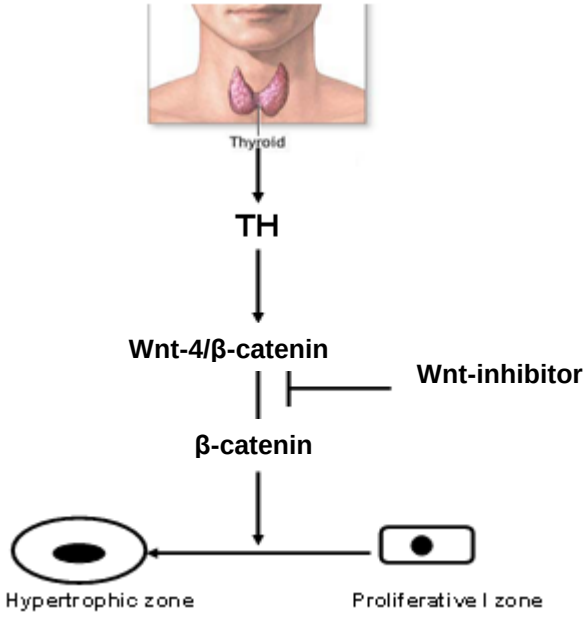


Fig 1.11: Molecular mechanism of thyroid hormone (TH) in the growth plate. TH activates Wnt-4/β-catenin signalling in growth plate chondrocytes. Then, β-catenin signals and promotes terminal differentiation of growth plate chondrocytes from the proliferative zone into the hypertrophic zone.

1.4.5 Local Control

1.4.5.1 Paracrine/Autocrine

The effect of GH on the chondrocytes in the growth plate is still controversial. GH stimulates the systemic production of IGF-1 from the hepatocytes and activates the undifferentiated chondrocytes in the growth plate in order to influence the IGF-1 function (somatomedin hypothesis). This hypothesis has been recently argued; hepatocyte IGF-1 knockout mice alone did not show growth retardation (164). Furthermore, vitro evidence has suggested that GH has a dual effect on the longitudinal growth in the growth plate (dual-effector theory). The finding is that GH stimulates longitudinal growth directly and enhances the local secretion of IGF-1 by stimulating transcription of the IGF-1 gene by autocrine/paracrine mechanisms (137). GH and IGF-1 stimulate the maturation of chondrocytes in growth plates at different levels. Therefore, GH plays an important role in pre-chondrocytes maturation at an early stage, whereas IGF-1 increases cell maturation at a later stage (137). The function and action of both GH and IGF-1 are independent and different. They have a synergistic effect when the two compounds are administered together. In transgenic animal models, it was found that GH but not IGF-1 stimulates the growth up to about two times comparing with their normal littermates (137). Thus, GH can be considered an ignition for growth. Hunziker et al. (143) reported that the final height of the growth plate in hypophysectomised rats, treated with GH and IGF-1, was similar, although IGF-1 took longer periods of time. GH deficiency during childhood is associated with low bone density and replacement restores the BMD at normal level (149). In addition, a mutation of GHR gene in humans (Laron Dwarfism) and animals results in a defect in postnatal growth. Studies show that mice missing GHR have shorter proliferative columns and fewer hypertrophic chondrocytes than normal. Therefore, these mice display a defect in the longitudinal growth of bones. GH has a local effect on the growth plate. It has been found that the longitudinal growth of the tibial bone can be enhanced by injection of GH into its growth plate. This action might be mediated by increased local production of IGF1. Subsequently, it increases chonodrogenesis through a paracrine and autocrine fashion (149).

1.4.5.2 Fibroblast Growth Factors

FGFs are polypeptide proteins, which have a major role in proliferation and differentiation of different cell types in a human body (165). The expression and the function (Tab.1.6) of each individual FGF are variable according to the site of the action (166). FGFs have high affinity to the glycosaminoglycan heparin binding sites on cells. FGF-1 and FGF-2 are acidic and basic in nature respectively, and both are known to play a critical role in growth and differentiation of the musculoskeletal system. FGF-1 induces mainly chondrocyte proliferation. On the other hand, the mitogenic effect of FGF-2 is more potent than FGF-1. The FGFs transmit their signals through a family of four memebrane spanning tyrosine kinases (FGF-R 1-4). Endochondral ossification and intramembranous ossification can be disrupted by a mutation in these receptors. For instance, achondroplasia, hypochondroplasia and thanatophoric dysplasia are associated with dominant missense mutations in the FGFR-3. It has been demonstrated that FGFs have an anabolic role in bone (167). Deletion of FGFR-3 in mice can lead to increase in the longitudinal development compared with the normal, while in humans activation of FGFR-3 due to point mutation at residue 380 amino acids (glycine to arginine) results in short proximal limbs known as achondroplasia (166). On the other hand, Krejci et al. (168) have suggested that the FGFR-3 has an inhibitory response on the chondrocyte proliferation after activation of STAT1 pathway. FGF-23 is an recognised member of the FGF family, and play an important role in phosphate homeostasis and skeletogenesis. A defect in FGF-23 can lead to different diseases such as autosomal dominant hypophosphatemic rickets, oncogenic osteomalacia , X-linked hypophosphatemia (169).

FGF type	Expression
FGF-2	Osteoblasts, periosteum of bone, MSC of skull sutures
FGF (7,8,17, 18)	Perichondrium surrounding the growth plate
FGF (4, 9)	MSC of skull sutures
FGF(18 ,20)	Osteoblasts

Tab 1.6: Fibroblast growth factors (FGFs) and their expression in humans.

1.5 Skeletal Morbidity in Children with Acute Lymphoblastic Leukaemia

ALL is the commonest paediatric cancer representing about one third of all childhood malignancies. As the survival rate of childhood with ALL has improved dramatically (170) and the five-year survival rate is 93.5% (171), the short- and long-term side effects related to both the disease and treatment have gained interest. Among these, skeletal morbidity has been evidenced in a consistent fraction of ALL during chemotherapy, burdened by a significant high rate of skeletal morbidity such as musculoskeletal pain (MSP), fractures and ON (71;72). No clear mechanism has been identified for causing skeletal morbidity in ALL children, but different risk factors, including the disease itself, chemotherapy, abnormal mineral haemostasis, physical inactivity and acquired GH insensitivity could be responsible for these complications in ALL children (172). ALL affects children at an age when peak BMD is being achieved. Bone mass increases rapidly during growth and puberty and reaches its peak in the third decade of life. Children treated for cancer during the period of normal accrual of peak bone mass may be at risk of reduced peak bone mass due to the disease process itself or the treatment received. These skeletal complications have been reported mainly at three different times; at diagnosis, during chemotherapy and following chemotherapy. This review will focus on the many risk factors that affect bone health in children treated for ALL at each period and possible managements (Fig.1.12.

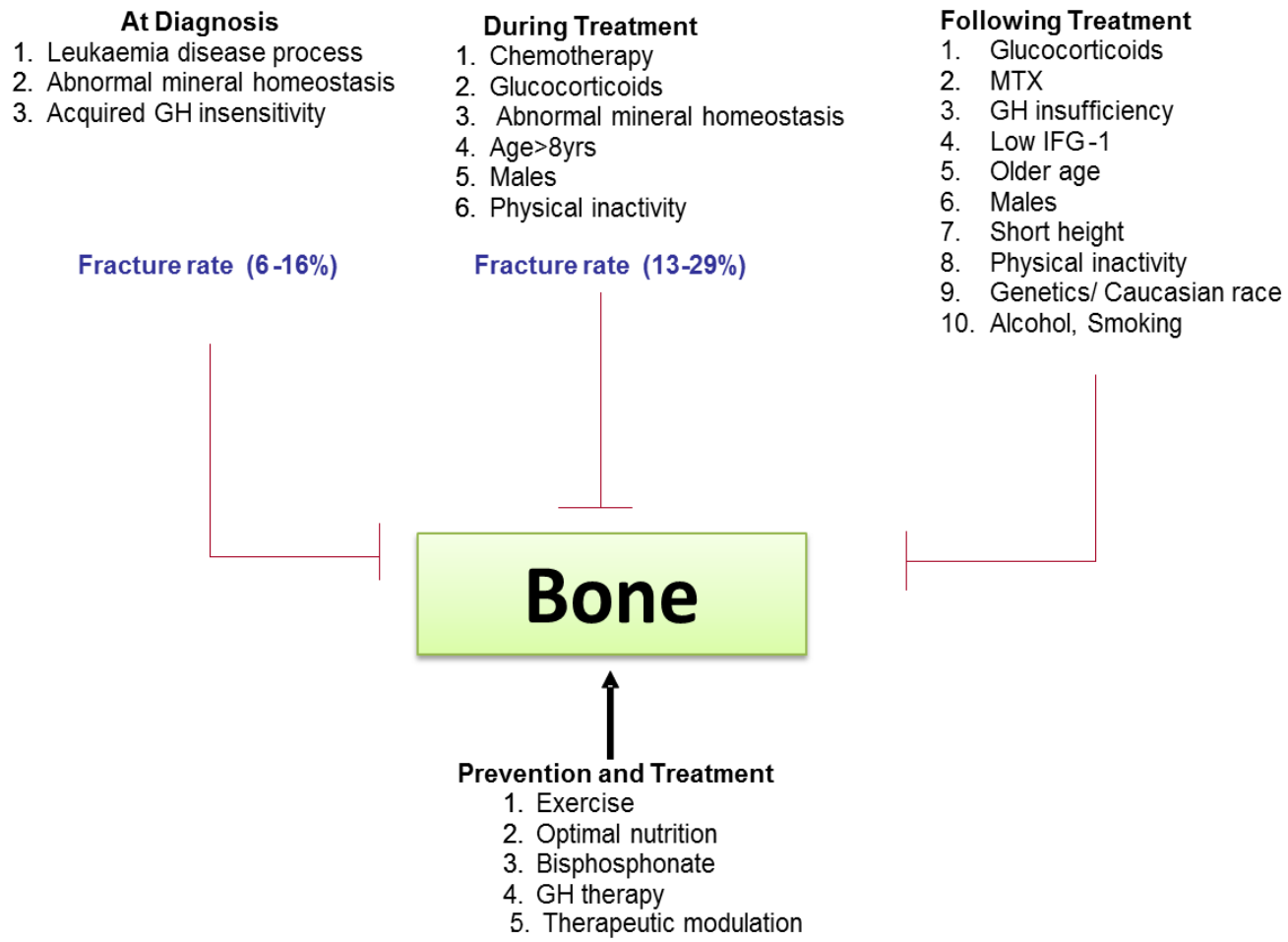


Fig .12: shows Influences on bone in ALL children at three different times – at diagnosis, during treatment and after completion of chemotherapy – and the incidence of fractures at presentation and during treatment. Exercise and optimum nutritional supplement can be used as a preventative method, whereas bisphosphonate, GH therapy and therapeutic modulation can be applied as an interventional option of skeletal morbidity in ALL children.

1.5.1 Types of Skeletal Morbidities

1.5.1.1 Musculoskeletal Pain

MSP in ALL children can be defined a spectrum of skeletal clinical presentation including bone pain, joint swelling, joint tenderness, limping, osteomyelitis and septic arthritis. It is important that radiological images show no evidence of fractures or ON.

1.5.1.2 Fractures

Approximately 10-25% of all paediatric injuries are caused by fractures. It is reported that the incidence of fractures during childhood is higher in boys than girls. Around 80% of fractures are located in the upper extremities, whereas 20% and less than 5% are distributed in lower extremities and appendicular skeleton, respectively (173).

1.5.1.3 Osteonecrosis

ON can be defined as insitu death of bone tissue due to interruption of blood supply. It is frequently reported in children and young adults with ALL. ON can cause serious complications due to its effect on the large joints such as hips and knees which might lead to a long term disability. Although, several joints can be affected at the same time, the weight-bearing joints (hips and knees) are most commonly involved (174). A recent report found that the rate of ON in ALL children aged above 10years is higher by about 5 times in the dexamethasone randomized group than in the prednisolone randomized group (175). Mattano et al (2012) reported that the incidence of ON can be decreased by giving dexamethasone in an alternate week schedule in stead of continuous 3-week schedule within the intensification course (176). Other risk factors of ON including genetics, age over 10yeras, fat cells and drugs such as such as asparaginase might contribute to the risk. Although the pathophysiology of ON is not fully understood, the mechanism can be assumed by Fig.1.13. ON can be diagnosed clinically or/and radiologically (x-ray and MRI). However, ON can be difficult to diagnosis because around 20% of cases have clinical symptoms whereas 70% of asymptomatic children can be only confirmed by MRI. Management of ON is mainly supportive, but few cases can treated surgically including total replacement of the

affected joint. Several trails have shown that ON can be treated with bisphophonates, but their efficacy and long-term complications remains uncertain (177).

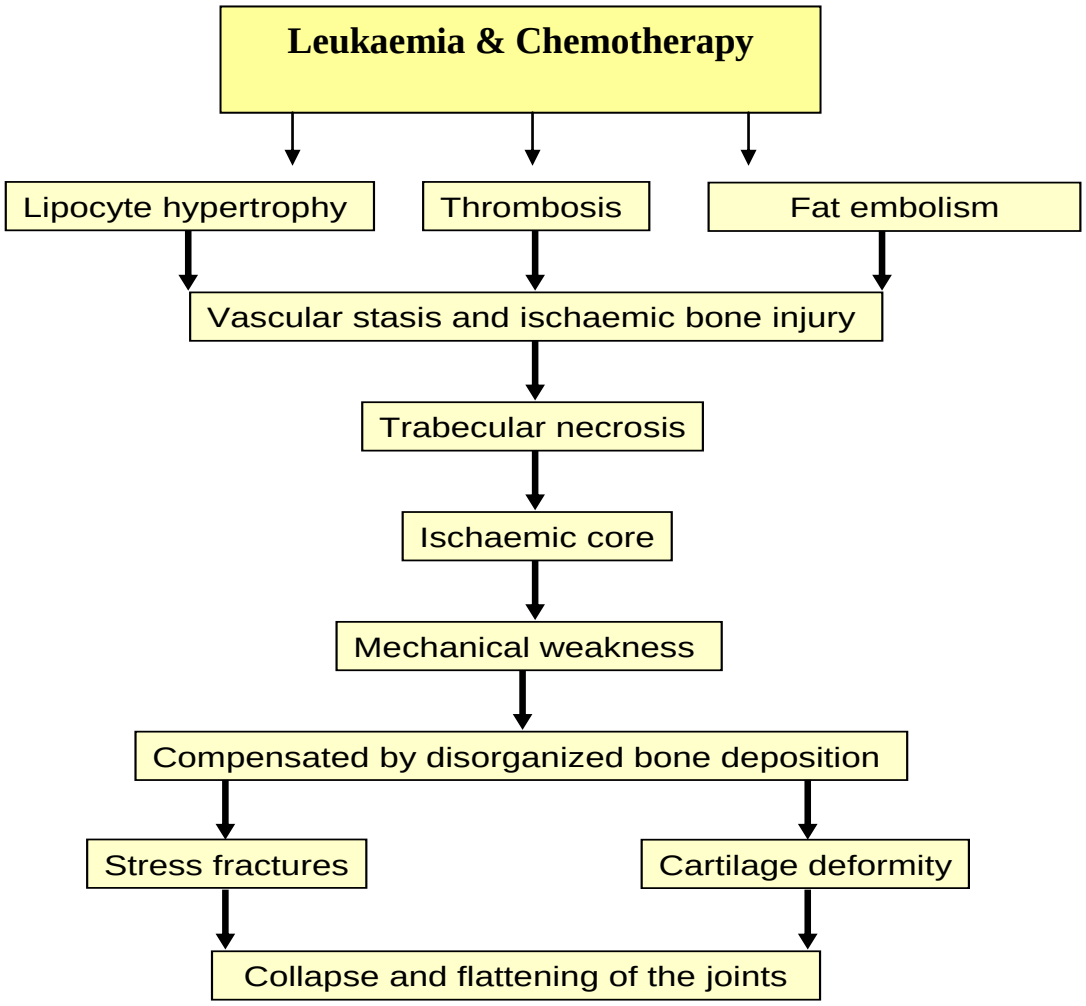


Fig 1.13: The pathogenesis and pathology of osteonecrosis (ON).

1.5.2 Skeletal Morbidity at Diagnosis

Skeletal morbidity is frequently reported as initial symptoms in children with ALL and can reach up to 38%, and the fracture rate ranges from 6–16%. At diagnosis 11% of children with ALL have BMD z score -2.00 or less (178). The major contributory factor of skeletal morbidity at diagnosis might be related to the disease process itself. It has been reported that ALL children are presented with vertebral compression in the first month of chemotherapy and

even before the start of chemotherapy (73;179-181). The abnormal bone metabolism and abnormal mineral haemostasis are considered the two main factors in skeletal morbidity in ALL children at diagnosis (Tab.1.7).

Skeletal Morbidity At Diagnosis					
Study	**Numbers**	**MSP**	**Fractures**	**ON**	**Risk Factors**
Halton et al.1995 (179)	40	36%	10%		
Maman et al .2007 (180)	765	34%			B cell precursor Lower WBC Lower Blast cells
Sinigaglia et al. 2008 (181)	112	38%	6%	-	
Halton, et al. 2009 (73)	186	-	16%		Low LS-BMD Back pain

Tab 1.7: Skeletal morbidity at diagnosis of ALL and their risk factors. Skeletal morbidity includes musculoskeletal pain (MSP), fractures and ON.

1.5.2.1 Bone Metabolism

Leukaemia itself can cause low rate of bone turnover and acquired GH insensitivity (172). At diagnosis, the biochemical markers of bone formation and bone resorption are relatively low compared with the healthy control population (182). Moreover, Halton et al. (179) and Atkinson et al. (183) reported that children with ALL at diagnosis had low OCN level, low plasma 1,25-dihydroxyvitamin D and hypercalciuria indicating an effect of the leukemic process on bone turnover. It is also reported that LS-BMD and bone formation markers are reduced significantly at diagnosis (184). GH resistant state may also occur at the time of diagnosis, as suggested by increasing urinary excretion of GH and decreasing levels of GH binding proteins (GHBPs). In addition, both IGF-1 and IGFBP-3 are also low at the time of diagnosis (182). Bone metabolism may also be negatively affected by several serum factors secreted by leukemic cells such as the osteoblast inhibiting factor and PTHrP. It is reported that ALL children (n,9) aged 2–7years had higher levels of leptin (pro-inflammatory adipocytokines) and lower levels of adiponectin (anti-inflammatory cytokine) at diagnosis. A review published by Davies (172) concluded that a direct infiltration of leukemic cells into bone, which expand in the bone marrow spaces, may lead to damage of spongiosa. Age at

diagnosis (>10years) and low BMD z score in lumbar spine are considered independent risk factor for low BMD z score in lumbar spine and fractures (178). In previous studies, no correlation was observed between leukocyte count at diagnosis and markers of bone formation (172). However, Halton et al. (179) reported that there was a positive correlation between the MSP at the time of diagnosis and CD10-positive leukaemia and leukocyte counts less than $20X10^9$ cells/L. Furthermore, a retrospective analysis in 783 children with ALL shows that musculoskeletal manifestation at the time of diagnosis is more likely to occur among those children with B cell precursor, low peripheral blood blasts and white blood cells (WBC) counts (180).

1.5.2.2 Mineral Homeostasis

Most ALL children have abnormalities mineral homeostasis at diagnosis; therefore, the leukemic cell process might have a major role in this mechanism (179). Atkison et al. (183) showed that over 70% of ALL children at the time of diagnosis had low plasma level of vitamin D.

1.5.3 Skeletal Morbidity during Treatment

A combination of chemotherapy, GCs, abnormalities in mineral homeostasis, physical inactivity and ongoing inflammation may lead to a further compromise in bone health during continuation therapy (172). Fractures during chemotherapy are reported to be six times commoner in these children compared with the healthy population (184). Skeletal morbidities in ALL children are more likely to occur in the peripheral skeleton and in older children (71;72). These complications may lead to further immobilisation and predispose the skeleton to further bone loss and osteoporosis. Furthermore, the type of leukaemia might have an influence on bone health in ALL children, supporting that T-cell type ALL group has lower level of total mean BMD z-score and higher level of ICTP during diagnosis than B-cell type ALL patients (185). Single nucleotide polymorphisms (SNP) have been shown to influence BMD in children with ALL. Haplotypes of the 5'-end of vitamin-D receptor (VDR) are associated with decreased BMD in ALL children (186) (Tab.1.8).

Skeletal Morbidity During Treatment					
Study	**Numbers**	**MSP**	**Fractures**	**ON**	**Risk Factors**
Strauss et al. 2001 (71)	176		28%	7%	Older >9years Males Dexamethasone
Hogler et al.2007 (72)	122	12%	13%	12%	Age >10years
Maman et al .2007 (187)	765	34%			B cell precursor Lower WBC Lower Blast cells
Hartman et al .2009 (188)	51		21%		
Mussa et al. 2010 (189)	44	34%	18%	7%	Low BTT
Winkel et al. 2009 (186)	69		13%		VDR 5'-promoter SNPs
Rayar et al. 2011 (178)	124		18%		Males Age>10years Dexamethasone Low LS-BMD z scores
Cockle et al. 2011 (190)	22		36%		Low BMD

Tab 1.8: The incidence and risk factors of skeletal morbidity that occurred during chemotherapy. Skeletal morbidity includes musculoskeletal pain (MSP), fractures and ON. Bone transmission time (BTT) is dependent on BMD and BTT is measured by QUS. Vitamin-D receptor (VDR) and single nucleotide polymorphism (SNP).

1.5.3.1 Chemotherapy

Chemotherapy nowadays is considered to be more curative in the majority of the ALL children and this success can be attributed to the development of improved chemotherapeutic regimens including the use of potent GCs such as dexamethasone. GCs are becoming an essential therapeutic intervention in childhood ALL protocols. GCs can, however, affect skeletal development through a variety of mechanisms (153). Studies show that there are alterations in bone turnover, short-term growth and bone mass during the course of chemotherapy. These alterations in bone-turnover favoured net bone resorption and more

recent studies revealed that such changes were more likely to occur with dexamethasone than prednisolone even after adjusting for the former drug's greater potency (68). These observations are reflected in clinical reports of skeletal morbidity occurring more often in children receiving dexamethasone (71). Dexamethasone is the only GC used in the UKALL2003 protocol as it shows that dexamethasone has better CNS penetrance and anti-leukaemic effect compared with prednisolone (191). However, children treated with dexamethasone have a greater risk of whole skeletal morbidity (MSP, fractures and ON) compared with those given prednisolone (71). Markedly low bone mass may not be a universal finding in children on chemotherapy (172); however, it seems that it is not the absolute bone mass but the fall in bone mass which is associated with a higher risk of fractures (183). Other drugs that could play a crucial role in adversely affecting bones include methotrexate; this may cause osteopathy and vincristine, which may affect bones indirectly through its mixed sensorimotor neuropathy effects.

1.5.3.2 Bone Metabolism

Serum levels of bone formation markers such as BAP and PICP declined immediately after the administration of chemotherapy (182). Most fractures occurred in the second year of chemotherapy with median duration of 18 months. Males and dexamethasone (71) and physical inactivity have been demonstrated to be independent risk factors for skeletal morbidity and reduced bone mineralisation. There was a significant reduction in BMD z score in ALL children particularly those over 11years of age. This may be because younger children have a greater rate of dexamethasone clearance than older ones (192). The low BMC z score over the six months of chemotherapy and by the end of 24months of chemotherapy can be used as a positive predictive value of 64% and 39% for fracture, respectively (183). A rapid decline in bone properties assessed by QUS was more observed in the first six months of chemotherapy and these problems persist throughout therapy (189). Chemotherapy has also an indirect effect on bone metabolism by suppressing factors that are important for bone maturation such as IGF-1 and IGF-BP3 (182).

Furthermore, bone metabolism during the course of chemotherapy might be affected with the level of adipokines. Surprisingly, it is found that leptin deceased significantly whereas, adiponectin increases during maintenance phase. However, these adipokines gradually

returned to the normal levels during treatment (193). Another small cohort study in ALL (n,14, age ranges 3.4-16.7years) children shows that serum leptin increases during the course of chemotherapy particularly at 12months and 24months after diagnosis (194). The conflict in these results might be related to different study factors such as age, number and protocols. However, it is well known that leptin level is positively correlated with FM (77). The latter study also reported that there was a significant increase in fat mass percent (FM%) by 6,12 and 24months of treatment (194).

1.5.3.3 Abnormal Mineral Homeostasis

Skeletal morbidities in ALL children might be related to the abnormal metabolic basis for Mg, Ca, Pho and vitamin D during the course of chemotherapy. Abnormal mineral homeostasis including hypocalcaemia, hypercalciuria and hypomagnesaemia are reported frequently in children with ALL during treatment (183;195). Atkinson et al. (195) demonstrated that 50% of ALL children had hypomagnesaemia and 56% of those cohort had hypocalcaemia. The subsequent study by the same group found that the incidence of hypomagnesaemia and low vitamin D in ALL children was 84% and 70%, respectively (183). Moreover, the level of vitamin D (≤60nmol/l), reported in the majority (81%) of children with ALL (190). The mechanism for development of abnormal mineral status might be related to a combination of leukaemic disease process and the influence of chemotherapeutic agents. This can be supported by plasma levels of Mg, BAP and OCN, which improved significantly after completion of treatment. High dose of GCs causes an immediate change in bone turnover as reflected by declining plasma OCN and increasing serum Mg significantly. In case of hypermagnesemia, this study hypothesised that release of intracellular Mg into circulation as large numbers of cells are destroyed following steroids. Cyclical steroid therapy and amino glycoside were correlated positively with excessive real loss of Mg. Therefore, the most likely contributing factor for hypomagnesaemia in ALL children was the excessive renal loss of Mg as the nutritional intake and Mg intestinal absorption were normal (183). Moreover, GCs might interfere with intestinal absorption of Ca and increase renal excretion of Ca, which results in secondary hyperparathyroidism. This induces bone turnover in favour of bone resorption.

1.5.3.4 Dietary Factors and Physical Inactivity

ALL children do not meet the recommended daily intake of energy, protein Ca, Pho, iron and folate (195). However, treatment with GCs in children with ALL increases energy intake dramatically, and this effect might cause obesity characteristics among these patients (196). A recent study has shown also that the level of energy intake and physical inactivity in ALL children on dexamethasone was higher than healthy controls. Therefore, a high BMI z score in this group of children might result from using dexamethasone in ALL protocols (197). Furthermore, Hinds et al. (198) showed that dexamethasone therapy during the course of chemotherapy increases the level of fatigue and sleep time. Recently, three candidate gene polymorphisms (AHSG, IL6, POLDIP3) were discovered in paediatric patients receiving treatment for ALL, which are associated with sleep disturbance, but not with fatigue (192). No differences were observed in serum albumin levels, as proxy for nutritional status, between those children who had skeletal morbidity and with no skeletal morbidity (192). The physical activity decreases significantly in paediatrics cancer and more particularly during inpatient stay (199).

1.5.4 Bone Mineral Decrements Following Treatment

It is still controversial whether survivors of childhood ALL maintain low BMD after the end of treatment. Low BMD in survivors of childhood ALL is described in some studies (200-204). ALL are at high risk of impaired bone mass accretion hence the peak age of disease onset corresponds to the period of rapid growth and bone mass accumulation. Therefore, ALL survivors might suffer from low BMD compared with the healthy controls. Kaste et al. (205) showed that around 68% of ALL survivors had low BMD below the age and sex adjusted population mean; the incidence of vertebral compression in ALL survivors is 3.5% and 80% of them are males. Thomas et al. (206) found 24% of long-term ALL survivors had low BMD. A recent study has shown that the impact of chemotherapy in ALL survivors is more profound in LS-BMD than FN-BMD (203). There are several possible etiological factors for decreasing BMD including chemotherapy and GCs, cranial irradiation and genetic and familial causes. Other factors including poor nutritional intake and low level of physical inactivity might influence low BMD in ALL survivors (Tab.1.9). However, there is uncertainty whether survivors of childhood cancer continue to have a low BMD in the long term. A large cohort

study (n=7,414), found no significant increase in the fracture rates among survivors when compared with healthy siblings at median follow up of 23 years (207).

1.5.4.1 Chemotherapy/Glucocorticoids

Chemotherapy has a negative impact on bone health in ALL children survivors; ALL children who received higher doses of methotrexate and 6-Mercaptopurine (6-MP) have lower BMD z scores (205). Furthermore, high dose of methotrexate is associated with low BMD in lumbar spine (182;208). These findings are supported by a recent study, which reported that children who received chemotherapy with no CNS irradiation in their protocol have a slight reduction in LS-BMD and apparently normal FN-BMD (203). This might be explained by the high sensitivity of trabecular bone to the chemotherapy in the spine compared with the hips. On the other hand, Mandel et al. (187) showed that even FN-BMD are affected in those children who received high dose of methotrexate and GCs compared with age matched normal controls (187). Indeed, the negative effect of GCs on bone metabolism is well recognised (182).

1.5.4.2 Cranial Irradiation

Cranial irradiation is considered to have a detrimental effect on BMD and bone growth in ALL survivors (187). This could be explained by disruption in hypothalamic pituitary axis, which leads to GHD. Some data show that cranial radiation exposure of >24gray(Gy) is associated with low BMD (206). This effect is dose dependent (205). However, within the “modern era” of leukaemia therapy, the CNS irradiation dose has shown a minimum effect on BMD as the incidence of GH dysfunction in those children who received CNS irradiation dropped significantly (203). Decreased dose and frequency of cranial irradiation in ALL protocols might explain the lack of effect of CNS irradiation on BMD compared with the previous study (187). Recently, Tonorezos et al. (209) reported that a history of cranial irradiation particularly among ALL female survivors was associated with higher leptin per kilogram fat mass. Moreover, female long-term survivors of childhood ALL who were treated without cranial radiation have a higher leptin level compared to controls (210).

Following Treatment					
Study	**Number**	**BMD**	**Bone Scan**	**Age at Study**	**Risk Factors**
Kaste et al. 2001 (205)	141	58% (Low BMD)	QCT	10-30years	Male sex, Caucasian race Cranial irradiation, High doses of antimetabolites
Tillmann et al. 2002 (208)	28	Reduced LS-BMD	DXA		Males, Low physical activity (iv) high dose of methotrexate
Mandel et al. 2004 (187)	106	Reduced FN-BMD	DXA	8-30years	High dose of methotrexate, High dose of GC Protocol C
Brennan et al. 2005 (211)	53	Reduced Radial BMD Normal TB-BMD Normal LS-BMD	p.QCT DXA	6-17years	
Kaste et al.2006 (212)	57	59.6% (Low BMD)	QCT	14-35years	Older age at diagnosis, Nutrition, Alcohol
Thomas et al. 2008 (206)	74	24% (Low BMD)	DXA	23-37years	Males, Short height, GH insufficiency Low IFG-1 Z score, Smoking
Rai et al. 2009 (213)	424	31%(Low BMD)	QCT DXA	9-40years	
Polgreen et al. 2012 (214)	319	Low BMC z score	DXA	9-18yeras	Physical inactivity, Hypogonadism, Lower LM, Higher IL-6
Aldhafiri et al. 2012 (215)	51	12% BMC z score <-1.0 8% BMC z score <-2.0	DXA	9-17years	

Tab 1.9: Bone mineral density (BMD) in survivors of childhood acute lymphoblastic leukaemia. The table also shows bone assessments (DXA and QCT), age range at the study time and risk factors for BMD deficits. Total body BMD (TB-BMD), Lumbar spine BMD (LS-BMD), Femoral neck BMD (FN-BMD).

1.5.4.3 Genetic/Familial

Generally, bone health is highly influenced by genetic and ethnic factors. Many genetic polymorphisms are thought to contribute BMD (216). For example, six different missense mutations of the LRP5 gene might lead to the osteopetrosis (217), whereas inactivating LRP5 gene mutations cause the osteoporosis-pseudoglioma syndrome (218). Furthermore, LRP5 gene polymorphisms might have an effect on vBMD during childhood, possibly through effects on trabecular bone formation (219). Bone density may be influenced by genetic polymorphism of the corticotrophin-releasing hormone receptor-1 (CRHR1) gene in survivors of ALL children (220). ALL children who are carriers for polymorphism of the VDR at 5'-end (Cdx-2/GATA) haplotype 3 have lower BMD than non-carriers (186). Caucasian race is another independent factor associated with low BMD in ALL survivors (212).

1.5.4.4 Gender and Age

Some studies have reported that males of ALL survivors are at higher risk of developing low BMD than females (205;208;212;220). This could be explained due to a delay in puberty progression in boys which might lead to a delay in the skeletal maturation. Furthermore, the skeletal response to oestrogens on bone accretion is greater than androgen. Another cause might be related to a longer duration of chemotherapy in boys (3years) than girls (2years) in UKALL2003. Furthermore, the toxic effect of cyclophosphamide on gonads is higher in boys than girls, which might also lead to a further delay in puberty and cause adverse effects on bone mineral accretion. Oral contraceptive pills in mature ALL survivors might also have a positive impact on bone health in females (205). Although males have higher incidence of low BMD than females, shorter females are at high risk of developing low BMD than shorter males (206). Older age at diagnosis is another independent factor associated with unfavourable BMD. Children diagnosed after 3.5years of age had lower BMD compared with those diagnosed before (208). Further studies are needed to reveal the responsible ethological factors for skeletal morbidities covered by these independent factors.

1.5.4.5 Physical Inactivity

Decreased physical activity has a negative impact on bone health (221). There are conflicting data as to whether physical activity is reduced (208;212) or not (222) in ALL survivors. Tillmann et al. (208) reported that the levels of physical activity in childhood ALL survivors are

lower than the control group, and females seem to be more at risk (223). Decreased physical activity may be due to increased weight or obesity in ALL survivors particularly those younger than 19years (224). On the other hand, a study has shown that no differences in the level of physical activity between the survivors of childhood ALL and healthy control (222).

1.5.4.6 Nutritional Factors

Poor nutritional intake, reduced vitamin D level and excessive alcohol consumption may have an impact on bone development in ALL survivors. Around one third of ALL survivors are not receiving the recommended dietary intake of Ca, vitamin D, Mg and potassium (224). However, most American children and adolescents did not meet recommended dietary intake of Ca (221). Furthermore, around 9% and 61% of the US healthy adolescent population have vitamin D deficiency (<15ng/dl) and insufficiency (15-29ng/dl) respectively (225). These results are quite similar to ALL survivors with the prevalence of vitamin deficiency at 11.5% and vitamin D insufficiency at 52% (226). Excessive alcohol consumption has been correlated with low BMD in ALL survivors (212).

1.5.5 Management

Attention to improve bone health in ALL children should occur at each follow up evaluation including primarily optimum nutritional intake, and regular exercise. Bisphosphonates, GH therapy and calcitonin can be alternatively beneficial in cases where fractures have already occurred (227). Management of skeletal morbidity in ALL children can be categorised into two groups; preventative measures such as optimum nutrition and regular exercise and therapeutic options such as bisphosphonates.

1.5.5.1 Prevention

1.5.5.1.1 Nutrition

Adequate nutritional supplementation during the course of chemotherapy may be beneficial as a high intake of dairy products during childhood and adolescence is positively related to bone accretion at maturity (228). However, the optimum effect of Ca intake on bone mass accrual is synergistic with a good level of physical activity (229;230). Vitamin D supplementation has improvement in BMD for a period of 6 months in patients with

secondary osteoporosis due to CP (231). Administration of 1,25$(OH)_2$ vitamin D in ALL children during the first year of chemotherapy is able to increase LS-BMD (232). Mg supplementation may also ameliorate skeletal morbidities in ALL children as some trails show that Mg is able to increase the rate of bone formation (233;234). Lifestyle modification such as avoiding alcohol, caffeine and smoking play an important role in improving BMD particularly in ALL survivors. Therefore, adequate nutritional supply should be encouraged among all family members during the course of chemotherapy.

1.5.5.1.2 Physical Activity

The effect of physical activity to improve bone density and reduce BMD deficiency has been proved in multiple studies (235;236). Exercise regimens that increase muscle bulk or increase mechanical loading on the skeleton may prove beneficial for skeletal health (237). Hartman et al. (188) suggest that performing exercises at home is associated with relatively poor adherence in children with ALL. On the other hand, a recent study that recruited newly diagnosed children with ALL (n,9) concluded that an in-patient and home exercise programme during early therapy are well tolerated by ALL children and their parents (238). In young adult ALL survivors a simple, inexpensive and safe home based exercise programme improves the fitness level and decreases FM% (223). Mechanical loading can be achieved by a fixed regimen of weight bearing exercises or with the help of a WBV platform. These vibration stimuli may have beneficial effects on muscle function (239) and bone mineral density (240-242) and endocrine hormonal profile (243). A number of vibrating platforms are now available and some report a beneficial effect on bone mass in children with CP and young women (244;245). They may be as effective as weight bearing exercise, but have the advantage of shortening the time required for exercise by delivering the mechanical loading over a shorter period. It is also possible that they may have added beneficial effects on body composition.

1.5.5.2 Therapeutic Option

1.5.5.2.1 . Bisphosphonate

Recently, interventions that may improve bone health have included the use of bisphosphonate therapy, but this has only been used in those children with ALL, who suffer fractures during chemotherapy (246). Bisphosphonate particularly pamidronate can be used as therapeutic option for MSP score and ON (247). A recent study has shown that BMD has improved in children with secondary osteoporosis treated with short-term zoledronic acid (248). However, there is no clear guidance for the optimal treatment regimens in terms of choice of drug, route of administration, dosing schedule and treatment duration in those children who were diagnosed with secondary osteoporosis. Moreover, a number of different doses and preparations have been used till now and it is unclear whether they have a differential effect on functional outcomes such as pain and fractures (249). Treatment with bisphosphonates cause specific radiological features characterized by several horizontal lines of high density of cortical bone at the metaphysis of the distal radius and ulnar. Fortunately, these findings do not need further investigations when the patients presented at casualty department (250). Bisphosphonates therapy in children may initially be associated with an acute reaction such as fever, muscle pain, headache and vomiting and hypocalcaemia when administered intravenously. Some animal trials have reported that high dose of bisphosphonates has a negative impact on growth, but this has not been observed in children. Bisphosphonates should not be used during pregnancy and all women of reproductive age should have a pregnancy test because of concerns about teratogenicity which has not yet been confirmed in humans (251). Skeletal complications such as osteonecrosis (ON) of jaws have been described in adults but not children or adolescents (252). Administration of zoledronic acid in rats during tooth development might lead to several types of dental problems such as prevention of tooth eruption (253). Furthermore, another study in children suggested association between pamidronate infusion treatment and prolonged QT interval (254). This complication can lead to syncopes, severe arrhythmia and even sudden death. Whilst a safe upper limit for bisphosphonate therapy has not been established, the adverse effect of greatest concern in children is over-suppression of bone modelling and remodelling. Latrogenic osteopetrosis and pathologic fractures have been

described in a child treated for over two years with a relatively high dose of pamidronate (255).

Using bisphosphonates as prophylaxis has not been considered in this population, possibly because of concerns about violation of the chemotherapy protocols. Furthermore, risks, benefits and long-term outcome of bisphosphonate use in this population should be addressed in a larger prospective, randomised trial.

1.5.5.2.2 Growth Hormone Therapy

GHD mostly occurs after cranial irradiation, and in ALL survivors may lead to low bone mass. A recent study has shown that GH therapy for two years in ALL survivors (age range 13-21years) increases total body BMD (TB-BMD) and LM and decreases FM% (256). On the other hand, five-year therapy with GH in ALL survivors (age range 19-32years) with GHD shows no beneficial effect on BMD (257). These conflicting results can be explained by a variety of factors such as duration of treatment and age related to GH therapy response.

1.5.5.2.3 Therapeutic Modulation

Prophylactic cranial irradiation can be replaced by intrathecal and systemic chemotherapy in a standard chemotherapy in order to radiation-associated adverse effects such as second cancers, cognitive deficits and endocrinopathy. Previous clinical trials in paediatrics assessed the possibility of omitting the prophylactic cranial irradiation completely from treatment (258;259). Recently, Pui et al. (171) have concluded that prophylactic cranial irradiation could be totally omitted without compromising overall survival. GCs in treatment protocols could be replaced by a selective GC receptor modulator, AL-438 as it has similar anti-inflammatory efficacy and less side effects on bone (260).

1.6 Exercise as a Therapeutic Application of the Mechanostat Theory

1.6.1 Mechanostat Theory

Bone is a dynamic tissue that adapts to its loading environment. In all mammals, birds and reptiles of any size, age and sex, healthy load-bearing bones have more strength than are required in order to provide a protection from non-traumatic fractures. According to Wolff's law, bone mass and bone shape (geometry) are dynamically remodelled in order to meet the functional demands of the mechanical loading environment. Mechanostat theory is a theoretical mechanism by which load bearing bones maintain shape and strength in response to muscle strain and mechanical usage (50). Frost (49) proposed that bone modelling and remodelling can be variably altered by bone strain within specific ranges. The maximal mechanical forces induce bone modelling by activation of osteoblasts and osteoclasts on different bone surfaces and repair bone architectures in remodelling by formation and resorption on the same bone surface, and these processes are influenced by a number of other hormonal and environmental modulators (261). On the other hand, bone unloading can cause disuse osteoporosis leading to fractures and deterioration of body function (262). Therefore, mechanical loading stimulates bone formation through mechanotransduction, whereas unloading induces bone resorption by increasing in the levels of osteoclastic activities.

There are some important terminologies of bone biomechanics which need first to be defined in order to understand the mechanostat such as stress and strain. Stress can be defined as force per unit area and can be classified into three different types: tension stress, compression stress and shear stress. Tension stress is developed when bone material is compressed and becomes longer, whereas in the compression stress, the applied force on bone area is in the opposite direction of the tension stress and bone material becomes shorter. Shear stress is developed when the direction of the parallel applied force is not in the same direction. The formula for stress is (Force (N)/area(m^2); therefore, the stress unit is Pascal (N/m^2). Strain is defined as the percentage change in bone length (bone deformation). For example, if a bone length is stretched or compressed to 1% of its original length, it has strain of 0.001(1000μ-strain) or 1% deformation. The formula for strain is (elongated bone-original bone length/original bone length) (263).

The mechanostat initiates (Fig.1.14) bone modelling and remodelling mechanisms according to the level of mechanical strain on bone. Micro-damage fractures in bones can result from repeated bone strains. These small damages can be recognised and repaired normally particularly when strains remain below the micro-damage threshold (MDT). However, strains above MDT can lead to pathological fractures as a result of an ineffective repair mechanism and an accumulation of micro-damages (50). This suggests that in case of impairment of bone material properties (osteoporosis) can be compensated by improving bone mass when the mechanical bone strain level exceeds the ‘‘modelling’’ threshold (Fig1.16). Moreover, it was concluded that oestrogen receptors play an important role in the osteoblasts’ adaptive response to mechanical strain. Therefore, the changes in BMD at the hips in premenopausal women with high impact vertical jump exercise were greater in premenopausal women compared with postmenopausal women (264).

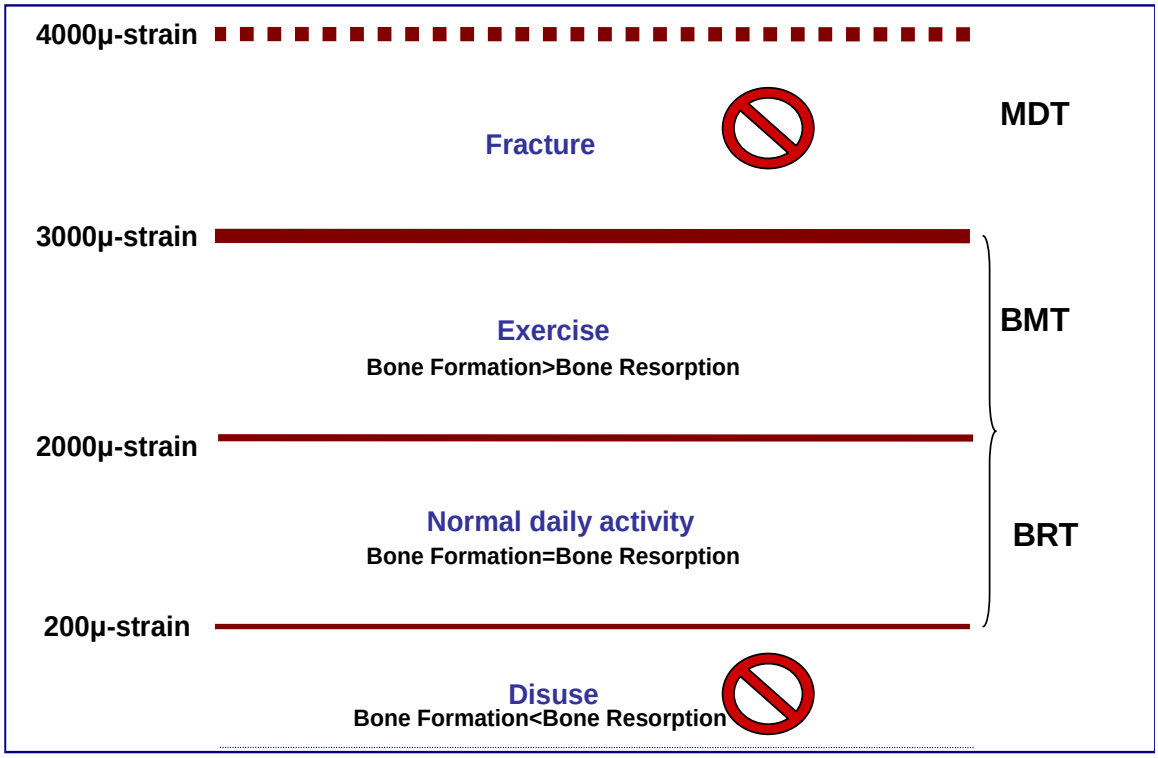


Fig 1.14: The mechanostat means the bone strength and mass are stimulated by bone strain from mechanical load. The mechanostat tries to keep mechanical strains between bone remodelling threshold (BRT) and bone modelling threshold (BMT) in order to prevent spontaneous fractures. Disuse and mild overload activate remodelling and modelling respectively. However, fractures can occur when the mechanical strain exceeds micro-damage threshold (MDT). BM stands for bone mass. This diagram demonstrates the relationship between bone mass and bone strains. Bones adapt their mass and structure in response to the demand of mechanical loading. Bone mass and strength increase when the bone strain levels exceed the bone modelling threshold BMT and vice versa. The thickness of the lines reflects bone mass.

1.6.2 Mechanotransduction in Bone

Mechanotransduction is defined as the conversion of mechanical stimuli into intracellular signals. Mechanotransduction in bone has four stages: mechanocoupling, biochemical coupling, the sensor of the effector cell (osteocytes) and the effector cell response (osteoblasts and osteoclasts) (265). These processes play an important role in keeping the dynamic balance between bone formation and bone resorption. The mechanotransduction is dependent on the integrin family and actin cytoskeleton (Fig.1.15). The integrins are heterodimeric transmembrane proteins, comprising of α- and β-subunits (266). The integrins are cell-substrate adhesion proteins that initiate intracellular signalling and may serve as mechanosensor receptor in bone (267). The integrins are transmembrane receptors that physically connect ECM at one end and the actin cytoskeleton within osteocytes to the intracellular signalling molecules (265). Mechanically induced bone formation in mice can be reduced significantly by deletion of the β-subunit in cortical osteocyte of ulna (268). CD44 (non-integrin) are also extracellular transmembrane receptors, which are attached the ECM to the cytoskeleton and are located in the osteocyte membrane (40). These receptors modulate the mechanotransduction through their further attachments to the extracellular membrane as well as the actin cytoskeleton. Mechanical loading applied to bone leads to flow of intracanalicular fluid surrounding the osteocyte. This mechanical force causes the deformation of the osteocytes membranes. Such bone cell deformation can stimulate intracellular biochemical responses. These signals produced by the molecule will be transmitted through an extensive network of dendritic processes which connect all osteocytes. Mechanotransduction induces new bone formation proceeded by expression of the transcription factor cFos and PG. Furthermore, FFS can induce cyclooxygenase-2 (COX-2) and NO (266). These molecules are an important biochemical mediator of mechanical loading in bone (265). The intracellular Ca^{2+}, inositol triphosphate (IP3) or cyclic adenosine monophosphate (c-AMP) are involved in the intracellular signals transduction pathways. It has been found that PG mainly PGE2 is secreted in bone tissue and cells during stress and stimulates new bone formation by promoting both proliferation and differentiation of osteoblastogenesis. Blocking COX2 or PGs in rats significantly reduces mechanically induced bone formation (269). NO may be involved as the osteoclast cell inhibitors during bone remodelling induced by mechanical loading (53). Furthermore, animal studies have shown that the production of NO is linearly proportional to the loading rate of mechanical stimuli. The mechanosensitivity of the osteocytes is increased by differentiation of osteoblasts to osteocytes. It has been shown that bone cells response to FSS is dependent mainly on the

frequency of mechanical loading rather than strain rate. Therefore, the osteogenic effect of high frequency, low magnitude mechanical stimuli might be similar to the osteogenic response to high impact activity (41;53). Recently, Rumney et al. (270) showed that increasing amounts of mechanical loading placed up osteoblasts (SaOS-2) stimulates ATP release and c-fos expression suggesting that these molecules are important for bone remodelling. The Wnt/β-catenin signalling pathway through LRP5 receptor has a complex role in skeletal mechanotransduction. Activation of this pathway leads to accumulation of intracellular β-catenin where it stimulates gene transcription (18). A reduction in the Wnt signalling level is likely to play a role in end-stage osteoblast differentiation. It has been found that stretching in human osteoblasts for 15min is able to down-regulate the Wnt signalling pathway and to up-regulate β-catenin levels (271). Furthermore, the Wnt/β-catenin signalling pathway in osteocytes can be activated by pulsating fluid flow at FSS of 0.7 ± 0.3 Pascal at 5 Hz. Conversely, this pathway can be blocked by adding focal kinase inhibitor FAK inhibitor, phosphatidyl inositol-3 kinase inhibitor and NO synthase inhibitor (272).

Several factors can have an effect on mechanotransduction process in bone. These factors are type and frequency of mechanical loading, age and gender. In fact, cyclical and intermittent mechanical loading, which gives the skeleton time off periods, is more effective than continuous mechanical loading in inducing the bone formation (273). Mechanotransduction is declined by aging; the rate of bone formation in nine months rats is 18times that in 19months rats (274). Furthermore, gender affects bone mechanotransduction as the bone loss in male rats is higher than female rats when they are exposed to hind limb unloading for a period of two weeks (275).

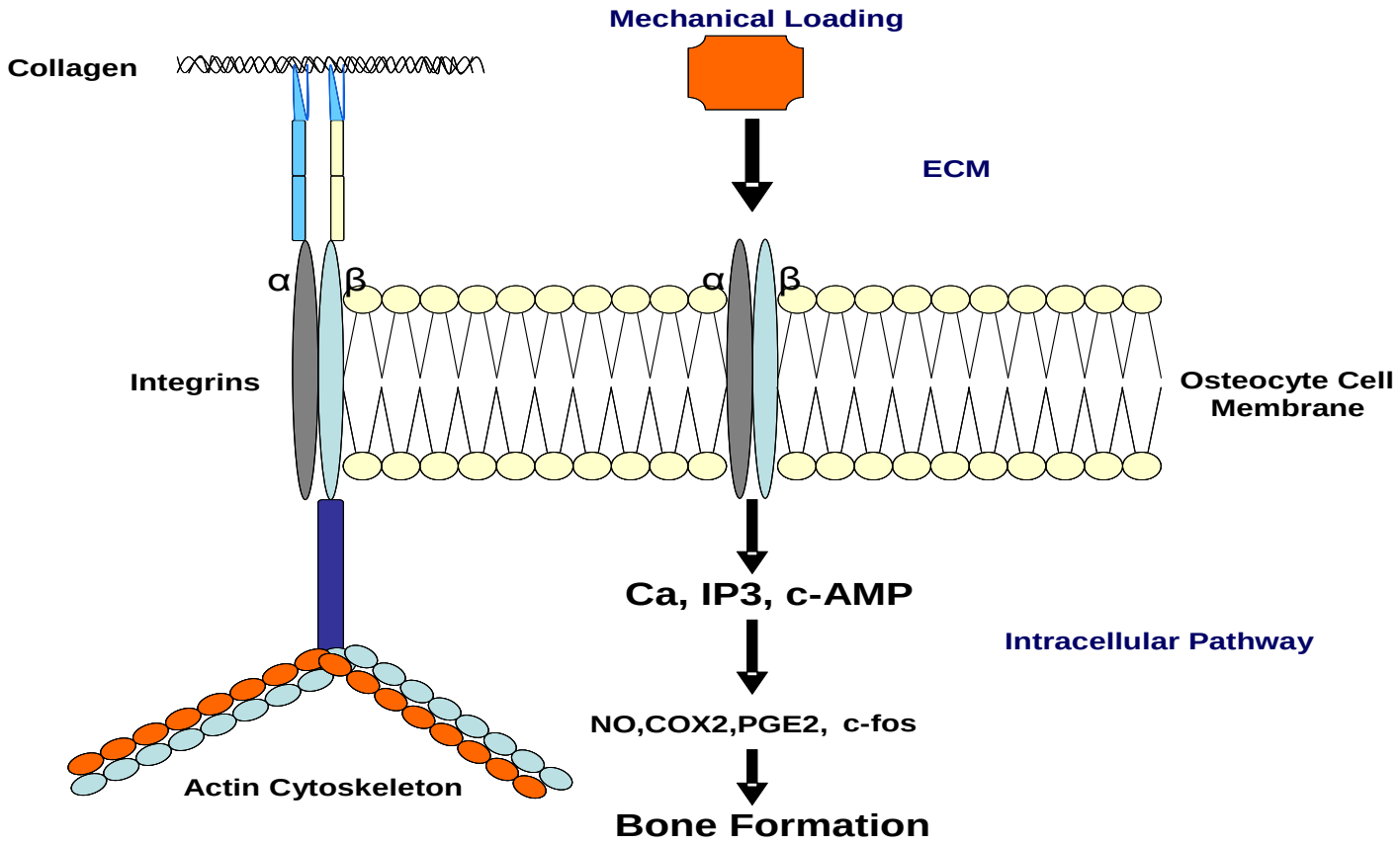


Fig 1.15: Integrins are heterodimeric transmembrane proteins, comprised of α- and β-subunits. Integrins connect extracellular matrix (ECM) at one end and the actin cytoskeleton within osteocytes to the intracellular signalling molecules.The mechanotransduction consists of two compartments: extracellular signal pathway and intracellular signal pathway. Mechanical stresses are distributed through the ECM and sensed by the receptors (intigrin α- and β-subunits) that are located on the osteocyte membrane. Then, the transducer molecules up regulate the production of NO, PGE2 ,c-Fos, and COX-2 . These products stimulate bone formation.

1.6.3 Fluid Shear Stress

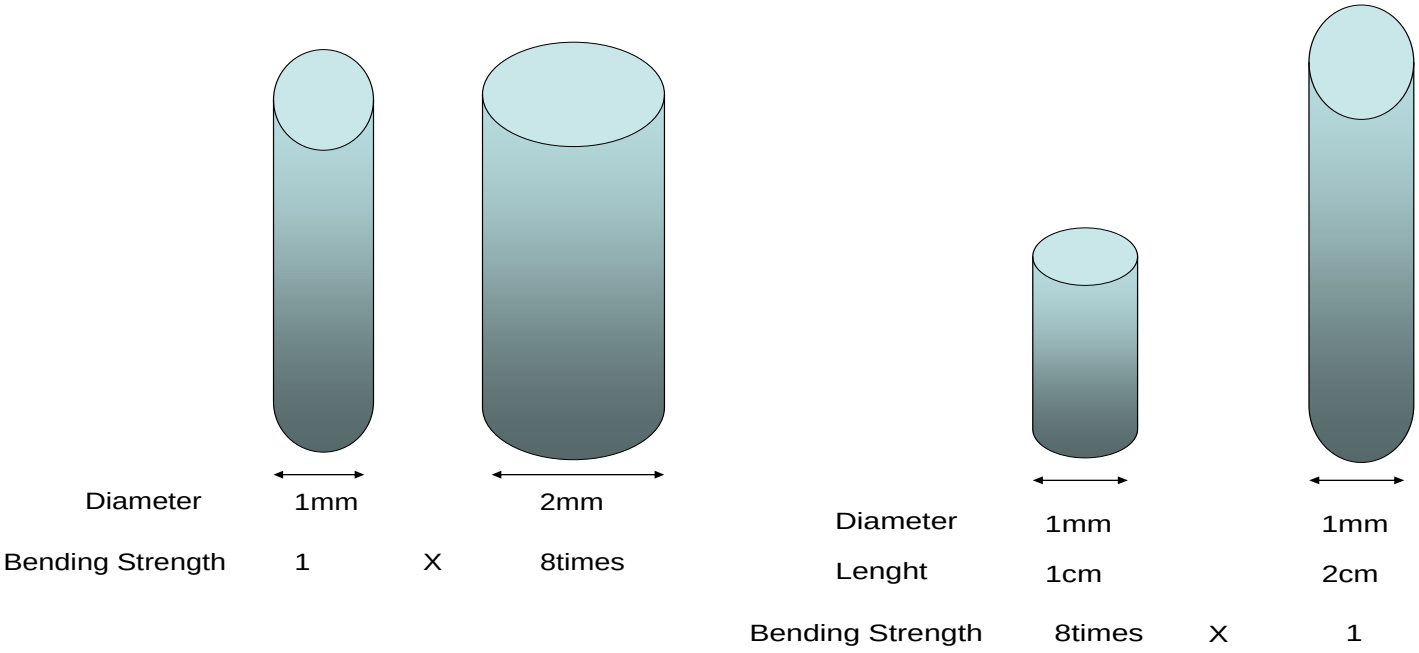
Osteocyte cells are the most abundant cell in bone and may indeed function as ‘’mechanosensors’’ in bone. These cells are in contact with neighbouring osteocytes via long slender cell processes. The cell processes are located in canaliculi, which are filled with interstitial fluid. However, the matrix that surrounds the osteocytes and the cell processes is not calcified and thus they construct a three dimensional networks. Mechanical loading placed upon bone creates substrate deformation; the interstitial fluid is squeezed in the three dimensional network and moves along canaliculi. Therefore, the osteocytes cells respond indirectly to the mechanical stimuli and convert these stimuli into cellular signals (mechanotransduction). Moreover, it is known that the osteocytes are mechanosensors in adult bone tissue and the mechanosensitivity of these cells is mediated by the lacuno-canalicular porosity (276). The fluid flow is sensed and transduced by osteocyte cell processes. The flow-derived stimulus that transports along canaliculi forms FSS and orchestrates the activities of the osteocytes in the remodelling process by stimulating the osteogenic factors such as NO and PGE2. These products are potent regulators of osteoblasts and osteoclasts during bone remodelling (41;277;278). The production of c-fos (osteogenic factor) from osteoblasts is greater when these cells are subjected to the combined effects of substrate strain and fluid flow than fluid flow alone (270;270). BMP7 is another product which induces endosteal bone formation in rats responding to mechanical loading (279). Recently, it was found that pulsating fluid flow in human bone cells ($VDR^{+}/^{+}$) up-regulates gene expression of BMP7, but it does not increase BMP7 in human bone cells with $VDR^{-}/^{-}$ (280). It was also noted that osteocytes are more sensitive to flow-derived stimuli compared with other bone cells (42). Bacabac et al. (277) concluded that both the frequency and amplitude of mechanical stress has a potential effect on bone formation at both tissue and molecular levels. Furthermore, several studies have shown that the NO production by MC3T3-E1 osteoblast like cells in vivo and by osteocytes in vitro was dependent on the rate of FSS, fluid viscosity, amplitude and frequency of stress (278). In addition, Lee et al. (281) have reported PGE2 production from osteoblasts, which increased during hypoxic states such as disuse and fractures.

In addition, mechanical loading can reduce osteocyte apoptosis by inhibition of TNF-α, which is one of the cytokines involved in the inflammatory processes and leads to osteocyte apoptosis, but has no effect on osteoblasts and periosteal fibroblasts (41;277;278). Application of mechanical loading on osteoblasts reduces the recruitment and differentiation

of osteoclasts by augmentation of OPG and reduction of RANKL expression, which in turn reduces the possibility of bone loss (282;283). On the other hand, mechanical unloading, which can be exposed under conditions of microgravity (eg. in space) and a long bed rest, could induce the osteocyte apoptosis. This can be either due to an increase of the TNF-α or decrease of the production of osteoclastic inhibitors (41;42).

1.6.4 Bone Geometry

Bone strength is also dependent on bone shape or geometric properties (Fig.1.16). Bone geometry is controlled by bone modelling and remodelling processes throughout life. These two processes active during the ageing process and are responsible for increasing periosteal diameter and endosteal through subperiosteal apposition and endosteal resorption, respectively (284).



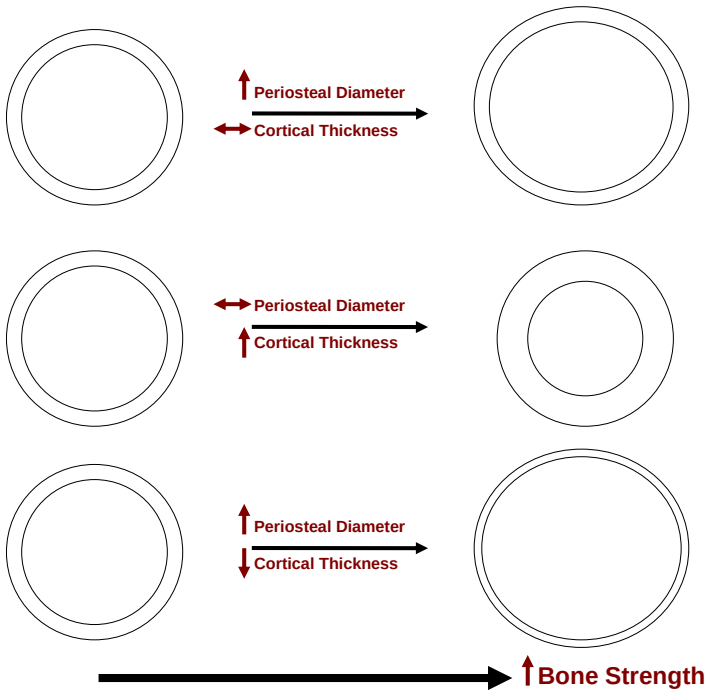


Fig 1.16: Bone geometry; bone strength increases by increasing bone diameter with unchanged cortical thickness. Bone strength also increases by increasing cortical thickness with no change in bone diameter. Finally, bone strength rises by increasing bone diameter, even with thinner cortical thickness (284).

1.6.5 Bone Responses to Conventional Exercise

There is overwhelming scientific evidence that people who have active lifestyles are healthier. Not only does exercise improve physical and mental health, it also increases bone and muscle strength, coordination and balance. Generally, conventional exercise is categorised into three main groups: isotonic (high intensity) – e.g. lifting, jumping and jogging – and isometric (low intensity) – e.g. stretching, pushing against a wall – and proprioceptive-facilitative exercises. The latter needs some factors which are important in their performance: time, force, space and flow. Examples of this kind of exercise are walking on a line (dynamic) and standing on one foot (static). It is widely believed that the high intensity exercise has an important role in maximising peak bone mass and reducing rate of bone loss. Therefore, athletes have higher bone mass than non-athletic people (285;286) and in highly active children compared with those with more sedentary lifestyle (287;288). Muscles are attached to bones anatomically and physiologically, therefore, the development of muscle strength should be matched by the development of bone strength in order to prevent pathological fracture. This explains why athletes have stronger bones compared with non-athletes. In addition to physical activity, ground force reaction (GFR) plays a vital role in development of musculoskeletal system. That is why astronomers suffer from muscle atrophy and bone loss when they are exposed to zero gravity (GFR=0) (49;50;289;290). A systematic review reported that weight bearing exercises in children and adolescents including aerobics, circuit training, jogging, jumping, volleyball and other sports showed a positive impact on bone mass. These improvements were observed in TB-BMD, LS-BMD and FN-BMC (291). However, the mechanostat's function as an osteogenic stimulator may also depend on other factors like the intensity and duration of the exercise, optimal nutritional status and hormones (GH, IGF-1) (291). For instance, low-intensity exercise with or without Ca supplementation produces no changes in bone mass (292). This study has recently been supported by Constantini et al. (293), concluding that physical activity is positively correlated with BMD in vitamin D deficient girls and suggesting exercise is superior to other major environmental factors affecting bone health. Exercise has been shown to increase bone mass at all ages, but the most effective exercise intervention occurs during the timing of puberty height velocity (HPV) "Window of Opportunity'', which is between 11–12 years in girls and 13–14 years of age in boys (294), whereas in pre-pubertal children (Tanner I) there is no change in bone mass and structure after exercise (295). However, Gunter et al. (296) reported that short term high impact exercise (jumping) in pre-puberty had a tremendous effect on bone mass in the interventional group compared with the control, and not only after finishing the exercise; the

responses remained significant three years post-exercise. However, the change in the quality of bone materials (properties) may persist for longer in the exercise group. The geometric bone changes (structural/properties) in early puberty girls doing jumping exercise for seven months may improve significantly compared with the control (295).

Fast and slow sprint exercises (30 seconds) are able to induce GH secretion and the concentration of GH is higher in the fast group (297). These exercises also decreased total ghrelin concentrations but did not alter IGF-1 release (297). Furthermore, heavy resistance exercise protocols increase GH and testosterone in both males and females with variable results in both groups (298). Another study showed that serum IGF-1 in rats can be enhanced by swimming training in trained diabetic and trained control compared with sedentary diabetic (299). Therefore, conventional exercise is likely to increase the anabolic hormones (GH, IGF-1 and testosterone) and decrease catabolic hormone (cortisol).

In conclusion, changes in bone shape and size are related positively to mechanical loading and these changes are observed more commonly around the time of puberty. High impact exercise can improve bone health even in the presence of detrimental environmental factors such as vitamin D deficiency. However, there are some problems related to doing conventional training in children, for example, low compliance rate, stress fractures and injuries. Although conventional exercise might hold several benefits for the skeleton including reduction of bone resorption, increased bone formation and increased peak bone mass, this mechanical loading might have a negative impact on the musculoskeletal system and may be associated with a risk of skeletal fragility and tissue injuries. For instance, there is a higher incidence of stress fracture in marathon runners, military recruits and ballet dancers. Alternatively, very low mechanical signals, induced through WBV platforms, can improve bone quality and quantity. Therefore, the use of low level mechanical signals to strengthen bone in children with low bone mass may be relevant not only to the treatment of existing skeletal fragility, but, by enhancing peak bone mass and retaining it through adulthood, this reduces the risk of osteoporosis and fractures later in life.

1.7 Whole Body Vibration

Vibration exercise is a new neuromuscular training method which has been introduced in a variety of clinical situations as an alternative method to improve muscle mass and bone density. Although vibrations used to be detrimental to humans, it has been shown that this form of exercise has no serious harm, is potentially safe and efficient as well as achievable with a high compliance. The first application of vibrations training in athletes was in 1985 by Nazarov and Spivak (300). Since then several scientific studies have emerged in this area (243;301;302). Vibration stimuli exert positive effects on muscle strength and bone mineral density. Deformations of bone (tissue strain) can be stimulated by mechanical loading such as weight lifting. The strain is dependent on magnitude. However, recently, high-frequency vibration has been reported to have an anabolic effect on the bone health of children with CP (245). Gusi et al. (303) proposed that the eight months course of WBV training is able to inhibit bone loss and reduce the incidence of osteoporosis in high-risk people.

1.7.1 Types of WBV Devices

Vibration exercise is mostly practised as WBV. Currently, WBV can be delivered by two broad categories of exercise devices that are currently available on the market (Fig.1.17). Firstly, WBV platforms reciprocate vertical displacements on the left and right side of a fulcrum, whilst type (sinusoidal vibration or side alternating vibration) generates higher lateral than vertical acceleration and has a potential movement around the hip and lumbo-sacral joints (less vibration to the trunk) (304). Secondly, WBV platforms have a plate oscillating up and down in vertical direction, both legs extend and stretch at the same time with a direct acceleration to the trunk. In contrast, this type is likely to produce greater strain in the vertical axis than in the lateral axis (303). Abercrombly et al. (305) reported that a greater peak acceleration cannot be tolerated in the vertical vibration mode as compared with the side alternating. Furthermore, the WBV platforms can be classified into two categories according to their peak acceleration; low intensity WBV platforms when produces gravity (g) force less than 1g regardless of frequency and high intensity WBV platforms (g force is more than 1g) (306). An example for a low-intensity WBV platform is the Juvent 1000 platform and a high-intensity WBV platform is Galileo platform. Other WBV devices available on the market are PowerPlate® and Fit Vibe®.

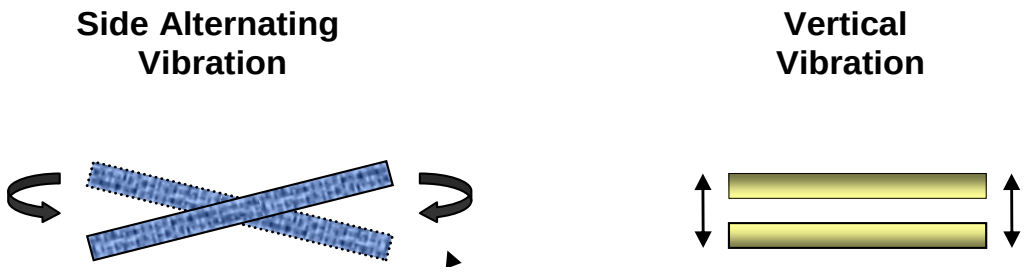


Fig 1.17: Two different movements of WBV plates: side alternating vibration and the whole plate oscillating up and down plate (vertical vibration).

1.7.2 Physical Principle and Recommendation

The physical definition of vibration is a forced oscillation, where energy is transferred from a vibratory platform (actuator) to a human body (resonator). The vibrations are produced by motors under the platforms (307). The intensity of WBV depends on the frequency (f) (Hertz, Hz) and peak to peak displacement (D) (millimetres, mm) (displacement from the lowest to the highest point of the total vibration excursion) and the amplitude (A) (mm), which is the maximum displacement from equilibrium (the half of total displacement) (Tab.1.10). The peak acceleration (a_{peak})(ms^{-2}) is calculated by either these two formulas ($a_{peak=}2x\pi^2Xf^2XD$) or ($a_{peak=}=A(2\pi f)^2$) and peak acceleration is expressed as multiples of earth's gravity (a_{peak}/9.81g) to produce a force equal to gravitational force (306;308).The g force produced by the vibratory plate is positively correlated with the frequency and peak-to-peak displacement (308-310). In typical WBV sessions, the subjects stand on the device in a still position or perform dynamic movements. There are different forms of WBV devices such as no standing free, holding on to a railing or lying on tilt tables. It is recommended to report the type of footwear (barefoot, socks, shoes, others) and body posture and standing (straight or knee and hip flexed) (311). It is also recommended that the subjects should have a firm stance on the WBV platform in order to achieve well-defined vibration parameters (243;301;302;307). In the various studies, the frequency of vibrating platforms usually ranges from a few Hz to 50Hz and the extent of amplitude ranges from micro millimetres to a few millimetres. The range of peak acceleration is from 0.1g to 10g. The time of exposure of vibration sessions ranges from few seconds to a half hour and takes place from once a week to everyday per week. In most studies, vibration sessions have several bouts of vibration exposure which are separated by rest intervals. The entire duration of WBV interventions ranges from a few weeks to one year

(306;311;312). A study has shown that there is not much difference in the rate of cortical bone formation and trabecular bone formation in women with low BMD, who were exposed to the WBV for 2minutes/day (30 Hz, 0.3g) and the others for 10minutes /day. On the other hand, no response was found in the women who used the same device more than 2minutes/day as well as in the control group. Therefore, it was presumed that the biologic response to WBV initiated triggered rather than accumulated (244).

The amount of vibration energy transmitted through the human body is dependent on musculoskeletal stiffness (mainly tendons) and damping properties (muscle and bone). As the human body is not rigid, the transmission of vibratory stimuli through muscles tendons which work as spring-like elements produces mechanical energy. Therefore, the compression and expansion of these elements occur at the time of vibration up stroke and down stroke, respectively. Vibrations produce an accumulative energy in the mass spring resonator, where the frequency of the actuator is equal to the frequency of the resonator. This accumulation of energy can result in a greater increase in the vibration amplitude in the resonator compared with the actuator. This will lead to catastrophe due to increased internal forces within the resonator. Nevertheless, this amplitude amplification can be prevented by damping elements of the muscles and bones. This will lead to absorption of energy and thus generate heat. Standing on the vibrating platforms properly can help to prevent resonance and provide firm stance. For instance, posing weight on the forefoot can reduce the transmission of vibration to the trunk and the head (307).

Whilst WBV is a promising alternative to load-bearing exercise and muscle training, the onset of beneficial action may be slower compared with pharmaceutical treatment of osteoporosis and this needs further evaluation. In addition, WBV programmes may be compromised by poor adherence to the exercise regimen and they have been reported to raise the incidence of fracture particularly in elderly individuals (22). To sum up, the maximum benefits of WBV training may be dependent on appropriate frequency, amplitude, magnitude, posture and stance.

1.7.3 Mechanism

The exact mechanism of WBV is still poorly understood. However, the mechanism of WBV might be related to the hypergravity effect that challenges the body to work harder to compensate gravitational loading (the acceleration reaches up 15g where 1g is the acceleration due to the earth's gravitational field or 9.81m/s^2) (310). A large number of studies hypothesise that transmission of mechanical vibrations applied to the tendon of a skeletal muscle in man stimulates an involuntary tonic stretch reflex contraction of this muscle and reciprocal relaxation of its antagonists, which in turn causes frequent muscle contractions (313). Vibrations cause muscle deformation, which leads to elicit dynamic stretch sensitive receptors of muscle spindles (307). Vibration stimuli can be sensed by three muscle spindle receptors: primary receptors (Ia afferent fibers), secondary receptors (II afferent fibers) and Golgi tendon organs (Ib afferent fibers) (314). Vibrations are more effective in the primary endings and the secondary endings than Golgi tendon organs (314). This will produce impulses transmitted in the CNS through trains of group Ia afferent pathway, which correspond to the frequency of the vibration. The afferent impulse produces two results within the CNS; firstly, it causes tendon reflex depression alpha motor neurones (efferent) concerned with phasic monosynaptic reflexes (primary endings or Ia afferent). Secondly, it causes the tonic contractions of muscle (tonic vibration reflex) by activation of a polysynaptic pathway (secondary endings or II afferent) (315)(Fig.1.18). Roelants et al. (316) concluded that WBV (f=35Hz and D=5mm) caused a different muscle activation in the legs to a magnitude that ranged from 13% to 82% of maximal muscle contraction. Nevertheless, vibrations are not able to elicit tonic vibrations reflexes when the amplitudes are very small (A<1mm) (314). Therefore, the anabolic effect of WBV on bone might be produced by different mechanisms. The anabolic effect of vibratory stimuli on muscle and bone is not well-recognised (317). Several animal and human studies have shown that mechanical loading at low magnitude and higher frequency might have more osteogenic response than the mechanical loading applied at natural frequency. Mechanical vibration at high frequency (45Hz) can reduce early bone loss and stimulate bone formation in post-ovariectomised rats (318). The anabolic effect of WBV on bone can be explained by several hypotheses (Fig 1.19). Firstly, the muscle contractions produced by WBV might stimulate bone formation rate through mechanotransduction, FSS mechanism or stochastic resonance phenomenon. The second hypothesis is that WBV can increase muscle strength, which in turn needs to be

adapted by strong bones through the mechanostat theory. Thirdly, WBV induces anabolic hormones (e.g. GH, IGF-1 and testosterone) and inhibits catabolic hormones (e.g. cortisol); such hormones have a positive effect on bone and muscle mass (endocrine response). Lastly, WBV might improve the rate of blood flow in muscle and bone which supplies the nutrients required to build bones and muscles (vascular response).

Parameters	Definitions	Units
Frequency (f)	The repetition rate of the cycles of oscillations per second	Hz
Peak-to-peak displacement (D)	Displacement from the lowest to the highest point of the total vibration excursion	mm
Amplitude (A)	The extent of vertical displacement from equilibrium position	mm
Peak acceleration (a_{peak})	The acceleration power or the force of the movement	$g(m/s^2)$

Tab 1.10: The biomechanical parameters used in WBV training.

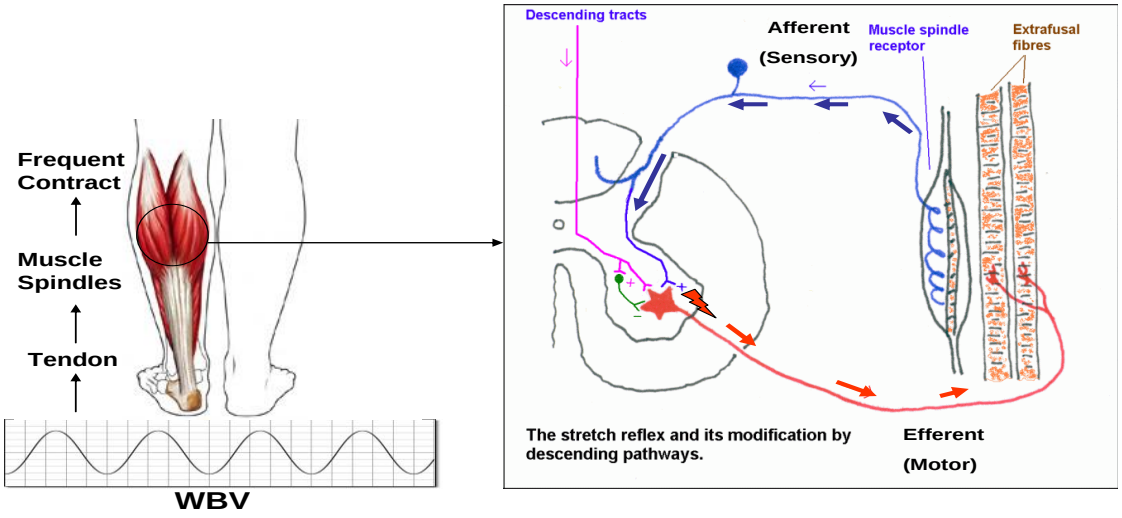


Fig 1.18: WBV stimulates muscle spindle and motor neurons which initiates a frequent muscle contraction.

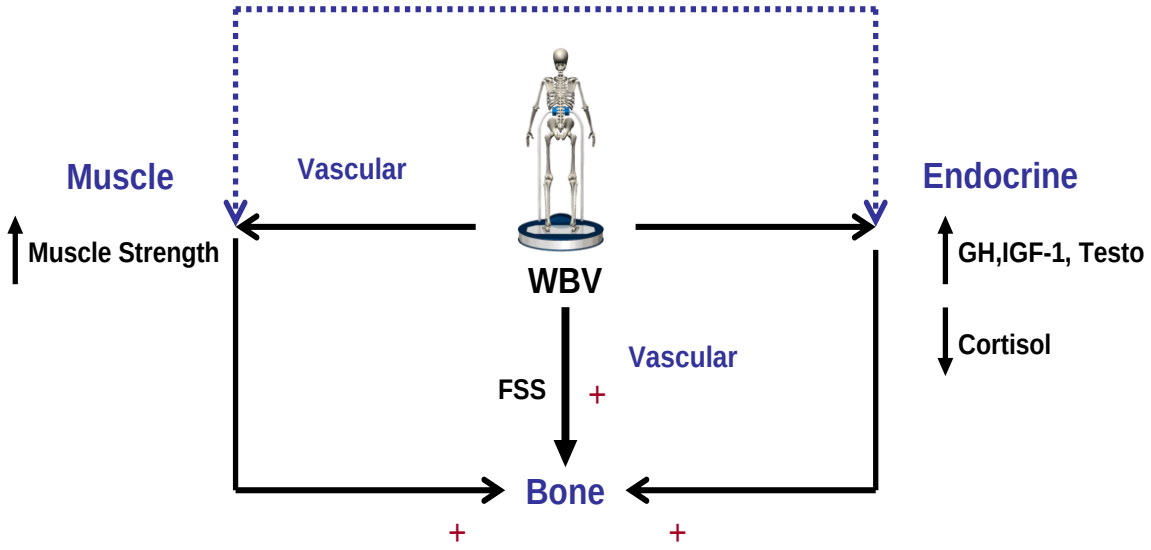


Fig 1.19: The Musculoskeletal and Endocrine Responses of WBV. The anabolic effect of WBV on bone can be explained by four pathways. Firstly, WBV stimulates bone directly through fluid shear stress (FSS). Secondly, WBV increases dynamic muscle strength. Thirdly, WBV induces the release of growth hormone (GH), Insulin-like growth factor-1 (IGF-1) and testosterone (Testo) secretions and decreases serum cortisol levels. Fourthly, WBV might increase blood flow to muscle and bone which lead to improve bone density.

1.7.4 Musculoskeletal Response

1.7.4.1 Bone

There are several reports about the anabolic effect of WBV on BMD, improving bone strength and bone properties (244;245;302;319;320). The exact mode of action of WBV on bone is still not wholly understood; but it might be explained by some theories. Garman et al. (321) suggested that very small-amplitude oscillatory accelerations can induce trabecular bone formation in vitro without or negligible bone deformation (strain). They also assumed that the movement of osteocyte nuclei within lacuna was greater in this scenario compared with the direct strain on the calcified matrix. Verschueren et al. (22) also suggested that the response of bone tissue to high frequency stimuli could be related to FSS rather than a direct response to bone strain. Tanaka et al. (322) also hypothesised that the response of cortical bone formation to the vibratory stimuli could be related to the mechanical noise released from a vibration and this noise is known as stochastic resonance. This phenomenon can enhance

the osteogenic response of bone tissue by stimulating mechanoreceptors such as muscle spindle (322;323).

Nevertheless, Judex et al. (324) have a different opinion about the underlying mechanism of WBV. They proposed that the FSS, which depends on the strain magnitude, is unlikely to be the underlying mechanism of high frequency-low magnitude stimuli since two high frequency regimes (45 and 90Hz) with the same strain magnitude generated different anabolic effects on bone tissue. They suggested that the possible mechanism could be related to the direct sensitivity of bone cells to the high-frequency oscillatory motion itself. The mechanosensor bone cells (osteocytes) respond to the low magnitude, high frequency vibrations by increasing COX-2 secretion and decreasing RANKL and PGE2 releases. These soluble factors released from the osteocytes prevent osteoclastogenesis (325) and, therefore, might induce bone formation rate. WBV training for eight weeks (3times/week) in post-menopausal women was associated with in a significant reduction in NTX when compared with sham vibration exposure (326).

The anabolic effect of mechanical strain on bone tissue depends on their frequency. In other words, the mechanical stimuli with low frequency require higher amplitude (strain) in order to influence new bone formation. Therefore, 1Hz mechanical load needs at least 1000μ-strain to induce cortical bone formation. On the other hand, the same result can be achieved by 50μ-strains at 30Hz, whereas only 5μ-strains (0.1g) can stimulate trabecular bone mass (Fig.1.20) (61). Low magnitude, high frequent vibrations might improve trabecular bone formation even when applied for very short duration. According to these data, bone modelling and remodelling might be initiated by active biological bone formation products rather than stimulated by the repair of micromanages. The osteogenic mechanism of mechanical stimulation could be related to inducing additional osteoblasts instead of increasing their activity (245;302). Gilsanz et al. (244) recommended that the time of exposure to the WBV exercise (30Hz, 0.3g) should be at least 2min/day in order to stimulate cortical and trabecular BMD. According to the previous study (244), the low magnitude and high frequent strains in women with low BMD did not increase the cross sectional diameter of the femurs, even though they increased the cortical bone formation in these bones. It means that the WBV

stimuli may have greater effects on the endocortical bone formation than the periosteum (Fig.1.21).

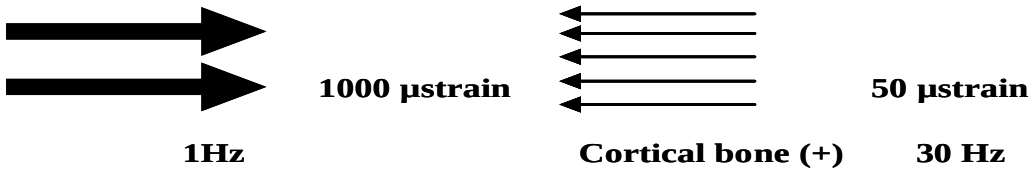


Fig 1.20: This diagram illustrates that the anabolic activity of cortical bone can be stimulated by only 2% of the peak strain (1000 µ-strain) that occurs when the frequency increases from 1Hz to 30Hz. The loading rate decreases the loading strain.

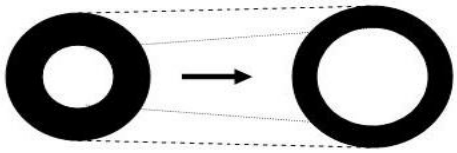


Fig 1.21: The cross sections of long bone resembling hollow tubes. The effect of the WBV exercise results from increasing the endoosteal surfaces of long bones.

In recent years it has been shown that trabecular bone formation increases with increasing vibration magnitude from 0.1 to 0.9 g. Chirstiansen and Silva. (327) reported that trabecular bone volume response to WBV did not show a dose-dependent response to increasing vibration magnitude in mice. They also demonstrated that trabecular bone volume of the skeletal sites closest to the ground such as the proximal tibial metaphysis and distal femoral metaphysis may be likely affected due to decreases in vibration magnitude with distance from the source of stimuli. However, another study concluded that the mice vibrated at 0.3g did not induce any changes in trabecular bone volume at the proximal tibial metaphysis and distal femoral metaphysis. Therefore, these results did not correspond with a dose-dependent response to increasing magnitude. However, this conclusion is not constant with another study established by Judex et al. (324). They showed a significant rise at the same sites at 45 Hz, 0.25g, 10min/day for days a week. The reason for such discrepancy is not obvious, even though it may be related to different gender, age or methods.

An animal study showed that low magnitude, high frequency stimuli (90Hz,0.15g, 10minutes/day/4weeks) have potential effects on stimulating bone growth by increasing bone formation and reducing disuse osteoporosis (324). Another research demonstrated that the low magnitude WBV (45Hz,0.3g,15minutes/day/3weeks) can suppress the osteoclastic activity only in trabecular bone and can induce the bone formation activity only in the cortical bone (328). On the other hand, Garman et al. (321) showed that the extremely low magnitude oscillatory accelerations (45Hz,0.6g,20minutes/day/3weeks) did not influence the osteoclastic activity neither in trabecular bone nor cortical bone, but they can stimulate the metaphysial trabecular bone formation by about 70% in the in the absence of weight bearing. It was also found that the NO and COX-2 production from the MC3T3-E1 cultured cells (osteoblasts) in vitro were linearly correlated with the applied peak frequency (277;329).

Moreover, Tanaka et al. (318) demonstrated that mechanical loading at higher frequency applied on MC3T3-E1 cells has more osteogenic response than the mechanical loading applied at normal frequencies. This study suggested that the high frequency, low magnitude mechanical loading increased the sensitivity of osteoblasts by stochastic phenomena. Furthermore, the OCN mRNA expression was upregulated by sinusoidal vibratory stimuli, with low amplitude and high frequency. Additionally, MMP-9 gene expression, which increases in osteoblasts during osteogensis, was also elevated in low amplitude and high frequency mechanical stimuli.

It has been demonstrated in recent meta-analysis data that WBV has a positive effect on BMD, particularly in children, adolescents and post-menopausal women (330). This shows that WBV significantly increased LS-BMD and trabecular vBMD in children and adolescents. In postmenopausal women, the effect of WBV was more pronounced in hip BMD. However, Raun et al (240) reported that LS-BMD is more sensitive to WBV than FN-BMD. They explained that the transmission of vibration is in the same direction of lumbar spine. No positive effect of WBV in adults might be explained by a higher rate of WBV transmissibility to the ankle and hip in children than in adults (331). It is also assumed that a growing skeleton in children and adolescents makes WBV more sensitive in these populations than adults and postmenopausal women. Furthermore, the metabolic rate in adult's skeleton is less active than children's and adolescent's skeleton, which might lead to less responsiveness of

mechanical stimuli produced by WBV. Another possibility for not improving bone density in adults is related to the study methodology either in a small sample size or insufficient statistical power calculation in this population (330). According to this report, the optimal target population for WBV is firstly children and adolescents and secondly postmenopausal women. On the other hand, Wysocki et al. (306) reported that there is little evidence on the benefits of WBV for improving BMD in study populations. Another systematic review and meta-analysis of RCT reported that WBV does not have effect on BMD in postmenopausal women (332). These two reviews did not involve studies that recruited children or healthy adults with normal BMD. Other studies show that the anabolic effects of high frequency, low level magnitude strains would be more beneficial in subjects with low body weight and with low BMD. Supporting data show that mice with low BMD have a greater response to the vibratory stimuli than mice with high BMD (244).

Tab.1.11 summaries a number of randomised clinical studies of WBV. Diversity in WBV protocols was reported, frequency ranges from 12Hz to 90Hz, peak to peak displacement ranges from <50 um to 12mm and g force range from 0.2g to 5.09g. Those studies reporting significant improvement in BMD can be categorised into two groups; firstly, studies applying frequency at more than 30Hz and peak to peak displacement less than 100μm and whose g force is usually less than 1g; secondly, studies using frequency at less than 30Hz and peak to peak displacement more than 1mm and whose g force is usually over 1g. Therefore, no recommendations can be presented for improved bone parameters. However, the optimal frequency ranges from 20–30Hz for a minimum of eight weeks' duration of WBV exercise is likely to promote BMD (333). A higher frequency of WBV does not necessarily increase the anabolic threshold of bone tissue. A recent study has shown that low-intensity (0.3g) vibrations at 60Hz increases bone mass in children with CP, but no positive results were observed at a higher frequency (90Hz) (334).

Although several clinical trials of WBV are promising as regards a new modality for prevention and treatment of osteoporosis, more research is required to understand the efficacy and the optimal dose of WBV training. Irregularities in study design and WBV protocol and poor quality trail are responsible for inconsistent results. There is no fixed protocol for the parameters of WBV. The optimal frequency, amplitude, acceleration, duration

and time of exposure and direction of vibrations to stimulate bone formation in humans are not yet clear. It is also possible that combining WBV training with other forms of exercise such as high-load resistive exercise may be more effective in inhibiting bone loss due to prolonged bed rest than high-load resistive exercise alone (335).

Study	Group	Study	Parameters	Assessment	Results
Torvinen et al. 2003 (336)	Healthy adults (19-38years) (n,56)	RCT	f=25-45Hz D=2mm G=2-8g Direction=Vertical Time=4min/3-5timies/week Duration=8months	p.QCT Bone markers	No changes in BMD and bone markers Increases vertical jump height
Russo et al. 2003 (337)	Post-menopausal Women (n,29)	RCT	f=12-28Hz D=- G>1g Direction=- Time=6min/twice/week Duration=6months	p.QCT Bone markers	Inhibits loss of cortical BMD in tibia in the WBV group
Rubin et al. 2004 (319)	Post-menopausal Women (n,78)	RCT	f=30Hz D=55um G=0.2g Direction=Vertical Time=20min/day Duration=1year	DXA	Inhibited loss of FN-BMD and LS-BMD in the WBV group
Ward et al. 2004 (245)	Children with disability (n,20)	RCT	f=90Hz D=100um G= 0.3g Direction= sinusoidal Time=10min/5times/wk Duration=6months	QCT	Improvement in trabecular BMD in tibia the WBV group
Verschueren et al . 2004 (22)	Postmenopausal women (n,70)	RCT	f=35-40Hz D=1.7-2.5mm G= 2.28-5.09g Direction= - Time=10min/3times/week Duration=6months	DXA Bone Markers	Improvement in FN-BMD, no change in bone markers
Gilsanz et al. 2006 (244)	Women (15–20 years) with low BMD(n,48)	RCT	f=30Hz D <50 um G= 0.3g Direction= sinusoidal Time=10min/day Duration=12months	QCT	Increased cortical bone in femur Increased trabecular bone in lumbar spine

Gusi et a.l 2006 (303)	Postmenopausal women (n,28)	RCT	f=12Hz D=6mm G=5g Direction= Sinusoidal Time=1-6min/3times/week Duration=8months	DXA	Improvement in FN-BMD, no change in LS-BMD
Raun et al. 2008 (240)	Post-menopausal Women (n,116)	RCT	f=30Hz D=10mm G=0.3g Direction=? Time=10min/5times/week Duration=6months	DXA	Increased in LS-BMD and FN-BMD in the WBV group
Beck et al. 2010 (338)	Postmenopausal women (n,47)	RCT	f1=30Hz f2=12.5Hz D1=50um D2=4mm G1= 0.3 G2=1 Direction1=Vertical Direction2=Sinusoidal Time1=15min/2time/week Time2=6mins/2times/week Duration=8months	DXA QUS	Inhibited loss of FN-BMD and LS-BMD in the WBV groups
Wren et al. 2010 (339)	Children with CP (n,31)	RCT	f=30Hz D=- g= 0.3g Direction= - Time=10min/day Duration=6months	CT	Increased cortical BMD in the WBV group.
Ruck et al. 2010 (340)	Children with CP (n,20)	RCT	f=12-18Hz D=2-4mm G= 2.6g Direction= sinusoidal Time=9min/5times/week Duration=6months	DXA	No changes in BMD

Bemben et al. 2010 (341)	Postmenopausal women (n,55)	RCT	f=30-40Hz D=2-4mm G=2-2.8g Direction=- Time=2min/3times/week Duration=8months +Resistance training	DXA Bone Markers	No changes in BMD and bone markers
Verschueren et al . 2011(342)	Postmenopausal women (n,113)	RCT	f=30-40Hz D= G=1.6-2.2g Direction=? Time=15min/3times/week Duration=6months +Vitamin D	DXA	Hip BMD increased significantly in the intervention and the control group
Turner et al. 2011 (343)	Postmenopausal women(n,39)	RCT	f=12Hz D=50um G=0.3g Direction=Vertical Time1=20min/1time/week Time2=20min/3times/week Duration=8weeks	Bone Markers BAP NTX	Time2 exposure caused a reduction marker of bone (NTX) resorption when WBV
Von-Stengel et al. 2011 (344)	Post-menopausal Women (n,108)	RCT	f1=12.5Hz- f2=35Hz D1=12mm D2=1.7 G1= G2= Direction1=Vertical Direction2=Sinusoidal Time=15min/3time/week Duration=12months	DXA	LS-BMD increased in the two WBV groups; more pronounced in the first group.
Slatkovska et al. 2011 (345)	Post-menopausal Women (n,202)	RCT	f=30-90Hz D<50um G=0.3g Direction=? Time=20min/day Duration=12months	DXA p.QCT	No changes in BMD

Reyes et al. 2011 (334)	Disabled children (n,65)	RCT	f1=60Hz- F2=90Hz D1= D2=- G1= 0.3g G2=0.3g Time=5min/day Duration=6months WBV was delivered to the radii and femurs	QUS	Increased BMD at the radius with frequency 60Hz
Ligouri et al. 2012 (346)	Healthy Adult (n,10)(6male) Age 18-22yeras	RCT	f=15-26Hz D=4.16 G=? Direction= Sinusoidal Time=20mins/3times/week Duration=12week	DXA	Increased BMD at the lateral and posterioranterior view of the spine

Tab 1.11: Clinical trials of WBV: characteristics of participants, study type, WBV parameters, bone assessment and the results. . Frequency (f), peak to peak displacement (D)

1.7.4.2 Muscle

Vibrations usually increase motor activity and electromyography (EGM) activity during contractions. WBV with frequencies 30, 40 and 50Hz has shown to elicit a greater response in the EMG activity in the vastus lateralis muscle compared with the control group with the highest response at 30Hz (347). In contrast to the previous study, vibrations at frequency 65Hz and amplitude 1.2mm applied to the biceps tendon with two different loads could not activate EMG (348). Therefore, an increase in vibration amplitude and frequency may not be beneficial in enhancing maximal effort contractions and submaximal muscle contractions can induce a greater acute enhancement in neuromuscular performance (EMG activity) (349). It is reported that EMG activity is more common with side alternating vibrations (305). Furthermore, metabolic power rate can be increased by vibration exercise due to the increased muscular activity (304). Recently, Zange et al. (350) have shown that the amount of ATP consumption in an isometric contracting calf muscle can be boosted by about 60% when WBV training has been added (f=20Hz and D=4mm). Hence, the amount of ATP turnover in muscle can be increased during vibrations, this might lead to enhanced intramuscular temperature. Cochrane et al. (351) reported that WBV training (26Hz and D=66mm) can also elevate intramuscular temperature.

The effects of WBV in muscular performance and muscle mass have shown conflicting results. Several studies have shown that WBV has a positive influence on muscular performance and body balance. Torvinen et al. (352) reported that vertical jump height increased significantly by about 8% after eight months of WBV training in healthy young adults. Another study by the same group concluded that the four-month WBV training enhanced vertical jump height and jumping power in young, healthy, nonathletic adults (353). In postmenopausal women aged 58-74years, six months of WBV is an efficient training method and is effective as conventional exercise in enhancing muscle movement and jump efficiency (354). Additionally, WBV training (3months) leads to improvement in muscle function in cystic fibrosis patients (355). Ten weeks of WBV inhibit the loss of muscle strength and mass associated with age-induced sarcopenia in older women (356). Moreover, the short term effect of WBV increases the vertical jump height and improves flexibility performance (357;358). The combination of WBV with high intensity resistance exercise is more effective for increasing muscle strength than resistance training alone (341). Moreover, a meta-

analyses study has concluded that WBV training has a beneficial effect in enhancing muscle strength in older populations (332). The effectiveness of WBV on muscle performance might be dependent on WBV parameters (359). Torvinen et al. (357) demonstrated that WBV amplitude is positively correlated with muscle performance while others suggested that frequency is the most important variable in WBV (360;361). The combination of these two parameters determines the acceleration magnitude of magnitude. Another study reported that the short-term effect of WBV training with the different g forces (control,2.16g,2.80g,4.87g and 5.83g) and the frequency (0,30,40,35 and 50Hz) and the amplitude (0,2-4mm,2-4mm,4-6mm and 4-6mm) produced no changes in the muscle performance in young healthy men, but in women the 2.80g (35Hz) and 5.83g (50Hz) showed a significant change in counter jump movements (353;360).

Dynamic muscle strength increased significantly following six-month training of WBV combined with vitamin D supplementation in postmenopausal women; however, there was no significant difference compared with the control group who also received only vitamin D (342). In this study there is no clear evidence that the improvement in muscle strength resulted either from WBV or vitamin D supplementation as there is a positive correlation between serum concentration of vitamin D and muscle power and force (362). Creatine kinase (CK) is a marker of muscular lesion and commonly elevated after intensive exercise (running/walking downhill) due to rhabdomyolysis (363). Recently, it was shown that side alternating WBV training in combination with dynamic exercise (f=26Hz and A=15mm) can double the level of serum CK in 25% healthy adults (364). It is well known that dynamic exercise can increase accumulation of lactate in muscle and blood (365). It is also reported that lactate concentration can be elevated after WBV training (364;366;366).

Vibratory stimuli have a potential to stimulate proprioceptors on the sole of the foot and anterior cruciate ligament. These receptors play an important role in the body balance and postural stability (367). WBV (six weeks) improves balance and mobility in elderly people (368). It is reported that WBV training increases walking speed, stride duration and length and cadence in Parkinson’s diseases (369;370) and has beneficial effects on muscle strengthening, balance and walking ability in the elderly (371;372) and in individuals with spinal cord injury (373).

1.7.4.3 Hormonal Response

The endocrine system may play an important role in determining the individual's response to the exercise. WBV exercise has been shown to increase acutely testosterone and GH in healthy young individuals (n,14) after a single bout of 10minutes (243). It is reported that there is a significant increase in testosterone and GH and a decrease in the serum concentration of cortisol in healthy young men after 10minutes of WBV exercise (6minutes, 26 Hz, peak-to-peak displacement of 4 mm; acceleration,17g) (243). In healthy adults (n,9) the level of GH increased significantly after the first bout of WBV, whereas the second bouts (with a 2-h interval) of WBV are associated with blunting of GH responsiveness to the second stimulus (366). However, both Di Loreto et al. (374) and Cardinale et al. (375) did not find any acute changes in testosterone and cortisol in healthy individuals (n, 9, 10 respectively) undergoing 5 and 20minutes of WBV exercise, albeit with relatively small amplitude and frequencies of 27 and 30 Hz respectively. In addition, Di Loreto et al. (374) measured glucose, insulin, glucagon, adrenaline, noradrenaline, GH and IGF-1 and reported a small fall in glucose levels and a rise in adrenaline. Recently, hormonal fluctuations have been observed in elderly individuals following a single session of WBV (376). IGF-1 levels were elevated immediately, 1hr and 2hr post WBV plus static squat (slight knee flexion) vs. levels observed with static squat alone (376). Immediately following the WBV session, cortisol levels were higher in those who static squat alone; however, by 1 hr and 2 hr post treatment, cortisol concentrations were reduced below pretreatment levels with WBV plus static squat and static squat alone (376). In contrast, no differences were observed in testosterone or GH concentrations between treatment groups (376). It is reported that no reduction in body weight, total body fat or subcutaneous fat was observed after 24 weeks WBV training in non-athletic young females (377). However, eight months training of WBV in combination with resistance training in postmenopausal women is effective in reduction of total FM% (378). Tab.1.12 summarises the studies showing acute hormonal responses to WBV.

Study	Populations	Parameters	GH	IGF-1	Testost	Cortisol	Glucose
Bosco et al. 2000 (243)	14(males) 25±4.6years	f=26Hz D=4mm Duration=10mins	Increased	-	Increased	Decreased	-
Di Loreto et al. 2004 (374)	10(males) 39±3years	f=30Hz D= Duration=25mins	No change	No change	-	No change	Decreased
Cardinale et al. 2006 (375)	9(males) 22±2years	f=30Hz D=1.5-3mm Duration=20mins	-	No change	No change	-	
Erskine et al. 2007 (379)	17(males) 22.3±2.7years	f=30Hz D=4mm Duration=20mins	-	Not measured	No change	No change	-
Fricke et al. 2009 (380).	20 (10females)	f=26Hz D=2mm Duration=5mins	Increased (males) Decreased (females)	-	-	-	Decreased
Cardinale et al. 2010 (376)	20 (11females) 70years(66-85)	f=30Hz D=4mm Duration=5mins	No change	Increased	No change	Increased/ Decreased	-
Sartorio et al. ,2010 (366)	9(males) 23±2years	f=35Hz D=5mm Duration =15mins	Increased	-	-	-	-

Tab 1.12: Acute hormonal responses to WBV exercise. Frequency (f), peak to peak displacement (D)

1.7.4.4 Vascular Responses

The systematic effects of vibratory exercise include increasing heart rate; systolic blood pressure and oxygen uptake have been recorded after exercise. Unexpectedly, diastolic hypotension has also been reported in the vibratory exercise group and the reason for that could be arterial vasodilatation (381). WBV (f=26Hz, D=6mm) increases the blood volume in the peripheral blood circulation of quadriceps and gastrocnemius muscles from 6.5cm/s to 13cm/s (382). Furthermore, side-alternating vibrations in health adults with frequency 26Hz and D=6mm for 9minutes result in a dramatic increase in popliteal blood flow by 100% as measured by a Doppler ultrasound machine (382). Several studies show the skin blood flow as assessed by laser-Doppler flowmetry can be enhanced by WBV training (381;383;384). It has also been reported that a 5minutes bout of WBV (f=50Hz,D=4mm)) in health adult people (n,14,9males) increases superficial skin temperature of lower legs, total hemoglobin and deoxyhemoglobin (385). Thus WBV's influence on peripheral circulation might improve BMD and muscle strength through increasing the amount of nutrients required by these tissues. It is reported that WBV increases the oxygen uptake, heart rate and blood lactate levels to values comparable to moderate exercise (381;386).

1.7.5 Clinical Applications

WBV is currently being examined as a prevention and treatment for several conditions (Tab.1.13). However, other studies have produced conflicting results with regard to the utility of WBV for the treatment of these conditions.

Conditions	Examples
Osteoporosis	Postmenopausal women (344) Cerebral palsy (339) Patients with low BMD (244)
Muscular performance	Osteogenesis imperfecta (387) Multiple sclerosis (388)
Balance and stability	Elderly people (368) Parkinson's disease (369) knee osteoarthritis (389)
Pain management	Low back pain (390) Fibromyalgia (391)
Glycaemic control	Improving glycaemic control in type 2 diabetes patients (392)
Rehabilitation	Improvement in walking distance and decreasing in the requiring for a sit-stand test in chronic obstructive pulmonary disease (393) Reduce muscle pain and enhance recovery after football exercise (394)

Tab 1.13: The clinical applications of WBV

1.7.6 Possible Concerns

Although many studies show some beneficial effects of WBV on musculoskeletal system and hormones, there are several concerns regarding safety features.. There are some occupational hazards associated with vibrations such as low back pain, the Raynaud's phenomena, plantar fasciitis, blurred vision, tinnitus and intraocular dislocation (306). Vibrations with the frequency between 5 and 15Hz can lead to low back pain and may contribute to circulatory problems such as Raynaud's phenomena (302). However, the level of chronic back pain in post-menopausal women can also be reduced by applying WBV training (240). The exposure limits have been recommended by agencies such as the International Safety Organisation (ISO). It shows that when the magnitude of WBV is below

0.56g and the frequency between 20-90Hz, these are not pathogenic to the musculoskeletal system, whereas the magnitude of WBV that exceeds 1g might be harmful to the body (328). Therefore, it is extremely important to develop correct recommendations for WBV parameters as over exposure of WBV might lead to injury (361). Erythema and itching in the legs have been recorded in some cases after a vibratory exercise session, which disappears within a few minutes. The reason for that could be related to increasing blood flow in the lower limbs (381), which might lead to stimulation of mast cells and histamine release. However, long adverse effects of the low magnitude, high frequency loading treatment have not been observed or reported (395).

1.7.7 Future Direction

WBV exercise could provide a therapeutic intervention to the patients who have a high risk of fractures due to low BMD such as postmenopausal women, children with muscular disorders and CP and patients who receive GCs and chemotherapies. Applying WBV in ALL children receiving chemotherapy may have a beneficial effect as the anabolic bone effect of WBV is more pronounced in children and adolescents particularly with low BMD. However, the optimal dose of the WBV exercise and the systematic response to the different doses require further research in order to maximise the beneficial effects of vibratory exercise.

1.8 Diagnostic Approaches to Musculoskeletal System

1.8.1 Methods for Measurements of Paediatric Bone

The role of DXA, p.QCT and QUS, Leonardo mechanography and maximal isometric grip force (MIGF) in the clinical assessment of bone, muscle and body composition parameters in children are considered (Tab.1.14). It is important to understand some terms related to these methods; firstly, bone area (BA, cm^2) represents bone size or volume and can be either in two-dimensional projections (cm2) or in a three-dimensional projection (cm^3). Secondly, bone mineral contents (BMC) are defined as amount of bone material in the measured area (g). Thirdly, bone mineral density (BMD) represents mass per volume (BMC/BA). If the BA is measured in two-dimensions (cm^2) and in three-dimensions (cm^3), this will result in aBMD (g/cm^2) and vBMD (g/cm^3) respectively.

1.8.1.1 Dual Energy X-Ray Absorptiometry

DXA was developed in the late 1980s and became the most common method used to screen osteoporosis in post-menopausal women. Subsequently, DXA measurements of BMC and BMD rapidly increased in paediatric research and clinical practice (396). The fundamental principle of DXA is the measurement of transmission of X-rays, produced at high and low energies at a stable X-ray source. The calculated mass attenuation of the soft tissue and bone can be differentiated by these two energies (397). The radiologist is required to evaluate precisely patient position and region of interest (ROI) in each measurement. Lumbar spine should be centralised and straight, with visualisation of last rib pair and upper of sacrum. Personal belongings such as jewellery should be removed, if possible. L1-L4 is ROI and will be selected automatically. The image should include the entire vertebral body with a minimum adjacent soft tissue. For hip evaluation, the femoral shaft should be parallel with the long axis of the image with only a small part of the minor trochanter visualised. There should be no overlap between the trochanters and the femoral neck or acetabulum. The ROIs are the femoral neck, trochanter, intertrochanteric and total hip. Whole body DXA measures TB-BMC and aBMD (398). There is some controversy about including head in TB-BMC or BMD in paediatric bone measurement. This is due to a large contribution of heads in bone density. Lumbar spine and total body less head (TBLH) are the most preferable skeletal sites for assessing BMC and aBMD in growing children, whereas the FN-BMC and FN-BMD are not

highly accurate and reproducible in this age group of children. It is very crucial in children with normal growth or delayed growth; BMC and BMD results should be adjusted for absolute height or height age, or compared with paediatric reference data that provide age-, gender- and height-specific z scores (399). Two types of standard deviations (SD) are often described in DXA results T-score and z score. The T-score compares the patients BMD to the optimal peak bone density for the same gender. Clinical diagnosis of osteoporosis is based on T-score BMD in postmenopausal women and in men age 50 and over. The T-score is restricted in adult bone DXA, not applicable for children. Instead, a z score of BMD is more appropriate in paediatrics. A z score is also SD score of BMD based on same age peers and sex, height and weight (400). However, this approach is not completely appropriate for those children suffering from abnormal growth pattern, delayed sexual maturation and chronic inflammation. Furthermore, Areal bone density will be underestimated in smaller bones in short individuals and overestimated in larger bones in tall individuals of the same age and gender. A numbers of methods have been developed to accommodate differing bone size. According to the ISCD, paediatric bone density will be significantly low if BMD z score is less than or equal to -2.0, adjusted for age, gender and body size, as appropriate (399). The ISCD recommended that lumbar spine and TBL-BMC and aBMD of DXA results in children with linear growth or maturational delay should be adjusted for absolute height or height age, or compared with paediatric reference data that provide age, sex and height-specific z-scores in order to have appropriate results (396). Moreover, Kalkwarf et al. (401) evaluated a large number of children by DXA and found the LMS curve method (statistical method) might be more appropriate in identification of low BMC or BMD in children rather than SD. This method has a greater accuracy in providing a wide range of reference data particularly at low and upper ends of distribution. These z scores are only suitable when comparing average size children in population based studies. Therefore, in children (5-19years) with chronic diseases such as IBD, to reduce the effect of bone size on BMC values, the predicted TB-BMC and LS-BMC (L2-L4) can be calculated for bone area by regression models. The TB-BMC and LS-BMC z scores corrected by bone area (size) can be obtained by these models (402). This method is currently used in our team's research and is dependent on the calculation of predicted and percentage predicted bone area (PPBA) for age and gender. Therefore, a short child with small bones would have a low PPBA. TB-BMC z score and LS-BMC BMC z score (L2-L4) was calculated by using a regression formula for males and females in order to minimise the

effect of bone size on BMC values (402). Bone mineral apparent density (BMAD) is another frequent method for bone size adjustment used in paediatric DXA. BMAD was developed to minimise the size-related effects of DXA aBMD measurements. BMAD at lumbar spine can be calculated by estimating the vertebral depth as the square root of the area measured by DXA (Bone area). Then, the vertebral volume is calculated by simply multiplying the height x width(BA) x depth (403). For example, in the case of a 7.4 years child with BMC 19.24g and bone area 17.45, the calculated aBMD age matched is 19.24/17.45=0.743g/cm^2, whereas the BMAD=19.24/(17.45xsequre root 17.45)= 0.26g/cm^3. Recently, Zemel et al. (404) have shown that DXA BMC/BMD z scores adjusted for height for age z-score (HAZ) provided the least biased approach for estimating the effect of short (or tall) stature on measures of BMD. Adjustments using HAZ were the least biased compared with age z score, height age z score, height z score and BMAD. Therefore, this approach can be applied to assess the effect of short or tall stature on BMC/BMD z score.

Recently, ISCD (2007) stated that osteoporosis should not be diagnosed in children based solely on DXA BMD (399). The Society's position is that the diagnosis of osteoporosis in children necessitates the co-existence of a clinically significant fracture and a low BMD or BMC. Long bone fractures of the lower limbs, compression fractures of vertebrae and two or more long bone fractures of upper limbs are considered significant clinical history of fracture. Low BMC or BMD is defined as a BMC or aBMD z-score that is less than or equal to -2.0, adjusted for age, gender and body size, as appropriate. Whilst it is prudent not to label children with a sole abnormality of a low BMC or BMD with osteoporosis, it is becoming increasingly clear that children with chronic diseases can suffer fractures without necessarily having a particular low size-adjusted BMC or BMD (405). Perhaps a fall in bone mass may be a better indicator of fractures in children with chronic disease and requires further investigation (406)

DXA is characterised by relatively fast procedure, low dose of ionising radiation and low cost. The total radiation exposure for whole body scan is below 13micro-sievert, which is significantly below the dose limits of 5000 micro-sievert recommended by the Federal Drug Administration Regulation for exposures from medical research procedures in children (400). On the other hand, it has several limitations; firstly, DXA measurements are expressed as a

two dimensional aBMD (g/cm^2), which depends on bone size and cannot assess the true vBMD (g/cm^3); secondly, DXA cannot differentiate between cortical and trabecular bone; thirdly, inaccuracies result from changes in body composition; and, finally, limited reference data for paediatrics (400). True vBMD depends on both BMC and bone size in a three dimensional projection. BMC can be measured precisely by DXA, whereas the bone size in three dimensions cannot be assessed by DXA as it is unable to measure bone depth.

1.8.1.2 Peripheral quantitative computed tomography

p.QCT has been developed since the 1970s (400) and the first publication was in the early 1990s (407). p.QCT allows paediatric investigators to measure true vBMD, bone geometry (periosteal, endosteal diameters and cortical thickness), bone strength (strain index and fracture load) and body compositions (lean and fat) in the peripheral skeleton (400). p.QCT images are generally obtained from the forearm or lower limb. In the forearm, images are taken at 4% and 20% of radial length (distal to proximal). Total BMC, trabecular vBMD and total cross sectional area are assessed mainly at 4% of radial length, whereas cortical thickness, cortical bone area, cortical vBMD, stress-strain index (SSI) (mm3) and periosteal and endosteal circumferences are measured mainly at 20% of radial length. In the lower limbs images are taken at four different positions (4%, 14%, 38%, 66%) of the tibiae length (from distal to proximal) (400). Each site has specific bone measurements; at 4% position the distal metaphysis of the tibia is more accurate to determine trabecular vBMD (g/cm3), strength data (fracture load (N) and SSI) is more appropriate at 14%-38% of the tibial length. Cortical thickness, cortical bone area, cortical vBMD and body composition data (muscle and fat) are more accessible at 66% of tibial length (408). p.QCT has several advantages over DXA. Firstly, it has ability to provide a three-dimensional image, and thus, it can measure true vBMD rather than aBMD. Secondly, it can differentiate between cortical and trabecular bone. Thirdly, it can measure bone size and geometry (400). On the other hand, total cross-sectional bone area, cortical bone area, periosteal and endosteal circumferences and cortical thickness can be evaluated by p.QCT (409). Measurement of bone geometry properties plays a vital role in determination of bone strength. Therefore, this technique can provide fracture load of measured bone. In addition, p.QCT is characterised by low dose of radiation. However, p.QCT underestimates cortical vBMD when the cortical thickness is below 2mm. Technically; it provides error reading when there is movement during the procedure (400).

1.8.1.3 Quantitative Ultrasound

In 1984, QUS was firstly developed to assess calcaneal bone status in adults. QUS has gained popularity in both clinical and research fields and has become an acceptable method to screen osteoporosis in elderly people (410). Two measures are important in QUS; SOS and BUA. BUA and SOS rise with greater bone connectivity and higher in normal than osteoporotic subjects. It is found that BUA parameter is mainly influenced by trabecular separation and bone connectivity, whereas SOS is mainly influenced by trabecular separation (411). SOS is the ratio between the traversed distance and the transit time (m/s). SOS (m/s) is dependent on several factors, including bone density, bone structure and elasticity. Attenuation is defined as the energy which is lost during transmitting ultrasound waves through materials. There is a linear relationship between total attenuation and the frequency. The slope of attenuation relying on the frequency in decibel per megahertz (dB/ MHz/cm) has been known in clinical practice in BUA (400).There is a positive relationship between the SOS and age, physical activity, weight and height. Zadik et al. (412) also observed that the SOS at radius increased sharply during the first five years in both genders. After that, there was a much slower decrease over the next five years. During puberty, the SOS again increased substantially at 11years and 14years of age for girls and boys respectively. A diagnostic sensitivity of QUS can be influenced by significant changes in cortical bone distribution during growth; however, it can be improved by calculating z score. The major advantages of QUS are low cost, greater speed, increased portability, accessibility and no ionised radiation. Moreover, QUS can be applied successfully and precisely in infants from 24 weeks gestation through to full term as the SOS increases with gestational age (413). On the other hand, the actual bone deficit cannot be identified by QUS such as BMD and BMC (400). Furthermore, the variability between the QUS and DXA measurements make the sensitivity of QUS in assessing bone abnormalities relatively low. It is also reported that the correlation between hip BMD and calcaneal QUS is more positively with BUA rather than SOS especially after 12 months training in healthy adult men (414) .

	DXA	p.QCT	QUS
Region of interest	Total body Spine Hips Arms Legs	Radius Tibia	Radius Tibia Phalanx Calcaneus
Parameters	BA BMC aBMD BMC/BMD Z-score BMD T-score BMAD	BA BMC vBMD Bone geometry Bone strength	SOS BUA Percentile Z-score
Advantages	Accessible Accepted Low cost Low dose of radiation	Non-invasive Measures true v-BMD (three dimensional) Measures bone strength Measures bone geometry Differentiate between cortical and trabecular bone Low dose of radiation	Fast/easy Low cost No ionized radiation Portable
Disadvantages	Cannot measure v-BMD	Difficult in young children Variability of trabecular BMD throughout the metaphysic at different positions of the measurements Movement artifacts, Dose not differentiate clearly between the inner margin of cortical bone and the outer margin of trabecular bone, particularly close to the growth plate The resolution can be improved by using thinner slices Wide ranges in trabecular BMD measured at different times (baseline-6minths) (415). No reference data for tibial scans	Can not measure BA,BMC, BMD

Tab 1.14: The difference between three bone assessment methods (DXA, p.QCT and QUS) in terms of region of interest, bone parameters, advantages and disadvantages. BA bone area, BMC, bone mineral content, areal bone mineral density (aBMD), volumetric bone mineral density (vBMD), BMAD bone mineral apparent density, SOS speed of sound, BUA broadband ultrasound attenuation.

1.8.2 Muscle Function Assessment

Muscles are connected to bones anatomically and physiologically. Therefore, the development of muscle mass should be matched with the development of bone mass and properties in order to prevent fracture (237). This explains why athletes have stronger bones compared with non-athletes. In addition to physical activity, gravity (GFR) plays also a vital role in the development of the musculoskeletal system. In the present studies, the assessment of muscle function can be done by Leonardo mechanography and MIGF.

1.8.2.1 Leonardo Mechanography

Leonardo Mechanography (Novotecc Medical GmbH, Pforzheim, Germany) is a device used to assess the dynamic (kinetic) parameters deriving from motor performance. Leonardo Mechanography has two parts: the mechanography software (Version 4.2-b05.53-RES was used in this thesis) and the Leonardo ground reaction force platform (GRFP) hardware (Fig.1.22). The ideal place for the GPFP is concrete (solid and even surface). These two components are connected to each other by USB cable and the software analyses data after patients jump on the GPFP. There are three types of jump: single two leg jump (s2LJ), multiple one leg jump (m1LJ) and heal rise test (HRT) chair rising test (CRT) (416). There are three phases for the measurement: However, before a subject steps onto GPFP, it is important to make a zero adjustment for the platform by pressing a zero adjust button on the computer screen. Phase1: the subject steps on the GPFP and stands straight and still for at least two seconds with each bare foot (wearing only socks) on each force measurement plate. At the end of phase 1, a single beep will be heard to ready the subject for the next phase. In phase 2, the subject is asked to perform a jump. Phase 3: after the measurement itself, the subject is asked to stand still on the GPFP for a few seconds until it is indicated by a double beeping sound (416). The measurement can be repeated three times and the highest reading of the highest jump is selected. The main measurement outcome of the Leonardo mechanography is described in Tab.1.15 A few studies measure muscle performance using mechanography (237;355;362;380;417-420).

A recent study has shown that coefficient of variation (CV) for the Leonardo mechanography measurements in children ranged from 3.4–7.5% s2LJ, m1LJ and HRT, whereas it was higher for CRT (16%) (416). The CV results can be either influenced by jumping with shoes or jumping with bare feet. This indicates that it is necessary to standardise the measurement method condition. Shosed foot seems to have low CV compared with bare foot apart from the Vmax test (421). A positive correlation between GFR assessed by the Leonardo mechanography and body size in paediatrics are observed. Therefore, mechanography is a novel device that assesses muscle function in children and adolescents (417).

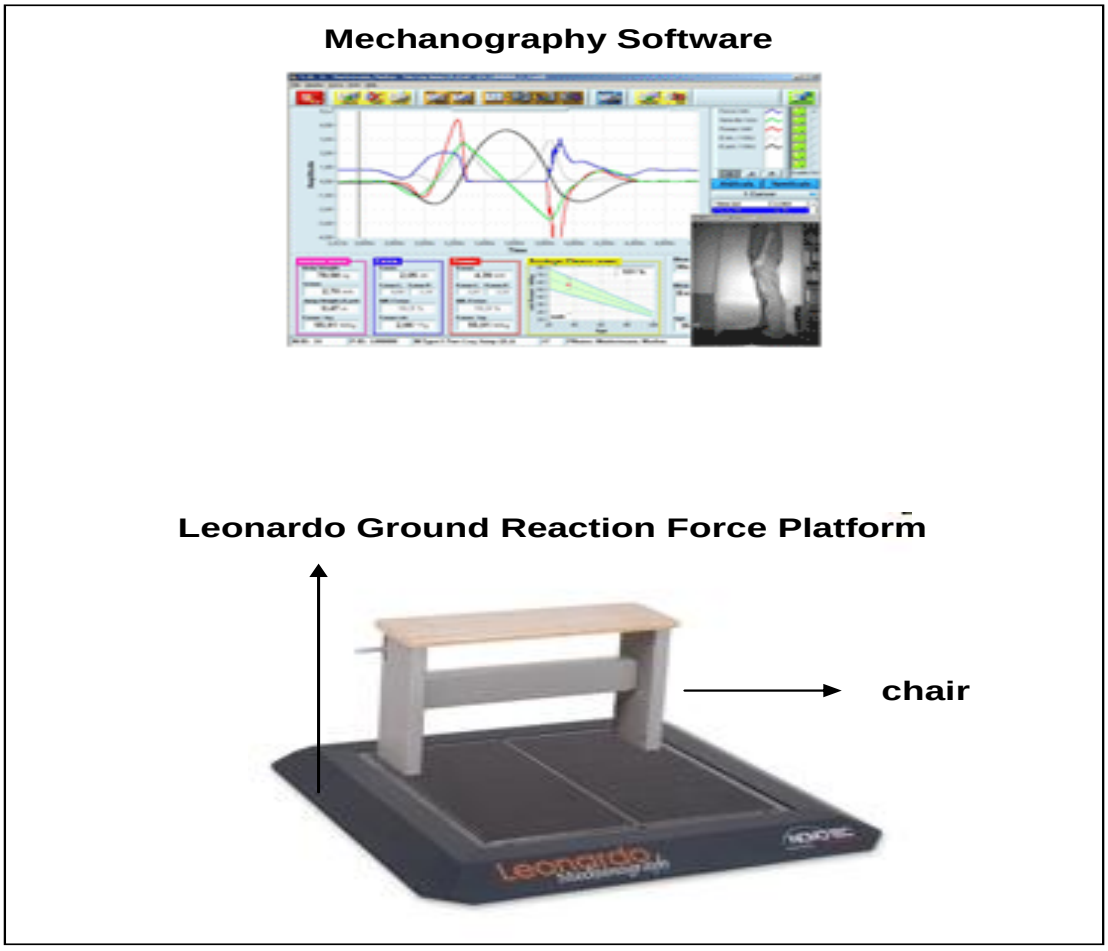


Fig 1.22: Leonardo mechanography consists of two parts: the mechanography software installed in the computer and the Leonardo ground reaction force platform (GRFP) hardware with chair.

Measurement	Abbreviation	Units	Preferable Test
Esslinger Fitness Index*	E.F.I	%	s2LJ
Jump Efficiency	Efficienc	%	s2LJ
Jump Height	JH	m	s2LJ
Velocity maximum	V max	m/s	s2LJ,HRT,CRT
Maximum force	F max tot	kN	All tests
Maximum force in the left leg	F max L	kN	m1LJ, s2LJ
Maximum force in the right leg	F max R	kN	m1LJ, s2LJ
Maximum force differences between legs	diff. F max	%	m1LJ, s2LJ
Maximum force related to body weight	F max tot rel	Fg*	All tests
Maximum power	P max tot	kW	s2LJ,HRT,CRT
Maximum power in the left leg	P max L	kW	s2LJ
Maximum power in the right leg	P max R	kW	s2LJ
Maximum power differences between legs	Diff. P max	%	s2LJ
Maximum power related to body weight	P max / kg	W/kg	s2LJ,HRT,CRT
Energy store by pre-tension muscle	E Store / kg	mJ/kg	All tests

Tab 1.15: Esslinger fitness Index (E.F.I) represents correlation between maximum power related to body weight and age for both sexes compared with reference data population. A value of 100% is equal to the average of the reference group (422). Jump efficiency is efficiency of movement and is the maximum power relative to maximum force. Jump height measured by metre (m), the maximum jump velocity is the maximum lift off velocity measured by meter/second (m/s). The unit of maximum force to lift off of the jump is kilo-Newton (kN), the unit of maximum power to lift off of the jump phase of the jump is kilo-Watt (kW). Maximum force/power differences between legs during lift off phase of the jump (kN/kW). The maximum force related to body weight is Fg (Xtimes the body weight of the subject) and the Maximum power related to body weight measured by Watt/kilogram (W/kg).The unit of Energy store by pre-tension muscle can be defined when the subject bends his knees before the lift off movement; at this stage, the muscle increases length as well as force, which is equivalent to a spring storing energy when compressed or extended. This energy is used to increase the force and power output for the lift off of the jump. The unit is mega joules per kilogram (mJ kg). Each outcome can be mainly determined by different (416).

1.8.2.2 Maximal Isometric Grip Force

MIGF is a simple reliable method used to determine the maximum handgrip strength. Handgrip strength is an important test to evaluate physical fitness. There are several types of devices used to measure MIGF such as the Jamar the Saehan, the Biodex dynamometers, Myogrip and Martin Vigorimeter. No much difference is observed between the Jamar and the Biodex dynamometers, and Myogrip in the validity and reliability of these instruments (423;424). In adults, the coefficient variance (CV) of the Jamar dynamometer (6%) is lower than the Biodex dynamometers (17%) (425). The Jamar dynamometer is a reliable instrument to evaluate MIGF in children aged from 6–12years (426). MIGF is dependent on age, gender, BMI and ethnicity (427). BMD in post-menopausal women has a positive correlation with MIGF (428) and some research suggests MIGF can be used as a predictive value for osteoporosis (429). Low MIGF can be applied as a predictive value for poor hospital outcome in children with critical condition (430). It is reported that men have a higher MIGF than women and with the peak measurement at 35 years of age and then decreases continuously. Positive influencing factors with MIGF are forearm circumference, hand size, body mass and height (431). The reference data for MIGF should be adjusted for age and gender rather than height (427;432). In our studies, A Jamar handgrip dynamometer (Preston, Jackson, MI, USA) was used to measure forearm muscle force in dominant and non-dominant arm and the highest measurements were recorded. The MIGF (N) data were converted into age and height based SDS in children (433). The participants were asked to squeeze as hard as possible. In adult measurements (Chapter 4), the elbow was fully extended, whereas in children with ALL study (chapter5), the hand was flexed at 90°.

1.8.3 Body Composition Assessment

There are several techniques that are used to measure body composition in paediatrics. However, some of these techniques cannot be practically used such as hydrostatic underwater weighing to determine body FM and body LM. We used three main approaches to assess body composition in this thesis: anthropometry, Bioelectrical impedance and DXA.

1.8.3.1 Anthropometry

The actual measurements of height and weight and BMI can be converted into SDS for chronological age using 1990 UK standards (434;435). The anthropometric measurements SDS are more practical in the paediatric population than the 3rd centile; this would reduce the false positive rate reading (436). BMI has been recommended for evaluating overweight and obesity in children and adolescents in the clinical setting and it is a useful proxy measure of adiposity (437). Calculates of BMI based on weight and height (BMI=weight/height^2) have been available for many years (438).The advantages of BMI is its cheap cost and relative ease of use; it can also be used as screen test for obesity in children. The limitations of BMI include high reading of BMI, which does not always mean obesity. Because the BMI calculation depends on weight and height, BMI does not differentiate between FM and LM. For example; the BMI of athletes is high because of the level of LM not from FM (439). Therefore, additional research on alternatives or adjuncts to BMI is needed.

1.8.3.2 Bioelectrical Impedance

Bioelectrical impedance is an extremely popular method for assessing body composition. The mechanism of bioelectrical impedance is based upon the conduction of an applied electrical current at multiple frequencies in the body. The flow of a low frequency current is dependent on the amount of water found in the human body. The highest percentage of water in the human body is found in LM, and the least amount in fat and bone (Fig.1.23). Therefore, muscle tissue has low resistance to electrical currents and bone and fat tissue have high resistance to electrical currents (440). This technique finally estimates total body water (TBW), fat free mass (FFM) and FM% (441). The technique is simple, non-invasive, relatively cheap and applicable to individuals of almost all ages. However, bioelectrical impedance results can be influenced by the level of hydration and the density of the FFM, which are not stable among individuals (440). Furthermore, this technique does not provide accurate measurements of body composition in overweight or obese children because of the large errors in individual estimates (442;443). Because of these limitations, this technique is not recommended for measuring body composition by researchers, clinicians and practitioners (444). There are different devices used to measure bioelectrical impedance such as Tanita and Xitron Hydra. In our study, we assessed body compositing by using foot-to-foot Tanita

TBF-300 (Tanita Corp., Tokyo, Japan) that delivers a current with a single frequency of 50 kHz. The measurements were performed in a standing position barefoot, with two footpad electrodes in contact with soles and heels on both feet and following the manufacturer's instructions.

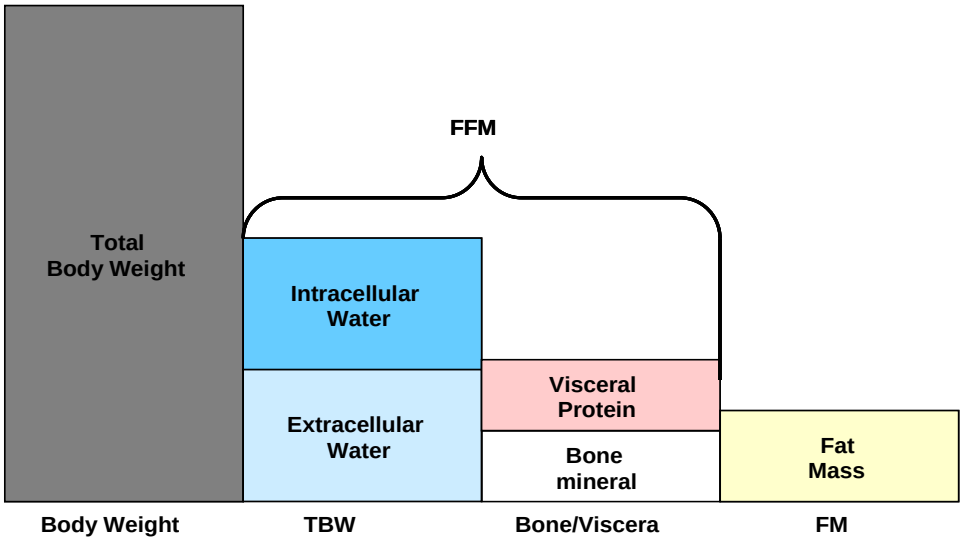


Fig 1.23: Schematic diagram of Bioelectrical impedance assessments; total body weight, total body water (intracellular and extracellular water) (TBW), free fat mass (FFM) and fat mass (FM).

1.8.3.3 Dual Energy X-Ray Absorptiometry

DXA is an accurate and precise method of measuring soft tissue body composition. The basic principle of DXA was described earlier. Compared with bioelectrical impedance analysis, DXA has been validated in many populations (445). DXA can provide a recommended method for assessing body composition in obese children adolescents aged 5–21 years. There are some concerns that size and shape of obese children might exceed the scanning area, which leads to some tissue being missed out of the scanning area (446). However, this problem can be reduced by applying a half body DXA scan (447). The body composition parameters assessed by DXA includes FM%, FM and LM in different area of human body (total body, trunk, arms and legs).

1.9 Aims

ALL is the most common childhood malignancy. With the survival rates improving to over 90% (171), research has focused on the recognition and reduction of treatment-related morbidity. Skeletal morbidity, characterised by MSP, fractures and ON is frequently reported in ALL children at diagnosis, during chemotherapy and thereafter. Morbidity in these children has a negative impact on the quality of life, which might lead to immobility and also require surgical interventions. This thesis presents four studies which examine the extent of skeletal morbidity in children with ALL and then explore the use of physical activity as a means of improving bone health. The specific aims of the four studies performed are outlined as follows (Fig.1.24).

1.9.1 Skeletal Morbidity in Children Receiving Chemotherapy for ALL (Chapter Two)

Aim: To determine the incidence rate and risk factors of skeletal morbidity (MSP, factures and ON) in children treated for ALL, we performed a retrospective study of ALL children treated with chemotherapy at the Royal Hospital for Sick Children, Glasgow between 1997 and 2007 on two consecutive protocols (UKALL97/01 and UKALL2003).

1.9.2 Skeletal Morbidity in Children Receiving Chemotherapy for ALL and Its Association with Mineral Homeostasis and Duration of Inpatient Stay (Chapter Three)

Aim: To investigate the influence of in-patient stay, age and mineral status (Ca, Pho, Mg) over the first 12 months of chemotherapy on subsequent skeletal morbidity, we collected data retrospectively from ALL children treated with UKALL 2003 from 2003 to 2009.

1.9.3 A Comparison of the Effect of Two Types of Vibration Exercise on the Endocrine and Musculoskeletal System (Chapter Four)

Aim: To assess the short-term and medium-term effects of sinusoidal and vertical WBV delivered through the Galileo platform (GP) and the Juvent1000 platform (JP), respectively on a range of outcome measures related to the endocrine and musculoskeletal system. We performed this study in the normal healthy adult men aged 20-50years.

1.9.4 A Randomised Controlled Trial of the Effect of Vibrational Exercise on the Bone Health of Children with ALL (Chapter Five)

Aim: To explore the feasibility using WBV in children receiving chemotherapy for ALL and assess its effect on bone health and body composition. The study was performed prospectively in children with ALL, who presented to the Royal Hospital for Sick Children between 2005 and 2009. In this study ALL children randomised into either receiving WBV or acting as control.

Study 1

Hypothesis: Leukaemia and chemotherapy might have a detrimental effect on bone health.
Aim: To determine the incidence rate and risk factors of skeletal morbidity in children treated for ALL.

Study 2

Hypothesis: Physical inactivity and abnormal mineral homeostasis might have a negative impact on bone.
Aim: To investigate the influence of in-patient stay and mineral status on subsequent skeletal morbidity .

Study 3

Hypothesis: Different WBV training might have different effects on the endocrine and musculoskeletal system.
Aim: To compare the effects of two regimens of WBV on endocrine status and musculoskeletal system.

Study 4

Hypothesis: WBV training in ALL children might have a beneficial effect on bone health
Aim: To explore the feasibility using WBV in ALL children and assess its effect on bone health and body composition

Fig 1.24: The summary of the hypothesis and aims for the studies in the present thesis.

Chapter 2

Skeletal Morbidity in Children Receiving Chemotherapy For Acute Lymphoblastic Leukaemia

2.1 Abstract

Background: Children receiving chemotherapy for ALL may be susceptible to skeletal morbidity.

Aim: To determine the incidence and risk factors for skeletal morbidity in ALL children.

Patients and Methods: The medical records of all (n,186,male:110) children presenting to a single centre with ALL between 1997 and 2007 and treated on UKALL97, UKALL97/01 or UKALL2003 were studied. Skeletal morbidity included MSP, fractures and ON. MSP was classified as any event of limb pain, muscle pain, joint symptoms or back pain that required radiological examination. Fractures and ON were confirmed by X-rays and MRI respectively.

Results: Skeletal morbidity, presenting as MSP, fractures or ON were reported in 88(47%) children of whom 56(63%) were boys. Of 88 children, 49(55%), 27(30%) and 18(20%) had MSP, fracture(s) or ON respectively. 6(7%) had both fractures and ON. The median (10^{th},90^{th}centiles) age at diagnosis of ALL in those children without skeletal morbidity was 3.9years(1.4,12)which was lower than in those with skeletal morbidity at 8.2years(2.2,14.3)($p<0.00001$,95%CI:1.7,4.4). Children with ALL diagnosed over 8years of age were at increased risk of developing fracture(s) ($p=0.01$,OR=2.9, 95%CI:1.3,6.5) whereas the risk of ON was higher in those who were diagnosed after 9 years of age($p<0.0001$,OR=15,95%CI:4.1,54.4). There was no gender-difference in the incidence of skeletal complications. Children who received dexamethasone had a higher incidence of skeletal morbidity than those who were treated with prednisolone($p=0.027$,OR=2.6,95%CI: 1.1,5.9).

Conclusion: The occurrence of skeletal morbidity in ALL children may be influenced by age and the type of GCs. These findings may facilitate the development of effective bone protective intervention.

2.2 Introduction

Acute lymphoblastic leukaemia (ALL) is the commonest childhood cancer with a 5year survival rate of over 80% (448). With the sustained improvement in survival rates, attention has been directed towards recognition and prevention of disease and treatment-related morbidity. Over the last two decades, skeletal morbidity is increasingly being recognized as a major problem in these children and may occur at diagnosis as well as during or following treatment (449). It may present as MSP, fractures, ON, pain or loss of mobility and deformity, with resultant adverse consequences on quality of life. Different underlying causes and mechanisms may explain several MSP symptoms in ALL children. In this study MSP included any event of limb pain, joint and muscle symptoms or back pain that required diagnostic imaging and no evidence of fractures and ON. The most common causes for evaluating MSP were trauma, infection and joint swelling such as haemarthrosis. A clear understanding of this group of complications may enable the institution of rational strategies that can improve bone health. Whilst the aetiology of skeletal morbidity in this group of children may be multifactorial and include the disease itself, chemotherapy, poor nutrition, mineral abnormalities, physical inactivity, low LM and ongoing inflammation (172), GC therapy is well known to be associated with poor skeletal development in many clinical conditions in childhood and may play a vital contributory role in the skeletal morbidity in children receiving chemotherapy for ALL (450). In ALL, GC therapy, as well as other intensive therapy, is associated with abnormalities of markers of bone turnover that favour bone resorption (182) and these abnormalities may be more pronounced in those children who receive dexamethasone rather than prednisolone (68). An increased predisposition towards skeletal morbidity in children receiving dexamethasone has been observed by Strauss et al. (71) but not by other investigators (72). Over the last decade, the chemotherapy protocols for the treatment of ALL in the United Kingdom have evolved from a clinical trial where children received either dexamethasone or prednisolone (UKALL97/01) to solely dexamethasone (UKALL2003). The aim of the current study was to perform a retrospective survey of the extent of skeletal morbidity encountered in children treated on these protocols and to investigate the relationship of this morbidity to therapy and patient related factors.

2.3 Methods

2.3.1 Patients

The medical records of 186 consecutive patients (male,110) who were diagnosed between January 1997 and December 2007 to the Royal Hospital for Sick Children in Glasgow with a diagnosis of ALL were examined for information regarding skeletal morbidity including MSP, fractures and ON until 6 months after the last diagnosis. The median follow up for boys and girls was 5.7years (1.9,10) and 5.9years (2.3,10), respectively (NS). The median (10^{th},90^{th} centiles) age at presentation for the whole cohort was 5.3years (1.7,13.7) and there was no significant difference between the median age at diagnosis of boys at 5.6years(1.7,12.6) and girls at 5.3years(1.7,13.7). Skeletal morbidity included MSP, fractures and ON. MSP was defined as any event of limb pain, joint and muscle symptoms or back pain that required diagnostic imaging. Fractures were confirmed by X-ray whereas ON was confirmed by XR and MRI imaging. Location of skeletal morbidity, gender distribution, age and details of chemotherapy at presentation of skeletal morbidity were also recorded. Of the 186 children, 12 did not survive and 10 children proceeded to bone marrow transplantation.

Between 1997 and 2001, children were randomized to receive prednisolone or dexamethasone according to UKALL97. However, dexamethasone was the steroid administered to all patients during intensification blocks of therapy on this protocol irrespective of steroid randomisation. From 2001 to 2003, all children received dexamethasone as GC therapy according to UKALL97/01 and from 2003 onwards, children were treated with dexamethasone as part of UKALL2003. Of the 186 children, 31 were randomized to receive prednisolone; 6 were treated by high-risk ALL (HRL); 2 were treated by infant leukaemia regimen; 82 received dexamethasone as part of UKALL97 or 97/01 and 65 (26, 10, 28 regimen A, B and C respectively and 1 treated by infant leukaemia regimen) children received dexamethasone as part of UKALL2003. Therefore, the total number of children who were treated with dexamethasone was 146. Dexamethasone was converted into prednisolone equivalence by multiplying its dose by a conversion factor of 6.67 based on the relative anti-inflammatory responses. The total dose of GCs for both protocols was calculated as prednisolone and prednisolone equivalence (Tab.2.1). The total amount of GCs was lower in the prednisolone treated children than the prednisolone-equivalence dose

(dexamethasone treated children) in the UKALL97/01 (Tab.2.1). Comparing steroid doses between the UKALL97/01 and UKALL2003 protocols, shows that the calculated doses of prednisolone in the UKALL97/01 was slightly lower than the doses of pred-Equivalent (dexamethasone) in the UKALL2003. Boys had a longer duration of GCs and, therefore, a higher cumulative dose of GCs.

2.3.2 Statistical Analysis

Results are presented as medians and 10^{th} and 90^{th} centiles. Statistical analysis was performed with XL STAT V7·0 (Addinsoft, Paris, France), Minitab15 (Minitab, Coventry, UK) and MS Excel 2003 (Microsoft Corp, Redmond, WA). Difference between groups was assessed using the Mann Whitney U test and the Pearson Correlation, logistic regression and the Chi squared test were employed to assess any association between groups of variables. The study was approved by the local Ethics Committee as an audit of a standard treatment protocol.

UKALL97, 97/01				
Gender	**Girls A, B**	**Boys A, B**	**Girls C**	**Boys C**
Duration (week)	112	164	118	169
Total Pred (mg/m^2)	7728	10328	6728	9328
Total Dex Pred-Equivalent (mg/m^2)	1230 8204	1652 11019	1067 7117	1490 9938
Pred Pred-Equivalent (mg/m^2/week)	69 73	62 67	57 60	55 58
UKALL2003				
Gender	**Girls A, B**	**Boys A, B**	**Girls C**	**Boys C**
Duration (week)	112	164	118	170
Dex (D1) (mg/m^2) Pred-Equivalent	1080 7203	1470 9805	1010 6736	1430 9538
Dex(D2) (mg/m^2) Pred-Equivalent	1160 7737	1550 10338		
Pred-Equivalent (D1) Pred-Equivalent (D2) mg/m^2 /week	64 69	60 63	57	56

Tab 2.1: The cumulative dose of prednisolone and/or dexamethasone in the UKALL97, UKALL97/01 and the UKALL 2003 protocol. Each protocol was classified into A, B, C regimens. Dexamethasone doses were converted to prednisolone equivalents by multiplying the dexamethasone dose by a conversion factor of 6.67 which is based on the relative anti-inflammatory properties of the two drugs. In the UKALL97/01, children were randomized to receive either prednisolone or dexamethasone, whereas in the UKALL2003, children were randomized to treatment intensification; delayed intensification I (D1) and delayed intensification II (D2).

2.4 Results

2.4.1 Skeletal morbidity

Skeletal morbidity, as MSP alone, fracture(s) or ON were reported in 88(47%) of the 186 children, and 56(63%) were boys (Fig.2.1). Of the 88 children, MSP alone, i.e. without any evidence of fracture(s) or ON was present in 49(55%) of whom 29(59%) were boys. Fractures alone occurred in 21(23.8%) of whom 16(76%) were boys and ON alone in 12(13%) of whom 7(58%) were boys. A further 6(6.8%) children had both fractures and ON. Therefore, the total incidence of MSP, fractures and ON was 49/186 (26%), 27/186(14.5%) and 18/186(9.7%), respectively.

2.4.2 Timing and age

Whilst the median age at diagnosis of ALL was 5.3years(1.7,13.6) for the whole cohort (Fig.2.2), the median age at diagnosis of those children without skeletal morbidity was 3.9years(1.5,12) and lower than in those with skeletal morbidity 8years(2.2,14.3) ($p<0.00001$,95%CI:1.7,4.4). The median age at diagnosis of ALL in those children with MSP was 6.4years(2,14). The median age at diagnosis of ALL in those children with a fracture was 8.3years(2.1,13.8) and lower than that for children with ON at 12.2years(6.8,14.9)($p=0.0077$,95%CI:1.1,6.3). Furthermore, the median age of children at diagnosis of fracture(s) was 10years(4.8,16) and at diagnosis of ON was 13.8years(9.6,18)($p=0.002$,95%CI:1.6,6.6). The first fracture occurred after a median duration of chemotherapy of 18.7months(4.3, 35) whereas the first event of ON occurred at a median of 29 months(8.8, 48) after the start of chemotherapy (NS). Six (33%) out of the 18 children with ON had Total Body Irradiation (TBI) and the remaining 12(67%) were just treated with standard anti-leukaemia chemotherapy (SAC). The median age at diagnosis of ALL in those children with ON who were treated with TBI was 11.4years(5,14) and lower than for children with ON treated with SAC at 12.2years(9,14)($p=0.4$,95%CI-5.7,1.8). The median age of children at diagnosis of ON in the former and the latter was 13.8years(10,18) and 14years(10,18)($p=0.9$,95%CI:-3.5,3.5), respectively. The median time from diagnosis of ALL to first diagnosis of ON was 17months(3,49) and 46months(29,73) in the SAC and TBI respectively ($p=0.02$,95%CI:8.0,39.0). Children over 8years at diagnosis had a significant

(p=0.01, OR=2.9, 95% CI:1.3,6.5) risk of developing fractures whereas the risk of ON was seen in children over 9years at diagnosis (p<0.0001,OR=15.9, 95%%CI:4.14:54.37).

2.4.3 Sex Distribution

No sex differences were observed in any form of skeletal morbidity. The incidence of MSP in boys and girls was almost equal 29/110(26%) and 20/76(26%), respectively whereas the incidence of fractures in boys at 21/110(19%) was over twice compared to girls at 6/76(8%). In addition, there was no difference in the incidence of ON between boys at 11/110(10%) and girls at 7/76(9%). The median age at time of fracture(s) in girls was 10.2years(3.7,12.4) which was almost similar to boys at 9.9years(5.2,16.2). The median age at diagnosis of ON in boys occurred at 14.6years(10.6,18.5) and in girls at 13.1years of age(9.6,15.8)(NS).

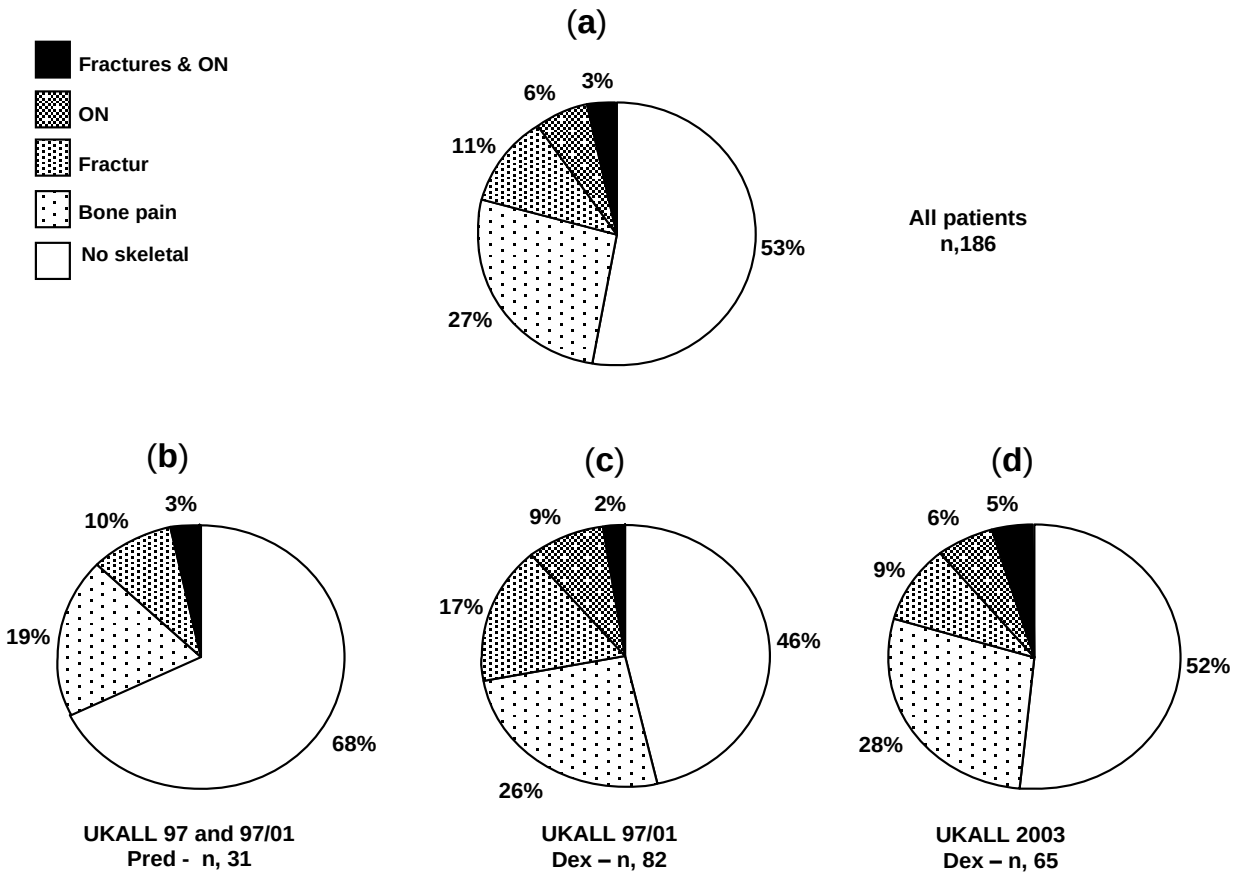


Fig 2.1: The distribution of skeletal morbidity by protocol and distribution of children who suffered from musculoskeletal pain (MSP) only, fractures, osteonecrosis (ON) or a combination of ON and fractures. (a) each skeletal morbidity in the whole cohort who received 5 different protocols (UKALL97 and UKALL97/01(n,113),UKALL2003(n,64), HRLALL(n,6) and infant leukaemia regimen(n,3)).(b) the distribution of each skeletal morbidity in those children who were treated by prednisolone (UKALL97). (c) The distribution of each skeletal morbidity in those children who were treated with dexamethasone (UKALL97/01). (d) The distribution of each skeletal morbidity in those children who were treated by dexamethasone (UKALL 2003).

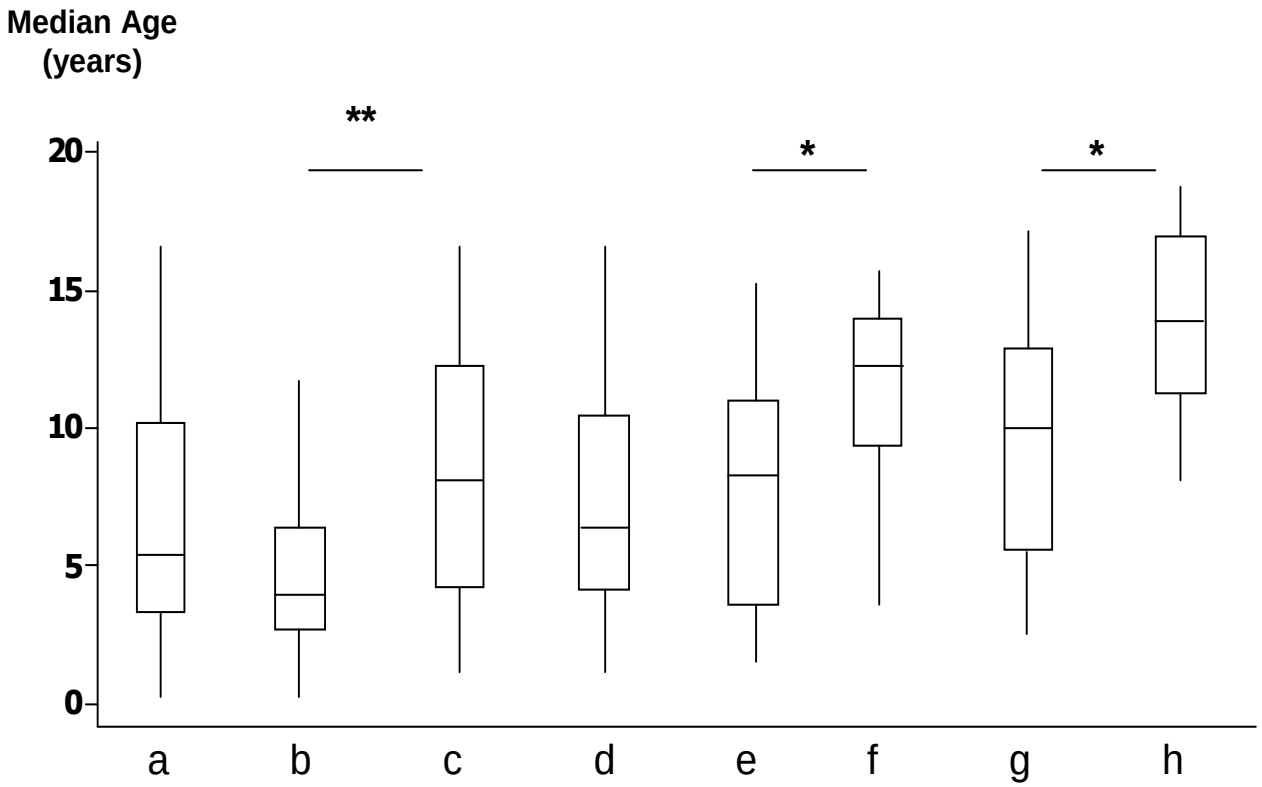


Fig 2.2: Median, 25th, 75th centiles, minimum and maximum values for age at (a) diagnosis of ALL for the whole group (b) diagnosis of ALL for those who had no skeletal morbidity, diagnosis of ALL for those who had skeletal morbidity, (d) diagnosis of ALL who had musculoskeletal pain (MSP), (e) diagnosis of ALL who had fractures and (f) diagnosis of ALL who had osteonecrosis (ON) (g) diagnosis of fractures (h) diagnosis of ON. *p=0.01, **p<0.0001.

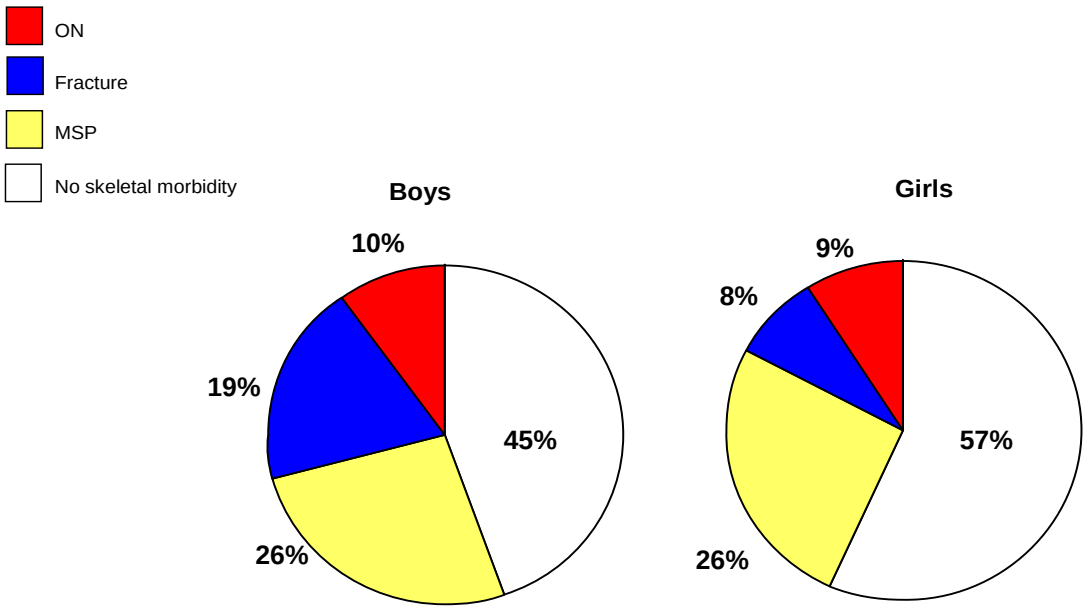


Fig 2.3: The distribution of skeletal morbidity in ALL children in boys and girls. No sex differences were observed in any form of skeletal morbidity

2.4.4 Glucocorticoids

Children treated with prednisolone had a lower incidence of skeletal morbidity than those treated with dexamethasone by about 2.6times (p=0.027, 95%CI:1.1,5.9) (Fig.2.1). MSP alone as a feature of skeletal morbidity occurred in 49 children in total. This group consisted of 6/31(19%) children who were treated with prednisolone and 40/146 children (27%) treated with dexamethasone; 3/6 children (50%) were treated with HRL and no skeletal morbidity was observed in children who received the infant protocols (n,2). The incidence of fractures in those children who were treated with prednisolone and dexamethasone was 3/31(9.6%) and 24/146(16%), respectively. The incidence of ON in those children who were treated with dexamethasone was higher at 16/146(11%) than in those children who were treated with prednisolone and HRL regimen at 1/29(3.5%).

2.4.5 Site of Skeletal Morbidity

The 88 children with complaints of skeletal morbidity had 246 x–ray examinations. In order of frequency, 134(54%) examinations were performed in lower limbs (foot, tibiae, fibulae, knees and femora), 63(26%) in axial bone (spine and pelvis) and the remaining 49(11%) in the upper limbs (hands, radii, ulnae, humeri) (Fig.2.4-.5). In the 28 cases who had fractures, 16(57%) children had a single fracture, 7(25%) children had two fractures and the remaining 5(18%) had more than two fractures. Of a total of 43 fractures, 22(51%) occurred in the lower limbs of which 10 occurred in the feet. Fractures in the upper limbs occurred in 17(40%) cases of which 14 occurred in the hands (n,3) and forearms (n,11). In the remaining 4(9%) cases, fractures occurred in the spine. Amongst the 35 cases of ON, 13, 9 and 7 cases occurred in the knees, hips and shoulders, respectively. ON was multifocal in 10/18(55%) children who were affected.

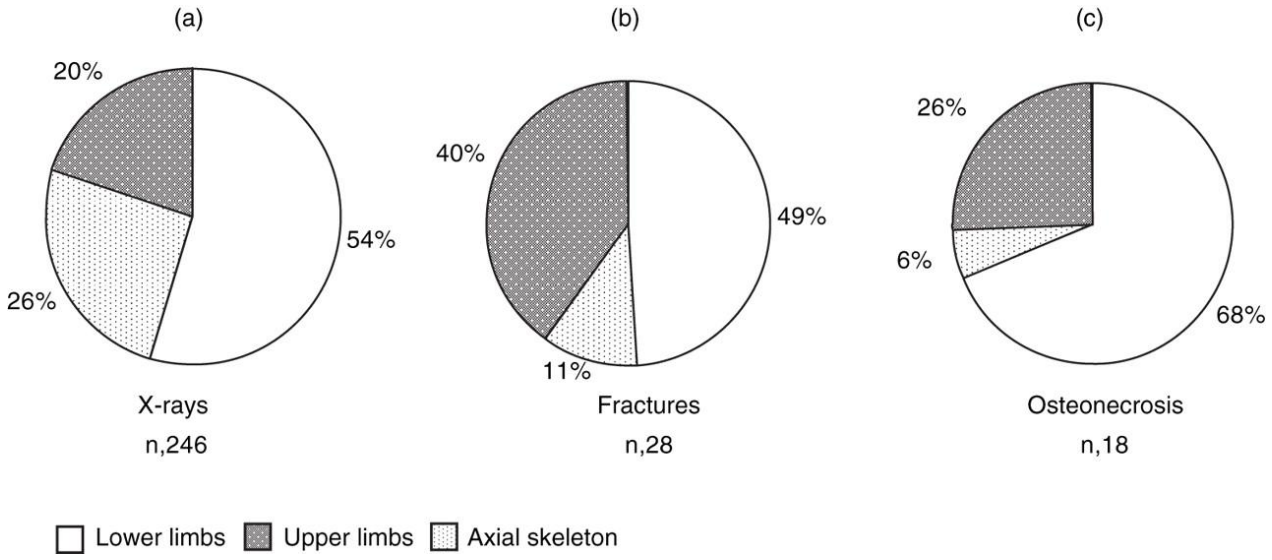


Fig 2.4: The proportion of sites (lower limbs, upper limbs and axial skeleton) affected by skeletal morbidity as judged by (a) X-rays performed for MSP (b) the distribution of fractures and (c) the distribution of ON.

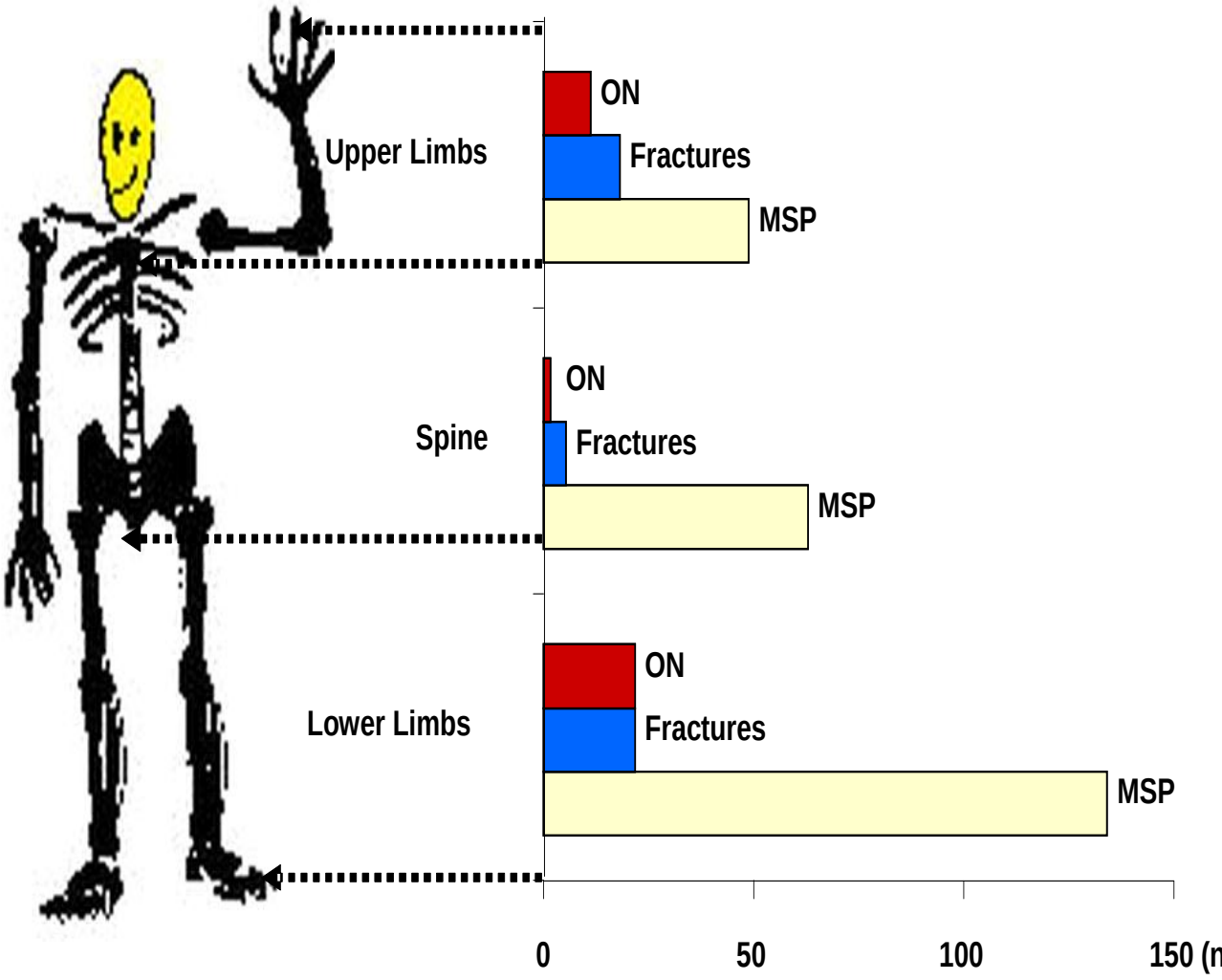


Fig 2.5: The location of skeletal morbidity (MSP, facture and ON) in lower limbs, spine and upper limbs.

2.5 Discussion

This current review represents the largest systematic single centre study of skeletal morbidity in children who received chemotherapy for ALL in the United Kingdom. By defining skeletal morbidity as those events of MSP that required radiological imaging our study may have underestimated the incidence of skeletal morbidity. However, despite this limitation, the study showed that MSP was sufficiently severe to require imaging in about half of children with ALL. In about half of this group of children with MSP, there were no abnormal findings on imaging

and in the other half; there was clear evidence of a fracture or ON. Thus, a quarter of children had skeletal morbidity which was confirmed on imaging. These results show a markedly higher incidence of skeletal morbidity than those reported by Mitchell et al (451), who reported the incidence of osteopenia and ON in over 1600 children with leukaemia who received dexamethasone or prednisolone in ALL97 and ALL99. This study was aiming to improve event free and overall survival and did not look specifically to the incidence of skeletal morbidities particularly MSP and fractures. However, our results are generally similar to those reported for fractures and ON by previous investigators (71-73;183;184). Trabecular bone, as found in the axial skeleton, is more sensitive to GIO induced osteoporosis (452). However, the current study, as well as previous studies, tends to suggest that fractures are more likely to be identified in the appendicular skeleton in children receiving ALL chemotherapy. This observation may represent a selection bias as diagnostic imaging may only have been undertaken in those children where there was clinical concern. Given that vertebral fractures can be difficult to diagnose in children and that symptoms may overlap with general muscular and postural pain (453), it is possible that fractures in the axial skeleton may not be identified without systematic radiological screening of the spine. Detailed vertebral morphometry studies performed recently in 186 children with ALL suggested that 16% may have a vertebral deformity consistent with a compression fracture (73;453). However, these vertebral compressions were assessed within 30 days of diagnosis, and could be viewed as presenting features rather than complications of therapy.

Our finding of a clear association of fractures with dexamethasone administration has only been described by one other group of investigators (71;453). GCs are an essential component of treatment for ALL. Compared to prednisolone, dexamethasone has enhanced lymphoblast cytotoxicity and penetration of the central nervous system, even at a dosage that is equipotent for GC effect. This finding has led to the substitution of dexamethasone for prednisolone in the treatment of ALL and to improved event-free and overall survival (451). However, dexamethasone is associated with increased toxicity (451) with more marked adverse effects on growth, bone turnover and surrogate biochemical markers of FM and insulin sensitivity in children with ALL (68;454). The extent of GC effect may be variable and the concept of ‘one equipotent dose for GC effect’ needs to be reconsidered as it may be an end-organ specific phenomenon. Our observation that ON was almost exclusively associated

with the use of dexamethasone is also notable and may reflect on the aetiology of this condition. Despite relatively minor differences in prednisolone equivalent doses in the dexamethasone group vs the prednisone group (less than 10%), we observed a threefold greater incidence of musculoskeletal morbidity in the dexamethasone treated group.

Our observation that ON was more commonly associated with the use of dexamethasone is also notable and may reflect on the aetiology of this condition but needs to be confirmed in larger studies. The incidence and distribution of symptomatic ON in the current study was similar to that reported in previous studies (71;455). The lack of sexual dimorphism, earlier timing, association with large joints, particularly in the weight bearing appendicular skeleton, and almost exclusive association of ON with dexamethasone emphasizes the fact that the aetiology of ON is different to that of fractures. Studies on animals with steroid-induced ON in the femoral head show hypertrophy and hyperplasia of marrow fat cells and lipid deposition in osteocytes (456). In addition, hyperlipidaemia secondary to GC administration has also been linked to the occurrence of ON (457). Although, the onset of ON in ALL children treated with TBI occurred later compared to those who were treated with SAC, the age of this cohort at diagnosis of ON was almost similar in both treated groups. This might be explained by the fact that the TBI group was diagnosed at an early age.

There are other drugs in the chemotherapy protocol that have been reported to adversely affect bone health. Most notably, these include methotrexate (182) which can induec an osteopathyand vincristine which may indirectly affect bone health through reduced physical activity. L-Asparaginase induced coagulopathy has previously been reported to be associated with ON (458) and in larger cohorts of patients it would be useful to explore the combined effect of dexamethasone and the more effective forms of asparaginase that have been introduced over the last decade. Therefore, the calculating of cumulative dose of these drugs in this study may have a negative impact on the final outcomes.

There were a number of other important findings in this study that may shed further light on the different aetiology of fractures and ON. ON was more likely to occur in older children and MSP, as the sole finding without any radiological findings of skeletal morbidity, and fractures were more likely to occur in younger children. ON in SAC group was more likely to occur

earlier, whereas, fractures were more likely to occur at a later stage of treatment. Not only do these findings point to a difference in the aetiology of these GC-related adverse effects but they also help in developing a rationale for the timing and nature of bone-protective interventional strategies. According to these findings, therapy duration and onset of puberty might be an influential risk for fractures and ON respectively. The early onset of ON in girls and late onset in boys in our study may also support the correlations between ON and puberty.

The observation that fractures tend to occur later in maintenance therapy maybe explained by the multifactorial aetiology of low bone mass in these children, the accumulation of the different effects of GCs, and an imbalance between low bone strength and increasing activity. However, the timing was later than that described by others (71;72) and may reflect differences in the chemotherapy protocols. The predisposition of the older child to skeletal morbidity is recognised; children may be more sensitive to interference with skeletal development during the peripubertal and adolescent growth phases, when bone mass increases due to a disproportionately greater increase in bone formation than resorption. This process of bone mass accretion is linked to the direct and indirect effects of sex steroids on bone through the growth hormone axis and muscle development. It is, therefore, possible that children are more likely to fracture around the peripubertal period if the normal process of bone acquisition is disrupted. It is also possible that older children are more likely to localize MSP to specific parts of the skeleton.

A sex difference in fracture incidence with a higher incidence in boys has been described before (71). Whilst our study also shows this difference, the results were not statistically significant. Interestingly, a recent study in children with asthma reported that boys receiving oral GCs were more likely to suffer osteopenia than girls (459).

It is important to critically evaluate the results of the whole study. First, the retrospective design of this study relied on data that had been gathered from the medical case notes. Another limitation of this study is that recorded fractures were only those which were “clinically apparent” as some fractures will have been missed or were “occult” (STOPP).

Furthermore, the cumulative doses of GCs calculated were from the ALL protocol and did not represent the real dose administered to each individual patient.

In summary, this report represents the first detailed study of the skeletal morbidity associated with contemporary protocols of chemotherapy for the treatment of childhood ALL in the UK. At least a quarter of children develops confirmed ON or fractures, typically at around the end of the first year and the second year of chemotherapy, respectively. These data may facilitate the institution of appropriately timed regimens that reduce skeletal morbidity.

Chapter 3

Skeletal Morbidity in Children Receiving Chemotherapy for Acute Lymphoblastic Leukaemia and Its Association with Mineral Homeostasis and Duration of Inpatient Stay

3.1 Abstract

Background: Reduced activity, older age and abnormal bone mineral status are considered as important determinants of poor bone health in children with acute lymphoblastic leukaemia (ALL). The independent contribution of these factors towards skeletal morbidity requires further investigation.

Aim: To investigate the influence of activity, age and mineral status over the first 12 months of chemotherapy on subsequent skeletal morbidity.

Patients and Methods: The medical records of 56 children presenting with ALL between 2003 and 2007 and treated on UKALL2003 were reviewed for the number of in-patient days over the first 12 months of chemotherapy as a surrogate marker of inactivity and lack of well-being. Data for serum Ca, Mg Pho, albumin were also collected over this period. Skeletal morbidity was defined as any episode MSP or fractures.

Results: The median duration of in-patient days over the first 12months of treatment in children with no skeletal morbidity was 58days(40,100) whereas the median number of in-patient days during the first 12months in those children with any skeletal morbidity, MSP only or fractures only was 83days(54,131), 81days(52,119) and 91days(59,158), respectively (p=0.003). Children with skeletal morbidity and fractures particularly had lower levels of serum Ca, Mg and Pho compared to those without skeletal morbidity over the first 12 months of chemotherapy. There was a higher risk of skeletal morbidity in those who were diagnosed after the age of 8 years(p=0.001,OR=16,CI:3,80). Multiple regression analysis showed that the incidence of skeletal morbidity only had a significant independent association with age at diagnosis (p=0.001) and the number of inpatient days(p=0.03) over the first 12 months (r=23). All children who were diagnosed after the age of 8 years with an inpatient stay of greater than 75 days in the first 12months of the chemotherapy (n,14) children had some form of skeletal morbidity (OR=64).

Conclusion: The incidence of skeletal morbidity in children receiving chemotherapy for ALL is associated with a higher likelihood of being older and having longer periods of in-patient stay. The close link between age and changes in bone mineral status may be one explanation for the increased bone morbidity in ALL children.

3.2 Introduction

As the survival rate for acute lymphoblastic leukaemia (ALL) has reached over 80%for children (448) increasing attention has being paid to the skeletal morbidities that are often reported in these children (72;460). Although bone morbidity may be present soon after presentation, most studies suggest that symptomatic skeletal morbidity, including fractures occur after the first 12months of therapy. The timing of the skeletal morbidity may point to an insult that occurs in the initial part of the chemotherapy period. Physical activity plays an important role in maintenance of bone health (237) and it is possible that children on chemotherapy are inactive during the early stages when they are undergoing particularly intensive chemotherapy (237;461). It is also possible that intensive chemotherapy that is delivered over the first few months of the chemotherapy protocol has a greater adverse effect on bone health, either through direct (179) or indirectly, through an effect on mineral homeostasis (195). Amongst the drugs that play an important contributory role, GCs and, particularly, dexamethasone, are considered important (182). GCs may exert their effects on bone health through a number of mechanisms including abnormalities of mineral homeostasis (179;195).

Increased Mg losses through the kidneys and lower serum Mg concentration have previously been described to be associated with lower bone mineral content in animal studies (183;462;463). There are a number of reasons why serum Mg may be lower in children on ALL chemotherapy since increased urinary excretion of Mg has been reported as a cause for low serum Mg in children with ALL. For instance, aminoglycoside antibiotics and steroids induce excessive renal loss of Mg eventually leading to the observed hypomagnesaemia (195). In addition, it is unclear whether this link between serum Mg and low bone mineral content is causal or an association which simply reflects the clinical state of the child. Previous studies have not investigated a link between serum Mg and actual skeletal morbidities.

Previous studies have reported that most skeletal morbidity in ALL children receiving chemotherapy occurs during the second year when children receive maintenance therapy (72;460). Therefore, our hypothesis was that the abnormal mineral homeostasis and long duration of inpatient stay which occurred over the first 12 months of the treatment might be

crucial in the development of subsequent skeletal morbidity in the second year of chemotherapy.

3.3 Methods

3.3.1 Patients

Children who were diagnosed with ALL between July 2003 and December 2007 and treated by any one of the three arms of UKALL2003 (Regimen A,B and C) (464) at the Royal Hospital for Sick Children in Glasgow were eligible for the study. The 64 children with these criteria included 4 children who received a stem cell transplant, 3 children who did not survive and 1 child who was less than one year of age; these children were excluded from subsequent analysis. Information about skeletal morbidity was collected from the medical records from July 2003 to September 2009 with a median follow up period of 4years (2.5,6). Those children with skeletal morbidity were divided into those with MSP and/or fractures. MSP was defined as any event of limb pain, joint and muscle symptoms or back pain that required diagnostic imaging also including those children who had ON. MSP and fractures were all confirmed by radiological images (X-rays and MRI).

Over the first of 12 months of chemotherapy, each single serum measurement for Ca (Ca) phosphate (Pho), magnesium (Mg) and albumin concentration was collected retrospectively and then the average serum concentration of Ca, Pho, Mg and albumin for each child was calculated as the median (10^{th} and 90^{th} centiles) of all the individual measurements for that child from month 1 to month 12. Information on the number of inpatient days over the first 12 months of chemotherapy for each child was collected from the medical records was calculated from the first day of diagnosis to 12 months post chemotherapy and used as a surrogate marker of the clinical state of the child as well as relative inactivity.

3.3.2 Statistical Analysis

Results are presented as mean for normally distributed data and as median and 10th-90th centiles for data which were not normally distributed. Intergroup differences were assessed using t-tests or ANOVA for normally distributed data and Mann-Whitney tests or Kruskal-Wallis tests for other data. Binary logistic regression was used to assess the association

between skeletal morbidity in general with other factors and ordinal logistic regression was used to assess the relationships between MSP and fractures to other factors. Statistical analysis was performed with Minitab16 (Minitab, Coventry, UK), with significance set at a level of 5%. The confidence intervals (95%CI) and Odds Ratio (OR) were also calculated. The study was approved by the local Ethics Committee as a retrospective case note analysis of a standard treatment protocol.

3.4 Results

3.4.1 Patient Characteristics

Details of the 56 children studied are outlined in Tab.3.1. The children who received chemotherapy Regimen C had the highest number of in-patient days over the first 12 months at a median of 96days(61,140), followed by regimen B at 74days(60,84) and then regimen A at 58days(43, 86). The difference between regimen A and regimen B was highly significant (p=0.0001). The median serum Mg in children who received regimen A was 0.85mmol/L (0.77,0.93) and higher than that in Regimen B at 0.76mmol/L(0.72,0.83) and Regimen C at 0.79mmol/L(0.74,0.84)(p=0.01 and p=0.003, respectively). The total number of X-rays in all patients with skeletal morbidity (n,34) was 147 and the total number of fractures which occurred in 16 children was 21(Fig.3.1).

Characteristics	No SM*	SM*	MSP	Fractures	P Value
Total Events	22(m,12)	34(m,21)	18(m,10)	16(mm,11)	
Events in 2nd first 12 months	22(m,12)	22(m,14)	11(m,5)	11(m,9)	
Events in children diagnosed <8years	20(61%)	13(39%)	9(27%)	4(12%)	
Events in children diagnosed >8years	2(9%)	21(91%)	9(39%)	12(52%)	
Age at Diagnosis of ALL(years)	4.7(2.6,7.6)	9(3.5,13.5)	7.5(4.2,14)	10(3,13)	0.001
First Record Of SM (months)	*	16(4,35)	15.5(3.2,36)	18.7(6.3,35.5)	
Regimen A(n,26)	14(54%)	12(46%)	6(23%)	6(23%)	
Regimen B(n,8)	0	8(100%)	4(50%)	4(50%)	
Regimen C(n,22)	8(36%)	14(64%)	8(37%)	6(27%)	
No of Assays (Ca)	95(71,144)	131(86,187)	128(82,163)	140(95,198)	0.003
No of Assays (Pho)	5(70,144)	130(81,181)	126(76,156)	142(98,193)	0.005
No of Assays (Mg)	76(54,112)	104(57,158)	95(51,140)	114(72,165)	0.02
No of Assays (Alb)	76(50,120)	107(66,147)	104(61,132)	116(78,170)	0.02

Tab 3.1: The incidence of skeletal morbidities (SM), musculoskeletal pain (MSP) and fractures during chemotherapy. The median of age at diagnosis of ALL(10th,90th) in those children with SM, the median age at SM and the time of SM were measured in each categories. Distribution of SM in each protocols (A,B,C). the median number of assays of Ca, Pho, Mg and Alb (albumin) were measured over the first 12 months of chemotherapy. p value differences between No SM and SM.

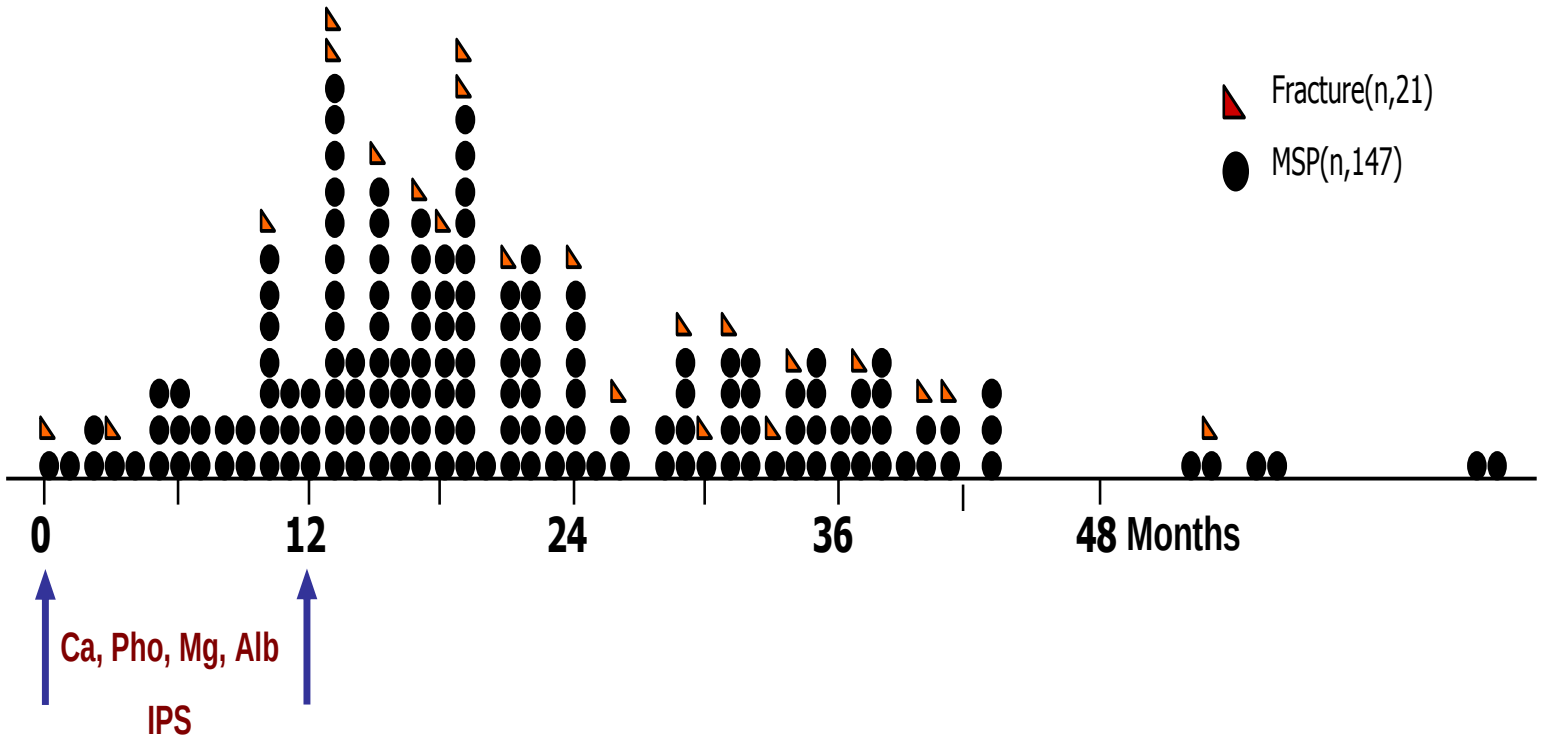


Fig 3.1: the distributions of skeletal morbidities, musculoskeletal pain (MSP) and fractures from time of diagnosis (0 month to 48 months). From 0-12months of chemotherapy, serum calcium (Ca), phosphate (Pho), magnesium (Mg), albumin (Alb) and in patient stay (IPS) were collected retrospectively.

3.4.2 Total Duration of In-patient Stay over the First 12 Months of Chemotherapy

The median duration of in-patient days over the first 12-months of treatment in children with no skeletal morbidity was 58days (40,100) and the median number of in-patient days during the first of 12 months in those children with skeletal morbidity was 83days (54,131)(p=0.003); in those children with MSP-only and fractures-only, median duration of in-patient days were 81 days (52,119) and 91days (59,158), respectively. The median number of in-patient days over the first 12-months in those children (n,22) who only had skeletal morbidity in the second year was 82days(53,101) and remained significantly higher than children with no skeletal morbidity in the second year (p=0.01)(Fig.3.2).The children who had a total in-patient stay of more than 75 days (n,28) during the first 12-months were more likely to have skeletal morbidity (OR=5.9%,CI:1.5,15.7). This relationship existed even when children who had skeletal morbidity in the same period were excluded from the analysis (OR=3.8, 95%CI:1.1,13). There was no association between age and inpatient stay (p=0.54) (Fig.3.3).

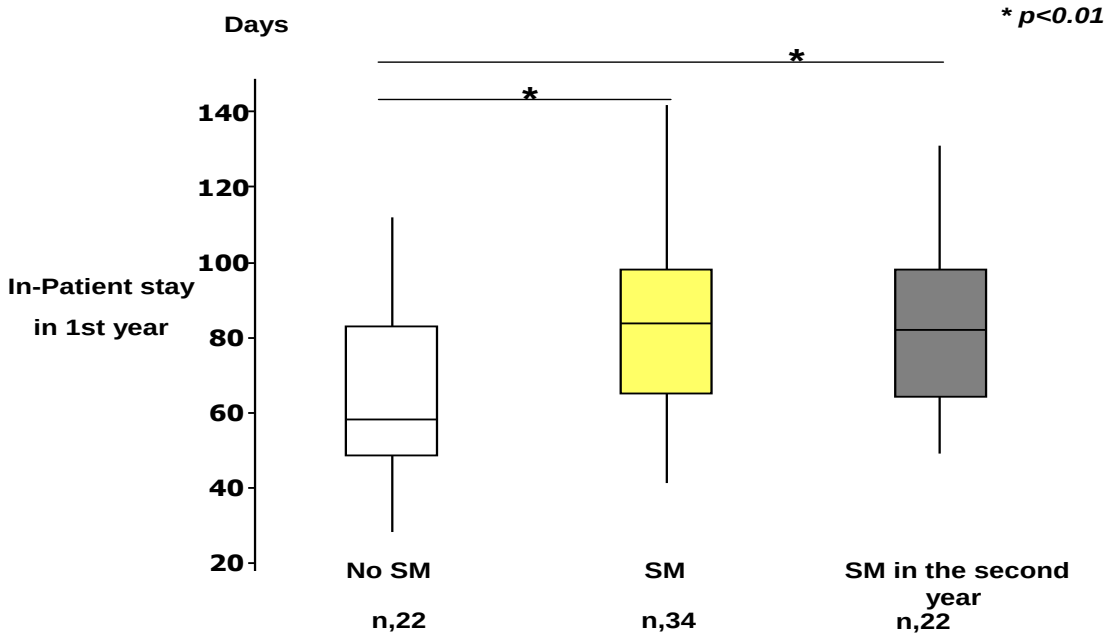


Fig 3.2: Median duration of inpatient days over the first 12 months of chemotherapy for children with no skeletal morbidity (SM), for children with SM and for those children who only had SM after the first first 12 months * p<0.05.

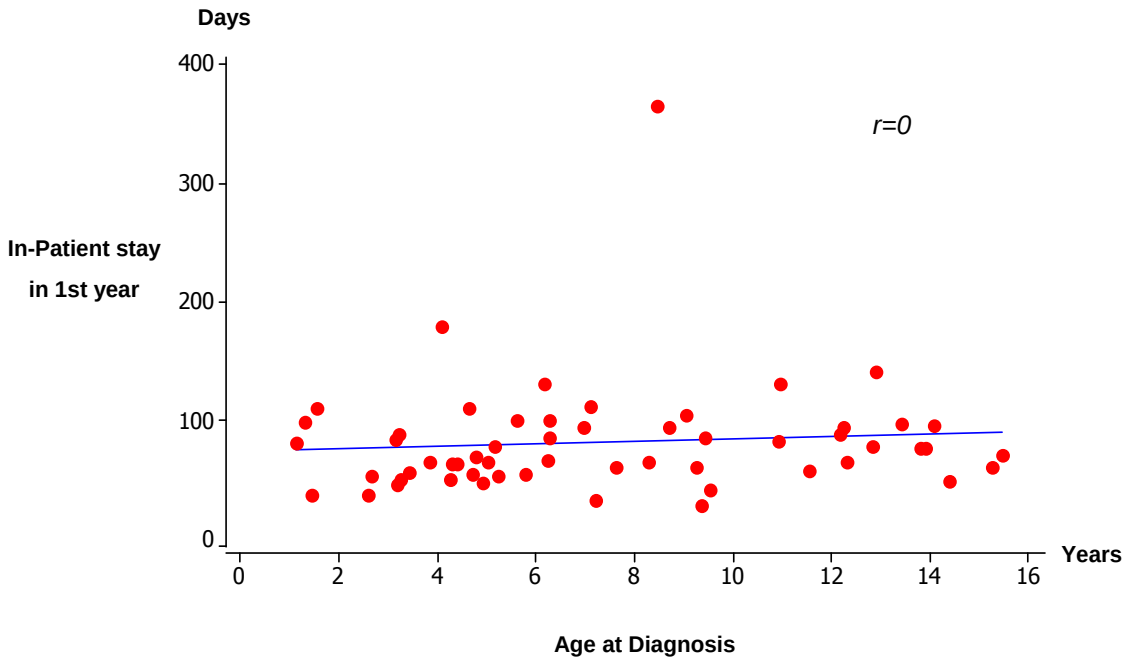


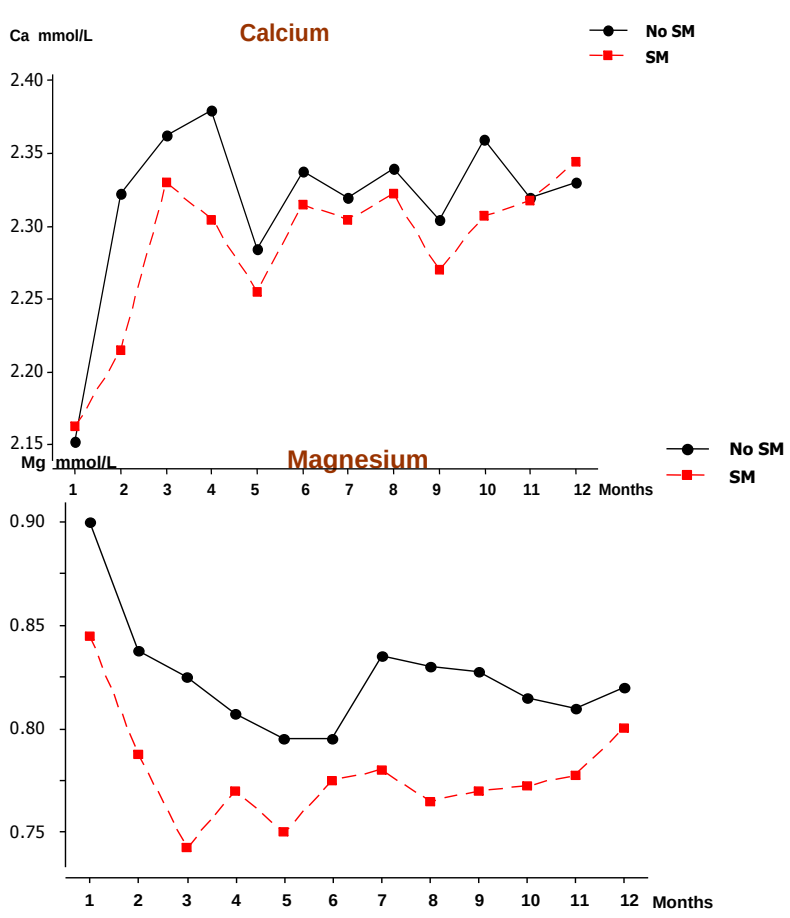
Fig 3.3: The relationship between in patient stays over the first year of the chemotherapy and age at diagnosis of ALL (r=0).

3.4.3 Mineral Status over the First 12 Months of the Chemotherapy

Over the first 12 months of chemotherapy, children with skeletal morbidity and fractures had lower trend levels of serum Ca, Pho and Mg compared to those without skeletal morbidity (Fig.3.4). However, unlike Ca and Pho, serum Mg remained persistently low in those children with skeletal morbidity 0.79mmol/l(0.77,0.88) compared to those with no skeletal morbidity 0.82mmol/L(0.77,0.9)(p=0.02,) (Fig3.5). Furthermore, skeletal morbidity rates were higher in those children with Mg levels below 0.80mmol/L compared to children with Mg levels above 0.79mmol/L(r=0.13,p=0.004,). There were no differences in the median serum albumin between the groups (Tab.3.2). There was a negative association between serum Mg and the age at diagnosis of leukaemia (r=0.18,p=0.001)(Fig.3.6). The median serum Mg was lower in those children diagnosed after the age of 8years at 0.78mmol/L(0.74,0.81) compared to those diagnosed before the age of 8years at 0.83mmoml/L(0.77,0.92)(p=0.001). The rate of hypomagnesaemia in the whole cohort over the first 12 months of chemotherapy occurred in 54% in children.

3.4.4 Age at Diagnosis and Inpatient Stay

Age at diagnosis of leukaemia was associated with a higher risk of skeletal morbidity with an OR of 16 (95%CI:3,80) in those who were diagnosed after the age of 8years. The data were further analysed by stratifying the children depending on whether they were over or under 8years at diagnosis and whether they had a total number of in-patient days that exceeded 75 days or not (Fig.4). Five (26%) of the first group of children (n,19) who were diagnosed before the age of 8years with in-patient stay of less than 75 days had skeletal morbidity. Eight (57%) children in the group who were diagnosed before the age of 8years with total in-patient stay greater than 75 days in the first year (n,14) had skeletal morbidity (OR=4,95%CI:1.5,15)). The third group included children who were diagnosed after the age of 8years with a total in-patient stay of less than 75 days in the first 12 months of chemotherapy (n,9); 78% of this group had skeletal morbidity(OR=16,95%CI:3,80). The fourth group included children who were diagnosed after the age of 8years with an inpatient stay of greater than 75 days in the first 12 months of chemotherapy (n,14); all children had some form of skeletal morbidity . Hence, the OR for the last group can be calculated manually by (4X16=64) (Fig.3.7).



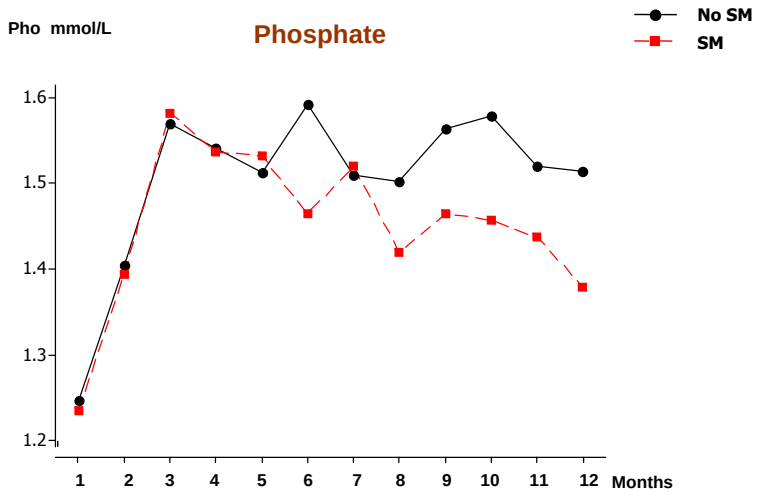


Fig 3.4: The changes of serum calcium (Ca), phosphate (Pho) and magnesium (Mg) over the first 12months of chemotherapy in children with no skeletal morbidity (No SM; black circles) and with skeletal morbidity (SM; red squares).

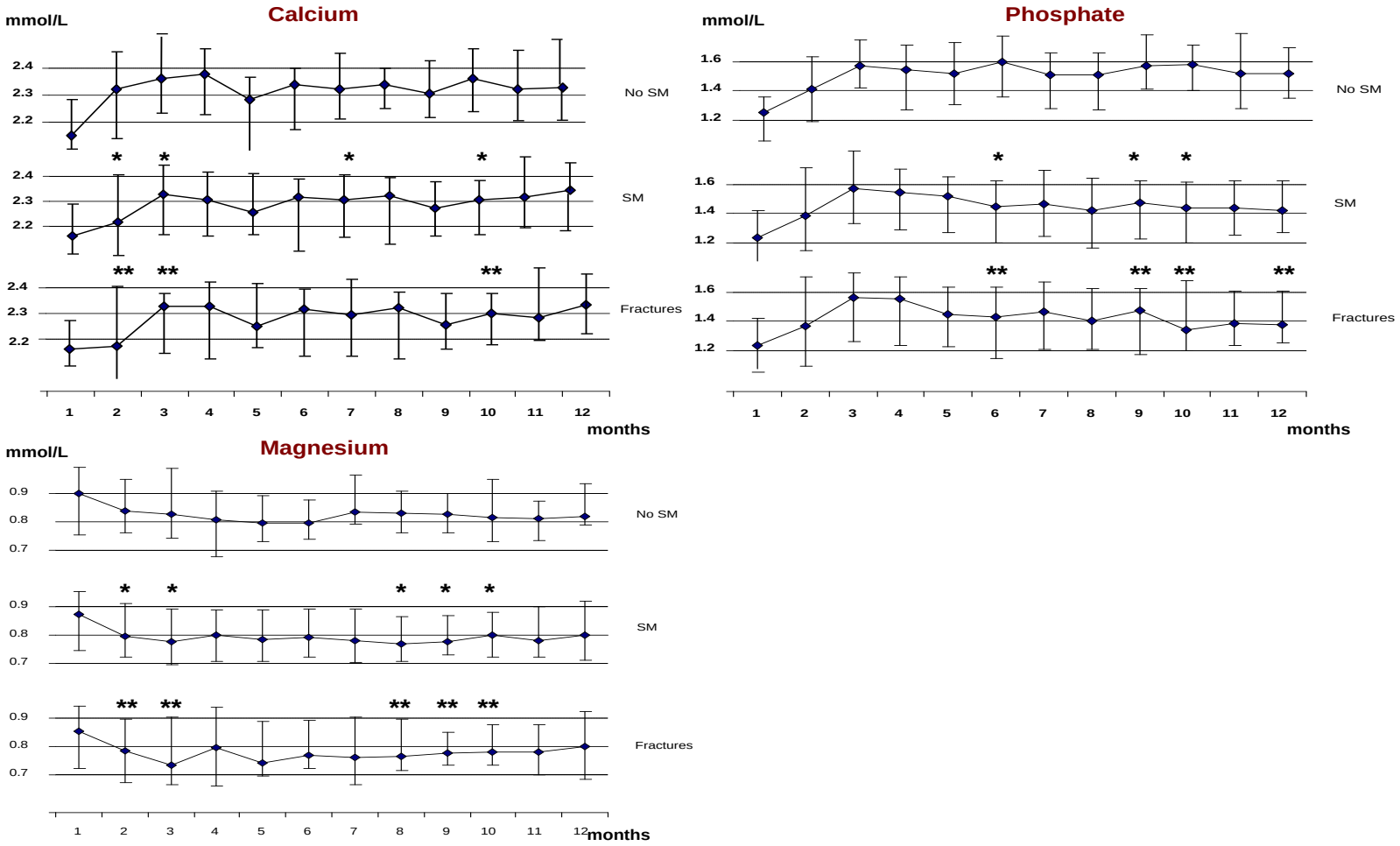


Fig 3.5: Median serum Ca, Pho and Mg from month 1 to month 12 of chemotherapy in children with no skeletal morbidity (SM) (n,22), skeletal morbidity (SM) (n,34) and with fractures (n,16). *p<0.05, no SM v SM and p** <0.05 no SM V v fractures.

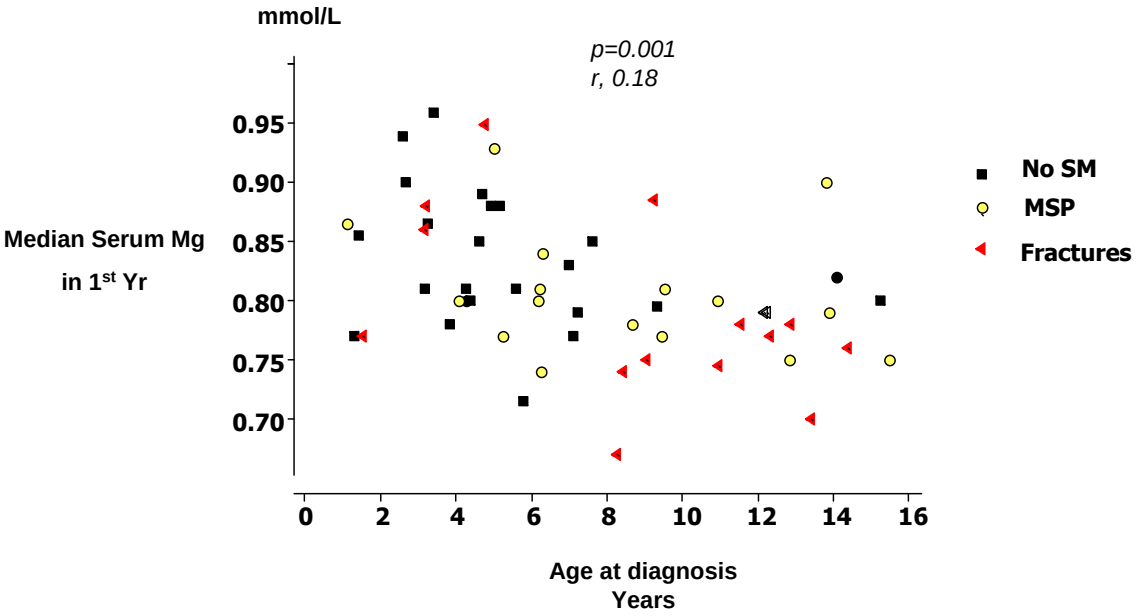


Fig 3.6: The relationship between median serum magnesium (Mg) and age at diagnosis of ALL in children without skeletal morbidity (No SM), musculoskeletal pain (MSP) and fractures.

	No SM	MSP	Fractures	Ref Range
Ca	2.29(2.2,2.34)	2.26(2.21,2.32)	2.25(2.15,2.34)	2.2-2.7mmol/L
Pho	1.4(1.3,1.5)	1.42(1.3,1.5)	1.4(1.25,1.52)	0.8-1.5mmol/L
Mg	0.82(0.77,0.9)	0.8((0.75,0.87)	0.77(0.72,0.88)*	0.75-0.8mmol/L
Albumin	35(31,38)	33(30,37)	34(29,38)	32-45g/L

Tab 3.2: Median (10th and 90th centiles) serum Ca, Pho, Mg and albumin over the first 12 months of chemotherapy in children with no skeletal morbidity (SM), musculoskeletal pain only (MSP) and fractures.* Difference between no SM and Fractures, $p<0.05$.

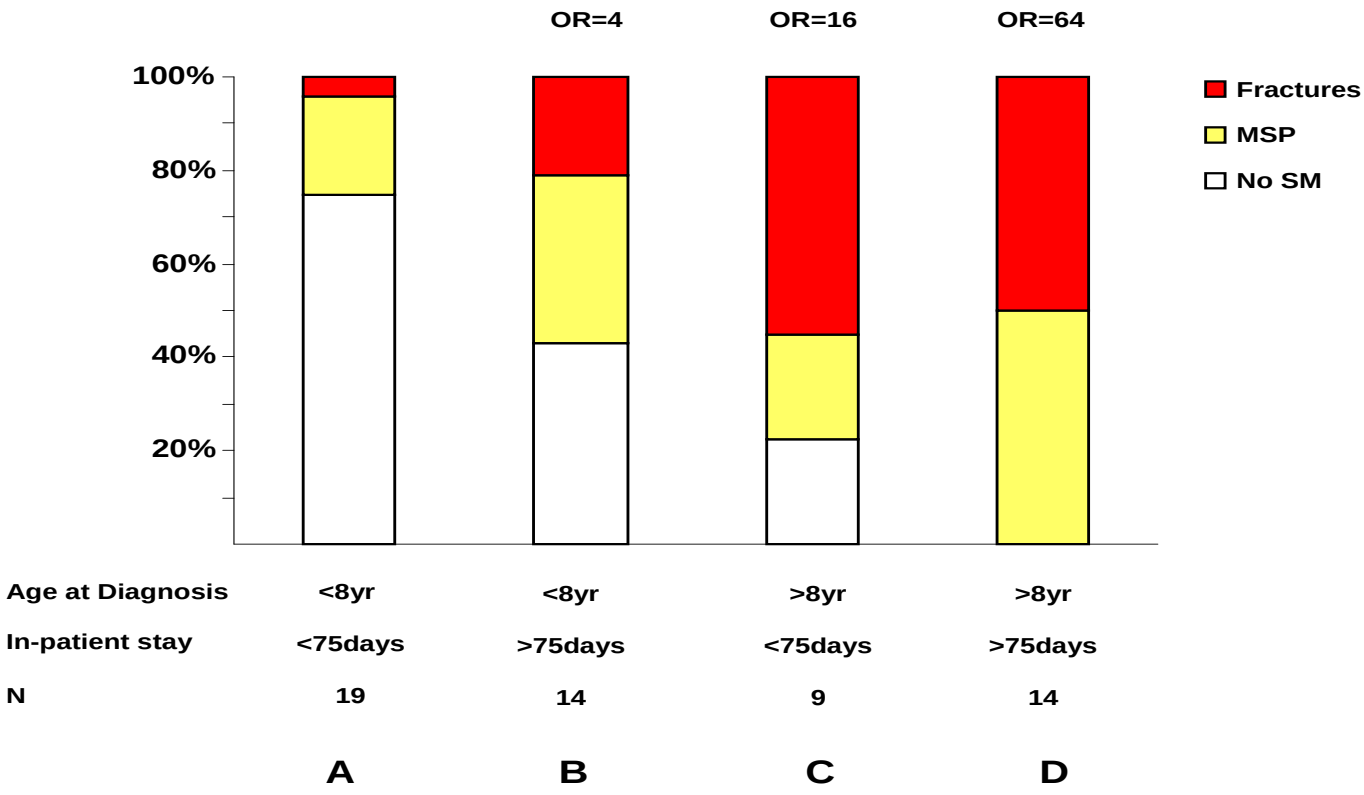


Fig 3.7: Likelihood of skeletal morbidity (SM) in relation to age and total in-patient stay. (A) Children diagnosed at less than 8years of age and who had a total duration of inpatient stay of less than 75 days; (B) Children who were diagnosed at less than 8years of age and who had a total duration of inpatient stay of more than 75 days; (C) Children who were diagnosed at more than 8years of age and who had a total duration of inpatient stay of less than 75 days; (D) Children who were diagnosed at more than 8years of age and who had a total duration of inpatient stay of more than 75 days. OR = Odds Ratio compared to Group A.

	Univariate Analysis	**Multivariate Analysis**
Age at Diagnosis	OR=1.3 p=0.002	OR=1.3 p=0.003
In-patient stay	OR=1.1 p=0.01	OR=1.1 p=0.01
Serum Mg	OR=1.1 p=0.01	OR=1.1 p>0.05

Tab 3.3: The regression models (Univariate and multiple regression analysis) shows the correlation between skeletal morbidity (SM) and 3 independent factors, age at diagnosis, in-patent stay over the first year of chemotherapy and serum magnesium (Mg).

3.4.5 Multivariate Analysis

Univariate regression model showed a significant association between skeletal morbidity and the age at diagnosis of ALL(r=0.18,p=0.001), the number of inpatient days in the first 12 months of chemotherapy (r=0.07,p=0.02) and serum Mg over this period (r=0.06,p=0.036). However, multiple regression analysis showed that the incidence of skeletal morbidity was only associated to age at diagnosis (r=0.23,p=0.001) and the number of inpatient days (r=0.23,p=0.03) over the first 12 months (Tab.3.3).

3.5 Discussion

Although skeletal morbidity has been reported to be common in children with ALL (71;72;460) the pathophysiology of bone disease in this population remains unclear. Bone mineral status, physical activity and age are amongst the factors that have been suggested as possible contributors towards an increased risk (72;195;208). However, to our knowledge, this is the first study which has investigated the association between actual skeletal morbidity and these factors.

Previous studies in children receiving chemotherapy for ALL have raised the possibility that the observed increased incidence of bone morbidity may be related to low serum Mg concentrations (183;195). In other clinical scenarios, elevated serum Mg has been reported to be associated with PTH suppression in peritoneal dialysis patients (465) and elderly patients with fractures of long bones have been reported to have lower serum Mg compared to those without fractures (466). Furthermore, Mg depletion in normal subjects may cause osteoporosis due to hypocalcaemia, impaired PTH secretion, and low serum concentrations of 1,25-dihydroxyvitamin D(1,25-$(OH)_2$D) (467). Rude et al. (468) demonstrated that Mg therapy in gluten-sensitive enteropathy patients resulted in a rise in PTH and an increase in bone density. In animal models, Mg is mitogenic for osteoblasts and may increase osteoblast activity (469); its depletion has been reported to be associated with in vitro inhibition of osteoblast proliferation and raised concentrations of pro-inflammatory cytokines (463;470). Mg supplementation of ovariectomized rats suppresses the increased bone resorption resulting from ovariectomy by inactivating osteoclasts (234). Recently, a study has reported that short-term oral Mg supplementation in postmenopausal osteoporotic women increases

serum osteocalcin levels and decreases urinary deoxypyridinoline levels indicating a reduction in bone turnover (233).

In children on ALL chemotherapy, cyclical dosing with steroids may disturb the intracellular Mg concentration as well as lead to excessive renal loss of Mg (183;195). Indeed, our study observed that hypomagnesaemia occurred in 54% in children on chemotherapy and this was similar to previously published data (471). Our study also showed the likelihood of skeletal morbidity was associated with a lower serum Mg concentration. However, on multivariate analysis, serum Mg per se was not the contributory variable but it was the age of the patient which was the important variable. Children with fractures tended to be older and had lower serum Mg as compared to the other groups. Our observation that serum Mg showed a significant correlation with age is consistent with previous reports of a relationship between age and serum Mg in healthy individuals (472;473). Mg supplementation can increase bone density and arrest bone loss in osteoporotic subjects (474) and its supplementation in children with ALL and hypomagnesaemia is associated with a variable rise in serum Mg and as well as a rise in OCN levels (195). It is possible that this variability response is age dependent and needs further exploration.

Musculoskeletal complications have been associated with several risk factors such as GCs and methotrexate therapy, cranial irradiation decreased physical activity and nutritional deficiency leading to altered in Ca, vitamin D and Mg metabolism (460;475).

The current study also showed a significant association between skeletal morbidity and the number of inpatient days (marker of inactivity) over the first 12 months of chemotherapy. This association existed even after excluding all skeletal morbidity that occurred during the first 12months of chemotherapy. On the other hand, it may provide a useful integrated measure of well-being as children on chemotherapy who may have a number of underlying reasons for being unwell and inactive. Based on the mechanostat model, reduced motor activity will lead to low bone strength (38). Tillmann et al. (208) reported that ALL treated with chemotherapy alone had reduced lumbar volumetric BMD and were physically less active than their healthy controls. The current study showed that skeletal morbidity in the second year of

chemotherapy was about four times higher in those children who stayed over 75days in the first 12months of chemotherapy.

The present study has certain limitations that need to be taken into account. Firstly, the study was designed retrospectively. Secondly, blood samples were collected at irregular intervals; therefore phosphate levels may have simply reflected recent phosphate ingestion from food. Furthermore, the abnormal results of mineral homeostasis (hypocalcaemia, hypomagnesemia and hypophosphatemia) might have treated with their supplementations. The other parameters such as 25-OHD and PTH were not measured routinely during the course of chemotherapy. However, including these measurements in this analysis would have given a greater insight into mineral homeostasis during ALL treatment. The higher number of assays undertaken in may reflect the general wellbeing ALL children and therefore it may have skewed the results.

In summary, older age and higher total duration of inpatient stay during the first 12 months of chemotherapy are associated with an increased likelihood of skeletal morbidity in children with ALL. A predisposition for lower serum Mg may be an explanation for the increased susceptibility to skeletal morbidity in older child but this requires further study. Interventions that target the older child who is less active or spends a higher amount of time as an inpatient over the first 12months of chemotherapy may be most effective in improving bone health in children on ALL chemotherapy.

Chapter 4

A Comparison of the Effect of Two Types of Vibration Exercise on the Endocrine and Musculoskeletal System

4.1 Abstract

Background: Whole body vibration (WBV) is a novel training intervention but a comparison of different methods of WBV has rarely been performed.

Aim: To compare the short and medium term effects of two regimens of WBV on endocrine status, muscle function and markers of bone turnover.

Patients and Methods: Over a period of 16 weeks**,** 10 men with a median age of 33years (range,29,49), were randomised to stand on the Galileo platform (GP) or Juvent1000 platform (JP) three times/week. The total study duration was 16 weeks with measurements performed in a four- week period of run-in, 8 weeks of WBV and a 4 week period of washout. These measurements included an assessment of anthropometry, body composition, muscle function and biochemical markers of endocrine status and bone turnover. To assess immediate effects of WBV, measurements were also performed at 60minutes before and 5,30 and 60minutes after WBV.

Results: GP at 22 Hz was associated with an immediate increase in serum GH, rising from 0.07µg/l(0.04,0.69) to 0.52µg/l(0.06,2.4)(p=0.06), 0.63µg/l(0.1,1.18)(p=0.03), 0.21µg/l (0.07,0.65)(p=0.2) at 5minutes, 20minutes and 60minutes after WBV, respectively. An immediate effect was also observed in median serum cortisol which reduced from 316nmol/l (247,442) before WBV to 173nmol/l(123,245)(p=0.01),165nmol/l(139,276)(p=0.02) and 198nmol/l(106,294)(p=0.04) at 5minutes, 20minutes and 60minutes after WBV, respectively. Median serum CTX reduced significantly after 8 weeks of WBV training in the GP group from 0.42ng/ml(0.29,0.90) pre-WBV to 0.29ng/ml(0.18,0.44) at the end of WBV training (p=0.03). Over the 8 weeks, there was a reduction in median serum cortisol in the GP group from 333nmol/l(242,445)(pre-WBV) to 270nmol/l(115,323)(WBV)(p=0.04). None of the changes observed in the JP group reached statistical significance. Neither group showed any significant effect on muscle function, IGF-1, testosterone, leptin, C- reactive protein (CRP), creatine kinase (CK), insulin or other markers of bone turnover.

Conclusion: WBV can stimulate GH secretion, reduce circulating cortisol and reduce bone resorption. These effects are independent of clear changes in muscle function and depend on the type of WBV that is administered.

4.2 Introduction

In whole body vibration (WBV), the vibration platform delivers high frequency mechanical stimuli with small amplitude which are transmitted through the body where they introduce mechanical loading to the musculoskeletal system through bone, muscle and sensory receptors (395). Although WBV is an increasingly popular form of training that has been reported to have beneficial effects on bone health (245;395), muscle mass (309;357;476;477), and hormonal profile (243), the underlying mechanisms that explain these effects remain unclear (243;360). WBV can be delivered by two broad categories of exercise devices: devices that reciprocate vertical displacements on the left and right side of a fulcrum (sinusoidal vibration) and generate higher lateral than vertical acceleration and devices that have a plate which oscillates up and down in a vertical axis (vertical vibration) (304) and which produce greater strain in the vertical axis than in the lateral axis (303). Given that WBV may represent an effective non-pharmacologic, user-friendly, therapeutic intervention for osteoporosis and sarcopenia (310); there is a need for more critical evaluation and comparison of the systems that deliver this stimulation.

The devices that deliver WBV can be broadly categorised according to their peak acceleration; low intensity WBV platforms which produce a gravitational force less than 1 g regardless of frequency and high intensity WBV platforms (g force of more than 1 g) (306). An example for a low-intensity WBV platform is the Juvent 1000 platform and a high-intensity WBV platform is Galileo platform. Physical exercise is closely linked to a diverse range of hormonal effects and although there are some studies which have investigated the short-term effects of WBV on the endocrine axis, there is a scarcity of knowledge about the medium-term effects of WBV on endocrine targets that are reported to be responsive to physical exercise. In addition, there are currently no studies that have compared the effect of the two different methods of WBV on the endocrine profile as well as muscle function. This study was, therefore, performed to compare the effects of sinusoidal and vertical WBV delivered through the Galileo platform (GP) and the Juvent1000 platform (JP), respectively on a range of outcome measures related to the endocrine and musculoskeletal system. It was hypothesized that WBV with different magnitudes may elicit different patterns of musculoskeletal and endocrine responses. Therefore, different doses in each platform have

been tested in this study in order to obtain the optimal dose of the WBV exercise and the systematic response to these different magnitudes.

4.3 Method and Material

4.3.1 Subjects

All potential candidates had a physical examination to determine their general health and were excluded if they had any chronic illness, recent fractures, skeletal anomalies or implants. Following informed consent, 14 healthy men were recruited to the study but 4 subjects withdrew as one subject had a hamstring injury before starting WBV and the remaining 3 could not attend the exercise visits. A total of 10 men with a median age of 33years (range, 29, 49) completed the study. Ethics approval was obtained from the University of Glasgow Research Ethics Committee.

4.3.2 Study Procedures

An initial interview was conducted to describe the purpose and the aims of the study and the tests that would be performed. The total study duration was 16 weeks and was divided into three periods; a Run-In period of 4 weeks, a WBV period of 8 weeks followed by a 4 week Wash Out period (Fig.4.1). The measurements were performed at 5 time points (T0, T1, T2, T3, and T4). T0 was at the beginning of the Run-In period; T1, T2 and T3 were at the beginning, half-way and the end of the WBV period; and, T4 was at the end of the Wash-Out period. To assess the short-term effect of WBV, multiple samples were collected at T1, T2 and T3 at 60minutes before WBV and 5, 30 and 60minutes after WBV. To assess the medium-term effects, samples collected at T0 and the first sample collected at T1 were jointly analysed as pre-WBV, samples collected at T2 and T3 were jointly analysed as WBV and those collected at T4 were referred to as post-WBV. The blood samples were collected between 08.00 and 09.00am, after a minimum of 8 hours of fasting. The participants were not allowed to consume food and drinks during the treatment. All blood samples were collected via an indwelling venous cannula and centrifuged at 2600-2800 rev/minute for 10min, and the serum was subsequently stored at -70C.

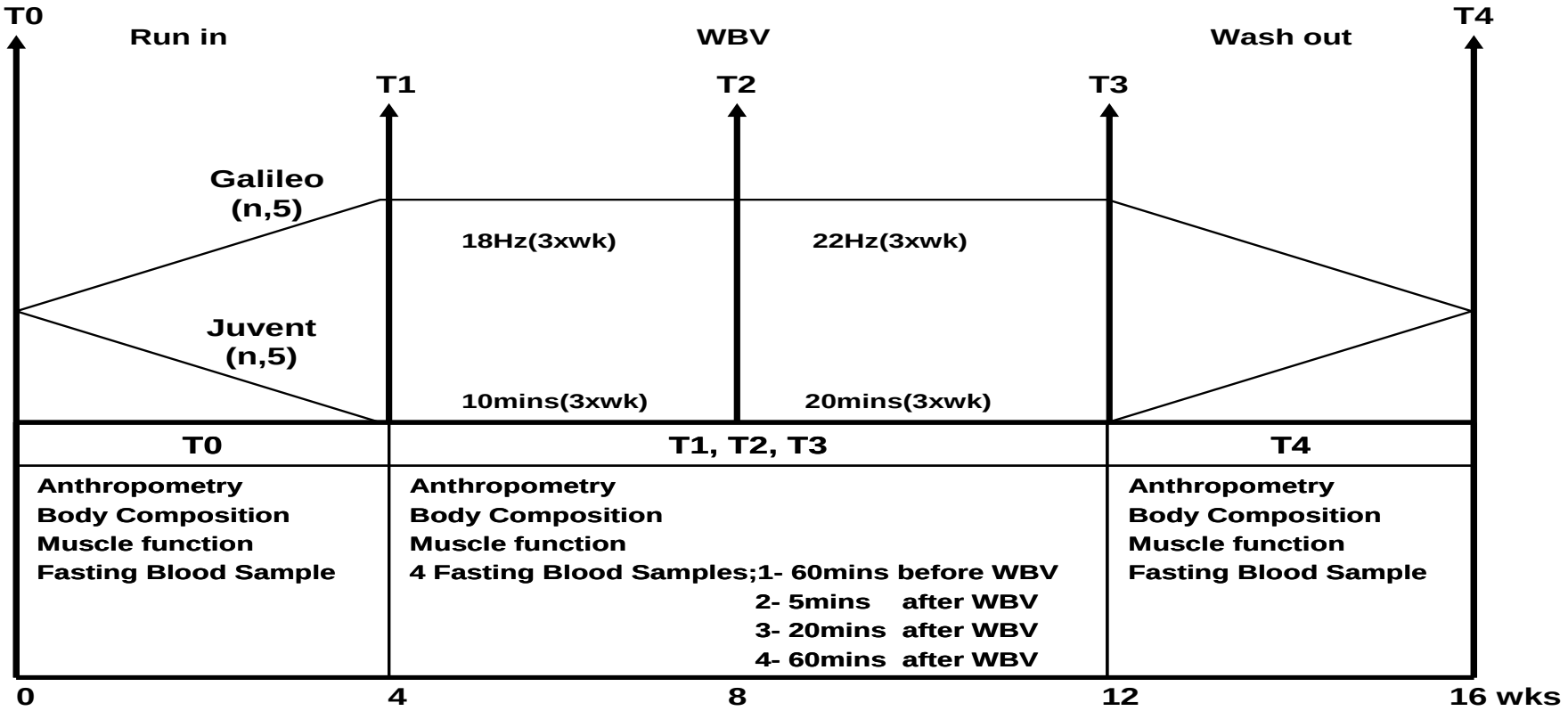


Fig 4.1: Study protocol consists of 5 time points (T0-T4) over a period of 16 weeks. At T0, the participants were randomised into two groups; GP and JP. At T0 and T4, weight, height, muscle function, body composition and a fasting blood sample were collected. WBV was performed for a period of 8 weeks from T1 to T3, 3times/week with different frequency and duration. The measurements at these time points were similar to T0 and T4. In addition, 4 fasting blood samples were also collected at T1, T2 and T3; the first sample was collected was 60minutes before WBV and the others were collected at 5, 20 and 60minutes after WBV**.**

4.3.3 Exercise Regimen

At T0, all participants were randomised to receive WBV by the Galileo device (Novotec, Pforzheim, Germany) or Juvent 1000 DMT (Juvent Medical Inc, Somerset, USA) on 3 days every week over the WBV period. For the Galileo platform (GP), the WBV intensity used for the first 4 weeks(from T1 to T2) was at the frequency of 18Hz, the peak-to peak displacement 4mm and the acceleration 2.6g whereas, the second 4 weeks(from T2 to T3); the frequency increased up to 22Hz, the peak-to peak displacement remained constant at 4mm and the acceleration, 3.8g. All exercise of GP was supervised and each exercise visit consisted of 3 bouts of WBV with each bout lasting for 3minutes with one minute rest in between the bouts. For the Juvent platform (JP), the WBV intensity was kept constant at 32-37Hz of frequency with a peak-to peak displacement 0.085mm and an acceleration of 0.3g for 10minutes in the first 4 weeks (from T1 to T2) whereas, and 32in the second 4 weeks (from T2 to T3), the duration of exposure doubled to 20minutes for each session. Details of the WBV regimen are outlined as recommended by the International Society of Musculoskeletal and Neuronal Interactions (311). The WBV parameters that were used in this study were chosen as they have previously been reported to exert a beneficial effect on musculoskeletal health (326;478). All participants were instructed to stand still on the vibration platforms without shoes and, in the case of GP, with slight flexion of the knees.

4.3.4 Body Composition and Muscle Function

Anthropometry included height, weight and body composition measurements were performed using a Harpenden Stadiometer and Tanita (TBF-300,Tokyo,Japan), respectively. Muscle force, power, velocity and jump height were assessed by Leonardo mechanography (Novotec Medical,Pforzheim, Germany). A two-leg jump was assessed as a counter movement with freely moving arms and the subjects were instructed to jump as high as possible. Vertical jump height (cm), power max total (kW), power max / kg (W/kg), efficiency (%) and Esslinger Fitness Index (EFI) (%) were all assessed. Each participant was asked to jump at least three times at each time point and the result of highest jump was included. Handgrip strength was assessed with the Jamar handgrip dynamometer (Preston, Jackson, MI, USA) using the dominant arm and the highest measurements were recorded.

4.3.5 Biochemical Assays

The short effect of WBV was assessed by measuring serum growth hormone (GH), cortisol, creatine kinase (CK) and glucose and the medium term effect was assessed by measuring markers of bone turnover, Insulin, Insulin-like growth factor 1 (IGF-1), Testosterone, Leptin and C-reactive protein (CRP). Serum bone-specific alkaline phosphatase (BAP) was measured by Ostase® BAP immunoenzymetric assay (immunodiagnostic systems Ltd (IDS Ltd, Boldon,UK) with an intra-assay CV of 5.5% to 7.3%. Serum osteocalcin (OCN) was measured using N-MID® osteocalcin ELISA (IDS Ltd, Boldon, UK) with an intra-assay CV of 3.3% to 9.7%. Serum cross linked C-telopeptide of type I collagen (CTX) was determined using serum crossLaps® ELISA (IDS Ltd, Boldon, UK) with an intra-assay CV of 1.9% to 4.2%. Serum tartrate-resistant acid phosphatase 5b(TRAP5b) was detected by using bone TRAP(r) Assay (IDS Ltd, Boldon,UK) with the intra-assay CV of 1.7% to 3.4%. Serum sclerostin (Scl) was detected by using TECO Sclerostin Elisa Kit (Pathway Diagnostic Ltd, Dorking, UK) with an intra-assay CV of 1.1 % to 3.9%. Serum GH and insulin were measured by the Siemens Immulite 2000 Erlangen, Germany. Between-run CV was less than 5% for both measurements. Serum IGF-1 concentration was determined using IGF-1 ELISA kit (Mediagnost IGF-1, Reutlingen, Germany), with an intra-assay CV of 5.5% to 9.5%. Serum cortisol concentration was evaluated using Architect Cortisol (Abbott Diagnostics, Abbott Park, USA) with an intra-assay CV of 6.8% to 10%. Serum testosterone concentration was determined using Abbott automated immunoassay platform (Abbott Diagnostics, Abbott Park, USA). Between-run CV was from 3% to 5%. Serum leptin concentration was determined by an in-house RIA with an intra-assay CV from 2.8% and 6%. Serum CK and glucose were measured using CK Kit and glucose reagent kit respectively (The ARCHITECT c System, Abbott Laboratories). Within-run coefficients of variation for CK and glucose were (from 3.4% to 4.1%) and (from 1.6% to 2.6%), respectively. CRP was assessed by CRP Vario (Sentinel Diagnostics, Abbott Diagnostics) with an intra-assay CV from 3.6% to 8.6%.

4.3.6 Statistical Analysis

Results are presented as median and ranges and inter-group differences were assessed using Mann-Whitney tests or Kruskal-Wallis tests. Statistical analysis was performed with Minitab16 (Minitab, Coventry, UK), with significance set at a level of 5%. The correlation

between the variables was measured by regression test and r-value. Furthermore, ANOVA test (General linear Model) has been done for repeated measures may be more appropriate for the serial measurements. The short term effect of biochemical markers was assessed by the changes occurred from the measurements before the WBV training (-60minutes) and after WBV training (5, 20, 60 minutes) respectively. The data for the three study periods, run-in, WBV and wash-out were analysed by studying data at T0 and T1 for run-in, data at T2 and T3 for WBV and data at T4 for wash-out.

4.4 Results

4.4.1 Baseline Characteristics

Tab.4. 1 shows the results for all the physical and biochemical measurements of the two groups over the study period. The reported median vibration training compliance for both study groups was 100%(18,100).

4.4.2 Body Composition and Muscle Function

In the GP group, median jump height at pre-WBV(T0-T1) was 41cm(32,51) and 44cm(41,55) during the WBV sessions (T2-T3)(p=0.08) as compared to 44cm(41,52)(p ,0.12) at post-WBV (T4). In the JP group, median jump height was 46cm(35,55) , 45cm(32,52) and 49cm(33,55) pre-WBV, during WBV sessions(p=0.6) and at post-WBV(p=0.9), respectively. There were no significant changes in the other parameters of muscle function (Fig.4.2). There were also no significant changes in body composition.

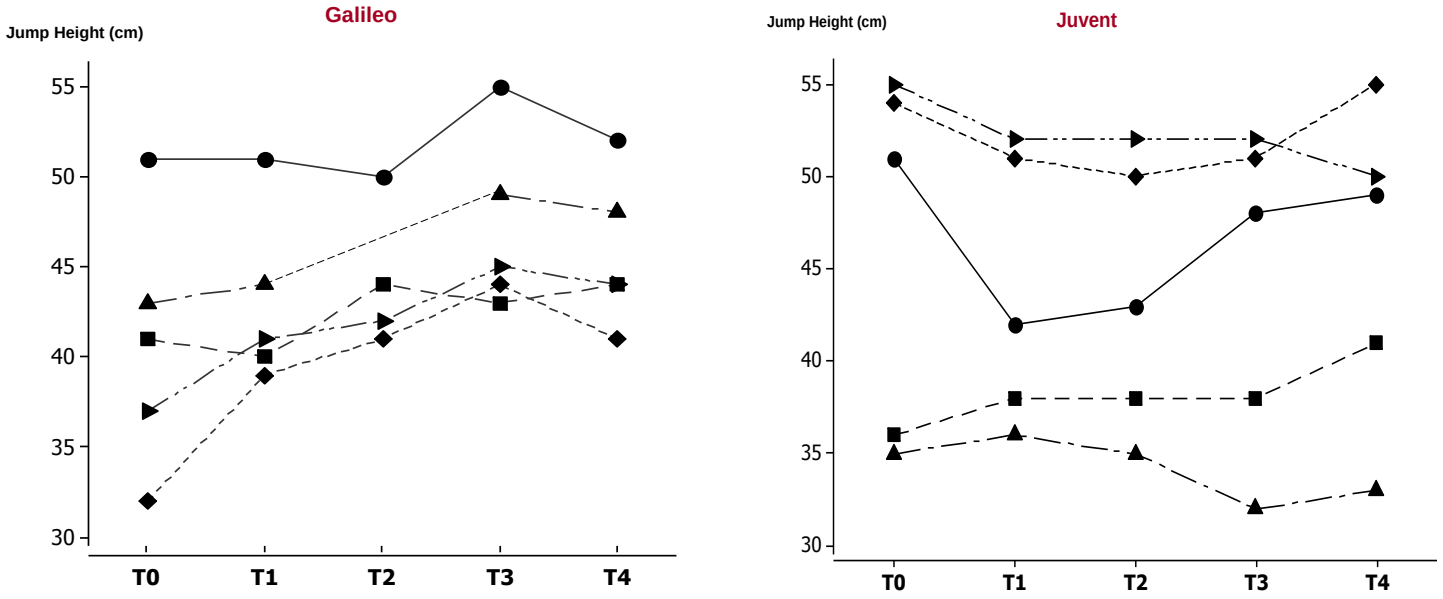


Fig 4.2: The changes in the vertical jump (T0-T4) height measured by mechanography in the Galileo group and Juvent group.

4.4.3 Short-term Effect of Exercise on Biochemical Markers

At 18Hz, GP was associated with a non-significant increase in serum GH, but at 22Hz, serum GH rose from 0.07µg/l(0.04,0.69) at 60minutes pre-WBV to 0.52µg/l(0.06,2.4) (p=0.055), 0.63µg/l(0.1,1.18)(p=0.026), 0.21µg/l(0.07,0.65)(p=0.2) at 5minutes, 20minutes and 60minutes post-WBV, respectively. In contrast, JP was not associated with any significant change in serum GH (Fig.4.3). At 18Hz, GP was associated with a reduction in serum cortisol from 316nmol/l(247,442) at 60minutes pre-WBV to 173nmol/l(123,245)(p=0.01), 165nmol/l(139,276)(p=0.02) and 198nmol/l(106,294)(p=0.04) at 5minutes, 20minutes and 60minutes post-WBV, respectively. At 22 Hz, GP was associated with a reduction in serum cortisol from 269nmol/l(115,323) at 60minutes before WBV to 214nmol/l(139,394)(p=0.5), 200nmol/l(125,337)(p=0.08) and 181nmol/l(104,306)(p=0.04) at 5minutes, 20minutes and 60minutes post-WBV, respectively. In the JP group there were no significant changes in serum cortisol (Fig.3). Serum CK, as a marker of the effect of exercise on muscle, did not show any significant change in either of the two groups with different frequency and durations. There was no significant change in serum glucose in both groups (Tab.4.2).

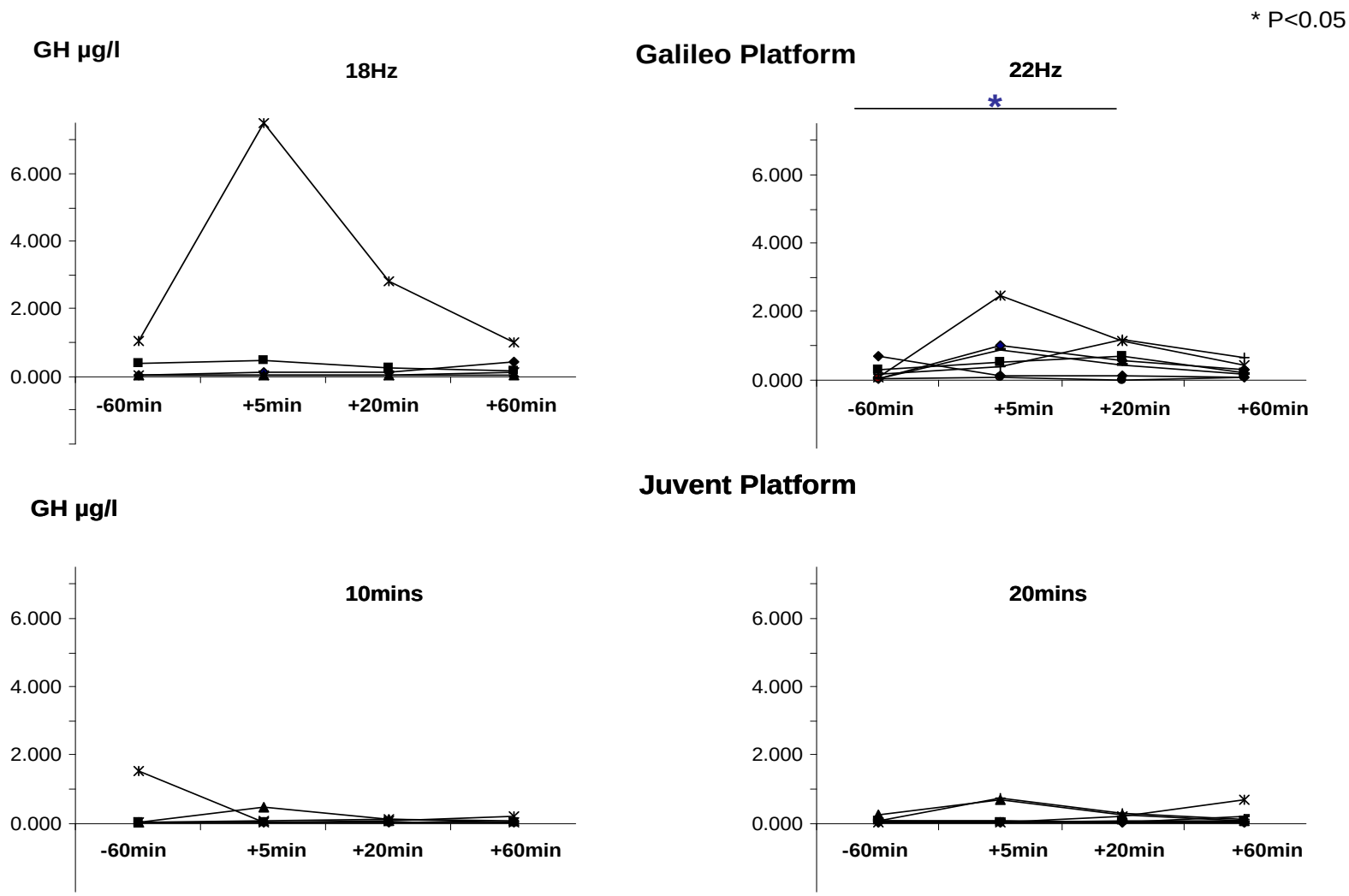


Fig 4.3: Short term effect of WBV on serum cortisol. GP group had WBV at frequency of 18Hz at (T1) and 22Hz at (T2,T3). JP group stood for 10minutes at (T1) and 20minutes at (T2,T3). *p<0.05.

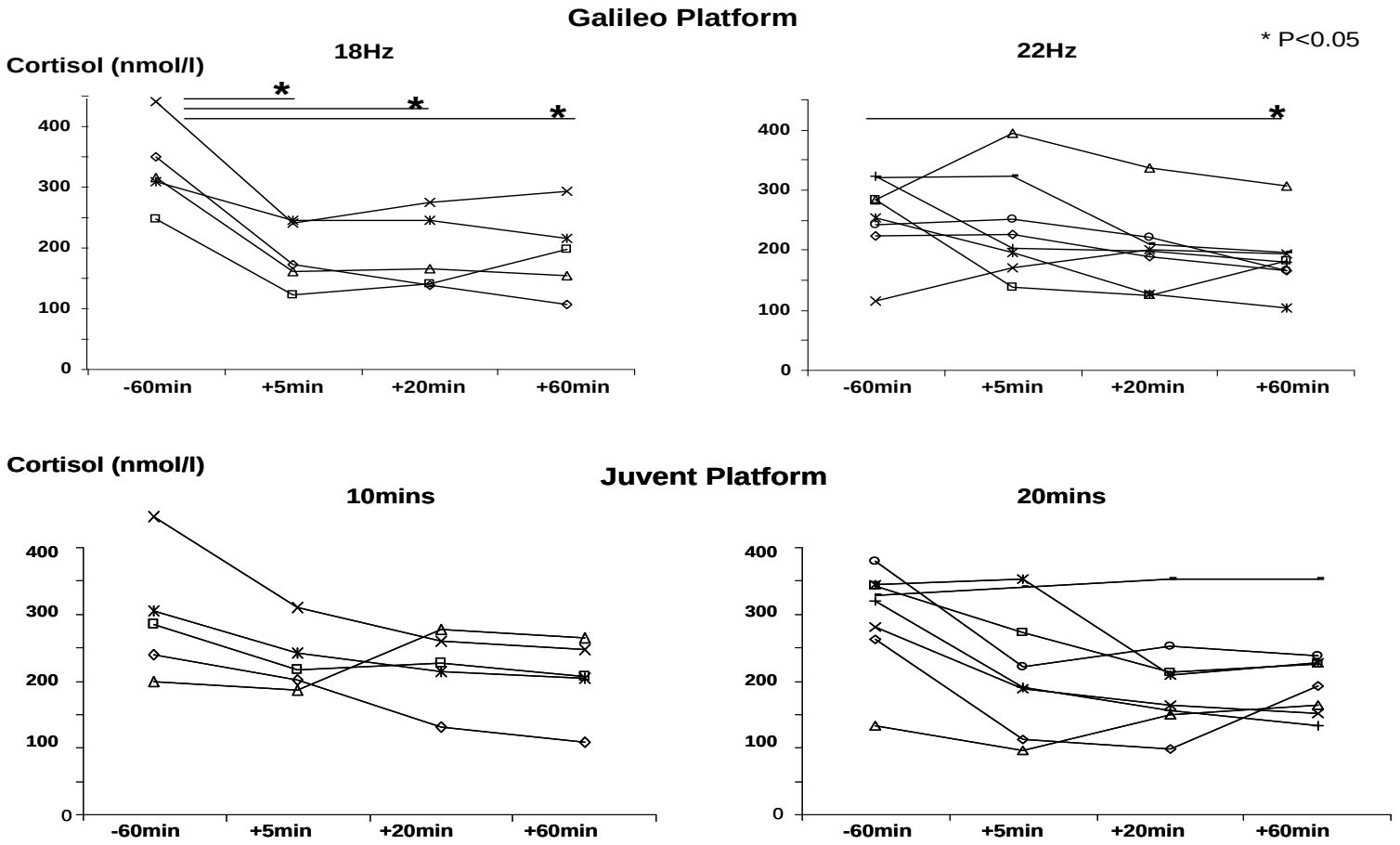


Fig 4.4: Short term effect of WBV on GH. GP group had WBV at frequency of 18Hz at (T1) and 22Hz at (T2,T3). JP group stood for 10minutes at (T1) and 20minutes at (T2,T3). *p<0.05.

4.4.4 Medium Term Effects on Biochemical Markers

Median serum CTX, a marker of bone resorption fell significantly over 8 weeks of WBV training in the GP group from 0.42ng/ml(0.29,0.90) pre-WBV to 0.29ng/ml(0.18,0.44) at the end of WBV training (p=0.029). After 4 weeks of stopping exercise, median serum CTX of the post-WBV measurement increased to 0.45ng/ml(0.40,0.66)(p=0.01). There were no significant changes in the JP group. WBV was not associated with any significant change in BAP, OCN, TRAP5 and Scl in either group (Tab.4.3). Over the 8 weeks, there was a reduction in median serum cortisol in the GP group from 333nmol/l (242,445)(pre-WBV) to 270nmol/l(115,323) (WBV)(p=0.04)(Fig.4.4). GH, IGF-1, testosterone, leptin, CK and insulin did not show any significant changes over the period of exercise (Tab.4.3).

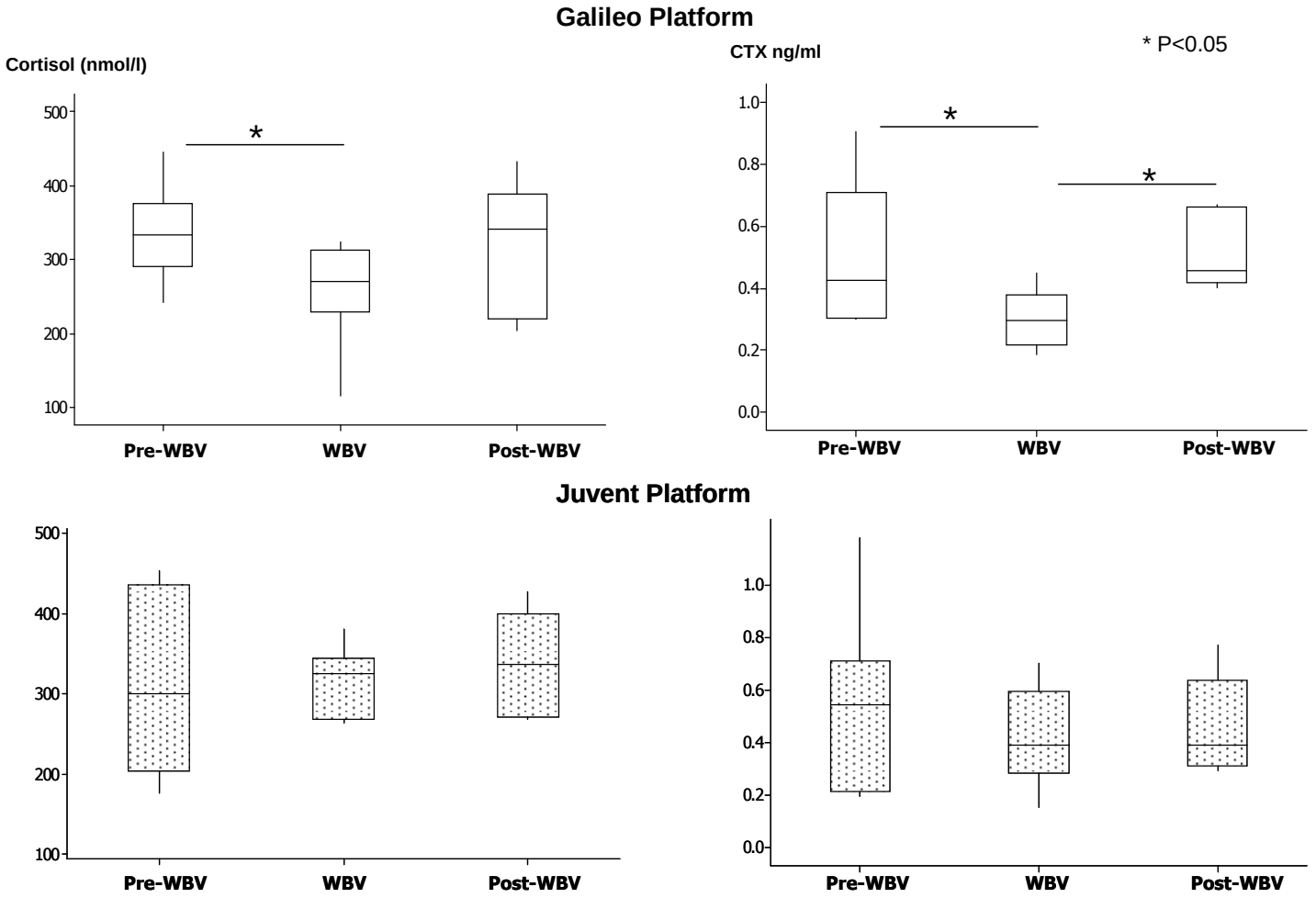


Fig 4.5: Medium term effects of WBV on serum cortisol and serum CTX. The data are presented for three study periods; run-in (T0 to T1), WBV (T2,T3) and wash-out (T4). *p<0.05.

4.5 Discussion

WBV with Galileo and Juvent was well tolerated with a high rate of compliance in the study population. Apart from very mild itching, which was experienced particularly over the shins and thighs and which has been reported previously (386), the exercise regimens were not associated with any adverse reactions. In addition, the current study showed that, over the short-term, exercise with GP was associated with increased serum GH and decreased cortisol concentration. The lowering of circulating cortisol was also observed over the medium term and this fall was also associated with a reduction in bone resorption.

The exact mechanism of WBV is still poorly understood. However, the most commonly cited mechanism of WBV is that it applies tonic vibration (313). Roelants et al. (316) concluded that WBV led to activation of lower limb muscles to a magnitude that ranged from 13% to 82% of maximal muscle contraction. However, vibration cannot elicit tonic reflexes when the amplitude is less than 1mm (314). Therefore, the anabolic effect of WBV on bone may be produced by other mechanisms. Furthermore, the effect of WBV on the musculoskeletal system may be dependent on a range of WBV parameters (frequency, amplitude and g force)(314). Torvinen et al. (357) demonstrated that WBV amplitude is positively correlated with muscle performance while others believed that frequency is the most important variable in WBV (360;360;361).

Vibratory exercise has been suggested to have a beneficial effect on muscle strength. Previous studies have suggested that over the short-term, WBV can increase vertical jump height (239;358;479;480). Although we did not assess the immediate effect of WBV on jump performance, there was no significant change in muscle function over the 8 weeks for either vibratory platform. We did observe a positive trend for an improvement in vertical jump height in those subjects who stood on the Galileo platform but it is possible that we may have observed a significant change with a larger sample size or over a longer period of study. Our study was based on previous research that has reported significant changes in biochemical parameters after WBV. Over a similar study period to ours, Wyon et al. (239) found that 6 weeks of WBV at 35 Hz for 5minutes twice a week increased vertical jump height and another group has recently reported that 4 weeks of WBV training (three times a week with a frequency 40-Hz, 4-mm) could improve muscle function when added to the conventional training of basketball players (479). However, improved muscle function over such periods is

not a universal finding as reported in a 14 week study of WBV training (30-to 35-Hz frequency and 4 mm) in female basketball players (481).

Previous studies of the acute hormonal responses to WBV exercise have so far reported variable results. However, the investigators reported a significant increase in testosterone and GH and a decrease in the serum concentration of cortisol in healthy young men after 10minutes of WBV exercise (6minutes, 26 Hz , peak-to-peak displacement of 4 mm; acceleration,17g) (243). The association between exercise and immediate GH secretion is well established (482), but the association between serum GH secretion and WBV in young men is variable with some reporting a rise (243;366) and some reporting a lack of association (374;376). In the current study, GH increased significantly only in the GP group when the frequency was 22Hz, suggesting that the effect on GH secretion may be dependent on exercise intensity.

The changes that we observed in serum cortisol immediately after WBV have also been reported by others (243;376). The exercise intensity can be measured by the volume of oxygen consumed while exercising at maximum capacity. This is known as VO2 max (the maximum amount of oxygen in millilitres used in one minute per kilogram of body weight) .Furthermore, several studies report that the moderate to high intensity exercise as characterised by VO_2 max of 60-90% is associated to an increase in serum cortisol, but low intensity exercise actually resulted in a reduction in circulating cortisol levels (483;484). The reported VO_2 max of WBV is less than 50% (381) and, therefore, WBV can be classified as low intensity exercise. Elevation of CK and lactate dehydrogenase following various forms of exercise are previously documented (485). Recently, it was shown that side alternating WBV training in combination with dynamic exercise can double the level of serum CK in 25% healthy adults (364). In this study, we assessed exercise intensity by measuring serum CK and there was no difference between the two study groups. In peripheral tissues, corticosteroid hormone action is determined, in part, through the activity of 11β-hydroxysteroid dehydrogenases (11β-HSD), two isozymes which interconvert hormonally active cortisol (F) and inactive cortisone (E). 11β-HSD type 2 (11β-HSD2) inactivates F to E in the kidney; whilst 11β-HSD type 1 (11β-HSD1) principally performs the reverse reaction activating F from E (486). Intense physical exercise has been reported to be associated with

an increase in the conversion of cortisone to cortisol by stimulating 11β-HSD1 activity (487) whereas low intensity exercise results in a reduction in circulating cortisol levels (483;484). In addition, GH may inhibit 11β-HSD1, increasing conversion of cortisol to cortisone (486) and in the raised GH following exercise may be one possible mechanism for the observed reduction in circulating cortisol over the short-term as well as the medium term. It might argue that the significant drop rate in the cortisol level following WBV in the GP is possibly related to its level in the circadian normal peak in the morning and falls over time in the afternoon. However, this change has not been observed following WBV in the JG.

WBV has been reported to be osteogenic in several animal models (302;321;395), however; in humans this is less clear. Recent studies suggest that whole-body vibration (WBV) can improve measures of bone health for certain clinical conditions and ages (245;319). Our results did not show any positive effects on osteoblast activity but there was a significant negative effect on osteoclast activity as observed by a decrease in serum CTX, a marker of bone resorption, in the Galileo group. Our results are consistent with previous reports of suppressed osteoclast activity after 15minutes of daily WBV in mice (328). WBV at 1.5g may also be associated with reduced pyridinoline crosslinks production in aging mice (488). More recently, WBV training for 8 weeks (3times/week) in post-menopausal women was associated with in a significant reduction in N-telopeptide-x when compared with sham vibration exposure (326). The reduction in bone resorption in these studies, as well as ours, may be due to the observed reduction in circulating cortisol. TRAP5b, another marker of bone resorption also reduced in both groups but the reduction was not significant. It is possible that the positive effect on bones is mediated via osteocyte signalling and we, therefore, measured Scl which increases in response to unloading through antagonizing Wnt/β-cantenin signalling (43). Our results suggest that in these healthy young adults, the reduction in biochemical markers of bone resorption was independent of changes in Scl.

A number of important limitations need to be considered. The most important limitation lies in the fact that the small size sample was underpowered for the outcomes because the size of the pilot study was unknown. As the number of participants was small, the effect of outliers may have been greater and skewed the results. The inclusion of a non-intervention group would have enabled a more detailed analysis of the cortisol results and distinguished whether

the rate of fall of cortisol was more than expected in the intervention groups. Furthermore, two months of WBV exposure may not have been sufficient to stimulate any musculoskeletal adaptations, particularly in muscle performance. A lack of familiarisation tests prior to baseline assessment may have led to results confounded by learning effects.

In summary, WBV, as delivered through the Galileo platform was associated with a measurable increase in circulating GH and a decrease in circulating cortisol. These changes were not associated with any changes in muscle function over this period but a significant fall in bone resorption was, nevertheless, observed. It is possible that some of the beneficial effects of WBV on bone health are mediated through its effects on bone turnover through alteration in GH and cortisol production rather than through muscle function.

		T0	T1	T2	T3	T4
Weight (kg)	**GP**	75(72,82)	74(71,82)	75(74,83)	75(70,82)	74.6(72,82)
	JP	74(72,86)	75(73,87)	74(70,87)	74(72,87)	73(70,86)
MGF (kg)	**GP**	43(35,51)	49(42,53)	47(43,57)	45(35,53)	45(35,48)
	JP	49(32,53)	45(32,62)	48(35,58)	45(31,49)	43(30,53)
BMI	**GP**	24(23,28)	24(23,28)	25(23,28)	24(22,28)	24(22,28)
	JP	24(23,29)	23(23,30)	23(23,30)	23(23,30)	23(23,30)
FAT%	**GP**	17(16,25)	18(16,23)	19(15,34)	17(15,23)	18(16,22)
	JP	19(17,29)	19(18,28)	19(17,30)	19(18,30)	19(17,27)
FM (kg)	**GP**	13(12,19)	14(12,18)	15(11,18)	12(11,17)	14(12,18)
	JP	14(12,25)	14(13,24)	14(12,26)	14(13,26)	14(12,23)
FFM (kg)	**GP**	62(56,63)	61(57,63)	63(56,65)	60(57,65)	60(58,64)
	JP	61(59,62)	60(58,62)	61(59,62)	60(43,61)	60(58,63)
TBW (kg)	**GP**	45(41,46)	45(42,46)	46(41,47)	44(42,47)	44(42,46)
	JP	44(43.5,45)	44(43,45)	44(43,45)	43(43,44)	44(42,46)
EFI(%)	**GP**	91(73,92)	90(72,98)	92(76,101)	91(78,97)	86(80,99)
	JP	93(86,107)	86(84,114)	92(87,111)	91(82,120)	86(83,114)
Jump Height (cm)	**GP**	41(32,51)	41(39,51)	43(41,50)	45(43,55)	44(41,52)
	JP	51(35,55)	42(36,52)	43(35,52)	48(32,52)	49(33,55)
F max (kN)	**GP**	2.13(1.65,2.81)	1.88(1.60,2.72)	1.94(1.75,2.43)	1.79(1.60,2.28)	1.92(1.63,2.21)
	JP	1.87(1.77,2.77)	2.11(1.73,2.63)	2.24(1.77,2.48)	2.27(1.70,2.55)	2.00(1.73,2.75)
P maxt (kW)	**GP**	3.51(3.18,3.93)	3.76(3.16,4.03)	3.84(3.41,4.1)	3.80(3.53,4.08)	3.71(3.54,3.95)
	JP	3.85(3.58,4.43)	3.72(3.50,4.77)	3.80(3.53,4.7)	3.90(3.27,4.98)	3.71(3.22,4.66)
Efficiency (%)	**GP**	78(56,87)	81(67,92)	82.5(76,88)	85(80,93)	82(80,90)

	JP	91(59,101)	87(63,00)	87(64,98)	91(61,95)	86(57,103)
CK (IU/L)	**GP**	203(68,300)	141(64,462)	185(97,390)	149(84,261)	168(126,270)
	JP	136(118,234)	135(77,150)	123(104,338)	125(83,185)	117(78,253)
Leptin (ng/ml)	**GP**	8.4(7,16.6)	9.6(4.7,11.9)	6.6(6.4,12.4)	13.1(5.4,13.2)	9.8(4.2,13.2)
	JP	9.2(6.7,14.9)	8.9(5,21.3)	10(3.7,23.9)	11(6.6,14.8)	8.2(5.1,14.7)
Insulin (uU/ml)	**GP**	6.4(4.6,21.8)	7.3(3.8,17.3)	4.4(2.2,9.7)	10.6(0.3,24.4)	7.1(5.2,10)
	JP	10.7(5.2,14.3)	7.7(4.6,10.2)	9.6(4.3,11.3)	7.55(4.8,16.1)	8.9(7.1,27.7)
Testo (nm/L)	**GP**	16(13,28)	18(12,26)	16(12,28)	16(10,30)	17(12,31)
	JP	20(9,21)	18(10,25)	22(12,24)	19(12,21)	19(9,22)
GH (µg/l)	**GP**	0.14(0.04,0.73)	0.05(0.04,1.03)	0.09(0.04,0.30)	0.04(0.04,0.69)	0.09(0.04,3.68)
	JP	0.07(0.04,0.22)	0.04(0.04,0.15)	0.05(0.04,0.23)	0.07(0.04,0.09)	0.04(0.04,0.31)
IGF1 (ng/ml)	**GP**	246(21,289)	240(207,479)	223(183,282)	231(166,347)	214(210,348)
	JP	200(179,228)	208(159,257)	200(132,233)	187(132,266)	173(118,209)
Cortisol (nmol/l)	**GP**	351(242,445)	316(247,442)	284(225,285)	255(115,323)	341(203,433)
	JP	367(175,444)	289(202,454)	272(133,342)	332(28,380)	337(268,380)
Glucose (mmol/L)	**GP**	5.0(4.5,5.4)	4.3(3.7,5.7)	4.8(4.7,5.7)	5.6(4,6.3)	5.1(4.4,5.8)
	JP	5.6(5.3,5.8)	5.3(4,5.6)	5.3(5,5.5)	5.3(5.1,6)	5.3(4.9,5.7)
BAP (µg/l)	**GP**	19(10,32)	20(10,25)	14(12,14)	14(14,36)	16(5,49)
	JP	13(12,33)	16(10,20)	12(11,26)	12(7,17)	13(9,28)
OCN (ng/ml)	**GP**	17(11,19)	17(14,23)	18(14,22)	18(5,24)	18(15,29)
	JP	19(10,28)	18(11,25)	18(12,30)	12(7,26)	17(9,26)
CTX (ng/ml)	**GP**	0.48(0.30,0.86)	0.35(0.29,0.90)	0.28(0.20,0.38)	0.29(0.18,0.44)	0.45(0.40,0.66)
	JP	0.65(0.19,1.18)	0.41(0.20,0.70)	0.31(0.15,1.09)	0.43(0.30,0.48)	0.38(0.29,0.77)
TRAP5b (ng/ml)	**GP**	3.2(1.9,6.2)	3.8(2.2,5)	2.4(1.9,4.7)	3.9(2.3,5)	4.1(0.5,6.8)

	JP	2.8(2.14,3.6)	1.9(1,3.9)	2.7(2.1,3.8)	1.62(1.61,2)	3(1.4,4)
Scl (ng/ml)	**GP**	0.38(0.16,0.60)	0.31(0.02,0.53)	0.22(0.18,0.32)	0.39(0.19,0.52)	0.37(0.20,0.54)
	JP	0.27(0.23,0.85)	0.37(0.19,0.84)	0.41(0.21,0.79)	0.30(0.07,0.55)	0.30(0.05,0.81)
Jump Height/FM	**GP**	3(1.7,4.2)	2.9(2,3.9)	2.8(2.2,4.2)	3.6(2.5,4.3)	3.1(2.2,4.3)
	JP	3.6(1.4,4.1)	3(1.5,3.6)	3.3(1.4,4.2)	3.4(1.4,3.9)	3.3(1.7,4.3)
Jump Height/FFM	**GP**	0.65(0.50,0.82)	0.7(0.6,0.8)	0.7(0.6,0.8)	0.7(0.6,0.9)	0.7(0.6,0.8)
	JP	0.83(0.58,0.90)	0.68(0.6,0.86)	0.69(0.58,0.85)	0.7(0.5,1.1)	0.8(0.5,0.9)
Leptin/FM ratio	**GP**	0.64	0.68	0.44	1.0	0.70
	JP	0.65	0.63	0.71	0.78	0.58

Tab 4.1: Physical and biochemical parameters at every time point (T0-T4) in GP group and JP group. Weight, grip force (GF), body mass index (BMI), Fat mass (FM), free fat mass (FFM), total body water (TBW), Esslinger Fitness Index (EFI), force maximum total (F max), power maximum total (P max), growth hormone (GH), insulin like growth factor-1 (IGF-1), bone specific alkaline phosphatase (BAP), osteocalcin (OCN), Serum cross linked c-telopeptide of type I collagen (CTX), Tartrate-resistant acid phosphatase 5b(TRAP5b), sclerostin (Scl) and creatine kinase (CK),Jump height adjusted forFM(Jump Height/FM),. Jump height adjusted forFFM (Jump Height/FFM), Leptin/FM ratio.

		GP			
		-60 minute	**+5 minute**	**+20 minute**	**+60 minute**
GH (µg/l)	**18Hz**	0.05(0.04,1.03)	0.11(0.05,1.5)	0.11(0.04,2.83)*	0.16(0.04,1.02)
	22Hz	0.07(0.04,0.69)	0.52(0.06,2.47)	0.63(0.10,1.18)	0.21(0.07,0.65)
Cortisol (nmol/l)	**18Hz**	316(247,442)	173(123,245)*	165(139,276)*	198(106,294)*
	22Hz	269(115,323)	214(139,394)	200(125,337)	181(104,306)*
CK (IU/L)	**18Hz**	141(64,462)	172(76,489)	137(65,422)	155(79,492)
	22Hz	167(84,390)	157(78,329)	153(77,350)	147(94,343)
Glucose (mmol/L)	**18Hz**	4.3(3.7,5.7)	4.5(3.4,4.8)	5.4(3.7,5.5)	5.3(3.7,5.8)
	22Hz	5.4(4,6.3)	5.4(4.6,6.1)	5.7(4.6,6.1)	5.6(4.6,5.9)
		JP			
		-60 minute	**+5 minute**	**+20 minute**	**+60 minute**
GH (µg/l)	**10mins**	0.04(0.04,1.52)	0.04(0.04,0.48)	0.05(0.04,0.12)	0.04(0.04,0.20)
	20mins	0.05(0.04,0.23)	0.05(0.04,0.72)	0.05(0.04,0.27)	0.08(0.04,0.68)
Cortisol (nmol/l)	**10mins**	289(202,454)	220(190,315)	231(133,282)	211(111,270)
	20mins	301(328,380)	206(97,352)	187(99,352)	209(133,353)
CK (IU/L)	**10mins**	135(77,150)	134(88,154)	140(89,145)	136(109,414)
	20mins	123(83,338)	138(92,348)	135(86,340)	142(85,341)
Glucose (mmol/L)	**10mins**	5.3(4,5.6)	4.9(4.1,5.6)	5.1(4.1,5.4)	4.7(4.2,5.8)
	20mins	5.3(5,6)	5.3(4.2,6.3)	5.1(4.2,5.9)	5.1(4.8,5.4)

Tab 4.2: Short term effect of WBV on biochemical parameters in GP and JP groups. These markers included serum GH, cortisol, CK and glucose. These measurement were taken at four discrete time points; 60minutes before WBV (-60minutes), 5minutes(+5min), 20minutes (+20minutes) and 60minutes (+60minutes), after completing the WBV training, respectively.*significant difference from -60min, $p < 0.05$

	TP	Pre-WBV (T0,T1)	WBV(T2,T3)	Post-WBV(T4)
Leptin (ng/ml)	**GP**	9(4.7,16.6)	9.5(5.4,13.2)	9.8(4.2,13.2)
	JP	9(5,21.3)	11(3.7,23.9)	8.2(5.1,14.7)
Insulin (uU/ml)	**GP**	6.8(3.8,21.8)	8.4(0.3,24.4)	7.1(5.2,1)
	JP	8.5(4.6,14.3)	7.6(4.3,16.1)	8.9(7.1,27.7)
Testos (nm/L)	**GP**	17(12,28)	16(10,29)	17(12,31)
	JP	19(9,25)	20(12,24)	19(9,22)
GH (µg/l)	**GP**	0.09(0.04,1.03)	0.07(0.04,0.69)	0.09(0.04,3.68)
	JP	0.05(0.04,1.52)	0.05(0.04,0.23)	0.04(0.04,0.31)
IGF1 (ng/ml)	**GP**	243(207,479)	227(166.6,347)	214(217,384)
	JP	204(159,257)	193(132,266)	173(118,209)
Cortisol (nmol/l)	**GP**	333(242,445)	269(115,323)[1]	341(203,433)
	JP	299(157,454)	324(133,380)	337(268,428)
Glucose (mmol/L)	**GP**	4.9(3.7,5.7)	5.4(4,6.3)	5.1(4.4,5.8)
	JP	5.3(4,5.8)	5.3(5,6)	5.3(4.9,5.7)
BAP (µg/l)	**GP**	19(9,31)	14(12,36)	16(5,49)
	JP	13(10,33)	12(7,26)	13(9,28)
OCN (ng/ml)	**GP**	17.(11,22)	17(5,24)	18(14,29)
	JP	18(10,27)	16(7,30)	17(9,25)
CTX (ng/ml)	**GP**	0.42(0.29,0.90)	0.29(0.18,0.44)[1]	0.45(0.40,0.66)[2]
	JP	0.54(0.19,1.18)	0.39(0.15,1.09)	0.38(0.29,0.77)
TRAP5b (ng/ml)	**GP**	3.5(1.9,6.2)	3.3(1.9,5)	4.1(0.5,6.8)
	JP	2.4(1,3.9)	2.3(1.6,3.8)	3(1.4,4)
Scl (ng/ml)	**GP**	0.35(0.02,0.60)	0.27(0.18,0.52)	0.37(0.20,0.54)
	JP	0.32(0.19,0.85)	0.31(0.07,0.79)	0.30(0.06,0.81)
CK (IU/L)	**GP**	172(64,462)	167(84,390)	168(126,270)
	JP	135(77,234)	123(83,338)	117(78,253)

Tab 4.3: Medium term effect of WBV on biochemical parameters in GP and JP groups. These parameters included serum leptin, insulin, testosterone, GH, IGF-1, cortisol, glucose, BAP, OCN, CTX, TRAP5b, Scl and CK. The measurements were assessed at three different times; run-in (T0, T1), WBV (T2, T3) and wash-out (T4). [1] significant difference between pre-WBV and WBV ($p<0.05$). [2] significant difference between WBV and post-WBV ($p<0.05$).

Chapter 5

A Randomised Controlled Trial of the Effect of Vibrational Exercise on the Bone Health of Children with Acute Lymphoblastic Leukaemia

5.1 Abstract

Background: There is a need to reduce the bone morbidity that is observed in children receiving chemotherapy for acute lymphoblastic leukaemia (ALL).

Aim: To assess the effect of Whole Body Vibration (WBV) on the bone health of children receiving chemotherapy for ALL.

Patients and Methods: In this four-month trial, 16 children with ALL (age 5 to 13.8 years; nine boys) were randomised either to receive side-alternating WBV (Galileo, Novotec, Pforzheim, Germany)(16-20Hz, 2mm (peak to peak displacement), 1-1.6g)(n,9) or to stand on a still platform as a control group (n,7) for 9minutes, once/week for four months. Measurements were performed at baseline, two-month and four-month assessing bone health (DXA and p.QCT), body composition and muscle function by imaging and biochemical assessment. DXA BMC data were corrected for bone area and presented as BMC z-score.

Results: The median compliance rate measured as a ratio of actual completed minutes and expected minutes of WBV was 55%(17,100). The median percentage change of TB-BMC z-score in the WBV group from baseline to four-month was -10%(-25,10)(p=0.1), whereas it was -87%(-203,4)(p=0.07) in the control group. The median LS-BMC z-score (L2-L4) in the WBV group was -0.4(-1.3,0.3) and -0.3(-1.4,1.5) at baseline and four-months, whereas the respective data in the control group were 0.04(-0.6,2.4) and -0.1(-1.1,1), respectively. The median percentage change in LS-BMC z-score from baseline to four-month was -19%(-349,365)(p=0.1) and -75%(-1016,178)(p=0.1) in the WBV and control groups, respectively.

Conclusion: WBV is tolerated by children receiving chemotherapy. WBV may prevent the deterioration in bone mineral density that is seen in children on chemotherapy and requires further study.

5.2 Introduction

Acute lymphoblastic leukaemia (ALL) is the commonest paediatric cancer representing about one third of all childhood malignancies. As the survival rate of ALL has improved dramatically (448), the adverse effects that may occur during chemotherapy, as well as after completion of therapy have gained greater attention. Adverse bone health has now been well documented in children receiving chemotherapy and may be manifested overtly as skeletal fractures (71-73;460) or sub-clinically, as changes in markers of bone turnover and bone density, which are characteristic of an overall state of bone loss (68;182;489). The osteoporosis that occurs in these children has a multifactorial aetiology and includes bone marrow involvement in the disease process, hypogonadism, nutritional and mineral deficits, reduced physical activity and long-term use of drugs such as GCs, methotrexate and vincristine, which can cause a combination of myopathy, osteopathy and neuropathy (172;490). Whilst tailoring the use of such chemotherapy agents to match the risk profile of each patient may reduce the likelihood of adverse effects in the future, there is a need to explore interventions that are targeted specifically at improving bone health.

Physical activity, which is known to influence bone mass accretion, particularly in childhood (491), is often reduced in children on chemotherapy as well as after completion of therapy (198;199). In addition, there is an association between length of inpatient stay and subsequent fractures (492). Exercise regimens that increase muscle bulk or increase mechanical loading on the skeleton may prove beneficial for skeletal health (237), but performing exercise at home in children with ALL is associated with poor adherence (188). Mechanical loading can be achieved by a fixed regimen of weight bearing exercises or with the help of a whole-body vibration (WBV) platform, which delivers vibration stimuli that have been investigated to have beneficial effects on muscle function (493) and bone mineral density (242). It is also possible that WBV is as effective as weight bearing exercise, whilst shortening the time required for the exercise. In this study, we explore the feasibility of using WBV in children receiving chemotherapy for ALL and assess its effect on bone health and body composition.

5.3 Subjects and Methods

5.3.1 Patients

The study was performed in children with ALL who presented to the Royal Hospital for Sick Children, Glasgow, UK between 2005 and 2009. Those children who were less than four years old or who had started chemotherapy less than four months previously were excluded as were children who had completed their chemotherapy more than two years previously. Of the 79 (Fig.5.1) children who presented during the study period, 23 were recruited into the study and randomised into either receiving WBV or acting as control and 56 children were excluded for a range of reasons. In the WBV group, two children withdrew from the study even before starting the WBV regimen. Another child had an accidental fall at home after one session of WBV regimen and suffered a vertebral fracture and was withdrawn from the study and, finally, one child suffered a seizure at home after three sessions of WBV and withdrew from the study; the seizure was, itself, attributed to the neurotoxic effects of chemotherapy. In the control group, three children declined to participate in the study because they were not adherent.

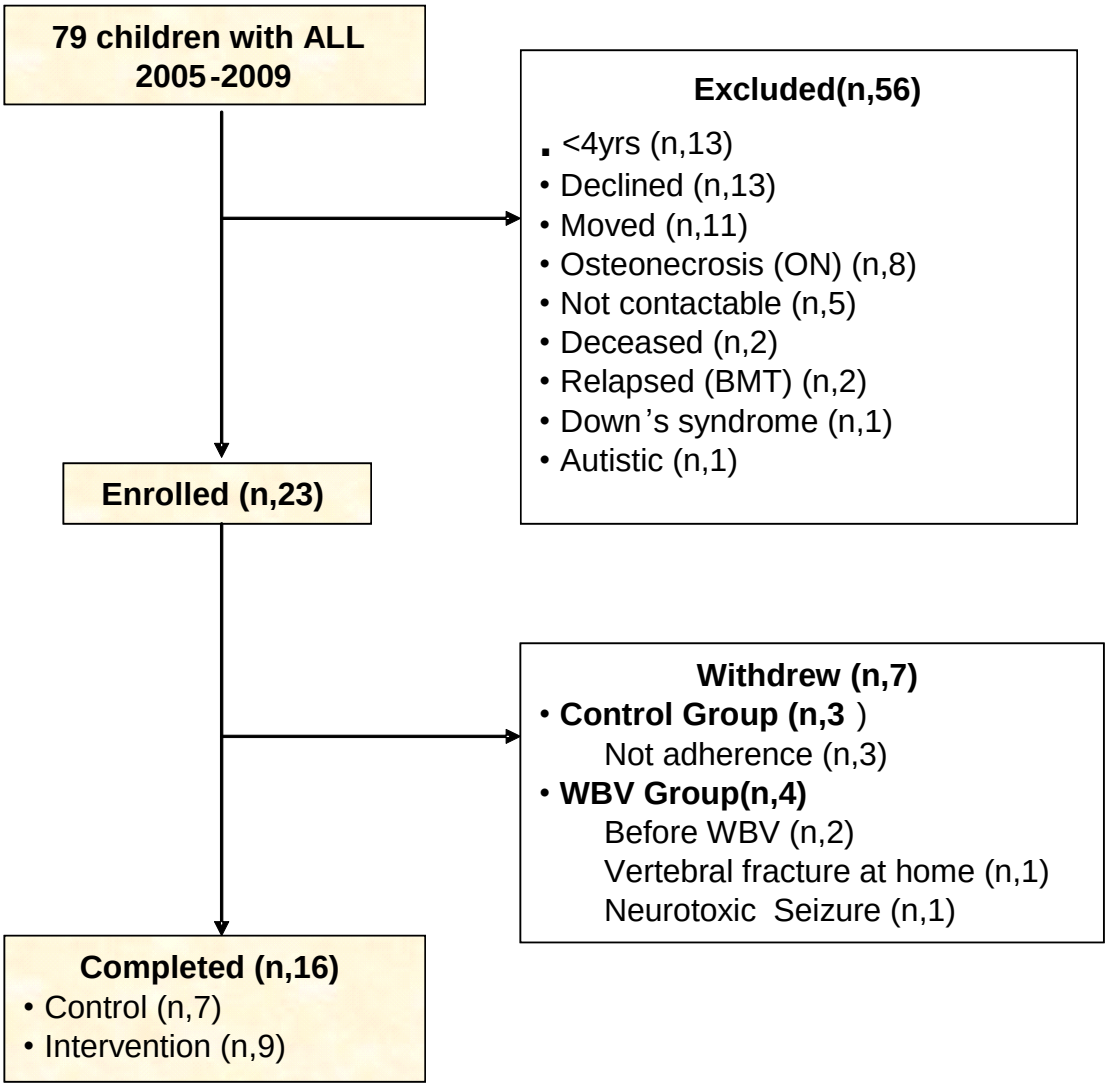


Fig 5.1: Consort diagram for the study. The total number of children who were diagnosed with ALL between 2005 and 2009 was 79. 56children were excluded from the study because of the listed reasons; 23children were eligible of whom 7. The remaining 16children were randomised into the WBV group (9) and the control group (7).

5.3.2 Chemotherapy Treatment

ALL children received therapy as part of the Medical Research Council trial of childhood ALL treatment -UKALL2003. The treatment plan included sustained induction, and consolidation with two or three blocks of intensive chemotherapy, followed by chemotherapy maintenance for two years for girls and three years for boys through any one of three arms (Regimen A, B, C). During induction therapy dexamethasone was administered for 28 days and then tapered over the next seven days (Fig.5.2). Regimen A was reserved for children younger than 10 years old and those with a white cell count less than $50x10^9$/L. Regimen B was reserved for children who were over 10 years old or had a white cell count greater than $50x10^9$ /L. Those children, who had a poor response to the two previous regimens, were positive for minimal residual disease at day 29 or had unfavourable cytogenetic results were allocated to regimen C. The duration of treatment for regimens A and B was shorter in girls (112-114weeks) compared with boys (164-166weeks), whereas the treatment duration in regimen C was longer in both girls (118weeks) and boys(170weeks). The total dose of dexamethasone received over that period was 1080mg/m^2, 1470mg/m^2, 1010mg/m^2, 1430mg/m^2 for girls receiving regimens A or B, boys receiving regimens A or B, girls receiving regimen C and boys receiving regimen C, respectively.

Randomisation was performed at recruitment following stratification into two-month blocks based on time from diagnosis. Amongst the 16 children who completed the study, 9 were randomised to the WBV group and 7 to the Control group. There were no significant differences between the two groups (Tab 5.1). The total duration of the study period in each child was 20 months and consisted of a four-month intervention period followed by a 16-month period of observation (Fig.5.3). Measurements were performed at baseline, two-month and four-month after start of the intervention. Information on any skeletal morbidity including MSP and fractures was collected retrospectively, as well as prospectively from the point of recruitment. The study was approved by the National Research Ethics Service and informed consent was obtained from all parents and children, where appropriate.

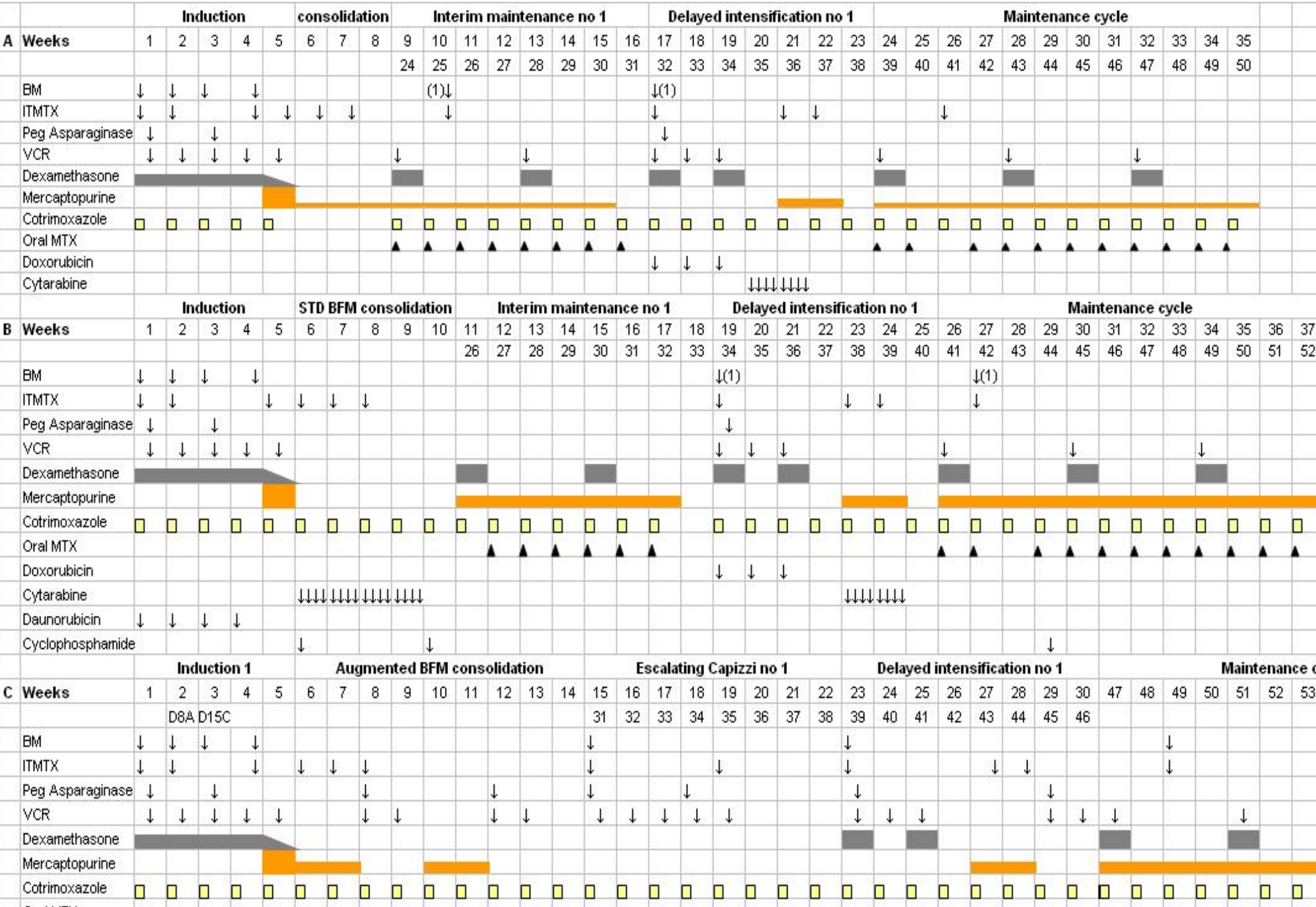

A
Induction
consolidation
Interim maintenance no 1
Delayed intensification no 1
Maintenance cycle
Weeks
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35
24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50
BM
ITMTX
Peg Asparaginase
VCR
Dexamethasone
Mercaptopurine
Cotrimoxazole
Oral MTX
Doxorubicin
Cytarabine
B
Induction
STD BFM consolidation
Interim maintenance no 1
Delayed intensification no 1
Maintenance cycle
Weeks
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37
26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52
BM
ITMTX
Peg Asparaginase
VCR
Dexamethasone
Mercaptopurine
Cotrimoxazole
Oral MTX
Doxorubicin
Cytarabine
Daunorubicin
Cyclophosphamide
C
Induction 1
Augmented BFM consolidation
Escalating Capizzi no 1
Delayed intensification no 1
Maintenance c
Weeks
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 47 48 49 50 51 52 53
D8A D15C
31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46
BM
ITMTX
Peg Asparaginase
VCR
Dexamethasone
Mercaptopurine
Cotrimoxazole

Fig 5.2: The UKALL2003 (regimen A, B and C).

Regimen A – Single Delayed Intensification. There are five stages of treatment: Induction (from 1 to 5 weeks), Consolidation (from 6 to 8 weeks), Interim Maintenance Number 1 (from 9 to 16 weeks), Delayed Intensification Number One (1DI) (from 17 to 23 weeks) and Maintenance Cycle (from 24 to 35 weeks) in each cycle repeated eight times in girls and12 times in boys. Those who were randomised to double Delayed Intensification (2DI) had extra Interim Maintenance Number 2 following (1DI) (from 24 to 31 weeks) and Delayed Intensification Number Two (2DI) (from 32 to 38 weeks) and Maintenance Cycle (from 39 to 50 weeks) in each cycle repeated six times in girls and 11 times in boys. The duration of treatment is 112 weeks in girls and 164 weeks in boys.

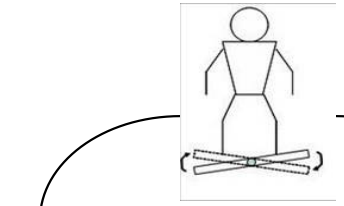
Regimen B – children have the same treatment cycles as regimen A. Additionally, there is standard BFM consolidation (duration five weeks) following induction to 10 weeks. Interim Maintenance Number one (from 11 to 18 weeks), Delayed Intensification Number One (1DI) (from 19 to 25 weeks) and Maintenance Cycle (from 26 to 37 weeks) in each cycle repeated eight times in girls and 12 times in boys. Those who were randomised to double Delayed Intensification (2DI), had extra Interim Maintenance Number 2 following (1DI) (from 26 to 33 weeks) and Delayed Intensification Number Two (2DI) (from 34 to 40 week) and Maintenance Cycle from 39 to 50 weeks in each cycle repeated six times in girls and 11 times in boys. The duration of treatment is 114 weeks in girls and 166 weeks in boys.

Regimen C – there are additional stages of treatment including induction (duration, five weeks), Augmented BFM consolidation (duration, nine weeks), Interim Capizzi maintenance I (duration, eight weeks), Delayed intensification I (duration, eight weeks); Interim Capizzi maintenance II (duration, eight weeks), Delayed intensification II (duration, eight weeks) and finally Maintenance cycles. The duration of treatment is 118 weeks in girls and 170 weeks in boys.

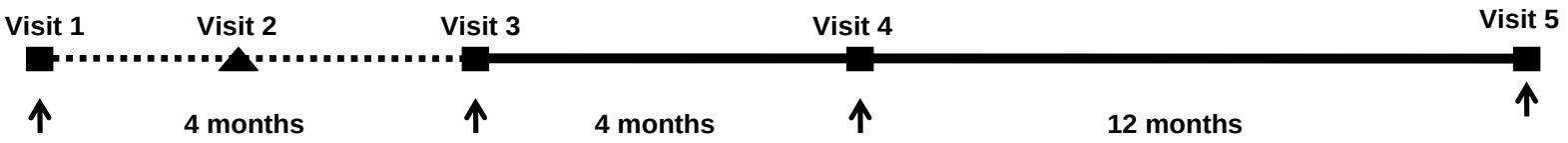
Bone marrow aspirates (BM), intrathecal methotrexate (IT MTX), pegylated L-asparaginase (Oncaspar), vincristine (VCR), dexamethasone, 6-mercaptopurine, Cotrimoxazole, oral methotrexate (MTX), Doxorubicin (Adriamycin), Cytarabine (ara-C), Daunorubicin (only regimen B and C), Cyclophosphamide (only regimen B and C), IV methotrexate (Regimen C).

Group	Sex	Age (years)	Puberty Stage	Comp (%)	In-Patient Stay	Regimens	Chemo duration	D-MIGF SDS	ND-MIGF SDS
WBV1	M	5.0	1	17	0	C	44	1.5	0.1
WBV2	F	5.0	1	34	11	A	14	1.7	0.2
WBV3	M	6.0	1	89	1	A	16	1.7	0.1
WBV4	F	10.0	2	45	0	A	8	1.9	0.2
WBV5	M	12.6	2	100	1	B	20	2.1	0.1
WBV6	M	13.3	3	40	0	A	14	2.2	0.1
WBV7	M	14.9	4	89	0	C	24	2.4	0.1
WBV8	F	15.5	4	94	0	C	16	2.1	0.2
WBV9	F	15.6	4	55	27	C	20	2.1	0.2
C1	M	5.3	1	-	0	A	18	1.8	0.1
C2	F	6.7	1	-	0	A	6	1.7	0.2
C3	M	6.8	1	-	0	A	20	1.8	0.1
C4	F	7.3	1	-	3	A	8	1.9	0.2
C5	M	7.7	1	-	11	C	16	1.7	0.1
C6	F	7.9	1	-	18	A	8	1.6	0.2
C7	M	13.8	2	-	3	C	18	2.2	0.1
Median (WBV)		**12.6**		**55**	**0**		**16**	**2.1**	**0.1**
Median (C)		**7.3**		**-**	**3**		**16**	**1.8**	**0.1**
P Value		**0.2**			**0.6**		**0.3**	**0.3**	**0.9**

Tab 5.1: Patient characteristics assessed at baseline (BL). 9children in the WBV group (WBV1-9) and 7children in the control (C1-7) group. The age and puberty (Tanner stages) were assessed at BL. The compliance rate (comp %) and inpatient stay were evaluated from BL to four-month. The regimens (A,B,C) and duration of chemotherapy (months) were assessed in these two groups. Maximal isometric grip force (MIGF) of the dominant hand (D) and non-dominant hand (ND) were measured at BL. The median values of these measurements and the differences (p value) between the WBV group and the control group were calculated.



Children either stand on a vibratory or still platform (9minutes) once a week every week for 18 weeks (4 months)



Baseline	2months	4months	8months	20months
Clinical assessments	+	+	+	+
Anthropometry	+	+	+	+
Blood tests	+	+	+	+
MIGF	+	+	+	+
DXA	-	+	+	+
p.QCT	-	+	+	+
	Primary outcomes		**Follow up assessments**	

Fig 5.3: Study protocol. Measurements were performed at BL, 2month and 4month, 8months and 20month after start of the intervention. The WBV group stood on a vibratory platform (9minutes) once a week for four months and the control group spent the same time standing on a still platform. The measurements included clinical assessment, anthropometry measurements, Maximal isometric grip force (MIGF), blood samples to assess bone markers; DXA and p.QCT were performed at BL, 2, 4,8 and 20month. No DXA and p.QCT at 2month visit.

5.3.3 WBV Exercise

The WBV schedule was adapted from a previous report that had used the same WBV system in children with neuromuscular disease and bone fragility disorders (306;387). Details of the WBV regimen are outlined as recommended by the International Society of Musculoskeletal and Neuronal Interactions (311). During the intervention period, the children who were randomised to WBV stood on the Galileo vibratory platform with both feet (Novotec, Pforzheim, Germany) once a week for a total of 9minutes, which was divided into three sessions of 3minutes of WBV with a one minute period of rest in between WBV. The participants were standing freely on the device, but the young children usually had support from their parents mainly in the first week. The participants were asked to remove their shoes and stand on the platforms wearing socks. The feet were placed at an equal distance from the centre of the platform and the legs were flexed at the knees and hips at 10 degrees. The device has a motorised board that produces side to side vibrations (sinusoidal) around a fulcrum in the mid-section of the platform**.** Several studies had frequency settings that changed, either during an individual vibration session or during the intervention study period (395). In the present study, the intensity of WBV exercise increased gradually through increasing the frequency in order to improve exercise tolerance in ALL children and to prevent falls during platform use. Therefore, during the first and second treatment months, sessions were performed using a vibration frequency (f) of 16Hz and 18Hz, respectively, and in the last two months the frequency was set at 20Hz. The amplitude of displacement (A) was set at 1mm (total peak to peak displacement (D) was 2mm) and remained static over the study period. The peak acceleration (a_{peak}) is calculated by either these two formulas ($a_{peak=}2x\pi^2Xf^2XD$) or ($a_{peak=}= A(2\pi f)^2$) and peak acceleration is expressed as multiples of the earth's gravity (a_{peak} /9.81g) (306;308). Therefore, the calculated gravitational force for three different frequencies of 16, 18 and 20 Hz were 1, 1.3 and 1.6g, respectively. The control group spent the same time standing on a non-vibratory platform (natural frequency). All the sessions in the WBV and control group were supervised by the research team and occurred when the children were attending the haematology clinic for routine care.

5.3.4 Anthropometry

Height was measured using a Harpenden stadiometer and weight using a standard clinical balance. The weight, height and BMI were expressed as SDS.

5.3.5 Bone Densitometry

DXA scan (Lunar Prodigy,GE Medical Systems, Waukesha, Wisconsin, USA) was performed to assess bone parameters and body composition. To minimise the effect of bone size on BMC values, the predicted total body and lumbar spine (L2-L4) were calculated for bone area as previously described (402). Bone mineral content (BMC) data measured by DXA were corrected for bone area and presented as BMC z-score. Tibial volumetric BMD and surrogate markers of bone strength were also determined by peripheral quantitative CT scan (p.QCT) (Stratec XCT 2000, Software version 6.00, Pforzheim, Germany) at the 4% and 66% site and stress-strain index (SSI) (mm^3) at (38%). In addition, lean and fat areas were assessed at the 66% site. The pQCT scans with any movement artifacts and other potential problems were excluded and repeated in order to have sufficient quality to be included in this study.

5.3.6 Biochemical Assays

All blood sample collections coincided with routine clinic visits; samples were centrifuged at 2600-2800 rev/minute for 10min, and the serum was subsequently stored at -70C. Serum bone-specific alkaline phosphatase (BAP) was measured by Ostase® BAP immunoenzymetric assay (Immunodiagnostic Systems Ltd (IDS Ltd, Boldon, UK) with an intra-assay CV of 5.5% to 7.3%. Serum osteocalcin (OCN) was measured using N-MID® osteocalcin ELISA (IDS Ltd, Boldon, UK) with an intra-assay CV of 3.3% to 9.7%. Serum cross linked C-telopeptide of type I collagen (CTX) was determined using serum crossLaps® ELISA (IDS Ltd, Boldon, UK) with an intra-assay CV of 1.9% to 4.2%. Serum sclerostin (Scl) was measured using TECO Sclerostin Elisa Kit (Pathway Diagnostic Ltd, Dorking, UK) with an intra-assay CV of 1.1% to 3.9%.

5.3.7 Motor Performance and Physical Activity

Handgrip strength was assessed as maximal isometric grip force (MIGF) with the Jamar handgrip dynamometer (Preston, Jackson, MI, USA) using the dominant and non-dominant arm and the highest measurements were recorded. The MIGF (N) data were converted into age and height based SDS (433). The test was performed in sitting position with elbow flexed at 90° and the children were asked to squeeze as hard as possible. The total inpatient

stay during the study period (baseline to four-month) was collected in both groups as a surrogate marker of physical activity.

5.3.8 Statistical Analysis

The outcome variables were expressed as percentage change between the baseline visit and the four-month visit in each of the two groups. Data were presented as median and ranges and inter-group differences were assessed using Mann-Whitney tests, 1-Sample Wilcoxon, Chi-square test or Kruskal-Wallis tests. Descriptive statistics and significance were determined using the Minitab16 software (Minitab, Coventry, UK), with significance set at a level of 5% ($P<0.05$).

5.4 Results

5.4.1 Patient Characteristics

At baseline, age, gender, chemotherapy duration and regimens were not significantly different between the two groups (Tab 5.2-3). No fracture was reported in the WBV group and the control group prior to the study. Six children in the WBV group and three children in the control group had a history of skeletal pain requiring a radiological assessment. Over the 24 months following recruitment into the study, two children (one in each group) had a history of MSP assessed by X-rays from baseline to four-month. Five (55%) children in the WBV group and three (42%) controls had a history of MSP evaluated by x-rays and two sustained fractures (one in the WBV group and one in the control). The onset of fracture from the baseline visit of the study was 15-months in the WBV and six-months in the control (Tab 5.4). The median duration of in-patient days from baseline to four months was 0day(0,27) and 3days(0,18) in the WBV group and the control group($p=0.6$), respectively.

5.4.2 Compliance

Of nine children randomised to WBV for four months, four children had WBV once a week, two had WBV once every two weeks and the remaining three had WBV less than once every two weeks. The median duration of WBV per week was 5minutes (1.5, 9). The median compliance rate measured as a ratio of actual completed minutes and expected minutes of

WBV was 55% (17,100). No immediate adverse effects were reported apart from very mild, transient itching of the legs immediately following the WBV in three children (33%).

5.4.3 Bone Densitometry

The median TB BMC z-score (Tab. 5.5-6) in the WBV group was -0.6 (-1.3, 2.3) at baseline and remained similar at -0.6 (-1.2,2) at four months (Fig.5.4). In the control group, median TB BMC z-score was 0.03(-0.1,1) at baseline and -0.01(-0.1, 0.2) at four-month. The median percentage change of TB-BMC z-score in the WBV group from baseline to four-month was -10%(-25,10)(p=0.1) and in the control group -87%(-203,4)(p=0.07). For TB-BMC z-score, 3/9 children (33%) in the WBV group and 2/ 7 (28%) children in the control group showed an improvement from baseline to four-month (p=0.8). The median LS-BMC z-score in the WBV group changed from -0.4(-1.3,0.3) at baseline to -0.3(-1.4,1.5) at four-month, whereas in the control group, median LS BMC SDS was 0.04(-0.6,2.4) at the baseline and decreased to -0.1(-1.1,1) at four-month. The median percentage of LS-BMC z-score in the WBV group from baseline to four-month was -19%(-349,365)(p=0.1) and in the control group -75%(-1016,178)(p=0.1). In LS-BMC z-score, 2 children out of 9(22%) in the WBV group and 1 out of 7 (14%) children showed a positive improvement from baseline to four-month (p=0.7). There was no correlation between the percentage changes of TB-BMC z-score and LS BMC z-score with age of children at the time of four-month. The median percentage change of FN-BMD in the WBV group and in the control group from baseline to four-month was -2.8%(-5.6,4.1) and 11.1%(0.8,19)(p=0.008, 95%CI(3,20)).

On assessment by p.QCT(Tab.5.7-8), the median percentage change of total area (TA) at the 66% site from baseline to four-month was 5%(-4,16)(p=0.04,95%CI(0.01,12) and 2.3%(1.3,12.6)(p=0.03,95%CI(1.5,12)) in the WBV group and the control group, respectively. No significant changes were observed in the trabecular BMD (at 4%), and the stress-strain index (SSI) (mm^3) at (38%) and cortical BMD at 66% of the tibial length comparing between the two groups at each time point of study visit.

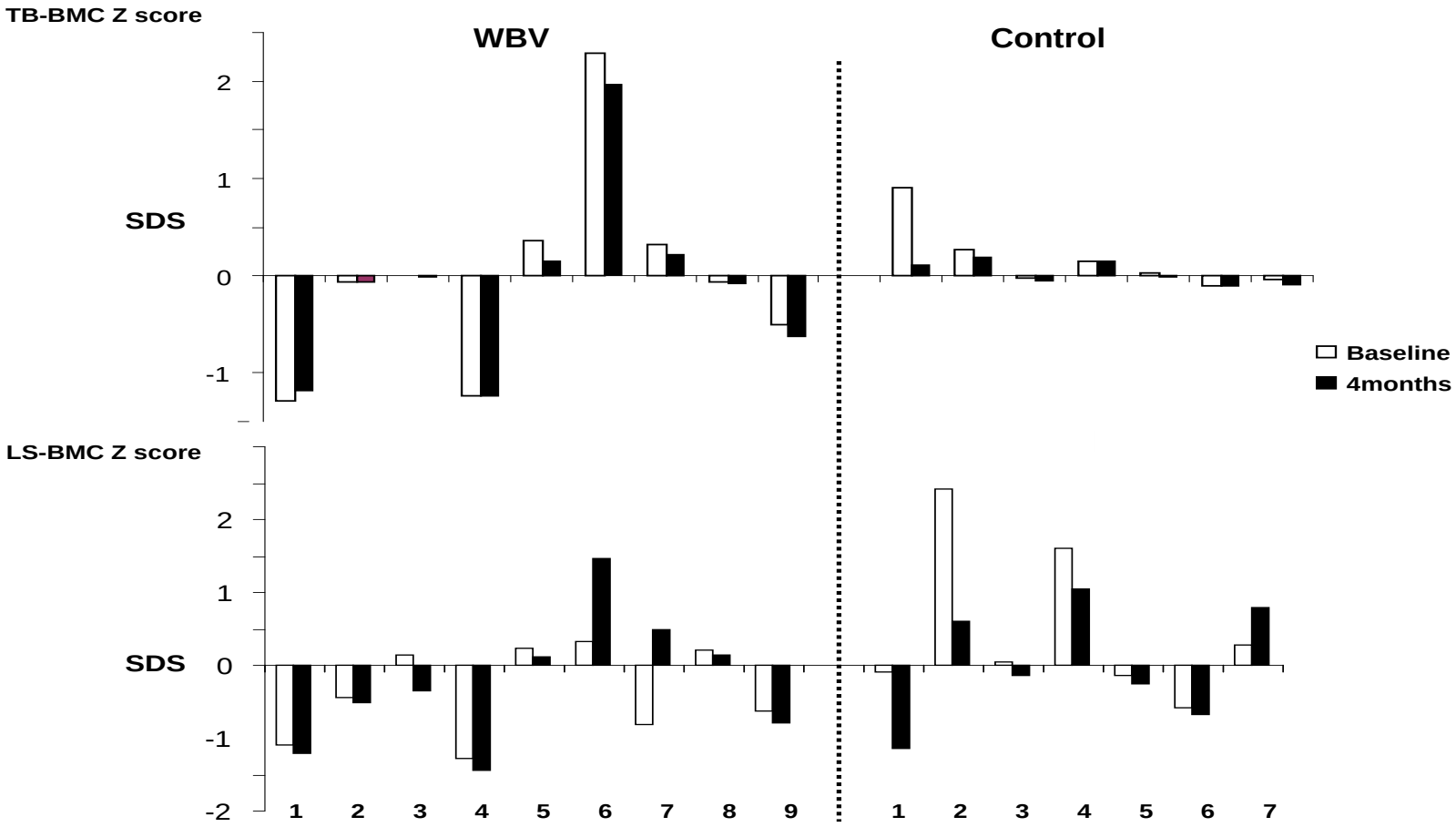


Fig 5.4: The individual measurements of total body bone mineral content z score (TB-BMC-z score) and lumbar spine BMC z scores (LS- BMC z score) at baseline (white box) and four-month (black box). There were 9children in the WBV (1-9) group and 7children in the control group (1-7).

5.4.4 Body Composition

In the WBV group, the median percentage change from baseline to four-month in total body FM% (TB-FM%) was 4% (-5, 44)(p=0.1), whereas in the control group, the median percentage in the same time was 0.2% (-10,8)(p=0.8) (Tab 5.9-10). The median percentage change from baseline to four-month in (TB-FM) in the WBV group was 10%(-7,49) (p=0.04, 95%CI(1.3,27)) and in the control group this was 8% (-10,17)(p=0.4) (Tab.5.9-10). The median percentage change of total body LM (TB-LM) in the WBV group was 4%(-3,10)(p=0.2) and in the control group this was 3.6%(0.2,8) (p=0.01,95%CI(1.8,6.7)). The median percentage change from baseline to four-month in the leg-FM and leg-LM in the WBV group was 12.7% (-13,27) (p=0.04,95%CI(0.4,20)) and 4.4% (0.4,11) (p=0.004, 95%CI(1.7,9)), respectively, and in the control group, the median percentage change in leg-FM and LM in the same time period was 8%(-7,22) (p=0.07) and 6%(3,17) (p=0.02,95%CI(4.5,15), respectively. In the WBV group, the median percentage change of arm FM%, arm FM and LM from the baseline to four-month was 9%(-8,33) (p=0.04,95%CI(0.4,19), 16%(-6,37) (p=0.01,95%CI(6,28), and 5%(-3,12) (p=0.02,95%CI(1,9)), respectively. In the control group, the percentage change of arm FM%, arm FM and LM in the same time was 3%(-12,10)(p=0.4),7%(-16,36)(p=0.2) and 3.4%(-9,17) (p=0.2), respectively. On comparing the two groups, no significant difference was observed in any parameters of body composition at four-month.

5.4.5 Motor Performance

The median measurement of the height-adjusted MIGF SDS of the dominant hand at baseline was -1.3(-2.8, -0.5) and -0.9(-3.2,0.8) for the WBV group and the control group respectively, and at four-month it increased to -0.9(-3.6,2.6)(p=0.1) and -0.4(-2.2,1)(p=0.1) in the WBV group and the control group, respectively. No difference was observed between the WBV group and control group at the four-month visit.

5.4.6 Biochemical Markers

In the control group, median serum BAP was 50µg/l(30,57), 64µg/l(45,67) and 65µg/l(39,81) at baseline, two-month and four-month, respectively and in the WBV group, median BAP was 39µg/l(27,82), 44µg/l(20,89) and 47µg/l (18,70) over the same period. The median serum OCN in the control group were 18ng/ml(6.8,37), 18ng/ml(9,31) and 28ng/ml(18,76) at

baseline, two-month and four-month, respectively, and in the WBV group, median serum OCN was 14.8ng/ml(11,18), 28ng/ml(8.6,101) and 16ng/ml (6.6,46) over the same period. In the control group, median serum CTX was 18ng/ml(7,37), 18ng/ml (9,31) and 28ng/ml (18,76) at baseline, two-month and four-month, respectively, and in the WBV group, the median OCN was 15ng/ml(11,18), 28ng/ml(8.6,101) and 16ng/ml(6.6,46) over the same period. Median serum CTX measurement in the control group was 1.3ng/ml (1,1.8), 1.7ng/ml (0.26,2.1) and 1.6ng/ml(0.72,1.86) at baseline, two and four-months respectively; whereas the median serum CTX measurement in the WBV group 0.8ng/ml(0.07,1.4) at baseline and remained at 0.8ng/ml(0.59,1.4) and 0.9ng/ml(0.52,1.54) at two and four-month (Fig.5.5). In the control group, median serum Scl was 0.28ng/ml(0.22,0.42) and 0.35ng/ml(0.23,0.57) at baseline and four months, respectively (p=0.6) and in the WBV group, median Scl was 0.45ng/ml(0.22,0.48) and 0.22ng/ml(0.15,0.46) (p=0.3) at the same time point.

5.4.7 Follow Up Assessments

The first follow up was conducted four months after cessation of the intervention. Ten children were completed eight-month; seven children were in the WBV group and the remaining three were in the control group. In the WBV group, there was a significant improvement in the median percentage change of FN-BMD from four months to eight months (1.6%(-4, 5) (0.03 95%CI(-8,-0.4)) compared with the change that occurred from baseline to four-month [-2.8% (-7,4)]. No significant changes were observed in any of other bone parameters and body composition at eight-month compared with four-month. The second follow up was conducted at 20-month from the baseline visit (16 months after cessation of the intervention). Six children were completed 20-month; four children were in the WBV group and the remaining were in the control group. No significant changes were observed in any of other bone parameters and body composition at 20-month compared with four-month and eight-month (Tab.5.5-7).

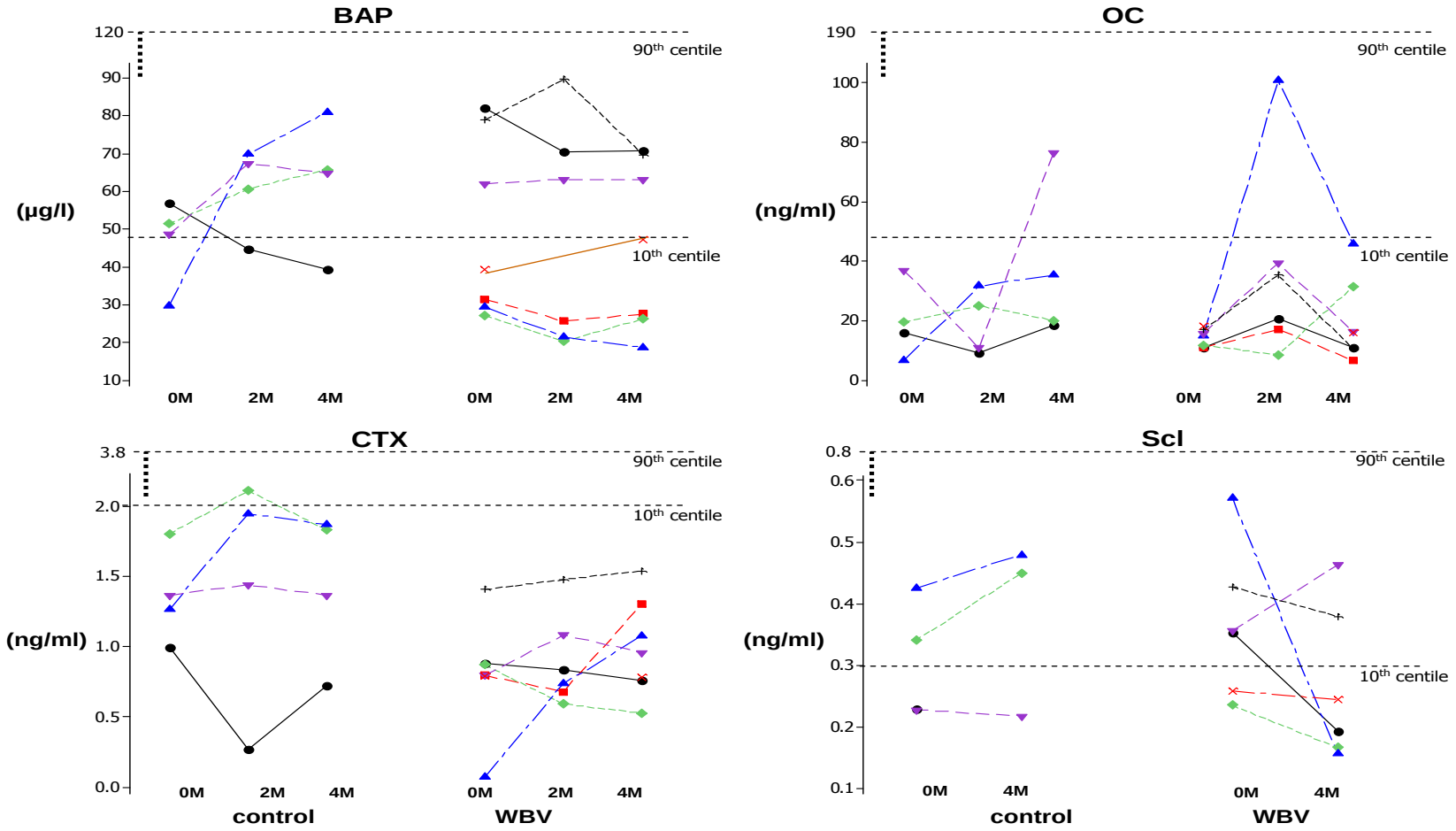


Fig 5.5: The level of serum bone markers. The measurements are bone-specific alkaline phosphatase (BAP) and osteocalcin (OCN), cross linked C-telopeptide of type I collagen (CTX) and sclerostin (Scl). The reference ranges (10^{th},90^{th}centiles) for BAP, OCN and CTX in children aged from 4years to 16years are (48µg/l,121µg/l), (47ng/ml, 191ng/ml) and (2ng/ml, 3.8ng/ml), respectively. The reference ranges for Scl in males and females are (021ng/ml, 1.27ng/ml) and (0.31ng/ml,0.81ng/ml),respectively.

Group	Total Body (TB)			Lumbar spine (LS)			Femoral neck (FN)			Leg			Arm		
	BMC (g)	BA (cm)	BMC z score	BMC (g)	BA (cm)	BMC Z score	BMC (g)	FN-BA (cm)	BMD (g/cm^2)	BMC (g)	BA (cm)	BMD (g/cm^2)	BMC (g)	BA (cm)	BMD (g/cm^2)
WBV1	616	790	2.29	12	20.2	0.31	2.1	3.1	0.69	165	236	0.70	52	96	0.55
WBV2	515	694	0.31	10	20.3	-0.82	1.9	3.1	0.63	106	196	0.54	37	80	0.47
WBV3	741	970	0.36	15	22.3	0.23	2.3	3.8	0.60	216	345	0.62	63	116	0.54
WBV4	1209	1390	-0.06	21.7	25.6	0.21	2.9	3.8	0.77	447	498	0.88	95	164	0.58
WBV5	1190	1548	-1.29	19.4	29	-1.09	2	4	0.49	398	544	0.73	97	174	0.56
WBV6	1349	1524	-0.50	23.5	30	-0.63	2.6	4	0.64	452	559	0.80	137	211	0.65
WBV7	1740	2033	-1.24	29.8	39	-1.28	4.1	5	0.83	685	738	0.92	233	326	0.71
WBV8	2118	1999	-0.01	31.3	31.3	0.13	3.9	4.5	0.86	734	674	1.08	265	310	0.85
WBV9	1948	1976	-0.06	42.4	40.8	-0.43	3.4	4.5	0.77	696	713	0.97	181	252	0.72
C1	613	891	0.03	13.4	21.8	-0.12	1.8	3.2	0.58	156	265	0.59	50	96	0.52
C2	624	812	0.14	12.5	19	1.61	1.7	3.2	0.54	162	261	0.62	41	83	0.49
C3	703	852	0.26	11.6	17.9	2.42	1.9	3.1	0.62	194	243	0.61	60	108	0.55
C4	1083	1245	-0.02	19.2	25.3	0.04	2.5	3.7	0.68	395	459	0.86	87	149	0.58
C5	651	875	0.89	10.2	19.6	-0.10	2.1	4.2	0.50	173	292	0.59	48	97	0.50
C6	1053	1254	-0.03	17.6	23.7	0.28	2.4	3.7	0.64	343	452	0.76	83	148	0.56
C7	1352	1583	-0.11	18.7	26.7	-0.58	3.1	4.1	0.75	511	512	1	103	171	0.60
Median(WBV)	**1209**	**1524**	**-0.06**	**21.7**	**29**	**-0.43**	**2.6**	**4**	**0.69**	**447**	**544**	**0.80**	**97**	**174**	**0.58**
Median(C)	**703**	**891**	**0.03**	**13.4**	**22**	**0.04**	**2.1**	**3.7**	**0.62**	**194**	**292**	**0.62**	**60**	**108**	**0.55**
P Value	**0.2**	**0.2**	**0.4**	**0.08**	**0.04**	**0.2**	**0.1**	**0.3**	**0.1**	**0.1**	**0.1**	**0.2**	**0.1**	**0.1**	**0.2**

Tab 5.2: Bone parameters measured by DXA at BL in the WBV group and the control group**.**

Total body bone mineral content (TB-BMC), total body bone area (TB-BA), total body bone mineral content z score (TB-BMC z-score), lumbar spine bone mineral content (LS-BMC), lumbar spine bone area (LS-BA), lumbar spine bone mineral content z score (LS-BMC z-score), Femoral neck bone mineral content (FN-BMC), femoral neck bone area (FN-BA), femoral neck bone mineral density (FN-BMD) Leg bone mineral content (Leg-BMC), leg bone area (Leg-BA), leg bone mineral density (Leg-BMD)Arm bone mineral content (Arm-BMC), arm bone area (Arm-BA), arm bone mineral density (Arm-BMD) Median for each measurements was calculated in both groups. P value presented the difference between the WBV group and the control group.

Group	Trab vBMD (mg/cm^3)	SSI mm^3	Total bone CSA (mm^2)	Cort vBMD (mg/cm^3)	Cort CSA (mm^2)	FA (mm^2)
Position	4%	38%	66%	66%	66%	66%
WBV1	228	298	359	924	109	1094
WBV2	117	343	373	937	71	1565
WBV3	175	378	446	983	100	1318
WBV4	226	602	494	1024	215	4092
WBV5	222	592	428	1058	171	3269
WBV6	139	825	460	1077	192	2912
WBV7	156	1221	653	1084	267	1366
WBV8	169	1120	584	1171	326	1708
WBV9	147	1352	659	1079	249	4448
C1	117	501	463	935	93	1550
C2	304	399	324	1039	116	1699
C3	119	372	555	900	57	1530
C4	324	589	644	968	132	2530
C5	-	-	-	-	-	-
C6	194	647	435	1040	172	3150
C7	271	882	465	986	209	4498
Median(WBV)	**169**	**602**	**460**	**1058**	**192**	**1708**
Median(C)	**233**	**545**	**464**	**977**	**124**	**2114**
P Value	**0.3**	**0.5**	**0.9**	**0.1**	**0.2**	**0.7**

Tab 5.3: Bone parameters measured by p.QCT at BL in the WBV group and the control group.

Trabecular volumetric bone mineral density (Trab v BMD), stress-strain index of tibial length (SSI), total bone cross sectional area (CSA), cortical volumetric BMD (Cort vBMD), cortical cross sectional (Cort CSA), fat area (FA). P value presented the difference between the WBV group and the control group.

. Group	Number of X-ray before BL	Site of X-ray	Last X-ray before BL Months	Fracture before BL	Number of X-ray after BL	Site of X-ray	Onset of X-ray after BL months	Onset of fracture after BL months
WBV1	-	-	-	-	-	-		-
WBV2	1	Foot	4	-	1	Foot	22	-
WBV3	-	-	-	-	1	Elbow	6	-
WBV4	1	Ulna	3	-	2	Foot	14	-
WBV5	-	-	-	-	1	Hand	2	-
WBV6	4	Knees	24	-	-	-	-	-
WBV7	3	Ankle/Feet/ Radius	10	-	3	Foot/Lumbar spine	15	15
WBV8	1	-	2	-	-	-	-	-
WBV9	3	Ankles/Elbow	6	-	-	-	-	-
C1	2	Radius/Ulna	7	-	-	-	-	-
C2	-	-	-	-	1	Shoulder	6	6
C3	-	-	-	-	-	-	-	-
C4	1	Foot	8	-	-	-	-	-
C5	-	-	-	-	1	Foot	17	-
C6	-	-	-	-	-	-	-	-
C7	1	Lumbar spine	17	-	1	Knee	1	-
Median (WBV)			5	-	-		14	-
Median (C)			8	-	-		6	-
P Value								

Tab 5.4: The rate of skeletal morbidity in the WBV group and the control group occurred before and after BL. The history of X-rays prior and after BL includes the number of X-ray, the sites of X-ray and the onset (months) of X-rays and fracture from BL.

%(BL-4)	WBV1	WBV2	WBV3	WBV4	WBV5	WBV6	WBV7	WBV8	WBV9	Median WBV	C1	C2	C3	C4	C5	C6	C7	Median C
TB-BMC	6.9	46.9	7.4	0.9	1.9	-0.6	0.7	1.5	-2.5	**1.5**	8.9	8.1	6.0	5.2	-0.6	7.1	5.4	**6.0***
TB BA	4.6	-17.1	5.8	1.2	-0.3	1.1	0.4	1.6	-2.1	**1.1**	6.7	3.9	6.7	5.8	0.2	8.5	3.3	**5.8***
TB BMC z score	-14.0	-33.0	-59.0	-10.0	9.0	-25.0	1.0	0.0	3.0	**-10**	-124.0	1.0	-32.0	-104.0	-87.0	-203.0	4.0	**-87**
LS BMC	7.2	4.6	-0.2	2.5	-0.1	0.6	8.0	16.5	-0.8	**2.5**	5.4	10.6	14.6	-4.0	-1.9	14.9	1.4	**5.4**
LS BA	-4.36	-7.09	0.94	1.84	1.72	2.60	8.47	16.50	0.66	**1.7**	3.39	6.88	14.74	-0.08	8.38	2.28	1.87	**3.3**
LS BMC z score	365.8	159.3	-53.4	-30.8	-11.0	-25.5	-11.9	-348.8	-18.7	**-18.7**	-100.3	-35.6	-74.8	-452.2	-1016.2	177.5	-16.3	**-74.8**
FN BMC	12.0	-7.7	-2.6	-1.3	-0.5	0.8	5.7	-5.3	-3.2	**-1.3**	12.8	21.1	3.1	0.8	-23.1	16.2	15.0	**12.8**
FN BA	7.7	-4.2	0.3	4.2	-0.7	3.2	6.6	-2.0	3.3	**3.2**	5.6	3.7	4.2	2.4	-35.4	4.6	0.5	**3.7**
FN BMD	4.2	-3.7	-2.8	-4.9	0.0	-2.6	-0.7	-7.1	-6.3	**-2.8**	6.7	16.7	-0.8	-1.8	19.1	11.1	14.5	**11**
Leg BMC	15.0	22.5	10.7	-1.1	6.5	5.2	0.5	1.8	-5.2	**5.2***	18.6	9.3	-14.8	2.5	1.7	10.1	9.5	**9.3***
Leg BA	7.2	16.3	5.2	10.6	1.8	5.0	0.9	1.3	-4.9	**5.0**	11.7	2.7	9.5	3.5	4.8	7.7	8.8	**7.7**
Leg BMD	7.3	5.3	5.3	-2.6	4.6	0.0	-0.5	0.5	-0.4	**0.5**	5.8	6.6	1.1	-0.9	-2.9	2.0	0.5	**1.1***
Arm-BMC	23.6	7.4	12.1	4.0	9.4	-7.4	0.0	-0.3	46.4	**7.4**	9.1	28.1	3.1	7.6	2.9	-0.6	5.8	**5.8***
Arm BA	15.6	6.3	10.3	1.8	8.6	-4.3	1.8	2.3	-24.1	**2.3**	10.4	21.7	4.6	2.7	3.1	-2.0	1.8	**3.1**
Arm BMD	6.9	0.6	2.0	1.9	1.0	-2.9	-1.7	-2.6	-0.3	**0.6**	-0.8	5.2	-0.5	5.3	-0.2	1.1	4.0	**1.1**

Tab 5.5: Bone parameters measured by DXA showing the percentage changes from BL to 4-month (%(BL-4)) and the median of these percentage changes in the WBV group and the control group.* represents the significant difference ($p<0.05$) between BL and 4month.

	Time	WBV1	WBV2	WBV3	WBV4	WBV5	WBV6	WBV7	WBV8	WBV9	Median WBV	C1	C2	C3	C4	C5	C6	C7	Median C
TB-BMC	**BL**	617	515	742	1209	1191	1349	1741	2118	1949	**1209**	613	625	704	1083	651	1053	1352	**703.8**
	4	660	757	797	1220	1214	1341	1754	2150	1901	**1220**	668	676	746	1139	647	1128	1425	**745.9**
	8	750	591	805		1159		1710	2284	2077	**1159**			768	1175	707			**767.7**
	20			951		1277		1887	2355		**1582**			928		780			**854.1**
TB BA	**BL**	790	694	970	1390	1548	1524	2033	1999	1976	**1524**	891	812	852	1245	875	1254	1583	**891**
	4	826	575	1026	1407	1544	1541	2042	2031	1934	**1541**	951	844	909	1317	877	1360	1636	**951**
	8	897	780	1035		1521		2008	2165	2077	**1521**			933	1325	943			**943**
	20			1181		1612		2147	2141		**1877**			1098		1003			**1051**
TB BMC	**BL**	2.2	0.31	0.36	-0.06	-1.29	-0.50	-1.24	-0.01	-0.06	**-0.06**	0.03	0.14	0.27	-0.03	0.90	-0.03	-0.11	**0.03**
z Score	**4**	1.9	0.21	0.14	-0.07	-1.17	-0.62	-1.23	-0.01	-0.05	**-0.05**	-0.01	0.14	0.18	-0.05	0.11	-0.10	-0.10	**-0.01**
	8	1.5	-0.46	0.11		-1.29		-1.25	-0.02	-0.06	**-0.06**			0.16	-0.03	0.06			**0.06**
	20			-0.25		-1.22		-1.18	0.01		**-0.72**			0.05		0.04			**0.04**
LS BMC	**BL**	12.0	9.9	15.2	21.7	19.4	23.5	29.9	31.3	42.4	**21.7**	13.4	12.6	11.7	19.2	10.3	17.6	18.8	**13.4**
	4	12.8	10.4	15.1	22.3	19.4	23.7	32.3	36.5	42.1	**22.3**	14.1	13.9	13.4	18.5	10.1	20.2	19.0	**14.1**
	8	13.8	10.5	17.0		18.9		29.6	37.0	43.8	**18.9**			11.2	18.3	10.6			**11.2**
	20			18.5		22.2		36.4	40.7		**29.3**			14.4		12.3			**13.3**
LS BA	**BL**	20.2	20.3	22.3	25.6	29.1	30.4	39.1	31.3	40.8	**29.1**	21.8	19.0	18.0	25.3	19.7	23.7	26.7	**21.8**
	4	19.3	18.9	22.5	26.1	29.6	31.2	42.4	36.5	41.1	**29.6**	22.6	20.4	20.6	25.3	21.3	24.3	27.2	**22.6**
	8	20.3	19.5	25.2		29.4		38.0	36.1	41.5	**29.4**			17.8	25.1	20.4			**20.4**
	20			25.9		31.9		45.1	37.1		**34.5**			21.0		23.8			**22.4**
LS BMC	**BL**	0.32	-0.82	0.23	0.22	-1.10	-0.64	-1.28	0.14	-0.44	**-0.44**	-0.13	1.62	2.42	0.04	-0.10	0.28	-0.58	**0.04**
z score	**4**	1.47	0.49	0.11	0.15	-1.22	-0.80	-1.43	-0.34	-0.52	**-0.34**	-0.26	1.04	0.61	-0.15	-1.13	0.78	-0.68	**-0.15**
	8	1.02	-0.05	-0.51		-1.29		-1.14	-0.19	-0.41	**-0.41**			2.37	-0.15	-0.44			**-0.15**
	20			-0.37		-1.20		-1.31	0.08		**-0.79**			0.79		-1.36			**-0.28**
FN BMC	**BL**	2.2	1.9	2.3	3.0	2.0	2.6	4.2	3.9	3.5	**2.6**	1.9	1.8	2.0	2.6	2.2	2.4	3.1	**2.2**
	4	2.4	1.8	2.3	2.9	2.0	2.6	4.4	3.7	3.4	**2.6**	2.1	2.1	2.0	2.6	1.7	2.8	3.6	**2.1**
	8	2.5	1.2	2.3		2.1		4.1	3.8	3.6	**2.5**			2.2	2.9	1.3			**2.2**
	20			2.4		2.0		4.3	4.4		**3.3**			2.7		2.1			**2.4**
FN BA	**BL**	3.1	3.1	3.8	3.8	4.0	4.0	5.0	4.6	4.5	**4.0**	3.2	3.2	3.1	3.8	4.3	3.7	4.2	**3.7**
	4	3.3	3.0	3.8	4.0	4.0	4.2	5.3	4.5	4.7	**4.0**	3.4	3.3	3.3	3.9	2.8	3.9	4.2	**3.4**
	8	3.4	2.0	3.9		4.1		5.1	4.5	4.7	**4.1**			3.3	3.9	2.3			**3.3**

	20			3.8		4.2		5.0	4.6		4.4			3.7		3.6			**3.7**
FN BMD	**BL**	0.70	0.63	0.60	0.77	0.50	0.65	0.84	0.86	0.77	**0.70**	0.58	0.54	0.63	0.68	0.51	0.65	0.75	**0.63**
	4	0.73	0.61	0.59	0.73	0.50	0.63	0.83	0.80	0.73	**0.73**	0.62	0.64	0.62	0.67	0.60	0.72	0.86	**0.64**
	8	0.73	0.62	0.59		0.51		0.79	0.83	0.76	**0.73**			0.65	0.73	0.59			**0.65**
	20			0.62		0.48		0.86	0.95		**0.74**			0.73		0.56			**0.65**
Leg BMC	**BL**	165	107	216	447	399	452	685	734	696	**447**	156	163	194	396	174	344	512	**194**
	4	190	131	239	442	425	475	688	748	660	**442**	185	178	166	406	177	379	560	**185**
	8	234	118	249		415		728	753	728	**415**			180	438	212			**212**
	20			309		460		723	831		**591**			243		245			**244**
Leg BA	**BL**	236	196	345	498	544	559	738	674	713	**544**	265	261	243	459	292	452	512	**292**
	4	253	228	363	551	554	587	745	683	678	**554**	296	268	266	475	306	487	557	**306**
	8	293	203	376		573		661	695	730	**573**			286	488	331			**331**
	20			434		586		757	714		**650**			348		355			**352**
Leg BMD	**BL**	0.70	0.55	0.63	0.89	0.73	0.81	0.93	1.09	0.98	**0.81**	0.59	0.62	0.62	0.86	0.60	0.76	1.00	**0.62**
	4	0.75	0.58	0.66	0.87	0.77	0.81	0.92	1.10	0.97	**0.81**	0.62	0.66	0.62	0.86	0.58	0.78	1.01	**0.66**
	8	0.80	0.58	0.66		0.72		0.91	1.08	1.00	**0.80**			0.63	0.90	0.64			**0.64**
	20			0.71		0.78		0.96	1.16		**0.87**			0.70		0.69			**0.69**
Arm-BMC	**BL**	53	38	63	96	98	137	234	265	182	**98**	51	41	61	88	49	84	104	**61**
	4	65	41	71	100	107	127	234	264	266	**107**	55	53	62	95	50	83	110	**62**
	8	69	27	75		109		223	276	184	**109**			65	104	57			**65**
	20			90		124		245	288		**185**			98		67			**82**
Arm BA	**BL**	96	80	116	164	174	211	326	310	252	**174**	96	83	108	149	97	148	171	**108**
	4	111	85	128	167	189	202	332	317	191	**189**	106	101	113	153	100	145	174	**113**
	8	116	56	135		196		317	324	261	**196**			116	1	110			**110**
	20			156		211		342	333		**272**			151		128			**140**
Arm BMD	**BL**	0.55	0.47	0.55	0.59	0.56	0.65	0.72	0.86	0.72	**0.59**	0.53	0.50	0.56	0.59	0.50	0.57	0.61	**0.56**
	4	0.59	0.47	0.56	0.60	0.57	0.63	0.70	0.83	0.72	**0.60**	0.52	0.52	0.56	0.62	0.50	0.57	0.63	**0.56**
	8	0.60	0.47	0.55		0.56		0.70	0.85	0.70	**0.60**			0.56	164.00	0.52			**0.56**
	20			0.58		0.59		0.72	0.86		**0.65**			0.65		0.52			**0.58**

Tab 5.6: Bone parameters measured by DXA in the WBV group and the control group at baseline (BL), 4, 8 and 20-month. The median of bone measurements was calculated at each time point in both groups.

%(BL-4)	WBV2	WBV2	WBV3	WBV4	WBV5	WBV6	WBV7	WBV8	WBV9	Median WBV	C1	C2	C3	C4	C5	C6	C7	Median C
Trab vBMD (4%)	28.9	17.0	11.8	-1.4	-8.0	-2.1	-1.9	2.8	12.8	**2.8**	33.6	-39.2	9.0	-15.2	-	5.9	22.2	**7.5**
SSI (38%)	25.4	-2.6	13.6	13.6	0.7	0.6	0.3	-0.9	-1.6	**0.6**	-10.4	-26.0	-20.4	20.0	-	-12.4	3.9	**-11.5**
Total Bone CSA (66%)	62.0	-4.6	3.6	10.5	1.6	4.9	1.3	10.5	-3.1	**3.6**	4.1	21.5	-25.1	-32.5	-	5.1	17.7	**4.6**
Cort vBMD (66%)	-9.1	0.8	-2.0	-2.3	0.0	-1.3	0.6	-2.0	0.8	**-1.3**	-0.2	-3.6	7.5	10.6	-	0.8	-6.5	**0.3**
Cort CSA (66%)	-17.2	53.3	4.2	-6.5	-1.3	-6.0	-1.3	-3.9	-1.0	**-1.3**	13.9	-9.1	127.5	49.9	-	13.4	2.0	**8.0**
FA (66%)	-5.0	23.2	-21.5	3.3	2.3	9.5	19.4	34.3	-9.3	**3.3**	-	1.0	11.3	37.6	-	12.3	25.8	**-5.7**

Tab 5.7: Bone parameters measured by p.QCT showing the percentage changes from baseline to 4-month (%(BL-4)) and the median of these percentage changes in the WBV group and the control group**.** * the difference ($p<0.05$) between BL and 4month.

Paramerters	Visit	WBV1	WBV2	WBV3	WBV4	WBV5	WBV6	WBV7	WBV8	WBV9	Median WBV	C1	C2	C3	C4	C5	C6	C7	Median C
Trab vBMD (4%)	BL	228	118	176	227	223	140	156	170	147	**170**	118	304	119	324		194	272	**233**
	4	294	138	197	224	205	137	153	174	166	**174**	157	185	130	275		206	332	**195**
	8	236	54	178		174		152	374	223	**178**			133	376				**255**
	20							154	136	208	**154**			143					**143**
SSI (38%)	BL	298	343	378	602	592	825	1221	1120	1352	**602**	501	399	372.3	589		647	882	**545**
	4	374	334	429	684	596	830	1226	1110	1331	**684**	448	295	296	707		567	916	**507**
	8	396	426	468		611		1297	1302	1391	**611**			326			716		**521**
	20					518		729	1504	1459	**1094**			333					**333.2**
Total bone CSA (66%)	BL	359	373	446	494	428	460	653	584	659	**460**	463	324	555	644		435	465	**464**
	4	582	356	463	545	435	482	662	645	638	**545**	482	394	416	434		457	547	**446**
	8	505	389	512		424		657	569	605	**512**			475	463				**469**
	20			474		462		687	680		**577**			452					**452**
Cort vBMD (66%)	BL	924	937	983	1024	1058	1077	1084	1171	1079	**1058**	935	1039	900	968		1039	986	**977**
	4	839	944	963	1000	1058	1064	1091	1147	1088	**1058**	933	1002	967	1071		1047	922	**985**
	8	984	936	957		1097		1095	1180	1095	**1095**			925	1053				**989**
	20							1003	1089	1154	**1089**			962					**962**
Cort CSA (66%)	BL	109	71	100	215	171	192	267	326	249	**192**	93	116	57	132		172	209	**124**
	4	90	109	105	201	169	180	264	313	247	**180**	106	105	130	198		149	213	**140**
	8	62	103	119		174		263	328	251	**174**			109	218				**164**
	20			153		175		263	321		**219**			145					**145**
FA (66%)	BL	1094	1565	1318	4092	3269	2912	1366	1708	4448	**1708**	1550	1699	1530	2530		3150	4498	**2114**
	4	1039	1929	1034	4228	3345	3187	1630	2293	4033	**2293**	1062	1716	1703	3481		2761	3338	**2238**
	8	916	2200	1124		3449		1385	2119	4281	**2119**			1190	3445				**2317**
	20			1778		3675		1629	2345		**2345**			2197					**2197**

Tab 5.8: The p.QCT measurements in the WBV group and the control group at BL, 4, 8 and 20month. The median of bone measurements was calculated at each time point in the both groups.

Parameters	WBV1	WBV2	WBV3	WBV4	WBV5	WBV6	WBV7	WBV8	WBV9	Median (WBV)	C1	C2	C3	C4	C5	C6	C7	Median C
TB FM%	4.5	6.0	10.6	0.7	4.0	-5.4	43.8	7.9	-4.9	**4.5**	-1.1	8.4	-10.3	0.2	6.6	-0.4	1.8	**0.2**
TB FM	6	15	25	5	10	0	50	19	-7	**10***	-1	17	-10	9	12	6	8	**8**
TB LM	4.0	7.0	9.7	3.8	1.8	10.3	-2.9	6.5	-2.7	**4.0**	0.2	3.6	3.4	8.3	2.6	6.8	5.0	**3.6***
Trunk FM%	-13.2	7.8	16.4	0.5	3.9	-6.8	92.6	7.6	-2.1	**3.9**	-1.5	11.0	-11.6	-1.8	13.9	1.6	2.5	**1.6**
Trunk FM	-11.7	20.2	36.6	7.2	9.6	-0.2	97.5	17.0	-9.0	**9.6**	-7.0	12.4	-11.3	10.3	18.2	5.2	10.2	**10.2**
Trunk LM	5.7	8.8	12.5	6.3	1.8	12.7	-7.6	4.8	-5.0	**5.7**	-5.3	-3.6	4.7	13.7	1.0	2.3	4.7	**2.3**
Leg FM%	9.0	2.9	4.7	0.6	3.8	-3.5	17.5	8.0	-6.5	**3.8**	-4.8	3.4	-7.6	1.4	2.1	-3.5	0.2	**0.2**
Leg FM	12.7	13.7	15.4	2.3	9.5	3.7	27.6	25.3	-13.0	**12.7***	1.6	22.4	-7.3	9.2	8.3	8.6	6.2	**8.3**
Leg LM	4.4	8.6	7.8	1.2	1.7	10.9	3.7	10.7	0.4	**4.4***	8.9	15.5	3.4	6.1	5.0	16.7	5.7	**6.1***
Arm FM%	33.3	11.8	16.5	1.6	5.9	-8.6	11.3	0.0	9.4	**9.4***	3.2	10.5	-12.6	6.7	1.7	-2.2	3.9	**3.2**
Arm FM	37	16	37	9	16	-6	19	8	22	**16***	7	37	-17	2	17	3	10	**7**
Arm LM	2.0	0.1	12.6	6.2	4.3	10.9	5.0	8.5	-3.2	**5.0**	3.4	16.8	-0.1	-9.4	13.9	7.0	2.2	**3.4**

Tab 5.9: Body composition measured by DXA. The percentage changes of body composition of DXA results from BL to 4 months (%(BL-4)) and the median of these percentage changes in.the WBV group and the control group.

Total body fat mass percent (TB FM%), total body fat mass(TB FM), total body lean mass (TB LM). trunk fat mass percent (Trunk FM%), trunk fat mass(Trunk FM), trunk lean mass (Trunk LM), leg fat mass percent (Leg FM%), leg fat mass(Leg FM), leg lean mass (Leg LM), arm fat mass percent (Arm FM%), arm fat mass(Arm FM), arm lean mass (Arm LM).

* the difference ($p<0.05$) between BL and 4month.

Parameters	Visit	WBV1	WBV2	WBV3	WBV4	WBV5	WBV6	WBV7	WBV8	WBV9	Median (WBV)	C1	C2	C3	C4	C5	C6	C7	Median C
TB FM%	**BL**	13.3	25.0	21.6	42.5	47.6	44.2	13.7	31.5	51.2	**31.5**	17.9	32.2	25.3	41.9	21.2	45.3	49.8	**32.2**
	4	13.9	26.5	23.9	42.8	49.5	41.8	19.7	34.0	48.7	**34.0**	17.7	34.9	22.7	42.0	22.6	45.1	50.7	**34.9**
	8	15.9	25.7	20.6		48.1		14.3	37.7	50.9	**25.7**			22.4	44.2	24.4			**24.4**
	20			25.3		48.7		19.1	39.7		**32.5**			26.4		27.2			**26.8**
TB FM	**BL**	2564	4855	4728	18730	24254	20187	6168	15867	39637	**15867**	4122	7827	6154	14866	4916	16906	25451	**7827**
	4	2713	5604	5902	19641	26684	20241	9232	18871	36670	**18871**	4081	9165	5522	16196	5488	17933	27537	**9165**
	8	3420	5487	5140		26142		6711	21502	38888	**6711**			5610	18140	6094			**6094**
	20			7275		28781		10201	24133		**17167**			7914		7539			**7727**
TB LM	**BL**	16125	14557	17129	25338	26746	25522	38725	34455	37776	**25522**	18929	16462	18174	20629	18291	20406	25510	**18929**
	4	16770	15570	18797	26294	27217	28162	37610	36700	36750	**27217**	18973	17062	18795	22343	18765	21788	26776	**18973**
	8	18147	15870	19871		28208		40073	35524	37499	**28208**			19457	22935	18906			**19457**
	20			21518		30265		43246	36636		**33451**			22099		20142			**21121**
Trunk FM%	**BL**	9.1	21.9	19.5	40.3	49.0	43.9	9.4	31.4	52.3	**31.4**	13.4	30.0	25.9	39.9	14.4	45.1	51.3	**30.0**
	4	7.9	23.6	22.7	40.5	50.9	40.9	18.1	33.8	51.2	**33.8**	13.2	33.3	22.9	39.2	16.4	45.8	52.6	**33.3**
	8	10.5	25.2	16.8		48.0		11.8	38.8	53.3	**25.2**			23.1	42.3	17.2			**23.1**
	20			23.4		49.4		19.2	40.1		**31.8**			27.8		19.5			**23.7**
Trunk FM	**BL**	775	1913	1848	7718	12136	9290	2121	7992	21396	**7718**	1481	3445	2966	6394	1568	8033	11220	**3445**
	4	684	2300	2524	8271	13307	9272	4188	9353	19480	**8271**	1377	3873	2632	7053	1854	8453	12360	**3873**
	8	1002	2990	1932		12578		2747	11039	20028	**2990**			2709	7978	1869			**2709**
	20			3008		14558		5015	12016		**8516**			3723		2384			**3054**
Trunk LM	**BL**	7551	6838	7631	11421	12612	11876	20485	17476	19550	**11876**	9562	8042	8479	9623	9343	9762	10653	**9562**
	4	7985	7442	8585	12136	12836	13390	18938	18317	18580	**12836**	9055	7751	8877	10944	9440	9984	11149	**9440**
	8	8520	8875	9385		13633		20500	17424	17542	**13633**			8997	10880	8967			**8997**
	20			9869		14895		21167	17916		**16405**			9679		9837			**9758**
Leg FM%	**BL**	21.1	34.7	27.4	49.2	50.2	48.6	21.1	35.1	54.1	**35.1**	27.0	40.6	27.8	49.1	33.3	50.8	53.7	**40.6**

	4	23.0	35.7	28.7	49.5	52.1	46.9	24.8	37.9	50.6	**37.9**	25.7	42.0	25.7	49.8	34.0	49.0	53.8	**42.0**
	8	24.1	33.1	27.4		52.0		19.7	40.5	52.2	**33.1**			24.8	50.5	36.1			**36.1**
	20			30.5		51.3		22.4	42.5		**36.5**			28.5		39.2			**33.9**
Leg FM	**BL**	1232	2060	2070	8276	8723	7627	2937	5753	13824	**5753**	1837	2995	1921	6180	2488	6384	10600	**2995**
	4	1388	2342	2389	8467	9552	7909	3747	7211	12033	**7211**	1867	3666	1781	6751	2695	6930	11253	**3666**
	8	1657	1688	2293		9639		2964	7863	14387	**2964**			1802	7372	3165			**3165**
	20			3080		10037		3950	8929		**6440**			2610		3820			**3215**
Leg LM	**BL**	4453	3881	5498	8545	8651	8068	10968	10650	11725	**8545**	4962	4377	4984	6415	4982	6179	9141	**4984**
	4	4650	4215	5927	8646	8797	8946	11370	11792	11768	**8797**	5403	5054	5155	6806	5230	7211	9658	**5403**
	8	5226	3419	6078		8895		12119	11572	13180	**8895**			5457	7240	5605			**5605**
	20			7004		9532		13715	12070		**10801**			6559		5923			**6241**
Arm FM%	**BL**	11.4	23.7	20.0	42.8	47.6	47.5	15.0	31.2	48.1	**31.2**	19.0	35.2	28.6	43.3	23.7	46.2	46.6	**35.2**
	4	15.2	26.5	23.3	43.5	50.4	43.4	16.7	31.2	52.6	**31.2**	19.6	38.9	25.0	46.2	24.1	45.2	48.4	**38.9**
	8	17.9	25.9	21.3		50.7		12.5	33.8	47.2	**25.9**			22.6	48.3	25.1			**25.1**
	20			25.5		51.2		13.3	39.1		**32.3**			26.7		31.6			**29.2**
Arm FM	**BL**	211	405	403	1888	2473	2319	680	1548	3431	**1548**	370	706	711	1568	407	1705	2549	**711**
	4	290	469	551	2061	2880	2172	808	1678	4186	**1678**	397	967	592	1601	475	1753	2807	**967**
	8	382	304	508		3035		585	1939	3465	**585**			590	2006	583			**590**
	20			729		3273		764	2479		**1622**			1003		812			**908**
Arm LM	**BL**	1589	1302	1609	2523	2719	2559	3849	3418	3696	**2559**	1575	1300	1779	2055	1312	1985	2925	**1779**
	4	1621	1303	1812	2679	2837	2838	4043	3708	3577	**2837**	1629	1519	1778	1862	1495	2124	2990	**1778**
	8	1755	869	1871		2950		4080	3802	3874	**2950**			2020	2145	1737			**2020**
	20			2133		3115		4960	3860		**3488**			2758		1762			**2260**

Tab 5.10: Body composition measured by DXA in the WBV group and the control group at (BL), 4, 8 and 20 months. The median of each measurement was calculated at each time point from the BL measurements in both groups.

5.5 Discussion

Over the last decade WBV has received considerable attention as a possible method for promoting bone mass (494). However, there is little published evidence that WBV benefits bone mass, particularly in children (495). The most promising effect in children was observed in those with CP (245;319) but the magnitude was relatively low and a more recent report has been unable to confirm these findings (332;496). In children, osteoporosis secondary to chronic disease is not unusual, particularly in those who are receiving chemotherapy for ALL (183;189). The aetiology of this osteoporosis is multifactorial, but as inactivity may play a large role, it was deemed appropriate to consider a study of WBV in these children. Thus, the current report represents the first attempt at studying this interventional regimen in this group of children.

The peak incidence of ALL in childhood is between 2 and 5 years of age and a number of children were excluded as they were considered to be too young to stand on the vibrating platform. Given that previous studies have suggested that it is the older child with ALL who may be predisposed to osteoporosis (71;192;460) and the current study suggested that compliance was greater in the older child, WBV may be a suitable method for delivering exercise to the older child with ALL. However, a conclusive exploration of the effect of WBV on bone health in this group of patients will require a larger sample size.

The number of children in whom BMD fell when assessed by DXA was greater in the control group compared with the WBV group raising the possibility that WBV may prevent the deterioration in BMD, which is often described in these children during chemotherapy and can be a predictor of subsequent fractures (183;188;189). This finding can be supported by other studies, which have shown that WBV has a positive effect on reducing the risk of osteoporosis by increasing LS-BMD in postmenopausal women (344;497). Markers of bone turnover did not show any significant difference between the two groups as well as within the group.

The changes in total and leg body composition which were assessed by DXA were counter to what would have been expected. The increase in TB-FM and leg-FM were more prominent in the WBV group, whereas in the control group, the percentage change of TB-LM and leg-LM from the baseline to the four-month visit was more pronounced. One of the contradictory

finding in this study was the total LM increased in the control group whereas the total FM and leg FM increased in the WBV group. This could be related to puberty difference, age and chemotherapy responses between these two groups rather than WBV training. However, previous animal studies have reported that WBV is associated with a reduction in adipocyte numbers and FM (498;499). However, assessment by p.QCT did show an increase in lean area, which was greater in the control compared with the WBV group, but it was not significantly different.

The lack of stark changes in bone density (BMD) as well as muscle mass may have a number of explanations. Firstly, it is possible that a more profound effect may be exerted with a more intensive and longer period of WBV. Other studies suggest that WBV training may only be effective at improving leg muscle strength (354;377), BMD (242) and fall risk (368) when the subjects participated in training sessions at least three times a week. However, this may not be practically feasible in children ongoing chemotherapy especially when the chemotherapy may last for almost three years. It is possible that targeted exercise such as WBV does not need to be delivered throughout the chemotherapy period. Previous studies suggest that the peak incidence of fractures is at 18 months after start of chemotherapy, particularly in those children who are older and spend more time in hospital. Delivering WBV for a few months prior to this critical point may be sufficient. Previous studies by our group have observed that children on ALL chemotherapy often have reduction in serum Mg (492) and it is possible that inadequate bone mineral status may hinder a beneficial effect of exercise. However, there were no differences in the bone mineral status of the two groups (data not shown). The drugs these children receive for chemotherapy include agents such as vincristine and methotrexate, which may induce a transient neuropathy (500;501) or alter mechanotransduction within bone (502), thus altering the mechanism through which WBV exerts an effect on bone.

The mechanism of the osteogenic effect of WBV stimuli is not entirely clear. It is possible that the response of bone tissue to high frequency stimuli could be related to fluid shear stress rather than a direct response to bone strain (503). Additionally, the response of cortical bone formation to the vibratory stimuli could be related to the mechanical noise that is released by the vibrations (322). This phenomenon can enhance the oestrogenic response of bone tissue by increasing the mechanosensitivity of osteocytes. However, a daily intervention of low-level

high frequency vibration for one year can also increase the bone density in the hind limb of an adult sheep (503). In a previous study of WBV in young healthy adults, we reported a fall in serum cortisol and a fall in circulating markers of bone resorption, independent of any detectable changes in muscle function (504), suggesting that some of the effects of WBV on bone health may be independent of the effect of muscle function. However, the adults who were studied had WBV on multiple days of the week and were in good general health and it is likely that these factors influenced the observed association between WBV and the endocrine effects.

The limitations created by a small sample size, a lack of power calculation and no health control can have profound effects on the results. The discrepancy between the WBV group vs control group, and the effect on biasing results in relation to: more patients in pubertal age range in the WBV group, more patients on high risk regimens in WBV group and the problems of patients being at different points in their chemotherapy schedule at times of assessment, even though the two groups were similar for chemotherapy weeks. Furthermore, this was a longitudinal study, but time points were compared with cross-sectional reference data within the groups as there was no control, this introduces a bias and may have masked some actual changes in bone outcome parameters. It is reported that serum levels of bone formation markers such as BAP and PICP declined during chemotherapy (182). Our results in Fig 5.5 showed low levels of bone markers particularly in the serum levels of CTX and OC. This might have a negative impact on WBV training to improve bone health in ALL children during chemotherapy. Frequent morbidity related to leukaemia had a negative impact on patients' compliance and adherence to the study protocol. As the study established and evaluated in the hospital during follow up, the adherence to the protocol was also interrupted by infrequent visits to the oncology clinic and other issues such as holidays.

In conclusion, this preliminary study suggests that WBV may reduce the fall in BMD that is often observed in children on chemotherapy and this effect needs further exploration in a larger group of children.

Chapter 6

Conclusion and Discussion
Future Directions

6.1 Discussion and Conclusion

The main aim of is the line of work undertaken as part of this thesis was to reduce the rate of skeletal morbidity in ALL children as these complications have been frequently described in this group. These skeletal complications can present at various times during the course of leukaemia. The aetiological process of skeletal complications in ALL children can be categorized into two main groups; Firstly, direct causes such as leukaemia itself and chemotherapy. Secondly, independent factors such age, gender, nutritional status and physical activity might have an indirect effect on bone health in ALL children. Therefore, the direct causes make these complications inevitable. The early recognition of such skeletal morbidities and their risk factors are vital to facilitate the institution of rational strategies for monitoring and optimising bone health in ALL children. Minimising these complications has multiple advantages such as reducing a disease comorbidity, which might have positive impacts on the patient and parents. Furthermore, less skeletal complications lead to a reduction in the amount of radiation exposure to the patients and the radiological staff. Some of these complications such as ON can be treated only by a surgical intervention. Immobility which resulted from these complications might have a further problem on bone health and increase the rate of BMD loss. A large number of investigations such as X-rays, DXA and MRI are required for assessing these skeletal complications and which in turn might have a burden on the cost of health care.

Four different studies have been conducted in this thesis. The first two studies (chapter 2&3) were collected retrospectively and aiming to explore the incidence rate of skeletal morbidity in children with leukaemia and their risk factors such as age, GCs, physical inactivity and abnormal mineral homeostasis. In the third study (chapter 4), the objective was to prospectively compare and assess the effect of two different types of WBV on endocrine and musculoskeletal systems in healthy adult men.The final study (chapter 5) was aiming to investigate the effect of WBV mainly on bone health in children with ALL.

The first study confirms the finding from previous reports (177;178;240;255;545) that skeletal morbidity was common in children with ALL during therapy. This study also showed that the majority of skeletal morbidity occurred at around the second year of starting chemotherapy. These data suggest that bone health of those groups of children could be improved through

introduction of some preventive measures in the first year of chemotherapy. In addition, the risk of developing fractures and ON was higher in older children than younger children. A recent report showed that older age and low LS-BMD z score during continuation therapy were considered to be independent predictors of fractures in ALL children (178). In comparison to previous studies, this study also suggests that fractures in ALL children were more likely to be identified in the lower limbs. On the other hand, the most commonly fractured site in children is the forearm (173). A recent study conducted by Kohler et.(210) showed that cortical bone changes in radius did not differ between ALL children and control, however, tibial cortical cross sectional was reduced significantly in ALL children. This can be explained either by that the pathophysiology of fractures in ALL children compared to normal children population might be different or the effect of leukaemia disease and chemotherapy were more pronounced in the lower limbs. This observation may under estimate vertebral fractures because their symptoms can overlap with general muscular and postural pain (453). Although, the fracture rate was higher in upper limbs compared to spine, the total number of required X-ray was higher in spine. This can support that the routine radiological images might be not sensitive tools to confirm such type of fractures. The prospective STOPP study demonstrated that vertebral fractures occur frequently in children with ALL and may be asymptomatic. They required a detailed morphological such as lateral thoracolumbar spine radiographs to confirm diagnosis (73). The previous group also showed that there is a clear association between skeletal morbidities and dexamethasone. Recently, Rayar et al. (178) found that 30% of fractures during chemotherapy was in ALL children treated with dexamethasone compared to 10% in ALL children treated with prednisolone. Furthermore, type of GCS and low BMD z score during the chemotherapy were independent predictors of fractures. Alos et al (505) reported that vertebral fractures at diagnosis, back pain and a reduction in LS-BMD z-score can be applied as a predictor for developing fractures at 12 months of chemotherapy. We were also interested in describing the spectrum of morbidities associated with factures and ON in our patients. Multiple fractures (≥2) and multifocal ON were affected in 34% and 55%, respectively.

Although the first study reported that the skeletal morbidities are commonly associated with ALL children (460), the pathophysiology and mechanism of these complications remains unclear. It has been claimed before that abnormal mineral status and physical inactivity might

be possible contributors towards an increased risk of developing these abnormalities (72;195;208). Therefore, the second study was designed to investigate a possible metabolic basis for these complications or to determine their associations with abnormal mineral homeostasis during the course of chemotherapy and physical inactivity. Abnormal bone metabolism is known to frequently occur in children with leukaemia. However, no studies have thoroughly examined the association between actual skeletal morbidities and changes in mineral homeostasis. We found that children with MSP and fractures generally had lower levels of serum Ca, Pho and Mg compared with those without skeletal morbidities over the first 12 months of chemotherapy. It is of note that serum levels of these markers were mostly abnormal during the first three months of chemotherapy (Fig 3.4). Although the majority of serum levels of these parameters remained within the normal values over the study period, the trends generally were lower in those children with MSP and fractures. There is no clear explanation for this finding, however, further studies are need to assess the daily intake of Ca, Pho and Mg and the urinary execration of these minerals in ALL children during the course of chemotherapy. This data and previous studies inform that serum Mg correlated significantly with age in healthy individuals (472;473). However, on multivariate analysis, serum Mg per se was not the contributory variable, but it was the age of the patient which was the important variable. Therefore, these finding might shed light on the management of skeletal morbidities in those children by Mg supplementation in particularly older children. Mg supplementation in ALL children having hypomagnesaemia is associated with a variable rise in serum Mg and a rise in OCN levels (195). Increasing OCN might explain why oral Mg supplementation improves insulin sensitivity (506). On the other hand, oral daily oral single dose of Mg supplementation in normal, non-Mg-deficient, young adult men is able to suppress bone turnover by reducing the biochemical markers of bone formation (OCN) and resorption (ICTP), compared with the control subjects (507). Several studies show that oral Mg supplementation might have a beneficial effect on bone health; however, introducing this element in the chemotherapy protocol might require a longitudinal RCT study, which certainly takes decades to prove this effect.

This study also showed a significant association between skeletal morbidities and the number of inpatient days (marker of inactivity). Previously, it was reported that ALL treated with chemotherapy alone had reduced lumbar volumetric BMD and were physically less active

than their healthy controls (208). Every physical activity during childhood is highly recommended to improve bone health (491), is often reduced in children on chemotherapy as well as after completion of therapy (198;199). Exercise can reduce the risk of fractures, increase bone strength and reduce the number of falls. Moreover, exercise regimens that increase muscle bulk or increase mechanical loading on the skeleton may prove beneficial for skeletal health (237), but performing conventional exercise at home in ALL children might be associated with poor adherence (188). Mechanical loading can also be delivered by vibratory exercise via WBV platforms. It is also possible that WBV is as effective as weight bearing exercise, whilst shortening the time required for the exercise.

In the third study, we examined the effect of two different types of WBV on healthy adult men. This study was, therefore, performed to compare the effects of sinusoidal and vertical WBV delivered through the GP and the JP, respectively, on a range of outcome measures related to the endocrine and musculoskeletal system. Based on the result of this study, WBV was well tolerated with a high rate of compliance in the study population. Apart from very mild itching, which was experienced particularly over the shins and thighs, the exercise regimens were not associated with any adverse reactions. In addition, the current study showed that, over the short term, exercise with GP was associated with increased serum GH and decreased cortisol concentration. The lowering of circulating cortisol was also observed over the medium term and this fall was also associated with a reduction in bone resorption. Therefore, it can be hypothesized that WBV delivered at a certain magnitude was able to produce a positive impact on bone health either directly by suppressing osteoclasts or indirectly through stimulating GH and reducing cotrisol levels.

Therefore, the hypothesis of the fourth study was based on the previous study as WBV exercise delivered by GP might be able to reduce the risk of developing skeletal morbidities in ALL children receiving chemotherapy. Compared with the previous study, the time of exposure to WBV training (once a week) was less frequent, whereas the total duration of the intervention was longer (four months). However, the WBV schedule was adapted from a previous report that had used the same WBV system in children with neuromuscular disease and bone fragility disorders (306;387). The current report represents the first attempt at studying the WBV regimen in this group of children. The main finding in this study was that

the number of children in whom BMD fell when assessed by DXA (LS and TB-BMD) was greater in the control group compared with the WBV group, raising the possibility that WBV may prevent the deterioration in BMD, which is often described in these children during chemotherapy and can be a predictor of subsequent fractures (183;188;189). This finding can be supported by several studies, which have shown that WBV has a positive effect on reducing the risk of osteoporosis by increasing LS-BMD in postmenopausal women (344;497). Markers of bone turnover did not show any significant difference between the two groups as well as within the group.

Although the rate of bone loss was higher in the control group compared with the WBV group, this change did not reach significant levels. This could suggest that WBV training once a week for a period of 4months was not beneficial in improving BMD. The lack of positive results may be explained by several reasons. First, although the study was based on a reasonable concept, it may not be possible to maintain a positive result by undertaking WBV exercise at this dose. A large number of studies suggested that WBV training may be a feasible and effective way to improve isometric/dynamic leg muscle strength (316), BMD (242) and decreased fall risk (368) when the subjects participated in training sessions at least three times a week. Furthermore, the adherence and compliance to the exercise programme was frequently interrupted in this group of children mainly due to the disease itself and chemotherapy adverse effects such as fever, neurtropaenia and MSP. The median age of the WBV group was slightly older than the control group. This may be an important factor for the lack of significant results as several studies have reported that the older age children with ALL children had higher incidence of skeletal morbidity (492). Another possible explanation for this negative finding is that GCs have suppressed bone formation in our cohort study as dexamethasone is the mainstay of the therapy for UKALL2003.

These data suggest that short term training of WBV in ALL children may not able to produce in long-term beneficial effects on bone development. However, further studies are necessary to assess the effect of long term and frequent training of WBV on bone outcomes and fracture rate in ALL children.

6.2 Future Direction

Finally, I would like to present directions for future research in the field. Further research is needed to determine if WBV exercise in ALL children may reduce the risk of developing skeletal morbidities and fractures and increase BMD. In order to do this, it is essential to know the highest risk age group and the duration and the time of WBV exposure. There is a need for systematic monitoring of bone health similar to that being currently performed in the STOPP studies (73). Future studies should select those children over 8years as these groups have the highest risk factor for developing skeletal morbidities. Furthermore, exercise may be most effective if started at the time of diagnosis in parallel with chemotherapy but user acceptability of WBV may not be high at this point. Also, where sufficient data are available, there is a need to compare outcomes between WBV and conventional exercise for improvement in children's bone health. Whereas in my studies, the effect of WBV on the musculoskeletal and endocrine systems was assessed, for any further work, also it may be useful to consider the interactive effect of nutritional optimisation and Mg supplementation on bone health during chemotherapy.

Biblograhy

(1) Rho JY, Kuhn-Spearing L, Zioupos P. Mechanical properties and the hierarchical structure of bone. Medical Engineering & Physics 1998 Mar;20(2):92-102.

(2) Knott L, Bailey AJ. Collagen cross-links in mineralizing tissues: A review of their chemistry, function, and clinical relevance. Bone 1998 Mar;22(3):181-7.

(3) Olszta MJ, Cheng XG, Jee SS, Kumar R, Kim YY, Kaufman MJ, et al. Bone structure and formation: A new perspective. Materials Science & Engineering R-Reports 2007 Nov 28;58(3-5):77-116.

(4) Favus M, American Society for Bone and Mineral Research. Primer on the metabolic bone diseases and disorders of mineral metabolism. Washington, D.C. : American Society for Bone and Mineral Research; 2003.

(5) Lotz J, Gaertner T, Hahn M, Prellwitz W. Collagen type I metabolism after bone surgery. Archives of Orthopaedic and Trauma Surgery 1999 May;119(3-4):212-6.

(6) Risteli J, Elomaa I, Niemi S, Novamo A, Risteli L. Radioimmunoassay for the pyridinoline cross-linked carboxy-terminal telopeptide of type I collagen: a new serum marker of bone collagen degradation. Clinical Chemistry 1993 Apr;39(4):635-40.

(7) Unsold C, Pappano WN, Imamura Y, Steiglitz BM, Greenspan DS. Biosynthetic processing of the pro-alpha 1(V)(2)pro-alpha 2(V) collagen heterotrimer by bone morphogenetic protein-1 and furin-like proprotein convertases. Journal of Biological Chemistry 2002 Feb 15;277(7):5596-602.

(8) Roach HI. Why does bone matrix contain non-collagenous proteins? The possible roles of osteocalcin, osteonectin, osteopontin and bone sialoprotein in bone mineralisation and resorption. Cell Biology International 1994 Jun;18(6):617-28.

(9) Ninomiya JT, Tracy RP, Calore JD, Gendreau MA, Kelm RJ, Mann KG. Heterogeneity of human bone. Journal of Bone and Mineral Research 1990 Sep;5(9):933-8.

(10) Sommarin Y, Wendel M, Shen ZX, Hellman U, Heinegard D. Osteoadherin, a cell-binding keratan sulfate proteoglycan in bone, belongs to the family of leucine-rich repeat proteins of the extracellular matrix. Journal of Biological Chemistry 1998 Jul 3;273(27):16723-9.

(11) Yang Y, Cui QA, Sahai N. How does bone sialoprotein promote the nucleation of hydroxyapatite? A molecular dynamics study using model peptides of different conformations. Langmuir 2010 Jun 15;26(12):9848-59.

(12) Mousny M, Omelon S, Wise L, Everett ET, Dumitriu M, Holmyard DP, et al. Fluoride effects on bone formation and mineralization are influenced by genetics. Bone 2008 Dec;43(6):1067-74.

(13) Grabowski P. Physiology of bone. Endocr Dev 16[32], 48. 2009.
Ref Type: Journal (Full)

(14) Harada S, Rodan GA. Control of osteoblast function and regulation of bone mass. Nature 2003 May 15;423(6937):349-55.

(15) Suda T, Takahashi N. Contributions to osteoclast biology from Japan. Proceedings of the Japan Academy Series B-Physical and Biological Sciences 2008 Dec;84(10):419-38.

(16) Schoppet M, Preissner KT, Hofbauer LC. RANK ligand and osteoprotegerin - Paracrine regulators of bone metabolism and vascular function. Arteriosclerosis Thrombosis and Vascular Biology 2002 Apr;22(4):549-53.

(17) Hadjidakis DJ, Androulakis II. Bone remodeling. Women'S Health and Disease: Gynecologic, Endocrine, and Reproductive Issues 2006;1092:385-96.

(18) Boland GM, Perkins G, Hall DJ, Tuan RS. Wnt 3a promotes proliferation and suppresses osteogenic differentiation of adult human mesenchymal stem cells. Journal of Cellular Biochemistry 2004 Dec 15;93(6):1210-30.

(19) Koch H, Jadlowiec JA, Campbell PG. Insulin-like growth factor-I induces early osteoblast gene expression in human mesenchymal stem cells. Stem Cells and Development 2005 Dec;14(6):621-31.

(20) Giustina A, Mazziotti G, Canalis E. Growth hormone, insulin-like growth factors, and the skeleton. Endocrine Reviews 2008 Aug;29(5):535-59.

(21) Mukherjee A, Rotwein P. Insulin-like growth factor-binding protein-5 inhibits osteoblast differentiation and skeletal growth by blocking insulin-like growth factor actions. Molecular Endocrinology 2008 May;22(5):1238-50.

(22) Vanderschueren D, Vandenput L, Boonen S, Lindberg MK, Bouillon R, Ohlsson C. Androgens and bone. Endocrine Reviews 2004 Jun;25(3):389-425.

(23) Vanderschueren D, Venken K, Ophoff J, Bouillon R, Boonen S. Clinical review: Sex steroids and the periosteum - Reconsidering the roles of androgens and estrogens in periosteal expansion. Journal of Clinical Endocrinology & Metabolism 2006 Feb;91(2):378-82.

(24) Krum SA, Miranda-Carboni GA, Hauschka PV, Carroll JS, Lane TF, Freedman LP, et al. Estrogen protects bone by inducing Fas ligand in osteoblasts to regulate osteoclast survival. Embo Journal 2008 Feb 6;27(3):535-45.

(25) Raisz LG, Rodan GA. Pathogenesis of osteoporosis. Endocrinology and Metabolism Clinics of North America 2003 Mar;32(1):15-+.

(26) Vaananen HK, Zhao H, Mulari M, Halleen JM. The cell biology of osteoclast function. Journal of Cell Science 2000 Feb;113(3):377-81.

(27) Rao HW, Lu GW, Kajiya H, Garcia-Palacios V, Kurihara N, Anderson J, et al. alpha(9)beta(1): A novel osteoclast integrin that regulates osteoclast formation and function. Journal of Bone and Mineral Research 2006 Oct;21(10):1657-65.

(28) Clowes JA, Riggs BL, Khosla S. The role of the immune system in the pathophysiology of osteoporosis. Immunological Reviews 2005 Dec;208:207-27.

(29) Cenci S, Weitzmann MN, Roggia C, Namba N, Novack D, Woodring J, et al. Estrogen deficiency induces bone loss by enhancing T-cell production of TNF-alpha. Journal of Clinical Investigation 2000 Nov;106(10):1229-37.

(30) Krum SA, Brown M. Unraveling estrogen action in osteoporosis. Cell Cycle 2008 May 15;7(10):1348-52.

(31) Weitzmann MN, Pacifici R. The role of T lymphocytes in bone metabolism. Immunological Reviews 2005 Dec;208:154-68.

(32) Kanatani M, Sugimoto T, Sowa H, Kobayashi T, Kanzawa M, Chihara K. Thyroid hormone stimulates osteoclast differentiation by a mechanism independent of RANKL-RANK interaction. Journal of Cellular Physiology 2004 Oct;201(1):17-25.

(33) Abe E, Marians RC, Yu WQ, Wu XB, Ando T, Li YN, et al. TSH is a negative regulator of skeletal remodeling. Cell 2003 Oct 17;115(2):151-62.

(34) Baron R, Rawadi G. Minireview: Targeting the Wnt/beta-catenin pathway to regulate bone formation in the adult skeleton. Endocrinology 2007 Jun;148(6):2635-43.

(35) Kamioka H, Honjo T, Takano-Yamamoto T. A three-dimensional distribution of osteocyte processes revealed by the combination of confocal laser scanning microscopy and differential interference contrast microscopy. Bone 2001 Feb;28(2):145-9.

(36) van Hove RP, Nolte PA, Vatsa A, Semeins CM, Salmon PL, Smit TH, et al. Osteocyte morphology in human tibiae of different bone pathologies with different bone mineral density - Is there a role for mechanosensing? Bone 2009 Aug;45(2):321-9.

(37) Frost HM. The mechanostat: a proposed pathogenic mechanism of osteoporoses and the bone mass effects of mechanical and nonmechanical agents. Bone and Mineral 1987 Apr;2(2):73-85.

(38) Frost HM. Osteoporoses: A rationale for further definitions? Calcified Tissue International 1998 Feb;62(2):89-94.

(39) Tatsumi S, Ishii K, Amizuka N, Li MQ, Kobayashi T, Kohno K, et al. Targeted ablation of osteocytes induces osteoporosis with defective mechanotransduction. Cell Metabolism 2007 Jun;5(6):464-75.

(40) Klein-Nulend J, Bacabac RG, Mullender MG. Mechanobiology of bone tissue. Pathologie Biologie 2005 Dec;53(10):576-80.

(41) Tan SD, Kuijpers-Jagtman AM, Semeins CM, Bronckers ALJJ, Maltha JC, den Hoff JW, et al. Fluid shear stress inhibits TNF alpha-induced osteocyte apoptosis. Journal of Dental Research 2006 Oct;85(10):905-9.

(42) Sikavitsas VI, Temenoff JS, Mikos AG. Biomaterials and bone mechanotransduction. Biomaterials 2001 Oct;22(19):2581-93.

(43) Gaudio A, Pennisi P, Bratengeier C, Torrisi V, Lindner B, Mangiafico RA, et al. Increased sclerostin serum levels associated with bone formation and resorption markers in patients with immobilization-induced bone loss. Journal of Clinical Endocrinology & Metabolism 2010 May;95(5):2248-53.

(44) Frost HM. Wolff law and bones structural adaptations to mechanical usage - an overview for clinician. Angle Orthodontist 1994;64(3):175-88.

(45) Sims NA, Gooi JH. Bone remodeling: Multiple cellular interactions required for coupling of bone formation and resorption. Seminars in Cell & Developmental Biology 2008 Oct;19(5):444-51.

(46) Hernández-Gil I, Miguel Angel Alobera Gracia M, Mariano del Canto Pingarrón LBJ. Physiological bases of bone regeneration II. The remodeling process. Med Oral Patol Oral Cir Bucal 2006;11:151-7.

(47) Seeman E. Bone quality: the material and structural basis of bone strength. Journal of Bone and Mineral Metabolism 2008 Jan;26(1):1-8.

(48) Ruimerman R, van Rietbergen B, Hilbers P, Huiskes R. The effects of trabecular-bone loading variables on the surface signaling potential for bone remodeling and adaptation. Annals of Biomedical Engineering 2005 Jan;33(1):71-8.

(49) Frost HM. Defining osteopenias and osteoporoses: Another view (with insights from a new paradigm). Bone 1997 May;20(5):385-91.

(50) Frost HM. Bone's mechanostat: A 2003 update. Anatomical Record Part A-Discoveries in Molecular Cellular and Evolutionary Biology 2003 Dec;275A(2):1081-101.

(51) Davies JH, Evans BAJ, Gregory JW. Bone mass acquisition in healthy children. Archives of Disease in Childhood 2005 Apr;90(4):373-8.

(52) Bachrach LK, Hastie T, Wang MC, Narasimhan B, Marcus R. Bone mineral acquisition in healthy Asian, Hispanic, black, and Caucasian youth: A longitudinal study. Journal of Clinical Endocrinology & Metabolism 1999 Dec;84(12):4702-12.

(53) Chae HJ, Park RK, Chung HT, Kang JS, Kim MS, Choi DY, et al. Nitric oxide is a regulator of bone remodelling. Journal of Pharmacy and Pharmacology 1997 Sep;49(9):897-902.

(54) Shaw NJ. Osteoporosis in paediatrics. Archives of Disease in Childhood-Education and Practice Edition 2007 Dec;92(6):169-75.

(55) Brunner R, Doderlein L. Pathological fractures in patients with cerebral palsy. Journal of Pediatric Orthopaedics-Part B 1996;5(4):232-8.

(56) Houde S, Filiatrault M, Fournier A, Dube J, D'Arcy S, Berube D, et al. Deflazacort use in Duchenne muscular dystrophy: An 8-year follow-up. Pediatric Neurology 2008 Mar;38(3):200-6.

(57) Soderpalm AC, Magnusson P, Ahlander AC, Karlsson J, Kroksmark AK, Tulinius M, et al. Low bone mineral density and decreased bone turnover in Duchenne muscular dystrophy. Neuromuscular Disorders 2007 Dec;17(11-12):919-28.

(58) Bianchi ML, Morandi L, Andreucci E, Vai S, Frasunkiewicz J, Cottafava R. Low bone density and bone metabolism alterations in Duchenne muscular dystrophy: response to calcium and vitamin D treatment. Osteoporosis International 2011 Feb;22(2):529-39.

(59) Chenu C. Innervation of bone. M S-Medecine Sciences 2001 Dec;17(12):1276-80.

(60) Konttinen YT, Imai S, Suda A. Neuropeptides and the puzzle of bone remodeling - State of the art. Acta Orthopaedica Scandinavica 1996 Dec;67(6):632-9.

(61) Wang Y, Wan C, Gilbert SR, Clemens TL. Oxygen sensing and osteogenesis. Skeletal Biology and Medicine, Pt B 2007;1117:1-11.

(62) Hattori T, Muller C, Gebhard S, Bauer E, Pausch F, Schlund B, et al. SOX9 is a major negative regulator of cartilage vascularization, bone marrow formation and endochondral ossification. Development 2010 Mar 15;137(6):901-11.

(63) Greer FR, Krebs NF. Optimizing bone health and calcium intakes of infants, children, and adolescents. Pediatrics 2006 Feb;117(2):578-85.

(64) Canalis E, Mazziotti G, Giustina A, Bilezikian JP. Glucocorticoid-induced osteoporosis: pathophysiology and therapy. Osteoporosis International 2007 Oct;18(10):1319-28.

(65) Mazziotti G, Angeli A, Bilezikian JP, Canalis E, Giustina A. Glucocorticoid-induced osteoporosis: an update. Trends in Endocrinology and Metabolism 2006 May;17(4):144-9.

(66) Nilsson O, Marino R, De Luca F, Phillip M, Baron J. Endocrine regulation of the growth plate. Hormone Research 2005;64(4):157-65.

(67) Leonard MB. Glucocorticoid-induced osteoporosis in children: Impact of the underlying disease. Pediatrics 2007 Mar;119:S166-S174.

(68) Ahmed SF, Tucker P, Mushtaq T, Wallace AM, Williams DM, Hughes IA. Short-term effects on linear growth and bone turnover in children randomized to receive prednisolone or dexamethasone. Clinical Endocrinology 2002 Aug;57(2):185-91.

(69) Crofton PM, Ahmed SF, Wade JC, Elmlinger MW, Ranke MB, Kelnar CJH, et al. Bone turnover and growth during and after continuing chemotherapy in children with acute lymphoblastic leukemia. Pediatric Research 2000 Oct;48(4):490-6.

(70) Laan RFJM, Buijs WCAM, Vanerning LJTO, Lemmens JAM, Corstens FHM, Ruijs SHJ, et al. Differential effects of glucocorticoids on cortical appendicular and cortical vertebral bone mineral content. Calcified Tissue International 1993 Jan;52(1):5-9.

(71) Strauss AJ, Su JT, Dalton WMK, Gelber RD, Sallan SE, Silverman LB. Bony morbidity in children treated for acute lymphoblastic leukemia. Journal of Clinical Oncology 2001 Jun 15;19(12):3066-72.

(72) Hogler W, Wehl G, van Staa T, Meister B, Klein-Franke A, Kropshofer G. Incidence of skeletal complications during treatment of childhood acute lymphoblastic leukemia: Comparison of fracture risk with the general practice research database. Pediatric Blood & Cancer 2007 Jan;48(1):21-7.

(73) Halton J, Gaboury I, Grant R, Alos N, Cummings EA, Matzinger M, et al. Advanced vertebral fracture among newly diagnosed children with acute lymphoblastic leukemia: results of the Canadian Steroid-Associated Osteoporosis in the Pediatric Population (STOPP) research program. Journal of Bone and Mineral Research 2009 Jul;24(7):1326-34.

(74) Van Staa TP, Cooper C, Leufkens HGM, Bishop N. Children and the risk of fractures caused by oral corticosteroids. Journal of Bone and Mineral Research 2003 May;18(5):913-8.

(75) Chitre MM, Hayes W. 3-year results of a member and physician intervention to reduce risk associated with glucocorticoid-induced osteoporosis in a health plan. Journal of Managed Care Pharmacy 2008 Apr;14(3):281-90.

(76) Fan CM, Georgiou KR, King TJ, Xian CJ. Methotrexate toxicity in growing long bones of young rats: a model for studying cancer chemotherapy-induced bone growth defects in children. Journal of Biomedicine and Biotechnology 2011.

(77) Lustig RH. Autonomic dysfunction of the beta-cell and the pathogenesis of obesity. Reviews in Endocrine & Metabolic Disorders 2003 Mar;4(1):23-32.

(78) Ducy P, Amling M, Takeda S, Priemel M, Schilling AF, Beil FT, et al. Leptin inhibits bone formation through a hypothalamic relay: A central control of bone mass. Cell 2000 Jan 21;100(2):197-207.

(79) Oshima K, Nampei A, Matsuda M, Iwaki M, Fukuhara A, Hashimoto J, et al. Adiponectin increases bone mass by suppressing osteoclast and activating osteoblast. Bone 2006 Mar;38(3):S29.

(80) Gafni RI, Baron J. Childhood bone mass acquisition and peak bone mass may not be important determinants of bone mass in late adulthood. Pediatrics 2007 Mar;119:S131-S136.

(81) Stein GS, Lian JB. Molecular mechanisms mediating proliferation/differentiation interrelationships during progressive development of the osteoblast phenotype. Endocrine Reviews 1993 Aug;14(4):424-42.

(82) Seibel MJ. Molecular markers of bone turnover: Biochemical, technical and analytical aspects. Osteoporosis International 2000;11:18-29.

(83) Fishman WH. Alkaline phosphatase isozymes - recent progress. Clinical Biochemistry 1990 Apr;23(2):99-104.

(84) Magnusson P, Degerblad M, Saaf M, Larsson L, Thoren M. Different responses of bone alkaline phosphatase isoforms during recombinant insulin-like growth factor-I (IGF-I) and during growth hormone therapy in adults with growth hormone deficiency. Journal of Bone and Mineral Research 1997 Feb;12(2):210-20.

(85) Magnusson P, Larsson L, Magnusson M, Davie MWJ, Sharp CA. Isoforms of bone alkaline phosphatase: Characterization and origin in human trabecular and cortical bone. Journal of Bone and Mineral Research 1999 Nov;14(11):1926-33.

(86) Puchacz E, Lian JB, Stein GS, Wozney J, Huebner K, Croce C. Chromosomal localization of the human osteocalcin gene. Endocrinology 1989 May;124(5):2648-50.

(87) Koshihara Y, Hoshi K. Vitamin K-2 enhances osteocalcin accumulation in the extracellular matrix of human osteoblasts in vitro. Journal of Bone and Mineral Research 1997 Mar;12(3):431-8.

(88) Lian J, Stewart C, Puchacz E, Mackowiak S, Shalhoub V, Collart D, et al. Structure of the rat osteocalcin gene and regulation of vitamin D-dependent expression. Proceedings of the National Academy of Sciences of the United States of America 1989 Feb;86(4):1143-7.

(89) Blumsohn A, Hannon RA, Eastell R. Apparent instability of osteocalcin in serum as measured with different commercially available immunoassays. Clinical Chemistry 1995 Feb;41(2):318-9.

(90) Garnero P, Grimaux M, Seguin P, Delmas PD. Characterization of immunoreactive forms of human osteocalcin generated in vivo and in vitro. Journal of Bone and Mineral Research 1994 Feb;9(2):255-64.

(91) Seibel MJ, Meier C. Biochemical Markers of Bone Turnover GÇô Basic Biochemistry and Variability. In: Adler RA, editor. Osteoporosis. Humana Press; 2010. p. 97-130.

(92) Ferron M, Wei JW, Yoshizawa T, Del Fattore A, DePinho RA, Teti A, et al. Insulin signaling in osteoblasts integrates bone remodeling and energy metabolism. Cell 2010 Jul 23;142(2):296-308.

(93) Lee NK, Sowa H, Hinoi E, Ferron M, Ahn JD, Confavreux C, et al. Endocrine regulation of energy metabolism by the skeleton. Cell 2007 Aug 10;130(3):456-69.

(94) Oury F, Sumara G, Sumara O, Ferron M, Chang HX, Smith CE, et al. Endocrine regulation of male fertility by the skeleton. Cell 2011 Mar 4;144(5):796-809.

(95) Merry AH, Harwood R, Woolley DE, Grant ME, Jackson DS. Identification and partial characterisation of the non-collagenous amino-and carboxyl-terminal extension peptides of cartilage procollagen. Biochemical and Biophysical Research Communications 1976;71(1):83-90.

(96) Taubman MB, Goldberg B, Sherr CJ. Radioimmunoassay for human procollagen. Science 1974;186(4169):1115-7.

(97) Brandt J, Krogh TN, Jensen CH, Frederiksen JK, Teisner B. Thermal instability of the trimeric structure of the N-terminal propeptide of human procollagen type I in relation to assay technology. Clinical Chemistry 1999 Jan;45(1):47-53.

(98) Seibel MJ, Meier C. Biochemical Markers of Bone Turnover GÇô Basic Biochemistry and Variability. In: Adler RA, editor. Osteoporosis. Humana Press; 2010. p. 97-130.

(99) Lowry M, Hall DE, Brosnan JT. Hydroxyproline metabolism by the rat kidney: distribution of renal enzymes of hydroxyproline catabolism and renal conversion of hydroxyproline to glycine and serine. Metabolism-Clinical and Experimental 1985;34(10):955-61.

(100) Smith R. Collagen and disorders of bone. Clinical Science 1980;59(4):215-23.

(101) Prockop DJ, Kivirikko KI, Tuderman L, Guzman NA. Biosynthesis of collagen and its disorders. New England Journal of Medicine 1979;301(1):13-23.

(102) Leigh SD, Ju HSJ, Lundgard R, Daniloff GY, Liu V. Development of an immunoassay for urinary galactosylhydroxylysine. Journal of Immunological Methods 1998 Nov 1;220(1-2):169-78.

(103) Yamada S, Aoto Y, Suou T, Hirayama C. Urinary hydroxyproline and hydroxylysine excretions in relation to hepatic hydroxyproline content in chronic liver disease. Clinical Biochemistry 1989 Oct;22(5):389-93.

(104) Eyre DR. The specificity of collagen cross-links as markers of bone and connective tissue degradation. Acta Orthopaedica Scandinavica 1995 Oct;66:166-70.

(105) Eyre DR, Wu JJ. Collagen cross-links. Collagen 2005;247:207-29.

(106) Simsek B, Karacaer O, Karaca I. Urine products of bone breakdown as markers of bone resorption and clinical usefulness of urinary hydroxyproline: an overview. Chinese Medical Journal 2004 Feb;117(2):291-5.

(107) Colwell A, Russell RGG, Eastell R. Factors affecting the assay of urinary 3-hydroxy pyridinium crosslinks of collagen as markers of bone resorption. European Journal of Clinical Investigation 1993 Jun;23(6):341-9.

(108) Seibel MJ, Robins SP, Bilezikian JP. Urinary pyridinium crosslinks of collagen: specific markers of bone resorption in metabolic bone disease. Trends in Endocrinology and Metabolism 1992 Sep;3(7):263-70.

(109) Gineyts E, Borel O, Chapurlat R, Garnero P. Quantification of immature and mature collagen crosslinks by liquid chromatography-electrospray ionization mass spectrometry in connective tissues. Journal of Chromatography B-Analytical Technologies in the Biomedical and Life Sciences 2010 Jun 1;878(19):1449-54.

(110) Elomaa I, Virkkunen P, Risteli L, Risteli J. Serum concentration of the cross-linked carboxyterminal telopeptide of type I collagen (ICTP) is a useful prognostic indicator in multiple myeloma. British Journal of Cancer 1992 Aug;66(2):337-41.

(111) Aruga A, Koizumi M, Hotta R, Takahashi S, Ogata E. Usefulness of bone metabolic markers in the diagnosis and follow-up of bone metastasis from lung cancer. British Journal of Cancer 1997 Sep;76(6):760-4.

(112) Hakala M, Risteli L, Manelius J, Nieminen P, Risteli J. Increased type I collagen degradation correlates with disease severity in rheumatoid arthritis. Annals of the Rheumatic Diseases 1993 Dec;52(12):866-9.

(113) Rosen HN, Moses AC, Garber J, Iloputaife ID, Ross DS, Lee SL, et al. Serum CTX: A new marker of bone resorption that shows treatment effect more often than other markers because of low coefficient of variability and large changes with bisphosphonate therapy. Calcified Tissue International 2000 Feb;66(2):100-3.

(114) Robins SP. Collagen crosslinks in metabolic bone disease. Acta Orthopaedica Scandinavica 1995 Oct;66:171-5.

(115) Bianco P, Fisher LW, Young MF, Termine JD, Robey PG. Expression of bone sialoprotein (BSP) in developing human tissues. Calcified Tissue International 1991 Dec;49(6):421-6.

(116) Ganss B, Kim RH, Sodek J. Bone sialoprotein. Critical Reviews in Oral Biology & Medicine 1999 Feb;10(1):79-98.

(117) Hunter GK, Goldberg HA. Nucleation of hydroxyapatite by bone sialoprotein. Proceedings of the National Academy of Sciences of the United States of America 1993 Sep 15;90(18):8562-5.

(118) Fedarko NS, Fohr B, Robey PG, Young MF, Fisher LW. Factor H binding to bone sialoprotein and osteopontin enables tumor cell evasion of complement-mediated attack. Journal of Biological Chemistry 2000 Jun 2;275(22):16666-72.

(119) Seibel MJ, Woitge HW, Pecherstorfer M, Karmatschek M, Horn E, Ludwig H, et al. Serum immunoreactive bone sialoprotein as a new marker of bone turnover in metabolic and malignant bone disease. Journal of Clinical Endocrinology & Metabolism 1996 Sep;81(9):3289-94.

(120) Shaarawy M, Hasan M. Serum bone sialoprotein: a marker of bone resorption in postmenopausal osteoporosis. Scandinavian Journal of Clinical & Laboratory Investigation 2001;61(7):513-21.

(121) Halleen JM, Ylipahkala H, Alatalo SL, Janckila AJ, Heikkinen JE, Suominen H, et al. Serum tartrate-resistant acid phosphatase 5b, but not 5a, correlates with other markers of bone turnover and bone mineral density. Calcified Tissue International 2002 Jul;71(1):20-5.

(122) Schilling AF, Mulhausen C, Lehmann W, Santer R, Schinke T, Rueger JM, et al. High bone mineral density in pycnodysostotic patients with a novel mutation in the propeptide of cathepsin K. Osteoporosis International 2007 May;18(5):659-69.

(123) Stoch SA, Miller D, Van Dyck K, Jin B, Panebianco D, Liu Q, et al. Effect of cathepsin K inhibition on bone resorption markers in healthy postmenopausal women. Calcified Tissue International 2007;80:S152-S153.

(124) Lin CW, Jiang X, Dai ZQ, Guo XZ, Weng TJ, Wang J, et al. Sclerostin mediates bone response to mechanical unloading through antagonizing Wnt/beta-catenin signaling. Journal of Bone and Mineral Research 2009 Oct;24(10):1651-61.

(125) Wong M, Carter DR. A theoretical model of endochondral ossification and bone architectural construction in long bone ontogeny. Anatomy and Embryology 1990;181(6):523-32.

(126) Ducy P, Karsenty G. Genetic control of cell differentiation in the skeleton. Current Opinion in Cell Biology 1998 Oct;10(5):614-9.

(127) Chen D, Zhao M, Mundy GR. Bone morphogenetic proteins. Growth Factors 2004 Dec;22(4):233-41.

(128) Laor T, Jaramillo D. MR imaging insights into skeletal maturation: What is normal? Radiology 2009 Jan;250(1):28-38.

(129) Glorieux FH, Rauch F, Shapiro JR. Bisphosphonates in children with bone diseases. New England Journal of Medicine 2003 Nov 20;349(21):2068-9.

(130) Rauch F. Bone growth in length and width: The Yin and Yang of bone stability. Calcified Tissue International 2007;80:S16.

(131) Macrae VE, Farquharson C, Ahmed SF. The pathophysiology of the growth plate in juvenile idiopathic arthritis. Rheumatology 2006 Jan;45(1):11-9.

(132) Beier F. Cell-cycle control and the cartilage growth plate. Journal of Cellular Physiology 2005 Jan;202(1):1-8.

(133) Hindmarsh PC, Dattani MT. Use of growth hormone in children. Nature Clinical Practice Endocrinology & Metabolism 2006 May;2(5):260-8.

(134) Slonim AE, Bulone L, Damore MB, Goldberg T, Wingertzahn MA, McKinley MJ. A preliminary study of growth hormone therapy for Crohn's disease. New England Journal of Medicine 2000 Jun 1;342(22):1633-7.

(135) Wong SC, Kumar P, Galloway PJ, Blair JC, Didi M, Dalzell AM, et al. A preliminary trial of the effect of recombinant human growth hormone on short-term linear growth and glucose homeostasis in children with Crohn's disease. Clinical Endocrinology 2011 May;74(5):599-607.

(136) Maccarinelli G, Sibilia V, Torsello A, Raimondo F, Pitto M, Giustina A, et al. Ghrelin regulates proliferation and differentiation of osteoblastic cells. Journal of Endocrinology 2005 Jan;184(1):249-56.

(137) Ohlsson C, Bengtsson BA, Isaksson OGP, Andreassen TT, Slootweg MC. Growth hormone and bone. Endocrine Reviews 1998 Feb;19(1):55-79.

(138) Kofoed EM, Hwa V, Little B, Woods KA, Buckway CK, Tsubaki J, et al. Growth hormone insensitivity associated with a STAT5b mutation. New England Journal of Medicine 2003 Sep 18;349(12):1139-47.

(139) Herrington J, Smit LS, Schwartz J, Carter-Su C. The role of STAT proteins in growth hormone signaling. Oncogene 2000 May 15;19(21):2585-97.

(140) Smit LS, Meyer DJ, Billestrup N, Norstedt G, Schwartz J, CarterSu C. The role of the growth hormone (GH) receptor and JAK1 and JAK2 kinases in the activation of Stats 1, 3, and 5 by GH. Molecular Endocrinology 1996 May;10(5):519-33.

(141) Macrae VE, Horvat S, Pells SC, Dale H, Collinson RS, Pitsillides AA, et al. Increased bone mass, altered trabecular architecture and modified growth plate organization in the growing skeleton of SOCS2 deficient mice. Journal of Cellular Physiology 2009 Feb;218(2):276-84.

(142) Wang J, Zhou J, Bondy CA. IGF1 promotes longitudinal bone growth by insulin-like actions augmenting chondrocyte hypertrophy. Faseb Journal 1999 Nov;13(14):1985-90.

(143) Hunziker EB, Wagner J, Zapf J. Differential effects of insulin-like growth factor I and growth hormone on developmental stages of rat growth plate chondrocytes in vivo. Journal of Clinical Investigation 1994 Mar;93(3):1078-86.

(144) Macrae VE, Ahmed SF, Mushtaq T, Farquharson C. IGF-I signalling in bone growth: Inhibitory actions of dexamethasone and IL-1 beta. Growth Hormone & Igf Research 2007 Oct;17(5):435-9.

(145) Macrae VE, Farquharson C, Ahmed SF. The restricted potential for recovery of growth plate chondrogenesis and longitudinal bone growth following exposure to pro-inflammatory cytokines. Journal of Endocrinology 2006 May;189(2):319-28.

(146) Wang YM, Cheng ZQ, ElAlieh HZ, Nakamura E, Nguyen MT, Mackem S, et al. IGF-1R signaling in chondrocytes modulates growth plate development by interacting with the PTHrP/Ihh pathway. Journal of Bone and Mineral Research 2011 Jul;26(7):1437-46.

(147) Ohlsson C, Nilsson A, Isaksson OGP, Lindahl A. Effect of growth hormone and insulin-like growth factor-I on DNA synthesis and matrix production in rat epiphyseal chondrocytes in monolayer culture. Journal of Endocrinology 1992 May;133(2):291-&.

(148) Wong SC, Macrae VE, McGrogan P, Ahmed SF. The role of pro-inflammatory cytokines in inflammatory bowel disease growth retardation. Journal of Pediatric Gastroenterology and Nutrition 2006 Aug;43(2):144-55.

(149) Olney RC. Regulation of bone mass by growth hormone. Medical and Pediatric Oncology 2003 Sep;41(3):228-34.

(150) Wu SF, Aguilar AL, Ostrow V, De Luca F. Insulin resistance secondary to a high-fat diet stimulates longitudinal bone growth and growth plate chondrogenesis in mice. Endocrinology 2011 Feb;152(2):468-75.

(151) Malik S, Ahmed SF, Wilson ML, Shah N, Loganathan S, Naik S, et al. The effects of anti-TNF-alpha treatment with adalimumab on growth in children with Crohn's disease (CD). Journal of Crohns & Colitis 2012 Apr;6(3):337-44.

(152) Marcovecchio ML, Mohn A, Chiarelli F. Inflammatory cytokines and growth in childhood. Current Opinion in Endocrinology Diabetes and Obesity 2012 Feb;19(1):57-62.

(153) Mushtaq T, Bijman P, Ahmed SF, Farquharson C. Insulin-like growth factor-I augments chondrocyte hypertrophy and reverses glucocorticoid-mediated growth retardation in fetal mice metatarsal cultures. Endocrinology 2004 May;145(5):2478-86.

(154) Chrysis D, Zaman F, Chagin AS, Takigawa M, Savendahl L. Dexamethasone induces apoptosis in proliferative chondrocytes through activation of caspases and suppression of the Akt-phosphatidylinositol 3 '-kinase signaling pathway. Endocrinology 2005 Mar;146(3):1391-7.

(155) Emma F, Sesto A, Rizzoni G. Long-term linear growth of children with severe steroid-responsive nephrotic syndrome. Pediatric Nephrology 2003 Aug;18(8):783-8.

(156) Chagin AS, Savendahl L. GPR30 estrogen receptor expression in the growth plate declines as puberty progresses. Journal of Clinical Endocrinology & Metabolism 2007 Dec;92(12):4873-7.

(157) Juul A. longThe effects of oestrogens on linear bone growth. Human Reproduction Update 2001 May;7(3):303-13.

(158) Xu LT, Wang Q, Wang QJ, Lyytikainen A, Mikkola T, Volgyi E, et al. Concerted Actions of Insulin-Like Growth Factor 1, Testosterone, and Estradiol on Peripubertal Bone Growth: A 7-Year Longitudinal Study. Journal of Bone and Mineral Research 2011 Sep;26(9):2204-11.

(159) Borjesson AE, Lagerquist MK, Liu C, Shao RJ, Windahl SH, Karlsson C, et al. The role of estrogen receptor-alpha in growth plate cartilage for longitudinal bone growth. Journal of Bone and Mineral Research 2010 Dec;25(12):2414-24.

(160) Khosla S, Riggs BL. Androgens, estrogens, and bone turnover in men. Journal of Clinical Endocrinology & Metabolism 2003 May 1;88(5):2352.

(161) Wang L, Shao YY, Ballock RT. Thyroid hormone interacts with the Wnt/beta-catenin signaling pathway in the terminal differentiation of growth plate chondrocytes. Journal of Bone and Mineral Research 2007 Dec;22(12):1988-95.

(162) Robson H, Siebler T, Stevens DA, Shalet SM, Willlams GR. Thyroid hormone acts directly on growth plate chondrocytes to promote hypertrophic differentiation and inhibit clonal expansion and cell proliferation. Endocrinology 2000 Oct;141(10):3887-97.

(163) Wang L, Shao YY, Ballock RT. Leptin synergizes with thyroid hormone signaling in promoting growth plate chondrocyte proliferation and terminal differentiation in vitro. Bone 2011 May 1;48(5):1022-7.

(164) Pass C, Macrae VE, Ahmed SF, Farquharson C. Inflammatory cytokines and the GH/IGF-I axis: novel actions on bone growth. Cell Biochemistry and Function 2009 Apr;27(3):119-27.

(165) Mabilleau G, Aguado E, Stancu IC, Cincu C, Basle ME, Chappard D. Effects of FGF-2 release from a hydrogel polymer on bone mass and microarchitecture. Biomaterials 2008 Apr;29(11):1593-600.

(166) Glorieux FH PJJH. Pediatric bone: biology and diseases. San Diego, 2003.

(167) Lieberman JR, Daluiski A, Einhorn TA. The role of growth factors in the repair of bone - Biology and clinical applications. Journal of Bone and Joint Surgery-American Volume 2002 Jun;84A(6):1032-44.

(168) Krejci P, Salazar L, Goodridge HS, Kashiwada TA, Schibler MJ, Jelinkova P, et al. STAT1 and STAT3 do not participate in FGF-mediated growth arrest in chondrocytes. Journal of Cell Science 2008 Feb 1;121(3):272-81.

(169) Razzaque MS, St-Arnaud R, Taguchi T, Lanske B. FGF-23, vitamin D and calcification: the unholy triad. Nephrology Dialysis Transplantation 2005 Oct;20(10):2032-5.

(170) Ribera JM, Oriol A. Acute lymphoblastic leukemia in adolescents and young adults. Hematology-Oncology Clinics of North America 2009 Oct;23(5):1033-+.

(171) Pui CH, Campana D, Pei DQ, Bowman WP, Sandlund JT, Kaste SC, et al. Treating childhood acute lymphoblastic leukemia without cranial irradiation. New England Journal of Medicine 2009 Jun 25;360(26):2730-41.

(172) Davies JH, Evans BAJ, Jenney MEM, Gregory JW. Skeletal morbidity in childhood acute lymphoblastic leukaemia. Clinical Endocrinology 2005 Jul;63(1):1-9.

(173) Mäyränpää M. Fractures in children: epidemiology and associated bone health characteristics 2012.

(174) Vora A, Wade R, Mitchell C, Goulden N, Richards S. Incidence and Outcome of Osteonecrosis in Children and Young Adults with Acute Lymphoblastic Leukaemia Treated on a Dexamethasone Containing Protocol: Results of the Medical Research Council UK Trial ALL 2003. Blood 2008 Nov 16;112(11):337.

(175) Vrooman LM, Neuberg DS, Stevenson KE, Supko JG, Sallan SE, Silverman LB. Dexamethasone and Individualized Asparaginase Dosing Are Each Associated with Superior Event-Free Survival in Childhood Acute Lymphoblastic Leukemia: Results From DFCI-ALL Consortium Protocol 00-01. Blood 2009 Nov 20;114(22):136.

(176) Mattano LA, Devidas M, Nachman JB, Sather HN, Hunger SP, Steinherz PG, et al. Effect of alternate-week versus continuous dexamethasone scheduling on the risk of osteonecrosis in paediatric patients with acute lymphoblastic leukaemia: results from the CCG-1961 randomised cohort trial. Lancet Oncology 2012 Sep;13(9):906-15.

(177) Vora A. Management of osteonecrosis in children and young adults with acute lymphoblastic leukaemia. British Journal of Haematology 2011 Dec;155(5):549-60.

(178) Rayar M, Athale U, Nayiager T, Webber C, Barr R. Predictors of bony morbidity in children with acute lymphoblastic leukemia. Pediatric Blood & Cancer 2011;DOI 10.1002/pbc.24040(5).

(179) Halton JM, Atkinson SA, Fraher L, Webber CE, Cockshott WP, Tam C, et al. Mineral homeostasis and bone mass at diagnosis in children with acute lymphoblastic leukemia. Journal of Pediatrics 1995 Apr;126(4):557-64.

(180) Maman E SDSBISWS. Acute lymphoblastic leukemia in children: correlation of musculoskeletal manifestations and immunophenotypes. J Child Orthop 1[1], 63-68. 2007.
Ref Type: Journal (Full)

(181) Sinigaglia R, Gigante C, Bisinella G, Varotto S, Zanesco L, Turra S. Musculoskeletal manifestations in pediatric acute leukemia. Journal of Pediatric Orthopaedics 2008 Jan;28(1):20-8.

(182) Crofton PM, Ahmed SF, Wade JC, Stephen R, Elmlinger MW, Ranke MB, et al. Effects of intensive chemotherapy on bone and collagen turnover and the growth hormone axis in children with acute lymphoblastic leukemia. Journal of Clinical Endocrinology & Metabolism 1998 Sep;83(9):3121-9.

(183) Atkinson SA, Halton JM, Bradley C, Wu B, Barr RD. Bone and mineral abnormalities in childhood acute lymphoblastic leukemia: Influence of disease, drugs and nutrition. International Journal of Cancer 1998;35-9.

(184) van der Sluis IM, Heuvel-Eibrink MM, Hablen K, Krenning EP, Keizer-Schrama SMPF. Altered bone mineral density and body composition, and increased fracture risk in childhood acute lymphoblastic leukemia. Journal of Pediatrics 2002 Aug;141(2):204-10.

(185) Tragiannidis A, Dokos C, Sidi V, Papageorgiou T, Koliouskas D, Karamouzis M, et al. Alterations of bone mineral metabolism of children with different cell lineage types of acute lymphoblastic leukaemia under chemotherapy. Hippokratia 2011;15(1):43-7.

(186) Winkel MLT, van Beek RD, Keizer-Schrama SMPF, Uitterlinden AG, Hop WCJ, Pieters R, et al. Pharmacogenetic risk factors for altered bone mineral density and body composition in pediatric acute lymphoblastic leukemia. Blood 2009 Nov 20;114(22):1194.

(187) Mandel K, Atkinson S, Barr RD, Pencharz P. Skeletal morbidity in childhood acute lymphoblastic leukemia. Journal of Clinical Oncology 2004 Apr 1;22(7):1215-21.

(188) Hartman A, Winkel MLT, van Beek RD, Keizer-Schrama SMPF, Kemper HCG, Hop WCJ, et al. A randomized trial investigating an exercise program to prevent reduction of bone mineral density and impairment of motor performance during treatment for childhood acute lymphoblastic leukemia. Pediatric Blood & Cancer 2009 Jul 15;53(1):64-71.

(189) Mussa A, Bertorello N, Porta F, Galletto C, Nicolosi MG, Manicone R, et al. Prospective bone ultrasound patterns during childhood acute lymphoblastic leukemia treatment. Bone 2010 Apr;46(4):1016-20.

(190) Cockle JVASAMT. Bone density in children with acute lymphoblastic leukaemia at a regional centre and comparison to children at risk of low bone density. Endocrine Abstracts 2011;27:42.

(191) Inaba H, Pui CH. Glucocorticoid use in acute lymphoblastic leukaemia. Lancet Oncology 2010 Nov;11(11):1096-106.

(192) Vallance K, Liu W, Mandrell BN, Panetta JC, Gattuso JS, Hockenberry M, et al. Mechanisms of dexamethasone-induced disturbed sleep and fatigue in paediatric patients receiving treatment for ALL. European Journal of Cancer 2010 Jul;46(10):1848-55.

(193) Moschovi M TGVMKKMADACGPI. Serial plasma concentrations of adiponectin, leptin, and resistin during therapy in children with acute lymphoblastic leukemia. J Pediatr Hematol Oncol 2010;3(1):8-13.

(194) Davies JH, Evans BAJ, Jones E, Evans WD, Jenney MEM, Gregory JW. Osteopenia, excess adiposity and hyperleptinaemia during 2 years of treatment for childhood acute lymphoblastic leukaemia without cranial irradiation. Clinical Endocrinology 2004 Mar;60(3):358-65.

(195) Atkinson SA, Fraher L, Gundberg CM, Andrew M, Pai M, Barr RD. Mineral homeostasis and bone mass in children treated for acute lymphoblastic leukemia. Journal of Pediatrics 1989 May;114(5):793-800.

(196) Reilly JJ, Brougham M, Montgomery C, Richardson F, Kelly A, Gibson BES. Effect of glucocorticoid therapy on energy intake in children treated for acute lymphoblastic leukemia. Journal of Clinical Endocrinology & Metabolism 2001 Aug;86(8):3742-5.

(197) Jansen H, Postma A, Stolk R, Kamps W. Acute lymphoblastic leukemia and obesity: increased energy intake or decreased physical activity? Supportive Care in Cancer 2009 Jan;17(1):103-6.

(198) Hinds PS, Hockenberry MJ, Gattuso JS, Srivastava DK, Tong X, Jones H, et al. Dexamethasone alters sleep and fatigue in pediatric patients with acute lymphoblastic leukemia. Cancer 2007 Nov 15;110(10):2321-30.

(199) Winter C, Muller C, Brandes M, Brinkmann A, Hoffmann C, Hardes J, et al. Level of activity in children undergoing cancer treatment. Pediatric Blood & Cancer 2009 Sep;53(3):438-43.

(200) Arikoski P, Komulainen J, Voutilainen R, Riikonen P, Parviainen M, Tapanainen P, et al. Reduced bone mineral density in long-term survivors of childhood acute lymphoblastic leukemia. Journal of Pediatric Hematology Oncology 1998 May;20(3):234-40.

(201) Arikoski P, Voutilainen R, Kroger H. Bone mineral density in long-term survivors of childhood cancer. Journal of Pediatric Endocrinology & Metabolism 2003 Mar;16:343-53.

(202) Hoorweg-Nijman JJG, Kardos G, Roos JC, van Dijk HJ, Netelenbos C, Popp-Snijders C, et al. Bone mineral density and markers of bone turnover in young adult survivors of childhood lymphoblastic leukaemia. Clinical Endocrinology 1999 Feb;50(2):237-44.

(203) Le Meignen M, Auquier P, Barlogis V, Sirvent N, Contet A, Simeoni MC, et al. Bone mineral density in adult survivors of childhood acute leukemia: impact of hematopoietic stem cell transplantation and other treatment modalities. Blood 2011 Aug 11;118(6):1481-9.

(204) van Beek RD, Heuvel-Eibrink MM, Hakvoort-Cammel FG, van den Bos C, van der Pal HJH, Krenning EP, et al. Bone mineral density, growth, and thyroid function in long-term survivors of pediatric Hodgkin's lymphoma treated with chemotherapy only. Journal of Clinical Endocrinology & Metabolism 2009 Jun;94(6):1904-9.

(205) Kaste SC, Jones-Wallace D, Rose SR, Boyett JM, Lustig RH, Rivera GK, et al. Bone mineral decrements in survivors of childhood acute lymphoblastic leukemia: frequency of occurrence and risk factors for their development. Leukemia 2001 May;15(5):728-34.

(206) Thomas IH, Donohue JE, Ness KK, Dengel DR, Baker KS, Gurney JG. Bone mineral density in young adult survivors of acute lymphoblastic leukemia. Cancer 2008 Dec 1;113(11):3248-56.

(207) Wilson CL DKNKLWSCKSSMGDAGRLK-LNS. Fractures among long-term survivors of childhood cancer: A report from the Childhood Cancer Survivor Study. Cancer 2012;May 17. doi: 10.1002/cncr.27626. [Epub ahead of print].

(208) Tillmann V, Darlington ASE, Eiser C, Bishop NJ, Davies HA. Male sex and low physical activity are associated with reduced spine bone mineral density in survivors of childhood acute lymphoblastic leukemia. Journal of Bone and Mineral Research 2002 Jun;17(6):1073-80.

(209) Tonorezos ES, Vega GL, Sklar CA, Chou JF, Moskowitz CS, Mo QX, et al. Adipokines, body fatness, and insulin resistance among survivors of childhood leukemia. Pediatric Blood & Cancer 2012 Jan;58(1):31-6.

(210) Kohler JA, Moon RJ, Wright S, Willows E, Davies JH. Increased adiposity and altered adipocyte function in female survivors of childhood acute lymphoblastic leukaemia treated without cranial radiation. Hormone Research in Paediatrics 2011;75(6):433-40.

(211) Brennan BMD, Mughal Z, Roberts SA, Ward K, Shalet SM, Eden TOB, et al. Bone mineral density in childhood survivors of acute lymphoblastic leukemia treated without cranial irradiation. Journal of Clinical Endocrinology & Metabolism 2005 Feb;90(2):689-94.

(212) Kaste SC, Rai SN, Fleming K, McCammon EA, Tylavsky FA, Danish RK, et al. Changes in bone mineral density in survivors of childhood acute lymphoblastic leukemia. Pediatric Blood & Cancer 2006 Jan;46(1):77-87.

(213) Rai SN, Hudson MM, McCammon E, Carbone L, Tylavsky F, Smith K, et al. Implementing an intervention to improve bone mineral density in survivors of childhood acute lymphoblastic leukemia: BONEII, a prospective placebo-controlled double-blind randomized interventional longitudinal study design. Contemporary Clinical Trials 2008 Sep;29(5):711-9.

(214) Polgreen LE, Petryk A, Dietz AC, Sinaiko AR, Leisenring W, Goodman P, et al. Modifiable risk factors associated with bone deficits in childhood cancer survivors. Bmc Pediatrics 2012 Mar 28;12.

(215) Aldhafiri F, AL-Nasser A, Al-Sugair A, Khanna S , Ahmed SF, Al-Mutairi H , et al. Importance of Adjusting Dual Energy X-Ray Output For Body Size : An Example From Survivors Of Childhood Acute Lymphoblastic Leukemia. Pediatric Hematology Oncololgy 2012;(In Press).

(216) Styrkarsdottir U, Halldorsson BV, Gretarsdottir S, Gudbjartsson DF, Walters GB, Ingvarsson T, et al. Multiple genetic loci for bone mineral density and fractures. New England Journal of Medicine 2008 May 29;358(22):2355-65.

(217) Van Wesenbeeck L, Cleiren E, Gram J, Beals RK, Benichou O, Scopelliti D, et al. Six novel missense mutations in the LDL receptor-related protein 5 (LRP5) gene in different conditions with an increased bone density. American Journal of Human Genetics 2003 Mar;72(3):763-71.

(218) Gong YQ, Vikkula M, Boon L, Liu J, Beighton P, Ramesar R, et al. Osteoporosis-pseudoglioma syndrome, a disorder affecting skeletal strength and vision, is assigned to chromosome region 11q12-13. American Journal of Human Genetics 1996 Jul;59(1):146-51.

(219) Koay MA, Tobias JH, Leary SD, Steer CD, Vilarino-Guell C, Brown MA. The effect of LRP5 polymorphisms on bone mineral density is apparent in childhood. Calcified Tissue International 2007 Jul;81(1):1-9.

(220) Jones TS, Kaste SC, Liu W, Cheng C, Yang W, Tantisira KG, et al. CRHR1 polymorphisms predict bone density in survivors of acute lymphoblastic leukemia. Journal of Clinical Oncology 2008 Jun 20;26(18):3031-7.

(221) Wasilewski-Masker K, Kaste SC, Hudson MM, Esiashvili N, Mattano LA, Meacham LR. Bone mineral density deficits in survivors of childhood cancer: Long-term follow-up guidelines and review of the literature. Pediatrics 2008 Mar;121(3):E705-E713.

(222) Heath JA, Ramzy JM, Donath SM. Physical activity in survivors of childhood acute lymphoblastic leukaemia. Journal of Paediatrics and Child Health 2010 Apr;46(4):149-53.

(223) Järvelä LS KJNHHJLPKJAMHOJ. Effects of a home-based exercise program on metabolic risk factors and fitness in long-term survivors of childhood acute lymphoblastic leukemia. Pediatr Blood Cancer 2011 Dec 19. doi: 10.1002/pbc.24049. 2011.
Ref Type: Journal (Full)

(224) Tylavsky FA, Smith K, Surprise H, Garland S, Yan XW, McCammon E, et al. Nutritional intake of long-term survivors of childhood acute lymphoblastic leukemia: evidence for bone health interventional opportunities. Pediatric Blood & Cancer 2010 Dec 15;55(7):1362-9.

(225) Kumar J, Muntner P, Kaskel FJ, Hailpern SM, Melamed ML. Prevalence and associations of 25-hydroxyvitamin D deficiency in US children: NHANES 2001-2004. Pediatrics 2009 Sep;124(3):E362-E370.

(226) Simmons JH, Chow EJ, Koehler E, Esbenshade A, Smith LA, Sanders J, et al. Significant 25-hydroxyvitamin D deficiency in child and adolescent survivors of acute lymphoblastic leukemia: treatment with chemotherapy compared with allogeneic stem cell transplant. Pediatric Blood & Cancer 2011 Jul;56(7):1114-9.

(227) Ward L, Tricco AC, Phuong P, Cranney A, Barrowman N, Gaboury I, et al. Bisphosphonate therapy for children and adolescents with secondary osteoporosis. Cochrane Database of Systematic Reviews 2007;(4).

(228) Sandler RB, Slemenda CW, Laporte RE, Cauley JA, Schramm MM, Barresi ML, et al. Postmenopausal bone density and milk consumption in childhood and adolescence. American Journal of Clinical Nutrition 1985;42(2):270-4.

(229) Harvey N, Cole Z, Crozier S, Dennison E, Inskip H, Godfrey K, et al. (Premier Poster-Award Candidate) childhood physical activity and calcium intake predict bone size and density of the hip: the Southampton women's survey. Osteoporosis International 2010 Nov;21:S460-S461.

(230) Vandenbergh MFQ, Deman SA, Witteman JCM, Hofman A, Trouerbach WT, Grobbee DE. Physical activity, calcium intake, and bone mineral content in children in The Netherlands. Journal of Epidemiology and Community Health 1995 Jun;49(3):299-304.

(231) Iwasaki T, Takei K, Nakamura S, Hosoda N, Yokota Y, Ishii M. Secondary osteoporosis in long-term bedridden patients with cerebral palsy. Pediatrics International 2008 Jun;50(3):269-75.

(232) Diaz PR, Neira LC, Fiseher SG, Torres MCT, Milinarsky AT, Giadrosich VR, et al. Effect of 1,25(OH)2-vitamin D on bone mass in children with acute lymphoblastic leukemia. Journal of Pediatric Hematology Oncology 2008 Jan;30(1):15-9.

(233) Aydin H, Deyneli O, Yavuz D, Gozu H, Mutlu N, Kaygusuz I, et al. Short-term oral magnesium supplementation suppresses bone turnover in postmenopausal osteoporotic women. Biological Trace Element Research 2010 Feb;133(2):136-43.

(234) Toba Y, Kajita Y, Masuyama R, Takada Y, Suzuki K, Aoe S. Dietary magnesium supplementation affects bone metabolism and dynamic strength of bone in ovariectomized rats. Journal of Nutrition 2000 Feb;130(2):216-20.

(235) Chad KE, Bailey DA, Mckay HA, Zello GA, Snyder RE. The effect of a weight-bearing physical activity program on bone mineral content and estimated volumetric density in children with spastic cerebral palsy. Journal of Pediatrics 1999 Jul;135(1):115-7.

(236) Guadalupe-Grau A, Fuentes T, Guerra B, Calbet JAL. Exercise and bone mass in adults. Sports Medicine 2009;39(6):439-68.

(237) Vicente-Rodriguez G, Ara I, Perez-Gomez J, Dorado C, Calbet JAL. Muscular development and physical activity as major determinants of femoral bone mass acquisition during growth. British Journal of Sports Medicine 2005 Sep;39(9):611-6.

(238) Gohar SF, Comito M, Price J, Marchese V. Feasibility and parent satisfaction of a physical therapy intervention program for children with acute lymphoblastic leukemia in the first 6 months of medical treatment. Pediatric Blood & Cancer 2011 May;56(5):799-804.

(239) Wyon M, Guinan D, Hawkey A. Whole-body vibration training increases vertical jump height in a dance population. Journal of Strength and Conditioning Research 2010 Mar;24(3):866-70.

(240) Ruan XY, Jin FY, Liu YL, Peng ZL, Sun YG. Effects of vibration therapy on bone mineral density in postmenopausal women with osteoporosis. Chinese Medical Journal 2008 Jul 5;121(13):1155-8.

(241) Rubin C, Turner AS, Bain S, Mallinckrodt C, McLeod K. Anabolism - Low mechanical signals strengthen long bones. Nature 2001 Aug 9;412(6847):603-4.

(242) Rubin C, Judex S, Qin YX. Low-level mechanical signals and their potential as a non-pharmacological intervention for osteoporosis. Age and Ageing 2006 Sep;35:32-6.

(243) Bosco C, Iacovelli M, Tsarpela O, Cardinale M, Bonifazi M, Tihanyi J, et al. Hormonal responses to whole body vibration in men. European Journal of Applied Physiology 2000 Apr;81(6):449-54.

(244) Gilsanz V, Al Wren T, Sanchez M, Dorey F, Judex S, Rubin C. Low-level, high-frequency mechanical signals enhance musculoskeletal development of young women with low BMD. Journal of Bone and Mineral Research 2006 Sep;21(9):1464-74.

(245) Ward K, Alsop C, Caulton J, Rubin C, Adams J, Mughal Z. Low magnitude mechanical loading is osteogenic in children with disabling conditions. Journal of Bone and Mineral Research 2004 Mar;19(3):360-9.

(246) Lethaby C, Wiernikowski J, Sala A, Webber C, Barr RD. Use of alendronate to treat reduced bone mineral mass in children surviving acute lymphoblastic leukemia (ALL). Bone 2005 May;36:S85-S86.

(247) Kotecha RS, Powers N, Lee SJ, Murray KJ, Carter T, Cole C. Use of bisphosphonates for the treatment of osteonecrosis as a complication of therapy for childhood acute lymphoblastic leukaemia (ALL). Pediatric Blood & Cancer 2010 Jul 1;54(7):934-40.

(248) Simm PJ, Johannesen J, Briody J, McQuade M, Hsu B, Bridge C, et al. Zoledronic acid improves bone mineral density, reduces bone turnover and improves skeletal architecture over 2 years of treatment in children with secondary osteoporosis. Bone 2011 Nov;49(5):939-43.

(249) Bachrach LK, Ward LM. Clinical review: bisphosphonate use in childhood csteoporosis. Journal of Clinical Endocrinology & Metabolism 2009 Feb;94(2):400-9.

(250) Davies J, Gregory J. Radiographic long bone appearance in a child administered cyclical pamidronate. Archives of Disease in Childhood 2003;88(10):854.

(251) Fleisch H. Bisphosphonates in osteoporosis. European Spine Journal 2003 Oct;12:S142-S146.

(252) Filleul O, Crompot E, Saussez S. Bisphosphonate-induced osteonecrosis of the jaw: a review of 2,400 patient cases. Journal of Cancer Research and Clinical Oncology 2010 Aug;136(8):1117-24.

(253) Hiraga T, Ninomiya T, Hosoya A, Nakamura H. Administration of the bisphosphonate zoledronic acid during tooth development inhibits tooth eruption and formation and induces dental abnormalities in rats. Calcified Tissue International 2010 Jun;86(6):502-10.

(254) Rothenbuhler A, Marchand I, Bougneres P, Linglart A. Risk of corrected QT interval prolongation after pamidronate infusion in children. Journal of Clinical Endocrinology & Metabolism 2010 Aug;95(8):3768-70.

(255) Whyte MP, McAlister WH, Novack DV, Clements KL, Schoenecker PL, Wenkert D. Bisphosphonate-induced osteopetrosis: Novel bone modeling defects, metaphyseal osteopenia, and osteosclerosis fractures after drug exposure ceases. Journal of Bone and Mineral Research 2008 Oct;23(10):1698-707.

(256) van den Heijkant S, Hoorweg-Nijman G, Huisman J, Drent M, van der Pal H, Kaspers GJ, et al. Effects of growth hormone therapy on bone mass, metabolic balance, and well-being in young adult survivors of childhood acute lymphoblastic leukemia. Journal of Pediatric Hematology Oncology 2011 Aug;33(6):E231-E238.

(257) Follin C, Link K, Wiebe T, Moell C, Bjork J, Erfurth EM. Bone loss after childhood acute lymphoblastic leukaemia: an observational study with and without GH therapy. European Journal of Endocrinology 2011 May;164(5):695-703.

(258) Manera R, Ramirez I, Mullins J, Pinkel D. Pilot studies of species-specific chemotherapy of childhood acute lymphoblastic leukemia using genotype and immunophenotype. Leukemia 2000 Aug;14(8):1354-61.

(259) Vilmer E, Suciu S, Ferster A, Bertrand Y, Cave H, Thyss A, et al. Long-term results of three randomized trials (58831,58832,58881) in childhood acute lymphoblastic leukemia: a CLCG-EORTC report. Leukemia 2000 Dec;14(12):2257-66.

(260) Owen HC, Miner JN, Ahmed SF, Farquharson C. The growth plate sparing effects of the selective glucocorticoid receptor modulator, AL-438. Molecular and Cellular Endocrinology 2007 Jan 29;264(1-2):164-70.

(261) Fricke O, Schoenau E. The functional muscle bone unit: Probing the relevance of mechanical signals for bone development in children and adolescents. Growth Hormone & Igf Research 2007 Feb;17(1):1-9.

(262) Kondo H, Nifuji A, Takeda S, Ezura Y, Rittling SR, Denhardt DT, et al. Unloading induces osteoblastic cell suppression and osteoclastic cell activation to lead to bone loss via sympathetic nervous system. Journal of Biological Chemistry 2005 Aug 26;280(34):30192-200.

(263) Turner CH, Burr DB. Basic biomechanical measurements of bone - A tutorial. Bone 1993 Jul;14(4):595-608.

(264) Sugiyama T, Yamaguchi A, Kawai S. Effects of skeletal loading on bone mass and compensation mechanism in bone: a new insight into the "mechanostat" theory. Journal of Bone and Mineral Metabolism 2002;20(4):196-200.

(265) Huang CY, Ogawa R. Mechanotransduction in bone repair and regeneration. Faseb Journal 2010 Oct;24(10):3625-32.

(266) Pavalko FM, Chen NX, Turner CH, Burr DB, Atkinson S, Hsieh YF, et al. Fluid shear-induced mechanical signaling in MC3T3-E1 osteoblasts requires cytoskeleton-integrin interactions. American Journal of Physiology-Cell Physiology 1998 Dec;275(6):C1591-C1601.

(267) Litzenberger JB, Kim JB, Tummala P, Jacobs CR. beta (1) integrins mediate mechanosensitive signaling pathways in osteocytes. Calcified Tissue International 2010 Apr;86(4):325-32.

(268) Litzenberger JB, Tang WJ, Castillo AB, Jacobs CR. Deletion of beta 1 integrins from cortical osteocytes reduces load-induced bone formation. Cellular and Molecular Bioengineering 2009 Sep;2(3):416-24.

(269) Forwood MR. Inducible cyclo-oxygenase (COX-2) mediates the induction of bone formation by mechanical loading in vivo. Journal of Bone and Mineral Research 1996 Nov;11(11):1688-93.

(270) Rumney RMH, Sunters A, Reilly GC, Gartland A. Application of multiple forms of mechanical loading to human osteoblasts reveals increased ATP release in response to fluid flow in 3D cultures and differential regulation of immediate early genes. Journal of Biomechanics 2012 Feb 2;45(3):549-54.

(271) Jansen JHW, Eijken M, Jahr H, Chiba H, Verhaar JAN, van Leeuwen JPTM, et al. Stretch-induced inhibition of Wnt/beta-catenin signaling in mineralizing osteoblasts. Journal of Orthopaedic Research 2010 Mar;28(3):390-6.

(272) Santos A, Bakker AD, Zandieh-Doulabi B, de Blieck-Hogervorst JMA, Klein-Nulend J. Early activation of the beta-catenin pathway in osteocytes is mediated by nitric oxide, phosphatidyl inositol-3 kinase/Akt, and focal adhesion kinase. Biochemical and Biophysical Research Communications 2010 Jan 1;391(1):364-9.

(273) Saxon LK, Robling AG, Alam I, Turner CH. Mechanosensitivity of the rat skeleton decreases after a long period of loading, but is improved with time off. Bone 2005 Mar;36(3):454-64.

(274) Turner CH, Takano Y, Owan I. Aging changes mechanical loading thresholds for bone formation in rats. Journal of Bone and Mineral Research 1995 Oct;10(10):1544-9.

(275) David V, Lafage-Proust MH, Laroche N, Christian A, Ruegsegger P, Vico L. Two-week longitudinal survey of bone architecture alteration in the hindlimb-unloaded rat model of bone loss: sex differences. American Journal of Physiology-Endocrinology and Metabolism 2006 Mar;290(3):E440-E447.

(276) Bonewald LF, Johnson ML. Osteocytes, mechanosensing and Wnt signaling. Bone 2008 Apr;42(4):606-15.

(277) Bacabac RG, Smit TH, Van Loon JJWA, Doulabi BZ, Helder M, Klein-Nulend J. Bone cell responses to high-frequency vibration stress: does the nucleus oscillate within the cytoplasm? Faseb Journal 2006 May;20(7):858-64.

(278) McAllister TN, Frangos JA. Steady and transient fluid shear stress stimulate NO release in osteoblasts through distinct biochemical pathways. Journal of Bone and Mineral Research 1999 Jun;14(6):930-6.

(279) Cheline AJ, Reddi AH, Martin RB. Bone morphogenetic protein-7 selectively enhances mechanically induced bone formation. Bone 2002 Nov;31(5):570-4.

(280) Santos A BAWHBNBAK-NJ. Mechanical loading stimulates BMP7, but not BMP2, production by osteocytes. Calcif Tissue Int 2011;89(4):318-26.

(281) Lee CM, Genetos DC, Wong A, Yellowley CE. Prostaglandin expression profile in hypoxic osteoblastic cells. Journal of Bone and Mineral Metabolism 2010 Jan;28(1):8-16.

(282) Kim CH, You L, Yellowley CE, Jacobs CR. Oscillatory fluid flow-induced shear stress decreases osteoclastogenesis through RANKL and OPG signaling. Bone 2006 Nov;39(5):1043-7.

(283) Papachroni KK, Karatzas DN, Papavassiliou KA, Basdra EK, Papavassiliou AG. Mechanotransduction in osteoblast regulation and bone disease. Trends in Molecular Medicine 2009 May;15(5):208-16.

(284) Davison KS, Siminoski K, Adachi JD, Hanley DA, Goltzman D, Hodsman AB, et al. Bone strength: The whole is greater than the sum of its parts. Seminars in Arthritis and Rheumatism 2006 Aug;36(1):22-31.

(285) Andreoli A, Monteleone M, Van Loan M, Promenzio L, Tarantino U, De Lorenzo A. Effects of different sports on bone density and muscle mass in highly trained athletes. Medicine and Science in Sports and Exercise 2001 Apr;33(4):507-11.

(286) Dook JE, James C, Henderson NK, Price RI. Clinical investigations - Exercise and bone mineral density in mature female athletes. Medicine and Science in Sports and Exercise 1997 Mar;29(3):291-6.

(287) Daly RM, Rich PA, Klein R. Influence of high impact loading on ultrasound bone measurements in children: A cross-sectional report. Calcified Tissue International 1997 May;60(5):401-4.

(288) Haapasalo H, Kannus P, Sievanen H, Pasanen M, Uusi-Rasi K, Heinonen A, et al. Effect of long-term unilateral activity on bone mineral density of female junior tennis players. Journal of Bone and Mineral Research 1998 Feb;13(2):310-9.

(289) Goode AW, Rambaut PC. Space medicine - the skeleton in space. Nature 1985;317(6034):204-5.

(290) Tuner CH. Muscle-bone interactions, revisited. Bone 2000 Sep;27(3):339-40.

(291) Hind K, Burrows M. Weight-bearing exercise and bone mineral accrual in children and adolescents: A review of controlled trials. Bone 2007 Jan;40(1):14-27.

(292) Iuliano-Burns S, Saxon L, Naughton G, Gibbons K, Bass SL. Regional specificity of exercise and calcium during skeletal growth in girls: A randomized controlled trial. Journal of Bone and Mineral Research 2003 Jan;18(1):156-62.

(293) Constantini NW, Dubnov-Raz G, Chodick G, Rozen GS, Giladi A, Ish-Shalom S. Physical activity and bone mineral density in adolescents with vitamin D deficiency. Medicine and Science in Sports and Exercise 2010 Apr;42(4):646-50.

(294) Baxter-Jones ADG, Mirwald RL, Mckay HA, Bailey DA. A longitudinal analysis of sex differences in bone mineral accrual in healthy 8-19-year-old boys and girls. Annals of Human Biology 2003 Mar;30(2):160-75.

(295) Petit MA, Mckay HA, MacKelvie KJ, Heinonen A, Khan KM, Beck TJ. A randomized school-based jumping intervention confers site and maturity-specific benefits on bone structural properties in girls: A hip structural analysis study. Journal of Bone and Mineral Research 2002 Mar;17(3):363-72.

(296) Gunter K, Baxter-Jones ADG, Mirwald RL, Almstedt H, Fuller A, Durski S, et al. Jump starting skeletal health: A 4-year longitudinal study assessing the effects of jumping on skeletal development in pre and circum pubertal children. Bone 2008 Apr;42(4):710-8.

(297) Stokes KA, Sykes D, Gilbert KL, Chen JW, Frystyk J. Brief, high intensity exercise alters serum ghrelin and growth hormone concentrations but not IGF-I, IGF-II or IGF-I bioactivity. Growth Hormone & Igf Research 2010 Aug;20(4):289-94.

(298) Kraemer WJ, Gordon SE, Fleck SJ, Marchitelli LJ, Mello R, Dziados JE, et al. Endogenous anabolic hormonal and growth factor responses to heavy resistance exercise in males and females. International Journal of Sports Medicine 1991 Apr;12(2):228-35.

(299) Gomes RJ, de Mello MAR, Caetano FH, Sibuya CY, Anaruma CA, Rogatto GP, et al. Effects of swimming training on bone mass and the GH/IGF-1 axis in diabetic rats. Growth Hormone & Igf Research 2006 Oct;16(5-6):326-31.

(300) Nazarov V SG. Development of athlete's strength abilities by means of biomechanical stimulation method. Theory Prac Phys Culture 1985;12:445-50.

(301) Bosco C, Cardinale M, Tsarpela O, Colli R, Tihanyi J, von Duvillard SP, et al. The influence of whole body vibration on jumping performance. Biology of Sport 1998;15(3):157-64.

(302) Rubin C, Xu G, Judex S. The anabolic activity of bone tissue, suppressed by disuse, is normalized by brief exposure to extremely low-magnitude mechanical stimuli. Faseb Journal 2001 Oct;15(12):2225-9.

(303) Gusi N, Raimundo A, Leal A. Low-frequency vibratory exercise reduces the risk of bone fracture more than walking: a randomized controlled trial. Bmc Musculoskeletal Disorders 2006 Nov 30;7.

(304) Rittweger J, Schiesel H, Felsenberg D. Oxygen uptake during whole-body vibration exercise: comparison with squatting as a slow voluntary movement. European Journal of Applied Physiology 2001 Dec;86(2):169-73.

(305) Abercromby AFJ, Amonette WE, Layne CS, McFarlin BK, Hinman MR, Paloski WH. Vibration exposure and biodynamic responses during whole-body vibration training. Medicine and Science in Sports and Exercise 2007 Oct;39(10):1794-800.

(306) Wysocki A, Butler M, Shamliyan T, Kane RL. Whole-body vibration therapy for osteoporosis: State of the science. Annals of Internal Medicine 2011 Nov 15;155(10):680-U60.

(307) Rittweger J. Vibration as an exercise modality: how it may work, and what its potential might be. European Journal of Applied Physiology 2010 Mar;108(5):877-904.

(308) Lorenzen C, Maschette W, Koh M, Wilson C. Inconsistent use of terminology in whole body vibration exercise research. Journal of Science and Medicine in Sport 2009 Nov;12(6):676-8.

(309) Cardinale M, Bosco C. The use of vibration as an exercise intervention. Exercise and Sport Sciences Reviews 2003 Jan;31(1):3-7.

(310) Cardinale M, Wakeling J. Whole body vibration exercise: are vibrations good for you? British Journal of Sports Medicine 2005 Sep;39(9):585-9.

(311) Rauch F, Sievanen H, Boonen S, Cardinale M, Degens H, Felsenberg D, et al. Reporting whole-body vibration intervention studies: Recommendations of the International Society of Musculoskeletal and Neuronal Interactions. Journal of Musculoskeletal & Neuronal Interactions 2010 Sep;10(3):193-8.

(312) Rauch F. Vibration therapy. Developmental Medicine and Child Neurology 2009 Oct;51:166-8.

(313) Eklund G, Hagbarth KE. Normal variability of tonic vibration reflexes in man. Experimental Neurology 1966;16(1):80-&.

(314) Fallon JB, Macefield VG. Vibration sensitivity of human muscle spindles and Golgi tendon organs. Muscle & Nerve 2007 Jul;36(1):21-9.

(315) Degail P, Lance JW, Neilson PD. Differential effects on tonic and phasic reflex mechanisms produced by vibration of muscles in man. Journal of Neurology Neurosurgery and Psychiatry 1966;29(1):1-&.

(316) Roelants M, Verschueren SMP, Delecluse C, Levin O, Stijnen V. Whole-body-vibiration-induced increase in leg muscle activity during different squat exercises. Journal of Strength and Conditioning Research 2006 Feb;20(1):124-9.

(317) Judex S, Zhong N, Squire ME, Ye K, Donahue LR, Hadjiargyrou M, et al. Mechanical modulation of molecular signals which regulate anabolic and catabolic activity in bone tissue. Journal of Cellular Biochemistry 2005 Apr 1;94(5):982-94.

(318) Tanaka SM, Li JL, Duncan RL, Yokota H, Burr DB, Turner CH. Effects of broad frequency vibration on cultured osteoblasts. Journal of Biomechanics 2003 Jan;36(1):73-80.

(319) Rubin C, Recker R, Cullen D, Ryaby J, McCabe J, McLeod K. Prevention of postmenopausal bone loss by a low-magnitude, high-frequency mechanical stimuli: A clinical trial assessing compliance, efficacy, and safety. Journal of Bone and Mineral Research 2004 Mar;19(3):343-51.

(320) Stark C, Nikopoulou-Smyrni P, Stabrey A, Semler O, Schoenau E. Effect of a new physiotherapy concept on bone mineral density, muscle force and gross motor function in children with bilateral cerebral palsy. Journal of Musculoskeletal & Neuronal Interactions 2010 Jun;10(2):151-8.

(321) Garman R, Rubin C, Judex S. Small oscillatory accelerations, independent of matrix deformations, increase osteoblast activity and enhance bone morphology. Plos One 2007 Jul 25;2(7).

(322) Tanaka SM, Alam I, Turner CH. Stochastic resonance in osteogenic response to mechanical loading. Faseb Journal 2002 Dec;16(14):313-+.

(323) Cordo P, Inglis JT, Verschueren S, Collins JJ, Merfeld DM, Rosenblum S, et al. Noise in human muscle spindles. Nature 1996 Oct 31;383(6603):769-70.

(324) Judex S, Lei X, Han D, Rubin C. Low-magnitude mechanical signals that stimulate bone formation in the ovariectomized rat are dependent on the applied frequency but not on the strain magnitude. Journal of Biomechanics 2007;40(6):1333-9.

(325) Lau E, Al-Dujaili S, Guenther A, Liu DW, Wang LY, You LD. Effect of low-magnitude, high-frequency vibration on osteocytes in the regulation of osteoclasts. Bone 2010 Jun;46(6):1508-15.

(326) Walker SJ, Climstein M, Naughton G, Baker M, Singh MF, Torode M. A randomized controlled trial of whole body vibration exposure on markers of bone turnover in postmenopausal women. Gerontologist 2008 Oct;48:695.

(327) Christiansen BA, Silva MJ. The effect of varying magnitudes of whole-body vibration on several skeletal sites in mice. Annals of Biomedical Engineering 2006 Jul;34(7):1149-56.

(328) Xie LQ, Jacobson JM, Choi ES, Busa B, Donahue LR, Miller LM, et al. Low-level mechanical vibrations can influence bone resorption and bone formation in the growing skeleton. Bone 2006 Nov;39(5):1059-66.

(329) Bacabac RG, Smit TH, Mullender MG, Van Loon JJWA, Klein-Nulend J. Initial stress-kick is required for fluid shear stress-induced rate dependent activation of bone cells. Annals of Biomedical Engineering 2005 Jan;33(1):104-10.

(330) Slatkovska L, Alibhai SMH, Beyene J, Cheung AM. Effect of whole-body vibration on BMD: a systematic review and meta-analysis. Osteoporosis International 2010 Dec;21(12):1969-80.

(331) Bressel E, Smith G, Branscomb J. Transmission of whole body vibration in children while standing. Clinical Biomechanics 2010 Feb;25(2):181-6.

(332) Lau RWK, Liao LR, Yu FL, Teo T, Chung RCK, Pang MYC. The effects of whole body vibration therapy on bone mineral density and leg muscle strength in older adults: a systematic review and meta-analysis. Clinical Rehabilitation 2011 Nov;25(11):975-88.

(333) Mikhael M, Orr R, Singh MAF. The effect of whole body vibration exposure on muscle or bone morphology and function in older adults: A systematic review of the literature. Maturitas 2010 Jun;66(2):150-7.

(334) Reyes ML, Hernandez M, Holmgren LJ, Sanhueza E, Escobar RG. High-frequency, low-intensity vibrations increase bone mass and muscle strength in upper limbs, improving autonomy in disabled children. Journal of Bone and Mineral Research 2011 Aug;26(8):1759-66.

(335) Belavy DL, Beller G, Armbrecht G, Perschel FH, Fitzner R, Bock O, et al. Evidence for an additional effect of whole-body vibration above resistive exercise alone in preventing bone loss during prolonged bed rest. Osteoporosis International 2011 May;22(5):1581-91.

(336) Torvinen S, Kannus P, Sievanen H, Jarvinen TAH, Pasanen M, Kontulainen S, et al. Effect of 8-month vertical whole body vibration on bone, muscle performance, and body balance: A randomized controlled study. Journal of Bone and Mineral Research 2003 May;18(5):876-84.

(337) Russo CR, Lauretani F, Bandinelli S, Bartali B, Cavazzini C, Guralnik JM, et al. High-frequency vibration training increases muscle power in postmenopausal women. Archives of Physical Medicine and Rehabilitation 2003 Dec;84(12):1854-7.

(338) Beck BR, Norling TL. The effect of 8 months of twice-weekly low- or higher Intensity whole body vibration on risk factors for postmenopausal hip fracture. American Journal of Physical Medicine & Rehabilitation 2010 Dec;89(12):997-1009.

(339) Wren TAL, Lee DC, Hara R, Rethlefsen SA, Kay RM, Dorey FJ, et al. Effect of high-frequency, low-magnitude vibration on bone and muscle in children with cerebral palsy. Journal of Pediatric Orthopaedics 2010 Oct;30(7):732-8.

(340) Ruck J, Chabot G, Rauch F. Vibration treatment in cerebral palsy: A randomized controlled pilot study. Journal of Musculoskeletal & Neuronal Interactions 2010 Mar;10(1):77-83.

(341) Bemben DA, Palmer IJ, Bemben MG, Knehans AW. Effects of combined whole-body vibration and resistance training on muscular strength and bone metabolism in postmenopausal women. Bone 2010 Sep;47(3):650-6.

(342) Verschueren SMP, Bogaerts A, Delecluse C, Claessens AL, Haentjens P, Vanderschueren D, et al. The effects of whole-body vibration training and vitamin D supplementation on muscle strength, muscle mass, and bone density in institutionalized elderly women: a 6-month randomized, controlled trial. Journal of Bone and Mineral Research 2011 Jan;26(1):42-9.

(343) Turner S TMCMNGGDBMFMA. A randomized controlled trial of whole body vibration exposure on markers of bone turnover in postmenopausal women. J Osteoporos 2011;Article ID 710387, 10 pages.

(344) von Stengel S, Kemmler W, Bebenek M, Engelke K, Kalender WA. Effects of whole-body vibration training on different devices on bone mineral density. Medicine and Science in Sports and Exercise 2011 Jun;43(6):1071-9.

(345) Slatkovska L, Alibhai SMH, Beyene J, Hu HX, Demaras A, Cheung AM. Effect of 12 months of whole-body vibration therapy on bone density and structure in postmenopausal women: a randomized trial. Annals of Internal Medicine 2011 Nov 15;155(10):668-U45.

(346) Ligouri GC, Shoepe TC, Almstedt HC. Whole body vibration training is osteogenic at the spine in college-age men and women. Journal of Human Kinetics 2012 Mar;31:55-68.

(347) Cardinale M, Lim J. Electromyography activity of vastus lateralis muscle during whole-body vibrations of different frequencies. Journal of Strength and Conditioning Research 2003 Aug;17(3):621-4.

(348) Luo J, Clarke M, McNamara B, Moran K. Influence of resistance load on neuromuscular response to vibration training. Journal of Strength and Conditioning Research 2009 Mar;23(2):420-6.

(349) Moran K, McNamara B, Luo J. Effect of vibration training in maximal effort (70% 1RM) dynamic bicep curls. Medicine and Science in Sports and Exercise 2007 Mar;39(3):526-33.

(350) Zange J, Haller T, Muller K, Liphardt AM, Mester J. Energy metabolism in human calf muscle performing isometric plantar flexion superimposed by 20-Hz vibration. European Journal of Applied Physiology 2009 Jan;105(2):265-70.

(351) Cochrane DJ, Stannard SR, Sargeant AJ, Rittweger J. The rate of muscle temperature increase during acute whole-body vibration exercise. European Journal of Applied Physiology 2008 Jul;103(4):441-8.

(352) Torvinen S, Kannus P, Sievanen H, Jarvinen TAH, Pasanen M, Kontulainen S, et al. Effect of 8-month vertical whole body vibration on bone, muscle performance, and body balance: A randomized controlled study. Journal of Bone and Mineral Research 2003 May;18(5):876-84.

(353) Torvinen S, Kannus P, Sievanen H, Jarvinen TAH, Pasanen M, Kontulainen S, et al. Effect of four-month vertical whole body vibration on performance and balance. Medicine and Science in Sports and Exercise 2002 Sep;34(9):1523-8.

(354) Roelants M, Delecluse C, Verschueren SM. Whole-body-vibration training increases knee-extension strength and speed of movement in older women. Journal of the American Geriatrics Society 2004 Jun;52(6):901-8.

(355) Rietschel E, van Koningsbruggen S, Fricke O, Semler O, Schoenau E. Whole body vibration: a new therapeutic approach to improve muscle function in cystic fibrosis? International Journal of Rehabilitation Research 2008 Sep;31(3):253-6.

(356) Machado A, Garcia-Lopez D, Gonzalez-Gallego J, Garatachea N. Whole-body vibration training increases muscle strength and mass in older women: a randomized-controlled trial. Scandinavian Journal of Medicine & Science in Sports 2010 Apr;20(2):200-7.

(357) Torvinen S, Kannus P, Sievanen H, Jarvinen TAH, Pasanen M, Kontulainen S, et al. Effect of a vibration exposure on muscular performance and body balance. Randomized cross-over study. Clinical Physiology and Functional Imaging 2002 Mar;22(2):145-52.

(358) Cochrane DJ, Stannard SR. Acute whole body vibration training increases vertical jump and flexibility performance in elite female field hockey players. British Journal of Sports Medicine 2005 Nov;39(11):860-5.

(359) Luo J, McNamara B, Moran K. The use of vibration training to enhance muscle strength and power. Sports Medicine 2005;35(1):23-41.

(360) Bazett-Jones DM, Finch HW, Dugan EL. Comparing the effects of various whole-body vibration accelerations on counter-movement jump performance. Journal of Sports Science and Medicine 2008 Mar;7(1):144-50.

(361) Jordan MJ, Norris SR, Smith DJ, Herzog W. Vibration training: An overview of the area, training consequences, and future considerations. Journal of Strength and Conditioning Research 2005 May;19(2):459-66.

(362) Ward KA, Das G, Berry JL, Roberts SA, Rawer R, Adams JE, et al. Vitamin D status and muscle function in post-menarchal adolescent girls. Journal of Clinical Endocrinology & Metabolism 2009 Feb;94(2):559-63.

(363) Clarkson PM, Kearns AK, Rouzier P, Rubin R, Thompson PD. Serum creatine kinase levels and renal function measures in exertional muscle damage. Medicine and Science in Sports and Exercise 2006 Apr;38(4):623-7.

(364) Gojanovic B, Feihl F, Liaudet L, Gremion G, Waeber B. Whole body vibration training elevates creatine kinase levels in sedentary subjects. Swiss Medical Weekly 2011 Jul 7;141.

(365) Sahlin K, Harris RC, Nylind B, Hultman E. Lactate content and pH in muscle obtained after dynamic exercise. Pflugers Archiv-European Journal of Physiology 1976;367(2):143-9.

(366) Sartorio A, Lafortuna CL, Maffiuletti NA, Agosti F, Marazzi N, Rastelli F, et al. GH responses to two consecutive bouts of whole body vibration, maximal voluntary contractions or vibration alternated with maximal voluntary contractions administered at 2-h intervals in healthy adults. Growth Hormone & Igf Research 2010 Dec;20(6):416-21.

(367) Moezy A, Olyaei G, Hadian M, Razi M, Faghihzadeh S. A comparative study of whole body vibration training and conventional training on knee proprioception and postural stability after anterior cruciate ligament reconstruction. British Journal of Sports Medicine 2008 May;42(5).

(368) Bruyere O, Wuidart MA, Di Palma E, Gourlay M, Ethgen O, Richy F, et al. Controlled whole body vibration to decrease fall risk and improve health-related quality of life of nursing home residents. Archives of Physical Medicine and Rehabilitation 2005 Feb;86(2):303-7.

(369) Ebersbach G, Edler D, Kaufhold O, Wissel J. Whole body vibration versus conventional physiotherapy to improve balance and gait in Parkinson's disease. Archives of Physical Medicine and Rehabilitation 2008 Mar;89(3):399-403.

(370) Novak P, Novak V. Effect of step-synchronized vibration stimulation of soles on gait in Parkinson's disease: a pilot study. Journal of Neuroengineering and Rehabilitation 2006 May 4;3.

(371) Bogaerts A, Verschueren S, Delecluse C, Claessens AL, Boonen S. Effects of whole body vibration training on postural control in older individuals: A 1 year randomized controlled trial. Gait & Posture 2007 Jul;26(2):309-16.

(372) Wanderley FS, Alburquerque-Sendin F, Parizotto NA, Rebelatto JR. Effect of plantar vibration stimuli on the balance of older women: a randomized controlled trial. Archives of Physical Medicine and Rehabilitation 2011 Feb;92(2):199-206.

(373) Ness LL, Field-Fote EC. Whole-body vibration improves walking function in individuals with spinal cord injury: A pilot study. Gait & Posture 2009 Nov;30(4):436-40.

(374) Di Loreto C, Ranchelli A, Lucidi P, Murdolo G, Parlanti N, De Cicco A, et al. Effects of whole-body vibration exercise on the endocrine system of healthy men. Journal of Endocrinological Investigation 2004 Apr;27(4):323-7.

(375) Cardinale M, Leiper J, Erskine J, Milroy M, Bell S. The acute effects of different whole body vibration amplitudes on the endocrine system of young healthy men: a preliminary study. Clinical Physiology and Functional Imaging 2006 Nov;26(6):380-4.

(376) Cardinale M, Soiza RL, Leiper JB, Gibson A, Primrose WR. Hormonal responses to a single session of whole-body vibration exercise in older individuals. British Journal of Sports Medicine 2010 Mar;44(4):284-8.

(377) Roelants M, Delecluse C, Goris M, Verschueren S. Effects of 24 weeks of whole on body composit body vibration training ion and muscle strength in untrained females. International Journal of Sports Medicine 2004 Jan;25(1):1-5.

(378) Fjeldstad C, Palmer IJ, Bemben MG, Bemben DA. Whole-body vibration augments resistance training effects on body composition in postmenopausal women. Maturitas 2009 May 20;63(1):79-83.

(379) Erskine J, Smillie I, Leiper J, Ball D, Cardinale M. Neuromuscular and hormonal responses to a single session of whole body vibration exercise in healthy young men. Clinical Physiology and Functional Imaging 2007 Jul;27(4):242-8.

(380) Fricke O, Semler O, Land C, Beccard R, Thoma P, Schoenau E. Hormonal and metabolic responses to whole body vibration in healthy adults. Endocrinologist 2009 Jan;19(1):24-30.

(381) Rittweger J, Beller G, Felsenberg D. Acute physiological effects of exhaustive whole-body vibration exercise in man. Clinical Physiology 2000 Mar;20(2):134-42.

(382) Kerschan-Schindl K, Grampp S, Henk C, Resch H, Preisinger E, Fialka-Moser V, et al. Whole-body vibration exercise leads to alterations in muscle blood volume. Clinical Physiology 2001 May;21(3):377-82.

(383) Lohman EB, Petrofsky JS, Maloney-Hinds C, Betts-Schwab H, Thorpe D. The effect of whole body vibration on lower extremity skin blood flow in normal subjects. Medical Science Monitor 2007 Feb;13(2):CR71-CR76.

(384) Maloney-Hinds C, Petrofsky JS, Zimmerman G. The effect of 30 Hz vs. 50 Hz passive vibration and duration of vibration on skin blood flow in the arm. Medical Science Monitor 2008 Mar;14(3):CR112-CR116.

(385) Games K, Sefton J. Whole body vibration influences lower extremity circulatory and neurological function. Scandinavian Journal of Medicine & Science in Sports 2011;doi: 10.1111/j.1600-0838.2011.01419.x.

(386) Rittweger J, Mutschelknauss M, Felsenberg D. Acute changes in neuromuscular excitability after exhaustive whole body vibration exercise as compared to exhaustion by squatting exercise. Clinical Physiology and Functional Imaging 2003 Mar;23(2):81-6.

(387) Semler O, Fricke O, Vezyroglou K, Stark C, Stabrey A, Schoenau E. Improvement of individual mobility in patients with osteogenesis imperfecta by whole body vibration powered by Galileo-System. Bone 2007 Jun;40(6):S77.

(388) Hebert JR, Corboy JR, Manago MM, Schenkman M. Effects of vestibular rehabilitation on multiple sclerosis-related fatigue and upright postural control: a randomized controlled trial. Physical Therapy 2011 Aug;91(8):1166-83.

(389) Trans T, Aaboe J, Henriksen M, Christensen R, Bliddal H, Lund H. Effect of whole body vibration exercise on muscle strength and proprioception in females with knee osteoarthritis. Knee 2009 Aug;16(4):256-61.

(390) Rittweger J, Just K, Kautzsch K, Reeg P, Felsenberg D. Treatment of chronic lower back pain with lumbar extension and whole-body vibration exercise - A randomized controlled trial. Spine 2002 Sep 1;27(17):1829-34.

(391) Alentorn-Geli E, Padilla J, Moras G, Haro CL, Fernandez-Sola J. Six weeks of whole-body vibration exercise improves pain and fatigue in women with fibromyalgia. Journal of Alternative and Complementary Medicine 2008 Oct;14(8):975-81.

(392) Baum KVTSJ. Efficiency of vibration exercise for glycemic control in type 2 diabetes patients. Int J Med Sci 2007;4(3):159-63.

(393) Gloeckl R, Heinzelmann I, Baeuerle S, Damm E, Schwedhelm AL, Diril M, et al. Effects of whole body vibration in patients with chronic obstructive pulmonary disease - A randomized controlled trial. Respiratory Medicine 2012 Jan;106(1):75-83.

(394) Marin PJ, Zarzuela R, Zarzosa F, Herrero AJ, Garatachea N, Rhea MR, et al. Whole-body vibration as a method of recovery for soccer players. European Journal of Sport Science 2012;12(1):2-8.

(395) Verschueren SMP, Roelants M, Delecluse C, Swinnen S, Vanderschueren D, Boonen S. Effect of 6-month whole body vibration training on hip density, muscle strength, and postural control in postmenopausal women: A randomized controlled pilot study. Journal of Bone and Mineral Research 2004 Mar;19(3):352-9.

(396) Gordon CM, Bachrach LK, Carpenter TO, Crabtree N, Fuleihan GEH, Kutilek S, et al. Dual energy X-ray absorptiometry interpretation and reporting in children and adolescents: The 2007 ISCD Pediatric Official Positions. Journal of Clinical Densitometry 2008 Jan;11(1):43-58.

(397) Fewtrell MS. Bone densitometry in children assessed by dual x ray absorptiometry: uses and pitfalls. Archives of Disease in Childhood 2003 Sep 1;88(9):795-8.

(398) Binkovitz LA, Henwood MJ. Pediatric DXA: technique and interpretation. Pediatric Radiology 2007 Jan;37(1):21-31.

(399) Baim S, Binkley N, Bilezikian JR, Kendler DL, Hans DB, Lewiecki EM, et al. Official positions of the International Society for Clinical Densitometry and executive summary of the 2007 ISCD Position Development Conference. Journal of Clinical Densitometry 2008 Jan;11(1):75-91.

(400) Binkley TL, Berry R, Specker BL. Methods for measurement of pediatric bone. Reviews in Endocrine & Metabolic Disorders 2008 Jun;9(2):95-106.

(401) Kalkwarf HJ, Zemel BS, Gilsanz V, Lappe JM, Horlick M, Oberfield S, et al. The bone mineral density in childhood study: Bone mineral content and density according to age, sex, and race. Journal of Clinical Endocrinology & Metabolism 2007 Jun;92(6):2087-99.

(402) Ahmed SF, Horrocks IA, Patterson T, Zaidi S, Ling TC, McGrogan T, et al. Bone mineral assessment by dual energy X-ray Absorptiometry in children with inflammatory bowel disease:

Evaluation by age or bone area. Journal of Pediatric Gastroenterology and Nutrition 2004 Mar;38(3):276-80.

(403) Carter DR, Bouxsein ML, Marcus R. New approaches for interpreting projected bone densitometry data. Journal of Bone and Mineral Research 1992 Feb;7(2):137-45.

(404) Zemel BS, Leonard MB, Kelly A, Lappe JM, Gilsanz V, Oberfield S, et al. Height adjustment in assessing dual energy x-ray absorptiometry measurements of bone mass and density in children. Journal of Clinical Endocrinology & Metabolism 2010 Mar;95(3):1265-73.

(405) Wong SC, Khanna S, Rashid R, Ahmed SF. Auditing bone densitometry and fractures in children with chronic disease. Archives of Disease in Childhood 2008 Aug;93(8):705-7.

(406) Eastell R, Reid DM, Compston J, Cooper C, Fogelman I, Francis RM, et al. Secondary prevention of osteoporosis: when should a non-vertebral fracture be a trigger for action? Qjm-Monthly Journal of the Association of Physicians 2001 Nov;94(11):575-97.

(407) Schneider P, Borner W. Peripheral quantitative computed tomography for bone mineral measurement using a new special QCT-scanner. Methodology, normal values, comparison with manifest osteoporosis. Fortschritte Auf dem Gebiete der Rontgenstrahlen und der Neuen Bildgebenden Verfahren 1991 Mar;154(3):292-9.

(408) Moyer-Mileur LJ, Quick JL, Murray MA. Peripheral quantitative computed tomography of the tibia: Pediatric reference values. Journal of Clinical Densitometry 2008 Apr;11(2):283-94.

(409) Bennell KL, Creaby MW, Wrigley TV, Hunter DJ. Tibial subchondral trabecular volumetric bone density in medial knee joint osteoarthritis using peripheral quantitative computed tomography technology. Arthritis and Rheumatism 2008 Sep;58(9):2776-85.

(410) Lee HD, Hwang HF, Lin MR. Use of quntitative ultrasound for identifying low bone density in older people. Journal of Ultrasound in Medicine 2010 Jul;29(7):1083-92.

(411) Gluer CC, Wu CY, Jergas M, Goldstein SA, Genant HK. 3 Quantitative ultrasound parameters reflect bone structure. Calcified Tissue International 1994 Jul;55(1):46-52.

(412) Zadik Z, Price D, Diamond G. Pediatric reference curves for multi-site quantitative ultrasound and its modulators. Osteoporosis International 2003 Oct;14(10):857-62.

(413) McDevitt H, Ahmed SF. Quantitative ultrasound assessment of bone health in the neonate. Neonatology 2007;91(1):2-11.

(414) Rawal JEKSJPZLMPJDFWMHFHSMH. Relationship between calcaneal quantitative ultrasound and hip dual energy X-ray absorptiometry in young healthy men. Osteoporos Int DOI 10.1007/s00198-011-1853-1. 2012.
Ref Type: Journal (Full)

(415) Lee DC, Gilsanz V, Wren TAL. Limitations of peripheral quantitative computed tomography metaphyseal bone density measurements
LEE2007. Journal of Clinical Endocrinology & Metabolism 2007 Nov;92(11):4248-53.

(416) Veilleux LN, Rauch F. Reproducibility of jumping mechanography in healthy children and adults. Journal of Musculoskeletal & Neuronal Interactions 2010 Dec;10(4):256-66.

(417) Fricke O, Weidler J, Tutlewski B, Schoenau E. Mechanography - A new device for the assesment of muscle function in pediatrics. Pediatric Research 2006 Jan;59(1):46-9.

(418) Item F, Denkinger J, Fontana P, Weber M, Boutellier U, Toigo M. Combined effects of whole-body vibration, resistance exercise, and vascular occlusion on skeletal muscle and performance. International Journal of Sports Medicine 2011 Oct;32(10):781-7.

(419) Rittweger J, Felsenberg D, Maganaris C, Ferretti JL. Vertical jump performance after 90 days bed rest with and without flywheel resistive exercise, including a 180 days follow-up. European Journal of Applied Physiology 2007 Jul;100(4):427-36.

(420) Runge M. Multifactorial pathogenesis of falls and fractures and fall risk assessment. Osteoporosis International 2006 Mar;17:S121-S122.

(421) Veilleux LN, Robert M, Ballaz L, Lemay M, Rauch F. Gait analysis using a force-measuring gangway: Intrasession repeatability in healthy adults. Journal of Musculoskeletal & Neuronal Interactions 2011 Mar;11(1):27-33.

(422) Runge M, Rittweger J, Russo CR, Schiessl H, Felsenberg D. Is muscle power output a key factor in the age-related decline in physical performance? A comparison of muscle cross section, chair-rising test and jumping power. Clinical Physiology and Functional Imaging 2004 Nov;24(6):335-40.

(423) Li K, Hewson DJ, Duchene J, Hogrel JY. Predicting maximal grip strength using hand circumference. Manual Therapy 2010 Dec;15(6):579-85.

(424) Reis MMAPMM. Assessment of hand grip strength: validity and reliability of the saehan dynamometer. Fisioter Pesq 2011;18(2):176-81.

(425) Trutschnigg B, Kilgour RD, Reinglas J, Rosenthall L, Hornby L, Morais JA, et al. Precision and reliability of strength (Jamar vs. Biodex handgrip) and body composition (dual-energy X-ray absorptiometry vs. bioimpedance analysis) measurements in advanced cancer patients. Applied Physiology Nutrition and Metabolism-Physiologie Appliquee Nutrition et Metabolisme 2008 Dec;33(6):1232-9.

(426) Molenaar HM, Zuidam JM, Selles RW, Stam HJ, Hovius SER. Age-specific reliability of two grip-strength dynamometers when used by children. Journal of Bone and Joint Surgery-American Volume 2008 May;90A(5):1053-9.

(427) Budziareck MB, Duarte RRP, Barbosa-Silva MCG. Reference values and determinants for handgrip strength in healthy subjects. Clinical Nutrition 2008 Jun;27(3):357-62.

(428) Karkkainen M, Rikkonen T, Kroger H, Sirola J, Tuppurainen M, Salovaara K, et al. Physical tests for patient selection for bone mineral density measurements in postmenopausal women. Bone 2009 Apr;44(4):660-5.

(429) Di Monaco M, Di Monaco R, Manca M, Cavanna A. Handgrip strength is an independent predictor of distal radius bone mineral density in postmenopausal women. Clinical Rheumatology 2000;19(6):473-6.

(430) Ali NA, O'Brien JM, Hoffmann SP, Phillips G, Garland A, Finley JCW, et al. Acquired weakness, handgrip strength, and mortality in critically ill patients. American Journal of Respiratory and Critical Care Medicine 2008 Aug 1;178(3):261-8.

(431) Gunther CM, Burger A, Rickert M, Crispin A, Schulz CU. Grip strength in healthy Caucasian adults, reference values. Journal of Hand Surgery-American Volume 2008 Apr;33A(4):558-65.

(432) Luna-Heredia E, Martin-Pena G, Ruiz-Galiana J. Handgrip dynamometry in healthy adults. Clinical Nutrition 2005 Apr;24(2):250-8.

(433) Rauch F, Neu CM, Wassmer G, Beck B, Rieger-Wettengl G, Rietschel E, et al. Muscle analysis by measurement of maximal isometric grip force: New reference data and clinical applications in pediatrics. Pediatric Research 2002 Apr;51(4):505-10.

(434) Freeman JV, Cole TJ, Chinn S, Jones PRM, White EM, Preece MA. Cross sectional stature and weight reference curves for the Uk 1990. Archives of Disease in Childhood 1995 Jul;73(1):17-24.

(435) Cole TJ, Freeman JV, Preece MA. Body mass index reference curves for the Uk, 1990. Archives of Disease in Childhood 1995 Jul;73(1):25-9.

(436) Cole TJ. Do growth chart centiles need a face lift. British Medical Journal 1994 Mar 5;308(6929):641-2.

(437) Zemel BS, Riley EM, Stallings VA. Evaluation of methodology for nutritional assessment in children: Anthropometry, body composition, and energy expenditure. Annual Review of Nutrition 1997;17:211-35.

(438) Eknoyan G. Adolphe Quetelet (1796-1874) - the average man and indices of obesity. Nephrology Dialysis Transplantation 2008 Jan;23(1):47-51.

(439) Daniels SR. The use of BMI in the clinical setting. Pediatrics 2009 Sep;124:S35-S41.

(440) Chumlea WC, Guo SS. Bioelectrical-impedance and body-composition - present status and future-directions. Nutrition Reviews 1994 Apr;52(4):123-31.

(441) Lukaski HC, Johnson PE, Bolonchuk WW, Lykken GI. Assessment of fat-free mass using bioelectrical impedance measurements of the human-body. American Journal of Clinical Nutrition 1985;41(4):810-7.

(442) Goldfield GS, Cloutier P, Mallory R, Prud'homme D, Parker T, Doucet E. Validity of foot-to-foot bioelectrical impedance analysis in overweight and obese children and parents. Journal of Sports Medicine and Physical Fitness 2006 Sep;46(3):447-53.

(443) Lazzer S, Boirie Y, Meyer M, Vermorel M. Evaluation of two foot-to-foot bioelectrical impedance analysers to assess body composition in overweight and obese adolescents. British Journal of Nutrition 2003 Nov;90(5):987-92.

(444) Radley DCCBFNJOBTJGCWAWAGPJ. Validity of foot-to-foot bio-electrical impedance analysis body composition estimates in overweight and obese children. Int J Body Compos Res 2010;7(1):15-20.

(445) Cleary J, Daniells S, Okely AD, Batterham M, Nicholls J. Predictive validity of four bioelectrical impedance equations in determining percent fat mass in overweight and obese children. Journal of the American Dietetic Association 2008 Jan;108(1):136-9.

(446) Wells JCK, Haroun D, Williams JE, Wilson C, Darch T, Viner RM, et al. Evaluation of DXA against the four-component model of body composition in obese children and adolescents aged 5-21 years. International Journal of Obesity 2010 Apr;34(4):649-55.

(447) Breithaupt P, Colley RC, Adamo KB. Body composition measured by dual-energy X-ray absorptiometry half-body scans in obese children. Acta Paediatrica 2011 Dec;100(12):E260-E266.

(448) Brenner H, Kaatsch P, Burkhardt-Hammer T, Harms DO, Schrappe M, Michaelis J. Long-term survival of children with leukemia achieved by the end of the second millennium. Cancer 2001 Oct 1;92(7):1977-83.

(449) Sala A, Barr RD. Osteopenia and cancer in children and adolescents - The fragility of success. Cancer 2007 Apr 1;109(7):1420-31.

(450) Mushtaq T, Ahmed SF. The impact of corticosteroids on growth and bone health. Archives of Disease in Childhood 2002 Aug;87(2):93-6.

(451) Mitchell CD, Richards SM, Kinsey SE, Lilleyman J, Vora A, Eden TOB. Benefit of dexamethasone compared with prednisolone for childhood acute lymphoblastic leukaemia: results of the UK Medical Research Council ALL97 randomized trial. British Journal of Haematology 2005 Jun;129(6):734-45.

(452) Ward LM. Osteoporosis due to glucocorticoid use in children with chronic illness. Hormone Research 2005;64(5):209-21.

(453) Junkins EP, Stotts A, Santiago R, Guenther E. The clinical presentation of pediatric thoracolumbar fractures: A prospective study. Journal of Trauma-Injury Infection and Critical Care 2008 Nov;65(5):1066-71.

(454) Wallace AM, Tucker P, Williams DM, Hughes IA, Ahmed SF. Short-term effects of prednisolone and dexamethasone on circulating concentrations of leptin and sex hormone-binding globulin in children being treated for acute lymphoblastic leukaemia. Clinical Endocrinology 2003 Jun;58(6):770-6.

(455) Mattano LA, Sather HN, Trigg ME, Nachman JB. Osteonecrosis as a complication of treating acute lymphoblastic leukemia in children: A report from the children's cancer group. Journal of Clinical Oncology 2000 Sep 15;18(18):3262-72.

(456) Kawai K, Tamaki A, Hirohata K. Steroid-induced accumulation of lipid in the osteocytes of the rabbit femoral head. A histochemical and electron microscopic study. Journal of Bone and Joint Surgery-American Volume 1985;67A(5):755-63.

(457) Nishida K, Yamamoto T, Motomura G, Jingushi S, Iwamoto Y. Pitavastatin may reduce risk of steroid-induced osteonecrosis in rabbits: A preliminary histological study. Clinical Orthopaedics and Related Research 2008 May;466(5):1054-8.

(458) Hanada T, Horigome Y, Inudoh M, Takita H. Osteonecrosis of vertebrae in a child with acute lymphocytic leukaemia during L-asparaginase therapy. European Journal of Pediatrics 1989 Dec;149(3):162-3.

(459) Kelly HW, Van Natta ML, Covar RA, Tonascia J, Green RP, Strunk RC. Effect of long-term corticosteroid use on bone mineral density in children: A prospective longitudinal assessment in the Childhood Asthma Management Program (CAMP) study. Pediatrics 2008 Jul;122(1):E53-E61.

(460) Elmantaser M, Stewart G, Young D, Duncan R GBASF. Skeletal morbidity in children receiving chemotherapy for acute lymphoblastic leukaemia. Archives of Disease in Childhood 2010;95(10):805-9.

(461) Arikoski P, Komulainen J, Riikonen P, Parviainen M, Jurvelin JS, Voutilainen R, et al. Impaired development of bone mineral density during chemotherapy - A prospective analysis of 46 newly diagnosed children with cancer. Osteoporosis International 2000;11:S16.

(462) Rude RK, Gruber HE, Norton HJ, Wei LY, Frausto A, Kilburn J. Dietary magnesium reduction to 25% of nutrient requirement disrupts bone and mineral metabolism in the rat. Bone 2005 Aug;37(2):211-9.

(463) Rude RK, Gruber HE, Norton HJ, Wei LY, Frausto A, Kilburn J. Reduction of dietary magnesium by only 50% in the rat disrupts bone and mineral metabolism. Osteoporosis International 2006 Jul;17(7):1022-32.

(464) Vora A, Mitchell C, Hann I. UKALL2003. 2003.
Ref Type: Internet Communication

(465) Wei MX, Esbaei K, Bargman JM, Oreopoulos DG. Inverse correlation between serum magnesium and parathyroid hormone in peritoneal dialysis patients: a contributing factor to adynamic bone disease? International Urology and Nephrology 2006;38(2):317-22.

(466) Saito N, Tabata N, Saito S, Andou Y, Onaga Y, Iwamitsu A, et al. Bone mineral density, serum albumin and serum magnesium. Journal of the American College of Nutrition 2004 Dec;23(6):701S-3S.

(467) Fatemi S, Ryzen E, Flores J, Endres DB, Rude RK. Effect of experimental human magnesium depletion on parathyroid hormone secretion and 1,25-dihydroxyvitamin D metabolism. Journal of Clinical Endocrinology & Metabolism 1991 Nov;73(5):1067-72.

(468) Rude RK, Olerich M. Magnesium deficiency: Possible role in osteoporosis associated with gluten-sensitive enteropathy. Osteoporosis International 1996;6(6):453-61.

(469) Janning C, Willbold E, Vogt C, Nellesen J, Meyer-Lindenberg A, Windhagen H, et al. Magnesium hydroxide temporarily enhancing osteoblast activity and decreasing the osteoclast number in peri-implant bone remodelling. Acta Biomaterialia 2010 May;6(5):1861-8.

(470) Sgambato A, Wolf FI, Faraglia B, Cittadini A. Magnesium depletion causes growth inhibition, reduced expression of cyclin D1, and increased expression of p27(Kip1) in normal but not in transformed mammary epithelial cells. Journal of Cellular Physiology 1999 Aug;180(2):245-54.

(471) Wu B, Atkinson SA, Halton JM, Barr RD. Hypermagnesiuria and hypercalciuria in childhood leukemia: An effect of amikacin therapy. Journal of Pediatric Hematology Oncology 1996 Feb;18(1):86-9.

(472) Hoshino K, Ogawa K, Kitazawa R, Nakamura Y, Uehara R. Ionized magnesium level in whole blood of healthy Japanese children. Acta Paediatrica Japonica 1998 Apr;40(2):116-21.

(473) Munoz R, Laussen PC, Palacio G, Zienko L, Piercey G, Wessel DL. Whole blood ionized magnesium: Age-related differences in normal values and clinical implications of ionized hypomagnesemia in patients undergoing surgery for congenital cardiac disease. Journal of Thoracic and Cardiovascular Surgery 2000 May;119(5):891-8.

(474) Sojka JE, Weaver CM. Magnesium supplementation and osteoporosis. Nutrition Reviews 1995 Mar;53(3):71-4.

(475) Haddy TB, Mosher RB, Reaman GH. Osteoporosis in survivors of acute lymphoblastic leukemia. Oncologist 2001;6(3):278-85.

(476) Delecluse C, Roelants M, Verschueren S. Strength increase after whole-body vibration compared with resistance training. Medicine and Science in Sports and Exercise 2003 Jun;35(6):1033-41.

(477) Torvinen S, Sievanen H, Jarvinen TAH, Pasanen M, Kontulainen S, Kannus P. Effect of 4-min vertical whole body vibration on muscle performance and body balance: A randomized cross-over study. International Journal of Sports Medicine 2002 Jul;23(5):374-9.

(478) Muir J, Judex S, Qin Y, Rubin CT. Safety of whole body vibration, considered for the prevention and/or treatment of osteoporosis, relative to standards set by the international safety organization. Journal of Bone and Mineral Research 2006 Sep;21:S294.

(479) Colson SS, Pensini M, Espinosa J, Garrandes F, Legros P. Whole-body vibration training effects on the physical performance of basketball players. Journal of Strength and Conditioning Research 2010 Apr;24(4):999-1006.

(480) Lamont HS, Cramer JT, Bemben DA, Shehab RL, Anderson MA, Bemben MG. Effects of 6 weeks of periodized squat training with or without whole-body vibration on short-term adaptations in jump performance within recreationally resistance trained men. Journal of Strength and Conditioning Research 2008 Nov;22(6):1882-93.

(481) Fernandez-Rio J, Terrados N, Fernandez-Garcia B, Suman OE. Effects of vibration training on force production in female basketball players. Journal of Strength and Conditioning Research 2010 May;24(5):1373-80.

(482) Stokes K. Growth hormone responses to sub-maximal and sprint exercise. Growth Hormone & Igf Research 2003 Oct;13(5):225-38.

(483) Davies CTM, Few JD. Effects of exercise on adrenocortical function. Journal of Applied Physiology 1973;35(6):887-91.

(484) Hill EE, Zack E, Battaglini C, Viru M, Viru A, Hackney AC. Exercise and circulating cortisol levels: The intensity threshold effect. Journal of Endocrinological Investigation 2008 Jul;31(7):587-91.

(485) Kanter MM, Lesmes GR, Kaminsky LA, Lahamsaeger J, Nequin ND. Serum creatine kinase and lactate dehydrogenase changes following an eighty kilometer race. Relationship to lipid peroxidation. European Journal of Applied Physiology and Occupational Physiology 1988 Jan;57(1):60-3.

(486) Stewart PM, Toogood AA, Tomlinson JW. Growth hormone, insulin-like growth factor-1 and the cortisol-cortisone shuttle. Hormone Research 2001;56:1-6.

(487) Dovio A, Roveda E, Sciolla C, Montaruli A, Raffaelli A, Saba A, et al. Intense physical exercise increases systemic 11 beta-hydroxysteroid dehydrogenase type 1 activity in healthy adult subjects. European Journal of Applied Physiology 2010 Mar;108(4):681-7.

(488) Wenger KH, Freeman JD, Fulzele S, Immel DM, Powell BD, Molitor P, et al. Effect of whole-body vibration on bone properties in aging mice. Bone 2010 Oct;47(4):746-55.

(489) Kroger H, Vainio P, Nieminen J, Kotaniemi A. Comparison of different models for interpreting bone mineral density measurements using DXA and MRI technology. Bone 1995 Aug;17(2):157-9.

(490) Ahmed S, Elmantaser M. Secondary osteoporosis. Endocrine Development 2009;16:170-90.

(491) Janz KF, Gilmore JM, Burns TL, Levy SM, Torner JC, Willing MC, et al. Physical activity augments bone mineral accrual in young children: The Iowa bone development study. Journal of Pediatrics 2006 Jun;148(6):793-9.

(492) Elmantaser ME, Young D, Gibson B, Ahmed SF. Skeletal morbidity in children receiving chemotherapy for acute lymphoblastic leukemia and its association with mineral homeostasis and duration of inpatient stay. Journal of Pediatric Hematology Oncology 2011 Oct;33(7):516-20.

(493) Kawanabe K, Kawashima A, Sashimoto I, Takeda T, Sato Y, Iwamoto J. Effect of whole-body vibration exercise and muscle strengthening, balance, and walking exercises on walking ability in the elderly. The Keio Journal of Medicine 2007;56(1):28-33.

(494) Eisman JA. Good, good, good ... good vibrations: the best option for better bones? Lancet 2001 Dec 8;358(9297):1924-5.

(495) Slatkovska L, Alibhai SMH, Beyene J, Cheung AM. The efficacy of whole-body vibration in reducing bone loss in postmenopausal women: A meta-analysis. Journal of Bone and Mineral Research 2008 Sep;23:S473.

(496) Kavanaugh AA, South MA, Hamdy RC, Stone ME, Stone MH, Ramsey MW. The effect of 4 months whole body vibration of on bone mineral density of division I cross country/distance runners. Journal of Strength & Conditioning Research 2011;doi: 10.1097/01.JSC.0000395766.35826.ee.

(497) von Stengel S, Kemmler W, Mayer S, Engelke K, Klarner A, Kalender WA. Effect of whole body vibration exercise on osteoporotic risk factors. Results of the controlled randomized longitudinal ELVIS study after one year. Deutsche Medizinische Wochenschrift 2009 Jul 24;134(30):1511-6.

(498) Maddalozzo GF, Iwaniec UT, Turner RT, Rosen CJ, Widrick JJ. Whole-body vibration slows the acquisition of fat in mature female rats. International Journal of Obesity 2008 Sep;32(9):1348-54.

(499) Rubin CT, Capilla E, Luu YK, Busa B, Crawford H, Nolan DJ, et al. Adipogenesis is inhibited by brief, daily exposure to high-frequency, extremely low-magnitude mechanical signals. Proceedings of the National Academy of Sciences of the United States of America 2007 Nov 6;104(45):17879-84.

(500) Chauvenet AR, Shashi V, Selsky C, Morgan E, Kurtzberg J, Bell B. Vincristine-induced neuropathy as the initial presentation of charcot-Marie-tooth disease in acute lymphoblastic leukemia: A pediatric oncology group study. Journal of Pediatric Hematology Oncology 2003 Apr;25(4):316-20.

(501) Gomber S, Dewan P, Chhonker D. Vincristine induced neurotoxicity in cancer patients. Indian Journal of Pediatrics 2010 Jan;77(1):97-100.

(502) Elliot KJ, Millward-Sadler SJ, Wright MO, Robb JE, Wallace WHB, Salter DM. Effects of methotrexate on human bone cell responses to mechanical stimulation. Rheumatology 2004 Oct;43(10):1226-31.

(503) Rubin C, Turner AS, Mallinckrodt C, Jerome C, McLeod K, Bain S. Mechanical strain, induced noninvasively in the high-frequency domain, is anabolic to cancellous bone, but not cortical bone. Bone 2002 Mar;30(3):445-52.

(504) Elmantaser M , McMillan M, Smith K, Khanna S, Chantler D, Panarelli M , et al. A Comparison Of The Effect Of Two Types Of Vibration Exercise On The Endocrine And Musculoskeletal System. JMNI 2012.

(505) Alos N, Grant RM, Ramsay T, Halton J, Cummings EA, Miettunen PM, et al. High Incidence of Vertebral Fractures in Children With Acute Lymphoblastic Leukemia 12 Months After the Initiation of Therapy. Journal of Clinical Oncology 2012 Aug 1;30(22):2760-7.

(506) Rodriguez-Moran M, Guerrero-Romero F. Oral magnesium supplementation improves insulin sensitivity and metabolic control in type 2 diabetic subjects - A randomized double-blind controlled trial. Diabetes Care 2003 Apr;26(4):1147-52.

(507) Dimai HP, Porta S, Wirnsberger G, Lindschinger M, Pamperl I, Dobnig H, et al. Daily oral magnesium supplementation suppresses bone turnover in young adult males. Journal of Clinical Endocrinology & Metabolism 1998 Aug;83(8):2742-8.

i