The effect of HIV and its treatment on trabecular and cortical bone architecture in children, adolescents and premenopausal women

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I Cynthia Kahari, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

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Abstract

Introduction: Understanding factors influencing bone accrual in childhood and bone loss in early adulthood may inform approaches to improve skeletal health and reduce fracture risk in adulthood. This thesis aimed to determine the effect of chronic HIV infection on bone architecture, including bone density, bone size, and bone strength in children, adolescents and premenopausal women.

Methods: I designed the peripheral quantitative computed tomography (pQCT) protocols, performed pQCT scans, trained a team of pQCT radiographers and worked with them to collect pQCT data in Zimbabwean children and adolescents living with HIV (CWH) and children and adolescents living without HIV (CWOH). Participants included 8 – 16 year old children from Harare, recruited from clinics at Parirenyatwa and Sally Mugabe hospitals and schools within the same catchment area. In a second previously conducted study premenopausal women living with HIV (WLWH) and without (WLWOH), aged 18 years or older, had been recruited from clinics in Soweto, Johannesburg. In both studies questionnaires captured clinical and demographic information. Outcomes were tibial pQCT measured 4% trabecular and 38% cortical volumetric bone mineral density (vBMD), 4% and 38% cross-sectional area (CSA), and stress-strain index (SSI) in both children and women. Additional outcomes included radial pQCT measured 4% trabecular and 66% cortical vBMD, 4% and 66% CSA and SSI in women only. I graded each of the pQCT scan slices for image quality. I compared differences in means by HIV status in both children and women. In children, I used linear regression, incorporating an interaction term for pubertal status to test for differences in mean and in change (Δ) in bone outcomes over one year. I further used structural equation modelling (SEM) to evaluate whether impaired height mediates the effect of HIV on Δ bone outcomes per year. In women, I used piecewise regression analysis to estimate trajectories of Δ bone outcomes between women with and without HIV, exploring the change in trajectory upon starting anti-retroviral therapy (ART).

Results: In total, 247 (149 (60%)) WLWH and 609 children (303 CWH; 50% female) were included in these studies. More CWH were pre-pubertal (i.e. Tanner 1; 41% vs. 23%) than CWOH, yet they were similar in age. Median age at ART initiation was 4 (IQR 2–7) years, whilst median ART duration was 8 (IQR 6–10) years. Male and female CWH in later puberty had lower trabecular vBMD, CSA (at 4% and 38%) and SSI than those without HIV at baseline and also at follow-up, whilst cortical density was similar. Puberty modified the effect of HIV on pQCT bone outcomes. Height mediated the effect of HIV on Δbone outcomes in female CWH but not in males. WLWH had lower vBMD than women living without HIV at

both the radial and tibial trabecular rich sites but not at the cortical sites. The longitudinal analysis showed that WLWH who were initiated on ART lost total vBMD at the distal tibia but gained total vBMD at the distal radius, over the 24 months, suggesting that South African women who are in their thirties may still be gaining bone at the radius while bone accrual at the tibia is complete. Furthermore, the skeletal response to HIV infection and ART initiation may vary depending on bone compartment (i.e., trabecular or cortical bone) and skeletal site (i.e. weight-bearing tibia or non-weight-bearing radius). This compartment and site-specificity may suggest that declines in bone in WLWH is more likely due to ART initiation than due to HIV infection.

Conclusions: Despite long-term ART, I identified deficits in bone density, size and predicted strength in children, adolescents and women living with HIV in Zimbabwe and South Africa. These findings are concerning as this may translate into higher fracture risk later in life. With the growing numbers people on ART, Southern Africa might be expected to see an increase in fracture incidence and it is a question of concern, whether the healthcare services are ill prepared for that.

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I dedicate this thesis to my sons (David, Joseph and Daniel), just so you know that it is possible and to the little girl or boy child growing up in Gokwe, just so you know that your dreams are valid.

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Acronyms

aBMD	Areal bone mineral density
ART	Antiretroviral therapy
BMC	Bone mineral content
BMD	Bone mineral density
BMAD	Bone mineral apparent density
BMR	Bone to muscle ratio
BMU	Basic multicellular unit
BRC	Bone remodeling compartment
BRTI	Biomedical Research and Training Institute
BSIc	Bone strength index of compression
CI	Confidence interval
CRF	Case report form
CSA	Cross-sectional area
CSMI	Cross-sectional moment of inertia
CT	Computed Tomography
CV	Coefficient of variation
CWH	Children living with HIV
CWOH	Children living without HIV
CHEU	Children exposed to HIV but uninfected
DMPA	Depot-medroxyprogesterone acetate
DPHRU	Developmental Pathways for Health Research Unit
DXA	Dual energy X-ray absorptiometry
FMR	Fat to muscle ratio
FN	Femur neck
G-CSF	Granulocyte-macrophage colony-stimulating factor
GH	Growth hormone
HCH	Harare Central Hospital
HCV	Hepatitis C virus
HIC	High income country
HIV	Human immunodeficiency virus
HRpQCT	High resolution peripheral quantitative computed tomography
IFN	Interferon alpha
IL	Interleukin
IMVASK	The IMpact of Vertical HIV infection on child and Adolescent Skeletal development.
IRB	Institutional review board
LS	Lumbar spine
LSHTM	London School of Hygiene and Tropical Medicine
M-CSF	Macrophage-colony stimulating factor
MLWH	Men living with HIV
MRC	Medical Research Council (UK)
MRCZ	Medical Research Council of Zimbabwe
OPG	Osteoprotegerin
OPGL	Osteoprotegerin ligand
PBM	Peak bone mass
PGH	Parirenyatwa Group of Hospitals
PI	Principal Investigator
PLWH	People living with HIV

PMI	Polar moments of inertia
pQCT	Peripheral quantitative computed tomography
PTH	Parathyroid hormone
QA	Quality Assurance
QC	Quality Control
QCT	Quantitative computed tomography
RANK	Receptor activator of NFkB
RANKL	Receptor activator of NFkB Ligand
RMS	Root mean square
SD	Standard deviation
SE	Standard error
SM	Section modulus
SSA	Sub-Saharan Africa
SSI	Strength strain index
TB	Total body
TBLH	Total body less head
TDF	Tenofovir disoproxil fumarate
TH	Total hip
TNF	Tumour necrosis factor
UNAIDS	United Nations Programme on HIV/AIDS
UOB	University of Bristol
UZ	University of Zimbabwe
vBMD	Volumetric bone mineral density
WBS	Women's Bone Health Study
WLWH	Women living with HIV

PhD outputs
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Oral Presentations and Awards

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Peer Reviewed Publications

1 **Cynthia Mukwasi-Kahari**, Andrea M. Rehman, Mícheál Ó Breasail, Ruramayi Rukuni, Tafadzwa Madanhire, Joseph Chipanga, Lynda Stranix-Chibanda, Lisa Micklesfield, Rashida A. Ferrand, Kate A. Ward, Celia L. Gregson. Impaired bone architecture in peripubertal children with HIV, despite treatment with anti-retroviral therapy: a cross-sectional study from Zimbabwe. Journal of Bone and Mineral Research, 2023 Feb; 38 (2):248-260. doi:0.1002/jbmr.4752. Epub 2022 Dec 10, PMID: 36426511

2. Gregson CL, Rehman AM, Rukuni R, **Mukwasi-Kahari C**, Madanhire T, Kowo-Nyakoko F, et al. Perinatal HIV infection is associated with deficits in muscle function in children and adolescents: a cross-sectional study in Zimbabwe. Aids. 2023;2023.

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1 Chapter 1: Bone anatomy biology and introduction to the study setting

1.1 Background

The global decline in Human immunodeficiency virus (HIV)-associated child and adult deaths due to improved access to ART has enabled children living with HIV (CWH) to grow into adulthood and people living with HIV (PLWH) to live longer (1). As PLWH live longer, the importance of skeletal aging-related co-morbidities, such as osteoporosis and fractures, has increased. These are a rising cause for concern for PLWH of all ages because bone health in later life is dependent upon both bone accumulation throughout childhood and bone loss during adulthood. In children, HIV is associated with stunting (height-for-age Z-score <-2) and underweight (weight-for-age Z-score <-2), with the prevalence of stunting varying from 23% to 73% (2–4). Through adolescence, linear growth continues as bone accumulates to reach peak bone mass (PBM), which is a crucial factor in determining the future risk of fracture in adults (5,6). People who attain a low PBM are at an increased risk of fracture (7). Adult fracture risk can double with a 10% decrease in PBM accretion (8,9). In adults, the long-term impact of exposure to both HIV infection and antiretroviral therapy (ART) is of concern, as impaired bone accrual during childhood and increased bone loss in adulthood both have implications for adult fracture risk (10). Architectural properties of bone such as the shape, the size and the density of bone, as well as the differentiation into trabecular (spongy) and cortical (compact) bone, together confer the strength of bone and determine fracture risk (10). Studies assessing bone mineral density (BMD), bone size and bone strength are needed to increase our understanding of bone health in PLWH. This chapter provides a background description of bone biology and introduces the thesis setting, rationale and objectives.

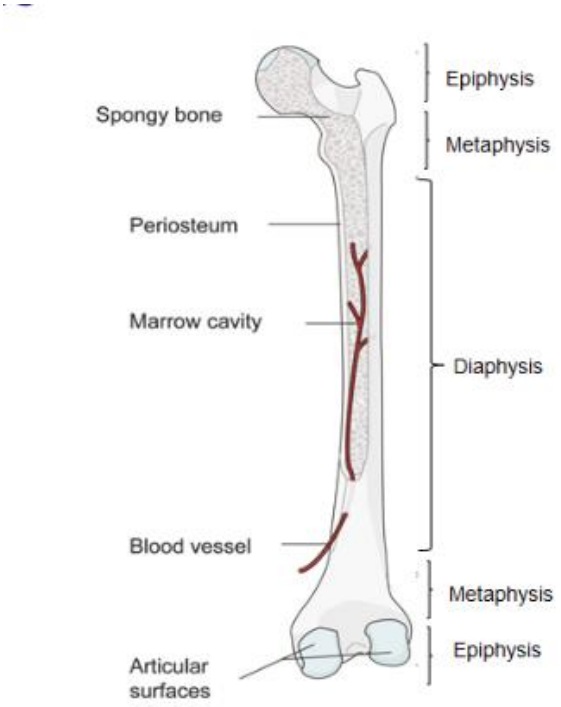
1.2 The anatomy of bone

1.2.1 Overview of the human skeleton

The human skeleton provides a structural framework for movement, attachment sites for skeletal muscle (via tendons) and protection for the brain, spinal cord and internal organs (11). The skeleton also has a role in regulating calcium and phosphorus, supporting haematopoiesis, defending against acidosis, and absorbing or capturing potentially toxic minerals (12). Bone is metabolically active and has many blood vessels, which allows oxygenated blood to flow through it to remove waste products and provide the nutrients it needs to constantly make new bone (modelling) and recycle damaged bone (remodelling). The mature human skeleton is made up of 206 bones (excluding the auditory ossicles and the sesamoid bones), of which 74 make up the axial skeleton, and 126 the appendicular

skeleton (13). The axial skeleton includes the skull, ribs, sternum, and spine whilst the appendicular skeleton includes the extremities (long bones), scapulae and pelvic girdle (14,15). The cervical, thoracic and lumbar spinal vertebrae, the lower extremities, some portions of the pelvis and the calcaneus are part of the weight-bearing skeleton whilst the remainder of the skeleton is non weight-bearing. Long bones, which are mostly in the appendicular skeleton, are the most often loaded structures and, because of this, the strongest weight-bearing bones in the body (16). Long bones are made up of the hollow, tubular shaft known as the diaphysis, the flared, cone shaped proximal and distal metaphysis and the wider, rounded proximal and distal epiphysis (Figure 1) (13). As will be discussed in more detail in section 1.4 in this thesis, in growing skeletons, the metaphysis contains the epiphyseal plate, the site of long bone elongation (17). In early adulthood, when the bone stops growing, the epiphyseal plate becomes an epiphyseal line. The outer walls of the diaphysis are dense and solid cortical or compact bone (12). The epiphysis and metaphysis are filled internally with trabecular or spongy bone surrounded by a thin layer of cortical bone (12,17). Flat bones are made up of a layer of spongy bone, covered on either side by a layer of compact bone. Overall, the human skeletal comprises about 80% cortical bone and 20% trabecular bone (18).

Figure 1.1: The structure of bone



Picture by Dresing K, Lumpp B. Bone anatomy and healing. AO Trauma ORP. 2015;1–10 (19), ***reproduced with permission.***

1.2.2 Bone architecture

Bone has a complicated structure because it needs to be rigid enough to withstand forces and load, but also flexible enough to deform and absorb energy. Bone needs to shorten and widen when compressed, but lengthen and narrow when stretched. It also needs to be able to withstand torsional and shear forces without breaking (15,20). At the macroscopic level, bone architecture is made up of two different types of osseous tissues: trabecular and cortical bone whilst the microscopic level has the tissue level and the material level (13).

1.2.2.1 Macroscopic architecture of bone

All bones have varying amounts of cortical and trabecular bone depending upon the demands placed upon them, according to their position and function (13). The proportions and structure of cortical and trabecular bone enable bones to be light weight but strong to enable movement.

1.2.2.1.1 Trabecular bone

Trabecular bone is also referred to as cancellous bone or spongy bone. It is mostly found in the epiphysis, metaphysis carpal bones, tarsal bones and vertebral bones, which are all part of the weight-bearing bones (14,19). Trabecular bone tissue is a meshwork of bone with several interconnecting spaces containing red bone marrow. In order to effectively accommodate mechanical loading of bone, the lattice-like three-dimensional structure of trabecular bone is organized in the direction from which the largest stresses are most frequently experienced (13,15). Trabecular bone can frequently withstand cyclical low-grade stresses due to the spongy and porous nature that allows it to store enormous quantities of energy before surrendering (15). Trabecular bone is responsible for transferring mechanical loads from the articular surface to the cortical bone. In comparison to cortical bone, trabecular bone contains less calcium, more water, has a greater surface area exposed to bone marrow and blood flow and has a higher turnover rate than cortical bone (21).

1.2.2.1.2 Cortical bone

The thin outer layer of all bones is made up of cortical bone, also known as compact bone (15). It is frequently found in the diaphysis of long bones throughout the appendicular skeleton. Cortical tissue is arranged in a cylindrical pattern with concentric layers in the periosteum and endosteum along the diaphyseal shaft of long bones. The periosteum is a thick membrane on the outer layer whilst the endosteum is a thin lining on the inner layer of bone. The basic construction unit for cortical bone is the osteon, also known as the Haversian system. Each osteon has a central canal that is surrounded by concentric layers or lamellae of bone and contains blood vessels and a tiny amount of connective tissue with interconnecting channels (19). Cortical bone is highly organized structurally, densely packed,

hard, and smooth in texture, with collagen fiber matrix and mineralized lamellar bone most conspicuously oriented in the direction of regular mechanical stress, allowing it to withstand rapid, high impact forces (13). Cortical bone has lower surface to volume ratio than trabecular bone (21). The cortex becomes more porous due to aging or disease, resulting in an increased surface area but reduced strength (21).

1.2.2.2 Microscopic architecture of bone

The microscopic and macroscopic properties, or levels, of bone, form a complex architecture that has shape, size, and density aimed at achieving maximum strength with the least weight (15,20). The two microscopic levels include the tissue level and material level. At the tissue level, bone is made up of woven and lamellar bone. At the material level, bone is made up of organic and inorganic components (13).

1.2.2.2.1 Woven bone and lamellar bone (Tissue level)

At various stages of the microscopic modeling and remodeling processes, bone appears as juvenile (woven) and mature (lamellar) tissue (15,22). The immature form of bone known as woven tissue is characterized by a high cell volume, random and spontaneous collagen organization, and relatively low tissue density (15). It has a disorganized and porous structure resulting from its quick formation (23). Throughout development, woven bone predominates, first constituting the whole skeleton at birth before gradually changing into mature lamellar bone during physical growth and skeletal maturation. The only other times that woven bone creation takes place is when woven bone makes a callus at the site of fracture, and it is assumed that this is a quick, protective, and regenerative reaction to severely compromised or injured hard tissue structures (15). Woven bone is considered a premature and provisional bone. The mature type of bone, however, known as lamellar tissue, eventually displaces woven tissue and takes the shape of trabecular or cortical bone. Lamellar tissue has exact and intentional parallel and concentric arrangement of lamellae sheets (13,15). To best withstand mechanical stresses, particularly torsional stress, lamellae sheets are created in opposing directions and with varying thickness and rotational position (15,19). Lamellar bone is stronger and more dense that woven bone (23).

1.2.2.2.2 Organic and inorganic material of bone (Material level)

Bone is a unique type of connective tissue that consists of extracellular organic material and a high concentration of mineralized inorganic material (20). One-third of the mass and two-thirds of the volume of bone are made up of organic material whilst the inorganic material makes up two-thirds of the mass and one-third of the volume (15). Collagen fibres constitute about 90% of the extracellular organic component, providing a framework for calcification and gives the bone its stiffness and flexibility so that it can bend without being brittle (20). In

contrast, the mineralized inorganic component of bone is predominantly composed of hydroxyapatite (calcium phosphate and calcium carbonate), an insoluble salt that gives bone its rigidity and hardness, especially in compression. How well bones hold together as a whole, depends on the contribution and interaction of the organic and inorganic materials (15). Changes in the density of inorganic minerals may affect the arrangements of bone's stiffness and flexibility, the ideal balance of which is still largely unknown (15). Accordingly, highly mineralized bone might become brittle whereas less mineralized bone may be harder but less stiff.

1.2.3 Bone cells

Bone cells constitute less than 2% of the bone mass. There are 5 types of bone cells: osteogenic cells, osteoblasts, osteoclasts, osteocytes, and extracellular lining cells; these interact to create, control, and preserve bone (20).

Osteoprogenitor cells: The osteogenic (osteoprogenitor) cell, found in the periosteum and endosteum, are undifferentiated cells that differentiate and develop into osteoblasts.

Osteoblasts: Osteoblasts are anabolic in nature and synthesize and calcify freshly produced collagen to form new bone (15,24). During the osteogenic process, osteoblasts undergo a unique transformation into bone lining cells (which surround the extracellular matrix) and osteocytes (which are embedded within the bone matrix) (15,20)

Osteocytes: Each osteocyte is located in a small cavity in the bone tissue called a lacuna, maintaining the mineral concentration of the matrix via the secretion of enzymes. They can communicate with each other and receive nutrients via long cytoplasmic processes that extend through channels within the bone matrix called canaliculi (25). Osteocytes are crucial for bone growth and renewal and are the most abundant, constituting 90% to 95% of all bone cells (26). Osteocytes create a strong network of sensory receptors to monitor environmental changes and provide reactive signals to osteoblasts, bone lining cells, and other osteocytes (27,28). Each osteocyte in this network has about 60 to 80 dendrite-like connections which are used for communication and offer a mechanosensitive platform for detecting mechanical stress and related micro-damage (20,22). This mechanically sensitive mechanism, known as mechano-transduction, allows bone to detect and convert mechanical energy into corresponding biochemical signals in order to support bone adaptation in response to the forces it endures (15,29).

Osteoclasts: Osteoclasts are a type of catabolic cells that break down, dissolve, and reabsorb bone material, frequently in response to degeneration or inactivity (30). Osteoclasts have a short life span, undergoing programmed cell death (apoptosis) within 2 to 4 weeks of osteoclastogenesis. Osteoblasts and osteoclasts work independently during bone modelling and co-operatively via a basic multi-cellular unit (BMU) during bone remodelling (31,32).

1.3 Bone modelling and bone remodelling

The periosteum and endosteum both contain a layer of cells that model (form new bone) and remodel (repair damaged bone) bone throughout life. The periosteum also contains blood vessels, nerves, and lymphatic vessels that nourish the compact bone. New bone tissue is formed constantly whilst old and/damaged bone is dissolved for calcium release or for repair. The purpose of bone modelling is to establish the peak bone mass during growth whereas that of bone remodelling is to maintain bone mass during adulthood (33). Neither bone modelling nor remodelling are directly assessed in this thesis but a background description of bone modelling in childhood is further discussed in chapter 3 of this thesis whereas bone remodelling is further discussed in chapter 6. The ongoing process of osteoclasts resorbing bone continually while osteoblasts are forming new bone, is responsible for the constant but subtle reshaping of bone. Some substances found in the mineralized (inorganic) part of bone offer significant resistance to X-ray beams and this forms the theoretical foundation for the instruments that assess bone density using X-rays.

1.4 Bone modelling during childhood

During childhood, bone grows substantially through bone modeling which is a coordinated action between bone resorption and new bone formation. As new bone is formed and some is resorbed, bone changes in size, density and shape (34). Bone modelling begins during the intra-uterine fetal stage of bone growth and continues until epiphyseal fusion in young adulthood (35). Bones grow in length (longitudinal) by bone formation at the diaphysis side of the epiphyseal plate, while they grow in width and thickness (appositional) by periosteal deposition and endosteal resorption of bone. The diameter of bone is increased by periosteal apposition, whilst the diameter of the medullary cavity is widened by endosteal resorption, shifting the cortex away from the neutral axis (i.e. the ’centre’ of the bone). Bones form primarily in 2 different ways which are described below.

1.4.1 Intramembranous ossification

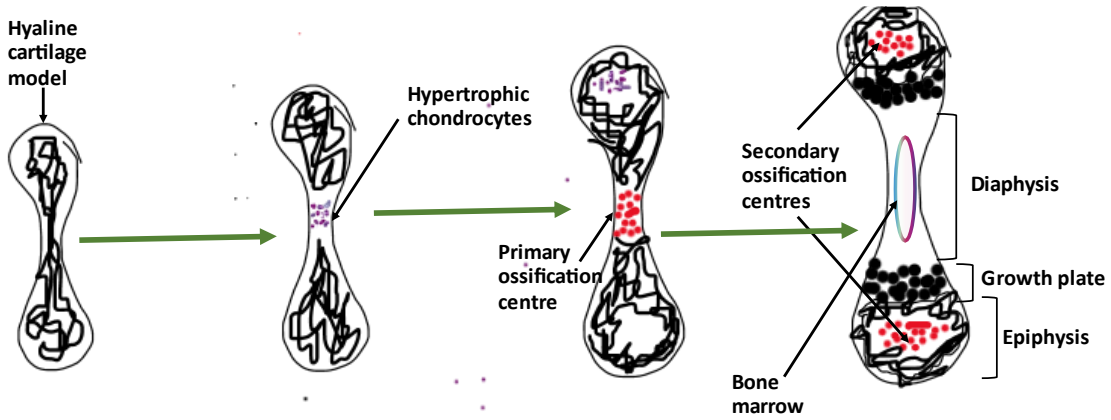
Intramembranous ossification is the process of bone formation from fibrous connective tissue rather than from cartilage. This process begins with the formation of a fibrous connective tissue template, which is then replaced by bone tissue. This process occurs during the development of the skull, facial bones, and clavicle (collarbone) (36). It also occurs during the healing of certain types of fracture (37). It's important to distinguish it from endochondral ossification (see 1.4.2 below). During intramembranous ossification, there is coordinated activity between osteoblasts and osteoclasts. Mesenchymal cells differentiate into osteoblasts, which then form bone tissue (38). This balance between osteoblastic bone formation and osteoclastic bone resorption is necessary for bone growth and remodeling

(37). Intramembranous ossification can be affected by genetic and environmental factors, and can be disrupted in certain pathological conditions such as cleidocranial dysplasia, where the collar bone and the skull bones fail to develop properly (39).

1.4.2 Endochondral Ossification

Endochondral ossification creates bone tissue from cartilage in long bones, the pelvis and all of the skeletal components of the vertebral column (16,40). Mesenchymal stem cells differentiate into chondroblasts which secrete a cartilaginous matrix resulting in a hyaline cartilage model (Figure 1.2). This cartilage model is the template for the formation of bone (22,36). In long bones, this model consists of a diaphysis, a primary ossification center in the middle of the diaphysis and a newly forming epiphyses. The outer layer surrounding the model is a dense membrane (perichondrium), containing fibroblasts and chondroblasts. Chondroblasts become embedded within the matrix and transform into chondrocytes as the cartilage template grows in size. At the diaphysis, cartilage is resorbed and lacunae (cavities) are formed. Spaces left by resorbed cartilage are invaded by ingrowing blood cells. As the chondrocytes apoptose and the perichondrium develops into the periosteum, osteoprogenitor cells differentiate into osteoblasts and begin to lay down bone matrix on the remains of the partially degraded, mineralized cartilage (37,38). Osteoblasts generate a layer of bone on the calcified cartilage model, giving rise to woven bone. At the end of long bones, secondary centres of ossification are formed between the epiphysis and metaphysis, resulting in growth plates (epiphyseal cartilage) (38). These ossification centres allow for expansion in bone length and diameter, and are responsible for longitudinal growth after the embryonic stage of development.

Figure 1.2: An illustration of stages of the endochondral ossification process during bone formation



1.4.3 The role of the RANK/RANKL/OPG system in bone modelling and remodeling

The development of osteoclastic precursors and the apoptosis of active osteoclasts determine the activity and quantity of osteoblasts. On the other hand, osteoblasts and stromal cells are essential for the formation of osteoclasts. Whilst osteoblasts are derived from mesenchymal stem cells, precursors of osteoclasts derive from cells of monocytes and macrophages which are cells of haematopoietic origin (41). The exact mechanism underlying the conversion of a monocyte into an osteoclast is unclear. Osteoclast differentiation must be induced by cell-to-cell interactions between the hematopoietic and osteoblastic cell lineages (40).

The osteoclastogenic process is regulated by the RANK/RANKL/OPG signaling pathway (41,42). Osteoprotegerin (OPG) is a potent inhibitor of osteoclast differentiation, whereas osteoprotegerin ligand (OPGL), also known as NF-related activation-induced cytokine (TRANCE) or the receptor activator of NF-κB (RANK) ligand (RANKL), is a potent inducer of osteoclast differentiation (42). RANKL enhances the development, survival, and binding of precursor osteoclast cells. It also activates mature osteoclasts and lengthens their life cycle. All these functions are due to the interaction between RANKL and RANK. Osteoclasts produce the transmembrane protein RANK, which binds to RANKL to activate osteoclasts. As a decoy receptor, OPG, a protein that prevents the growth of osteoclasts, sequesters RANKL and suppresses RANK signaling (40–42). Apoptosis of osteoblasts is then induced because osteoblastic activation is prevented. An imbalance of the RANKL-to-OPG ratio is thought to play a role in the mechanisms of bone resorption (43). Reduced or absent levels of RANKL or RANK, or both, result in high bone mass, e.g., in certain types of osteopetrosis, and in fewer mature osteoclasts, whereas increased osteoclastic activity and RANKL and RANK levels lead to accelerated bone loss e.g., in osteoporosis (42–44).

Cytokines are a broad and loose category of small proteins e.g. interleukins, interferons, etc. which are secreted by certain cells of the immune system and are important in cell signaling. The RANK/RANKL/OPG system, is regulated in many different ways, including through action by the following cytokines, amongst others:

1. Interleukins (IL) 1 and 6 and tumour necrosis factor (TNF) promote the production of RANKL, macrophage colony-stimulating factor (M-CSF), and granulocyte-macrophage colony-stimulating factor (G-CSF), which all act directly on osteoclast progenitors to promote proliferation and differentiation and prevent apoptosis of osteoclast (43).
2. Interleukin IL-1, TNF, parathyroid hormone (PTH) and 1,25-dihydroxy-vitamin D3 increase the production of osteopontin and integrins, which help osteoblast/stromal cells and

osteoclasts communicate with each other and increase the number of cytokines that control osteoclastogenesis (43).

3. Other cytokines e.g. Interferon alpha (IFN-α), IL-4, IL-10, IL-18, and transforming growth factor beta also slow down osteoclastogenesis.

1.5 Bone remodelling in adulthood

Bone remodelling is the process by which the bone regulates its own maintenance and repair, replacing old and damaged bone with new bone (45). Bone remodelling also contributes to the maintenance of mineral homeostasis by facilitating rapid access to calcium and phosphate (38). The bone remodelling cycle occurs within a basic multicellular unit (BMU) (32). The BMU contains osteoclasts, osteoblasts, in addition to a capillary blood supply. Since the BMU continues to function longer than the osteoclasts and osteoblasts that are contained inside it, these cells must be replenished continually. The osteocyte regulates osteoclast and osteoblast differentiation and therefore the replenishment of these cells (31). The BMU structure depends on whether it is in trabecular or cortical bone. In trabecular bone, the BMU is positioned on the surface of trabecular bone in such a way that a bone cavity known as resorption bays or Howship's lacunae (which house osteoclasts) can be filled back up after it has been resorbed (46). In cortical bone, osteoclasts within the BMU of cortical bone form a cutting cone that "burrows" into the cortex, removing damaged bone (38). Differentiated osteoblasts will then begin to lay down new bone behind the cutting cone in a concentrically aligned pattern on the tunnel walls, ensuring that there is room for a vascular supply within the new osteon's Haversian canal (24). In both cases, the bone marrow unit (BMU) is shielded from view by a canopy of cells that define the bone remodelling compartment (BRC), with close anatomical coupling of osteoblasts and osteoclasts (31). The remodelling cycle is highly controlled and predictable, with five overlapping stages happening over 4 to 6 months (31,38). The 5 stages of the bone remodelling cycle are activation, resorption, reversal, formation, and termination (Figure 1.3) (38).

1.5.1 The bone remodelling cycle

1.5.1.1 Activation

Mechanical or biochemical signals are needed for activation. An example of biochemical signal is secretion of hormones such as oestrogen or parathyroid hormone whereas mechanical signals result from injury to bone or change in loading (47,48). During the activation stage, mechanical or biochemical signals induce the bone lining cells (resting osteoblasts) to release cytokines such as M-CSF (Macrophage-colony stimulating factor) and RANK-L (receptor activator of NFkB) (47,48). These factors subsequently recruit and activate osteoclast precursors in order to begin the process of bone resorption (16). Once

osteoclast precursors are recruited from the bloodstream and stimulated, the lining cells detach from the underlying bone and form a raised canopy over the area that is going to undergo resorption, isolating the resorption pit from surrounding bone (Figure 1.3). At the same time, multiple mononuclear cells fuse to form multinucleated preosteoclasts and differentiate into multi-nucleated osteoclasts and attach to the bone surface (49). Bone remodelling can be targeted, which is aimed at a specific site when removing an area of damaged or old bone or non-targeted (24). In targeted remodelling, signal to start the process comes from the osteocytes, which use their large network of dendritic processes to send a message to other cells (50,51). Osteocytes which are the most abundant bone cells (about 10,000 cells per cubic millimetre) have about 50 processes per cell (31). These processes form a dense network which connects osteocytes with each other and with flattened lining cells on the surface of the endosteum, ensuring that no part of bone is more than several micrometres from a lacuna containing its osteocyte (31). Osteocyte apoptosis, which can be caused by things like bone matrix micro-damage that breaks up osteocyte canaliculi, leads to the release of paracrine factors that boost local angiogenesis and bring in more osteoclast and osteoblast precursors (52,53). Non-targeted remodelling, on the other hand, is not aimed at a specific site and it happens when hormones like PTH cause systemic changes in the body as a whole (38).

1.5.1.2 Resorption

After osteoclasts have attached themselves to the surface of bone, they begin to release acid phosphatases and hydrogen ions (24,38). These two substances work together to lessen the pH of the bone resorbing compartment, which in turn causes the resorption of bone material (38). During this phase, enzymes such as matrix metalloproteinases are also secreted to break down the organic parts of the bone matrix (54). Resorption lasts for a period of about two weeks. The resorption phase is ended by osteoclasts programmed cell death, and this prevents excessive resorption from taking place (55).

Figure 1.3: The five stages of the bone remodelling cycle summary

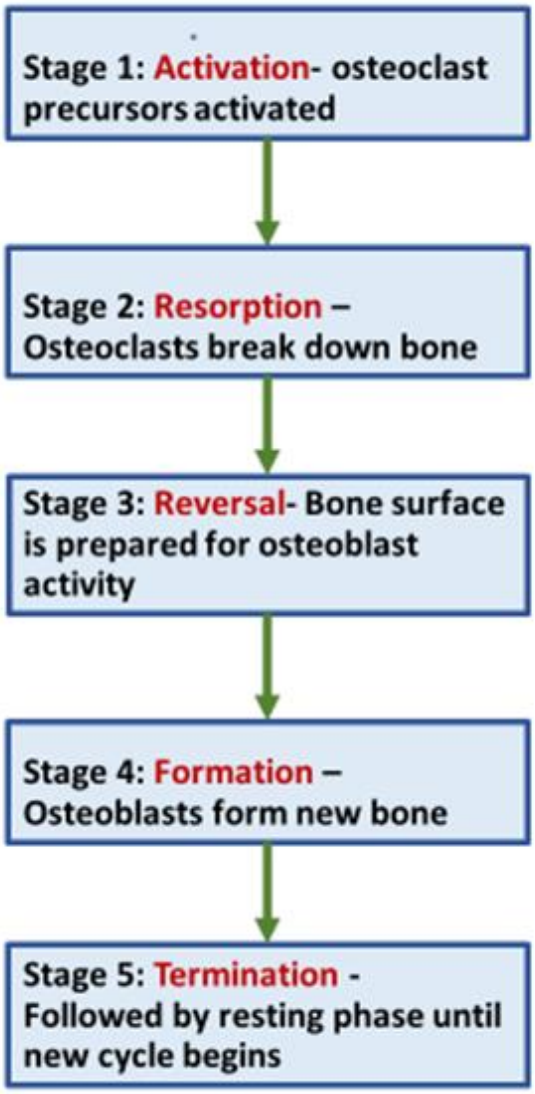


Figure 1.3 Summary of the five stages of bone remodelling in order of occurence

1.5.1.3 Reversal

The reversal stage lasts about four to five weeks. During this phase, the remaining fragments of the collagen matrix that have not been mineralised, are removed by a cluster of mononuclear cells of the osteoblastic lineage (38). These cells prepare the bone that has been newly resorbed for the deposition of newly formed bone matrix (32). The exact signal that couples bone resorption to subsequent bone formation, resulting in no net loss of bone, is not yet clearly understood. It is believed that cells of the reversal stage are a part of sending or receiving these signals (46,56). Literature suggests that osteoclasts are the source of the coupling factor via a regulatory receptor on their surface or by secreting cytokines e.g. interleukin 6 (IL-6) (57,58).

1.5.1.4 Formation

The formation stage lasts about four months (38). During this phase, pre-osteoblasts become osteoblasts and they secrete and deposit a type 1 collagen-rich matrix (osteoid) (24). Osteoid is mineralized gradually, forming new bone (38). During bone mineralization, hydroxyapatite crystals are deposited amongst collagen fibrils. The bone mineralization process is controlled by systemic regulation of the concentrations of calcium and phosphate, local calcium and phosphate concentrations (within the vesicles of the extracellular matrix) and by local inhibitors of mineralization (27,28). Osteoblasts will keep depositing new bone until the amount of bone that has been formed equals the amount of bone that has been resorbed (38).

1.5.1.5 Termination

The remodelling process ends. Osteoblasts enter a resting state, at which point they either change into bone lining cells by forming a single layer of cells on top of the newly formed mineralized matrix, undergo apoptosis or become osteocytes by becoming buried in the newly produced bone matrix (38). The entire structure of the BMU moves as a unit, with osteoblasts trailing behind osteoclasts and this is why the bone resorption and bone formation processes are described as being coupled to one another (48). The process of coupling is highly regulated, and osteocytes play a crucial part in the signalling of the completion of remodelling by the secretion of antagonists to osteogenesis. More specifically, these osteocytes secrete antagonists of the Wnt signalling pathway (59,60).

1.5.2 Key pathways in the bone remodeling cycle

The remodelling cycle is tightly regulated both locally and systemically, to achieve balanced resorption and formation. Two key pathways included are the RANKL/RANK/OPG and Wnt signalling.

1.5.3 RANKL/RANK/OPG signalling pathway

Before the activity of RANKL can take place, a permissive concentration of M-CSF is necessary (38). M-CSF is expressed by osteoblasts and stimulates the expression of RANK by osteoclasts resulting in binding of RANKL to its receptor, RANK, on osteoclasts (Figure 1.3) (42). When RANKL and RANK bind, signalling molecules like mitogen-activated protein kinase and TNF-receptor-associated factor 6 are activated. This leads to the activation of key transcription factors that control the production of osteoclasts (61). As described in chapter 3 (section 3.4), RANKL can be produced by osteoblasts, osteocytes and chondrocytes (42). However, it is the osteocytes that sense changes in load and micro-damage within the bone matrix, which initiate the bone remodelling cycle via production of RANKL. OPG, a decoy receptor for RANKL, inhibits osteoclastic bone resorption by binding to RANKL and preventing its binding to RANK, making the ratio of RANKL to OPG a crucial

factor in regulating the bone resorption process (43). Prostaglandins are also believed to increase the RANKL/OPG ratio thereby enhancing osteoclastogenesis (62).

Figure 1.4: The RANKL/RANK/OPG signalling pathway in bone remodeling

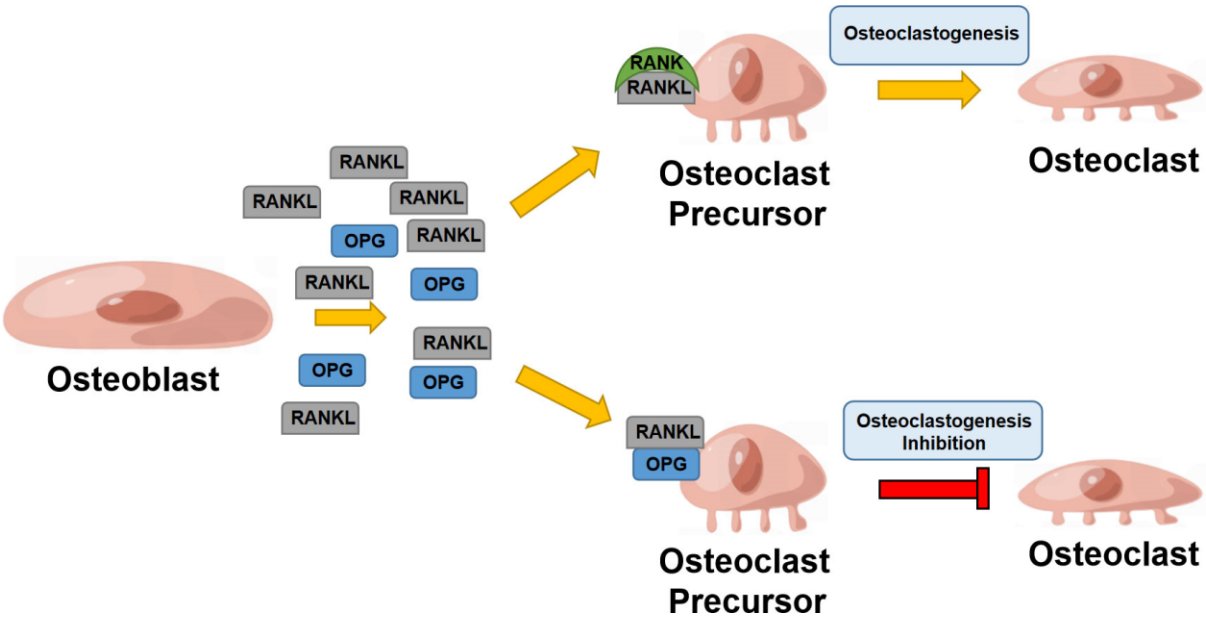


Figure 1.3 Conceptual illustration of the RANKL/RANK/OPG signalling pathway as illustrated by Zhang et al, 2022 (63). *(©Zhang et al, 2022* (63) *This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY); https://creativecommons.org/licenses/by/4.0/. The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication is cited, in accordance with accepted academic practice.)*

1.5.4 Wnt signalling

The Wnt signalling pathway is a major regulator of osteoblastic bone formation (38). Wnts are a family of 19 molecules in mammals, each consisting of 350—400 amino acids (64). The Wnt signalling route can be broken down into two primary sub-pathways, which are: canonical (b-catenin dependent) and non-canonical (b-catenin independent) (65,66). Of these, the canonical Wnt signaling pathway promotes osteogenesis. Through the canonical pathway, Wnts work on osteoblast precursor cells, causing them to differentiate into mature osteoblasts. Wnts also derail bone resorption by controlling the ratio of RANKL to OPG (67). However, during the process of bone remodelling, osteocyte expression of Wnt-inhibitors decreases, which makes it possible for osteoblastic bone formation to take place after bone resorption. When termination stage begins, the newly created osteocytes which have become entombed within the bone matrix, re-express Wnt inhibitors, leading to cessation of bone formation (66).

1.5.5 Factors regulating the bone remodelling cycle

Endocrine factors (e.g. growth hormone, sex hormones and parathyroid hormone) and paracrine factors (e.g. prostaglandins and cytokines) are also involved in regulation of the bone remodelling process.

1.5.5.1 PTH

Parathyroid hormone (PTH) binds to its receptors on osteoblasts, stimulating osteoblasts to increase their production of RANKL, OPG and M-CSF. M-CSF induces hematopoietic cell precursors to give rise to osteoclasts which then express the RANK receptor. Meanwhile, RANKL released by osteoblasts binds to RANK and this interaction induces differentiation of the osteoclasts such that they begin to resorb bone (Figure 1.4). Additionally, osteoblasts also produce OPG, which binds to RANKL therefore preventing RANKL to RANK binding. PTH can have both catabolic and anabolic effects on bone remodelling, depending on whether the PTH is released intermittently or continuously. Continuous PTH stimulates the bone resorption process and is a crucial for calcium homeostasis. Continuous exposure to excess PTH results in hypercalcaemia and induces both trabecular and cortical bone loss, though cortical bone is more severely affected (38). The catabolic effects of PTH on bone are due to its modulation of the OPG-RANKL-RANK signalling system whereby PTH increases RANKL and inhibits OPG to stimulate osteoclastogenesis. With intermittent PTH stimulation, the expression of osteocyte-derived Wnt inhibitors e.g. SOST is reduced, there is an increase in canonical Wnt signalling and therefore increase bone formation (68).

Figure 1.5: Endocrine regulators of bone remodelling

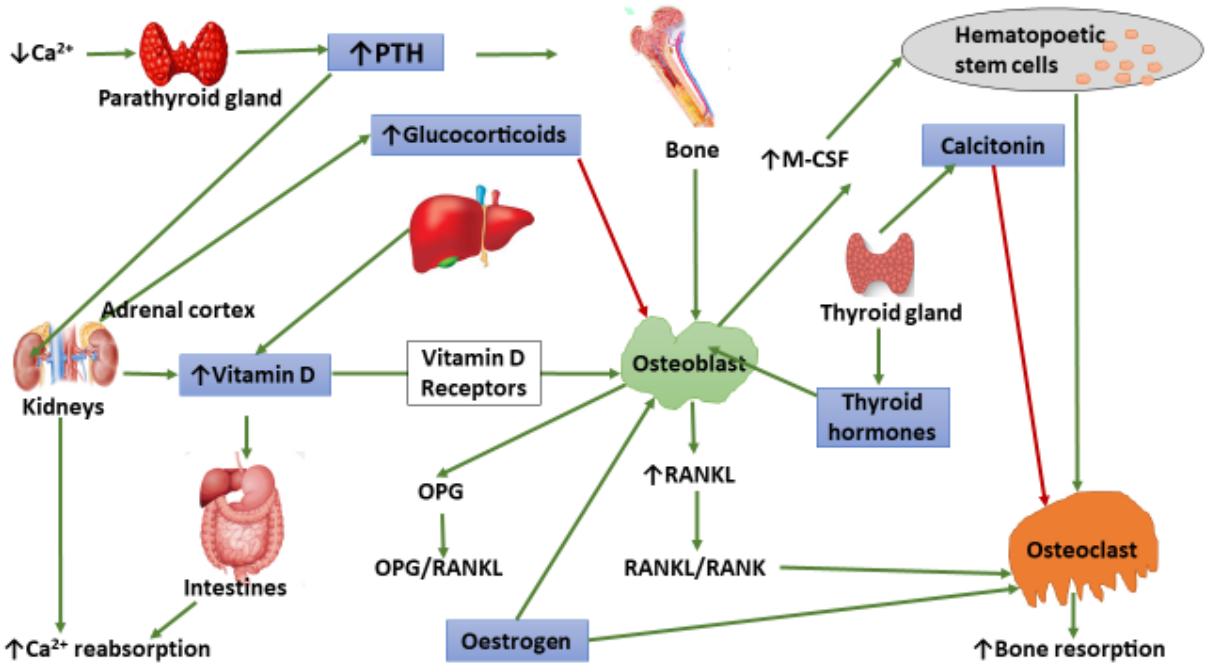


Figure by Cynthia Mukwasi-Kahari

1.5.5.2 Vitamin D. 1,25(OH)2

Vitamin D regulates the absorption of calcium and phosphate from the intestines. Vitamin D may also have direct action on skeletal cells e.g. osteoblasts which have vitamin D receptors (38). Vitamin D is essential for musculoskeletal health as it promotes calcium absorption from the bowel, enables mineralization of newly formed osteoid tissue in bone and plays an important role in muscle function

1.5.5.3 Calcitonin

Calcitonin is synthesised in the thyroid gland. At physiological concentrations, it binds with the calcitonin receptor on osteoclasts and acts to inhibit bone resorption (69,70). Calcitonin reduces the number of osteoclasts, their secretory activity and ruffled border formation. Calcitonin inhibits the actions of a coupling factor (sphingosine-1- phosphate) linking bone formation to bone resorption (38).

1.5.5.4 Thyroid hormone

Thyroid hormones enhance osteoblast differentiation and mineralization, speeding up bone formation (71). When there is a deficiency of thyroid hormone, the bone remodelling cycle is lengthened, with low bone turnover and this results in increased bone mass. In high concentrations of thyroid hormone, bone turnover is increased, thereby decreasing the bone remodelling cycle duration/length and bone loss increases (71).

1.5.5.5 Growth hormone and insulin-like growth factor 1

GH increases bone formation and resorption by enhancing expression of insulin-like growth factor 1 (a hormone that manages the effects of GH in the body). Though both processes are increased, bone formation predominates over bone resorption, increasing bone mass (72). When there is a deficiency of GH, bone resorption predominates over bone formation and this leads to increased bone loss.

1.5.5.6 Glucocorticoids

Increased glucocorticoids (cortisol and corticosterone) lead to bone loss. Glucocorticoids inhibit the differentiation and function of osteoblasts and increases osteoblast apoptosis. In addition, by inhibiting osteoblasts, glucocorticoids reduce OPG, increasing RANKL and RANK expression and thereby increasing bone resorption. However, prolonged glucocorticoid treatment eventually results in reduced osteoclast numbers and therefore reduced bone resorption (73). This means that prolonged glucocorticoids exposure slows down osteoclast bone resorption, leaving the suppression of osteoblastic bone formation as the predominant skeletal effect (74,75).

1.5.5.7 Sex hormones

Oestrogen is the major regulator of bone in both men and women (76). Oestrogen reduces the number and function of osteoclasts and increase osteoclasts apoptosis, inhibiting bone resorption. In addition, oestrogen maintains bone formations by inhibiting both osteoblast and osteocyte apoptosis (77). Aromatase converts androgens to oestrogens. In oestrogen

deficiency, there is increased bone remodelling. Even though both bone resorption and bone formation are increased in oestrogen deficiency, there is uncoupling of the bone remodelling process and this results in resorption outweighing formation therefore increased bone loss (77).

1.5.6 The effect of HIV and ART on bone cells during bone remodelling

In an effort to understand the effect of HIV on bone remodelling, various studies have assessed the effect of HIV and/or ART on osteoclast or osteoblast activity.

1.5.6.1 The effect of HIV and ART on osteoclasts

The monocyte/macrophage lineage from which osteoclasts come from is affected by HIV, making osteoclasts a target for HIV as well (78–80). HIV infection promotes osteoclast differentiation and osteolytic activity (80). In addition to RANKL levels being positively associated with HIV viral load, the RANKL: OPG ratio is reported to be significantly lower in PLWH who were not on ART (81). However, it is important to note that it is the circulating OPG levels (not the RANKL: OPG ratio) which has been reported to be associated with BMD (82,83). Changes in OPG and RANKL expression in PLWH could be due to aberrant expression by HIV-infected immune cells. In response to HIV, macrophages (84), B-cells (85), and T-cells (86), all increase RANKL and decrease OPG expression (87). This may suggest that elevated resorption of bone within the context of HIV possibly results from both HIV infection and the activation of HIV-infected immune cells.

ART initiation is also associated with increased osteoclast number and function either directly or indirectly via immune cell-mediated effects (87). ART suppresses HIV viral replication and results in immune reconstitution including CD4+ T-cells repopulation (88). This is crucial in elongating the life expectancy rate in PLWH. However, immune reconstitution has also been associated with proliferation of pro-inflammatory cytokines and this may lead to bone loss (88). After ART initiation, immune reconstitution leads to increased amounts of circulating RANKL and TNFα, increasing osteoclastogenesis in the process (89,90). There is a positive association between bone loss in PLWH and the amount of immune reconstitution (89). Following ART initiation, the greater the increase in CD4+ cell the greater the BMD loss in adults (89). ART is also believed to directly influence osteoclast activity. TDF exposure is associated with increased circulating RANKL (91). However, there are studies that reported no association between RANKL/OPG ratio or RANKL and bone loss regardless of ART type, suggesting no influence of ART on osteoclasts (91,92). These contradictory findings in relation to ART and bone cells may be due to differences in types ART drugs included in the studies. Furthermore, independent effect of each ART type on bone cells is not always clear since ART is taken as a combination of 2 or 3 drugs and

regimens can also be changed.. This is all in addition to the fact that few studies have reported on the effect of HIV and ART in women from sub-Saharan Africa, therefore more studies are required to understand the effect of HIV and ART on bone in the sub-Saharan African population.

1.5.6.2 The effect of HIV and ART on osteoblasts

Not much is known about how HIV affects osteoblasts. Some studies have reported no evidence of HIV infection in cultured osteoblasts or osteoblasts isolated from PLWH (93,94). However, viral products have been reported to impair osteoblast differentiation, even in PLWH who have well controlled viral load (93,95–97). ART also has a deleterious effects on osteoblasts number and function (98). For example, ART induces cell senescence and inhibits osteoblast differentiation (98). In addition, protease inhibitors have been reported to inhibit the function of mature osteoblasts (99–101). However most of these studies were conducted using PI containing ART (99,100). Despite the TDF associated bone loss which has been described in the literature (102,103), little is known about the effects of non PI containing ART on number and function of osteoblasts. TDF, which is widely used in sub-Saharan Africa, inhibits bone matrix formation by reducing suppressing collagen 1 expression and reducing the amount of calcification (59).

1.5.6.3 HIV, ART & Osteocytes in bone remodelling

The effects of HIV and ART on osteocytes remains largely unknown yet osteocytes constitute between 90 and 95% of bone cells and are crucial in regulation of the bone remodelling cycle (26,87).

1.5.7 Consequences of impaired bone remodelling cycle

In healthy people, the remodelling cycle has strict coupling between bone formation and bone resorption (38,87). When there is loss of such coupling, osteoporosis occurs and this is one of the possible causes of fragility fractures. Impaired bone architecture may be a result of failure to attain optimum peak bone mass during the growing years and increased bone resorption or reduced bone formation in adulthood. Menopause and aging are primary causes of bone loss (38). Secondary causes include systemic diseases such as Cushing's syndrome (104), diabetes (105), hyperthyroidism (106), hyperparathyroidism (107), hyperprolactinaemia (108), hypogonadism (109), rheumatoid arthritis (65), hypophosphatasia (110), chronic obstructive pulmonary disease (111), renal disease (112), liver disease (113), malabsorption, inflammatory bowel disease (114), myeloma, multiple sclerosis or treatment drugs such as glucocorticoids, anticoagulants, antiepileptics, gonadotrophic releasing hormone agonists/antagonists, chemotherapy and immune suppressants. HIV infection and its treatment are believed to impair the bone remodelling cycle (as discussed above) and lead to impaired bone architecture (115). Though the

mechanism by which that happens is not fully understood, PLWH are more likely to experience bone loss and fracture than their uninfected counterparts (115,116).

1.6 Importance of assessing bone health

Impaired bone accrual and increased bone loss lead to osteoporosis (117). Osteoporosis is a systemic skeletal disorder characterised by low bone mass and deteriorated microarchitecture of bone leading to enhanced bone fragility and increased fracture risk (118) The prevalence of osteoporosis and fragility fractures increase as the population ages. Both the sub-Saharan population and the population of PLWH in sub-Saharan Africa are increasing in age (119). However, the burden of fragility fractures is complicated by the fact that osteoporosis is usually under-diagnosed or left untreated resulting in increased morbidity and mortality in the population of those experiencing fragility fractures (120). The most common complication of osteoporosis is fragility fracture. The proportion of people experiencing fragility fractures has been estimated to be higher amongst PLWH than in those without HIV (116,121,122). The pooled prevalence from a systematic review of studies assessing fractures among PLWH was 6.6% (95% CI: 3.8–11.1); higher than in people without HIV by an odds ratio of 1.9 (95% CI: 1.1–3.2) (121,122). However, some of the studies comparing fragility fractures in PLWH and those without HIV have been inconclusive (123,124). In 1,281 PLWH who were on ART (77% male), fracture incidence was reported to be 3.3/1,000 patient-years (95% CI 2.0, 4.6) an incidence rate that was similar to general European population for the same age group, with 81% of the fractures resulting from trauma (123). Another study reported HIV to be associated with both low BMD and increased risk of fracture in 328 men living with HIV who were older than 49 years (125). A larger study also reported PLWH (n=5555) to be more likely to experience a wrist, vertebral or hip fracture than their uninfected counterparts (3.08 per 100 persons vs. 1.83; P,0.0001) (124). Risk factors for fractures include demographic factors (e.g. older age, low BMI, female gender, ethnicity), familial factors (e.g. history of previous fracture, parental history of low trauma fracture), lifestyle factors (smoking, alcohol intake, low physical activity), clinical factors which included diseases or medications know to affect bone such (e.g. rheumatoid arthritis and glucocorticoids) amongst other things. Among PLWH, HIV specific factors for fracture risk such as ART, severity of disease, viral load, inflammation, immune reconstitution may add to the risk for fractures. Low bone density and premature bone loss have been reported in PLWH in high income countries (126–129). In adults, prevalence of low bone mass in PLWH has ranged from 6 to 78% in cohorts from high income countries (126–129). However, BMD is not the only factor to consider as far as bone health and risk of fracture is concerned (130). There is some evidence to suggest that bone geometry e.g. bone size and shape, may predict future fracture risk independent of BMD measurements. PLWH are at increased risk of fracture compared to controls (131). Numerous studies have

also shown negative effects of ART on aBMD (132–134). The effects of ART have been demonstrated in CWH and also in adults living with HIV. However, not all studies demonstrated a negative effect of ART on aBMD and for those that do, the effect of different types of ART on trabecular and cortical bone, and also on bone size and bone strength remains to be fully understood

1.6.1 Peak bone mass accrual

Growth and development of the skeletal system during childhood and adolescence is an important period, determining future bone health in adulthood. Peak bone mass (PBM), the amount of bone mineral density (BMD) at skeletal maturity, is determined by bone accrual during childhood, adolescence and early adulthood (135). PBM is achieved by the end of the second or third decade of life, PBM occurring at different time points in different populations. It then plateaus from young adulthood, to then decline in women post menopause, and in all with age (35). Adult bone mass represents the difference between PBM achieved during growth and the amount lost as bone mass declines with age (8). The major clinical consequence of this bone loss and deterioration in bone architecture is susceptibility to fragility fracture. Peak bone mass is an important predictor of future osteoporosis and fragility fracture risk in early adulthood and later in life (20). Thus, a person with a lower PBM is at higher risk of fracture during adulthood (8).

Researchers have tried to determine to what extent PBM affects the risk of fracture by assessing BMD accrual, age and fracture occurrence. There is little variation in BMD in the age groups above 50 years of age, despite the bone loss which begins with ageing. Evidence from aBMD studies suggests that the average annual rate of bone loss in adulthood is relatively constant and tracks well within one person (136). If little variation in Z-scores or percentiles occurs during adult life, despite bone loss through later adulthood, it means that the PBM acquired at the end of the growth period has more impact in reducing future adult fracture risk than the efforts to prevent bone loss in the adulthood years. A 10% increase in PBM has been shown to delay the onset of osteoporosis (i.e., a T-Score ≤-2.5) by 13 years, whilst a 10% reduction in age-related bone loss delays the onset of osteoporosis by only 2 years (9). This supports the idea of putting much importance in maximising PBM accrual during childhood in order to reduce fragility fracture risk in late adulthood. In addition, epidemiological studies have shown that it is possible to predict that a 10% increase (about 1 SD) in PBM could reduce the risk of fracture by 50% in women after the menopause (8). Maximizing bone health during childhood and adolescence is potentially crucial for the prevention of future fractures. The concern across Africa is that as CWH reach

adulthood, their peak bone mass may be low making them less able to withstand further bone loss without crossing a 'threshold' of elevated fracture risk.
As the population living with HIV ages, osteoporosis may occur at a younger age than might otherwise be seen, due to compromised PBM. The fracture implications of impaired bone accrual and subsequent age-related bone loss in PLWH is not yet clear.

1.6.2 Factors affecting peak bone mass accrual

Peak bone mass is an essential concept in determining risk of future fracture, yet it is not fully understood. The term 'peak bone mass' is variably defined (137–139). Authors have used the terms peak bone mass and peak BMD synonymously (140,141) whereas other authors have made an effort to be more specific and reported peak bone mineral density instead of peak bone mass (142,143). Peak bone mass has been defined as the highest amount of bone mass achieved at skeletal maturation (5,144), as the highest values of both bone quantity and bone density reached in life (145) or as the maximum mineralization of bone tissue (146). However, peak bone mass encompasses growth of bones to include an increase in mineralization, elongation, volume, and strength and occurs at the end of bone growth, when bone has accrued to its maximum size, has reached its full mineralization and an individual has gained the maximum of bone tissue. Achieving PBM in childhood has more impact in reducing future adulthood fracture risk than efforts to preserve BMD through later adulthood life (9,147). Determinants of PBM accrual include non-modifiable factors such as genetic architecture, age, sex, race, height, as well as potentially modifiable factors such as physical activity (particularly weight-bearing activity), nutritional intake (e.g., calcium, protein, vitamin D), contraceptive use, smoking and alcohol intake. Other factors determining PBM accrual include body weight and presence or absence of disease.

1.6.2.1 Genes

Studies that have assessed bone in children and their parents have shown that PBM is highly heritable (148,149). Studies assessing BMD in twins have shown that 60 to 80% of the variance of adult bone mineral mass is heritable, and this effect is greater in trabecular rich sites, such as the lumbar spine, compared to cortical rich sites, such as the hip (150). Aside from heritable factors, environmental factors play a role and may account for 20 to 40% of peak bone mass variance (151). The role of genetic influences declines with age whilst that of environmental factors increases (152).

1.6.2.2 Growth hormone (GH) and insulin-like growth factor-1 (IGF-1)

GH and IGF-1 are crucial for bone accrual from birth through to puberty (151). IGF-1 positively influences bone growth (length and width). An increase in IGF-1 plasma levels is associated with an increase in bone size and corresponding changes in bone formation markers, alkaline phosphatase and osteocalcin (149). Growth plate chondrocytes and osteogenic cells, which produce the components of cortical and trabecular bone formation, are both directly influenced by IGF-1. In addition, IGF-1 receptors are found in the renal tubular cells and are linked to the production of the hormonal form of vitamin D (1,25 (OH)2 D) as well as the release of inorganic phosphate (Pi) from the luminal membrane of the tubular cells (151). This means that IGF-1 indirectly promotes intestinal absorption of Ca and Pi by increasing the synthesis and blood level of 1,25 (OH)2 D. IGF-1 increases extracellular Ca-Pi production and stimulates tubular Pi reabsorption, promoting bone matrix mineralization. At the bone level, IGF-1 also directly enhances the osteoblastic formation of the extra cellular matrix (149,151). The main source of circulating IGF-1 is the liver. Production of IGF-1 by the liver is influenced by GH and other factors such as amino acids from dietary proteins (153,154). The interaction between sex steroids and the GH-IGF-1 system during pubertal maturation is not clearly understood. It is possible that low oestrogen concentrations stimulate the production of IGF-1 by the liver, whereas high concentrations may inhibit IGF-1 production (155,156).

1.6.2.3 Physical activity

During childhood, a sedentary lifestyle can put individuals at risk of less than optimal PBM (35,147). Mechanical forces enhance bone formation, whilst inhibiting bone resorption. After sensing the fluid movement within their canaliculi and detecting the mechanical strain from physical activity, osteocytes activate bone lining cells to differentiate in pre-osteoblasts resulting in new bone formation (157,158). As a result, physical activity increases bone mineral mass accumulation in both children and adolescents. It has been suggested that the impact of physical activity on bone is stronger before than during or after pubertal maturation (159). Though some studies have been inconclusive on the effects of physically activity on bone in children (160) have shown increased BMD gains compared to those who are sedentary (161). It is possible that the higher PBM induced by physical activity during childhood may be maintained into old age, resulting in reduced fracture risk in old age. Higher PBM may contribute to the lower occurrence of fragility fractures observed in retired athletes (>60 years of age) compared to age matched counterparts who were not athletes (162).

Intensity, duration and frequency of exercise should also be taken into consideration. The impact of physical activity on bone will differ based on intensity, duration and frequency of exercise. Intensity refers to how vigorous or moderate a physical activity exercise is. Duration refers to how long one particular physical activity exercise is eg one hour cardio exercise versus 15 minute cardio exercise. Frequency refers to how many times the physical activity exercise is performed eg 7 days a week or 2 days a week. A person who exercises vigorously for 30minutes, 5 days a week may have different bone outcomes to a person who exercises moderately for 10 minutes, 2 days a week. Studies that have supported the importance of more intense exercise, including a study conducted in 4465 German children aged 0 to 10 years, reporting that just 10 minutes of moderate-to vigorous-intensity weight-bearing physical activity can significantly increase bone strength in children, whereas low-intensity weight-bearing physical activity is insufficient (163). Similarly, a review of school based exercise intervention studies in children concluded that in children, intensity of the exercise was the most important factor, even if the duration of physical activity is minimal (164). The ALSPAC study examined the relationships between childhood physical activity (PA), dietary calcium intake and bone size and density in 422 children (212 boys) and found that duration of moderate to very vigorous activity was positively related to hip bone area ($p<0.001$), mineral content ($p<0.001$), mineral density ($p=0.001$) and estimated volumetric density ($p=0.01$) (165). In adults, the British birth cohort reported that leisure time physical activity during adulthood has cumulative benefits on BMD in early old age, especially among men (166).

Weight-bearing physical activity during youth has been associated with higher aBMD sustained into adulthood. However, though evidence for long term effects of exercise is limited, long term studies are often not practical, or affordable. A study conducted in 57 American children showed that a school-based exercise intervention can have lasting benefits to aBMD 7 years after the intervention (167). Another study showed that those who were athletes whilst growing up, have better bone outcomes in their 60s. Another study examined the relationship between recalled weight bearing physical activity over the lifecourse and bone health in late adulthood and reported greater BMD at total hip in women who reported regular weight bearing physical activity compared with those who reported no weight bearing physical activity (168). However, it is important to note that such studies are prone to recall bias and other possible confounding factors. In studies utilising pQCT, exercise during growth was shown to increase the cortical bone size via periosteal expansion in 597 Swedish men who were 80 ± 4 years old (169). A randomized trial of activity and calcium supplementation conducted in 239 children aged 3–5 years randomized children to participate in either gross motor or fine motor activities for 30 minutes/day, 5 days

per week for 12 months showed that children in the gross motor group had greater tibia periosteal and endosteal circumferences by pQCT compared with children in the fine motor group at study completion ($p < 0.05$) and that calcium intake modifies the bone response to activity in young children (170).

A response that is mostly made up of an increase in periosteal apposition and diameter will provide more mechanical resistance than an increase in endosteal apposition and a decrease in endo-cortical diameter. There are site-specific differences in how the growing skeleton develops in response to physical activity (171). At cortical rich sites, loading from physical activity may lead to geometrical changes with larger bone and greater cortical area, whereas at trabecular rich sites, physical activity may increase the vBMD (171). In addition, it is possible that the increased intake of nutrients such as calcium and protein in those who are physically active, could also contribute to the positive effect on bone accrual that is observed in physically active children. However, intensive physical activity training in childhood may contribute to delayed pubertal onset and progress, negatively affecting PBM accrual (172). In females who start training hard after menarche, secondary oligomenorrhea or amenorrhoea can cause bone loss (173,174). It is also believed that the exercise-induced gain in bone mass, size and strength is due in part, to the skeletal system adapting to the increase in muscle mass and muscle strength (175).

1.6.2.1 Calcium intake, vitamin D and dietary protein

Adequate calcium intake, provided the dietary intake of vitamin D is adequate, positively affects BMD accrual during infancy, childhood and adolescence, supporting optimal peak bone mass achievement. Calcium intake recommendations vary widely for children depending on age and country, with recommendations ranging between 200mg and 1500mg (176,177) and an upper intake level for children and adolescents ranging from 1000mg/day (birth to 6 months) to 3000 mg/day (9–18years) (178). Milk consumption during childhood and adolescence is positively correlated with BMD (179). Interventional studies have reported greater BMD gains in children and adolescents on calcium supplementation over periods ranging from 12 to 36 months. In 144 pre-pubertal girls involved in a randomized controlled trial in Switzerland, there was an interaction between calcium intake and menarche on bone such that the positive effect of calcium supplementation on mean aBMD gain from baseline was greater in girls before but not after menarche (180).

Vitamin D is the major regulator of calcium homeostasis and promotes intestinal calcium absorption and renal calcium reabsorption facilitating skeletal mineralization. Existing evidence shows that vitamin D is associated with improved BMD (181,182).

In addition, some authors have suggested that amino acids from dietary proteins have an anabolic effect on bone health (183,184). Earlier reports from high income countries suggest that increased dietary protein is positively correlated with higher BMD (183–185). Another study reported that women with higher protein intakes were better off with higher calcium supplementation (186).

1.6.2.2 Weight, height and BMI

Bone accrual is closely related to height and weight because the bone density, size and strength need to be appropriate for body size (34). In adults, both height and weight are major indicators of PBM. With the prevalence of obesity (BMI>30 kg/m^2) among adolescents rising worldwide (187), and other studies suggesting an increased fracture incidence in obese children (188), several studies have reported on the relationship between weight and bone accrual in children (188–190). Some studies have concluded that in overweight children, aBMD is higher than in children of normal weight (191–193), whereas others have found lower than expected lumbar spine BMC in children and adolescents who are obese than in those with normal weight (194,195). It is important to note that aBMD measurement is affected by size of bone being measured therefore it is possible that in comparing studies that have assessed BMC vs those that have assessed aBMD, some differences might arise due to body size. Taller individuals naturally have higher BMC than shorter individuals. Height largely accounts for total body BMC at peak height velocity, which is the maximum rate of growth in height that occurs during puberty, typically at around 12 years of age for girls and 14 years for boys (196).

Body weight (both fat and lean mass) may possibly affect bone accrual through mechanisms related to both hormonal factors and their mechanical forces acting on bone. The growing skeleton responds to mechanical loading from eg lean muscle mass contraction and physical activity. According to Wolff's law and the 'mechanostat' theory, bone is withdrawn from skeletal places where mechanical stresses are low and added to those where demands are high, since load dictates bone formation and form follows function (197). However, people with the same BMI may have widely diverse body compositions and whilst lean mass has been reported to have a positive effect on BMD (195), the effect of fat accumulation on bone throughout growth is not clearly understood.

1.6.2.3 Puberty

The pubertal stage, defined as when one transitions from childhood to adulthood, plays a crucial role in longitudinal and appositional bone growth as well as bone mineral acquisition (198). The timing of pubertal onset varies by sex, ranging (on average) from 8 to 12 years of

age in girls and from 9 to 13 years of age in boys (199,200). In girls, pubertal timing usually defined by the age at menarche. Late age at menarche is associated with lower BMD (areal and volumetric) (201), reduced mechanical strength, and therefore higher risk of fragility fracture in adulthood (202–204). This may be associated with the effect of pubertal timing on PBM attainment. In premenopausal women, early menarche is associated with higher BMD. This association between pubertal timing and BMD could be due to earlier and therefore longer exposure to oestrogen (198).

In boys, puberty is indicated by age at voice breaking (200). Age at peak height velocity (PHV), which is the age when maximum longitudinal growth velocity occurs, can be used as an objective measure of the timing of puberty (205). Older age at puberty in boys, assessed either by Tanner stage (206) or by age at PHV (205), is negatively associated with aBMD in young adulthood. Bone accrual occurs throughout childhood, increasing rapidly during the pubertal 'growth spurt' with a peak for both bone mineral accretion and height velocity (207). About 40% of adult PBM accrual occurs during the pubertal growth spurt (139). Increases in height result in increase in bone size and this drives peak bone growth during puberty. Bone accrual continues after puberty, with men accruing more bone than women until bone accrual plateaus in the second and third decades of life (35). Certain bones e.g. femur, may continue to expand in size even after the end of linear growth. After longitudinal growth ends, bone remodeling takes over to maintain bone mass and therefore bone formation and bone resorption are balanced, that is until bone resorption outweighs bone formation in the later years of life (33,147).

During puberty, hormonal and endocrine changes lead to sex disparities in development such that by the end of puberty, males are more likely to achieve equal or higher BMC and aBMD than females, but at a later age and mostly due to greater bone size (198,208,209). In males, puberty starts later than in females, and the period of rapid bone growth lasts about four years, while in girls it only lasts about three (149). Some authors have suggested that bone accrual in females peaks at 12 to 15 years, compared to 14 to 16 years in males, levelling off at 16 to 18 years in females compared to 17 to 20 years in males (210). BMD accrual during growth and puberty, is mostly due to an increase in bone size, with minimal change in the amount of mineralized tissue within the bone envelope (149,211,212). Sex differences in bone mass at the end of puberty arise from a greater bone size increment in males (149,211,212).

1.6.2.4 Oestrogen

Oestrogen accelerates longitudinal bone growth at the beginning of puberty in females and is also a key determinant governing the closing of growth plates in both males and females (212). An increase in oestrogen production throughout puberty in females, suppresses periosteal apposition and endosteal resorption resulting in bones that are smaller and have a smaller diameter but that are not necessarily less dense (33). In females, BMD increases more by endosteal than periosteal apposition whereas in males there are greater increases in periosteal than endosteal apposition resulting in the increment of both external and internal perimeters of the cortical structure (149). By the end of puberty, cortical thickness is greater in males than in females. This difference between sexes is also seen in the frontal axis of the vertebral bodies, which is 10-15% larger in males than in females (149,213). These differences in the size, shape and mineral distribution of bones partly explain why adult women have a higher risk of fragility fractures than adult men. The increased endosteal apposition in females during puberty can be considered a biological adaptation allowing accumulation of BMD in expectation for pregnancy and lactation (149). In comparison to males of equal lean body composition, females' bone mass rises more quickly, which may indicate that growing females store more mineral than is necessary to maintain bone strength which may serve as a reserve for pregnancy and breastfeeding (214). Site-specific mineral deposition in non-weight-bearing areas may be indicative of this sex-specific adaptation (215). During puberty in females, cortical area increases more rapidly than before puberty, resulting in higher ratios of cortical area to muscle area in post pubertal females than in males due to endosteal apposition not periosteal expansion (216,217). In trying to adapt bone strength and BMD to the load placed on bone by muscle, strain threshold levels determine where bone modeling adds or strengthens bone and when bone remodeling conserves or removes it. Estrogens lower the remodeling threshold during puberty in females, affecting the sensitivity of the mechanostat and therefore causing BMD to increase more rapidly in females than in males with similar muscle strengths.

1.6.2.5 Pubertal timing

Pubertal timing predicts fractures occurring before peak bone mass attainment (218) and aBMD, such that late occurrence of puberty is associated with lower aBMD and higher risk of fractures (203,218). However, evidence supporting a role of pubertal timing in impaired bone accrual in PLWH in sub-Saharan Africa is still little. Critical stages of growth such as intrauterine phase and at peak height velocity are important determinants of adult osteoporosis risk (198). Pubertal delay, when a child's physical signs of sexual maturity don't appear by age 12 in girls or age 14 in boys (on average), may have long-lasting or permanent effects on bone mass, size, and strength into adult life (219). This necessitates

bone assessment together with a clinical assessment and interventions earlier in life, to prevent osteoporosis and fragility fractures in later life.

1.7 HIV and bone health within the sub-Saharan African context

1.7.1 HIV in sub-Saharan Africa

Worldwide, there are an estimated 37.7 million PLWH, 85% (32.2 million) of whom are in sub-Saharan Africa (1). In the sub-Saharan Africa region, the greatest burden of HIV-infection is concentrated in the Eastern and Southern Africa region (20.6 million) where country HIV prevalence in adults ranges between 15-28% (1). Although there is evidence that HIV as an epidemic is slowly stabilising in this region, the prevalence of HIV is continuing to rise because of the prolonged survival as a result of increased access to ART, combined with on-going new infections. In sub-Saharan Africa, HIV incidence is highest amongst adolescent girls and young women (aged 15 to 24 years) (1). In 2020, an estimated 27.5 million out of 37.7 million PLWH (73%) were accessing ART globally (1). Despite a huge variation in estimates of ART-coverage globally, Zimbabwe and South Africa have a fairly good uptake of ART, which was 93% and 72% respectively in the year 2020 (1). Drugs used in ART include 1. Nucleoside Reverse Transcriptase Inhibitors (NRTI) e.g. Tenofovir, Zidovudine and Lamivudine, 2. Non-nucleoside Reverse Transcriptase Inhibitors (NNRTI) e.g. Nevirapine and Efavirenz, 3. Protease Inhibitors e.g. Lopinavir/ritonavir and 4. Intergrase Inhibitors e.g. Dolutegravir. ART regimens in Zimbabwe and South Africa usually consist of a combination of three drugs, two nucleoside reverse transcriptase inhibitors (NRTI) and one protease inhibitor (PI) or non-nucleoside reverse transcriptase inhibitor (NNRTI). However, most patients will access an NNRTI-based regimen since this is significantly cheaper than a PI-based regime. HIV infection causes dysregulated immune function. Improved access to treatment and uptake of antiretroviral therapy (ART) has markedly increased life expectancy, hence HIV is now evolving to be a chronic disease presenting new risks of chronic ‘non-infectious’ comorbid and possible complications of ART. ART suppresses viral load, reducing inflammation and reconstituting immune function. However, long term use of ART has been shown to also result in renal and bone disease (220). Both HIV and ART can independently affect the skeletal system and are associated with increased fracture risk.

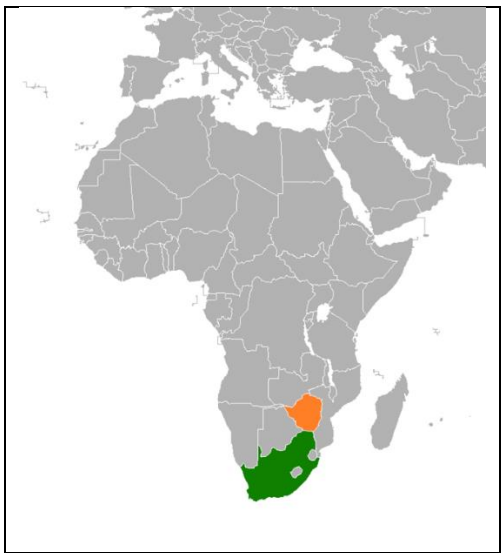
1.7.2 Background to Zimbabwe and South Africa

Zimbabwe: Zimbabwe is home to a population of 16 million people, located across 10 provinces and 63 districts. It is a land locked country in Southern Africa, sharing its borders with Zambia, Mozambique, Botswana and South Africa. The majority of people in Zimbabwe are living below the datum poverty line which is the amount of income of consumption

expenditure deemed necessary for a person to meet the basic needs of living in Zimbabwe (221). Zimbabwe's health system has experienced a decline in both services and performance over the past 2 decades, as it has been affected by economic and political instability, inadequate infrastructure, shortage of health workers and shortage of funding and resources (222). The HIV/AIDS epidemic has had a significant impact on the health care system and there are high rates of maternal and child mortality (223–225). The burden of disease is high, with a significant proportion of deaths being caused by HIV, malaria and TB. The prevalence of HIV in Zimbabwe in 2020 was 11.9% (1). The prevalence of HIV amongst children aged 0 – 14 years old in Zimbabwe in 2020 was 0.5% (1).

South Africa: South Africa is located at the most southern part of Africa and it has a population of 59 million people. It is bound by the South Atlantic and Indian Oceans to the south and it shares borders with Namibia, Botswana, Zimbabwe, Mozambique and Swaziland. Three fifths of the people in South Africa are living below the datum poverty line. The health care system in South Africa is a mixed system that includes both the private and public sector health providers. The public sector, which provides the majority of the health care services (both primary and specialised), has been facing a number of challenges in recent years, including inadequate funding, shortage of staff and equipment, leading to limited access to health particularly for those living in rural areas and under-served areas (226,227). In South Africa, in 2019, 19.1% of the population were living with HIV (1,228). The prevalence of HIV amongst children aged 0 – 14 years old in South Africa in 2020 was 0.6% (1).

Figure 1.6: Map showing Zimbabwe and South Africa



Key:- Orange; Zimbabwe, Green; South Africa

1.7.3 Bone health in PLWH in Zimbabwe and South Africa

Fracture rates in PLWH in Zimbabwe and South Africa are unknown, despite these two countries being high HIV prevalence populations. Amongst PLWH in Zimbabwe and South Africa, low DXA measured BMD (aBMD) is common in both children (229–231) and adults (232–234), potentially placing them at higher risk of fragility fracture.

1.7.3.1 Children and adolescents living with HIV

In South Africa, children living with HIV (CWH), with a mean age of 6.4 years, and established on ART for an average of 5.7 years, have been reported to have 0.17 lower TB BMC Z-scores compared to their HIV-uninfected peers (231). In Zimbabwean CWH, age at ART initiation was strongly negatively associated with both TBLH-BMCLBM and LS-BMAD Z-scores, with a 0.13 SD reduction in LS-BMAD seen for each year that ART initiation was delayed (230). Previous evidence from the same Zimbabwean cohort of children as reported in this PhD has shown CWH to have lower TBLH-BMC^{LBM} and LS-BMAD than children living without HIV (CWOH) which was more overt in later adolescence. TDF exposure and orphanhood were associated with lower TBLH-BMC^{LBM} Z-score (229). TDF exposure has been associated with low BMD in adults living with HIV (232,235). TB BMC Z-score was reported to be 0.55 higher in CWH who were switched to efavirenz compared with those remaining on Lopinavir/Ritonavir after adjustment for disease severity in South Africa (231). However, these studies used DXA, which cannot differentiate trabecular from cortical bone (236,237). Recently, cross-sectional results from a pQCT based study assessing bone in 7 to 14 year old CWH in South Africa reported lower trabecular vBMD in male CWH, and generally lower bone strength in CWH than in CWOH, after adjusting for sex, age and radial/tibial length(238). In comparison, this PhD thesis presents data from a larger cohort, followed over 12 months and presents stratification by sex and pubertal status.

1.7.3.2 Women living with HIV (WLWH)

A South African cross-sectional study conducted in PLWH [104 men (median age, 37) and 340 women (median age, 33)] recruited from a community health care clinic in Crossroads, Cape Town, reported a 17% prevalence of low (<-2 SD) lumbar spine (LS) and 5% prevalence of low total hip (TH) aBMD (239). However, this South African study lacked a comparison against people living without HIV. A recent large cross-sectional analysis of aBMD amongst community-based individuals (20–80 years) in rural South Africa, reported an association between HIV infection and lower femoral neck aBMD (240). The WBS longitudinal study in South Africa, examined the effects of HIV-infection and ART over time on aBMD in sub-Saharan Africa is the Women's Bone Health study (WBS) (232,234,241). Although aBMD was similar at baseline, the WLWH with a CD4 count low enough to be initiated on ART

demonstrated significant declines in aBMD of 2-3% over 12 months of starting ART, before and after body size adjustment, at both the femoral neck (FN) and lumbar spine (LS), compared to the women living without HIV, and those living with HIV but not on ART (232,241). The pQCT data collected on this WBS cohort is described in this PhD thesis.

To my knowledge, no previous study has examined bone architecture and strength in premenopausal WLWH using pQCT. It is important to understand how HIV and its treatment affect trabecular and cortical bone architecture at different time points in PLWH in Southern Africa.

1.7.4 Gaps in the current evidence

HIV-infection affects multiple system and organs in the body, including those involved in bone metabolism e.g. the gut (for calcium intake), kidneys (for regulation of calcium and phosphate homeostasis), liver and kidneys (for vitamin D metabolism) and parathyroid glands (for stimulation of bone formation and resorption) (242–244). In addition, HIV is associated with poor nutritional status and/or intake and low fat and lean mass. Physical activity, which is also important for its positive effect on skeletal development and maintenance (245), is lower in PLWH than without (246). The negative effects of TDF on bone have recently been demonstrated by one study which recruited a cohort of 400 breastfeeding mothers from Zimbabwe, South Africa, Uganda and Malawi and followed then over 74 weeks (132). BMD decline through week 74 postpartum was greater among breastfeeding WLWH randomized to receive maternal TDF during breastfeeding compared to those mothers whose infants received nevirapine prophylaxis (132).

As demonstrated in chapters 3 and 6, the available data on volumetric bone density, size and strength in the context of HIV and ART in sub-Saharan Africa is limited, largely because studies have been set in high-income countries and have focused mostly on males living with HIV. In addition, data on BMD in PLWH has largely focused on DXA measured aBMD in both adults and children. Most of these studies on aBMD, have also been cross-sectional in nature and therefore limited by study design. Furthermore, HIV-infection is associated with other risk factors for fragility fracture, including increased abdominal visceral fat (247), low physical activity levels, stunting, low calcium and vitamin D intake/absorption and low socio-economic status (248). These also need to be considered when assessing the effect of HIV-infection on bone density, size and strength as such risk factors may contribute more to decreases in bone mineral than HIV and/or ART alone.

1.8 Rationale, Hypothesis, Aims and objectives

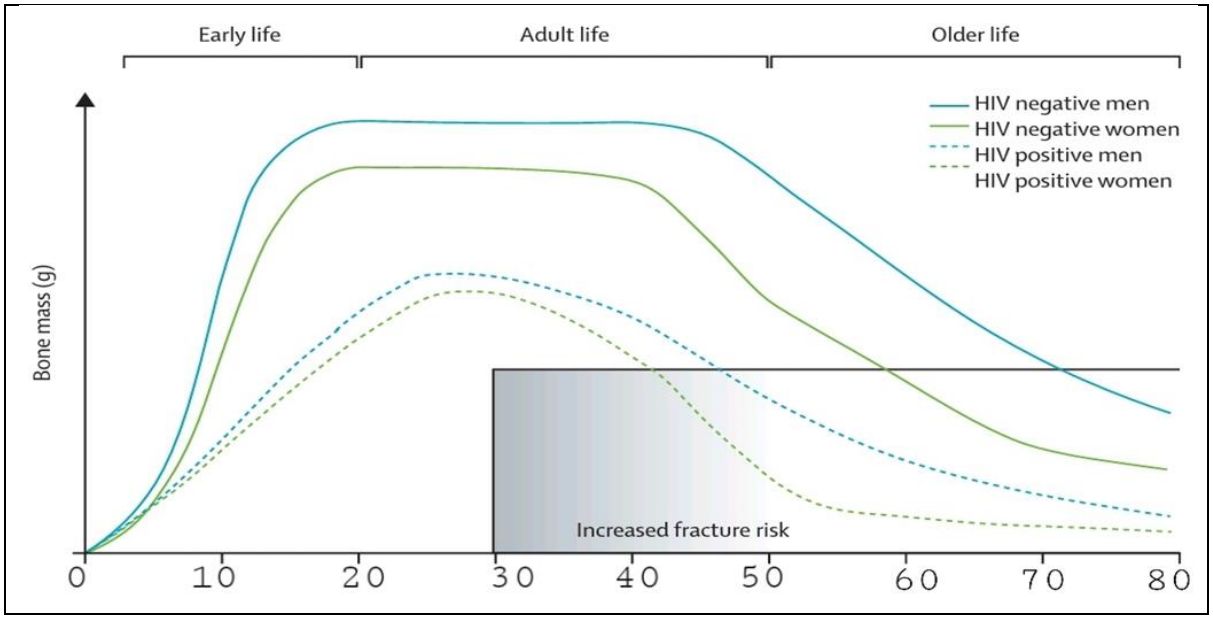
1.8.1 Rationale

The effects of HIV infection and ART on bone architecture are not fully understood. There is a high prevalence of HIV in southern Africa. Much of what is known about HIV effects on boneis derived from studies of different population groups from high-income country settings, which have limited generalisability to PLWH in southern African countries where most people with HIV are located. Due to improvements in ART, southern Africa also has an increased number of people living with chronic HIV which must be considered in determining healthcare priorities in the future. Longitudinal data is also valuable in understanding the mechanisms of bone changes with HIV and ART. Most studies performed to date are limited by the inherent limitations of the widely used DXA method of assessing areal BMD. This is particularly important in a population of PLWH because of the HIV-associated growth impairment in children and bone loss in adults. The potential benefits of pQCT which include 3-dimensional measurement of bone, separation of cortical and trabecular bone, and measures of geometry, provides greater understanding of bone loss and poor bone accrual in PLWH. This may lead to a better understanding of fracture risk in these patients and could inform clinical management and treatment choices.

1.8.2 Hypothesis

The main hypothesis is that PLWH have lower bone architecture (i.e bone density, bone size and bone strength) than people living without HIV. For perinatally infected children, this hypothesis means that CWH accrue less bone density, bone size and bone strength than their uninfected counterparts during childhood, attain a lower PBM and then go on to lose more bone than people living without HIV in adulthood (Figure 1.7). For PLWH who were infected in young adulthood life, the hypothesis is that they are likely to lose more bone than people living without HIV. Figure 1.2 is a hypothesis diagram, showing that in PLWH bone accrual is slower, PBM is lower and age-related bone loss starts earlier in the lifecourse than in people living without HIV.

Figure 1.7: Hypothesized bone mass accrual and bone loss in HIV infection



Ferrati et al, Lancet Child Adolesc Health 2020, Published Online,

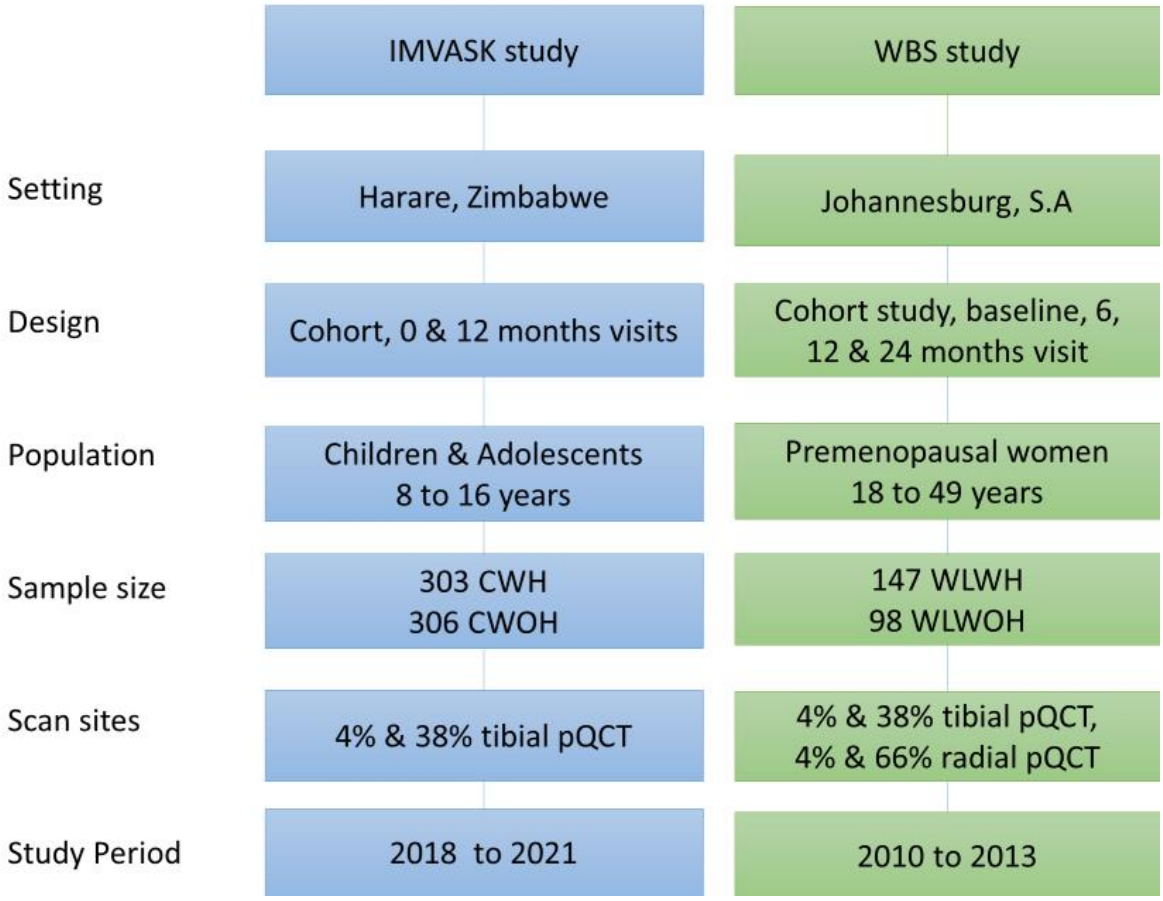
https://doi.org/10.1016/S2352-4642(20)30037-7

This graph is showing
A) HIV leads to slower accrual of bone, this may in part be a function of pubertal delay
B) PBM is lower in HIV
C) age-related bone loss starts earlier in the lifecourse in PLWH

1.8.3 Study Aim

The aim of this thesis was to understand the effect of HIV and its treatment on trabecular and cortical bone architecture at different time points, in two independent populations in sub-Saharan Africa. This study was focused on children from the IMVASK (The IMpact of Vertical HIV infection on child and Adolescent Skeletal development) and premenopausal women from the WBS (Women's Bone Health) studies completed in Harare, Zimbabwe and Johannesburg, South Africa respectively (see Figure 3). The study protocol and cross-sectional analysis of the baseline DXA results from IMVASK have been published (229,249). DXA results from the WBS study have been published at baseline (232), 12 months (233) and 24 months follow up (234). I led the data acquisition for all pQCT data for the IMVASK study. I processed and analysed the pQCT data from both cohorts.

Figure 1.8: A summary description of the IMVASK and WBS studies included in this thesis



IMVASK: The IMpact of Vertical HIV infection on child and Adolescent Skeletal development
WBS; Women's Bone Health study
S.A: South Africa
CWH: Children living with HIV, CWOH: Children living without HIV
WLWH: Women living with HIV
WLWOH: Women living without HIV

1.8.4 Study Objectives

IMVASK cross-sectional analysis (objectives addressed in chapter 4)

1. To compare the bone density, bone size and predicted bone strength parameters of trabecular and cortical bone in peripubertal males and females living with and without HIV in Harare, Zimbabwe.
2. To investigate whether any association of HIV with pQCT measured bone outcomes differed by pubertal stage by testing for interaction.
3. To determine the association between TDF exposure and bone outcomes amongst CWH.

IMVASK follow up analysis (objectives addressed in chapter 5)

4. To determine the effect of HIV on the change in bone density, size and strength over a 12-month period, as measured by pQCT.
5. To determine to what extent impairment in longitudinal growth explains any detrimental effects of HIV on pQCT-assessed bone outcomes.

WBS longitudinal analysis (objectives addressed in chapter 7)

6. To determine whether HIV infection and initiation of ART were associated with change in bone architecture (density, geometry and strength) assessed by pQCT at two skeletal sites over a two-year follow-up period.

1.9 Thesis outline

This thesis follows the format of the "research paper style" dissertation. The thesis is made up of manuscripts that have been published, are under review or ready for submission together with introductory, literature review and discussion chapters. Each paper establishes an independent chapter. The outline of the chapters is as follows:

Chapter 1 (the current chapter) presents a description of bone anatomy biology, introduces the background to the research study, justification and significance of the study, study aims and objectives and the outline of the thesis.

Chapter 2 presents a detailed description of the pQCT methods used in the studies described in this thesis.

Chapter 3 presents the review of literature undertaken for this thesis with a focus on children and adolescents, skeletal manifestations of HIV on the growing skeleton, the epidemiology of pediatric HIV and bone and what is known about bone in children and adolescents living with HIV.

Chapter 4 is a published manuscript describing cross-sectional analysis of bone architecture in children and adolescents living with and without HIV. The paper is titled "Impaired bone architecture in peripubertal children with HIV, despite treatment with anti-retroviral therapy: a cross-sectional study from Zimbabwe" published in the Journal of Bone and Mineral Research in Vol. 38, No. 2, February 2023, pp 248–260. DOI: 10.1002/jbmr.4752.

Chapter 5 is a manuscript which has been submitted to Journal of Bone and Mineral Research. The title of the paper is “Changes in pQCT measured bone density, size and strength in Zimbabwean children with and without HIV over one year: a cohort study”.

Chapter 6 presents the review of literature undertaken for this thesis with a focus on adult women, skeletal manifestations of HIV in adults, the epidemiology of HIV and bone and what is known about bone in premenopausal women living with HIV.

Chapter 7 consists of a manuscript which is yet to be submitted to a journal. The manuscript describes the longitudinal changes in bone density, size and strength in premenopausal women.

Chapter 8 is the main discussion of all study findings, conclusions and recommendations for future research.

2 CHAPTER 2: pQCT methods

2.1 Introduction

Factors impacting the mechanical strength of bone include bone size, the quantity and distribution of bone tissue within the periosteal envelope, the degree of mineralization and the structural organization of the organic matrix. In each individual, bone accrual occurs at different rates from foetal development through to attainment of peak bone mass (PBM) (250,251). Dual-energy x-ray absorptiometry (DXA) measured areal bone density (aBMD) is the most widely reported bone outcome due to the fact that DXA is the clinical gold-standard for the diagnosis of osteoporosis. It is used to measure aBMD which is the amount of bone mineral in grams divided by the scanned bone area in square centimetres (18). These aBMD measurements are used to define osteoporosis and to predict fracture risk (Blake, 2007). The World Health Organization (WHO) established, in the 1990's, a set of ranges for aBMD measurements as a working definition for osteoporosis, indicated by a T-score which shows the number of standard deviations the aBMD result is to the average peak bone density of a normal young adult, whilst a Z-score shows the number of standard deviations the aBMD result is to the average peak bone density of a person of the same age and gender. A T-score of -1 or greater is considered normal, that between -1 and -2.5 indicates low aBMD (osteopenia) and that of -2.5 or less indicates osteoporosis. DXA advantages include the fact that it is accurate; it uses low energy beams and low ionizing radiation to measure aBMD and is easily accessible. However, DXA utilizes two-dimensional planar imaging and therefore yields an areal bone density measure, not a volumetric BMD (vBMD). Bone density is determined by the degree to which a radiation beam is attenuated by all tissues, with bone density being calculated from the comparison to standards that convert attenuation co-efficients to density. The attenuation of a radiation beam not only depends on the physical density, but also on the size of the bone i.e the length of the path that the beam takes across the bone (252). In estimation of bone density by 2-dimensional imaging e.g. by DXA, small bones will have a lower aBMD than large bones, even if the physical density is the same. So sometimes a low aBMD value can just mean that a bone is small with normal vBMD (252). This is an issue in children, especially if there are disorders that delay or impact growth. In an attempt to address this, DXA measurements can be size adjusted, yielding lumbar spine bone mineral apparent density (LS-BMAD) and total-body less-head bone mineral content for lean mass adjusted for height (TBLH-BMC^{LBM}) (253)

Bone strength is defined as the maximum load that a bone tolerates before it bends or breaks (254). Bone strength is attributable to the interaction of material properties, the amount of material, and the morphological, organizational, and other quality issues of bone

tissue. The density, size and shape of bone together with how well it distributes pressures that generate strain are all important factors when determining bone strength. aBMD does not directly equate to bone strength, especially in the growing skeleton. When subjected to mechanical demands, a bone may undergo periosteal diameter expansion, increasing bone volume and resulting in reduced aBMD (since it is a ratio of mass to volume) but not the bending strength of the bone. Less material would be necessary to maintain the stiffness of the bone in the presence of a diameter expansion (254). The degree of bone strength and, consequently, the likelihood of developing fragility fractures as an adult, are factors influenced by architectural properties. Bones adapt to be able to tolerate strain and function under normal loading conditions. Factors that increase strain on bone include bone lengthening during growth, increased muscle mass, and increased physical activity (254). Even with endosteal resorption, periosteal apposition can help to reduce the stress and strain placed on long bones by partially maintaining the bone's cross-sectional area, which preserves bending strength. Mechanical load placed on bone induces periosteal changes and helps maintain bone strength. Structural properties bear more weight than material properties and bulk does not indicate actual bone strength. Low bone strength leads to fractures. Bone properties such as stiffness, static strength, toughness, and fatigue can change under different loading and physiologic conditions. These parameters are measured using cross-sectional 3-dimensional imaging methods which include pQCT. How the bone is loaded is also relevant. Bones respond differently to various types of loading, such as static or impact loading, depending on their architecture. Bones adapt based on their typical loading conditions. Understanding physiologic loads placed on bones e.g. from muscle-bone interactions may also be important in understanding bone strength (43–45).

Cortical and trabecular bone have different architectures and functions and therefore react differently when loaded. Cross-sectional 3-dimensional imaging methods, such as peripheral quantitative computed tomography (pQCT) offer an opportunity to better understand the skeletal system by measuring bone size and bone strength in addition to volumetric bone density (255,257–259). pQCT is a non-invasive three dimensional imaging method that uses x-rays and the principle of first generation CT scanners to measure volumetric BMD and geometry of bone in the peripheral skeleton (radius and tibia). pQCT provides a detailed assessment of cortical and trabecular bone compartments and therefore can increase our understanding of how shape and organisation of bone contribute to fracture risk over and above areal BMD measured by DXA. pQCT provides greater insight on mechanical properties of bone such as section modulus, cross-sectional moments of inertia (CSMI), and estimated bone strength. pQCT improves the estimation of bone strength by estimating vBMD, cortical thickness, cross-sectional area (CSA), CSMI and stress strain index (SSI)

which cannot be assessed using DXA (255,258–264). pQCT can provide detailed information distinguishing between trabecular (spongy bone) and cortical bone (dense, hard bone which forms the outer layers). pQCT also provides bone geometry measures such as cross-sectional area, which is essential in determining bone strength. This chapter aims to describe the pQCT methods used for this PhD. This chapter will be divided into 2 sections with the following headings

- Section 1: Background to pQCT as a method
- Section 2: pQCT methods used in the IMVASK study

2.2 Section 1: Background to pQCT as a method

2.2.1 Single slice XCT 2000 pQCT machine principle of operation

The basic principle of any CT scanner is that the internal structure of an object can be reconstructed from multiple projections of the object. pQCT uses 180° projections (with reconstruction by back projection) around the patient's limb to reconstruct the attenuation coefficients of tissue (265). The pQCT scanner uses the translate/rotate principle of operation and a pencil beam system. The x-ray tube generates an x-ray beam with a thickness of 2.5mm, which is filtered to absorb the low energy spectrum, leaving a narrowband beam at 37 keV (266). The voltage is adjusted to a fixed value of 60 kV and the anode current is regulated to a fixed value between 140 and 220 µA to maintain a constant intensity. An array of semiconductor detectors gathers data when the beam from the x-ray tube is being transmitted through it. This happens during the transmission of the emitted photons. The semiconductor detectors on the XCT 2000 pQCT (Stratec, Medizintechnik, Germany) assembly detect x-rays with an energy of roughly 38 keV with a detection rate of about 100%. Each detecting unit is made up of a semiconductor, a preamplifier with a high charge sensitivity, a shaping amplifier, and a comparator. The shielding is made in such a way that it prevents the detectors from being affected by natural background radiation.

How an object absorbs x-rays depends on strength of the x-rays are and what it is made of. This gives an absorption profile. The dead time and beam hardening in the raw data are fixed. A cross-sectional image of the original object can be created by mathematically combining numerous absorption profiles from various angles. The name for this method is "filtered back projection." Each point on the image, called a "voxel (volume element)," is equal to a specific attenuation coefficient. The attenuation coefficient, which describes how much the x-ray beam is diminished when it passes through a particular material, is typical for a given x-ray energy and for a given material. The attenuation coefficients can be converted to density values (mg/cm^3) through calibration using phantoms with a predetermined hydroxyapatite concentration. During calibration, only the mineral content of bone is taken

into consideration. The mechanical section allows the x-ray source and detectors to be moved in three different orientations. During the scout scan, the limb is scanned in 1 mm increments along the axis of the limb. The beam travels perpendicular to the limb's axis. The screen displays a color-coded digital image similar to that of an x-ray, with bright spots depicting bone and dark areas representing soft tissue. The distance between the scan line and the reference line is constant. After traversing the limb being scanned perpendicular to its axis, the gantry is rotated 12 degrees and the operation is repeated. The distance between the twelve CT-scan detectors and the x-ray source is 1 degree. Consequently, 15 rotations of the gantry will yield 180 projections. The required angular range for these projections is 180 degrees. The scan software computes a variety of densitometric and structural parameters. pQCT outcomes (Table 2.1) include bone mineral density [BMD (g/cm^3)], bone mineral content [BMC (g)], cortical thickness (mm), cross-sectional area (mm^2) and indices of bone strength such as the polar strength strain index (SSI_p;mm^3), cross-sectional moments of inertia (CSMI; mm^3), bone strength index of compression (BSIc; g^2/cm^4) and section modulus (Z, mm^3) (10,267).

Table 2.1: Bone density, size and strength pQCT bone outcomes and their meanings

pQCT parameters	Meaning
Bone density	
Total density	Total volumetric bone mineral density (mg/cm^3)
Trabecular density	volumetric bone mineral density (mg/cm^3) of trabecular bone
Cortical density	Volumetric bone mineral density (mg/cm^3) of the cortical bone
Bone size	
Total cross-sectional area	Total cross-sectional area (mm^2) of the bone
Trabecular cross-sectional area (CSA)	Cross-sectional area (mm^2) of the trabecular portion of the total bone area
Cortical area	Cross-sectional area (mm^2) of the cortical portion of the total bone area
Cortical thickness	The average thickness (mm) of the cortical shell
Periosteal circumference	Periosteal circumference (mm) or outer diameter of bone
Endosteal circumference	Endosteal circumference (mm) or inner diameter of bone
Bone strength	
Cross-sectional moment of inertia (CSMI)	Cross-sectional moment of inertia (mm^3): indicative of bending strength
Polar moment of inertia	Polar moment of inertia (mm^3): indicative of strength in torsion
Stress strain index (SSI)	Density weighted polar moment of inertia
Bone strength index (BSI)	Total area ×Total density^2

Zemel B, Bass S, Binkley T, Ducher G, Macdonald H, McKay H, et al. Peripheral Quantitative Computed Tomography in Children and Adolescents: The 2007 ISCD Pediatric Official Positions. J Clin Densitom. 2008;11(1):59–74 (268)

2.2.2 The pQCT bone density outcome measures

Volumetric BMD, as measured by pQCT, is a true measure of bone density; defined as the amount of bone mineral in milligrams divided by the scanned bone volume in cubic centimetres (269). vBMD is not distorted by size. pQCT distinguishes between trabecular and cortical density (Table 2.1). In pQCT terminology, the parameters that are applied for all sectional image analysis include contour mode, peel mode and cortical mode (266). Contour mode is the edge detection parameter that distinguishes bone from soft tissue. It provides automatic edge detection but the operator defines the threshold. The algorithm finds the first voxel of the outer bone edge based on the threshold set by the user. Then, this first voxel is compared to a set of its neighboring voxels, and those voxels are checked in a certain way to find the edge of the bone (270). Peel mode distinguishes trabecular bone from cortical subcortical bone, starting at the endosteal surface and using a user defined threshold (270).

2.2.2.1 Total vBMD

Total volumetric bone mineral density (vBMD) in mg/cm^3 represents the mass of mineral, which is the bone mineral content (in mg) per unit volume (in cm^3) of the entire cross-sectional bone slice (265,271). Total vBMD is therefore a product of the cortical and trabecular vBMD and the ratio between total cross-sectional area and cortical cross-sectional area. The ratio between total cross-sectional area and cortical cross-sectional area varies more than cortical vBMD during normal bone development, making it the main determinant of total vBMD in growing skeletons (271). Total vBMD may therefore not be a good indicator of bone strength in children.

2.2.2.2 Trabecular vBMD

Trabecular density is the average density of the region of interest defined as trabecular bone, calculated from attenuation of the x-ray beam in that region. It is not the actual material density of the trabecular bone itself, as it potentially includes bone marrow. Given that material density of human bone under normal physiologic conditions is estimated at 1200 mg/cm^3 and trabecular volume of, e.g. 25%, the apparent trabecular density would be 300 mg/cm^3 (25% of 1200mg/cm^3) (272). Trabecular vBMD is measured in mg/cm^3 by averaging the density of a region towards the centre of the bone, most defined as pragmatically 45% of metaphyseal CSA. Trabecular vBMD is affected by trabecular spacing, number and thickness, as well as the marrow density. Medullary area is calculated as the difference between total area and cortical area. pQCT uses an automatic threshold-based iterative edge detection-guided segmentation of marrow from the bone using the CALCBD analysis with an outer density threshold of e.g. 180

mg/cm^3. Within an individual, trabecular structure and therefore trabecular vBMD varies between adjacent pQCT slices along the length of the metaphysis for both the radius (251) and tibia (273). Trabecular vBMD is an independent indicator for fracture risk in healthy children: i.e lower trabecular vBMD has been associated with increased fracture risk in healthy children (274,275).

2.2.2.3 Cortical vBMD

Cortical vBMD is the amount of mineral per voxel (i.e per unit of cortical bone volume), and combines including both bone tissue and pores, i.e the material density and the cortical porosity (apparent volumetric density) (276). It is directly related to the amount of calcification of the bone matrix, which is known to change in a way that is proportional to the cortical bone tissue's elastic modulus (a measure of the material quality and intrinsic stiffness of bone) (277). Cortical bone is composed of 90 – 98% bone material and 2 – 10% osteons, therefore measured cortical density is 90 – 98% of the material density (265). In single slice pQCT, cortical parameters are usually not reported at the 4% site because the thin cortex can result in underestimation of bone values at this site. There is a tendency to underestimate the actual cortical vBMD measurement as a result of the fact that voxels at the periosteal and endocortical borders of the cortex are not completely filled with bone (the partial volume effect). The extent of this underestimation is greater in thinner cortices such as those at the 4% site, because they have a higher surface to volume ratio than thicker cortices. Consequently, even if the mass of the mineral per unit volume of the cortical compartment is identical, pQCT measured cortical vBMD increases with cortical thickness (271). This bias that is also accentuated in smaller bones therefore in smaller children, cortical vBMD may seem to increase with cortical thickness despite there being no change in material density (278,279). Using a smaller voxel size could possibly reduce this effect but that would mean an increased radiation exposure per scan and is limited by the technology (280,281). Cortical bone is mostly influenced by the rate of intracortical remodeling (271). As bone tissue matures, there is infilling of the Haversian systems, reducing cortical porosity and therefore also increase average cortical vBMD. Older cortical bone tissue has greater density than newly formed bone (282). Cortical bone is affected by the rate of bone turnover. It is crucial to remember this when interpreting differences in vBMD between participants, or when modelling changes in vBMD with age, as the resolution of pQCT does not distinguish between these various factors.

2.2.3 The pQCT bone size outcome measures

Bone geometry is the measure of the dimensions of bone and is used as a predictive indicator of both bone strength (250) and fracture risk (283). Quantitative computed tomography (QCT), high-resolution peripheral QCT (HRpQCT), and pQCT can measure bone geometry and help the effort to distinguish among individuals with and without fragility (low trauma) fracture (284,285). Bone geometry outcomes include cross-sectional area, cortical area, periosteal circumference and endosteal circumference (250). For cortical thickness, endosteal and periosteal circumference, a contour closing circular-ring model and user-defined inner and outer thresholds can be used.

2.2.3.1 Total Cross-sectional area

Total CSA is the area of the entire bone cross-section. Total cross-sectional area is directly measured, making it a reliable measure of outer bone size. At the diaphysis, total cross-sectional area includes both the cortical bone and marrow cavity. By finding the outer and inner cortical bone contour at a certain threshold, such as 710 mg/cm^3, the total cross-sectional area of the limb at the diaphysis can be calculated. Total CSA at the diaphysis is entirely determined by periosteal apposition (271). In the growing skeleton, as bone length increases, newly formed bone is continuously deposited at the junction between the growth plate and the metaphysis (251). At the opposite end of the metaphysis, all trabecular bone is removed as the cortex is integrated into the diaphysis. As the growth plate progresses in a distal direction, a section of newly formed metaphyseal bone undergoes a reduction of its diameter by periosteal resorption until it reaches the cross-sectional size of the diaphysis, a process known as metaphyseal inwaisting (251).Total CSA at the metaphyseal sites, therefore, depends on both periosteal apposition and endo-cortical resorption such that it increases as periosteal apposition occurs and decreases as a result of metaphyseal inwaisting (251,286,287). Metaphyseal CSA is the area of the entire cross-section of the metaphysis, including cortical and trabecular bone compartments. After cessation of growth, bone size changes minimally (250). In adults, aging bone strengthens by increasing cross-sectional area through endosteal bone reabsorption and periosteal bone apposition (33). This bone remodelling is more pronounced in males, but also occurs in females, particularly around the climacteric where varying degrees of cortical periosteal deposition and endosteal reabsorption produce different effects on cortical thickness, bone cross-sectional area and bone strength in the pre-, peri- and post-menopausal periods (33). The cross-sectional area (CSA) of the trabecular bone is determined after detecting the outer bone contour at a threshold of 180 mg/cm^3.

2.2.3.2 Cortical cross-sectional area

Cortical CSA is the surface area of the cortical bone cross-section, representing the area covered by pixels identified as "cortical" by the software, in mm^2. Cortical CSA is the total CSA minus the cross-sectional size of the marrow cavity. Cortical CSA calculation is also based on 710 mg/cm^3 threshold, such as total cross-sectional area at the diaphyseal site. Cortical CSA depends on both periosteal apposition and endocortical resorption (271).

2.2.3.3 Periosteal and endosteal circumference

The periosteal circumference is also a measure of the distance around the bone. Unlike cross-sectional area which is directly measured, periosteal circumference is mathematically calculated from total CSA by assuming the bone cross-section is circular.

2.2.3.4 Cortical thickness

The determination of cortical thickness using the circular ring model in pQCT is based on the assumption that the shape of the measured bone is circular (288). Cortical thickness is then calculated as the difference between the outer edge (periosteal circumference) and the inner edge of the limb (endosteal circumference) (251). Figure 2.1 shows a diagrammatic representation of the circular ring model assumption. The accuracy of cortical thickness measures based on the circular ring model for the tibia is sometimes disputed (278,289), with a suggestion that is should be applied with caution given that the shape of the tibia is more triangular than circular. A study which aimed to assess the accuracy of pQCT measures using dry human tibia specimens, reported that estimates of cortical thickness derived with pQCT assuming a circular ring model may be overestimated, and the overestimation increases with the thickness of the cortex (288).

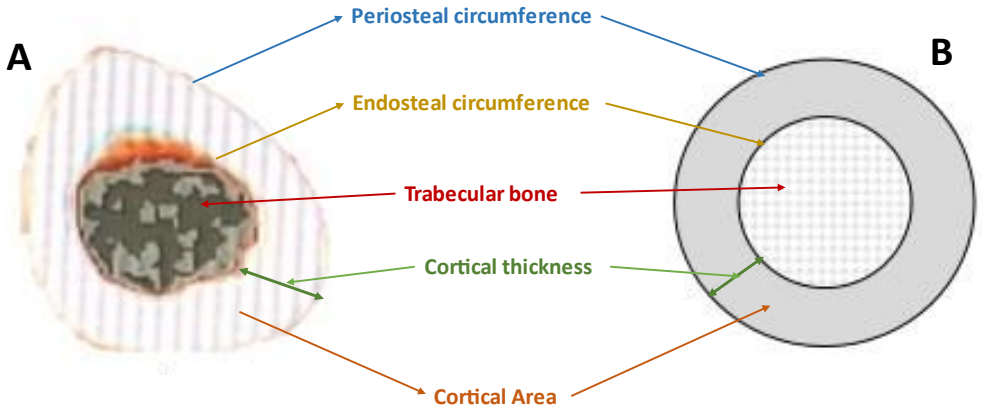
2.2.4 The pQCT bone strength measures

pQCT is used to predict bone strength by providing a measure of cross-sectional moment of inertia (CSMI), stress strain index (SSI) and bone strength index (BSI) (258). Animal and cadaver studies have shown a high correlation between the force required to break the bone and bending strength measured using pQCT measured BSI (r=0.94) (290). Compressive strength is estimated at the most trabecular rich parts at the epiphysis when the total bone density and total area are known (254). At the cortical sites, bending strength is estimated in many planes as the SSI and as section modulus (SM). Cross-sectional area is inversely related to strength in compression and SM is inversely related to maximum stress in bending (254).

However, bone size measures like cross-sectional area do not completely explain bone strength and resistance to fracture.

Figure 2.1: The circular ring model used in calculation of cortical thickness from a pQCT image

Actual example of bone shape from a pQCT image (A) vs assumed shape of bone using the circular ring model (B)



Cortical thickness = $(p_{circ}/2\pi)-(e_{circ}/2\pi)$

Where p_{circ} =periosteal circumference (mm), e_{circ}=endosteal circumference (mm) and π =the constant pi (mm^2)

Image courtesy of Cynthia Kahari

Bone geometry (size and distribution of bone material within a cross-section) and bone tissue material properties (e.g., elastic modulus, which determines bone material stiffness before yielding, and micro-structural factors, which affect bone post-yield behavior) affect bone's ability to resist different types of loading (axial compression, bending, torsion) (291). For diaphyseal bone to best resists loading from multiple directions, there should be optimum distance between the cortical bone mass and the neutral axes (291). The greater the distance of mineralised tissue from the axis of rotation, the greater the resistance to bending.

2.2.4.1 Cross-sectional moments of inertia

The CSMI describes this distribution of material, showing the architectural efficiency of the bone tissue distribution in the cross-sectional design of the cortical shell to resistance to bending and torsional loading (266,291). The CSMI depends on CSA and the distribution of bone in that area with respect to the axis of rotation. The CSMI is determined by calculating the integrated sums

of products of the area of each voxel in the defined cortical image by their squared perpendicular distance to the neutral bone axes passing through the center of mass of the bone image in mm^4 (Figure 2.2) (266).

2.2.4.2 Section modulus

Section modulus (SM), which is an estimator of torsional strength, is derived from the CSMI and the maximum distance between the center of the identified area and its outer boundary (291). SM is CSMI divided by the furthest point from the periosteum to a given bending axis (Figure 2.2). In terms of bone tissue's material properties, the elastic modulus (E) is a way to measure how stiff the material is and it depends on the degree of tissue mineralization and microstructure (291,292). pQCT cannot directly measure the elastic modulus of bone but pQCT derived cortical vBMD is used to estimate the mineralization of bone tissue, which is associated with elastic modulus. The ratio of the measured cortical vBMD and the normal physiological density of hydroxyapatite (1,200 mg/cm^3) provides an estimate of the modulus of elasticity (266,272,291).

Figure 2.2: Calculation of CSMI, SM and SSI in pQCT

$$\textbf{CSMI} = \sum_{voxels} (d^2 \times a)$$

$$\textbf{SM} = \sum_{voxels} \frac{(d^2 \times a)}{(dmax)}$$

$$\textbf{SSI} = \sum_{voxels} \frac{(d^2 \times a \times vBMDvox)}{(dmax \times vBMDmax)}$$

Where

d **: distance of each voxel from centre of gravity (mm)**

d_{max} **: maximum distance of any of the voxels of the cortical cross section from center of gravity (mm)**

a **: cross sectional area of a voxel (mm^2)**

$vBMD_{vox}$ **: measured volumetric bone mineral density of each voxel (mg/mm^3)**

$vBMD_{max}$ **: maximum cortical volumetric bone mineral density of human bone under normal physiologic conditions (mg/mm^3)**

Medizintechnik GnmH Stratec. XCT-2000 manual software version 6.20. Pforzheim, Germany. Medizintechnik GnmH Strat. 2007;66 (265)

2.2.4.3 Stress strain index

SSI is a bending strength estimator, which is predictive of the mechanical strength of bone, and is calculated using both geometrical and material properties by multiplying the SM by the ratio of the measured cortical density to the normal physiologic cortical density (1200 mg/cm^3) (293). Since the maximum theoretical value for cortical vBMD is 1200mg/mm^3, SSI is a standardized

form of expression of bone strength from both the geometrical and quality (cortical vBMD is related to a fixed, maximal value) points of view (266). Polar SSI (SSIp) predicts the torsional bone strength whereas axial SSI (SSIx) predicts the bending strength relative to the X or Y axis. SSIp which is independent of rotation is better reproducible since SSIx values are affected by different rotation of the limb during the measurement. The stress–strain index is defined as the cross-sectional moment of inertia (CSMI) weighted by density distribution and calculated by the (288). Measuring SSI and CSMI at the long bone epiphyses is incorrect because strength indices are usually derived from cortical bone geometry, and therefore require accurate assessment of the cortical compartment (265). The average cortical shell thickness at the epiphysis can be less than the voxel size used in clinical imaging (0.2 to 1.0 mm (XCT2000L)), making pQCT unable to accurately assess cortical compartments at these trabecular rich sites. In addition, these indices assess resistance to torsional and bending loads which are common in the diaphysis whilst the epiphysis is primarily loaded in axial compression (291). To resist the compression stress transmitted from the large cartilage areas of the joint, the trabecular network at the epiphysis is conveniently aligned to absorb energy from impacts to the cortical shells (266,291).

2.2.4.4 Bone strength index of compression

The bone strength index of compression (BSIc) is the total bone cross-sectional area multiplied by the square of the total density (291). BSIc can be assessed at trabecular rich sites such as the long bone epiphysis, providing a non-invasive bone compressive strength estimate measure which takes into account both bone material and its distribution (291). BSIc is appropriate for sites that are primarily loaded in compression and can be used to assess the distal parts of the radius or tibia in unilateral loading.

2.2.4.5 pQCT bone outcomes and fracture risk

The relationship between pQCT bone outcomes and fracture risk has not been as well studied as the relationship between DXA measured bone outcomes and fracture risk and this has been a limitation of pQCT becoming a clinically-accepted technique for prediction of fracture risk in adults. An earlier study utilising pQCT in 214 women, aged 45 to 85 years, showed that trabecular vBMD, total vBMD and cross-sectional moments of inertia were associated with fracture but this was not the case for cortical vBMD (294). This is contrary to the findings from a cross-sectional study of 62 men and women older than 50 years which showed that cortical density, cortical area and cortical thickness at the 20% tibial site were all associated with fractures (prevalent vertebral fracture or low trauma fracture), but this was not the case for 4% trabecular vBMD and total CSA at the 4% and 20% sites (295). In Australian females aged 51 to

83 years, after adjusting for age, sex and BMI, 4% total and trabecular vBMD, 4% CSA, 66% cortical density and 66% cortical thickness were all associated with prevalent low trauma fracture except for 66% CSA and SSI (296). In a 3 – 6 year longitudinal study of 1143 white men who had radial and tibial pQCT scans at the 4%, 33% and 66% sites, every standard deviation decrease in total CSA, cortical CSA, SSI, CSMI and section modulus was associated with approximately a 2-fold increase in fracture risk, after adjusting for age, site, BMI and femur neck aBMD (297). In 5 to 16 year old children, after adjusting for sex, age, black race, Tanner stage, height and injury type, 4% total vBMD, 20% cortical vBMD, 20% cortical area and 20% SSI were associated with an increased risk of fracture but this was not the case for trabecular vBMD (298). Bone strength index of compression but not SSI was shown to be associated with fracture risk in 77 women who were 50 to 78 years old. In 33 to 96 year old men, prior fracture was negatively associated with radial 4% total density (OR=0.61; 95%CI=0.47–0.78), 4% trabecular density (OR=0.62; 95%CI=0.48–0.79), 66% total density (OR=0.69; 95%CI=0.55–0.87) 66% cortical thickness (OR=0.68; 95%CI=0.54–0.86) and tibial 4% total density (OR=0.67; 95%CI=0.52–0.86), 4% trabecular density (OR=0.64; 95%CI=0.50–0.82), 66% cortical density (OR=0.74; 95%CI=0.58–0.94), 66% cortical area but were attenuated (for tibia) in models adjusted for age, height, weight, smoking, mobility, alcohol consumption (299). In both adults and children, none of the pQCT bone outcomes have been consistently shown to be associated with fractures, although all pQCT bone outcomes have been shown by one study or another. Though some of the studies mentioned here have a small sample size eg, the cross-sectional study of 62 men and women (295), and though studies did not agree on which ones of the pQCT bone outcomes, largely all of the pQCT bone outcomes discussed under this section have been shown to be predictive of especially prevalent fractures in adults. More investigation is needed to assess pQCT bone outcomes and incidental fracture risk in both adults and children.

2.2.5 Factors affecting pQCT image quality

Factors affecting pQCT image quality include placement of reference lines and participant motion during scan acquisition (300). Participant motion during scan acquisition and subsequent movement artifacts in the scan image present a challenge in pQCT imaging (267,301). Movement during scan acquisition can range from subtle muscle twitching or coughing/sneezing to a patient who is restless and cannot keep still. Movement degrades the quality of pQCT images leading to unusable scans and a need to re-scan the participant in order to obtain a

usable scan (267). pQCT operators need to know when to repeat a scan. In addition, a criterion for determining whether or not a scan can be included in data analysis is also required. Scans are qualitatively graded by visual inspection, to determine if there are any movement artifacts and the degree to which they affect the image quality (300). However, this approach is subjective and can result in varied gradations for a single rating. Having one person perform the visual assessment can limit inter-operator variability.

2.2.6 Reliability and validity of pQCT scans

Reliability is when you get the same finding each time a test is done whilst validity is when you get a true finding each time a test is done. If repeated measures yield the same outcome, its very reliable but if that outcome is different from the true outcome, then validity is poor. In simplified terms, reliability relates to how precise or reproducible a measurement is wheras validity relates to how accurate a measurement is.

Reliability: Precision is a measure of the reliability of repeated measurements in the same or different individuals over a long or short time and is determined by both the machine and the operator. Precision makes us more certain of the initial measurements and the ability to detect small changes with future measurements in the same patient (302). Precision errors are evaluated by performing repeated scans on a representative set of individuals to characterize the reproducibility of the technique (18,303).

The coefficient of variation (CV) shows what proportion of the mean is represented by the SD. CV can also be expressed as a percent coefficient of variation (%CV), after being multiplied by 100%. Precision error (PE) is expressed as the root mean square-standard deviation (RMS-SD) as an absolute measure with the same units as the measurement or the root-mean-square %CV (RMS-%CV) as an expression of precision error in percent relative to the mean value of the population (304). The RMS-SD and RMS-CV are preferred to the arithmetic mean SD and CV because the latter quantities tend to underestimate the Gaussian error (14). Studies assessing reliability and reporting the precision of pQCT bone outcomes have been done in humans, including both adults and children and these have reported pQCT to be a relatively precise technique. Evidence has also shown that pQCT reliability can be influenced by the time between repeat scans (305), age, measurement site and gender (306). Tibial measurements have been reported to be more precise than radial measurements (RMSCVs = 0.2–7.4 % [tibia] vs RMSCVs = 0.7–20.3 %[radius]) (306). In children aged 8 to 14 years, better precision for pQCT bone outcomes has also been reported for the tibia compared within the radius (289). The 4% site has been shown to have better reliability and validity for the total and trabecular

bone measurements than for the cortical bone measurements due to the limited spatial resolution of pQCT resulting in partial volume effect (307). However, most studies reporting validity of pQCT scans have been done either in mice or using specimens from human cadavers. In an experimental study pQCT measured SSI was shown to be significantly correlated with a biomechanical bone strength index, the maximum load at bone failure, assessed in a three-point bending test, using the right tibias of 15 male New Zealand White rabbits (308). Validity may also be affected by the fact that pQCT has low resolution. A study assessing pQCT measured tibial cortical thickness measurements in 15 dry human tibia specimens showed that cortical thickness measures derived using the circular ring model are overestimated, increasing with the thickness of the cortex (288). A possible explanation for this is that the low resolution in pQCT results in partial volume averaging which occurs when a single voxel contains tissues of different densities, such as the boundary between bone tissue and soft tissue, and therefore the attenuation coefficient assigned is some middle ground between the two (288).

To assess if machine calibration has not changed or drifted over time, a quality assurance phantom provided by the manufacturer is scanned daily and %CV can also be calculated for the phantom measurements (309). It is important to assess precision when pQCT measurements are used to follow up changes in bone outcomes over time.

The only way to know if a real biological change has occurred between two measurements in one participant is to assess if the change in the bone outcomes has exceeded the precision error (310). This is done by calculating the least significant change (LSC) as recommended by the International Society of Clinical Densitometry (ISCD) (311). The least significant change is calculated based upon the measured precision errors and a selected level of statistical confidence (312). This is important particularly for clinical work as contrasting observed bone changes of an individual patient to LSC estimates can assist in clinical decision-making (292). Studies reporting least significant change in children measured using pQCT are scarce. Duff et al defined least significant changes for pQCT bone outcomes in children (289)

Validity: Most studies reporting validity of pQCT scans have mostly been done either in mice or using specimens from human cadavers. In an experimental study pQCT measured SSI was shown to be significantly correlated with a biomechanical bone strength index, the maximum load at bone failure, assessed in a three-point bending test, using the right tibias of 15 male

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2.3 Section 2: pQCT methods used in the IMVASK and WBS studies

Trained radiographers performed the pQCT scanning using an XCT 2000 (Stratec Medizintechnik, Pforzheim, Germany) at Parirenyatwa Group of Hospitals in Harare Zimbabwe for the IMVASK study. For the WBS study, trained radiographers performed the pQCT scanning using an XCT 2000L (long version) bone densitometer (Stratec Medizintechnik, Pforzheim, Germany) at the Witz Developmental Pathways for Health Research Unit (DPHRU), in Soweto, Johannesburg. For the IMVASK study, pQCT scans were acquired by 4 radiographers, including me. We were all trained by two experienced pQCT experts who work together and use the exact same protocols. I then further trained the other three radiographers on pQCT acquisition using the same protocol. We had technical support from the pQCT experts throughout the study. All radiographers were trained to assess for movement artefact whilst the participant was still in the unit and repeat the scan if there was need. For the WBS study, pQCT scans were acquired by 2 radiographers who had both been trained by the same person (one of the pQCT experts on the IMVASK study).

2.3.1 Participant preparation

Participant preparation for the pQCT scan included explaining the procedure to the participant. To avoid movement artefact, participants were asked not to talk and not to move any part of their body during the scan acquisition process. In addition to emphasising the importance of them remaining still during the scan, radiographers also talked to the participant before the scan, to find out which one is their non-dominant leg and non-dominant arm (for WBS only), whether or not, they have had a fracture, injury or any metal inserted in any of their legs. The dominant tibia was defined as the participants' kicking foot. In the case of a participant unsure which leg they would kick a ball with, the leg contra-lateral to the dominant arm is the one which was scanned. If the participant had suffered a fracture at the non-dominant leg and/or non-dominant arm (WBS), then the dominant leg or dominant arm was scanned. The importance of dominance is based on the fact that the non-dominant limb is weaker and has less strength compared with the dominant limb (313,314). Literature suggests that an injury or fracture on the dominant limb has more initial impact on daily function, but it has a better chance for recovery than a fracture on the non-dominant side (315).

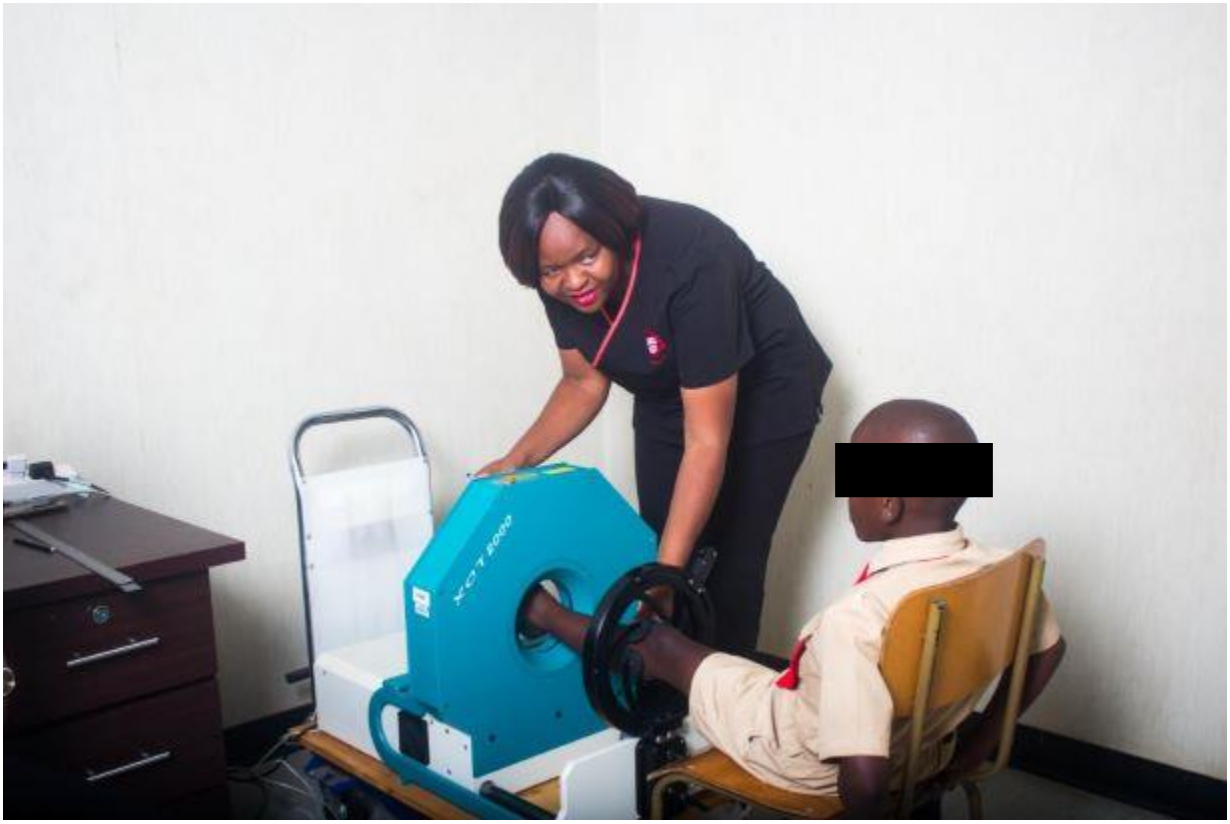
2.3.2 Tibial and radial length measurement

Limb length was measured on the non-dominant limb using a metal ruler, to the nearest 0.5mm. For the tibia, participants were asked to remove footwear and clothing from the lower limb to be measured. The participant was asked to sit on a chair, with the leg to be measured positioned at 90 degrees and crossed over the thigh of the other leg. Tibial length was defined as the length of the leg from the tibial plateau to the midpoint of the medial malleolus (middle of the bony prominence at inner side of ankle). For the radius scans, the participant sat on a chair and with the forearm bent at 90°. Forearm length was defined as the distance from the olecranon to the distal edge of the ulnar styloid process. The correct measurement of tibial and radial length is crucial for ensuring reproducibility of scans acquired for each participant and to ensure that in all participants, scan slices are obtained at the same percentage sites.

2.3.3 Participant positioning

The participant's limb was positioned securely and centrally in the gantry with the laser light distal to the medial malleolus, ensuring the limb was straight (Figure 2.4). The longitudinal axis of the limb was orientated perpendicular to the scanner gantry. For the tibia scans (IMVASK & WBS), the foot was secured in the foot holder and the limb holder ring was secured to prevent movement. Participant's leg was positioned such that the laser light was distal to the medial malleolus. In the event of a participant experiencing any discomfort from the limb supports in the holder, a small towel was placed across the supports. For the radius scans (WBS only), the participant's forearm was positioned securely and centrally in the gantry such that the laser light was distal to the ulnar styloid process.

Figure 2.3: An image showing a participant positioned for tibia pQCT scan in the IMVASK study

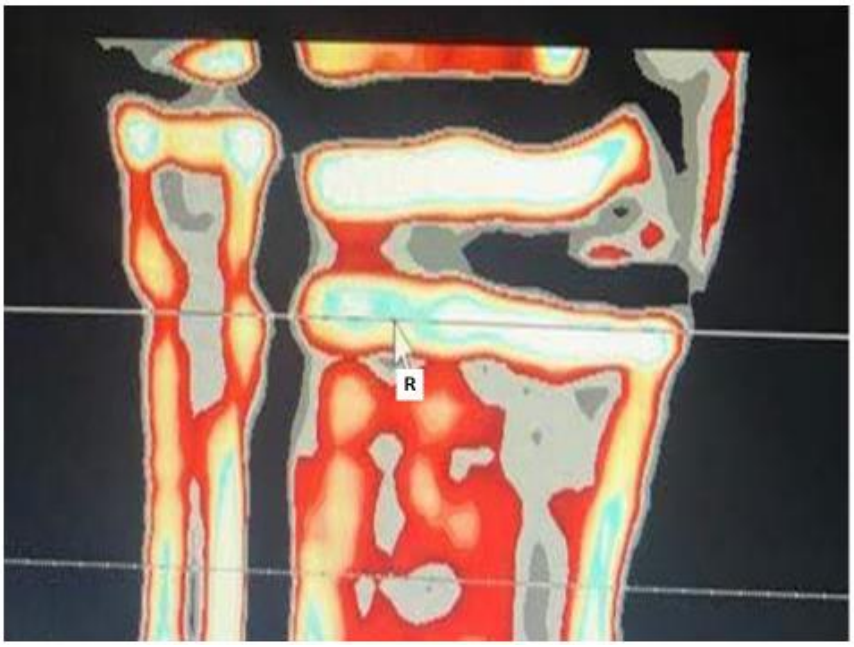


2.3.4 Scout view scan acquisition

Scan acquisition parameters were defined by the pQCT measurement mask which was selected by the radiographer before the scan. Entry of the measured tibia or radial length (object length, mm) allowed the automatic calculation of appropriate scan sites at predefined percentages of the object length proximal to the distal endplate or growth plate. For the IMVASK study, the measurement mask specified criteria for acquisition of 3 tibial slices with a fixed distance from the reference line. For the WBS study, the measurement mask for tibia specified criteria for acquisition of 4 slices with a fixed distance from the reference line whereas the measurement mask for radius specified criteria for acquisition of 2 slices from the reference line. For each measurement mask, one reference line can be defined, ensuring the mask specifies criteria for acquisition one or more slices with a fixed distance from one reference line. Different masks may have different numbers of slices with different slice distance. The positions of the reference lines are defined in the scout view. A pQCT scout view was obtained and used to locate the scan sites as a percentage of the tibial length measured from a physiologically defined reference line at the distal end of the tibia. This is a 3 cm coronal view which demonstrated the

distal joint surface of the tibia, fibula, and talus. Scan sites were determined as a percentage of tibia length, with the exact position determined by scout view placement of a reference line on the growth plate, or on the end plate for those with fused growth plates (316). As long bones grow in length, the growth plate moves upward and the wider metaphysis is reshaped into a diaphysis by continuous resorption by osteoclasts beneath the periosteum (317). It can be difficult to ensure that reference line placement is equivalent at all ages due to the normal developmental changes in the epiphysis during growth and the fact that the length of the metaphysis also differs between children (273). How those differences affect the scan location depends on the positioning of the reference line. To account for this and allow for consistency of a scan site, the reference line was always placed at the end plate, on the medial border of the articular surface for every participant. In those children whose end plate and growth plate were not yet fused, the reference line was placed at a point dissecting the medial border of the growth plate, as described by Ashby et al (318). The radiographer visually assessed the automatically placed reference line for correct positioning. Once the reference line was correctly positioned, the scan began. The scout scan was extended if there was need to ensure visualization of both the end plate and the growth plate.

Figure 2.4: A pQCT scout view image of the tibia with the reference line placed on the growth plate



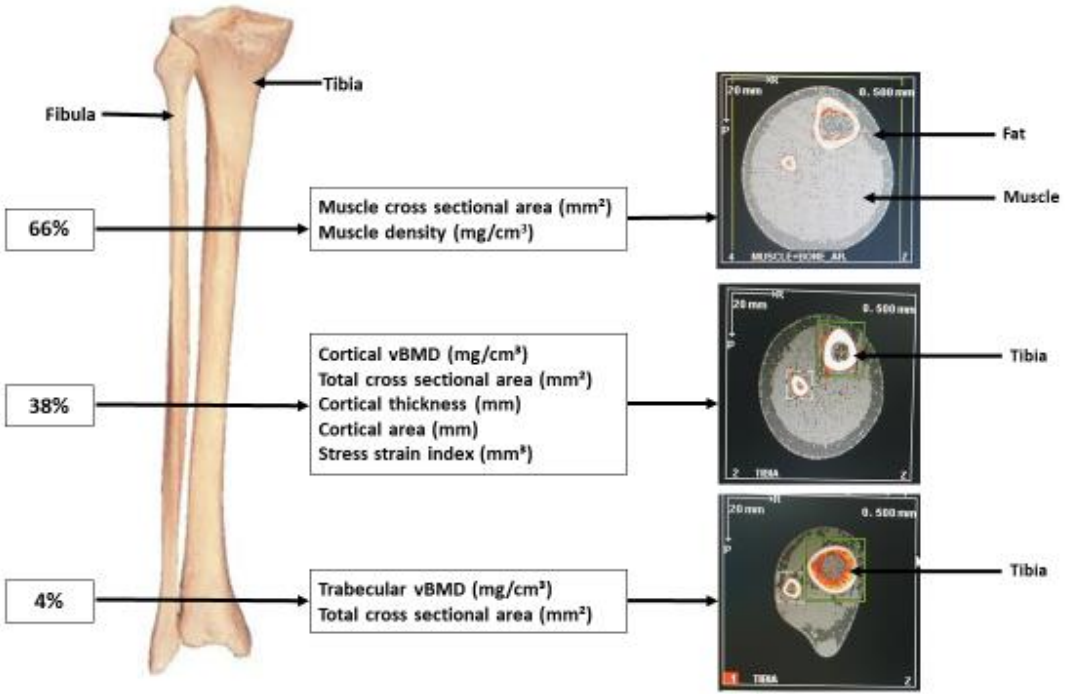
R: reference line

2.3.5 Scan slice acquisition

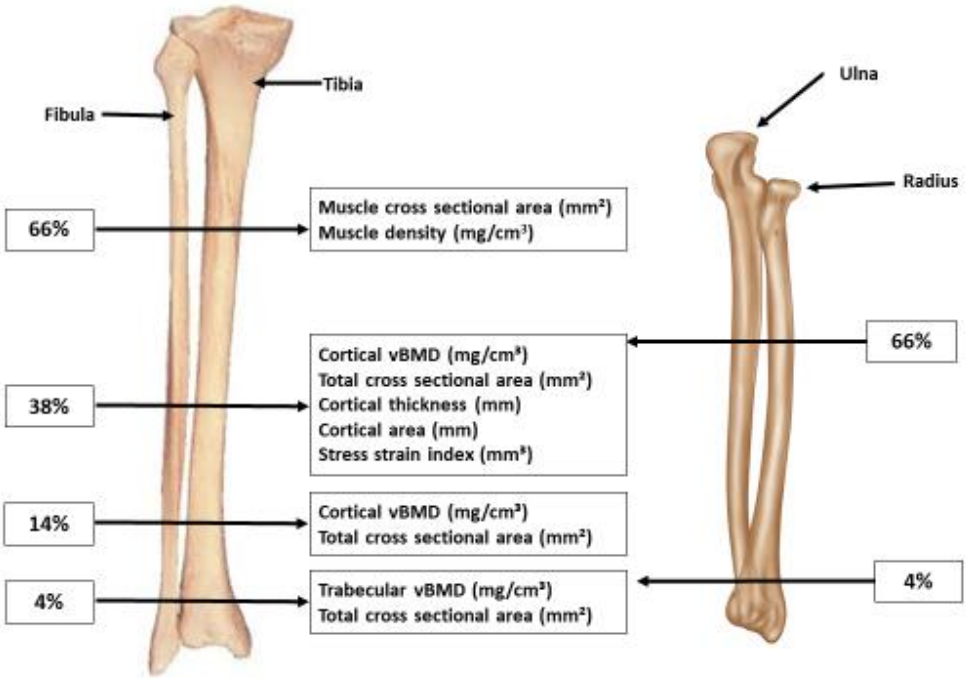
Scan sites (i.e. preselected percentages of the object length to give sites representing …..) were determined by the pQCT measurement mask selected by the operator prior to scanning. All scans were obtained using a voxel size of 0.5 mm and slice thickness of 2 mm. A CT scan speed of 30 mm/s was used for all slices, and a scout view speed of 40 mm/s was used for all scout views. Due to the resolution of XCT scanners specific sites are selected for the analysis of either trabecular or cortical bone. In the IMVASK study scans were acquired at 4%, 38%, and 66% of the tibia length (Figure 2.2.3). For the children recruited in the IMVASK study, the 4% tibial site was chosen for assessment of trabecular bone, the 38% tibial site was chosen for assessment of cortical bone and the 66% site was chosen for assessment of muscle. Muscle outcomes are reported in a separate analysis which is not part of this PhD. In the WBS tibia slices were acquired at 4%, 14%, 38%, and 66% of the tibia length and radius scan slices were acquired at 4% and 33% of the forearm length (Figure 2.2.4). For the women recruited in the WBS study, as in IMVASK, the 4% site was chosen for assessment of trabecular bone, the 38% site was chosen for assessment of cortical bone and the 66% site was chosen for assessment of muscle. Although the 14% and the 66% site can also offer cortical bone values, the 38% was also chosen to enable comparison with other studies as cortical bone measures are less commonly reported from the 14 and 66% sites. This is because the 38% site is the point of greatest cortical thickness therefore it is less confounded by partial volume averaging than the 14% and 66% sites, making the cortical bone assessment more accurate and precise at the 38% than at the 14% and 66% sites. For both the IMVASK and WBS studies, SSI_p at the 38% site was chosen as the bone strength measure to allow comparison with other studies as it is the most widely reported bone strength measure. All scan images were visually inspected, as pQCT is very sensitive to movement resulting in scan distortion and movement artefacts, if a scan slice was of poor quality the participant was asked if they would allow a rescan. A rescan was performed if the movement artifact was severe enough to cause the cortex to appear incomplete at one or more points of the scan.

Figure 2.5: An illustration of the pQCT scan slices in the IMVASK and WBS studies

An illustration of the IMVASK study pQCT scan slices of the tibia at 4%, 38% and 66%



An illustration of the WBS study pQCT scan slices of the tibia (4%, 14%, 38% and 66%) and radius (4% and 66%)



2.3.6 Follow-up pQCT Scan acquisition considerations

For the IMVASK study, follow up scans were acquired at 12 months whereas for the WBS study, follow up scans were acquired at 6, 12 and 24 months. The procedure and scan parameters for both baseline and follow up pQCT scans were identical, with the only exception being if the growth plate has fused. For participants being scanned for the second time on the follow up visit, the same scan parameters as the baseline scan were used. Participant data did not have to be entered manually but was selected from the pQCT machine database. The radiographer opened the previous scan to check how participant details (e.g. study number, sex, date of birth, age etc.) for initial scan compare to the pQCT data collection form for that day. Heights, weight and tibia length measurements were checked for consistency, e.g. for the growing children in IMVASK, one would expect height to increase and not decrease. Given that weight changes are more unpredictable, weight changes greater than 5kgs were investigated and verified to ensure no errors. In the event of any inconsistencies on participant details, the radiographer checked or verified with the study nurse and research assistant to determine whether this was a typing error, participant scanned under wring I.D or any other problem. The scout view scan of the first measurement was compared to the scout view scan of the second measurement to allow positioning of the reference line exactly at the same location as in the first scan.

2.3.7 pQCT scan analysis

All pQCT scan analysis was done by one person, (the PhD student). Scan analysis included checking if the automatic selection for the regions of interest was correct. The region of interest for the 4% and 38% slices included the tibia whereas for 66% slice, it included muscle and bone (Figure 2.6). For the participant scans where automatic drawing of a region of interest around the bones did not work, the PhD student manually re-drew the regions of interest. Correct region of interest was defined for those where it had either not been named or if it had not been named correctly. In case the automatic analysis does not give the correct result, the user analysed the measurement manually. For each scan slice, the region of interest completely included the bone to be analysed (tibia) but not the other bone (fibula). All scans were analysed using the manufacturer's software (Stratec XCT version 6.20). At the 4% tibia CALCBD was used to calculate total CSA and trabecular vBMD. CALCBD contour mode 1 (i.e. threshold algorithm) was used to exclude pixels in defined regions of interest (ROI) that fell below a threshold of 180 mg/cm^3, peel mode 1 (i.e. concentric peel) peeled away the outer 55% of the total bone CSA leaving an inner 45% CSA considered as the trabecular region of interest. At the 38% tibial site

and 66% radial site, CORTBD was used to define cortical vBMD and area, this algorithm removes all voxels within ROIs with an attenuation coefficient below a 710 mg/cm^3 threshold (with separation mode 1). Total CSA was defined at the 38% tibia using a 180 mg/cm^3 threshold. Cortical thickness was calculated using a circular ring model. A threshold of 280 mg/cm3 and cortmode 1, was used to obtain SSI for both the 38% tibial and 66% radial sites.

Figure 2.6: Thresholds applied to regions of interest and extraction of data based on the defined densities during pQCT scan analysis

Measurement site	
4% site	180 mg/ccm
38% site	710 mg/ccm
66% site	710 mg/ccm
66% site	40 mg/ccm

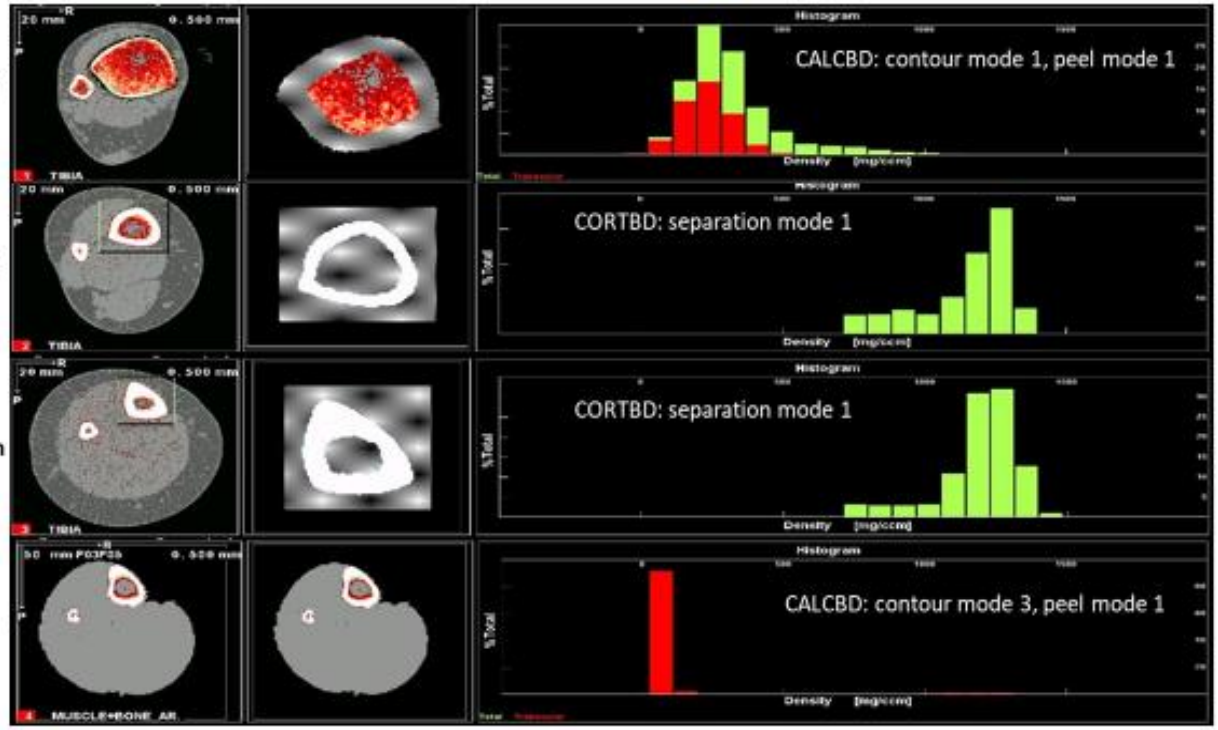


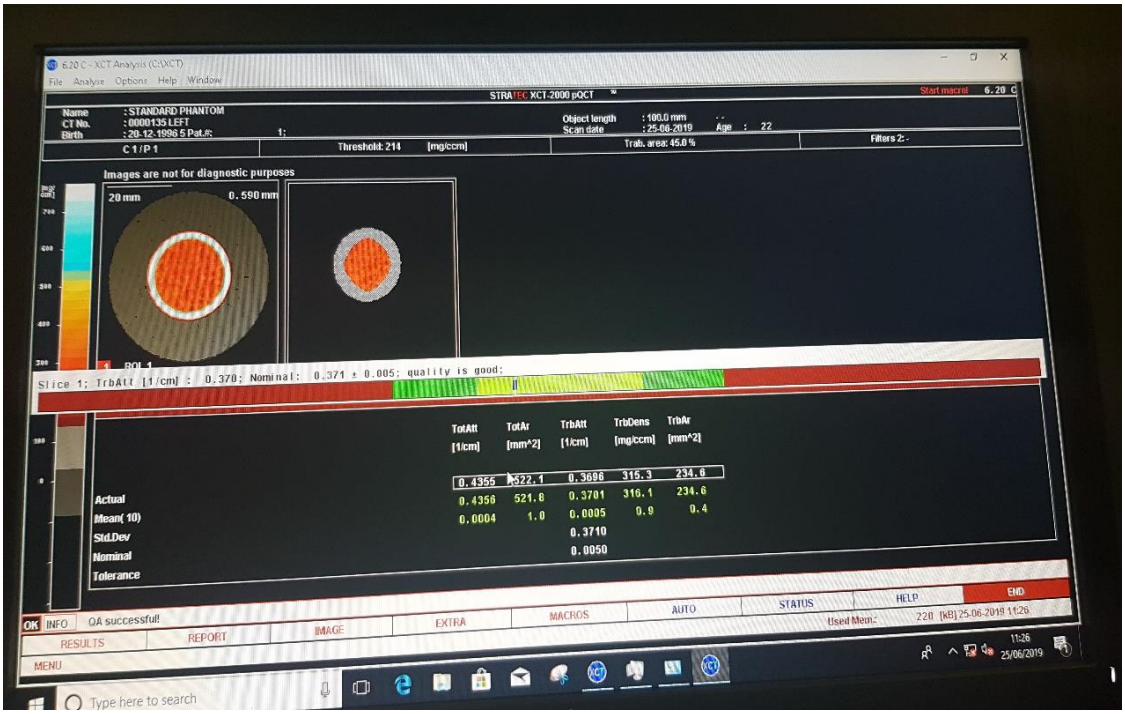
Image courtesy of Mícheál Ó Breasail, Bristol Medical School

2.3.8 Quality Control Procedures

2.3.8.1 Phantom scanning

Quality control procedures included performing quality assurance scan of the manufacturer provided standard phantom before scanning participants on each working day. The pQCT software compares the measured value with the rated value and if the difference between the measured and rated value is smaller than 1%, then quality assurance is successful (265). In case the difference is bigger, an error message is displayed. In case of an error message, the standard phantom scanning was repeated but if measurement failed again, assistance was sought from the service provider (manufacturer). In addition to the standard phantom every 3 weeks, the cone phantom was scanned. This phantom is used to check the linearity of the results and to confirm the precision of the repositioning of the device. During the procedure three different density ranges are measured. The precision of repositioning is proved according to the cross-sectional area of the cone (265). Furthermore, room temperature was monitored by a thermometer placed in the pQCT room and recorded on a temperature log. In the event that room temperature changes were more than 5°C during the day, another phantom scan was performed to calibrate and verify functionality as well as the accuracy and precision of the machine.

Figure 2.7: An image showing a successful QA scan from the IMVASK study



2.3.8.2 Image quality assessment and grading

pQCT scan images were assessed and graded for image quality in preparation for data extraction.

Participant motion presents itself in images as streaking and/or broken cortical bone shells (267,300). Determining the viability of a pQCT image, or if a repeat acquisition is required, was completed by rating the level of movement. Each scanogram or scout view was visually inspected and assessed for correct reference line placement. This is important because it affects values of the obtained pQCT bone outcomes. Scout views were graded as 0 if the scan was perfect and the reference line for the tibia went through the middle of the bone, 1 if the reference line was too distal or proximal and therefore slightly higher or lower than it should be and 2 if the reference line positioning was out of range and not in the correct position at all. Each of the pQCT scan slices were graded for movement artefacts. A score of 0 represented a scan with no movement whereas a score of 3 represented extreme movement such that significant image streaking and disruption of the cortical shell was present (Table 2.1). All radiographers were trained to assess movement, and this was done whilst the participant was still in the room. Representative images for each rating on the scale were posted on the wall for next to the radiographer's workstation. Images graded 3 were deemed to have unacceptable motion artefact for bone and soft tissue analysis and therefore required rescanning or exclusion from analysis.

Table 2.2: Description of scan slice grading criteria

Slice Grade	**Description**
0	**Perfect:** No red streaks on the scan and the bone is in perfect shape (not distorted)
1	**Slight movement:** Small amounts of red patches/streaks
2	**Medium streaky movement:** Large streaky red movement patches, the shape of the bone is distorted due to movement and there may be small breaks in the scan image
3	**Unusable:** Red streaky patches covering most of the scan image, the bone does not look circular in shape, pulled by the movement with large breaks in the scan image

2.3.8.3 Precision assessment

For 30 participants included in the IMVASK study, pQCT scans were performed twice on the same day, within a few minutes of each other and allowing for patient repositioning between each scan. The same operator performed repeated scanning within the same day and manually defined the region of interest around each tibia image, excluding possible movement artefacts. Tibia length was not re-measured. Mean, SD and CV were calculated for each of the sets of two measurements performed on each participant. The precision error for each of the pQCT bone outcomes was calculated as RMS-SD and RMS-%CV. The SDs were squared, summed, and then divided by the number of patients, (n=30) and then the square root was taken resulting in the RMS-SD in g/cm2. The CVs were squared, summed, and then divided by the number of patients, (n=30) and then the square root was taken resulting in the RMS-CV. The RMS-CV was then multiplied by 100 to get the RMS-%CV, which expresses it as a percentage. The LSC was calculated as the precision error multiplied by 2.77 as recommended by the International Society for Clinical Densitometry (ISCD) and reported in section 5.4.3, with a discussion of whether or not the changes observed in chapter 5 exceed the least significant change in section 5.7 of this thesis

2.3.9 pQCT Data management

Each pQCT scan performed on a participant was recorded onto the pQCT case report form (CRF) form and the pQCT scan and temperature log. The radiographer must cross check the data collection form before it leaves the pQCT unit to go to the data management people. The pQCT scan and temperature log remained in the pQCT unit for reference by radiographers. pQCT CRFs were stored in the pQCT unit but were immediately transported to the data management team by the following morning after the pQCT scan had been conducted. Hard copy forms of the pQCT scan and temperature log forms were filed in lever arch files and stored in a lockable cabinet in the pQCT unit. pQCT scan data was backed up on an encrypted hard drive for transportation to the data managers and also for backup in the event there was a problem with the pQCT computer.

3 CHAPTER 3: Literature review of studies assessing bone density, size and strength in children and adolescents living with HIV

3.1 Introduction

HIV-infection and fragility fractures are both global public health problems that can increase morbidity and therefore decrease quality of life for those who are affected, or increase mortality. Historically, opportunistic infections e.g. tuberculosis, were the leading cause of illness and death in people living with HIV (PLWH). In high income settings, HIV is now a chronic, controllable infection with a life expectancy that is close to normal because to effective ART. Improved access to ART has resulted in an increased life expectancy in PLWH such that perinatally infected children are growing through puberty and into adulthood. This has led to a new population of PLWH who are presenting with not just infectious but also non-communicable diseases which are comorbid complications of living with HIV. Health management of PLWH is slowly shifting focus from infectious disease to non-communicable disease as a cause of illness, reduced quality of life or death.

Skeletal complications such as an increased risk of fragility are amongst the complications of HIV. Due to the cumulative effect of HIV and ART on bone, perinatally infected children living with HIV (CWH) have HIV at a critical growth period and are affected for a longer period than PLWH who were infected in their adulthood, resulting in an increased lifetime risk for osteoporosis and fracture. It is unclear if CWH accrue bone at the same rate as children living without HIV (CWOH). It is also unclear whether bone loss in adults, due to ageing, is occurring at an earlier age in PLWH than in their uninfected counterparts, given that HIV is associated with premature ageing (319). Childhood and adolescence are key stages of skeletal maturation because 85–90% of adult bone mass is attained during this period (320). Impaired bone accrual during childhood and adolescence may result in a compromised peak bone mass (PBM), in adulthood, thereby increasing fracture risk. Eastern and Southern Africa are home to about 54% (20.6 million out of 37.9 million) of the world's population living with HIV (321). According to UNAIDS, 62% of CWH in this region are accessing ART. (321). The long-term effect of HIV and ART on bone growth and the causes of inadequate bone development in CWH are key topics of research

The aim of this chapter is to present a narrative review of the literature, discuss the knowledge gap and summarize the publications concerning bone density, size and strength in children and adolescents living with HIV. PUBMED, EMBASE, Web of Science and SCOPUS databases were searched from the year 2000, with the initial search being conducted in 2019. In addition to

PUBMED, EMBASE, Web of Science and SCOPUS databases, grey literature including studies presented at bone health conferences were also included in the search. The reference lists of journal articles included in this literature review were checked for relevant papers and citation tracking databases were used to identify additional articles that cited papers identified within the initial search. Citation tracking databases were used to identify additional or new articles relevant to the search therefore new publications relevant to the search were added to this review, up until 2023.

3.2 DXA based bone health assessment in children living with HIV

Studies assessing bone among ART naïve perinatally HIV-infected children and adolescents, have reported no deficits in TB or LS aBMD compared to healthy controls (322–324). This is consistent with no short-term differences in bone measures reported amongst 14 to 25 year old ART naïve and demographically similar seronegative participants and 199 horizontally infected young people (325). However, evidence from most studies show that CWH may be at higher risk for disruption to bone accrual due to effects of HIV on the immune system and the potential for drug-induced toxicity (229–231,323,326–330). Other inconsistences in the published literature on BMD in CWH are potentially explained by different methods used to assess BMD. This includes studies reporting aBMD as an outcome in children, studies reporting BMC instead of aBMD, and studies reporting a measure of aBMD adjusted for size, i.e. bone mineral apparent density (BMAD). Areal BMD is 2-dimensional and does not account for the depth and actual size of bone therefore smaller bones are more likely to have lower measured aBMD than bigger bones even though the vBMD is the same (65,84). BMAD is an attempt to counter the biases of 2 dimensional DXA aBMD measures. However, studies estimating LS BMAD in CWH have also applied different methods for calculating the LS BMAD (230,330,331). This lack of standardization makes it difficult to directly compare results between studies. In 8 to 16 year old American children of unspecified race, BMAD was reduced amongst CWH (n=15) compared to CWOH from a gender and age specifics normative database (331). An 8% prevalence of low LS BMAD Z-score (≤-2) was reported in perinatally infected Asian adolescents (10-18 years old) with poor immunological status before ART commencement (332). In Zimbabwean 6 to 16 year old children in 2019, we reported a 15% and 13% prevalence of low LS BMAD (Z-score ≤-2) and low TBLH-BMC^{LBM} in CWH compared to 1% and 3% in CWOH (230). Furthermore, we used the same methods and consistently demonstrated that Zimbabwean CWH who are from the cohort as studied in this PhD thesis, had lower TBLH-BMC^{LBM} and lower LS-BMAD than CWOH, which was more overt in later adolescence (229). The Zimbabwean study suggests that the HIV

infection itself, as well as exposure to TDF, may contribute to the impaired development of low bone density in CWH. South African CWH, with a mean age of 6.4 years, and established on ART for an average of 5.7 years, have been reported to have 0.17 lower TB BMC Z-scores compared to their HIV-uninfected peers (231).

3.2.1 Cross-sectional studies

Cross-sectional evidence from high and middle income countries suggests a higher prevalence of low BMD (Z-score ≤-2.0) in those living with HIV acquired perinatally or during adolescence (333); examples from Italy (323,334,335), the Netherlands (329), the United States (328,330), Brazil (327,336) and Thailand (337) are shown in Table 3.1. In a study of 101 HIV-infected Thai adolescents aged 12-20 years, who were all receiving anti-retroviral therapy, 24% met criteria for low TB BMD (337). Studies conducted in Brazil reported low TB and/or LS BMD in 32% of perinatally infected CWH (n=74, mean age 17 years) (336) and a 17% prevalence of low TB BMD in CWH (n=48, mean age 13years) (327), after adjusting for bone age, body mass, femur diameter, physical activity, calcium intake, duration of ART and PI use. In Italy (n=350, mean age 13 years) CWH had 7% and 4% prevalence of low TB aBMD and low LS aBMD, respectively, compared with 1% low TB aBMD and 1% low LS aBMD amongst CWOH, after adjusting for bone age and sex (328). Similarly, in the Netherlands (n=66, mean age 6.7 years), prevalence of low LS BMD amongst perinatally infected CWH was 8% (329). It is possible that decreases in bone accrual in perinatally infected CWH may become more pronounced with increasing age (330,338). Some of these studies adjusted the bone outcomes for factors such age, sex, ethnicity, height, weight and pubertal status. DiMeglio et al. reported a reduction in Z-score disparities between CWH and CWOH after adjusting for sex, weight, and pubertal stage (328). Studies assessing BMD in CWH acquired by means other than mother-to-child transmission are also scarce (325,333). However, these studies have demonstrated that HIV acquired via sexual transmission during adolescence or young adulthood may have a severe impact on bone integrity. Lower aBMD Z-scores were reported in young men aged 20-25 who were infected during adolescence than uninfected controls (333). aBMD measures in these young men living with HIV were similar to those of perinatally-infected men despite marked differences in the duration of HIV infection and ART exposure (333). Similarly, lower TB BMC Z-scores among those on ART were reported amongst 14-25 year old (n=199) men soon after acquiring HIV infection by sexual transmission (325). In general most cross-sectional studies conducted in CWH have a small sample size and lacked a comparison group of CWOH, necessitating further studies in this area.

Table 3.1: Studies reporting a cross-sectional analysis of DXA measured bone density in children living with HIV

First Author	Year Country	Sample Size	Gender Ethnicity	Age (years)	Scan sites & Bone Outcomes	Study Findings
Natukunda (339)	2023 Uganda	CWH: 159 CWOH: Nil	50% Males 100% Black	Range: 3 to 15 Median: 10 (7-12)	TBLH, LS aBMD	Prevalence of low BMD (Z-score<-2) was 18% TBLH, 13% (LS) and 15% (both)
Rukuni (229)	2021 Zimbabwe	CWH: 303 CWOH: 306	50% Males 100% Black	Range: 8 to 16 Mean: 12.4±2.5	TBLH, LS BMC, BMAD	Higher prevalence of low TBLH BMC & low LS BMAD in CWH than without
Gregson (230)	2019 Zimbabwe	CWH: 97 CWOH: 77	52% Females 100% Black	Range: 6 to 16 Mean: 12.7±2.5	TBLH, LS BMAD	Higher prevalence of low BMAD & TBLH-BMCLBM in CWH children [15% vs 1% (LS) & 13% vs 3% (TB)]
Shiau (326)	2018 South Africa	CWH: 219 CWOH: 180	49% Males NR	Range: 5 to 9 Mean: 6.7±1.4	TB BMC	Lower TB BMC Z-score in CWH than CWOH;− 0.95vs.− 0.79,p=0.05 & similar LS BMC Z-score − 0.22&− 0.38, p=0.08
Arpadi (231)	2016 South Africa	CWH: 219 CWOH: 219	55% Males NR	Range: 5 to 9 Mean: 7.0±1.3	TB BMC	0.17 Lower in CWH vs CWOH
Dimeglio (328)	2013 USA, Puerto Rico	CWH: 350 CHEU: 160	46% Males 30% Black	Range: 7 to 15 Median: 12 (10-14)	TB, LS aBMD	Higher prevalence of low BMD in CWH vs CHEU; 7% vs. 1%, P=0.008 (TB) & 4% vs. 1%, P=0.08 (LS)
Lima (327)	2013 Brazil	CWH: 48 CWOH: Nil	50% Males 50% White	Range: 7 to 17 Mean:12.7±2.7	TBLH, LS BMC, aBMD	8.5% & 16.7% prevalence of low BMC & aBMD in HIV, ↓ Lower BMD in CWH compared to NHANES IV
Bunders (329)	2013 Netherlands	CWH: 66 CWOH: Nil	Males, Females 62% Black	Range: NR Median: 6.7(4-10)	LS aBMD	8% prevalence of low LS aBMD & lower median LS BMD Z-scores in CWH vs CWOH
Schtscherbyna (336)	2012 Brazil	CWH: 74 CWOH: Nil	45% Males 37% White	Range: 13 to 21 Mean: 17.3±1.8	TB, LS aBMD	32.4% prevalence of low BMD Lower LS & TB BMD Z-scores in patients on Tenofovir
Puthanakit (337)	2012 Thailand	CWH 100 CWOH: Nil	50% Males NR	Range: 13 to 16 Median: 14.3(13-15)	LS aBMD	124% prevalence of low BMD based on LS aBMD Z-score
Jacobson (330)	2010 USA	CWH: 236 CWOH: 143	53% Males 54.7% Black	Range: 7 to 24 Median: 12.6	TB, LS BMC, aBMD	Lower TB & LS BMD & lower TB BMC in male CWH at Tanner 5 than CWOH
Zuccoti (334)	2010 Italy	CWH: 86 CWOH: 194	45% Males NR	Range: 4 to 22 Mean:	TB, LS BMC, aBMD	Lower TB BMD in CWH & on ART compared with CWOH. No difference between CWH & not on ART compared with CWOH
Pitukcheewanont (322)	2005 Italy	CWH: 58 CWOH:	45% Males NR	Range: 5 to 19 Mean: 12±4	TB, LS BMC, aBMD	Lower LS BMC (P <0.005) & lower LS aBMD (P <0.05) in CWH than in CWOH
Zamboni (335)	2003 Italy	CWH: 13 CWOH: 198	31% Males NR	Range: 4 to 12 Mean: 7.8±2.9	LS aBMD	Low bone formation & high bone resorption markers in CWH who had low CD4 & high IL-6
Arpadi (340)	2002 USA	CWH: 51 CWOH: 262	51% Males 41.5% Black	Range: 4 to 15 Mean: 8.2±2.6	TB, LS BMC	Lower TB BMC in CWH than CWOH
Mora (323)	2001 Italy	CWH: 40 CWOH: 317	45% Males 100% White	Range: 4 to 19 Mean: 11.5 ±2	TB, LS aBMD	Lower LS & TB aBMD values in CWH on ART
O'Brien (341)	2001 Italy	CWH: 19 CWOH: 583	NR 89% Black	Range: 6 to 16 Mean: 9.2±2.6	TB, LS BMC, aBMD	Lower TB BMC in CWH than CWOH

CWH; children living with HIV, CWOH; children living without HIV, CHEU; children who are HIV exposed but uninfected, TB; total body, TBLH; total body less head, LS; lumbar spine BMC; bone mineral content, BMD; bone mineral density, BMAD; bone mineral apparent density, NR; not reported, USA; United States of America

3.2.2 Longitudinal studies

Four studies have reported an increase in BMD in CWH over time (Table 3.2). A 12 months longitudinal study of 6 to 17 year old CWH (n=32) who were on long-term ART reported lower annual increase of TB BMD in CWH than CWOH (342). In a small study of 18 CWH who were perinatally infected and on ART (>80%), TB BMD in all CWOH increased or remained stable over a 1 to 3 year follow up period, whereas only 44% of the CWH showed an increase in TB BMD (p=0.09), after adjusting for weight Z-scores and height Z-scores (338). However, this study was underpowered. Another study reported increases in LS and FN aBMD Z-scores after a 2 to 3 year follow up in CWH (n=66), after adjusting for BMI, CD4, viral load and duration on ART (329). However, this study had no comparison group of CWOH. This was consistent with results reported in 11-16 years (mean age 13.6 years) perinatally infected CWH who had aBMD measurements at 0, 1 and 2 years, after adjusting for height and lean mass (343). A longitudinal study by Mora et al. found that in CWH, annual incremental changes in spine BMD were comparable to healthy children, whereas the annual increment for whole body BMD, was lower in CWH than in CWOH (342). Apart from the studies reporting an increase in BMD in CWH over time, other studies have reports declines in BMD over time (331,344). The decrease in aBMD reported in both these studies was associated with TDF exposure. An important point to note is the small sample sizes in existing longitudinal studies that have assessed bone in CWH (Figure 3.1). Most studies recruited fewer than 50 participants. This could contribute to inconsistences in results such that other studies were not powered enough to report a difference in change in bone between CWH and CWOH over time (324,345).

Figure 3.1: Graph to show sample sizes from cohort studies following up children living with HIV

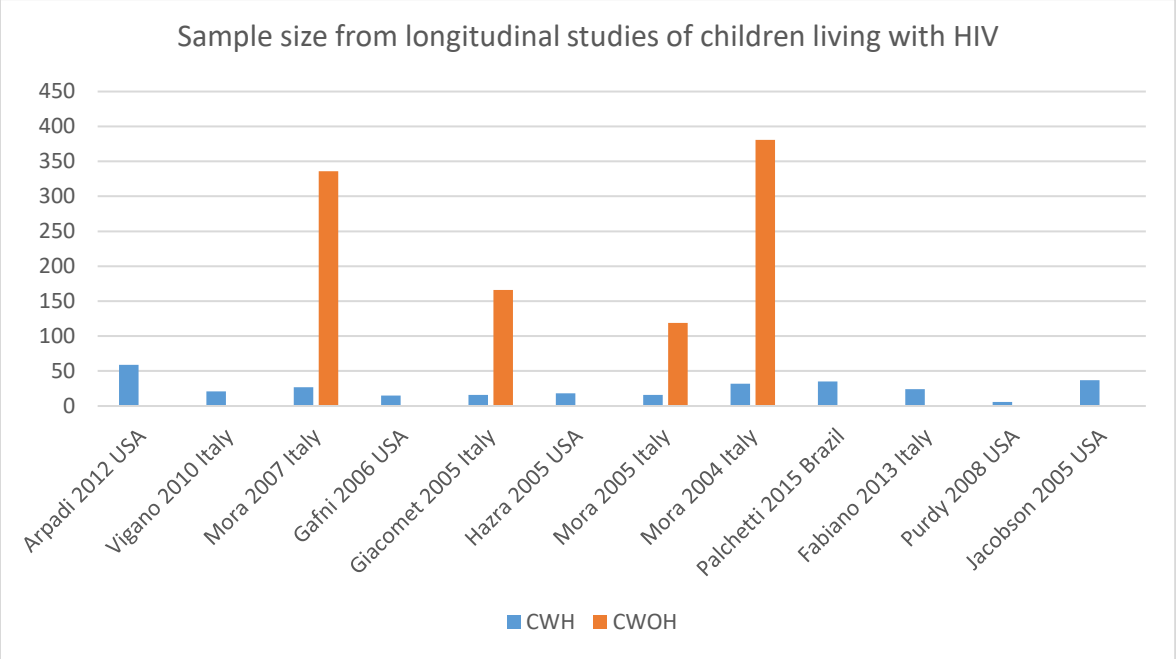


Table 3.2: Cohort studies reporting DXA measured follow up bone outcomes in children living with HIV

Author	Year Country	Sample size	Gender Ethnicity	Age (years) Follow-up	Scan sites Outcomes	ART use: Study Findings
Shen (346)	2021 S.A	CWH:220 CWOH:220	Males, Females Black	>7, 24 months	TB, LS BMC, aBMD	Yes: Lower mean WB-BMC, WB-BMD, WB-BMC Z-scores & LS-BMC and LS-BMD in CWH
Palchetti (347)	2015 Brazil	CWH: 35 CWOH: Nil	Males, Females NR	7 to 12, 24 months	TB, LS aBMD	Yes: Lower TB BMD in CWH on ART but similar LS BMD compared with CWOH
Fabiano (348)	2013 Italy	CWH: 24 CWOH: Nil	Males, Females NR	5 to 7, 96 months	LS BMC, aBMD	Yes: % BMC ↑linearly by 0.02%/year (p = 0.04)
Macdonald (343)	2013 Canada	CWH: 31 CWOH: 883	61% Males 26% Black	9 to 18, 24 months	TB, LS, FN BMC	Yes: BMC z-scores adjusted for height and lean mass were lower than controls at all sites except the lumbar spine (-0.57 to -0.27, p<0.05)
Arpadi (349)	2012 USA	CWH: 59 CWOH: NR	NR 63% Black	6 to 16, 24 months	TB BMC, BMC	Yes: Increase in TB & LS BMD & BMC in both CWH & CWOH over 1 & 2 years. No differences in increase in BMD between CWH & CWOH.
Vigano (350)	2010 Italy	CWH: 21 CWOH: NR	48% Males 100% White	4 to 18, 96 months	TB, LS aBMD	Yes: No change in TB & LS BMD over 60 months after starting Tenofovir
Mora (351)	2007 Italy	CWH: 27 CWOH: 336	48% Males 100% White	4 to 17, 6 months	LS aBMD	Yes: Lower TB & LS BMD in CWH than CWOH, adjusted for age, sex, weight & height
Gafni (331)	2006 USA	CWH: 15 CWOH: Nil	67% Males NR	4 to 18, 96 weeks	LS, TH, FN aBMD, BMAD	Yes: Reduced LS, FN & TH BMD & BMD Z -scores from week 24 to week 48 after starting Tenofovir
Giacomet (324)	2005 Italy	CWH: 16 CWOH: 166	NR 100% White	6 to 18, 12 months	TB, LS aBMD, BMC	Yes: TB & LS BMD & BMC did not differ from expected during treatment with Tenofovir
Hazra (344)	2005 USA	CWH: 18 WHO: Nil	61% Males 55% Black	8 to 16, 48 weeks	LS aBMD	Yes: Decrease in LS BMD observed in 5 of 15 participants at week 48 after starting Tenofovir
Mora (352)	2004 Italy	CWH: 32 CWOH: 381	53% Males	6 to 18, 12 months	TB. LS aBMD	Yes: Lower TB & LS BMD in CWH than CWOH, Lower annual increment for TB BMD in CWH than CWOH
Purdy (353)	2008 USA	CWH: 6 CWOH: Nil	67% Males NR	11 to 17, 48 weeks	TB, LS aBMD, aBMD	Yes: 5 out of 6 CWH had decrease in BMD after receiving Tenofovir
Jacobson (338)	2005 USA	CWH: 37 CWOH: 9	49% Males 40% Black	9 to 14, 4 years	TB aBMD, BMC	Yes: Lower TB BMD than population but not in comparison to siblings. Only 44% of CWH increased/plateued BMD vs 100% in CWOH

CWH; children living with HIV, CWOH; children living without HIV, NR; not reported TB; total body, TBLH; total body less head, LS; lumbar spine, BMC; bone mineral content, BMD; bone mineral density, BMAD; bone mineral apparent density, USA; United States of America

3.2.3 Bone density and ART in CWH

Reduced bone density has also been associated with ART initiation. There were decreases in BMD by 2-6% in children or young adults living with HIV who were previously ART naive and had just been initiated on ART (22). Similarly, this is consistent with reports from ART-experienced CWH who were switching to new drug regimens due to poorly controlled HIV (29). TDF has been associated with deteriorating bone mass (354). Recent aBMD data from our IMVASK cohort of Zimbabwean CWH and CWOH also suggest that among CWH, both TDF exposure and orphanhood were associated with lower TBLH-BMC^{LBM} Z-scores. Even in early life, fetal exposure to TDF used as either part of maternal combination ART or as prophylaxis against mother to child transmission has also been associated with lower bone outcomes (355). The potential mechanisms for adverse bone effects for TDF are not yet clear. Some authors have suggested that TDF directly affects bone formation by affecting osteoblast proliferation and increasing apoptosis (102,103), whereas others suggest that it induces functional vitamin D deficiency (356).

Different conclusions on the effect of ART amongst studies may be due to differences in time between initiation of ART and bone measurements. Evidence seems to suggest that a decline in BMD due to ART is more evident in the initial phases of treatment (320,331). ART is usually administered in combination and therefore the attributing effect to one type may introduce complications especially in underpowered studies. An earlier systematic review of studies assessing bone in CWH showed that most studies performed in CWH have small sample sizes on different ART regimens, implying the possibility of insufficient power to detect differences in bone accrual by ART type (320). Different ART types potentially resulting in different bone outcomes is of clinical importance and therefore further investigation is necessary. The long term effects of both HIV infection and ART exposure in children and adolescents during a critical period of bone accrual is a cause for concern, especially in perinatally-infected children where stunting and being underweight are common.

3.3 pQCT based bone health assessment in children living with HIV

3.3.1 Bone density

Contrary to studies that have used pQCT and/or HRpQCT in healthy children (Table 3.4), studies that have measured vBMD in CWH are few (Table 3.3). Three studies have utilized variable methods (i.e. QCT and pQCT) and they present variable findings. In 5 to 16 year old Thai CWH (n= 58), matched for age and gender to healthy controls, CWH had lower areal BMD

and BMC by DXA but similar vBMD by QCT, showing the bias introduced by bone size (322). Interestingly, in 31 Canadian CWH, of diverse ethnic backgrounds, cortical vBMD appeared to be higher in CWH than CWOH and was positively associated with non-nucleoside reverse transcriptase inhibitor (NNRTI) use (343). Using HRpQCT, a lower total and trabecular vBMD was reported in young men (20-25 years) who had acquired HIV either perinatally or during adolescence. A recent South African study using pQCT to assess cortical and trabecular vBMD as well as bone size and indices of bone strength in CWH showed that Trabecular vBMD was -17 mg/cm^3 (standard error (SE); 7.2, p=0.019) lower for male CLWH compared to male controls after adjustment for age and radial length (238).

3.3.2 Bone size

In a study conducted in 9-18 year old CWH in Canada (n=31), no differences in total and cortical bone area Z-scores at the tibia were observed between CWH and healthy CWOH from a referent database (343). In 172 South African CWH, tibial cortical area was smaller in both male and female CWH compared with CWOH (238). Using HRpQCT, both tibial and radial cortical thickness were lower in 20 – 25 year old young men living which HIV than in those without HIV (333). The 20 – 25 year old study population, though past adolescence, included those acquired HIV perinatally or during adolescence and this still adds to the importance of assessing bone size in perinatally infected CWH.

Table 3.3: Studies utilising pQCT, QCT or HRpQCT to assess bone health in children living with HIV

Author	Year and Country	Design, Follow up	n (CWH)	Sex and Ethnicity	Age Range (years)	Modality & scan site
Shiau (238)	2020 South Africa	Cross-sectional	CWH: 172 CWOH: 98	51% Males 100% Black	7 to 14	pQCT Radius,
Macdonald (343)	2013 Canada	Cohort 24 months	CWH: 31 CWOH: 883	61% Males 26% Black	9 to 18	pQCT Tibia, 50% site
Pitukcheewanont (322)	2005 Italy	Cross-sectional	CWH: 58 CWOH: Nil	45% Males NR	6 to 16	QCT Lumbar spine

CWH; children living with HIV, CWOH; children living without HIV, NR; not reported

3.3.3 Bone strength

To date there is conflicting evidence concerning the association between HIV-specific disease characteristics and bone strength. In a recent South African study, pQCT measured polar SSI was lower in CLWH than controls (778 vs. 962 mm^3, p<0.01) after adjustment for age and sex

and this finding was consistent in both males and females (238). In 31 Canadian CWH, pQCT measured bone strength (SSI_p) Z-scores were not significantly different from zero and were not increasingly compromised from baseline to follow up (343). Explanations for these inconsistencies between the South African and Canadian study include differences in populations studied and differences in sample size together with whether or not a study is powered adequately. The study in Canadian children was underpowered (343). There is inadequate understanding of the effect of HIV and its treatment on bone strength in CWH.

Table 3.4: Studies using pQCT and HRpQCT to assess bone density, bone size and bone strength in healthy children and adolescents

Author	Year	Country	Sample size	Sex	Age (years)	Imaging techniques	Scan site
Valkama (357)	2020	Finland	855	M,F	1 to 2	pQCT	Tibia
Vlok (358)	2019	Australia	1271	M, F	11 to 12	pQCT	Tibia
Kawalilak (292)	2017	Canada	32	M,F	8 to 13	HR-pQCT	Radius, Tibia
Gabel (359,360)	2017	Canada	393	M,F	9 to 20	HR-pQCT	Radius, Tibia
Chevalley (361)	2017	Switzerland	152	M	22.6	HR-pQCT	Radius, Tibia
Cheuk (362)	2016	Hong Kong	52	M,F	13 to 16	HR-pQCT	Radius
Burt (363)	2014	Canada	59	M,F	16 to 19	HR-pQCT	Radius, Tibia
Nishiyama (364)	2012	Canada	398	M, F	9 to 22	HR-pQCT	Radius, Tibia
Kalkwarf (298)	2011	USA	424	M, F	5 to 16	pQCT	Radius
Chevalley (203)	2011	Switzerland	176	M	7 to15	HR-pQCT	Radius, Tibia
Burrows (365)	2010	Canada	279	M, F	15 to 20	HR-pQCT	Tibia
Ashby (318)	2009	UK	629	M, F	6 to 19	pQCT	Radius
Kirmani (366)	2009	USA	121	M, F	6 to 21	HR-pQCT	Radius
Moyer-Mileur (367)	2008	USA	416	M, F	5 to 16	pQCT	Tibia
Macdonald (368)	2006	Canada	424	M, F	9 to 11	pQCT	Tibia
Macdonald (369)	2005	Canada	128	M, F	11 to 13	pQCT	Tibia
Schoenau (217)	2002	Germany	469	M, F	6 to 40	pQCT	Radius
Neu (281)	2001	Germany	473	M, F	6 to 40	pQCT	Radius
Rauch (370)	2001	Germany	278	M, F	6 to 40	pQCT	Radius
Schoenau (216)	2000	Germany	318	M, F	6 to 22	pQCT	Radius
Binkley and Specker (371)	2000	USA	101	M, F	3 to 4	pQCT	Tibia

M, F; Males, Females
USA; United States of America

3.4 Pathogenesis of impaired bone accrual in children living with HIV

Low BMD in CWH is likely due to multiple factors which include traditional risk factors like low calcium and vitamin D intake, low physical activity levels, pubertal delay as well as HIV-related factors like HIV infection itself, chronic immune activation, and the direct effects of ART. HIV viral proteins have been shown to decrease osteoblast function while enhancing osteoblast apoptosis (372). The effect of HIV on the function of osteoblasts and osteoclasts is influenced by a number of factors, including pro-inflammatory cytokines such as tumour necrosis factor-α (TNF-α), expression of RANKL (receptor of activated NF-κB ligand) and osteoprotegerin (OPG), vitamin D and calcium metabolism and endocrine function (373,374)**.** RANKL is a key cytokine required for osteoclast formation and activity. In the presence of permissive concentrations of macrophage colony-stimulating factor (MCSF), a survival factor for cells of the monocyte–macrophage–osteoclast lineage, RANKL binds to its receptor (RANK) on osteoclast precursors and promotes their differentiation into preosteoclasts, which fuse together into mature bone resorbing osteoclasts (42). OPG is a physiological decoy receptor of RANKL and the RANKL to OPG ratio is a key determinant of osteoclast differentiation and bone resorption in the body. As a result, osteoclasts are identified by the expression of the receptor activator (RANK). HIV proteins have been shown to shift the OPG/RANKL ratio resulting in an increase in RANKL-mediated osteoclastic activation and therefore greater bone resorption (375). In support of this, perinatally infected CWH have been shown to have a higher RANKL/OPG ratio than CWOH (351). The RANKL/OPG ratio is also higher in adults living with HIV, but not on ART, who have low BMD (375). In PLWH, there are correlations between increased serum RANKL levels, reduced OPG/RANKL ratios, increased HIV viral load and low BMD Z-scores (372,375). Activation of T-cells by HIV infection may also affect bone physiology by releasing RANKL and pro-inflammatory cytokines, e.g., IL-1 and TNF-a, which both stimulate osteoclast activity and cause stromal cells to make osteoclastogenic IL-7. In addition, though traditional risk factors for impaired bone health are prevalent in CWH, HIV disease-specific factors, such as duration of HIV infection, HIV viral load, and CD4 count also represent independent risk factors for reduced BMD in PLWH and in CWH specifically (144,376,377). Several studies have also reported associations between aBMD and plasma HIV RNA concentration (negative), CD4 count (positive), and advanced stage of HIV disease (144,328,337,338). CWH in the Netherlands, who had higher CD4 count and those who had higher viral load had lower LS BMD Z-scores (329). Macdonald et al reported a positive correlation between CD4 count and BMD (343). The role of persistent residual immune activation despite viral suppression on bone formation and

resorption in childhood and the mechanisms causing reduced bone density, size and strength in the context of HIV infection still needs to be studied and further clarified.

3.5 Risk factors for impaired bone growth in children living with HIV

Several traditional risk factors for low bone mass are seen in CWH. Common factors include stunting, underweight and delayed puberty (328,337,338).

3.5.1 Stunting, underweight and wasting

Childhood HIV infection can manifest as underweight and/or stunting (poor linear growth) (Table 3.5). Several studies, assessing growth patterns in African countries, have consistently reported high prevalence of underweight, stunting and wasting as measured by weight for age Z-scores (WAZ), height for age Z-scores (HAZ) and BMI for age Z-scores, respectively, in the context of HIV (3,4,378–383). In Zimbabwe CWH have higher odds of stunting, with the odds being eight times greater in those who have acquired HIV in utero, and four times higher in those who have acquired HIV around the time of birth (384).

Children born with HIV have impaired growth which can be detected as early as 6 weeks of age and lasts through infancy and childhood (384). CWH have a lower birth weight and length than those born without HIV (385). In Zimbabwe, 20% of the babies born between 1997 and 2001, who were perinatally infected with HIV were underweight compared with 3.3% babies born without HIV. In the same Zimbabwean cohort, 59% of the babies perinatally infected with HIV were stunted compared with 18.9% in those who had no HIV infection at birth (385). Similar growth deficits were evident in CWH from developing countries, before the initiation of ART. Overall, stunting and underweight are more common in low resource settings, even in CWOH (386,387). In Africa, CWH start ART later in life than other parts of the world, often when the disease is already in a more advanced stage, and this can potentially affect growth (388). Studies from Senegal, Uganda, Ethiopia, and Zimbabwe all report high prevalence of stunting , underweight, or both (3,388,389). Youth in Zimbabwe who test HIV positive for the first time between the ages of 6 and 13 have high rates of short stature (23%) and underweight (27%), with 35% having a head circumference Z-score ≤2 (3). In a cross-sectional analysis comparing 7-16 year old American CWH (n=399) to children exposed to HIV but uninfected and to CWOH, CWH had a 5% lower percentage total body fat, a 2.8% lower percentage extremity fat, a 1.4% higher percentage trunk fat, and a 10% higher trunk-to-extremity fat ratio than children exposed to HIV but uninfected. The same study reported CWH to have lower total body fat compared with CWOH from the NHANES study (390). In Canada, lower muscle cross-sectional area as

measured by pQCT, was reported in CWH (n=32) than CWOH (343). Studies investigating DXA measured lean mass and fat mass in CWH in Africa are scarce, with available studies having either a small sample size or lacking a control population.

An improvement in growth has been reported in CWH who have an early HIV diagnosis and less severe HIV symptoms (389). Children in Central Africa have been reported to experience significant increases in WAZ when ART is started, reducing the number of underweight children from 56% in 2004–2005 to 36% in 2012–2013 (391). Notably most studies have assessed growth impairment in CWH younger than 10 years old, with only a few studies measuring growth specifically in 10–19-year-olds, those that have reported persistent growth problems (388,392). Table 3.5 below summarises a few studies that have focused on assessing growth patterns in CWH in sub-Saharan Africa and have reported prevalence of stunting, underweight and wasting. It is possible that some of the differences in aBMD observed in children living with and without HIV may be explained by the effects of bone size on areal BMD measurements, since CWH are shorter or more stunted and therefore more likely to have smaller bones.

Table 3.5: Prevalence of stunting, underweight and wasting in children living with HIV; findings from selected studies focusing on growth patterns in children living with HIV in sub-Saharan African countries

Author	Country Study Design	CWH (n) CWOH (n)	Age range (years)	Underweight	Stunting	Wasting
Mwambenu 2022 (393)	South Africa Cohort	CWH:288	13 - 18	29%	56%	NR
Seth 2018 (394)	India Cohort	CWH: 180	0 - 14	NR	78%	NR
Poda 2017 (378)	Burkina Faso Case control	CWH: 164 CWOH: 164	0 - 5	77%	65%	63%
McHugh 2016 (3)	Zimbabwe Cross-sectional	CWH: 385	6 - 15	27%	23%	16%
Feucht 2016 (4)	South Africa Cohort	CWH: 159	0 - 5	50%	73%	19%
Tekleab 2016 (379)	Ethiopia Cohort	CWH: 202	0 - 5	40%	71%	16%
Joel 2014 (380)	Botswana Cohort	CWH: 1604	1 - 18	NR	28%	NR
Mwiru 2014 (381)	Tanzania Cohort	CWH: 3144	0 - 15	40%	52%	30%
McGrath 2012 (382)	Kenya Cohort	CHEU: 401	0 - 2	29%	58%	18%
Sutcliff 2011 (383)	Zambia Cohort	CWH: 193	0 - 16	59%	72%	NR

CWH; children living with HIV, CWOH; children living without HIV, CHEU; children who are HIV exposed but uninfected, NR; Not reported

3.5.2 Low Calcium and Vitamin D intake

Inadequate intake of vitamin D and vitamin D deficiency are highly prevalent in CWH (337,338). In South Africa, a 2-year vitamin D and calcium supplementation randomized placebo controlled trial in 6 to 16 year olds (n=56) did not report improvements in bone mass despite achieving adequacy in plasma concentrations of 25-OH D among those CWH who were receiving supplementation (349,395). However, their small sample size could have meant the study was not powered enough to detect changes within the 2 years of follow up. Vitamin D is the main regulator of calcium homeostasis and mineralization, which gives bone mass and strength. Vitamin D deficiency is more common in PLWH, due to restricted sun exposure due to sickness, malabsorption, infections, and ART (396,397). Evidence from a systematic review of the effects of vitamin D supplementation on bone and muscle showed that vitamin D deficiency is common among CWH around the world (398). Randomized clinical trials from the USA (n=59) and Thailand (n=26) showed that giving CWH vitamin D improves BMD (349,399). In addition, vitamin D affects muscle development which in turn affects bone as bone adapts to the muscle forces acting upon it (400). Post-hoc analysis of a small (n=56) study of young Americans with HIV suggested vitamin D supplementation may improve muscle power (401). These trials are small and none were conducted in SSA, where 90% of CWH live. 1,25-dihydroxyvitamin D3 aids bone mineralization by increasing intestinal and renal calcium absorption. Given the low calcium intake reported amongst people living with HIV, vitamin D supplementation may improve calcium intake and therefore improve bone accrual in CWH. Vitamin D insufficiency disrupts T-cell subset distribution and macrophage activity (402). Vitamin D deficiency may worsen HIV-mediated immunological activation (which causes comorbidities) and increase HIV-related all-cause mortality (402,403). Vitamin D deficiency is a factor that can be changed to reduce immune activation and the risk of long-term problems. Taking a high dose of vitamin D decreased T-cell and monocyte activation in American 8 to 25 year old children and youth living with HIV (n=51) (404). Vitamin D deficiency is linked to the growth of pro-inflammatory bacterial species, and vitamin D supplementation has recently been shown to change the composition of the gut microbiota (405). There is growing evidence that the gut microbiome affects both immune and bone homeostasis. Vitamin D supplementation may have some effect on bone through its effect on the gut microbiome (406).

3.5.3 Delayed Puberty

Adolescents of the same chronological age may have delayed skeletal maturity (407,408). Timing of peak bone accrual is closely related to pubertal development. Delayed puberty is associated with a lower PBM (409). A study conducted comparing growth and puberty in

Nigerian CWH and CWOH reported delayed pubertal development (as demonstrated by breast and pubic hair stages of sexual maturation) and increase in mean age at menarche of 1.4 years (from 11.8 years in the HIV uninfected to 13.2 years) among CWH. This is similar to findings from other sub-Saharan African countries. It is also similar to findings in studies assessing growth and puberty from high income countries (393,410–413). Other studies have suggested that pubertal delay in CWH is caused by underlying malnutrition, chronic inflammation and opportunistic infections. In South African CWH, the duration and magnitude of the pubertal growth spurt was found to be lower than the WHO reference population, with the age at PHV in girls being delayed by about 12 months (PHV=5.9 cm/year), while in boys the age at PHV was delayed by 24 months (PHV=5.7 cm/year) (393). Evidence from high income settings (USA) shows that in addition to pubertal onset occurring later for the CWH than CHEU, CWH who had an HIV-1 RNA viral load >10,000 copies/mL or a CD4% <15% had 4-13months later pubertal onset compared to those with HIV-1 RNA viral load <10,000 copies/mL or CD4% ≥15% (411). In an earlier study of CWH in Italy, pubertal onset was delayed by 2 years in females and 1 year in male CWH (413).

3.5.4 Low Physical activity

In CWH, assessments of physical activity, an important determinant of muscle mass and bone accrual, have been limited. Some authors have suggested that perinatally infected CWH could have deficits in muscle power, body mass, and muscle function which then influence their activities of daily living and the overall quality of life resulting in low physical activity in these children (414,415). Whilst chronic conditions affecting health in childhood and adolescence such as perinatal HIV infection usually limit participation in physical activity and sport, as a consequence of real or perceived limitations imposed by the disease, ART also induces muscle and metabolic abnormalities that may contribute to the reduction in exercise performance in CWH (416,417). In contrast to studies reporting low physical activity in CWH, DiMeglio et al. reported greater (self-reported on questionnaire) physical activity to be associated with higher LS BMD in a study including children from USA and Puerto Rico (328), Lima et al. did not find bone indices to be associated with accelerometer-assessed levels of physical activity in Brazilian children (418). Furthermore, Mulligan et al. found no association between low bone mass and regular exercise measured by questionnaire (325).

3.6 Implications of impaired bone architecture in children living with HIV

Impaired bone architecture in children living with HIV presents both a possible risk of not reaching PBM and an increased risk of fragility fractures. Children with lower bone density, size

or strength are at greater risk of fracture (298). In 5 – 16 year old CWOH in USA, those who had low pQCT measured vBMD, cortical area and SSI of the distal radius were associated with an increased fracture risk (298). To my knowledge, studies evaluating fracture prevalence and incidence in CWH are only four (419–422). Table 1 presents studies that have reported prevalence and incidence of fractures in CWH and shows whether or not this was in comparison to CWOH or to children exposed but uninfected with HIV (CHEU). A recent study showed that the adjusted risk of having at least one fracture of any type was 1.54 times higher (hazards ratio (HR): 1.54; 95% CI: 0.97, 2.44) in CWH than in CHEU (419). However, when these children were stratified by age, the IRR of any fracture amongst those who were less than 6 years old was 7.23 times higher (Incidence rate ratio (IRR): 7.23; 95% CI 0.98, 53.51) in CWH than in CHEU whereas there was no difference between CWH and CHEU (IRR: 1.20; 95% CI: 0.77, 1.87) in the greater than 6 year old age group (419). In an earlier prospective cohort study of CWH who were between 5 and 20 years of age, showed similar rates of fracture in CWH and CHEU (421). This study however, relied on passive clinical event collection possibly resulting in underreporting of fracture events in both groups. In a similar subsequent study, fracture rates increased over time in those who were living with HIV, even after adjustment for age (420). More recently, a fracture prevalence of 7% in CWH and 5% in CWOH has been reported in Zimbabwean children (422). Despite the fracture prevalence being similar in CWH and CWOH, fractures were associated with low size-adjusted bone density in CLWH (422). Given that evidence from high income countries has shown that fracture rates increase over time in CWH (420), further bone architecture and bone fracture studies in Zimbabwean children and/or children living in sub-Saharan African counties is necessary.

Table 3.6: Studies reporting fracture prevalence and/or incidence in children living with HIV

Author Year	Country Design (Follow up)	Sample size	Age (years)	Type of fracture	Prevalence (%)	Incidence (95% CI)
Rukuni 2023	Zimbabwe Cross-sectional	CWH: 303 CWOH: 306	Range: 8 – 16 Mean (SD):12.5(2.5)	Traumatic & atraumatic	CWH: 7% CWOH: 5%	
Jacobson 2020	USA, Puerto Rico Cohort (5 years)	CWH: 412 CHEU: 206	Range: 7 – 16 Median(min, max:18(8, 22)	Traumatic & atraumatic	CWH: 17% CHEU: 12%	IRR:7.2 (1.0, 53.5)[a] IRR:1.2 (0.8, 1.9)[b]
Mirani 2015	USA Cohort (12 months)	CWH: 303 CWOH: 306	Range: 8 – 16 Mean (SD): 17.4(5.4)	Traumatic & atraumatic		IRR:5.1 (1.7, 15.3)
Siberry 2012	USA Cohort (5 years)	CWH: 1326 CHEU: 648	Range: 5 – 20 Mean(Min, max):7(5, 10)	Traumatic & atraumatic		IRR:1.1 (0.2, 5.5)

CWH; children living with HIV, CWOH; children living without HIV, CHEU; children who are HIV exposed but uninfected, SD; Standard deviation
[a] Incidence rate ratio for children under 6 years of age
[b] Incidence rate ratio for children over 6 years of age
IRR; Incidence rate ratio per 100 person years

3.7 Conclusion

This chapter narrates literature reporting bone density, size and strength in CWH. Although the studies discussed in this chapter vary with respect to imaging methods used, adjustment techniques for body size or growth retardation and highlighted risk factors, most studies reported lower bone outcome measures in CWH than CWOH. Decreasing aBMD after TDF initiation has been a consistent finding. However, studies assessing bone architecture beyond bone density are scarce. Questions remain regarding short- and long-term effects of TDF exposure in CWH. vBMD, bone size and bone strength measures in children and adolescents living with HIV have not been evaluated extensively. In light of the potential importance of bone size and bone strength, assessments of these in the context of HIV and ART are warranted, as these may have implications for future fracture risk.

4 CHAPTER 4: Manuscript 1- Impaired bone architecture in peripubertal children with HIV, despite treatment with anti-retroviral therapy: a cross-sectional study from Zimbabwe

4.1 Introduction

The first question asked by this PhD is how bone architecture in children living with HIV compares to that of children living without HIV. To address this question, I compared bone density, bone size and predicted bone strength parameters of trabecular and cortical bone in children from Harare, Zimbabwe who were living with and without HIV. I investigated whether any association of HIV with pQCT measured bone outcomes differed by pubertal stage by testing for interaction. Furthermore, among CWH, I did an analysis to determine the association between TDF exposure and bone outcomes. I developed the specific research questions, developed the pQCT methods section of the protocol used in this study, with guidance from my supervisors and wrote the pQCT standard operation procedures. I collected the pQCT data included in this analysis, together with a team of radiographers that I trained and led on the pQCT scanning for this data collection. I performed the pQCT quality control and graded all the pQCT scans included in this study. I developed the statistical analysis plan together with the Stata analysis do files and I performed the statistical analysis, with support from my supervisor, Andrea M. Rehman. I produced the tables of results and graphs, wrote the first draft of the paper, and led on all revisions.

Cover sheet

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RESEARCH PAPER COVER SHEET

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SECTION A – Student Details

Student ID Number	1806477	**Title**	Mrs
First Name(s)	Cynthia		
Surname/Family Name	Kahari		
Thesis Title	The effect of HIV and its treatment on trabecular and cortical bone architecture in children, adolescents and premenopausal women		
Primary Supervisor	Andrea Rehman/Melissa Neuman		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

SECTION B – Paper already published

Where was the work published?	Journal of Bone and Mineral Research (JBMR)		
When was the work published?	Feb 2023 ; first published online in Nov 2022		
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion	N/A		
Have you retained the copyright for the work?*	**No**	Was the work subject to academic peer review?	**Yes**

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Where is the work intended to be published?	
Please list the paper's authors in the intended authorship order:	

Stage of publication	Choose an item.

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I developed the specific research questions, designed the pQCT methods of this study protocol with guidance from supervisors and advisors. I collected the pQCT data included in this analysis, together with a team of radiographers that I trained and led on the pQCT scanning for this data collection. I performed the pQCT quality control and graded all the pQCT scans included in this study. I developed the statistical analysis plan together with the Stata analysis do files and I cleaned the pQCT data and performed the statistical analysis, with support from my supervisor Andrea M. Rehman. I produced the tables of results and graphs, wrote the first draft of the paper, and led on all revisions.

SECTION E

Student Signature	[redacted]
Date	31 Jul 2023

Supervisor Signature	[redacted]
Date	18/8/2023

4.1.1 Title

Impaired bone architecture in peripubertal children with HIV, despite treatment with anti-retroviral therapy: a cross-sectional study from Zimbabwe

4.1.2 Authors

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Running title: Impaired bone architecture in children with HIV

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4.2 Abstract

HIV infection has multi-system adverse effects in children including on the growing skeleton. We aimed to determine the association between chronic HIV infection and bone architecture (density, size, strength) in peripubertal children. We conducted a cross-sectional study of children aged 8-16 years with HIV (CWH) on combined antiretroviral therapy (ART), and children without HIV (CWOH) recruited from schools, frequency matched for age strata and sex. Outcomes, measured by tibial peripheral quantitative computed tomography (pQCT), included 4% trabecular and 38% cortical volumetric bone mineral density (vBMD), 4% and 38% cross-sectional area (CSA), and 38% stress-strain index (SSI). Multivariable linear regression tested associations between HIV status and outcomes, stratified by sex and puberty (Tanner 1-2 *vs*. 3-5), adjusting for age, height, fat mass, physical activity, socio-economic and orphanhood statuses. We recruited 303 CWH and 306 CWOH; 50% female. Whilst CWH were similar in age to CWOH (overall mean±SD 12.4±2.5years), more were pre-pubertal (i.e Tanner 1; 41% vs. 23%). Median age at ART initiation was four (IQR 2–7) years, whilst median ART duration was eight (IQR 6–10) years. CWH were more often stunted (height-for-age Z-score<-2), than those without HIV (33% *vs* 7%). Both male and female CWH in later puberty had lower trabecular vBMD, CSA (4% and 38%) and SSI than those without HIV, whilst cortical density was similar. Adjustment explained some of these differences; however, deficits in bone size persisted in CWH in later puberty (HIV*puberty interaction p=0.035[males; 4% CSA] and p=0.029[females; 38% CSA]). Similarly, puberty further worsened the inverse association between HIV and bone strength (SSI) in both males (interaction p=0.008) and females (interaction p=0.004). Despite long-term ART, we identified deficits in predicted bone strength in those living with HIV, which were more overt in the later stages of puberty. This is concerning as this may translate to higher fracture risk later in life.

Keywords: ANALYSIS/QUANTIFICATION OF BONE, DISEASES AND DISORDERS OF/RELATED TO BONE, EPIDEMIOLOGY

4.3 Introduction

Improved access and earlier antiretroviral therapy (ART) initiation in children with HIV (CWH) has markedly increased survival, enabling many children to now reach puberty and adulthood (423). The global decline in HIV-associated child and adult deaths has largely been achieved in Eastern and Southern Africa, home to 89.2% (2.5 million out of 2.8 million) of the world's CWH (424). There is increasing recognition that, despite ART, HIV has adverse effects on multiple organ systems in children resulting in long-term multisystem comorbidities. These are of growing concern as the improved survival due to ART means that increasing numbers of CWH are now entering adolescence and adulthood (2). In sub-Saharan Africa (SSA), CWH are commonly underweight (weight-for-age Z-score <-2) and/or stunted (height-for-age Z-score <-2), with the prevalence of stunting varying from 23% to 73% (2–4). Linear growth continues through adolescence as bone accrues to achieve peak bone mass (PBM) (5). PBM is a critical determinant of adult osteoporotic fracture risk (6); a 10% reduction can double fracture risk in adulthood (although this has not yet been studied in African populations) (8,9). The long-term impact of exposure to both HIV infection and ART in perinatally-infected children is of concern as impaired linear growth may be associated with sub-optimal PBM accrual, with implications for adult fracture risk (425).

We recently reported lower dual energy x-ray absorptiometry (DXA) measured bone outcomes of total-body less-head (TBLH) bone mineral content (BMC) for lean mass adjusted for height (TBLH-BMC^{LBM}) and lumbar spine bone mineral apparent density (LS-BMAD) in CWH than in children without HIV (CWOH), which was more overt in later adolescence (229). Tenofovir disproxil fumarate (TDF) exposure has been associated with low aBMD in adults living with HIV (232,235). Recently, among CWH, both TDF exposure and orphanhood were associated with lower TBLH-BMC^{LBM} Z-score (229).

In a smaller cross-sectional study in Zimbabwe, age at ART initiation was strongly negatively correlated with both LS-BMAD and TBLH-BMC ^{LBM} Z-scores, with a 0.13 SD reduction in LS-BMAD seen for each year that ART initiation was delayed (230). However, both these studies used DXA, which cannot differentiate trabecular from cortical bone (236,237). Peripheral quantitative computed tomography (pQCT) offers an opportunity to study bone architecture, quantifying volumetric BMD, and bone size which enables prediction of bone strength (267,268). A recent South African study compared pQCT measured bone architecture between 172 CWH and 98 CWOH aged 7 to 14 years, reporting lower trabecular vBMD in male CWH, and generally lower bone strength in CWH than in CWOH (238).

We hypothesized that HIV infection would be associated with adverse effects on bone architecture leading to compromised pQCT bone outcomes i.e vBMD, bone size and predicted strength (see supplementary Fig. 1). We therefore sought to compare the bone density, bone size and predicted bone strength parameters of trabecular and cortical bone in peripubertal males and females living with and without HIV in Harare, Zimbabwe. We investigated whether any association of HIV with pQCT measured bone outcomes differed by pubertal stage by testing for interaction. Furthermore, among CWH, we determined the association between TDF exposure and bone outcomes.

4.4 Methods

4.4.1 Study setting

A cross-sectional study was conducted using baseline pQCT measurements from the IMpact of Vertical HIV infection on child and Adolescent Skeletal development (IMVASK) study, as per published protocol (ISRCTN12266984) (229,249). CWH, established on ART for at least two years, were quota sampled stratified by sex- and age- strata (8-10, 11-13 and 14-16 years) from HIV clinics at Parirenyatwa and/or Sally Mugabe Hospitals in Harare, Zimbabwe. These are the two large public hospitals in Harare, providing HIV care services to over 2,000 children. CWOH were randomly recruited, using school registers, from three primary and three secondary schools within the same community suburbs served by the hospitals providing HIV care, and were frequency-matched by sex and age strata. Inclusion criteria were: age 8 to 16 years, living in Harare. CWH were included if they were aware of their HIV status and had been taking ART for at least 2 years (because of stabilization in bone outcomes after starting ART). At the time of enrolment into the study, CWOH underwent HIV testing to confirm their status. Children with acute illness requiring hospitalization, those lacking guardian consent and those who were recently diagnosed with HIV were excluded from this study.

4.4.2 Study Procedures

Data were collected between May 2018 and Jan 2020. Demographic and clinical data were collected using an interviewer-administered questionnaire together with participant's medical records. Demographic and clinical data collected included age, sex, socio-economic status, guardianship, orphanhood, age at HIV diagnosis, age at ART initiation, ART regimen, diet and physical activity. SES was derived using the first component from a principal component analysis (426) combining details including number in household, head of household age, highest maternal and paternal education levels, household ownership, monthly household income, access to electricity, water, a flush toilet and/or pit latrine and ownership of a fridge, bicycle, car, television, and/or radio and was split into tertiles for analysis. The International

Physical Activity Questionnaire (IPAQ) short version (427), classified intensity of physical activity as: low (MET [metabolic rate] minutes <600 per week), moderate (MET minutes =600 to 3000 per week) and vigorous (MET minutes >3000 per week). Diet was assessed using a tool developed for the Zimbabwean context based on a validated dietary diversity and food frequency tool from India and Malawi and international guidelines applicable to SSA (428,429). Daily dietary calcium (Ca) intake was classified as very low (<150mg/day), low (150-299mg/day) and moderate (300–450mg/day). Daily dietary vitamin D intake was classified as very low (<4.0mcg/day), low (4.0-5.9mcg/day) and moderate (6.0-8.0mcg/day).

Puberty was Tanner staged by a trained study nurse and/or doctor. For males, testicular volume, penile size (length and circumference) and pubic hair growth (quality, distribution and length) were assessed. For females, breast growth (size and contour) as well as pubic hair growth and age of menarche were assessed. Testicular, breast and penile growth were graded from I-V based on Tanner descriptions (199,200,430). Where there was a discordance between the stages for males and females, testicular and breast development stage respectively were used to assign Tanner Stage. Participants were grouped into Tanner stages 1 and 2 (pre- to early puberty) and Tanner stages 3 to 5 (mid/late puberty). Three standing height measurements were obtained to the nearest 0.1cm using a Seca 213 stadiometer (Hamburg, Germany), and three weight measurements were obtained to the nearest 0.1kg with a Seca 875 weight scale (Hamburg, Germany), with means calculated. Equipment was calibrated annually. Using British 1990 growth references, height-for-age Z-score <-2, weight-for-age Z-score <-2 and low weight-for-height BMI Z-score <-2 were used to define stunting, underweight and wasting respectively (431,432). Fat mass and fat-free soft tissue (lean) mass were measured by whole body DXA scan, using a Hologic QDR Wi machine with Apex software version 4.5 (Hologic Inc., Bedford, MA, USA). CD4 cell count was measured using a PIMA CD4 machine (Waltham, Massachusetts, USA). HIV viral load was measured using the GeneXpert HIV-1 viral load platform (Cepheid, Sunnyvale, California, USA).

4.4.3 pQCT scan acquisition

Non-dominant tibial pQCT scans were performed using an XCT 2000™ (Stratec Medizintecknik, Pforzheim, Germany), with voxel size 0.5 x 0.5 mm and slice thickness 2 mm (CT scan speed 30 mm/s; scout view scan speed 40 mm/s). Tibial length was measured from the distal medial malleolus to the tibial plateau with a metal ruler. Scan sites were determined as a percentage of tibia length, with the exact position determined by scout view placement of a reference line on the growth plate, or on the end plate for those with fused growth plates (433). As long bones

grow in length, the growth plate moves upward and the wider metaphysis is reshaped into a diaphysis by continuous resorption by osteoclasts beneath the periosteum (317). To allow for consistency of a scan site the reference line was placed at the growth plate in those children whose end plate and growth plate were not yet fused. vBMD was measured in mg/cm^3 at the 4% (metaphyseal) site for trabecular bone and the 38% (diaphyseal) site for cortical bone. Bone size measures included 4% and 38% total cross-sectional area [CSA, mm^2] and 38% cortical thickness (mm). Predicted bone strength was measured as 38% stress strain index [SSI, mm^3] indicating the bending and torsional strength of bone (265). The manufacturer's software (version 6.20) was used for image processing and analysis. At the 4% tibia CALCBD was used to calculate total CSA and trabecular vBMD. CALCBD contour mode 1 (*i.e.* threshold algorithm) was used to exclude pixels in defined regions of interest (ROI) that fell below a threshold of 180 mg/cm^3, peel mode 1 (*i.e.* concentric peel) peeled away the outer 55% of the total bone CSA leaving an inner 45% CSA considered as the trabecular region of interest. At the 38% tibial site CORTBD was used to define cortical vBMD and area, this algorithm removes all voxels within ROIs with an attenuation coefficient below a 710 mg/cm^3 threshold (with separation mode 1). Total CSA was defined at the 38% tibia using a 180 mg/cm^3 threshold. Cortical thickness was calculated using a circular ring model. A phantom was scanned daily for quality assurance. Thirty participants were scanned twice, with repositioning, to assess reproducibility. Coefficients of variation were calculated from the results of the 30 re-scanned participants. The short-term precision (Root Mean Square % CV) was 1.26% for trabecular vBMD, 0.37% for cortical vBMD, 2.08% for 4% total CSA and 0.93% for 38% CSA. All pQCT scan slices and scout views were qualitatively graded by a single radiographer. Movement artefacts were graded 0 to 3: (0) none, (1) slight, (2) medium streaking and (3) scan unusable. Grade 3 images were excluded from analysis.

4.4.4 Ethical considerations

Trained research assistants and/or a study nurse explained the study information to participants and guardians. Guardians gave written informed consent and participants gave age-appropriate written assent. This study was approved by the Parirenyatwa Hospital and College of Health Sciences joint research ethics committee (JREC/123/19), the Biomedical Research and Training Institute Institutional Review Board (AP150/2019), the London School of Hygiene and Tropical Medicine (17154) Ethics Committee and the Medical Research Council of Zimbabwe (MRCZ/A2494).

4.4.5 Statistical analysis

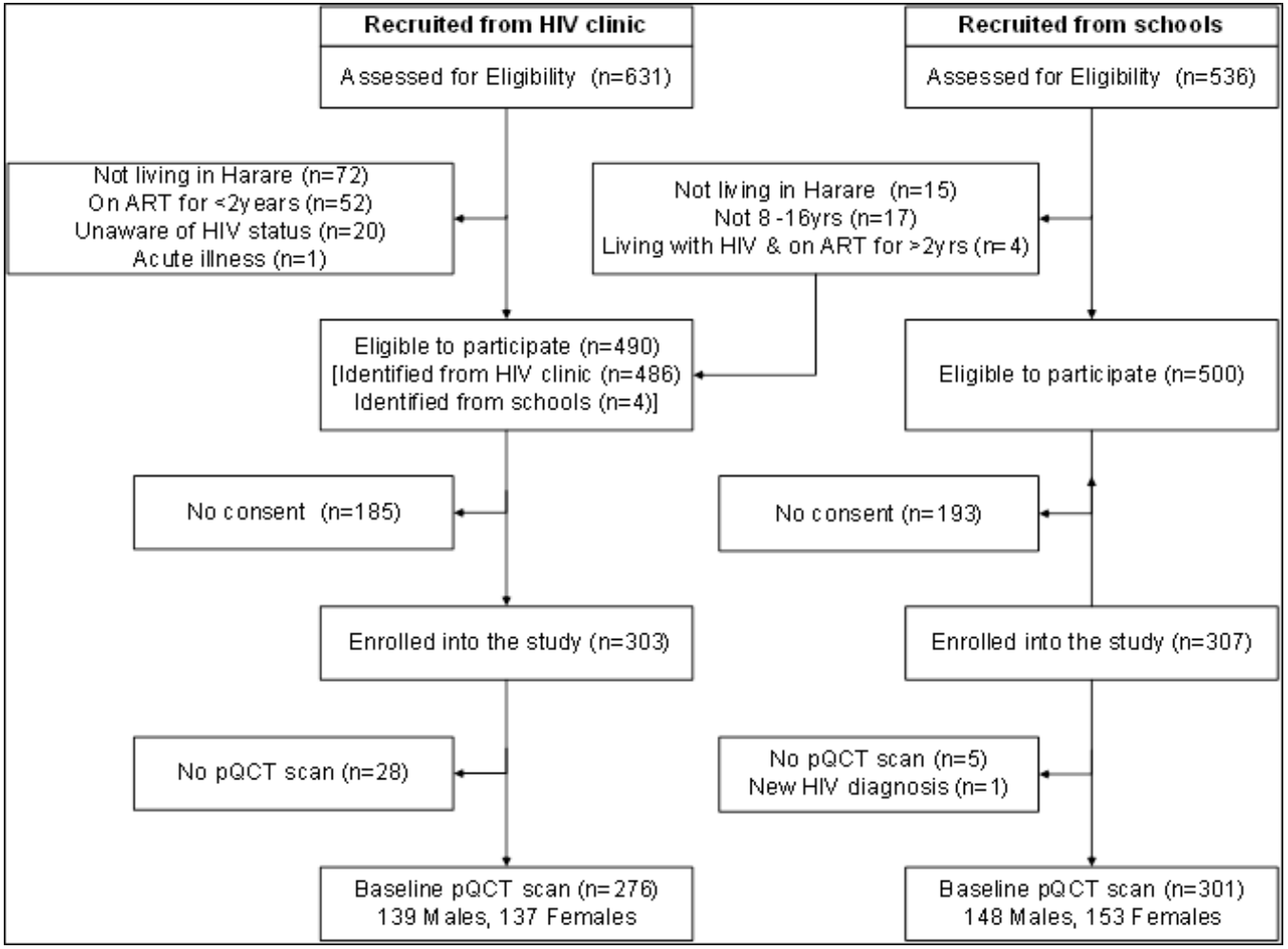
Statistical analyses were performed using Stata version 16.0 (Stata Corporation Inc., College Station, TX, USA). Data were cleaned and checked for consistency and outliers. Outcomes included 4% trabecular and 38% cortical vBMD (mg/cm^3), 4% and 38% CSA (mm^2), 38% cortical thickness (mm) and 38% stress-strain index (SSI) (mm^4). We used independent sample t-tests to compare continuous data between groups and percentages and chi-squared tests for categorical data. We determined differences between CWH and children without HIV, stratified by sex (given different bone accrual rates) in separate regression models, by comparing means using linear regression and generating mean differences and 95% confidence intervals. We compared an unadjusted model with a model adjusting for *a priori* confounders; age (years), height (cm), binary pubertal status (pre/early Tanner 1-2 *vs*. mid/late Tanner 3-5, to maximize statistical power) (434), fat mass (435), physical activity (436), SES (437) and orphanhood (229) (see Supplementary figure 1). As lean mass was co-linear with height (correlation coefficient = 0.90, p value= <0.001), lean mass adjustment was not made to avoid collinearity. If lean mass was included in linear regression, the variance inflation factors exceeded a value of 8. We assessed modification of the association of HIV with bone outcomes by pubertal stage, by incorporating a binary interaction term for puberty (pre/early *vs*. mid/late). In further analyses, restricted to CWH, we compared those who were exposed to TDF with those who were not. To account for missing data assumed missing at random, multiple imputation by chained equations (with 7 imputed datasets), allowing for imputation of categorical and continuous data simultaneously was performed (438). Imputation models included all pQCT bone outcomes, variables associated with missingness (Tanner stage only), and factors determined in complete case analysis to be associated with HIV (fat mass, SES, orphanhood, physical activity and sex). Imputation models were run on males and females combined, with regression analysis models using the imputed data run stratified by sex.

4.5 Results

4.5.1 Characteristics of the study population

The study recruited 610 participants, of whom 578 (94.8%) (276 with and 302 without HIV) had a pQCT scan (Figure 4.1). One school participant was excluded as they were newly diagnosed with HIV. Participants who did not have a pQCT scan were similar to those with a pQCT scan in terms of sex, pubertal status, SES, calcium and vitamin D intake, but children without a pQCT scan were more likely to be living with HIV and to report lower levels of physical activity compared to those who had a scan (Supplementary table 1).

Figure 4.1: Flow diagram to show participants included in the pQCT study



- *Figure 4.1 above shows participants enrolled and included in the study data analysis. All enrolled children were included in the final analysis, unless withdrawn from the study. Missing data was estimated by multiple imputation.*
  pQCT; Peripheral Quantitative Computed Tomography

Of the participants with a pQCT scan, CWH were similar in age to those without HIV. However, CWH were more likely to be in Tanner stages 1 and 2 compared with their uninfected counterparts; this was the case for both males (67.6% *vs*. 52.3%; p=0.009) and females (55.2% *vs*. 39.0%; p=0.005) (Table 4.1). Height increased with Tanner stage, in both CWH and CWOH (Supplementary Table 4.2). Compared with CWOH, CWH were mean 8.0cm (males) and 6.9cm (females) shorter, and 3.7kgs (males) and 6.9kgs (females) lighter (p<0.001 for all), with corresponding lower fat and lean mass.

Overall CWH had been diagnosed with HIV when aged 3.9±3.2 (mean ± SD) years, and started on ART at a median age of 3.7 (IQR 1.8–6.9) years, so that at the time of participation in this study, median ART duration was 8.1 (IQR 6.2–9.5) years. The majority (79%) had a suppressed viral load (<1000 copies/ml), and only 2.3% had a CD4 count <200 cells/mm^3. Among CWH 21% (n=63/303) were taking TDF at the time of the study. However, 33.6% (n=102) reported ever using TDF as part of their ART regimen, of whom 13.2% (n=40) reported taking it for more than 4 years. The average age at TDF initiation was 10 years.

CWH were more likely to have a lower SES than children without HIV. Compared with children without HIV, both male and female CWH were more likely to be orphans or to have only one surviving parent (Table 1). In both sexes, physical activity levels were lower in CWH compared to those without HIV. In both males and females, dietary calcium and vitamin D intakes were similar in those with and without HIV, with the majority having a low or very low calcium intake (Table 1).

Table 4.1: Demographic and anthropometric characteristics, by HIV status, in male and female children and adolescents

		Males (n=303)				Females (n=306)			
Clinical Characteristics		**n**	**CWH (n=152)**	**CWOH (n=151)**	**p value**	**n**	**CWH (n=151)**	**CWOH- (n=155)**	**p value**
Age (years)	Mean (SD)	303	12.5 (2.5)	12.4 (2.5)	0.773	306	12.4 (2.6)	12.6 (2.5)	0.518
Age Group (years)	8-10 years	303	52 (34.2)	50 (33.1)	0.920	306	50 (33.1)	48 (31.0)	0.890
	11-13 years		52 (34.2)	50 (33.1)			50 (33.1)	51 (32.9)	
	14-16 years		48 (31.6)	51 (33.8)			51 (33.8)	56 (36.1)	
Tanner Stage (%)	Tanner 1	292	57 (40.1)	45 (30.0)	0.026	299	60 (41.4)	25 (16.2)	<0.001
	Tanner 2		39 (27.5)	34 (22.7)			20 (13.8)	35 (22.7)	
	Tanner 3		22 (15.5)	24 (16.0)			33 (22.8)	29 (18.8)	
	Tanner 4		19 (13.4)	43 (28.7)			25 (17.2)	49 (31.8)	
	Tanner 5		5 (3.5)	4 (2.7)			7 (4.8)	16 (10.4)	
Socio-Economic Status (%)	Low, Tertile 1	303	54 (35.5)	38 (25.2)	0.121	306	61 (40.4)	50 (32.3)	0.014
	Middle, Tertile 2		51 (33.6)	54 (35.8)			54 (35.8)	44 (28.4)	
	High, Tertile 3		47 (30.9)	59 (39.1)			36 (23.8)	61 (39.4)	
Orphan Status (%)	Not an orphan	294	84 (58.3)	139 (92.7)	<0.001	299	83 (56.8)	144 (94.1)	<0.001
	One parent alive		53 (36.8)	7 (4.7)			52 (35.6)	7 (4.6)	
	Orphan		7 (4.9)	4 (2.7)			11 (7.5)	2 (1.3)	
Physical Activity in METS (%)	Low, <600	303	71 (46.7)	51 (33.8)	0.048	306	77 (51.0)	63 (40.6)	0.028
	Moderate, 600 -3000		35 (23.0)	50 (33.1)			42 (27.8)	38 (24.5)	
	High, >3000		46 (30.3)	50 (33.1)			32 (21.2)	54 (34.8)	
Calcium Intake (mg) (%)	<150 mg	303	67 (44.1)	69 (45.7)	0.848	306	68 (45.0)	67 (43.2)	0.951
	150-299 mg		30 (19.7)	32 (21.2)			32 (21.2)	34 (21.9)	
	300-449 mg		55 (36.2)	50 (33.1)			51 (33.8)	54 (34.8)	
Vitamin D Intake, mcg (%)	<4.0 mcg	303	24 (15.8)	18 (11.9)	0.535	306	16 (10.6)	19 (12.3)	0.422
	4.0 - 5.99 mcg		99 (65.1)	99 (65.6)			106 (70.2)	98 (63.2)	
	6.0 - 7.9 mcg		29 (19.1)	34 (22.5)			29 (19.2)	38 (24.5)	
Anthropometry									
Height, cm	Mean (SD)	301	139.7 (12.6)	147.7 (15.1)	<0.001	306	140.4 (13.1)	147.3 (11.8)	<0.001
Height for age Z-score	Mean (SD)	301	-1.8 (1.2)	-0.6 (1.0)	<0.001	306	-1.5 (1.1)	-0.5 (1.1)	<0.001
Stunting (%)	HAZ<-2, %	301	55 (36.7)	10 (6.6)	<0.001	306	40 (26.5)	12 (7.7)	<0.001
Weight, kgs	Mean (SD)	303	35.5 (17.5)	39.2 (14.5)	0.053	306	36.1 (13.5)	43.0 (15.8)	<0.001
Weight for age Z-score, mm	Mean (SD)	303	-1.2 (1.2)	-0.7 (1.0)	<0.001	306	-1.3 (1.1)	-0.3 (1.1)	<0.001
Underweight (%)	WAZ<-2, %	303	50 (32.9)	15 (10.0)	<0.001	306	30 (19.9)	8 (5.2)	<0.001
Body Mass Index, kg/cm²	Mean (SD)	303	16.5 (1.5)	17.2 (2.2)	0.004	306	17.4 (2.7)	18.9 (3.6)	<0.001
BMI for age Z-score	Mean (SD)	303	-0.8 (0.9)	-0.5 (1.0)	0.007	306	-1.5 (1.1)	-0.5 (1.1)	<0.001
Wasting (%)	BAZ<-2, %	303	11 (7.3)	16 (10.7)	0.313	306	5 (3.2)	12 (8.0)	0.071
Fat Mass, Kgs	Mean (SD)	288	6.8 (1.8)	8.3 (3.1)	<0.001	287	9.5 (4.3)	13.3 (6.1)	<0.001
Lean Mass, Kgs	Mean (SD)	288	26.2 (6.7)	30.1 (9.3)	<0.001	287	26.0 (6.9)	29.2 (7.3)	<0.001
HIV Characteristics									
Time since HIV diagnosis, years	Mean (SD)	152	8.7 (2.7)			151	8.6 (2.5)		
Age at HIV diagnosis, years	Mean (SD)	152	3.8 (3.1)			151	3.9 (3.3)		
Duration on ART, years	Mean (SD)	152	8.0 (2.7)			151	7.8 (2.5)		
Age at ART initiation, years	Mean (SD)	152	4.5 (3.1)			151	4.7 (3.4)		

Age at TDF initiation, years	Mean (SD)	152	10.1 (3.8)			151	10.6 (3.2)		
Current use of TDF	Yes	152	28 (18.4)			151	35 (23.2)		
Ever used TDF	Yes	152	50 (32.9)			151	52 (34.4)		
Years of exposure to TDF	None <4 years >4 years	152	102 (67.1) 30 (19.7) 20 (13.2)			151	99 (65.6) 32 (21.2) 20 (13.2)		
Viral Load in copies/ml (%)	<1000 ≥1,000	135	106 (78.5) 29 (21.5)			133	106 (79.7) 27 (20.35)		
CD4 Count in cells/µl(%)	<200 200-499 ≥500	148	4 (2.7) 30 (20.3) 114 (77.0)			140	4 (2.9) 20 (14.3) 116 (82.9)		
pQCT bone outcomes									
Trabecular Density, mg/cm³	Mean (SD)	287	197.5 (40.0)	210.1 (40.9)	0.009	290	202.0 (33.7)	213.8 (34.1)	0.003
Cortical Density, mg/cm³	Mean (SD)	287	1068.6 (39.2)	1070.8 (34.0)	0.616	290	1099.6 (38.7)	1094.9 (46.6)	0.354
4% Total Cross-sectional Area, mm²	Mean (SD)	287	670.1 (186.6)	772.6 (239.2)	<0.001	290	667.3 (174.5)	721.1 (193.6)	0.014
38% Total Cross-sectional Area, mm²	Mean (SD)	287	317.6 (70.1)	367.6 (83.6)	<0.001	290	302.3 (59.2)	348.4 (66.1)	<0.001
Cortical Thickness, mm	Mean (SD)	287	3.6 (0.6)	3.8 (0.6)	0.066	290	3.6 (0.6)	3.6 (0.6)	0.807
Stress Strain Index, mm³	Mean (SD)	287	979.8 (316.8)	1181.5 (394.1)	<0.001	290	922.8 (275.4)	1093.4 (325.9)	<0.001

**p values for categorical variables were calculated using the chi squared test, p values for continuous variables were calculated using the t test for 2 independent samples, SES; socioeconomic status, BMI; Body mass index, TB; Total body TDF; Tenofovir Disoproxil Fumarate, CWH; children living with HIV, CWOH; children living without HIV*

NB: Data presented are unadjusted

4.5.2 pQCT measured bone outcomes, stratified by sex

4.5.2.1 Bone density

In unadjusted analyses, trabecular vBMD was 12.6 mg/cm³ (6.2%) and 11.7 mg/cm³ (5.2%) lower in male and female CWH than in children without HIV, whilst no such differences were seen for cortical vBMD (Table 4.2). However, adjustment for *a priori* confounders completely attenuated these vBMD differences. Each of the variables included in the models had a small increment on the association between HIV and each of the pQCT bone outcomes. However, adjusting for height attenuated more of the effect of HIV on trabecular and cortical density than the other covariates.

4.5.2.2 Bone size

CWH (both males and females), had smaller metaphyseal and diaphyseal tibial bone size (CSA) than CWOH (Table 4.2). These size differences were largely explained by adjustment. Differences in cortical thickness were only evident between females with and without HIV after accounting for age, height and puberty, after which females with HIV appeared to have thicker cortices than females without HIV.

4.5.2.3 Bone strength

In both males and females, before any adjustment, SSI was lower in CWH than those without HIV; however, this was explained by adjustment for age, height, and puberty (Table 4.2).

Table 4.2: Differences in pQCT measured tibial bone outcomes in children living with and without HIV before and after adjustment

Males	**Unadjusted model (n=303)**		**Adjusted model (n=303)**	
Bone density	**MD (95% CI)**	**p value**	**MD (95% CI)**	**p value**
4% Trabecular Density, mg/cm³	-12.6 (-22.1, -3.1)	0.010	-7.5 (-18.5, 3.5)	0.179
38% Cortical Density, mg/cm³	-2.3 (-11.2, 6.5)	0.604	-1.4 (-11.8, 9.1)	0.796
Bone Size				
4% Total CSA, mm²	-93.4 (-144.7, -42.2)	<0.001	-22.1 (-54.8, 10.6)	0.184
38% Total CSA, mm²	-35.6 (-66.0, -5.3)	0.021	0.4 (-24.9, 25.7)	0.975
38% Cortical Thickness, mm	-0.1 (-0.3, 0)	0.063	0 (-0.1, 0.1)	0.973
Bone Strength				
38% Stress Strain Index, mm³	-189.1 (-270.1, -108.1)	<0.001	-40.2 (-95.1, 14.6)	0.150
Females	**Unadjusted model (n=306)**		**Adjusted model (n=306)**	
Bone density	**MD (95% CI)**	**p value**	**MD (95% CI)**	**p value**
4% Trabecular Density, mg/cm³	-11.7 (-19.9, -3.6)	0.005	-7.3 (-17.7, 3.07)	0.167
38% Cortical Density, mg/cm³	4.1 (-6.3, 14.4)	0.441	8.8 (-0.6, 18.1)	0.068
Bone Size				
4% Total CSA, mm²	-50.7 (-92.8, -8.6)	0.019	17.1 (-18.0, 52.2)	0.338
38% Total CSA, mm²	-41.1 (-55.2, -27.0)	<0.001	-6.2 (-18.7, 6.4)	0.336
38% Cortical Thickness, mm	0 (-0.2, 0.1)	0.817	0.2 (0.1, 0.4)	<0.001
Bone Strength				
38% Stress Strain Index, mm³	-156.0 (-226.0, -86.0)	<0.001	16.6 (-37.4, 70.6)	0.546

Adjusted for age (years), height (cm), pubertal status, fat mass, physical activity, socioeconomic status and orphanhood

MD (95% CI); Mean Difference (95 % Confidence Interval) with children without HIV as the reference group, such that negative values mean that those with HIV have lower values than those without HIV.

*All pQCT variables, Tanner stage and orphanhood were estimated by multiple imputatio*n

4.5.3 pQCT measured bone outcomes, stratified by sex and puberty

We next investigated the potential interaction between HIV infection and pubertal stage (pre/early [Tanner 1-2] *vs*. mid/late [Tanner 3-5]) on bone outcomes.

4.5.3.1 Bone density

Differences in trabecular vBMD between children with and without HIV were similarly small, in both pre/early and mid/later puberty, with no evidence of interaction for males or females (Figure 2 and Table 3). However, amongst girls, both before and after adjustment, evidence was detected for an interaction between HIV and pubertal stage, such that in the pre/early stages of puberty females with HIV had greater cortical vBMD than females without HIV, whereas no such difference was detected in the mid/later stages of puberty (Figure 4.2 and Table 4.3).

4.5.3.2 Bone size

Before adjustment clear evidence was detected to suggest that pubertal stage was modifying the effect of HIV on bone size, such that in both males and females, CWH in mid/late puberty had substantially smaller CSA than children without HIV, at the metaphysis and diaphysis, whereas this difference was not apparent in pre/early puberty (Figure 2 and Table 3). Whilst adjustment accounted for some of these differences, evidence for interactions remained for all, other than for metaphyseal bone size in females (Table 3). No evidence was detected to suggest that puberty modifies the effect of HIV on cortical thickness.

4.5.3.3 Bone Strength

In pre/early puberty, female CWH appeared to have higher bone strength than CWOH, whereas the reduced bone strength (SSI) seen in CWH, compared to those without HIV, was most overt in mid/late puberty, in both males and females (Figure 2 and Table 3). After full adjustment, strong evidence persisted to indicate that puberty was modifying the effect of HIV on bone strength in both males (interaction p=0.008) and females (interaction p=0.004), such that HIV-associated bone deficits appear worse towards the end of puberty (Tables 3).

Table 4.3: Differences in pQCT measured bone outcomes in children by HIV and pubertal status

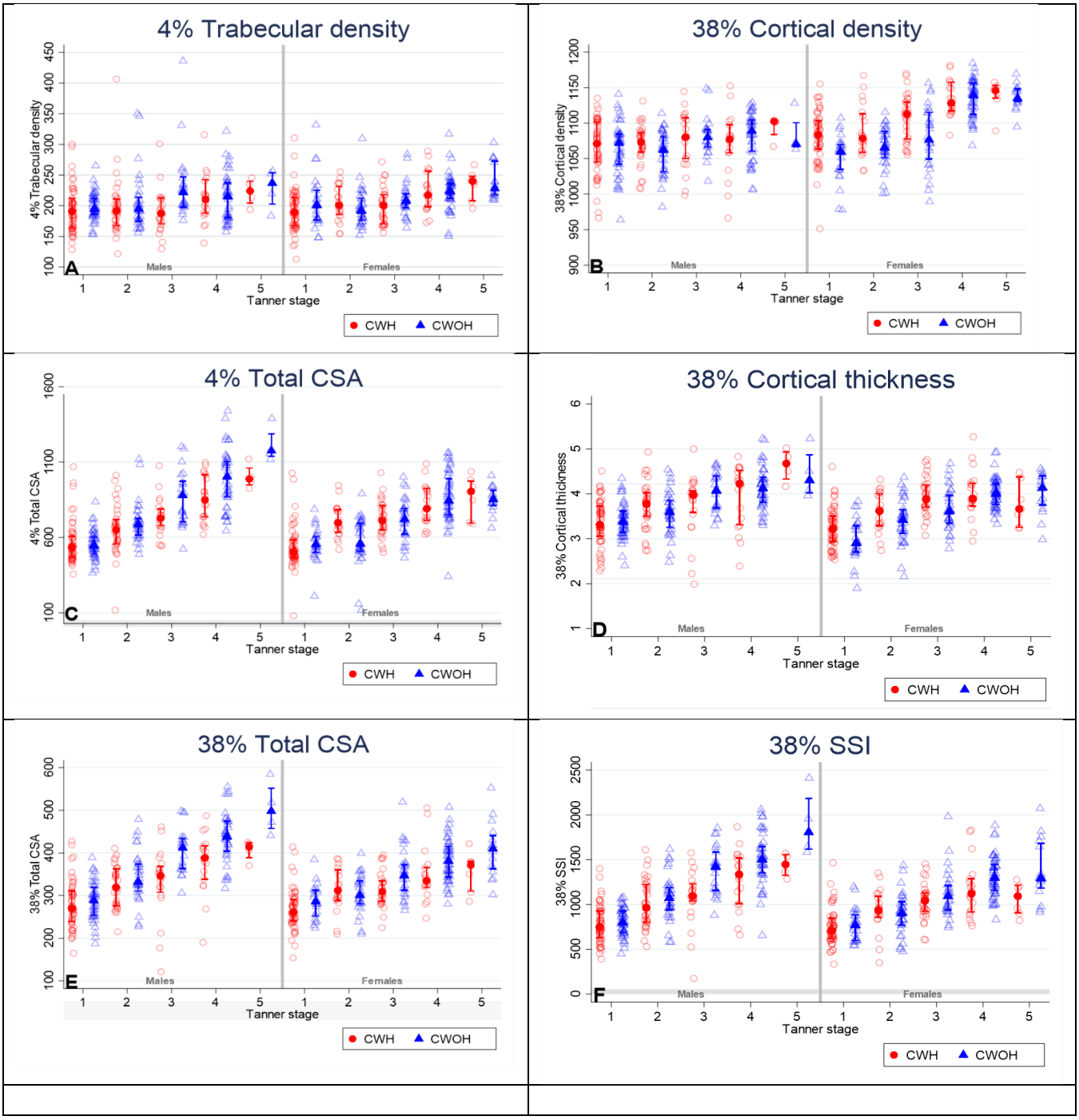
	Unadjusted model (n=303)			Adjusted model (n=303)		
Males	**Tanner Stages 1 and 2**	**Tanner Stages 3, 4 and 5**		**Tanner Stages 1 and 2**	**Tanner Stages 3, 4 and 5**	
Bone Density	**MD (95% CI)**	**MD (95% CI)**	**p value**	**MD (95% CI)**	**MD (95% CI)**	**p value**
4% Trabecular Density, mg/cm³	-6.1 (-17.4, 5.1)	-17.8 (-33.2, -2.4)	0.225	-3.0 (-16.7, 10.6)	-15.6 (-34.5, 3.4)	0.194
38% Cortical Density, mg/cm³	4.4 (-6.8, 15.5)	-7.5 (-21.8, 6.7)	0.192	2.2 (-10.6, 14.9)	-7.7 (-24.5, 9.2)	0.298
Bone SIze						
4% Total Cross-sectional Area, mm²	-2.2 (-46.8, 42.4)	-141.5 (-214.6, -67.4)	0.002	1.9 (-42.2, 46.0)	-65.1 (-126.4, -3.7)	0.035
38% Total Cross-sectional Area, mm²	7.4 (-33.9, 48.7)	-74.1 (-99.3, -48.9)	0.001	19.9 (-37.0, 76.9)	-34.5 (-76.7, 7.7)	0.016
38% Cortical Thickness, mm	0 (-0.1, 0.2)	-0.2 (-0.5, 0)	0.119	0.1 (-0.1, 0.2)	-0.1 (-0.3, 0.2)	0.369
Bone Strength						
38% Stress Strain Index, mm³	-23.5 (-98.9, 51.9)	-294.6 (-411.4, -177.7)	<0.001	14.3 (-50.2, 78.7)	-137.5 (-243.1, -32.1)	0.008
	Unadjusted model (n=306)			**Adjusted model (n=306)**		
Females	**Tanner Stages 1 and 2**	**Tanner Stages 3, 4 and 5**		**Tanner Stages 1 and 2**	**Tanner Stages 3, 4 and 5**	
Bone Density	**MD (95% CI)**	**MD (95% CI)**	**p value**	**MD (95% CI)**	**MD (95% CI)**	**p value**
4% Trabecular Density, mg/cm³	-5.8 (-18.0, 6.5)	-11.6 (-22.1, -1.1)	0.481	-8.0 (-22.5, 6.6)	-6.7 (-19.3, 5.9)	0.881
38% Cortical Density, mg/cm³	23.4 (11.5, 35.3)	0.1 (-12.1, 12.2)	0.007	19.0 (5.1, 32.9)	-1.2 (-15.0, 12.6)	0.016
Bone Size						
4% Total Cross-sectional Area, mm²	16.2 (-34.6, 67.0)	-49.3 (-100.1, 1.6)	0.087	45.6 (-1.5, 92.7)	-10.5 (-57.4, 36.5)	0.073
38% Total Cross-sectional Area, mm²	-17.9 (-34.8, -1.2)	-43.4 (-61.3, -25.7)	0.040	5.1 (-11.3, 21.6)	-17.1 (-33.7, -0.5)	0.029
38% Cortical Thickness, mm	0.16 (0, 0.4)	0.01 (-0.2, 0.2)	0.278	0.3 (0.1, 0.5)	0.2 (0, 0.4)	0.362
Bone Strength						
38% Stress Strain Index, mm³	-30.4 (-109.4, 48.6)	-170.6 (-253.6, -87.6)	0.016	81.2 (11.3, 150.9)	-46.1 (-119.3, 27.1)	0.004

Adjusted for age (years), height (cm), fat mass, physical activity, socioeconomic status and orphanhood

*MD (95% CI); Mean Difference (95 % Confidence Interval) with children without HIV as the reference group, such that negative values mean that those with HIV have lower values than those without HIV. HIV*puberty (binary) p values for interaction are shown*

All pQCT variables, Tanner stage and orphanhood were estimated by multiple imputation

Figure 4.2: Unadjusted comparison of pQCT measured bone outcomes between children living with and without HIV infection by sex and pubertal status



The marker is representing median and The bars are representing interquartile range

*NB: Data presented in this figure are unadjusted and HIV*puberty p values for interaction are shown*

CSA: Cross-sectional area, SSI: Stress strain index

4.5.4 pQCT bone outcomes in children with HIV, stratified by sex and exposure to TDF

In further analyses, restricted to CWH, those who were and were not exposed to TDF were similar in terms of sex, SES, physical activity and dietary intake of calcium and vitamin D (Supplementary table 3). However, those who were using TDF were older, taller, heavier and were more likely to be in the later stages of puberty compared with CWH who were not using TDF. After adjustment, using TDF was associated with lower trabecular vBMD in males (Table 4). No other skeletal deficits were evident between TDF exposed *vs*. non-exposed groups once analyses were adjusted.

Table 4.4: Differences in pQCT measured bone outcomes in children living with HIV by exposure to tenofovir

Males	**Unadjusted (n=151)**		**Adjusted (n=151)**	
Bone Density	**MD (95% CI)**	**p value**	**MD (95% CI)**	**p value**
4% Trabecular Density, mg/cm³	-12.9 (-27.7, 1.9)	0.088	-18.8 (-35.8, -1.8)	0.030
38% Cortical Density, mg/cm³	14.5 (-0.6, 29.6)	0.060	10.1 (-8.0, 28.2)	0.269
Bone Size				
4% Total CSA, mm²	109.6 (36.8, 182.5)	0.004	-2.4 (-70.6, 65.8)	0.945
38% Total CSA, mm²	85.8 (-31.6, 203.2)	0.151	64.9 (-77.4, 207.1)	0.369
38% Cortical Thickness, mm	0.1 (-0.1, 0.4)	0.352	-0.14 (-0.4, 0.1)	0.323
Bone Strength				
38% Stress Strain Index, mm³	122.3 (-10.0, 254.5)	0.070	-50.5 (-184.4, 83.4)	0.454
Females	**Unadjusted (n=152)**		**Adjusted (n=152)**	
Bone Density	**MD (95% CI)**	**p value**	**MD (95% CI)**	**p value**
4% Trabecular Density, mg/cm³	9.1 (-6.7, 24.9)	0.253	-5.4 (-23.7, 12.9)	0.557
38% Cortical Density, mg/cm³	14.7 (-1.1, 30.4)	0.067	-11.5 (-28.3, 5.3)	0.177
Bone Size				
4% Total CSA, mm²	175.1 (112.8, 237.4)	<0.001	45.1 (-24.9, 115.0)	0.204
38% Total CSA, mm²	47.2 (24.0, 70.4)	<0.001	5.6 (-20.4, 31.6)	0.668
38% Cortical Thickness, mm	0.2 (0.0, 0.5)	0.049	-0.1 (-0.4, 0.2)	0.423
Bone Strength				
38% Stress Strain Index, mm³	226.6 (119.3, 333.9)	<0.001	27.1 (-86.5, 140.6)	0.635

Adjusted for age (years), height (cm), pubertal status, fat mass, physical activity, socioeconomic status and orphanhood

MD (95% CI); Mean Difference (95 % Confidence Interval) with those who are not using TDF as the reference group, such that negative values mean that those using TDF have lower values than those who are not using TDF

All pQCT variables, Tanner stage and orphanhood were estimated by multiple imputation

4.6 Discussion

We report results from the largest study to date to use pQCT in a sample of children and adolescents growing up with HIV infection in SSA. In Zimbabwe, we have shown that CWH have deficits in both bone size and strength, compared with children who do not have HIV. These deficits included a 6% lower diaphyseal bone size in female CWH in the latter stages of puberty, even after accounting for body size and other confounders. Cortical thickness was greater in male CWH who are in the pre/early pubertal stage than in male CWOH. In females, cortical thickness was greater in CWH than in CWOH, regardless of pubertal status. Greater cortical thickness may contribute to the greater estimated bone strength reported in this study. In pre/early puberty, female CWH appear to have higher bone strength than CWOH. It is unclear whether this is due to the higher cortical density they showed at this pubertal stage. Despite CWH being shorter and lighter than CWOH, these results could be suggesting that CWH who are in pre/early puberty have adequate bone strength for their smaller stature. However, when they grow in height, the residual smaller bone size leads to reduced predicted bone strength so that a strength deficit begins to emerge in mid/late puberty. Although adjusted differences were less marked in females, reduced bone strength was still apparent in male CWH in the latter stages of puberty. These findings are of concern: adolescent pubertal growth is a crucial period for skeletal development, deficits in bone strength that manifest during later puberty are likely to persist into adulthood with implications for adult fracture risk.

To date, only two studies have reported using pQCT in children living with HIV (238,343). Our results are consistent with findings from a smaller cross-sectional pQCT study in South Africa of younger children (7 to 14 years), which suggested CWH have smaller bone size and lower predicted bone strength than CWOH, although this association was not adjusted for fat mass, physical activity or any indicator of SES. Our larger study was able to both stratify by pubertal stage and adjust for a number of relevant confounders and showed how differences in size and strength becomes more pronounced in later puberty. A longitudinal Canadian study of 9 to 18 year old CWH with diverse ethnic backgrounds, reported no change in pQCT bone outcomes over 2 years (343). However, this study was small (n=31 CWH) across sex and ethnic strata, suggesting insufficient power to detect change over time. Although notably, as seen in our study, Canadian CWH in early puberty had higher cortical BMD compared to CWOH (343). An extensive literature has established a higher prevalence of stunting and underweight in CWH than in CWOH (3,4,378–381), which has been confirmed by our study and our earlier studies in Zimbabwean

children (3,230). By adjusting for body size however, we have shown that the lower bone size in female CWH, especially in later puberty, is independent of lower height and weight.

This is the first study to use pQCT to examine the effects of TDF use on bone architecture in CWH. Our previous DXA findings in the same Zimbabwean cohort identified a strong association between TDF use and bone deficits, particularly affecting TBLH-BMC^{LBM}, such that children exposed to TDF for four or more years had on average a 0.5 SD lower TBLH-BMC^{LBM} Z-Score compared with CWH who had not received TDF (229). This effect size is clinically important, as a 0.5 SD reduction in bone density increases by 50% both childhood and, if sustained, future adult fracture risk (229). Here we show, after accounting for multiple confounders, that TDF use is particularly associated with trabecular bone loss. Whether this translates to increased fracture risk at trabecular rich skeletal sites, such as the wrist and vertebra, is currently unknown.

Our reported pQCT results further extend our previous DXA findings in the same Zimbabwean cohort, where we found a higher prevalence of low TBLH-BMC^{LBM} Z-score, a cortical rich region of interest, (10% vs. 6% p=0.066) and LS-BMAD Z-score, a trabecular rich site (14% vs. 6%; p=0.0007) in CWH compared with their uninfected counterparts (229). Further earlier work in a smaller slightly younger (6 to 16 years) Zimbabwean population identified similar prevalence of low LS-BMAD and TBLH-BMC^{LBM} in CWH (15% and 13% respectively, compared to 1% and 3% in those without HIV) (230). While DXA measured areal BMD does not differentiate trabecular and cortical compartments, the lower LS-BMAD previously reported indicates the possibility of a greater deficit in trabecular bone. This aligns with our unadjusted analysis that showed that trabecular vBMD and not cortical density was lower in CWH than in CWOH. However, this is inconsistent with our adjusted analysis, as we report no differences in both trabecular and cortical density in CWH and CWOH after full adjustment.

Pubertal delay is both a common consequence of HIV infection (229) and a risk factor for poor bone growth (439). Hence, as expected, we saw a greater number of CWH were pre-pubertal, than children without HIV. Pubertal delay could be the reason for compromised bone accrual in CWH. Without stratifying by pre/early vs. mid/late puberty, we would have missed important interactions between HIV and pubertal stage. In our study male CWH in mid/late puberty demonstrated the most adverse skeletal profile. The importance of stratifying by puberty was also demonstrated by a cross-sectional study in the US of 7 to 24 year olds, which similarly reported an HIV*puberty interaction on DXA measured spine and total body bone mineral content and

density; the lower bone mass in participants with HIV was more pronounced with advancing puberty (330). However, in this study the differences were more evident in males, whereas in our study we demonstrated differences in both males and females. It is not clear to what extent the difference in the source population (*e.g*. US vs. Zimbabwe), study sampling (frequency matched based on Tanner stage not age) and/or the age studied, might account for these different findings (330).

Our results suggesting females living with HIV particularly may be entering adulthood with bone size deficits is a concern. Women often experience periods of bone loss, *e.g*., pregnancy (440), lactation (441) and menopause. Lactation which is a known risk factor for bone loss in African women (441,442), with 83% of Zimbabwean women continuing to breastfeed beyond a 1 year post-partum (443). Coupled with HIV infection and ongoing ART, the risk of adult osteoporosis is high. Recent data from rural South Africa identified a 37% prevalence of femoral neck osteoporosis in women age ≥ 50 years living with HIV (240). Understanding fracture risk in African women living with HIV is an important research priority.

4.7 Strengths and limitations of this study

The major strength of this study is the novel use of pQCT in a large population of children in an understudied African population. The comparison with children without HIV, and the fact that the study sample is likely representative of children living in Harare, are study strengths. Our study has several limitations. The analysis is cross-sectional therefore we cannot infer causality. Further, pQCT is not a technique used in clinical practice which limits the ability to relate findings to routine clinical practice. We were unable to compare findings to normative pQCT reference data as there are no established normative pQCT data for children living in sub-Saharan Africa. The study was not powered to stratify by pubertal stage, therefore because males appeared to be transitioning through puberty more slowly than females, the study may not have included sufficient males at more advanced stages of puberty. Small numbers within Tanner stage categories limited our ability to test for interaction, as we needed to group participants in different pubertal stages based on numbers with each stage, rather than pubertal biology. Further follow-up may be helpful in assessing both the male and female children further.

4.8 Conclusion

Our results suggest the effect of HIV on bone size and strength differs by pubertal status. We report deficits in bone size and strength associated with HIV infection, which are seen more

clearly towards the end of puberty in both male and female children. We further show, for the first time, an indication of attenuated trabecular bone outcomes in males using TDF. We expect our findings to be broadly generalizable across populations living in southern Africa, where HIV prevalence is high. Hence these findings have implications for fracture risk in adulthood. The study of fracture incidence, in people living with HIV in sub-Saharan Africa, is now a research priority.

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Conflict of interest
The authors declare no conflicts of interest.

Author Contributions
Conception; CM-K, AMR, KAW and CLG. Design: CM-K, KAW and CLG. Data curation: CM-K, AMR, MO'B, RR, TM and JC. Formal analysis: CM-K, AMR and TM. Interpretation: CM-K, AMR, KW, CLG. Writing- Original draft: CM-K, AMR, KAW and CLG. Writing- Review & editing: CM-K, AMR, MO'B, RR, TM, JC, LSC, LKM, RAF, KAW and CLG. Supervision: AMR, LSC, LKM, RAF, KAW and CLG. Project administration: CM-K, RR, JC, RAF and CLG. All authors take responsibility for their contributions as outlined above

Acknowledgements
We are grateful to the IMVASK study team who assisted in collecting data for this study and also to the participants (and their parents/guardians) for making this study possible.

Data access statement
Data sharing: The data that support the findings of this study are available on request from the senior authors. The data are not publicly available due to privacy/ ethical restrictions.

Supplementary materials

1. Supplementary Table 1: Comparison of demographic and anthropometric characteristics in male and female children who did and did not have pQCT scans
2. Supplementary Table 2: Mean height of children with and without HIV, stratified by pubertal stage and sex
3. Supplementary Table 3: Comparison of demographic and anthropometric characteristics between children living with HIV by use of Tenofovir
4. Supplementary Figure 1: Hypothesised causal diagram showing the relationship between exposure, outcomes and the variables adjusted for in this paper

Supplementary Table 1: Comparison of demographic and anthropometric characteristics in male and female children who did and did not have pQCT scans

Characteristics		No pQCT scan (n=32)	pQCT scan (n=577)	p value
HIV infected	Yes	27 (84.4)	276 (47.8)	<0.001
Female sex	Yes	17 (53.1)	289 (50.2)	0.881
Age, years	Mean (SD)	13.1 (2.9)	12.4 (2.5)	0.115
Tanner Stage (%)	Tanner 1	10 (5.6)	177 (31.7)	0.811
	Tanner 2	6 (3.4)	122 (21.8)	
	Tanner 3	5 (2.8)	103 (18.4)	
	Tanner 4	10 (5.6)	126 (22.5)	
	Tanner 5	1 (0.6)	31 (5.5)	
Socio-Economic Status (%)	Low, Tertile 1	10 (32.3)	184 (33.3)	0.122
	Middle, Tertile 2	15 (48.4)	179 (32.4)	
	High, Tertile 3	6 (19.4)	189 (34.2)	
Orphan Status (%)	Not an orphan	18 (58.1)	432 (76.9)	0.005
	One parent alive	13 (41.9)	106 (18.9)	
	Orphan	0 (0)	24 (4.3)	
Physical Activity (%)	Low, <600 MET	19 (57.6)	243 (42.2)	0.026
	Moderate, 600 -3000 MET	11 (33.3)	154 (26.7)	
	High, >3000 MET	3 (9.1)	179 (31.1)	
Dietary calcium Intake (mg)	<150 mg	16 (48.5)	255 (44.3)	0.431
	150-299 mg	4 (12.1)	124 (21.5)	
	300-449 mg	13 (39.4)	197 (34.2)	
Dietary vitamin D Intake (mcg)	<4.0 mcg	3 (9.1)	74 (12.8)	0.215
	4.0 - 5.99 mcg	19 (57.6)	383 (66.5)	
	6.0 - 7.9 mcg	11 (33.3)	119 (20.7)	
Height, cm	Mean (SD)	144.4 (13.8)	143.8 (13.6)	0.879
Weight, kgs	Mean (SD)	37.7 (11.7)	37.1 (11.3)	0.797
Body Mass Index, kg/cm²	Mean (SD)	18.0 (3.0)	17.5 (2.8)	0.369

p values for categorical variables were calculated using the chi squared test, p values for continuous variables were calculated using the t test for 2 independent samples, SES = socioeconomic status. BMI; Body mass index, NB: Data presented in this table are unadjusted

Supplementary Table 2: Mean height of children with and without HIV, stratified by pubertal stage and sex

	Height (cm)					
	CWOH (n=306)			CWH (n=303)		
All	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**
Tanner 1	70	132.0	6.2	117	129.1	8.9
Tanner 2	69	141.3	8.1	59	140.0	7.7
Tanner 3	53	150.4	9.5	55	148.0	7.4
Tanner 4	92	160.6	7.2	44	152.8	7.3
Tanner 5	20	156.7	7.6	12	158.3	4.5
Males	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**
Tanner 1	45	131.8	5.8	57	129.8	10.1
Tanner 2	34	142.8	8.2	39	139.0	6.7
Tanner 3	24	153.7	10.7	22	147.0	7.6
Tanner 4	43	163.5	7.3	19	153.8	8.6
Tanner 5	4	167.6	3.8	5	160.9	3.7
Females	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**
Tanner 1	25	132.4	6.8	60	128.3	7.7
Tanner 2	35	139.9	7.8	20	141.9	9.2
Tanner 3	29	147.7	7.4	33	148.7	7.3
Tanner 4	49	158.1	6.1	25	152.0	6.3
Tanner 5	16	154.0	5.6	7	156.4	4.2

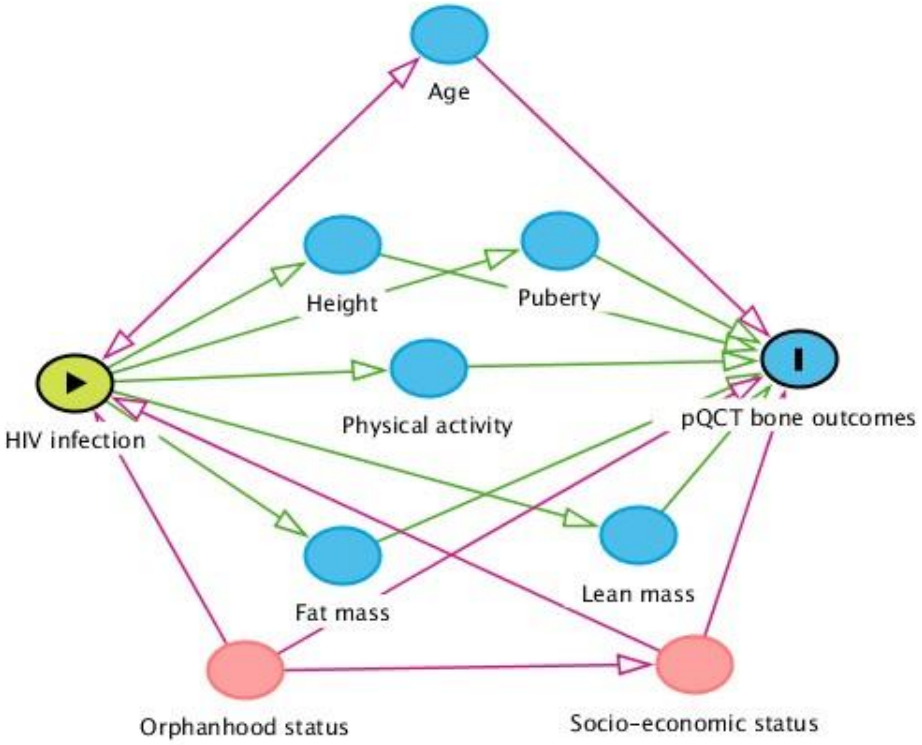
Table to show mean height of children with and without HIV, stratified by Tanner stage and sex. CWH; children living with HIV. CWOH; children living without HIV

Supplementary Table 3: Comparison of demographic and anthropometric characteristics between children with HIV by use of Tenofovir

Characteristic		**No TDF use(n=240)**	**TDF use(n=63)**	**p-value**
Sex (%)	Female	116 (48%)	35 (56%)	0.308
Age, years	Mean (SD)	11.9 (2.4)	14.5 (1.7)	<0.001
Tanner stage	Tanner 1	106 (47%)	11 (18%)	<0.001
	Tanner 2	49 (22%)	10 (16%)	
	Tanner 3	40 (18%)	15 (25%)	
	Tanner 4	26 (12%)	18 (30%)	
	Tanner 5	5 (2%)	7 (11%)	
Socio-Economic Status (%)	Low, Tertile 1	89 (37%)	26 (41%)	0.581
	Middle, Tertile 2	82 (34%)	23 (37%)	
	High, Tertile 3	69 (29%)	14 (22%)	
Orphan Status (%)	Not an orphan	141 (62%)	26 (42%)	0.003
	One parent alive	71 (31%)	34 (55%)	
	Orphan	16 (7%)	2 (3%)	
Physical Activity in METS, (%)	Low, <600	121 (50%)	27 (43%)	0.264
	Moderate, 600 -3000	56 (23%)	21 (33%)	
	High, >3000	63 (26%)	15 (24%)	
Calcium Intake in mg, (%)	<150 mg	106 (44%)	29 (46%)	0.943
	150-299 mg	50 (21%)	12 (19%)	
	300-449 mg	84 (35%)	22 (35%)	
Vitamin D Intake in mcg, (%)	<4.0 mcg	32 (13%)	8 (13%)	0.731
	4.0 - 5.99 mcg	160 (67%)	45 (71%)	
	6.0 - 7.9 mcg	48 (20%)	10 (16%)	
Height, cm	Mean (SD)	137.6 (12.4)	149.1 (9.0)	<0.001
Weight, Kgs	Mean (SD)	34.3 (16.7)	41.2 (9.4)	0.002
BMI	Mean (SD)	16.6 (2.0)	18.4 (2.6)	<0.001

p values for categorical variables were calculated using the chi squared test, p values for continuous variables were calculated using the t test for 2 independent samples, SES = socioeconomic status. BMI; Body mass index, NB: Data presented in this table are unadjusted

Supplementary Figure 1: Hypothesised causal diagram showing the relationship between exposure, outcomes and the variables adjusted for in this paper



Minimal sufficient adjustment sets for estimating the total effect of HIV infection on pQCT bone outcomes requires adjusting for age, orphanhood status and socio-economic status

5 CHAPTER 5: Manuscript 2- Changes in peripheral quantitative computed tomography measured bone density, size and strength in Zimbabwean children with and without HIV over one year: a cohort study

5.1 Introduction

The second question asked by this PhD is how children living with HIV (CWH) accrue bone compared to children living without HIV (CWOH). To address this question, I compared change (∆) in bone density, bone size and predicted bone strength parameters over one year in children from Harare, Zimbabwe who were living with and without HIV. I investigated to what extent impairment in longitudinal growth explains any detrimental effects of HIV on pQCT-assessed bone outcomes.

In this chapter, I report 12 months of longitudinal data from a cohort study of children living with and without HIV (8-16 years) at different stages of puberty. The results suggest puberty modifies the effect of HIV on change in bone size and height mediates the effect of HIV on change in pQCT bone density and bone size, particularly in females. However, there are deficits in bone size and strength associated with HIV infection over one year follow-up. Results from this analysis suggests some evidence of catch-up growth in CWH which is not sufficient to address deficits in bone density, size and strength. This is despite the fact that children living with HIV have seemingly gained more height, more bone size and more bone strength than their uninfected counterparts. These findings add to information on growth impairment in children living with HIV and therefore have implications for fracture risk in adulthood. Given the high burden of HIV in Southern Africa, and much improved access to anti-retroviral treatment, increasing numbers of children perinatally infected with HIV living are now living through adolescence and into adulthood, and are likely to enter adulthood with lesser bone development than their uninfected counterparts.

I developed the specific research questions, developed the pQCT methods section of the protocol used in this study, and wrote the pQCT standard operation procedures, with guidance from my supervisors. I collected the pQCT data included in this analysis, together with a team of radiographers that I trained and led on the pQCT scanning for this data collection. I performed the pQCT quality control and graded all the pQCT scans included in this study. I developed the statistical analysis plan together with the Stata analysis do files and I performed the statistical analysis, with support from my supervisor, Andrea M. Rehman. I produced the tables of results and graphs, wrote the first draft of the paper, and led on all revisions.

Cover sheet

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RESEARCH PAPER COVER SHEET

Please note that a cover sheet must be completed for each research paper included within a thesis.

SECTION A – Student Details

Student ID Number	1806477	**Title**	Mrs
First Name(s)	Cynthia		
Surname/Family Name	Kahari		
Thesis Title	The effect of HIV and its treatment on trabecular and cortical bone architecture in children, adolescents and premenopausal women		
Primary Supervisor	Andrea Rehman/Melissa Neuman		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

SECTION B – Paper already published

Where was the work published?			
When was the work published?			
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion			
Have you retained the copyright for the work?*	Choose an item.	Was the work subject to academic peer review?	Choose an item.

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SECTION C – Prepared for publication, but not yet published

Where is the work intended to be published?	Journal of Bone and Mineral Research (JBMR)
Please list the paper's authors in the intended authorship order:	Cynthia Mukwasi-Kahari, Celia L Gregson, Mícheál Ó Breasail, Ruramayi Rukuni, Tafadzwa Madanhire, Victoria Simms, Joseph Chipanga, Lynda Stranix-Chibanda, Lisa K. Micklesfield, Rashida A Ferrand, Kate A Ward, Andrea M.

	Rehman
Stage of publication	**Submitted**

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I developed the specific research questions, designed the pQCT methods of this study protocol, with guidance from my supervisors. I collected the pQCT data included in this analysis, together with a team of radiographers that I trained and led on the pQCT scanning for this data collection. I performed the pQCT quality control and graded all the pQCT scans included in this study. I developed the statistical analysis plan together with the Stata analysis do files and I performed the statistical analysis, with support from my supervisor, Andrea M. Rehman. I produced the tables of results and graphs, wrote the first draft of the paper, and led on all revisions.

SECTION E

Student Signature	[redacted]
Date	31 Jul 2023

Supervisor Signature	[redacted]
Date	18/8/2023

5.1.1 Title

Changes in peripheral quantitative computed tomography measured bone density, size and strength in Zimbabwean children with and without HIV over one year: a cohort study

5.1.2 Authors

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Running title: pQCT assessed bone growth in children with HIV

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5.2 Abstract

Understanding bone accrual in adolescents may inform approaches to improve skeletal health and reduce adult fracture risk. We investigated the effect of HIV on bone mineral accrual assessed by peripheral quantitative computed tomography (pQCT). Children with HIV (CWH) on anti-retroviral therapy (ART) for ≥2 years, and children without HIV (CWOH), aged 8-16 years (n=609), had tibial pQCT scans at 0 and 12 months. Linear regression estimated differences in mean and change in (∆) pQCT bone density (trabecular and cortical), size (total cross-sectional area [CSA]) and strength (SSI) between CWH and CWOH, stratified by sex, adjusting for socio-economic status (SES) and orphanhood and incorporating an interaction term for baseline pubertal status (Tanner 1-2[pre/early] vs 3-5[mid/late]). Structural equation modelling tested whether baseline height-for-age-Z-scores (HAZ) mediate the effect of HIV on ∆bone outcomes. CWH and CWOH were similar in age. CWH were more likely than CWOH to be orphans (44% vs 7%), of lower SES (43% vs 27%) and be stunted (30% vs 8%). At baseline and follow up, CWH had lower trabecular density, CSA and SSI than CWOH. After adjustment, bone density and strength increased similarly in CWH and CWOH. CWH in mid/late puberty at baseline had greater 12 months increases in CSA than CWOH, particularly males (mean difference [31.3(95%CI:-3.1, 65.6) mm^2 in mid/late puberty vs. -2.04(-23.8, 19.7) mm^2 in pre/early puberty; interaction p-value=0.013]. HAZ mediated the effect of HIV on ∆bone outcomes only in females as follows: indirect pathways from HIV to ∆trabecular density [-1.85(-3.5, -0.2) mg/cm^3], ∆cortical density [-2.01(-3.9, -0.01) mg/cm^3], ∆CSA [-2.59(-4.7, -0.5) mm] and ∆SSI [-18.36(-29.6, -7.2) mm^3]. In conclusion, puberty modifies the effect of HIV on change in bone size. CWH show bone deficits at follow up. Height explains bone mineral accrual in females. Investigations of bone mineral accrual earlier in life and post-puberty to peak bone mass are needed.

Keywords: ANALYSIS/QUANTIFICATION OF BONE, DISEASES AND DISORDERS OF/RELATED TO BONE, EPIDEMIOLOGY

5.3 Introduction

People living with HIV are at increased risk of fragility fractures, particularly hip fracture (7). Bone mineral density (BMD), size and strength are important determinants of fracture risk (444). Bone health in later life reflects both bone accrual in childhood and adolescence and bone loss in adult years. Understanding factors that influence bone accrual may inform approaches to improve skeletal health, for example through maximising acquisition of peak bone mass (PBM) to reduce future adult fracture risk. Most studies investigating BMD by dual energy x-ray absorptiometry (DXA) have reported lower BMD in children living with HIV (CWH) than in children without HIV (CWOH) (230,231,326). In a cross-sectional analysis, we previously demonstrated lower bone size and predicted bone strength in CWH compared to CWOH; deficits were larger in the later stages of puberty, suggesting lasting effects into early adulthood (445). Underlying aetiological mechanisms remain unclear, and furthermore, it is uncertain whether CWH 'catch-up' with their uninfected counterparts as the skeleton matures. (229). Treatment of HIV is associated with poor bone outcomes. In addition, traditional factors that contribute to poor growth and bone accrual such as low dietary calcium, low physical activity and social deprivation, are common in CWH (229). Although data on bone development in CWH has recently begun to emerge from sub-Saharan Africa where 90% of CWH live (321), available studies to date have either largely used DXA, which only measures two-dimensional areal bone density (229–231,326,446), or have been cross-sectional in study design (238).

Peripheral quantitative computed tomography (pQCT) uses very low-dose radiation to measure volumetric BMD and bone geometry of cortical and trabecular bone compartments. The skeleton constitutes 80% cortical bone and 20% trabecular bone (14). Use of pQCT offers an opportunity to increase our understanding of how HIV affects trabecular and cortical bone development during puberty.

Longitudinal data are necessary to understand bone development, particularly in the context of HIV-associated growth impairment (3,230). Longitudinal studies assessing the effect of HIV on bone accrual from sub-Saharan Africa are lacking despite the 90% of CHW living in this region. We have previously demonstrated that stunting disproportionately affects CWH compared to CWOH in Zimbabwe, where 32% CWH, age 8 -16 years, are stunted (229). It is unclear how much of the effect of HIV on bone accrual is explained by, and therefore proportionate to, impaired longitudinal growth. Hence, this study aimed to use pQCT to study a paediatric population in sub-Saharan Africa and determine the effect of HIV on the change in bone density, size and strength over a 12-month period, as measured by pQCT. We also

determined to what extent impairment in longitudinal growth explains any detrimental effects of HIV on pQCT-assessed bone outcomes.

5.4 Methods

5.4.1 Study setting

A cohort study, the IMpact of Vertical HIV infection on child and Adolescent Skeletal development (IMVASK), with two visits 12 months apart, was conducted as per published protocol (ISRCTN12266984) (229,249,445). Perinatally infected CWH attending either of the two main public hospitals in Harare, Zimbabwe, were quota sampled, stratified by sex and three-year age band (8-10, 11-13, 14-16 years). Inclusion criteria were being established on anti-retroviral therapy (ART) for at least 2 years and being aware of their HIV status. Stratified random sampling was used to recruit CWOH from three primary and three secondary schools randomly selected from the 109 primary schools and 44 secondary schools serving the population residing within the same catchment area as the hospitals. The number of children selected from each school was proportional to school size. A random number sequence was generated, and school registers were used to select participants of similar age and sex as the CWH using the same quota- based approach of 50 male and 50 female in each of the three age strata. CWOH were tested for HIV to confirm their status and excluded if positive. Those with acute illness requiring hospitalization, and who had no parental consent were excluded.

5.4.2 Study Procedures

Baseline data were collected from May 2018 to January 2020, and follow-up from May 2019 to January 2021. Study procedures at the baseline and follow-up visits were the same, as previously described (229,249). Questionnaires, completed by the interviewer in the company of the parents or guardians, were used to collect demographic and clinical data including age, sex, orphanhood, socio-economic status (SES), smoking status, alcohol use, medical history, steroid use, physical activity patterns and dietary data concerning calcium and vitamin D intake. SES was derived using the first principal component combining a list of factors (number in household, head of household age, highest maternal and paternal education levels, household ownership, monthly household income, access to electricity, water supply, household sanitation and ownership of a fridge, bicycle, car, television, and/or radio) and was split into tertiles for analysis. Physical activity, assessed using the International Physical Activity Questionnaire (IPAQ) short version (427), classified participants into low (<600 metabolic rate (MET) minutes per week), moderate (600 to 3000 MET minutes per week) and vigorous intensity (3000 MET minutes per week). A diet and

nutrition assessment food frequency tool, validated in India and Malawi (428) and then adapted to the Zimbabwean context with international guidelines applicable to sub-Saharan Africa, was used to quantify dietary calcium and vitamin D intake (447). The mandatory fortification of oils and margarine with vitamin D in Zimbabwe was included in the adaptation. Daily dietary calcium intake was classified as very low (<150mg/day), low (150-299mg/day) and moderate (300–450 mg/day). Daily dietary vitamin D intake was classified as very low (<4.0mcg/day), low (4.0-5.9mcg/day) and moderate (6.0-8.0mcg/day) (447).

Puberty was assessed at baseline and follow up by a trained study nurse or doctor using Tanner staging. For males, testicular volume, penile size (length and circumference) and pubic hair growth (quality, distribution and length) were assessed. For females, breast growth (size and contour) as well as pubic hair growth and age of menarche were assessed. Testicular, breast and penile growth were graded from 1-5 based on Tanner descriptions (199,200). Where there was a discordance between the indicators, testicular and breast development stage respectively for males and females were used to assign Tanner stage. Participants were grouped into Tanner stages 1 and 2 (pre/early puberty) and Tanner stages 3 to 5 (mid/late puberty). The mean of a total of 3 standing height measurements, to the nearest 0.1cm, using a Seca 213 stadiometer (Hamburg, Germany) and the mean of a total of 3 weight measurements, to the nearest 0.1kg, using a Seca 875 weight scale (Hamburg, Germany) were obtained. Height-for-age Z-score <-2, weight-for-age Z-score <-2, and low weight-for-height BMI Z-score <-2 were used to define stunting, underweight and wasting respectively (432,448). Anthropometry Z-scores were generated using British 1990 growth standards (448) because the World Health Organisation data for WAZ are not available beyond 10 years of age (20). CD4 cell count was measured using a PIMA CD4 (Waltham, Massachusetts, USA) machine and HIV viral load using a GeneXpert HIV-1 viral load platform (Cepheid, Sunnyvale, California, USA).

5.4.3 pQCT scan acquisition at baseline and follow-up

Non-dominant tibial pQCT scans were performed using a single XCT 2000^{TM} (Stratec Medizintecknik, Pforzheim, Germany), with voxel size 0.5 x 0.5 mm and slice thickness 2 mm (CT scan speed 30 mm/s; scout view scan speed 40 mm/s). Scan sites were determined as a percentage of tibia length, with the exact position determined by scout view placement of a reference line on the growth plate, or on the end plate for those with fused growth plates (433). As long bones grow in length, the growth plate moves upward and the wider metaphysis is reshaped into a diaphysis by continuous resorption by osteoclasts beneath the periosteum (317). To allow for consistency of a scan site the reference line was

placed at the growth plate in those children whose end plate and growth plate were not yet fused. If a growth plate had fused during the follow up interval, the reference line was placed on the end plate. Scan sites were at 4% (trabecular vBMD) and 38% (cortical vBMD) of the measured tibial length (distal medial malleolus to the tibial plateau). Other measurements included 4% and 38% total cross-sectional area [CSA], 38% cortical thickness and 38% stress strain index [SSI]. The same software (version 6.20m Stratec Medizintechnik) was used for image processing and analysis at both baseline and follow-up. Radiographers acquiring the scans were trained to repeat a pQCT scan the same day if it had motion artefact. As a result, the number of scans that were excluded because of motion artefact (n=8) were only for those participants that did not get a repeat scan or for which the repeat scan still had motion artefact.

A phantom was scanned daily for quality assurance. To assess reproducibility, 30 participants were scanned twice after repositioning. Short term precision (Root Mean Square % CV) was 1.26% for trabecular vBMD, 0.37% for cortical vBMD, 2.08% for 4% total CSA and 0.93% for 38% CSA. The least significant change, which is calculated as 2.77% × %CV was 3.49% for trabecular vBMD, 1.02% for cortical vBMD, 5.76% for 4% total CSA and 2.57% for 38% CSA. One radiographer qualitatively assessed and graded all pQCT images for movement artefacts from 0 to 3: (0) none, (1) slight streaking, (2) moderate streaking and (3) scan unusable. Scans graded as a 3 were excluded from analysis. Baseline and follow-up scans were assessed to check that scan positioning at baseline and follow-up were consistent. Scan errors such as disagreement in the side scanned (left or right tibia) or in the positioning of the reference line placement (growth plate or end plate) between baseline and follow-up scans were reasons to exclude participant scans from analyses.

5.4.4 Ethical considerations

This study was approved by the Parirenyatwa Hospital and College of Health Sciences joint research ethics committee (JREC/123/19), the Biomedical Research and Training Institute Institutional Review Board (AP150/2019), the Medical Research Council of Zimbabwe (MRCZ/A2494) and the London School of Hygiene and Tropical Medicine (17154) Ethics Committee. Written informed consent and/or age-appropriate assent was obtained from parents/guardians and participants.

5.4.5 Statistical analysis

Statistical analyses were performed using Stata version 17 (Stata Corporation Inc., College Station, TX, USA). Data were cleaned and checked for consistency and outliers. Primary

outcomes were annualised mean 4% Δ trabecular and 38% Δ cortical vBMD (mg/cm^3), 4% and 38% Δ CSA (mm^2), 38% Δ cortical thickness (mm) and 38% Δ stress-strain index (SSI) (mm^4). Annualised changes pQCT bone outcomes were defined as the pQCT bone outcomes at follow up minus the pQCT bone outcome at baseline, divided by number of day between the baseline and follow up visits and multiplied by 365.25. All analyses were stratified by sex as bone accrual rates differ between males and females (359). Independent t-tests were used to compare group means for continuous data and chi-squared tests were used for categorical percentages. Linear regression (with robust standard errors) was used to estimate mean differences (presented with 95% CI) between CWH and CWOH. Adjustments were made for SES in tertiles (437), and orphanhood (229). A hypothesised causal diagram (Supplementary figure 1) created in Dagitty (https://www.dagitty.net/) was used for determining the minimal sufficient adjustment sets for estimating the total effect of HIV on pQCT bone outcome. The minimum adjustment set included orphan status and SES. The baseline analysis was also used to inform which covariates would be included in the follow up analysis hence the reason why height was used in structural equation mdoels and puberty was used to test if there is modification of the exposure on the outcome.

Potential differences in the effect of HIV on Δbone outcomes by pubertal status were tested by incorporating an interaction term for baseline binary pubertal status (Tanner 1 & 2 vs Tanner 3, 4 & 5).

Structural equation modelling (SEM) for mediation analysis was used to evaluate whether the effect of HIV infection on change in bone outcomes was mediated by height impairment. SEM was used irrespective of the overall association, since it is possible for an exposure to exert an effect on an outcome indirectly through a mediator even if one cannot establish evidence of an association, through a hypothesis test, for a total effect of an exposure on an outcome (449). Mediation models were adjusted for SES and orphanhood. Ninety five percent confidence intervals (95% CI) for the ratio of direct effects/total effects were generated in Stata.

5.5 Results

5.5.1 Study population

At baseline 609 participants, 303 CWH (151 [49.8%] male and 152 [50.2%] female), and 306 CWOH (151 [49.8%] male and 152 [50.2%] female) were recruited, and 492 (80.7%) participants, 244 CWH (125 [51.2%] male and 119 [48.8%] female), and 248 CWOH (122 [49.2%] male and 126 [50.8%] female)) had a follow-up visit (Supplementary Figure 2). In the complete case analysis, 419 (68.8%) participants had usable pQCT scans at both baseline and follow-up and were not missing covariate data. The 190 (31.2%) participants

(108 CWH and 82 CWOH) not included in analyses were similar to those included in terms of age, sex, height, weight, pubertal stage, SES, physical activity levels, calcium and vitamin D intake; (Supplementary Table 1). Eleven of 419 children (3%) underwent complete fusion of epiphyses and had a change in reference line placement from the growth plate to end plate. Eight of the participants' tibial pQCT scans were excluded from analysis due to movement artefact and these are shown in the flow diagram as scan errors.

At baseline, males were aged mean 12.3 (standard deviation [SD] 2.5) years and females 12.4 (SD 2.5) years with no difference by HIV status (Table 1). Follow-up was challenged in 2020-2021 by national travel restrictions due to COVID-19 lockdown. Between-visit duration was on average 66 days longer among CWH compared to CWOH; therefore, at follow-up CWH were older than CWOH (Supplementary Table 1). Most males [n=132 (62%)] were in Tanner stages 1 or 2 at baseline, while fewer than half were so at follow-up [n= 94 (44%)]; this did not differ by HIV status. More female CWH were in Tanner stage 1 and 2 compared to CWOH (at both baseline and follow-up) (Table 1, Supplementary Table 1). Having one or both parents deceased was more common among CWH, compared to those without HIV (42% vs 7% in males; 46% vs 6% in females, Table 1). CWH were more likely to have a lower SES than CWOH (43% vs 25% in males; 42% vs 29% in females). Dietary calcium intake and levels of physical activity were generally low, with no difference by HIV status (Table 1).

Both height and weight were lower in CWH than CWOH, but during follow-up CWH experienced greater absolute gains in height compared to CWOH (Table 1). At baseline, male and female CWH were 6cm and 8cm shorter than CWOH, respectively and more likely to be stunted (33% vs 7% [males]; 26% vs 9% [females]). Stunting prevalence increased slightly at follow-up when CWH were more likely to be stunted than CWOH (43% vs 9% [males]; 33% vs 11% [females]) (Supplementary Table 2). Although overall the study population gained weight during follow-up, the proportion who were classified as having low weight-for-age increased; at follow-up this was more likely in CWH than CWOH (35% vs 14% in males; 27% vs 7% in females) (Table 1). During follow-up a greater proportion of CWH than CWOH advanced in Tanner stage, 62% vs 49% in males and 66% vs 59% in females. (Table 1).

5.5.2 The effect of HIV on pQCT bone outcomes

At baseline, all CWH had lower distal trabecular density, lower distal and diaphyseal tibial CSA and lower predicted bone strength (38% SSI) than CWOH, while diaphyseal cortical

density and thickness were comparable by HIV status, in both males and females (Table 2); these findings persisted at follow-up and remained robust to adjustment for SES and orphanhood (Table 2). In analyses of annualized change in bone parameters, adjusted for SES and orphanhood, the annual change in pQCT measures was similar by HIV status (Table 2). Baseline pubertal stage appeared to modify the effect of HIV on growth in bone size, specifically distal tibial CSA in males, such that in late puberty male CWH had a greater increase in tibial CSA than CWOH, with a mean adjusted difference of 31.3 mm^2 [95%CI -3.1, 65.6; p=0.074; interaction p=0.013] (Figure 1, Table 3). In females the same pattern was seen at the diaphyseal site for tibial CSA (rather than the distal site in males), such that female CWH in late puberty at baseline had greater increases in bone size than CWOH. No evidence was detected for an interaction between pubertal stage and HIV for bone density or strength measures.

5.5.3 The effect of HIV on change in pQCT bone outcomes mediated by linear growth (i.e. height)

In females, there was some evidence that the effect of HIV on annualized change in pQCT bone outcomes was mediated via HAZ (Table 4). In both males and females, there was strong evidence of an association between HIV and lower baseline HAZ (the mediator). The effect of HIV on HAZ, (β (95%CI); p-value) was (-0.88 (-1.2, -0.6); p<0.001 in males) and (-0.90 (-1.24, -0.57); p<0.001 in females). In males, there was no evidence of an indirect effect of HIV on change in pQCT outcomes via HAZ and hence no evidence for mediation by linear growth. In contrast, in females, there appeared to be an indirect effect of HIV via HAZ on Δ trabecular density, Δcortical density, Δdiaphyseal tibial CSA and predicted bone strength (SSI), whilst no evidence for a direct effect of HIV on these bone outcomes was seen, indicating that the effect of HIV on change in bone outcomes is totally mediated via HAZ. The ratio of direct effects over total effects (% mediated) exceeded 100% for Δtrabecular density (114%), Δ diaphyseal tibial CSA (181%) and Δ predicted bone strength (281%) suggesting that there are other pathways and mediators affecting these bone outcomes, that act in the opposite direction to HAZ. The percentage mediated for Δ cortical density was 57%.

In a sensitivity analysis modelling change in bone outcomes adjusted for duration of follow-up, findings were unchanged. Furthermore, excluding the 11 children who underwent epiphyseal fusion did not change findings.

5.6 Discussion

To our knowledge, this is the first study to investigate longitudinal changes in detailed pQCT measured bone parameters in CWH in sub-Saharan Africa. The study showed that CWH

have consistently lower trabecular bone density, bone size and bone strength than CWOH over one year of follow-up. Our findings suggest that in children aged 8-16 years, puberty modifies the effect of HIV on bone growth, such that in the later stages of puberty both male and female CWH increase their bone size more than CWOH, although at the end of follow-up, and despite more CWH transitioning pubertal stage, bone size remains smaller in CWH. Furthermore, we have demonstrated that, in females living with HIV, changes over one year in bone size, density and strength are partly explained by baseline HAZ, suggesting 3-dimensional bone growth is in part, proportionate to height.

There were greater increases in height over the one-year of this study in CWH than CWOH. In addition, over the same period, a greater proportion of CWH than CWOH advanced in Tanner stage. We have previously reported pubertal delay in the same cohort (229) and other studies have shown height is strongly determined by pubertal growth (450). Pubertal transition is classically characterised by a 'growth spurt' (*i.e.* peak height velocity). The differences in height change in our cohort could be explained by more CWH than CWOH, still undergoing pubertal transition from early to late puberty. Despite that, male CWH who were in the later stages of puberty gained more bone size at the 4% site than CWOH: however, male CWH still had lower mean bone size than CWOH at the follow up visit. These results suggest that, although CWH demonstrate a degree of catch-up growth in bone size as they transition through puberty, this catch-up growth may be insufficient to reach full physiological potential. These findings are concerning since deficits in bone accrual at the end of puberty are likely to persist into adulthood (219).

We have shown that CWH still had lower bone outcomes at follow up despite the greater increase in height over the study period. Moreover, we have demonstrated that the effect of HIV on Δbone size, Δbone strength and Δcortical density in females, is due to the indirect effect of HIV on height rather than the effect of HIV on changing bone turnover and mineral accrual. The calculated ratio mediated proportions yielded percentages exceeding 100% suggest that there are other pathways (operating through other mediators) that are acting in the opposite direction to HAZ-scores that affect the 4% trabecular density, 38% tibial CSA and predicted strength. The sum of the proportion mediated may exceed 100% if there are other mediators affecting these bone outcomes but that acting in a direction opposite to the mediator under consideration (HAZ) (451).

To date, only one other study conducted in Canada has assessed longitudinal changes in pQCT bone outcomes in 31 CWH, which reported that Z-scores for pQCT bone outcomes did not change over 2 years, suggesting that bone outcomes were not increasingly

compromised in CWH (38). Our findings demonstrate lower bone outcomes in CWH than in CWOH at both baseline and follow up, despite evidence of some catch up growth as CWH transition through puberty. Bone formation occurs at a faster rate before the age of 4 years, and during puberty than in any other time of one's life. Our cohort were enrolled into the study at minimum age of 8 years. It is unclear whether or not the lower bone density size, and strength we observed in CWH is a result of factors influencing bone in the early years of life and whether or not CWH will eventually catchup to their uninfected counterparts.

Childhood HIV infection can manifest as stunting (poor linear growth). Our study reports a higher prevalence of stunting in CWH than in CWOH for both males [33% (CWH) vs 7% (CWOH)] and females [26% (CWH) vs 10% (CWOH)]. This is similar to several studies that have assessed growth patterns in African countries which have consistently reported high prevalence of stunting as measured by height for age Z-scores in CWH (3,4,378–383). In Zimbabwe CWH have higher odds of stunting; eight times greater in those who have acquired HIV in utero, and four times greater in those who have acquired HIV around the time of birth (384). Improvements in growth have been reported in CWH who have an early HIV diagnosis and less severe HIV symptoms or who start ART early (389). However, in Africa, CWH start ART later in life than other parts of the world, often when the disease is already in a more advanced stage, potentially affecting growth (388).

Combining our results, we hypothesise that the trajectory of skeletal growth for CWH has been preset at a lower trajectory than in CWOH before the age of 8 years possibly even before the initiation of antiretroviral therapy by age 4 years, in the critical window of the first 1000 days of life. Figure 2 illustrates this hypothesis. As the children transition through puberty, it appears that the development of density and strength runs in parallel to CWOH meaning they are growing at the same rate but that the set point has been predetermined by something else earlier in life and by the end of our study's follow up period, there are still deficits in all parameters of bone. There is evidence of catchup growth as puberty proceeds with bone area increasing to accommodate height growth. Whether or not that is sufficient to enable CWH to get back on the trajectory they would have been had they not had HIV is unknown. It is also not known whether it is possible to reset that trajectory through interventions in the early life management of CWH. Moreover, when those children are reaching the end of puberty it is unclear whether children with HIV continue to accrue bone for a longer period of time to an older age than CWOH such that they do achieve the peak bone mass at a later stage. Studies are needed to examine the pattern of growth in later adolescence and timing of the acquisition of peak bone mass in CWH to assess whether deficits are likely to continue through adulthood and increase their risk of fracture.

5.7 Strengths and limitations

The strengths of this study include the novel use of pQCT's three-dimensional outcomes to evaluate bone measurements using a large sample. The inclusion of a comparison group of children without HIV, and longitudinal follow-up are also strengths. The participants are likely to be representative of children living in Harare due to our robust sampling methods. The use of pQCT may also be considered a potential limitation, as this is not a technique used in clinical practice, which limits the ability to relate our findings to routine clinical practice. There are currently no normative pQCT reference data for children living in sub-Saharan Africa, so it is not possible to determine how our measurements relate to a population norm. There is no validated reference growth database for children living in Zimbabwe or in sub-Saharan Africa. The use of growth reference values from British children in defining stunting, underweight and wasting in this study is therefore a limitation. Participants with missing data were excluded to allow structural equation modelling analysis, thereby limiting the number of participants and potentially reducing power in this study. Though structural equation modelling has its limits, it also has advantages over standard multivariable regression analysis as it offers an explicit assessment of measurement errors, an estimation of latent (unobserved) variables and best fit of data compared with multivariable regression analysis (449,452,453). Using a standard multivariable regression analysis approach would still have resulted in a sample size comparable to the sample size used in the current SEM approach. This is because a larger number of the excluded participants were those who had no/incomplete follow up visit due to loss to follow up (n=123) and those who had no pQCT at baseline visit (n=33), regardless of whether or not they had a complete follow up visit. Only 34 participants were excluded for missing data on the remaining variables. Participants excluded from analyses were similar to those included in terms of age, sex, height, weight, pubertal stage, SES, physical activity levels, calcium and vitamin D intake. However, it is possible that there may be some unmeasured confounding variables which may account for the findings observed in both the main analysis and the mediation analysis. Small numbers within Tanner stage categories limited our ability to test for interaction, as we needed to group participants in different pubertal stages based on numbers within each stage, rather than pubertal biological characteristics for each stage.

The only % change in pQCT bone outcomes that exceeded the least significant change in this study were those for 38% CSA in all participants regardless of sex or HIV status and for the 4% CSA site for the male CWH. Trabecular density (at the 4% site) and 38% cortical density had % changes that did not exceed the least significant change calculated in this study. In this study, though other pQCT bone outcomes were not reported in an attempt to reduce type 1 error, we still compared multiple pQCT bone outcomes (six) between the

CWH and CWOH and it is possible that the multiple statistical comparisons may have resulted in type 1 error.

The study was underpowered to assess the relationship between changes in height with changes in bone outcomes over a year, to determine whether changes were proportionate across compartments. Height was used in statistical models rather than tibia length, although highly correlated, lower limb growth occurs earlier in puberty than spinal growth. As the exact timing of peak height velocity could not be established, it is not possible to separate anticipated concurrent changes in bone from those that are lagged. Follow-up of longer than one year may provide further insights into trajectories of bone mineral accrual.

5.8 Conclusion

Puberty modifies the effect of HIV on change in bone size and height mediates the effect of HIV on change in pQCT bone outcomes, particularly in females. However, there are deficits in bone size and strength associated with HIV infection over one year follow-up. Results from this study suggests some evidence of catch-up growth in CWH which is not sufficient to address deficits in bone density, size and strength, despite that CWH have seemingly gained more height, more bone size and more bone strength than CWOH. These findings add to information on growth impairment in CWH and therefore have implications for fracture risk in adulthood. The trajectory of skeletal growth seems to have been preset at a lower trajectory than in CWOH before 8 years of age. If no catch up bone accrual occurs, CWH will be at higher risk of fracture in later life. Research is needed to assess the effect of HIV on bone in the early years and to establish whether CWH catchup on bone accrual or not.

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Conflict of interest

The authors declare no conflicts of interest.

Author Contributions

Conception; CM-K, AMR, KAW and CLG. Design: CM-K, KAW and CLG. Data curation: CM-K, AMR, MO'B, RR and JC. Formal analysis: CM-K, TM, VS and AMR. Interpretation: CM-K, AMR, KAW, CLG. Writing- Original draft: CM-K, CLG and AMR. Writing- Review & editing: CM-K, CLG, MO'B, RR, TM, VS, JC, LSC, LKM, RAF, KAW and AMR. Supervision: AMR, LSC, LKM, RAF, KAW and CLG. Project administration: CM-K, RR, JC, RAF and CLG. All authors take responsibility for their contributions as outlined above

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Data sharing statement

Data sharing: The data that support the findings of this study are available on request from the senior authors.

5.9 Figures and tables

5.9.1 Main figures and tables

Table 5.1: Clinical characteristics and anthropometry of children living with and without HIV, stratified by sex

	Males (n=213)			Females (n=206)		
	CWOH (n=113)	CWH (n=100)	p-value	CWOH (n=111)	CWH (n=95)	p-value
Baseline Clinical characteristics						
Age, yrs, mean(SD)	12.2 (2.4)	12.5 (2.5)	0.29	12.6 (2.5)	12.1 (2.4)	0.14
Age group, %			0.96			0.23
8 - 10yrs	40 (35%)	34 (34%)		35 (32%)	36 (38%)	
11 - 13yrs	39 (35%)	34 (34%)		33 (30%)	33 (35%)	
14 - 16 yrs	34 (30%)	32 (32%)		43 (39%)	26 (27%)	
Tanner stage, %			0.59			<0.001
Tanner 1	35 (31%)	38 (38%)		19 (17%)	43 (45%)	
Tanner 2	31 (27%)	28 (28%)		22 (20%)	15 (16%)	
Tanner 3	18 (16%)	15 (15%)		22 (20%)	20 (21%)	
Tanner 4	27 (24%)	16 (16%)		35 (32%)	14 (15%)	
Tanner 5	2 (2%)	3 (3%)		13 (12%)	3 (3%)	
Pubertal status, %			0.26			<0.001
Early Puberty	66 (58%)	66 (66%)		41 (37%)	58 (61%)	
Late Puberty	47 (42%)	34 (34%)		70 (63%)	37 (39%)	
Socio-economic status, %			0.009			0.077
Low, Tertile 1	28 (25%)	43 (43%)		32 (29%)	40 (42%)	
Middle, Tertile 2	36 (32%)	30 (30%)		37 (33%)	31 (33%)	
High, Tertile 3	49 (43%)	27 (27%)		42 (38%)	24 (25%)	
Orphanhood, %			<0.001			<0.001
Not an orphan	105 (93%)	58 (58%)		105 (95%)	51 (54%)	
One parent alive	5 (4%)	36 (36%)		4 (4%)	36 (38%)	
Orphan	3 (3%)	6 (6%)		2 (2%)	8 (8%)	
Physical Activity, %			0.061			0.16
Low, <600	37 (33%)	43 (43%)		43 (39%)	49 (52%)	
Moderate, 600 -3000	40 (35%)	21 (21%)		32 (29%)	24 (25%)	
High, >3000	36 (32%)	36 (36%)		36 (32%)	22 (23%)	
Calcium Intake, %			0.98			0.81
Low, <150 mg	49 (43%)	42 (42%)		48 (43%)	38 (40%)	
Moderate, 150-299 mg	24 (21%)	22 (22%)		24 (22%)	24 (25%)	
High, 300-449 mg	40 (35%)	36 (36%)		39 (35%)	33 (35%)	
Change in clinical characteristics during one year follow up						
Tanner stage			0.072			0.32
No	58 (51%)	38 (38%)		46 (41%)	32 (34%)	
Yes	55 (49%)	61 (62%)		65 (59%)	63 (66%)	
Baseline Anthropometry						
Height, cm, mean(SD)	146.2 (14.5)	140.6 (12.1)	0.003	147.7 (11.6)	139.4 (13.0)	<0.001
Height for age Z-score, mean(SD)	-0.7 (1.0)	-1.7 (1.1)	<0.001	-0.5 (1.1)	-1.5 (1.0)	<0.001
Stunting, %	8 (7%)	33 (33%)	<0.001	10 (9%)	25 (26%)	<0.001
Weight, kgs, mean(SD)	37.1 (11.0)	33.2 (7.7)	0.004	42.8 (13.1)	34.3 (10.3)	<0.001
Weight for age Z-score, mean(SD)	-0.8 (1.0)	-1.6 (1.2)	<0.001	-0.2 (1.1)	-1.3 (1.1)	<0.001
Underweight, %	12 (11%)	29 (29%)	<0.001	7 (6%)	19 (20%)	0.003
Body mass index, kg/cm²	16.9 (2.2)	16.5 (1.4)	0.17	19.1 (3.8)	17.2 (2.5)	<0.001
Body mass index Z-scores, mean(SD)	-0.6 (1.0)	-0.8 (0.8)	0.086	0.0 (1.2)	-0.6 (0.9)	<0.001
Wasting, %	9 (8%)	7 (7%)	0.79	3 (3%)	7 (7%)	0.12
Change in anthropometry						
Height, cm, mean(SD)	5.0 (2.4)	6.8 (4.7)	<0.001	3.7 (3.3)	4.9 (3.0)	0.005
Weight, kgs, mean(SD)	4.0 (2.4)	4.3 (3.1)	0.53	3.9 (3.5)	4.1 (3.3)	0.6
Body mass index, kg/cm², mean(SD)	0.7 (0.8)	0.4 (0.8)	0.029	0.9 (1.2)	0.8 (1.3)	0.83

p values for categorical variables were calculated using the chi squared test, p values for continuous variables were calculated using the t test for 2 independent samples, CWH; children living with HIV, CWOH; children living without HIV NB: Data presented are unadjusted

Table 5.2: pQCT bone outcomes of children living with and without HIV, stratified by sex

	Males (n=213)				Females (n=206)			
Baseline	***Mean (SD) CWOH (n=113)**	***Mean (SD) CWH (n=100)**	****Adjusted MD (95% CI)**	**p-value**	***Mean (SD) CWOH (n=111)**	***Mean (SD) CWH (n=95)**	****Adjusted MD (95% CI)**	**p-value**
4% Trabecular density, mg/cm³	208.0 (42.1)	197.3 (37.7)	-10.52 (-22.8 , 1.7)	0.092	210.7 (31.5)	198.5 (33.1)	-12.63 (-22.9 , -2.4)	0.016
38% Cortical density, mg/cm³	1070.3 (33.5)	1067.8 (37.1)	-3.56 (-14.4 , 7.3)	0.518	1098.6 (45.7)	1095.7 (40.2)	-8.21 (-21.9 , 5.5)	0.239
4% Total Cross-sectional area, mm²	752.7 (230.2)	679.2 (177.9)	-99.79 (-163.0 , -36.6)	0.002	731.7 (171.1)	661.1 (166.9)	-104.89 (-157.8 , -51.9)	<0.001
38% Total Cross-sectional area, mm²	341.79 (78.01)	313.00 (64.35)	-39.04 (-60.9 , -17.2)	0.001	330.25 (60.4)	286.52 (57.6)	-51.18 (-69.8 , -32.5)	<0.001
38% Cortical thickness, mm	3.8 (0.6)	3.8 (0.6)	-0.13 (-0.3 , 0)	0.153	3.8 (0.6)	3.7 (0.6)	-0.18 (-0.4 , 0)	0.062
38% Stress strain index, mm³	1162.0 (386.9)	1021.3 (311.1)	-197.54 (-304.9 , -90.2)	<0.001	1124.0 (326.4)	923.1 (284.2)	-241.28 (-338.3 , -144.3)	<0.001
Follow up	***Mean (SD) CWOH (n=113)**	***Mean (SD) CWH (n=100)**	****Adjusted MD (95% CI)**	**p-value**	***Mean (SD) CWOH (n=111)**	***Mean (SD) CWH (n=95)**	****Adjusted MD (95% CI)**	**p-value**
4% Trabecular density, mg/cm³	203.8 (34.5)	192.1 (36.1)	-10.87 (-21.6 , -0.1)	0.048	210.9 (33.1)	196.2 (32.6)	-14.52 (-25.0 , -4.1)	0.007
38% Cortical density, mg/cm³	1077.1 (38.8)	1074.0 (38.8)	-4.80 (-16.8 , 7.2)	0.429	1115.5 (45.6)	1109.0 (39.2)	-11.17 (-24.7 , 2.4)	0.106
4% Total Cross-sectional area, mm²	783.0 (221.8)	728.9 (187.3)	-81.91 (-144.8 , -19.0)	0.011	748.6 (152.7)	685.0 (151.1)	-94.32 (-142.0 , -46.6)	<0.001
38% Total Cross-sectional area, mm²	368.1 (79.1)	342.1 (70.0)	-36.31 (-59.2 , -13.5)	0.002	346.6 (56.5)	306.6 (57.5)	-48.16 (-66.1 , -30.2)	<0.001
38% Cortical thickness, mm	4.0 (0.6)	3.9 (0.7)	-0.17 (-0.4 , 0)	0.074	4.0 (0.6)	3.8 (0.6)	-0.21 (-0.4 , 0)	0.033
38% Stress strain index, mm³	1306.8 (410.2)	1175.6 (358.3)	-190.72 (-308.4 , -73.0)	0.002	1229.4 (314.6)	1034.3 (295.1)	-240.50 (-336.9 , -144.1)	<0.001
Change over 12 months	***Mean (SD) CWOH (n=113)**	***Mean (SD) CWH (n=100)**	****Adjusted MD (95% CI)**	**p-value**	***Mean (SD) CWOH (n=111)**	***Mean (SD) CWH (n=95)**	****Adjusted MD (95% CI)**	**p-value**
4% Trabecular density, mg/cm³	-3.8 (19.5)	-4.4 (17.2)	-0.35 (-6.0 , 5.3)	0.903	0.1 (13.4)	-2.1 (13.9)	-1.61 (-5.9 , 2.7)	0.463
38% Cortical density, mg/cm³	6.2 (18.3)	5.1 (18.4)	-1.90 (-7.6 , 3.8)	0.508	14.8 (16.7)	10.6 (14.5)	-3.61 (-8.6 , 1.4)	0.157
4% Total Cross-sectional area, mm²	27.5 (65.9)	41.3 (53.2)	13.23 (-5.3 , 31.8)	0.161	15.7 (57.6)	18.5 (61.8)	6.42 (-12.4 , 25.3)	0.503
38% Total Cross-sectional area, mm²	24.7 (15.7)	24.7 (18.6)	0.10 (-5.2 , 5.4)	0.970	14.6 (16.4)	16.4 (17.0)	1.44 (-3.8 , 6.7)	0.590
38% Cortical thickness, mm	0.2 (0.2)	0.1 (0.2)	-0.05 (-0.1 , 0)	0.101	0.2 (0.2)	0.1 (0.2)	-0.04 (-0.1 , 0)	0.252
38% Stress strain index, mm³	136.5 (77.9)	130.8 (95.6)	-7.12 (-33.7 , 19.5)	0.599	94.2 (73.5)	90.4 (89.8)	-6.52 (-32.2 , 19.2)	0.617

***Unadjusted, **Adjusted for socioeconomic status and orphanhood CWH: Children living with HIV, CWOH: Children without HIV, SD (Standard Deviation)*

MD (95% CI); Mean Difference (95 % Confidence Interval) with CWOH as the reference group, such that negative values mean that those with HIV have lower values than those without HIV

Table 5.3: pQCT bone outcomes of children living with and without HIV, stratified by sex and pubertal status

	Unadjusted (n=419)					*Adjusted (n=419)				
Males (n=213)	**Tanner Stages 1 and 2**		**Tanner Stages 3, 4 & 5**		**Interaction**	**Tanner Stages 1 and 2**		**Tanner Stages 3, 4 & 5**		**Interaction**
Characteristic	**MD (95% CI)**	**p-value**	**MD (95% CI)**	**p-value**	**p-value**	**MD (95% CI)**	**p-value**	**MD (95% CI)**	**p-value**	**p-value**
Δ 4% Trabecular Density, mg/cm³	-3.10 (-9.0 , 2.8)	0.298	3.59 (-5.6 , 12.8)	0.441	**0.208**	-2.91 (-9.6 , 3.7)	0.389	3.52 (-7.6 , 14.7)	0.531	**0.174**
Δ 38% Cortical Density, mg/cm³	3.49 (-2.1 , 9.1)	0.218	-7.13 (-16.1 , 1.9)	0.118	**0.051**	3.05 (-3.2 , 9.3)	0.337	-6.77 (-17.5 , 3.9)	0.212	**0.042**
Δ 4% Total Cross-sectional Area,mm²	-4.17 (-23.4 , 15.1)	0.669	40.81 (12.0 , 69.6)	0.006	**0.011**	-2.04 (-23.8 , 19.7)	0.853	31.27 (-3.1 , 65.6)	0.074	**0.013**
Δ 38% Total Cross-sectional Area,mm²	-0.78 (-6.7 , 5.1)	0.792	0.89 (-6.9 , 8.7)	0.820	**0.728**	-0.72 (-7.4 , 5.9)	0.831	1.47 (-7.9 , 10.8)	0.754	**0.728**
Δ 38% Cortical Thickness, mm	-0.05 (-0.1 , 0)	0.185	-0.03 (-0.1 , 0.0)	0.372	**0.653**	-0.05 (-0.1 , 0)	0.275	-0.03 (-0.1 , 0.1)	0.480	**0.713**
Δ 38% Stress Strain Index, mm³	-3.16 (-33.6 , 27.3)	0.838	-4.41 (-41.0 , 32.2)	0.811	**0.958**	-3.05 (-37.5 , 31.3)	0.861	4.42 (-39.3 , 48.1)	0.841	**0.966**
Females (n=206)	**Tanner Stages 1 and 2**		**Tanner Stages 3, 4 & 5**		**Interaction**	**Tanner Stages 1 and 2**		**Tanner Stages 3, 4 & 5**		**Interaction**
Characteristic	**MD (95% CI)**	**p-value**	**MD (95% CI)**	**p-value**	**p-value**	**MD (95% CI)**	**p-value**	**MD (95% CI)**	**p-value**	**p-value**
Δ 4% Trabecular Density, mg/cm³	0.98 (-4.5 , 6.5)	0.723	-4.53 (-10.0 , 1.0)	0.106	**0.163**	0.25 (-6.1 , 6.6)	0.938	-2.97 (-9.6 , 3.6)	0.374	**0.144**
Δ 38% Cortical Density, mg/cm³	-1.98 (-8.6 , 4.7)	0.556	-3.26 (-9.2 , 2.7)	0.278	**0.781**	-1.65 (-9.3 , 6.0)	0.669	-0.49 (-7.6 , 6.6)	0.892	**0.845**
Δ 4% Total Cross-sectional Area,mm²	-14.76 (-40.5 , 11.0)	0.259	-2.10 (-20.7 , 16.5)	0.824	**0.444**	-17.88 (-47.2 , 11.4)	0.229	1.72 (-20.9 , 24.3)	0.880	**0.546**
Δ 38% Total Cross-sectional Area,mm²	-6.51 (-13.4 , 0.3)	0.063	4.76 (-1.1 , 10.6)	0.110	**0.013**	-7.04 (-14.9 , 0.9)	0.080	3.32 (-3.7 , 10.3)	0.349	**0.022**
Δ 38% Cortical Thickness, mm	-0.09 (-0.2 , 0.0)	0.072	0.02 (-0.0 , 0.1)	0.501	**0.064**	-0.11 (-0.2 , 0)	0.061	0.02 (-0.1 , 0.1)	0.639	**0.096**
Δ 38% Stress Strain Index,mm³	-31.62 (-66.2 , 3.0)	0.073	9.68 (-20.5 , 39.9)	0.526	**0.072**	-37.27 (-76.9 , 2.4)	0.065	9.57 (-26.7 , 45.9)	0.602	**0.097**

Adjusted for socioeconomic status and orphanhood

MD (95% CI); Mean Difference (95 % Confidence Interval) with CWOH as the reference group, such that negative values mean that those with HIV have lower values than those with HIV

Table 5.4: pQCT bone outcomes of children living with and without HIV after mediation analysis with HIV as exposure, height for age Z-scores as the mediator and adjusted for SES and orphanhood status

	Adjusted for SES and orphanhood status			
Males (n=213)	**Direct effect of HIV on pQCT outcome, β (95% CI);p value**	**Effect of Height on pQCT bone outcome, β (95% CI);p value**	**Indirect effect of HIV on pQCT outcome, β (95% CI);p value**	**Total effect of HIV on pQCT outcome, β (95% CI);p value**
Δ 4% trabecular density	0.59(-5.38, 6.56); 0.846	1.09(-1.34, 3.50); 0.383	-0.95(-3.09, 1.20); 0.389	-0.35(-5.94, 5.24); 0.902
Δ 38% cortical density	0.11(-5.79, 6.00); 0.972	2.29(-0.09, 4.68); 0.060	-2.01(-4.22, -0.20); 0.075	-1.90(-7.46, 3.65); 0.502
Δ 4% total CSA	11.47(-8.00, 30.94); 0.248	-2.01(-9.90, 5.89); 0.618	1.76(-5.19, 8.71); 0.620	13.2(-4.98, 31.44); 0.154
Δ 38% total CSA	0.68(-4.85, 6.21); 0.809	0.66(-1.58, 2.90); 0.563	-0.58(-2.56, 1.40); 0.565	0.10(-5.07, 5.28); 0.969
Δ 38% cortical thickness	-0.07(-0.13, 0.01); 0.046	-0.02(-0.04, 0.01); 0.226	0.01(-0.01, 0.04); 0.237	-0.05(-0.11, 0.01); 0.095
Δ 38% SSI	-1.37(-29.28, 26.53); 0.923	6.55(-4.77, 17.87); 0.257	-5.74(-15.87, 4.38); 0.266	-7.12(-33.28, 19.05); 0.594
Females (n=206)	**Direct effect of HIV on pQCT outcome, β (95% CI);p value**	**Effect of Height on pQCT bone outcome, β (95% CI);p value**	**Indirect effect of HIV on pQCT outcome, β (95% CI);p value**	**Total effect of HIV on pQCT outcome, β (95% CI);p value**
Δ 4% trabecular density	0.24(-4.22, 4.71); 0.916	2.05(0.35, 3.76); 0.018	-1.85(-3.54, -0.17); 0.031	-1.61(-5.86, 2.64); 0.457
Δ 38% cortical density	-1.6(-6.78, 3.58; 0.545	2.22(0.24, 4.20); 0.028	-2.01(-3.95, -0.07); 0.043	-3.61(-8.53, 1.31); 0.151
Δ 4% total CSA	11.07(-8.58, 30.71); 0.269	5.15(-2.35, 12.66); 0.179	-4.65(-11.65, 2.34); 0.192	6.41(-12.10, 24.94); 0.417
Δ 38% total CSA	4.01(-1.36, 9.44); 0.143	2.87(0.80, 4.93); 0.006	-2.59(-4.69, -0.49); 0.015	1.44(-3.72, 6.59); 0.585
Δ 38% cortical thickness	-0.03(-0.09, 0.04); 0.459	0.01(-0.01, 0.04); 0.303	-0.01(-0.04, 0.01); 0.313	-0.04(-0.10, 0.03); 0.244
Δ 38% SSI	11.84(--14.03, 37.72); 0.370	20.31(10.43, 30.20); <0.001	-18.37(-29.62, -7.12); 0.001	-6.52(-31.75, 18.71); 0.612

Adjusted for socioeconomic status and orphanhood

****The effect of HIV on Height, (β (95% CI); p value) was '-0.88 (-1.19, -0.57); <0.001 (males) and '-0.90(-1.24, -0.57); <0.001 (females)***

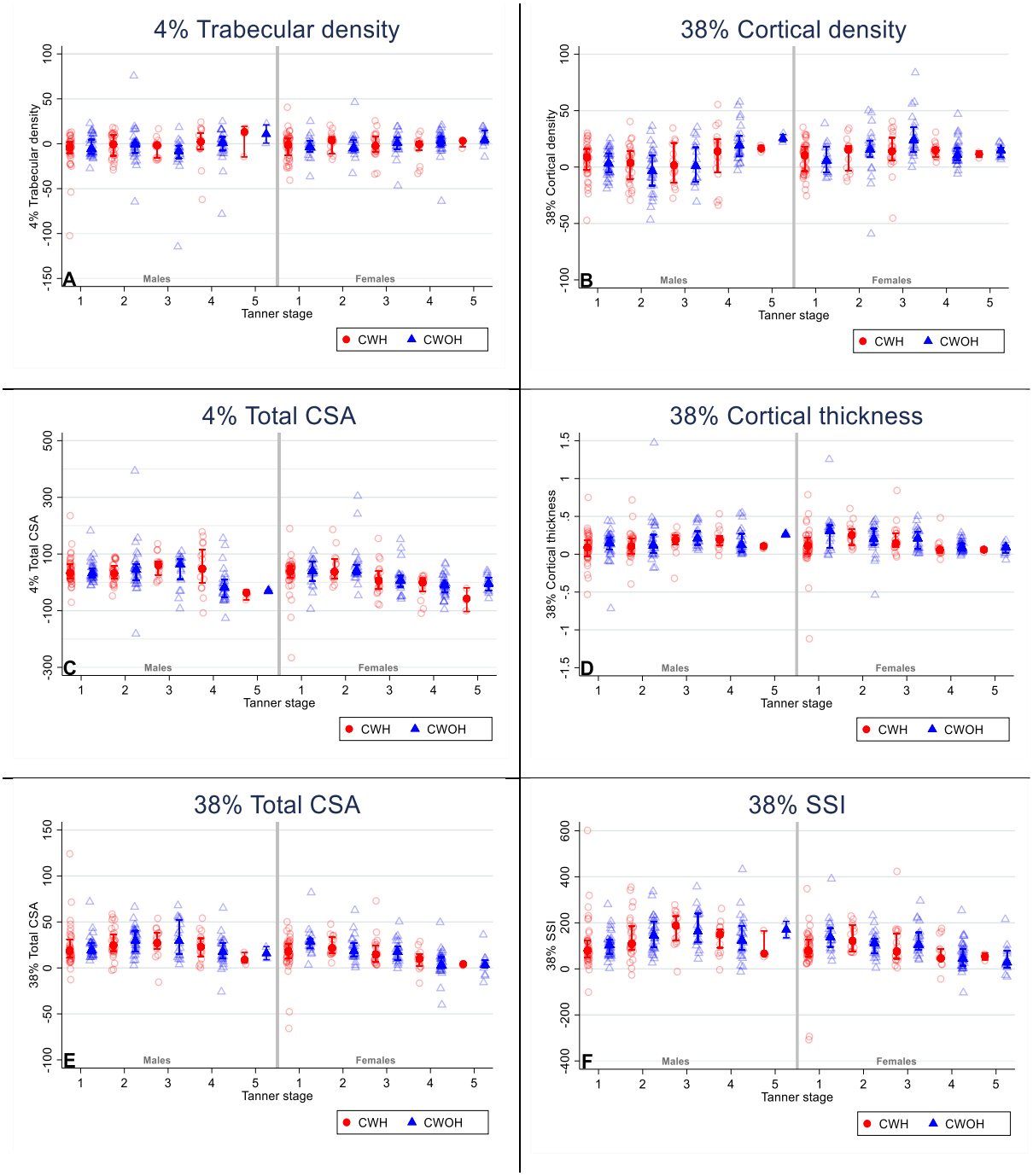
Direct effect is the pathway from HIV to the pQCT outcome

The indirect effect is the pathway from HIV via height to the pQCT outcome

The total effect is the sum of the direct and indirect effects

MD (95% CI); Mean Difference (95 % Confidence Interval) with CWOH as the reference group, such that negative values mean that those with HIV have lower values than those with HIV

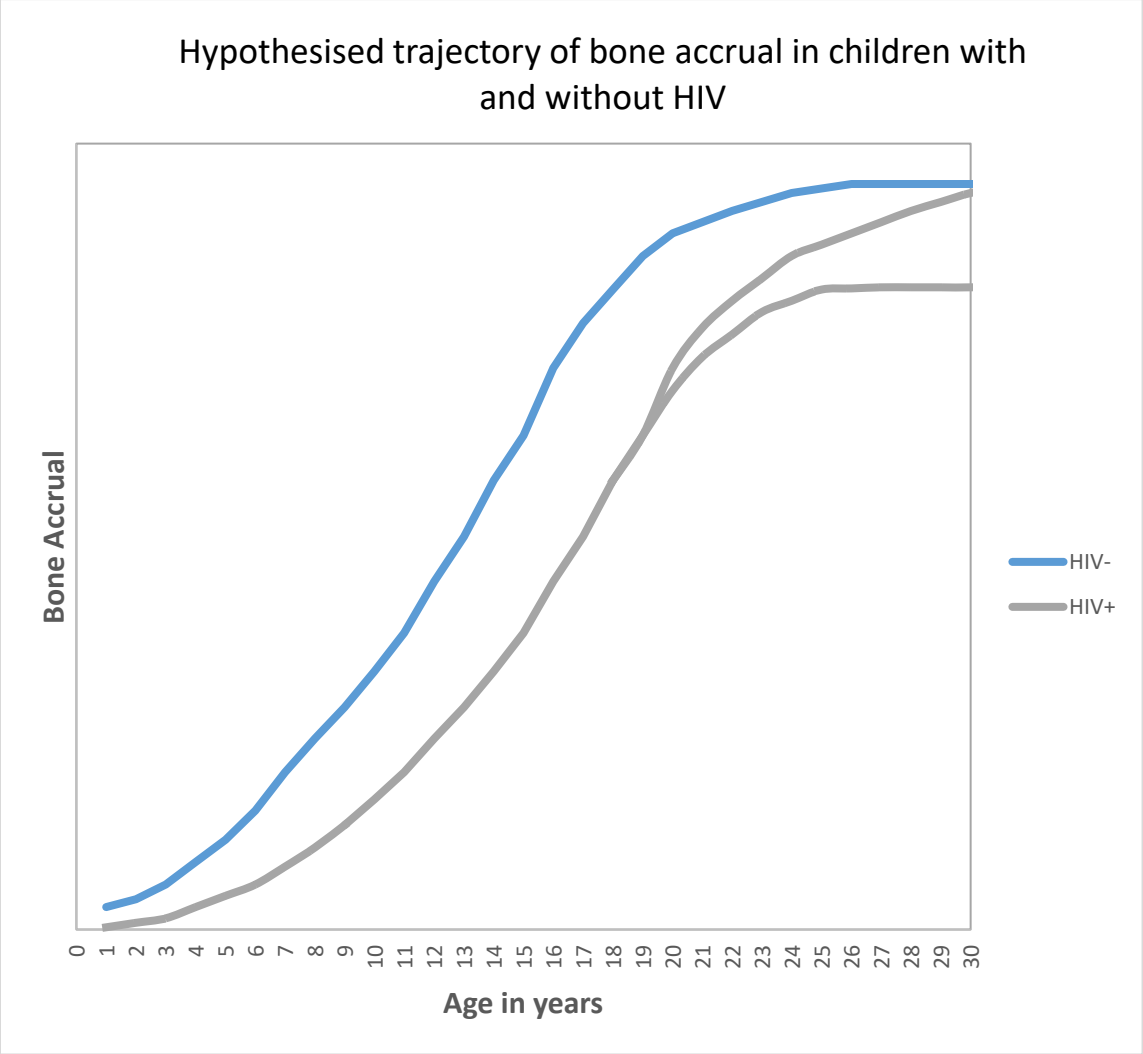
Figure 5.1: Unadjusted comparison of change in pQCT measured bone outcomes, over 12 months, between children living with and without HIV infection by sex and pubertal status



-Figure 1 above shows unadjusted comparison of change in pQCT measured bone outcomes, over 12 months, between children living with and without HIV infection by sex and pubertal status

-CWH: Children living with HIV, CWOH: Children without HIV, CSA: Cross-sectional area, SSI: Stress strain index

Figure 5.2: Conceptualised diagram showing bone accrual in children with and without HIV



-Figure 2 above is a hypothesised diagram, hypothesising that CWH are pre-set at a lower trajectory than CWOH before the age of 8 years, accrue bone at the same rate as CWOH and may or may not catch-up after pubertal years
-CWH: Children living with HIV, CWOH: Children without HIV, CSA: Cross-sectional area, SSI: Stress strain index

5.9.2 Supplementary figures and tables

1. Supplementary Table 1: Clinical characteristics and anthropometry of participants who were and were not included in the final statistical analysis
2. Supplementary Figure 1: Hypothesised causal diagram showing the hypothesis that HIV infection may lead to reduced pQCT bone outcomes
3. Supplementary Figure 2: Flow diagram to show participants included in this study

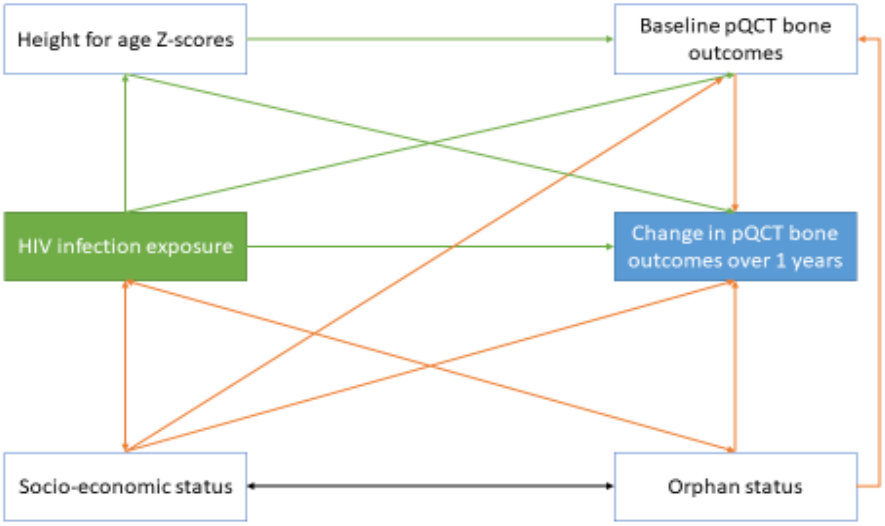
Supplementary Table 1: Clinical characteristics and anthropometry of participants who were and were not included in the final statistical analysis

Baseline clinical characteristics	Not included in analysis (n=190)	Included in analysis (n=419)	p-value
Age, yrs, mean(SD)	12.7 (2.6)	12.4 (2.5)	0.083
CWH	108 (57%)	195 (47%)	0.023
Female sex, %	100 (53%)	206 (49%)	0.43
Tanner stage, %			0.64
Tanner 1	52 (30%)	135 (32%)	
Tanner 2	32 (19%)	96 (23%)	
Tanner 3	33 (19%)	75 (18%)	
Tanner 4	44 (26%)	92 (22%)	
Tanner 5	11 (6%)	21 (5%)	
Socio-economic status, %			0.21
Low, Tertile 1	22 (30%)	143 (34%)	
Middle, Tertile 2	31 (42%)	134 (32%)	
High, Tertile 3	20 (27%)	142 (34%)	
Orphan hood, %			0.56
Not an orphan	131 (75%)	319 (76%)	
One parent alive	38 (22%)	81 (19%)	
Orphan	5 (3%)	19 (5%)	
Physical Activity, %			0.34
Low, <600	90 (47%)	172 (41%)	
Moderate, 600 -3000	48 (25%)	117 (28%)	
High, >3000	52 (27%)	130 (31%)	
Calcium Intake, %			0.21
Low, <150 mg	94 (49%)	177 (42%)	
Moderate, 150-299 mg	34 (18%)	94 (22%)	
High, 300-449 mg	62 (33%)	148 (35%)	
Change in clinical characteristics			
Age, yrs, mean(SD)	1.3 (0.3)	1.2 (0.2)	0.004
Tanner stage			0.89
No	23 (40%)	174 (42%)	
Yes	35 (60%)	244 (58%)	
Anthropometry			
Height, cm, mean(SD)	143.9 (14.5)	143.7 (13.3)	0.92
Height for age Z-score, mean(SD)	-1.3 (1.2)	-1.0 (1.2)	0.026
Stunting, %	41 (22%)	76 (18%)	0.29
Weight, kgs, mean(SD)	37.4 (11.2)	37.0 (11.4)	0.72
Weight for age Z-score, mean(SD)	-1.1 (1.3)	-0.9 (1.2)	0.083
Underweight, %	36 (19%)	67 (16%)	0.35
Body mass index, kg/cm²	17.6 (2.6)	17.5 (2.8)	0.65
Body mass index Z-scores, mean(SD)	-0.5 (1.1)	-0.5 (1.0)	0.65
Wasting, %	18 (10%)	26 (6%)	0.13
Change in anthropometry			
Height, cm, mean(SD)	4.8 (5.4)	5.1 (3.6)	0.66

Height for age Z-score, mean(SD)	-0.1 (0.6)	-0.0 (0.4)	0.61
Stunting, %	2 (3%)	20 (5%)	0.45
Weight, kgs, mean(SD)	4.0 (3.6)	4.1 (3.1)	0.90
Weight for age Z-score, mean(SD)	-0.1 (0.7)	-0.0 (0.5)	0.37
Underweight, %	4 (6%)	19 (5%)	0.69
Body mass index, kg/cm², mean(SD)	0.7 (1.0)	0.7 (1.1)	0.94
Body mass index Z-scores, mean(SD)	-17.9 (1.9)	-17.9 (2.1)	0.86
Low BMI, %	1 (1%)	11 (3%)	0.54

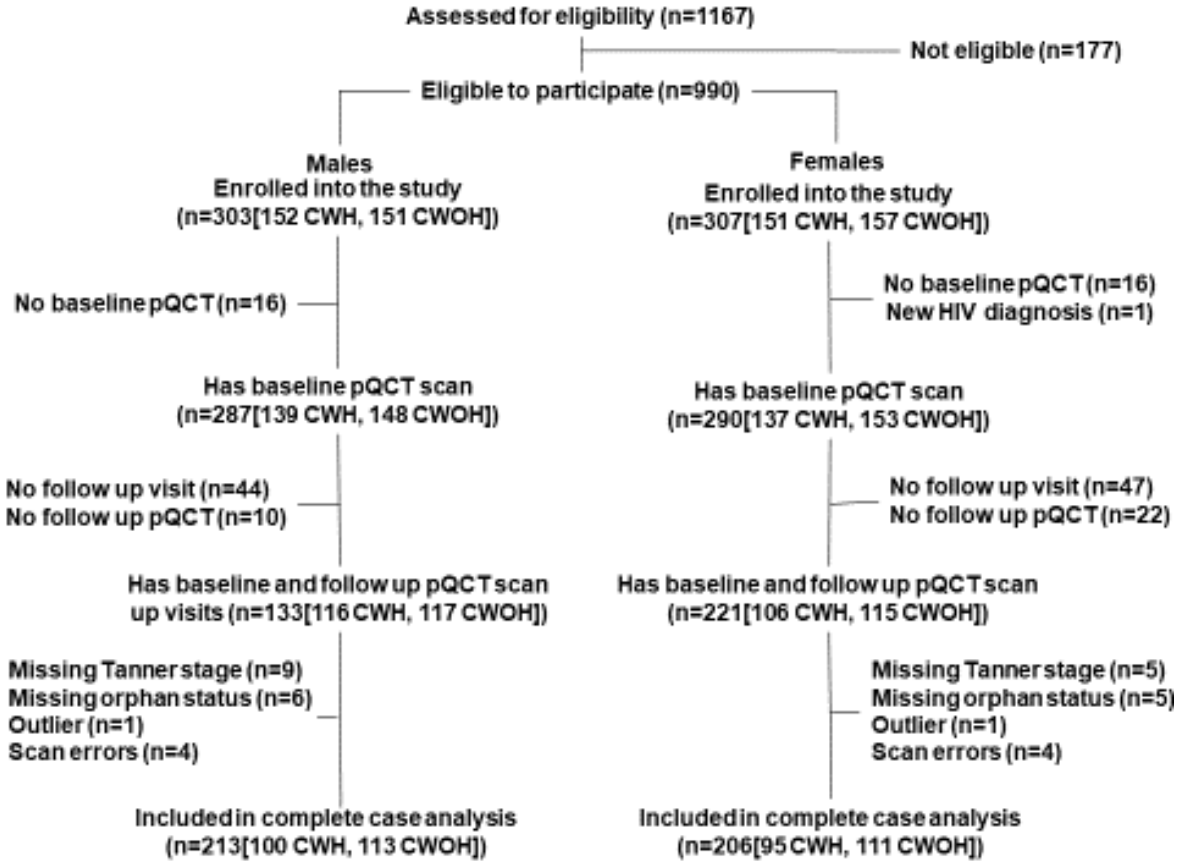
p values for categorical variables were calculated using the chi squared test, p values for continuous variables were calculated using the t test for 2 independent samples, NB: Data presented in this table are unadjusted

Supplementary Figure 1: Hypothesised causal diagram showing the hypothesis that HIV infection may lead to reduced pQCT bone outcomes



Minimal sufficient adjustment sets for estimating the total effect of HIV on pQCT bone outcome: Orphan status, SES

Supplementary Figure 2: Flow diagram to show participants included in this study



CWH: Children living with HIV, CWOH: Children without HIV
Those who were not eligible to participate were not living in Harare (n=87), on ART for less than two years (n=52), younger than 8 years (n=17), Living with HIV but unaware of their HIV status (n=20) and had acute illness (n=1)

6 CHAPTER 6: Literature review of studies assessing fractures bone density, bone size and bone strength in adults living with HIV, with a particular focus on women.

6.1 Introduction

The rise in chronic non-infectious comorbid complications amongst people living with HIV (PLWH) in sub-Saharan Africa includes cardiac, renal and musculoskeletal complications (243,454). Evidence from high income countries has shown low DXA measured areal BMD in adults living with HIV (455–457). Bone accrual patterns throughout childhood and into adulthood have shown women living with HIV (WLWH) to accrue bone at a lower rate than women without HIV (WLWOH). Menopause stage has been associated with low bone density. However, in WLWH, bone loss is accelerated during menopause compared to WLWOH (458). Few studies of bone health in WLWH have focused on women living in an African country (239,458–460). Studies comparing premenopausal WLWH to premenopausal WLWOH are rare and most studies that have included women are still dominated by men (239,460). As described in chapter 1, the prevalence of HIV in sub-Saharan Africa is higher in women than in men (1). In sub-Saharan Africa in the year 2020, females accounted for 63% of all new HIV infections compared with 32% outside sub-Saharan Africa, and yet are being underrepresented in currently available bone studies from this region. HIV infection can result in loss of bone mass due to both immune effects and the deteriorating health of the person (461). Increased cytokines in chronic HIV infection lead to increased bone turnover, stimulating osteoclasts and enhancing bone resorption (462,463). Low CD4 count is associated with reduced BMD (464). In addition, opportunistic infections such as tuberculosis, undernutrition, and reduced physical activity are factors that negatively influence bone mass (462–464). In addition to bone density, bone size and bone strength are important indicators of bone architecture as they also predict fracture risk independent of bone density (283). If WLWH have lower bone density, size and strength than their uninfected counterparts, this puts them at higher risk of fragility fracture.

This narrative literature review presented in this chapter assesses the available evidence from studies assessing bone architecture (density, size and strength) and fractures in women living with and without HIV. The search strategy included searching PUBMED, EMBASE, Web of Science and SCOPUS databases from the year 2000, with the initial search being conducted in 2019. In addition to PUBMED, EMBASE, Web of Science and SCOPUS databases, grey literature including studies presented at bone health conferences were also included in the search. The reference lists of journal articles included in this literature review were checked for relevant papers and citation tracking databases were used to identify additional articles that cited papers identified within the initial search. Citation

tracking databases were used to identify additional or new articles relevant to the search therefore new publications relevant to the search were added to this review, until 2023.

6.2 Prevalence of bone fractures in adult PLWH

Several studies have compared the prevalence of fractures between adult PLWH and those without HIV (465–474). Prevalence of fractures in PLWH ranges from 4 to 27% (Table 6.1). A 27% prevalence of low trauma fractures was reported in a study of 163 PLWH in Italy, with hepatitis C virus (HCV) co-infection, and low femoral BMD being the risk factors for fracture (467). However, women were underrepresented in this study, with only 17% of the 163 participants being female. In most studies reporting prevalence of fractures in PLWH, women are also not well represented, with percentages of women making up as few as 0% [Italy (472)] or 1.8% [USA (475)] of the study population. In addition to women being fewer than men, some of these studies do not present results stratified by sex (465,467,469,470,475–479). The variation in prevalence of fractures between PLWH and without confirm that there are more factors (e.g. sex, geographical location etc.) involved in the association between HIV and fractures even beyond an osteoporosis definition of a World Health Organisation (WHO) based aBMD cut off of femoral neck T-scores >-2.5. Traditional risk factors also contribute to differences in the prevalence of very low BMD and therefore prevalence of fractures between PLWH and their uninfected counterparts (480).

6.3 Incidence of bone fractures in adult PLWH

There is a 50% greater incidence of fractures in PLWH than people without HIV infection (121). In a longitudinal analysis of 4640 American men and women (median age 39 years), higher fracture incidence rates were reported within 2 years of initiating ART (0.53/100 person-years) than the years that followed (0.30/100 person-years) (6). A large population-based study in Spain reported age and sex adjusted hazard ratios (HR) for adults living with HIV/AIDS of 6.2 (95% confidence interval [CI] 3.5–10.9; p<0.001) for hip fracture, with HIV infection being associated with major osteoporotic fractures especially in the older population (7). In MLWH (n= 328) and men at risk of HIV through injection drug use or high-risk sexual behavior (n=231) aged 49 years or older, there was an association between HIV infection and both a reduced BMD and an increased incidence of fracture (125). However, this study did not adjust for other known risk factors for low trauma fractures. Higher incidence rates have been reported in studies that have assessed fracture occurrence in people living with HIV who have HCV co-infection (13), suggesting that HCV co-infection is an additional risk factor for fractures in adult PLWH. A study performed in 1221 MLWH and 1408 MLWOH who were 40 years or older, reported that whilst fracture incidence increased with age in both men living with and without HIV it was higher among men living with HIV, especially

those aged 50–59-year-old MLWH (481). This highlights the necessity of screening for risk factors for fracture in men living with HIV who are above 50 years of age (481). In Spain, a population-based cohort of over 1.1 million participants and 2,500 PLWH demonstrated a strong association between HIV infection and hip fracture incidence, with an almost 5-fold increased hip fracture risk in PLWH, independent of sex, age, smoking, alcohol, and comorbidities. The same study reported a 75% higher risk of all clinical fractures and a 60% increase in non-hip fractures among PLWH compared with their uninfected counterparts (116). A nationwide case control study of fracture in Denmark (52% female) found that HIV infection was associated with an almost 3-fold increase odds of any fracture (OR: 2.89 (95%CI 1.99, 4.18) and an almost 9-fold higher odds of hip fracture (OR 8.99 (95% CI 1.39 to 58.0) (482). However it is important to note that while some large cohort studies have found higher incidence of fractures in PLWH than those without HIV (483,484), others have not (123,125). No significant increase in fracture risk was reported in 328 MLWH followed for 2 years despite higher prevalence of low aBMD in MLWH than MLWOH (125). Given that men in this study were below 60 years, it is unclear whether the effect of HIV infection on BMD would have become more pronounced as men reach even older ages, or whether the increased risk of fracture due to HIV would be significant with older age, longer follow-up or a larger sample size (125). However, there were no increases in fracture rates in 1,728 American WLWH who were followed for 5 years (485) and in 1,281 European PLWH and on ART, who were followed for 10 years, compared to the general population (123).

A study conducted in 3251 Japanese PLWH initiating TDF, WLWH had a 3 times greater fracture rate than MLWH, with the first low trauma fracture occurring earlier in WLWH (123 days after ART initiation) than in MLWH (1438 days after ART initiation) (486). An earlier study, comparing risk of fracture in a US cohort of 2292 men and 869 women living with HIV, found that though MLWH had a 1.5 times higher incidence of fractures than WLWH from 36 to 46 years, the difference by sex was reduced in older age groups (487). These studies have shown that WLWH are at a higher risk of fractures than MLWH (486,487)

There are no studies reporting prevalence or incidence of fractures in adult PLWH from sub-Saharan Africa (Table 6.1). The studies presented in Table 6.1, reporting prevalence and/or incidence of fractures in PLWH are all from high income countries. The sample sizes vary from 92 to 95827 (Table 6.1), and most studies (126,468) recruited MLWH. This shows the need for more bone studies in WLWH and more importantly in PLWH in sub-Saharan Africa.

Table 6.1: Prevalence and incidence of bone fractures in adults living with HIV

Author	Year	Site	Design	Sample size	Age (years)	Female sex	Prevalence (%)	Incidence*
Komatsu (486)	2018	Japan	Cohort	3251	40	6%		0.1(0.03, 0.1)
Ciullini (465)	2018	Italy	Cross-sectional	141	43 (37, 52)[b]	12.80%	14%	
Gonciulea (481)	2017	USA	Cohort	1221	40 (40, 46)	0%		12.8 (11.1, 14.8)
Borges (466)	2017	Europe, Argentina, Israel	Cohort	11820	49 (42, 56)[b]	25%	4%	4.2 (3.8, 4.6)
Battalora (479)	2016	USA	Cohort	1006	43 (36, 49)[b]	17%	8%	8.4 (6.8, 10.3)
Mazzotta (467)	2015	Italy	Cross-sectional	163	42 ± 10[a]		27%	
Sharma (468)	2015	USA	Cohort	1713	40 (34, 46)[b]	100%	18%	
Gazzola (469)	2015	Italy	Cross-sectional	194	49 (40, 51)[b]	27%	12%	
Prieto-Alhambra (482)	2014	Denmark	Case control	124 655	43 ± 7[a]	52%		2.9 (1.9, 4.2)
Porcelli (477)	2014	Italy	Cross-sectional	131	51 (36, 75)[b]	29%	27%	
Short (488)	2014	UK	Cross-sectional	168	45 (38, 51)[b]	None	14%	
Borderi (470)	2014	Italy	Cross-sectional	202	51 (31, 67)[b]	32%	23%	
Maalouf (475)	2013	USA	Cohort	56660	44 (37, 52)[b]	1.8%	1%	2.07
Peters (471)	2013	UK	Cross-sectional	222	46 ± 9[a]	40%	20%	
Torti (472)	2012	Italy	Cross-sectional	160	53 (42, 71)[b]	0%	27%	
Lo re (489)	2012	USA	Cohort	95827	39 (33, 46)[b]	37%		1.95 (1.3, 2.9)
Bedimo (476)	2012	USA	Cohort	56660	NR[c]	2%		0.3 (0.3, 0.3)
Yin (478)	2012	USA	Cohort	4640	39 (33, 45)[b]	17%		0.1 (0.1, 0.1)
Womack (490)	2011	USA	Cohort		45 ± 9[a]			1.24 (1.1, 1.4)
Guaraldi (473)	2011	Italy	Case control	2854	46	37%	15%	
Hasse (491)	2011	Swiss	Cohort	8444	45 (39, 51)[b]	29%		0.7 (0.6, 0.8)
Young (484)	2011	USA	Cohort	5826	40 (31, 46)[b]	21		0.3 (0.2, 0.3)
Yin (474)	2010	USA	Cross-sectional	92	60 ± 1[b]	100%	7%	
Collin (123)	2009	France	Cohort	1281	36[d]	23%		0.3 (0.1, 0.9)
Arnsten (125)	2007	USA	Cohort	559	55 ± 5[a]	None		3.1 (1.9, 4.6)
Gallant (492)	2004	South America, Europe, USA	RCT	602	36 (18, 64)[b]	25%		0.6 (0.6, 1.7)

**Incidence per 1000 persons/year, [a]Mean (Standard deviation), [b]Median (Interquartile range), [c]Not reported, [d]Median only*

6.4 DXA based studies that have assessed aBMD in people living with HIV

HIV has been associated with low DXA measured bone density (68–71). A lower BMD at baseline and follow up, a greater bone loss and/or greater rate of bone loss, have all been reported in people living with HIV compared with their uninfected counterparts (126,128,129,494,500,506,512–517). An American cohort study reported a mean loss of 2% total body (TB) BMD over 96-weeks, in 796 people living with HIV (86% male) with a median age of 39 years (518). Table 6.2 shows studies that have assessed bone in a study population including both men and women living with HIV. In studies of PLWH which have included both men and women (Table 6.2), men constitute the greater percentages of participants in the sample sizes than women, resulting in women being underrepresented in these studies. Of all the studies included in Table 6.2, only 4 studies have been performed in Africa or have been multi-country studies including a sample of participants living in an African country (239,459,460,519). One South African cross-sectional study [n=104 (men) and n=340 (women)] reported a 17% prevalence of low (<-2 SD) lumbar spine (LS) and 5% prevalence of low total hip (TH) BMD irrespective of ART status (239). An earlier, larger Nigerian cross-sectional study (n=1005), reported a prevalence of 32% for osteoporosis and 47% for osteopenia (460). However, both this Nigerian study and the South African study lacked a comparison group of people living without HIV (520,521). The Nigerian study also failed to sex stratify, despite female sex being a strong determinant of BMD (460). Furthermore, the Nigerian study reported T scores in a relatively young population where Z-scores would have been more appropriate (460). Figure 6.1 and Table 6.3 show that even DXA based studies which included both men and women living with HIV were biased towards men than women.

Figure 6.1: Graph to show sex distribution from studies assessing aBMD in both men and women living with HIV

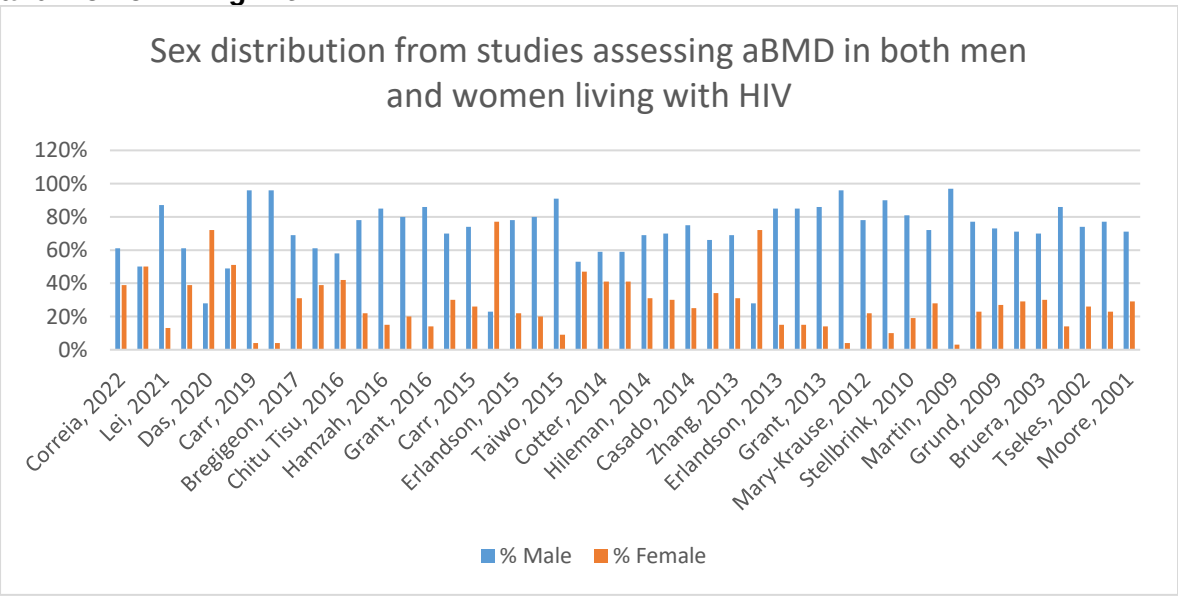


Table 6.2: DXA based studies assessing bone mineral density in men and women living with HIV

Author	Year	Country	Study Design	n PLWH	n PLWOH	% Male	Age (years)	Sex stratified analysis
Correia (522)	2022	Brazil	Cross-sectional	104	None	61%	45 (43, 47)[b]	Yes
Mwaka (519)	2021	Uganda	Cross-sectional	199	None	50%	40 ± 9[a]	No
Lei (523)	2021	UK	Cohort	84	None	87%	55 (51, 59)	No
Aguilar	2021	Brazil	Cross-sectional	106	None	61%	46 ± 11[a]	Yes
Das (524)	2020	India	Cross-sectional	50	None	28%	43 ± 7[a]	No
Cascio (525)	2020	Italy	Cross-sectional	83	None	49%	44 ± 5[a]	No
Carr (526)	2019	Australia	RCT	69	None	96%	50 ± 11[a]	No
Hoy (527)	2018	Australia, Spain	RCT	112	None	96%	50 ± 11[a]	No
Bregigeon (528)	2017	France	Cross-sectional	175	None	69%	53 (50, 56)[b]	Yes
Tinago (134)	2017	Ireland	Cohort	210	264	61%	39 (34, 46)[b]	No
Chitu Tisu (529)	2016	Romania	Cross-sectional	60	None	58%	34.8 ± 7.6[a]	No
Erlandson (530)	2016	USA	RCT	147	None	78%	47 (39, 54)[b]	No
Hamzah (531)	2016	UK	RCT	64	None	85%	43 ± 9[a]	No
Casado (456)	2016	Spain	Cohort	90	None	80%	48 (27 - 74)[d]	No
Grant (530)	2016	USA	Cohort	97	614	86%	40 (31, 44)[b]	No
Mazotta (467)	2015	Italy	Cohort	163	None	70%	44.2 ± 10[a]	No
Carr (455)	2015	Australia+	Cross-sectional	424	None	74%	34 ± 10.1[a]	No
Dave (239)	2015	South Africa	Cross-sectional	444	None	23%	20 - 49[c]	Yes
Erlandson (532)	2015	USA	RCT	147	None	78%	20 - 49[c]	No
Kooij (507)	2015	Netherlands	Cross-sectional	598	550	80%	53 (48, 59)[b]	No
Taiwo (533)	2015	USA	Cohort	259	None	91%	33 (26, 42)[b]	No
Madeddu (493)	2015	Italy	Cohort	67	None	53%	40 ± 7[a]	No
Cotter (534)	2014	Ireland	Cohort	210	264	59%	39 (33, 46)[b]	No
Pinnetti (495)	2014	Italy	Cross-sectional	918	None	59%	49 (44, 55)[b]	No
Hileman (535)	2014	USA	Cohort	47	41	69%	40 (25, 50)[b]	No
Porcelli (477)	2014	Italy	Cross-sectional	131	None	70%	51 (36, 75)[b]	No
Casado (496)	2014	Spain	Cross-sectional	285	None	75%	46 (30 - 80)[d]	No
Bonnet (536)	2013	France	Cohort	35	None	66%	42 ± 15[a]	No
Zhang (537)	2013	China.	Cohort	47	41	69%	37 ± 10[a]	No
Alonge (460)	2013	Nigeria	Cross-sectional	1005	None	28%	41 ± 10[a]	No
Erlandson (538)	2013	USA	Case Control	359	None	85%	38 ± 10[a]	No
Erlandson (539)	2013	USA	Cohort	269	None	85%	38 ± 10[a]	No
Grant (518)	2013	USA	Cohort	796	None	86%	39 (32, 44)[b]	No
Rozenburg (540)	2012	France	RCT	42	None	96%	45 (41, 53)[b]	No
Mary-Krause (498)	2012	France	Cross-sectional	892	78	78%	41 (37, 45)[b]	Yes
Hansen (499)	2011	Denmark	RCT	104	69	90%	26 ± 9[a]	No
Stellbrink (541)	2010	Europe	RCT	385	None	81%	37 (18 - 70)[d]	No
Bonjoch (509)	2010	Spain	Cohort	671	None	72%	42 (37, 48)[b]	No
Martin (542)	2009	Australia	RCT	357	None	97%	45 ± 8[a]	No
Duvivier (502)	2009	France	Cohort	71	None	77%	40 (33, 49)[b]	No
Grund (543)	2009	USA, Australia+	RCT	214	None	73%	44 (39, 50)[b]	No
De Socio (504)	2008	Italy	Case Control	17	34	71%	48 ± 7[a]	No
Bruera (544)	2003	Argentina	Cross-sectional	142	None	70%	20 - 45[c]	No
Mondy (511)	2003	USA	Cohort	125	None	86%	42 ± 1[a]	No
Tsekes (545)	2002	Greece	Cohort	23	None	74%	37 ± 9[a]	No
McDermott (546)	2001	USA	Cross-sectional	265	None	77%	39 ± 7[a]	Yes
Moore (547)	2001	UK	Cross-sectional	105	None	71%	40 (26, 60)[b]	No

[a]Mean (SD), [b] Median (Interquartile range), [c]Age range, [d]Mean (Age range), PLWH: People living with HIV, PLWOH: People living without HIV

Table 6.3 presents the DXA based studies assessing bone in WLWH only. Of all the studies included in Tables 6.3, only 6 studies have been performed in Africa or have been multi-country studies including a sample of participants living in an African country (132,232,234,241,458,548). In general, traditional risk factors for bone loss and fractures include older age, smoking, low physical activity, low dietary intake of calcium and vitamin D, smoking, alcohol use and certain diseases or medications (549). Smoking is associated with increased risk of bone fracture in both PLWH and those without HIV and this association is influenced by dose and duration (550). Smokers have twice the risk of fracture compared to non-smokers (488,551). HIV-specific factors such as CD4+ cell count, ART exposure, exposure to TDF (486), hepatitis C virus (HCV) co-infection (552), high viral load and low CD4 count (553), have been identified as risk factors for bone loss and fractures. HCV co-infection is an independent risk factor for both fragility and non-fragility fractures (552). Some studies have reported no change in BMD over time, with HIV and ART treatment (134,514). An Irish cohort study recently reported no between-group difference in rate of aBMD change over three years in men and women living with and without HIV (134). Possible reasons for these results include the fact that most participants in this study had been on ART for a fairly long time by enrolment (total ART exposure years median, 2.9 [0.7–5.4]). Those living with HIV in the Irish cohort were also younger than those living without HIV, though adjustment for age, sex, ethnicity, smoking status, and BMI minimally affected the estimated rates of BMD decline. These data are consistent with an earlier American cohort study which reported similar mean annual percentage change in TH and LS BMD in women living with and without HIV, though baseline BMD was lower in those living with HIV (514).

However, there are studies confirming reduced aBMD with ART initiation or use of TDF. Other studies have reported greater declines in aBMD in patients initiating ART than those established on ART (133,134,233). Teichmann et al. reported a higher risk of osteoporosis in PLWH who were on ART (513). However this study did not report the previous use of ART before the study or the ART regimen that the participants were on. Different types of ART regimen have also been shown to affect BMD differently (459,510,536,554). Tenofovir, which is widely used in sub-Saharan Africa is associated with bone loss (354,555–558). A Chinese study performed in 40 men and women living with HIV, with a mean age of 37 years, reported a decline over the first 48 weeks post ART initiation of 1.78-3.28%, though BMD then remained stable to 96 weeks (537). The type of ART regimen is essential, because it may possibly explain high or low BMD. NRTIs and PIs block RANKL, decrease calcitriol and inhibit osteoblasts whilst Zidovudine accelerates osteoclastogenesis and

Tenofovir impairs the mineralization of bone (559). Therefore it is essential to include regimen composition when assessing the effect of ART on bone.

Table 6.3 : DXA based studies that have assessed areal bone mineral density only in women living with HIV

Author Year	Country	Design, Follow-up	n (HIV+)	n (HIV-)	Menopausal status	Age (years)	Findings
Madanhire, 2023 (458)	South Africa	Cohort, 4.8 years	65	385	Postmenopause	Range: 40 - 60yrs Mean: 49.5 ± 5.7	In postmenopause, WLWH lost more TB BMD than WLWOH (-0.070 [-0.031, -0.108], p = 0.001) vs (-0.036 [-0.015, -0.057], p = 0.001
Sharma, 2022 (560)	USA	Cohort, NR	158	86	Pre, peri & postmenopausal	Range: 40 - 60yrs Mean: 50 ± 5	5-9% lower aBMD at LS (P=.001), FN (P=.04), TH (P=.003) & higher osteoporosis prevalence (T score<-2.5) in WLWH (13.5% vs 0%, P=.0003).
Matovu, 2022 (561)	Uganda	Cohort, 2 years	452	69	Premenopausal	Range: 18 - 35yrs Mean: 26 ± 4	Greater BMD decline (p<0·0001) in WLWH who are on TDF and DMPA than WLWH and on TDF alone or than controls
Stranix-Chibanda, 2021 (132)	*Zimbabwe, Malawi +	Cohort, 74 weeks	398	None	Premenopausal	Range: 18 - 35yrs Mean: 27 (23, 30)	2% (LS) and 5.3% (TH) decline in BMD in WLWH who are on TDF
Ellis, 2021 (562)	South Africa	Cohort, 2 years	120	None	Postmenopausal	Range: >45yrs Median: 50 (48, 55)	No BMD decline over 2 years
Matovu, 2021 (548)	Uganda	Cross-sectional	452	69	Premenopausal	Range: 18 - 35yrs Mean: 26 ± 4	Higher prevalence of low BMD (Z-score<-2) in WLWH 15-20%than without (2.9%) and 5.6% - 8% lower BMD in WLWH than without
Nabwire, 2020 (441)	Uganda	Cohort, 66 weeks	100	100	Premenopausal	Range: 18 - 40yrs Median: 23 (21, 27)	aBMD decreased in both groups during lactation, but WLWH had greater decreases at TH than WLWOH
Hamill, 2020 (234)	South Africa	Cohort, 2 years	71	39	Premenopause	Range: 20 - 51yrs Mean: 33.9 ± 6.6	At 24 months, despite gains in weight and fat mass, WLWH & on ART had lower aBMD than WLWOH
Kalyan 2018 (563)	Canada	Cross-sectional	73	290	Pre, peri & postmenopausal	Range: 25 - 60yrs Mean: 43 ± 9	Lower LS and TH aBMD Z-scores in WLWH [MD (95%CI): -0.39g/cm^2(-0.61, -0.17);p<0.001 (LS)] & [-0.29g/cm^2(-0.52, -0.07);=0.012 (TH)]
Hamill, 2017 (233)	South Africa	Cohort, 1 year	120	67	Premenopause	Range: 19 - 50yrs Mean: 33 ± 7	2-3% significant decrease in aBMD at femur neck & lumbar spine before and after size adjustment.
Sharma, 2015 (564)	USA	Cohort, 1.5 years	246	219	Pre, peri & postmenopausal	Range: 18 - 60yrs Mean: 46.9 ± 4.9	Lower baseline TH and FN BMD in WLWH but similar mean annual % change in TH and FN
Gomes, 2015 (494)	Brazil	Cross-sectional	537	None	Pre, peri & postmenopausal	Range: 40 - 60yrs Mean: 47.7 ± NR	Higher prevalence of low BMD in WLWH (14.6% vs 4.6% (LS) and 5.6% vs 3.3% (FN)
Hamill, 2013 (232)	South Africa	Cross-sectional	149	98	Premenopause	Range: 18 - 49yrs Mean: 32.1 ± 7.2	No significant differences in BMD at any site (p>0.05) between the groups by HIV status
Calmy, 2013 (515)	Switzerland	Cross-sectional	22	44	Premenopausal	Range: NR Mean: 44.3 ± 1.8	HIV+ patients with low BMD in the lumbar spine
Sharma, 2012 (516)	USA	Cohort, 5 years	318	122	Pre, peri & postmenopausal	Range: 18 - 65yrs Mean: 44 ± 8	Increased lean mass and fat mass associated with increased BMD at LS, TH and FN
Yin, 2010 (126)	USA	Cross-sectional	92	95	Postmenopausal	Range: >40yrs Mean: 56 ± 1	Higher prevalence of T scores below <-1.0 in WLWH 78 vs. 64%; P=0.05(LS), 45 vs. 29%; P=0.05(TH) and 64 vs.46%;P=0.05 (FN)

Yin, 2010 (127)	USA	Cohort	100	68	Premenopause	Range: 18 - 48yrs Mean: 40 ± 5	5% lower BMD in WLWH at baseline but no difference in annual percent decrease in BMD
Libois, 2010 (128)	Belgium	Cross-sectional	89	None	Premenopausal	Range: 19 - 45yrs Mean: 37 ±	31.5% prevalence of osteopenia/osteoporosis
Dolan, 2007 (517)	USA	Cross-sectional	152	100	Pre, peri & postmenopausal	Range: 18 - 60yrs Mean: 41 ± 1	Higher prevalence of osteoporosis (14%) amongst WLWH with low weight (p = 0.002)
Arnsten, 2006	USA	Cross-sectional	263	232	Pre, peri & postmenopausal	Range: NR Mean: 44 ± 5	Higher prevalence of low BMD in WLWH than without (27% vs 19%)
Anastos, 2007 (565)	USA	Cross-sectional	274	152	Pre, peri & postmenopausal	Range: NR Mean: 43 ± 7	Lower LS and FN BMD in WLWH than WLWOH and lower LS and FN BMD in WLWH who are on PI than those who are not on PI
Prior, 2007 (512)	Canada	Case Control	138	402	Pre, peri & postmenopausal	Range: >18yrs Mean: 38 ± 8	Higher prevalence of lifetime fragility fractures in WLWH (26.1% vs. 17.3; OR 1.7, 95% CI 1.1, 2.6)
Yin, 2005 (506)	USA	Cross-sectional	31	186	Postmenopausal	Range: 18 - 60yrs Mean: 38 ±	Higher prevalence of osteoporosis in WLWH than controls (42% vs 23% (LS) and 10% vs 1% TH
Dolan, 2004 (129)	USA	Cross-sectional	84	63	Pre & postmenopausal	Range: 18 - 60yrs Mean: 41 ± 1	Lower LS and TH BMD in WLWH. Higher osteopenia prevalence in WLWH (54% vs 30%, P=0.004
Teichmann, 2003 (513)	Germany	Cross-sectional	50	50	Pre & Perimenopausal	Range: 26 - 48yrs Mean: 39.4 ± 2.2	HIV+ patients with low BMD in the lumbar spine and femur

WLWH: Women living with HIV, WLWOH: Women living without HIV

LS; Lumbar spine, TH; Total hip, FN; Femur neck, TB; Total body

****Zimbabwe, Uganda, South Africa, Malawi***

Treatment regimens containing TDF lead to an approximately 1–3% greater BMD loss compared to non TDF containing regimens (354). A two year longitudinal study in South African WLWH reported that TDF initiation was associated with a loss in BMD of –1.8% (femoral neck) and –2.2% (total hip) which stabilised by 24 months. Increased bone turnover markers and increased bone loss have been reported in PLWH initiating ART containing TDF compared to those starting non-TDF-containing ART (34). In a study which compared BMD changes in PLWH who were randomised to one of four treatment regimens, ART initiation led to a large initial decrease in BMD, by week 96, with TDF containing ART regimen leading to greater decreases in spine than non TDF containing regimens (553). An earlier randomised clinical trial, reported greater decreases in spine and hip aBMD at 48 weeks in PLWH who were on TDF containing regimens than those who were not (541). The BMD losses observed with TDF exposure are similar in magnitude to the BMD losses sustained during the first 2 years of menopause (566). However, other studies that have followed up PLWH who were on TDF containing regimens for longer periods, showed no evidence of additional BMD loss with TDF, outside the 1–3% BMD loss that occurs early after ART initiation (530,567,568). Other studies have also shown that switching from TDF containing regimen to other ART regimens improves bone density (144,531,569).

In addition to TDF, other HIV-specific factors that have been associated with lower BMD include higher baseline HIV-1 viral load and lower CD4 cell count (464,511,553). HIV proteins can increase osteoclastic activity and promote osteoblast apoptosis (86,570). Furthermore, cytokines, such as IL-6 and TNF-α, may stimulate osteoclast activity (570). In resource limited settings like sub-Saharan Africa, TDF-containing ART regimens are prevalent. In addition to traditional risk factors for impaired bone health, and HIV-specific factors such as CD4+ cell count, ART exposure and hepatitis C virus (HCV) co-infection, premenopausal women have other factors such as pregnancy, lactation and hormonal contraception which need to be considered.

There are sex differences in the prevalence of bone loss and fractures are due to factors such as oestrogen, pregnancy (441), contraception (561), lactation (132) and menopause (560). Female sex is an independent risk factor for low bone mass in adults on ART (571–574) as differences in how men and women respond to HIV infection and its treatment resulting in different bone and fracture outcomes in WLWH have been reported (487). In a study of PLWH (WLWH=839, MLWH=1759), female sex was independently associated with both lower femoral neck and lumbar BMD over time and bone density at the femoral neck declined twice as quickly among women compared to men (571).

In an study of 499, American men and women, bone loss before ART initiation was greater in WLWH than in MLWH. In adjusted models, WLWH vs MLWH had lower BMD before ART (–0.39 g/cm^2 [lumbar spine], –0.05 g/cm^2 [hip]) (572). ART initiation accelerated HIV-associated bone resorption in a 24 week prospective cohort study in 20 American men and women living with HIV (89). An earlier cross-sectional study of 95 American MLWH who were compared to 17 MLWOH demonstrated that the greatest effect of ART on bone occurs during the first 2 years after ART initiation (575). This finding agrees with findings from WLWH in a South African cohort (241,576). Among PLWH who were aged 45 years and older, the risk of developing osteoporosis on a PI regimen was increased 5.9-fold in WLWH compared to 1.8-fold in MLWH. Regimens containing both a PI and TDF (vs neither) increased osteoporosis risk 7-fold among WLWH, but there was no increased risk among MLWH (573). Similarly, WLWH were reported to have a 1.7% greater decline in hip aBMD, after 48 weeks of TDF, than MLWH (572). In a 5-year cohort study of 66 Thai PLWH who were on ART, WLWH were 3 times more likely to experience ≥5% BMD loss at the lumbar spine than MLWH (574). These studies have shown that the effect of ART exposure on BMD loss is greater for women than men (571–574).

Injectable depot medroxyprogesterone acetate (DMPA) commonly referred to as ‘Depo’ amongst women in sub-Saharan Africa is a common form of contraception (577). DMPA lowers oestrogen levels, accelerating bone turnover and reducing aBMD (578). In 306 WLWOH, (102 using DMPA and 204 controls), DMPA users had lower aBMD (median duration of DMPA use 4.3 years (IQR 2.6 - 6.7 years) compared with the control group, and lower aBMD persisted for 2 to 5 years post cessation of DMPA use (578). In a cross-sectional analysis of Ugandan WLWH, higher prevalence of low BMD (Z-score ≤-2) was reported in WLWH with previous (12.7%) or current DMPA use (20.3%) compared with WLWOH (2.9%). Exposure to HIV and DMPA use were independently associated with lower mean BMD Z-scores at the lumbar spine, total hip and femoral neck, with the greatest difference being among WLWH who were currently using DMPA (5.6%-8.0%) versus WLWOH who are not using DMPA (548). More recently, after a 24 months follow up of the same Ugandan cohort, DMPA was found to accentuate the bone loss associated with TDF initiation (561). In this Ugandan study, WLWH who had just been initiated on ART containing TDF and who were also on DMPA experienced twice the bone loss [∆from baseline: –4·42 (–5.08 to –3.77); p<0.001] than WLWH who were on ART containing TDF but not on DMPA [–1.99 (–3.02 to –0.98); p<0.001] or women using DMPA but not on ART [–0.06 (–1.26 to 1.15); p=0.93] (561). This study reported a 1·8–2·5% annual loss of aBMD with concurrent use of TDF and DMPA, exceeding the magnitude of bone loss observed with TDF-containing ART initiation alone (0·4–1·1%) and the annual bone loss seen during normal menopausal

transition (1·0% and 1·6% per year), suggesting that the combination of ART and hormonal contraception in premenopausal WLWH is a cause for concern. Further studies are needed to determine to what extent the concurrent use of TDF and DMPA affects fracture risks and whether or not the effects of TDF and DMPA use are reversible when WLWH switch to non TDF containing regimens and /or stop using DMPA.

Pregnancy causes a mean 3% loss whilst lactation results in a 5–6% loss of aBMD, with subsequent recovery post weaning (579–581). During pregnancy and lactation, BMD is mobilised to provide calcium to the growing foetus and through breastfeeding. For pregnant WLWH and on ART, bone loss is even greater (132). In a Ugandan longitudinal cohort of women who were breastfeeding, WLWH and on ART containing TDF had similar reductions in spine BMD but greater hip BMD losses in the first 3 months of lactation and 2–3% lower recovery of BMD after they stopped breastfeeding than their uninfected counterparts who recovered fully (441). In a study conducted in women from Zimbabwe, Malawi, South Africa and Uganda, women with no prior TDF exposure were randomised during pregnancy to receive TDF containing ART or their infant received nevirapine for prevention of HIV transmission via breastfeeding. Results showed that the mothers who received TDF reported a 2% and 5.3% (spine and hip respectively) decline in BMD compared with mothers whose infants received nevirapine (132).

Menopause is associated with a 2–3% yearly loss of bone mass (560,582). In WLWH, early onset of menopause is more common in WLWH than in WLWOH (583,584) such that menopause occurs on average, 5 years earlier in WLWH than in those without (585,586). Postmenopausal WLWH have more bone loss than uninfected postmenopausal women, particularly at the lumbar spine (506,515,543). In sub-Saharan African settings, hormonal replacement therapy, which may positively affect bone by preventing bone loss and reducing fracture risk, is not widely available. A recent study in South African women reported WLWH to have lost more total body BMD (mean difference −0.013 [95% confidence interval −0.026, −0.001] g/cm^2, p = 0.040) despite gaining more weight (1.96 [0.32, 3.60] kg; p = 0.019) than women without HIV (458). The transition from pre to postmenopause was associated with greater total body BMD loss in WLWH (−0.092 [−0.042, −0.142] g/cm^2; p = 0.001) than those without (−0.038 [−0.016, −0.060] g/cm^2, p = 0.001; interaction p = 0.034) (458). In a recent study of 158 WLWH (aged 40 – 60 years), who were compared to 86 WLWOH in the US, postmenopausal WLWH had 5–9% lower lumbar spine, total hip and radial BMD than postmenopausal WLWOH (560). Another earlier study in the US reported 2.4 (spine) and 3.7 (radius) times higher rates of bone loss per year in 92 postmenopausal WLWH than their uninfected counterparts (n=95) (587). In addition to exacerbated bone loss with the interaction between menopause and HIV, there is an accelerated fracture risk among

postmenopausal WLWH compared with postmenopausal WLWOH or with MLWH (485) In a high income setting high prevalence of low BMD in postmenopausal WLWH has been reported, ranging from 23% to 78%; (126,560,588) A small study of Hispanic and African American women suggested that postmenopausal bone loss may be greater in women living with HIV, particularly in those with low BMI (587). In South Africa, a smaller 2-year longitudinal study of 120 South African postmenopausal women living with HIV and established on ART, showed little change in TB or LS BMD, potentially due to the small sample size and short follow-up period (562). In contrast, a more recent study in South African women showed that menopause related bone loss is greater in WLWH, suggesting women with HIV may be at greater risk of osteoporotic fractures (458).

To my knowledge the only longitudinal study to examine the effects of HIV-infection and ART naïve women over time on BMD in a population of only premenopausal non-lactating, non-pregnant women sub-Saharan Africa is the Women's Bone Health study (WBS) (232,234,241). WBS recruited women for baseline measurement between February and July 2010 at the MRC/Wits Developmental Pathways for Health Research Unit (DPHRU) in Johannesburg, South Africa (232,234,241). WBS included three groups of adult premenopausal women (age >18 years) who were either living without HIV, living with HIV with a preserved CD4 count and anticipated not to require ART at baseline, or living with HIV with a CD4 count low enough for them to start ART just after the baseline visit (232). Although similar at baseline the women living with HIV with a CD4 count low enough to be initiated on ART demonstrated significant declines in BMD of 2-3% over 12 months of starting ART, before and after body size adjustment, at both the femoral neck (FN) and lumbar spine (LS), compared to the women living without HIV and those living with HIV but not on ART (241). At 24 months follow up, despite gaining more weight and fat mass than WLWOH, WLWH who were exposed to ART remained lighter, with lower aBMD and higher bone turnover than WLWOH (234). Part of the data collected from this WBS cohort are included in this PhD study *(see chapter 7).*

Table 6.4: DXA based studies that have assessed areal bone mineral density in men living with HIV

Author	Year	Country	Design	MLWH	MLWOH	Age
Zhang (589)	2023	China	Cohort	76	None	32 ± 9[a]
Cornejo-Juarez (590)	2022	Mexico	Cross-sectional	105	None	46 ± 9[a]
Makras (591)	2021	Greece	Cohort	23	14	56 ± 10[a]
Atencio (592)	2021	Spain	Cross-sectional	245	None	36 (30, 40)[b]
Tsai (593)	2019	Taiwan	Cross-sectional	330	None	52 ± 8[a]
Kazakia (594)	2018	USA	Case control	8	11	56 ± 7[a]
Manavalan (377)	2016	USA	Cross-sectional	38	20	23 ± 0.3[a]
Sellier (595)	2016	France	Cross-sectional	53	50	49 ± 9[a]
Rey (133)	2015	France	Clinical Trial	39	None	39 (33, 46)[b]
Yin (333)	2014	USA	Cross-sectional	30	15	20 - 25[c]
Biver (596)	2014	Switzerland	Case control	28	112	60 - 70[c]
Grijsen	2013	Netherlands	Cross-sectional	147	30	NR
Martin (459)	2013	*SA, India, Malaysia +	Cohort	210	None	20 - 49[c]
Assoumou (597)	2013	France	Cohort	94	None	46 (41, 53)[b]
Pepe (598)	2012	Italy	Cross-sectional	50	27	49 ± 9
Buehring (508)	2012	USA	Cross-sectional	66	None	42 (23, 68)[b]
Haskelberg (558)	2012	Australia	Cohort	301	None	45 ± 8[a]
Grijsen (501)	2010	Netherlands	Cross-sectional	33	None	38 ± 9[a]
Calmy (503)	2009	Australia	Cross-sectional	153	None	48 (43, 55)[b]
Van Vonderen (599)	2009	**Europe+	RCT	50	None	39 (35,52)[b]
Bolland (600)	2008	New Zealand	RCT	33	None	50 ± 9[a]
Arnsten (125)	2007	USA	Cross-sectional	328	231	55 ± 5[a]
Bolland (601)	2007	New Zealand	Cohort	23	26	47 ± 9[a]
Bolland (602)	2006	New Zealand	Cross-sectional	59	118	50 ± 8[a]
Amiel (603)	2004	France	Cross-sectional	148	81	40 ± 8[a]
Lazanas (604)	2003	Greece	Cohort	108	20	38 ± 9[a]
Nolan (605)	2001	Australia	Cohort	183	None	43 ± 10[a]
Huang (247)	2001	USA	Cross-sectional	41	18	43 ± 7[a]
Tebas (575)	2000	USA	Cross-sectional	95	17	NR

[a]Mean (SD), [b] Median (Interquartile range), [c]Age range, [d]Mean (Age range)
MLWH: Men living with HIV, MLWOH: Men living without HIV
+There are other countries included in the study population which have not been shown on the table and these are listed below
**** South Africa, India, Malaysia, Thailand and Argentina***
***** Netherlands, Spain, Finland and the United Kingdom***

6.5 QCT based studies of bone density, size and strength in adults living with HIV

A few studies have assessed bone density, size and/or strength in PLWH using QCT, pQCT or HRpQCT (Table 6.5). Bone density has long been considered as the main indicator of whether or not bones will fracture. This is seen in how the World Health Organisation defines osteoporosis (606). However, the fact that low trauma fractures have been reported in individuals who do not have osteoporosis according to the BMD cut off standards, is evidence that bone density is not the only parameter to consider as an indicator of bone health (607,608). Bone architecture takes into consideration bone size and bone strength. Although bone density has been explored in studies utilising DXA, we know little about how they may influence vBMD, bone size and bone strength as measured by pQCT.

6.5.1 pQCT based studies

Out of the 17 studies presented in table 6.5, only 2 of them utilised pQCT (106,109–119). Regarding the effect of HIV infection on bone architecture in premenopausal women, one study performed in 40 to 52 year old women recruited from a US hospital demonstrated lower tibial trabecular and cortical vBMD, reduced cortical thickness and higher endosteal circumference, in women living with HIV and hepatitis C virus (HCV) coinfection, compared with a healthy reference population (120). A recent study in South African women, reported lower pQCT measured cortical vBMD in WLWH than without, highlighting the importance of optimizing bone health in WLWH, as they go through the menopausal transition (609).

6.5.2 HRpQCT based studies

Using high resolution pQCT, there was no difference between WLWH and WLWOH with regard to cortical and trabecular vBMD at the distal radius, trabecular vBMD at the tibia, and cortical porosity even though cortical area and cortical thickness were 11–12% lower in HIV-infected women (610). After adjusting for age and BMI, cortical area and thickness at the tibia remained lower in WLWH, suggesting differences in cortical microarchitecture that were independent of age and BMI (610). A recent report from Brazil reported lower HRpQCT measured trabecular & cortical vBMD in WLWH than without (611). In Canada, 8-19% lower tibial & radial trabecular vBMD in 50 WLWH who were compared to 50 WLWOH (612). Furthermore, a USA study reported lower radial trabecular vBMD in PLWH who are on TDF and PI containing regimen than PLWH who were on non TDF and PI containing regimens, showing the importance of not only HIV infection but ART regimen as well when assessing bone in PLWH (613). It is important to note that, the Brazilian (n=30 WLWH), Canadian (n=50 WLWH) and USA (n=43 PLWH) studies had small sample sizes and therefore may have been limited in detecting further differences between people living with and without HIV.

6.5.3 QCT based studies

Four of the studies presented in Table 6.3 utilised central QCT and both studies are from high income countries (247,543). Grund et al. conducted a randomized trial comparing intermittent, CD4 count-guided ART, with continuous ART in 214 PLWH and reported that QCT measured vBMD increased in both groups by 1 year but decreased (3%, trabecular vBMD) in both groups after 1 year (543). Huang et al reported a lower vBMD in PLWH with lipodystrophy than PLWH with no lipodystrophy & those without HIV (247,614).

Table 6.5: Studies utilising pQCT, QCT and HRpQCT to assess bone in adults living with HIV

Author Year	Country Design	Sample size	Age(yrs) Gender	QCT based measurement method: Study Findings
O'Breasail 2022 (609)	South Africa Cross-sectional	HIV+:96 HIV-:334	49 ± 5 ♀	**pQCT:** ↓ cortical vBMD in WLWH than without
Sharma 2022 (560)	USA Cohort	HIV+:158 HIV-:86	50 ± 5 ♀	**QCT:** ↓vBMD in WLWH [LS (157 ± 42 vs 173 ± 46;p=0.019) & TH (331 ± 62 vs 355 ± 58;p=0.035)
Oliveira 2022 (611)	Brazil Cross-sectional	HIV+: 30 HIV-: 36	58 ± 6 ♂	**HRpQCT:** ↓ trabecular & cortical vBMD in WLWH than without
Foreman 2020 (613)	USA Cross-sectional	HIV+:43	50 - 69 ♀♂	**HRpQCT:** ↓ radial trabecular vBMD in PLWH who are on TDF +PI regimen than other PLWH
MacDonald 2020 (615)	Canada Cross-sectional	HIV+:50 HIV-:50	50 ± 1 ♀	**HRpQCT:** 8-19% ↓ tibial & radial trabecular vBMD in HIV+ than controls
Kazakia 2018 (594)	USA Case Control	HIV+:8 HIV-:11	50 - 60 ♀	**HRpQCT:** ↓ tibial trabecular number, ↑tibial trabecular spacing in HIV+ than controls
Yin 2018 (616)	USA Cross-sectional	HIV+:156	NR ♂	**HRpQCT:** ↓ tibial trabecular vBMD in HIV+/HCV+ than in HIV+
Manavalan 2016 (377)	USA Cross-sectional	HIV+:38 HIV-:20	20 - 25 ♀	**HRpQCT:** 6-19% ↓ radial & tibial total vBMD, ↓ cortical thickness. 16% ↓ radial cortical area in perinatally vs adolescent infected
Sellier 2016 (595)	France Cross-sectional	HIV+:53 HIV-:50	49 ± 9 ♀	**HRpQCT:** 17% (tibia, 16% (radius) ↓ trabecular vBMD in PLWH
Lo Re 2015 (617)	USA Cross-sectional	HIV+:151 HIV-:263	47 (40, 52) ♂	**pQCT:** ↓cortical vBMD, ↓ cortical thickness & ↑periosteal & endosteal circumference in PLWH
Yin 2014 (333)	USA Cross-sectional	HIV+:30 HIV-:15	20 - 25 ♀	**HRpQCT:** ↓ total vBMD, ↓ cortical area, thinner cortices, similar cortical vBMD
Biver 2014 (618)	Switzerland Cross-sectional	HIV+:28 HIV-:112	60 - 70 ♀	**HRpQCT:** ↓ cortical thickness, 14% (tibia) & 16%(radius), ↓ total VBMD, 11-12% ↓ cortical area, ↑trabecular spacing in PLWH
Calmy 2013 (515)	Switzerland Case Control	HIV+:22 HIV-:44	44 (39, 46) ♂	**HRpQCT:** 14% ↓ tibial trabecular vBMD, ↓ cortical radial VBMD
Yin 2013 (610)	USA Cross-sectional	HIV+:92 HIV-:95	58 ♂	**HRpQCT:** 11-12% ↓ cortical area & cortical thickness in HIV infection. Similar CSA in both groups
Van Vonderen 2009 (599)	Netherlands RCT	HIV+:50	18 - 70 NR	**QCT:** Similar vBMD decrease over 2 years in all ART groups.
Grund 2009 (543)	USA, Australia, Spain RCT	HIV+:214	44 (39, 50) ♀♂	**QCT:** vBMD increases in both groups by 1year but decreases (3%, trabecular vBMD) in both groups after 1 year

Huang 2001 (247)	USA Cross-sectional	HIV+:411 HIV-:18	18 - 60 ♀♂	**QCT:** ↓vBMD in PLWH with lipodystrophy than PLWH with no lipodystrophy & those without HIV

6.6 Conclusion

In conclusion, studies presented in this literature review show that HIV infections may negatively affect bone in women. DXA based studies assessing bone have been largely performed in high income populations despite sub-Saharan Africa having the largest population of people living with HIV and/or mostly in men. Studies reporting on how HIV and ART affect bone are not conclusive. DXA based studies are limited by the two-dimensional nature of aBMD. All this shows the importance of assessing bone density, bone size and bone strength in adult premenopausal women living with HIV. When considering the high prevalence and incidence of fragility fractures in PLWH, it is crucial to note that whilst BMD is an established risk factor for fractures, there are a lot of people who have a fragility fracture despite a normal BMD value (607,608,619,620) Therefore even though it is necessary to assess BMD in PLWH, fragility fractures and low BMD are not the same outcome and using methods like pQCT which can give further information on bone geometry and predict bone strength may add more information to what is known already. Another existing gap in current literature is the lack of a sufficient number of longitudinal studies following up WLWH in sub-Saharan Africa over a long period of time, which would provide a more robust assessment of the effect of HIV on bone. Further studies are necessary to clarify the impact of both HIV and ART on bone density, bone size and bone strength. Therefore the next chapter presents results from a study investigating whether HIV infection and initiation of ART were associated with changes in trabecular and/or cortical bone architecture (density, geometry and strength) assessed by pQCT over a two-year period in premenopausal women from the Women's Bone Health study from South Africa.

7 CHAPTER 7: Manuscript 3-In premenopausal women, antiretroviral therapy initiation is associated with trabecular rather than cortical bone changes: a longitudinal study in South Africa

7.1 Introduction

This chapter addresses the question on whether HIV and its treatment affect bone architecture in women living with HIV. The hypothesis was that over time, there would be greater bone loss in WLWH and starting ART treatment than those living without HIV, or not starting ART treatment. I investigated whether HIV infection and initiation of ART were associated with changes in trabecular and/or cortical bone architecture (density, geometry and strength) assessed by pQCT over a two-year period in premenopausal women from the Women's Bone Health study from South Africa.

The results show that urban black South African women living with HIV have lower vBMD than their uninfected counterparts at both the radial and tibial trabecular rich sites. Furthermore, I have demonstrated that the skeletal response to HIV infection and ART initiation may vary depending on whether the site is primarily trabecular or cortical bone, and whether it is the radial or tibial site. Finally, our results also confirm that decline in bone in WLWH is more likely due to ART initiation than due to HIV infection. Given that trabecular rich sites are a common site for fractures e.g. Colles's fracture on the distal radius and that premenopausal women are likely going to be on ART for many years, we recommend further studies to assess the effect of ART on trabecular rich sites over a longer period of time and whether this is associated with an increase in fracture risk.

I performed the pQCT quality control and graded the pQCT scans included in this study. I developed the statistical analysis plan together with the Stata analysis do files and I performed the statistical analysis, with support from my supervisors. I produced the tables of results and graphs, wrote the first draft of the paper, and led on all revisions.

7.2 Cover sheet

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RESEARCH PAPER COVER SHEET

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SECTION A – Student Details

Student ID Number	1806477	**Title**	Mrs
First Name(s)	Cynthia		
Surname/Family Name	Kahari		
Thesis Title	The effect of HIV and its treatment on trabecular and cortical bone architecture in children, adolescents and premenopausal women		
Primary Supervisor	Andrea Rehman/Melissa Neuman		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

SECTION B – Paper already published

Where was the work published?			
When was the work published?			
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion			
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SECTION C – Prepared for publication, but not yet published

Where is the work intended to be published?	Bone
Please list the paper's authors in the intended authorship order:	Cynthia Mukwasi-Kahari, Andrea M Rehman, Micheál Ó Breasail, Matthew Hamill, Ann Prentice, John M Pettifor, Lisa K Micklesfield, Rashida A. Ferrand, Celia L Gregson, Kate A Ward

Stage of publication	**Not yet submitted**

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	pQCT data for this manuscript was collected as part of the WBS study before I started my PhD but none of the pQCT data was graded, cleaned or analysed. I developed the specific research questions. I performed the pQCT quality control and graded the pQCT scans included in this study. I developed the statistical analysis plan together with the Stata analysis do files and I performed the statistical analysis, with support from my supervisors. I produced the tables of results and graphs, wrote the first draft of the paper, and led on all revisions.

SECTION E

Student Signature	[redacted]
Date	31 Jul 2023

Supervisor Signature	[redacted]
Date	15/8/2023

In premenopausal women, antiretroviral therapy initiation is associated with trabecular rather than cortical bone changes: a longitudinal study in South Africa

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7.3 Abstract

The aim of the current study was to determine whether HIV infection and initiation of ART were associated with change in bone architecture (density, geometry and strength) assessed by pQCT at two skeletal sites over a two-year follow-up period. Tibia and radius pQCT scans were performed in 98 women living without HIV (WLWOH) and 149 women living with HIV (WLWH) at 4 time-points (baseline, 6, 12 and 24 months). Participants were aged ≥18 years and were recruited from clinics in Soweto, Johannesburg, South Africa. Primary outcomes were tibial and radial 4% total and 4% trabecular vBMD measured in mg/cm^3 and 4% cross-sectional area (CSA, cm^2). Secondary outcomes were cortical vBMD, CSA, cortical thickness and stress strain index (SSI, mm^3) at the 66% radial and 38% tibial sites. Differences between WLWH and WLWOH were determined by comparing means using piecewise linear regression analysis with WLWH as the reference group, such that negative values mean that WLWH have lower values than those with HIV. Overall, the participants had a mean age of 33.7 (7.2) years, with WLWH being older than WLWOH. WLWOH had a higher BMI than WLWH. At the 4% radial and tibial sites, WLWH had lower total and trabecular BMD (g/cm^3) than WLWOH, even after adjusting for age, height and weight. At the distal radius, total density increased and total cross-sectional area decreased in the WLWOH (p=0.001), and the WLWH who had been initiated onto ART (p=0.004), but there was no significant changes in either of these measures in the WLWH before ART initiation. There was no evidence of change in cortical density at the radial site in WLWOH but after adjustment WLWH, before ART initiation, showed a 11.7 mg/cm^3 decrease in cortical density. However, WLWH before ART initiation, showed an increase in radial cross-sectional area at the 66% site (p=0.028) whereas WLWH after ART initiation and WLWOH showed a decline in radial 66% cross-sectional area (p<0.001) after adjustment. For the tibia, there was a decrease in 4% total vBMD in WLWH after ART initiation before and after adjusting for age, height and weight. No significant changes were seen at the 38% tibia. In conclusion, urban black South African women who are in their thirties have lower vBMD than their uninfected counterparts at both the radial and tibial trabecular rich sites but not at the cortical sites. Our longitudinal results show that WLWH who were initiated on ART lost total vBMD at the distal tibia but gained total vBMD at the distal radius, over the 24 months, which suggests that South African women who are in their thirties may still be gaining bone at the radius while bone accrual at the tibia is complete. Further studies which follow up women living with HIV for longer than 24 months are needed to confirm if the skeletal response to HIV infection and ART initiation may vary depending on whether it is trabecular or cortical bone and whether it is radial or tibial site as suggested in this study.

7.4 Introduction

The sub-Saharan African region, host to the highest number of adults living with HIV, has PLWH now living longer due to earlier ART initiation and better treatment access (1). Low DXA measured areal BMD has been commonly reported in adults living with HIV, mostly in high income countries (1–16). From high income countries, comparable studies performed in women living with HIV (WLWH) are few and most studies that have included women still have the larger number in the sample being men. The prevalence of HIV infection is higher in men than in women in high income countries, but higher in women than in men in sub-Saharan Africa, yet women are underrepresented in current available studies despite carrying a greater burden of HIV. An increasing body of studies arising from African countries is now including bone health in WLWH, (132,239,441,458–460,548,561,609,624). A more recent study in South African women showed that menopause related bone loss is greater in WLWH than women living without HIV (WLWOH), suggesting women with HIV may be at greater risk of osteoporotic fractures compared to their uninfected counterparts (458).

In addition to menopause, other important considerations in WLWH are the effects of pregnancy, lactation and hormonal contraception use on bone health (132,441,548). In a cross-sectional analysis of Ugandan WLWH, a higher prevalence of low BMD (Z-score ≤-2) was reported in WLWH reporting previous (12.7%) or current depot-medroxyprogesterone acetate (DMPA) use (20.3%) compared with WLWOH (2.9%) (548). Exposure to HIV and DMPA use were independently associated with lower mean BMD Z-scores at the lumbar spine, total femur and femur neck, with the greatest difference (5.6%-8.0%) between WLWH who were currently using DMPA and WLWOH who are not using DMPA (548). At 24 months follow up of the same Ugandan cohort, DMPA was found to accentuate bone loss associated with TDF initiation (561). In a Ugandan longitudinal cohort of women who were breastfeeding, WLWH and on ART containing TDF had similar reductions in spine BMD but greater hip BMD loss in the first 3 months of lactation and 2–3% lower recovery of BMD after they stopped breastfeeding than their uninfected counterparts who recovered fully (441). In a study conducted in Zimbabwe, Malawi, South Africa and Uganda, women with no prior TDF exposure were randomised during pregnancy to receive TDF-ART or their infant received nevirapine for prevention of HIV transmission via breastfeeding. Results showed that the mothers who received ART reported a 2% and 5.3% (spine and hip respectively) decline in BMD compared with mothers whose infants received nevirapine (132). From this evidence it is possible that WLWH enter menopause with a

deficit in bone, lose more bone mass as oestrogen levels diminish, and have a greater risk of fragility fracture in later life than WLWOH.

People with low bone mass are at an increased risk of fracture (625,626). People living with HIV have a higher fracture rate than their uninfected counterparts (483,490,552). However, BMD is not the only risk for fracture. Other architectural properties of bone contribute to strength, for example size, shape and distribution, and are therefore important to consider (130). At the macro-architectural level, bone consists of trabecular and cortical bone, with the human skeleton comprising 80% cortical bone and 20% trabecular bone. Trabecular bone has higher bone turnover than cortical bone (21). Parameters describing architecture include cortical thickness, moment of inertia, amongst other measures. Peripheral quantitative computed tomography (pQCT) has potential benefits including 3-dimensional measurement of bone, separation of cortical and trabecular bone, and measurement of geometry (267). It is unclear whether HIV infection and ART treatment affect the bone architecture in the same way.

Using DXA, several longitudinal studies have compared people living with HIV to their uninfected counterparts (133,518,530,535,604), with a noteable South African study showing that although similar at baseline the WLWH with a CD4 count low enough to be initiated on ART demonstrated significant declines in BMD of 2-3% over 12 months of starting ART, before and after body size adjustment, at both the femoral neck (FN) and lumbar spine (LS), compared to the WLWOH and WLWH who were not on ART (232,241). To our knowledge, there are no longitudinal studies examining the effects of HIV-infection and ART over time on pQCT measured bone density, size and strength in non-pregnant, non-lactating premenopausal women in sub-Saharan Africa.

The aim of the current study was to determine how HIV infection and initiation of ART affect cortical and trabecular bone architecture (density, geometry and strength) assessed by pQCT at two skeletal sites over a two-year follow-up period. The hypothesis was that over time, the decline in bone density, size and strength in WLWH who started ART treatment at baseline would be greater than those living without HIV, or not starting ART treatment. Further we hypothesised that any bone changes would initially be seen at the trabecular rich sites. Assessing how HIV and ART affect the trabecular and cortical compartments may provide a better understanding of bone loss and fracture risk in PLWH and hopefully inform clinical practice and treatment choices.

7.5 Methods

7.5.1 Study design, setting and sample

The Women's Bone Health study was a longitudinal study of black premenopausal South African women, aged ≥18 years, recruited from clinics in Soweto, Johannesburg, South Africa. Data were collected at 4 time-points (baseline, 6, 12 and 24 months) at the MRC/Wits Developmental Pathways for Health Research Unit (DPHRU) based at the Chris Hani Baragwanath hospital in Soweto. WBS recruited a total study sample of 98 women living without HIV (WLWOH) and 149 women living with HIV (WLWH), who were either living with HIV with a preserved CD4 count and anticipated not to require ART at baseline (PPres, n=75), or living with HIV with a CD4 count low enough for them to start ART just after the baseline visit (Plow, n=74). Premenopause was defined as regular (continuous) menses and ascertained by questionnaire. Women who were pregnant, breastfeeding, had an acute medical condition or any disease or currently using medicines known to affect bone metabolism were excluded from the study. All women with at least one pQCT scan were included in these analyses (n=220).

7.5.2 Study procedures

HIV-negative status was confirmed using the Determine™ rapid HIV-antibody test (Alere San Diego, Inc., San Diego, CA, USA). Height was measured to the nearest 0.1 cm, using a stadiometer (Holtain, Crosswell, UK) that was permanently installed on the wall. Weight was measured to the nearest 100 grams using a digital scale (Tanita TBF-410 MA Body Composition Analyzer, Tanita Corporation of America, Inc. in Illinois, United States of America), with only light clothing being worn. Body mass index (BMI) was determined by dividing a participant's weight in kilograms by the square of their height in meters (kg/m^2). BMI was classified as underweight (<18.5 kg/m^2), normal (18.5–24.9 kg/m^2), overweight (25–29.9 kg/m^2), and obese (≥30.0 kg/m^2) (627). Whole body less head (WBLH) scans were completed on dual energy x-ray absorptiometry (DXA) using a Hologic QDR 4500A DXA (model: Discovery W (S/N 71201) software version 12.5:7 Hologic Inc., Waltham, MA, USA) to determine lean mass (fat free soft tissue mass) and fat mass in kilograms. HIV-negative status was confirmed using the Determine™ rapid HIV-antibody test (Alere San Diego, Inc., San Diego, CA, USA). Height was measured to the nearest 0.1 cm, using a stadiometer (Holtain, Crosswell, UK) that was permanently installed on the wall. Weight was measured to the nearest 100 grams using a digital scale (Tanita TBF-410 MA Body Composition Analyzer, Tanita Corporation of America, Inc. in Illinois, United States of America), with only light clothing being worn. Body mass index (BMI) was determined by dividing a participant's weight in kilograms by the square of their height in meters (kg/m2). BMI was classified as underweight (<18.5 kg/m2), normal (18.5–24.9

kg/m2), overweight (25–29.9 kg/m2), and obese (≥30.0 kg/m2) (569). Whole body less head (WBLH) scans were completed on dual energy X-ray absorptiometry (DXA) using a Hologic Discovery QDR W 4500A DXA Hologic Inc., Waltham, MA, USA) to determine lean mass (fat free soft tissue mass) and fat mass. Questionnaire was used to assess current use of any supplements, vitamin D supplement use, calcium supplement use, current hormonal contraception use, smoking status, history of any fracture and history of fragility fracture. CD4 count was assessed but data on HIV viral load was not collected. Laboratory tests included urine phosphate creatinine (mmol/mmol) and urine calcium creatinine (mmol/mmol) and collection of blood measured by chemi-luminescent immunoassay (Liason) kit (DiaSorin Inc., Stillwater, MN, USA) to assess serum 25(OH)D (nmol/l), serum phosphate (mmol/l), serum calcium (mmol/l), serum total alkaline phosphatase (U/L).Blood was collected in the morning after an overnight fast by venepuncture and processed as EDTA plasma for parathyroid hormone (PTH) analysis and as serum for other analytes relating to calcium, phosphorus and vitamin D metabolism (calcium, phosphate, magnesium, albumin, 25(OH)D) and bone turnover (total alkaline phosphatase (TALP); bone alkaline phosphatase (BALP); serum type 1 procollagen N-terminal (P1NP); and serum collagen type 1 cross-linked β-C-telopeptide (β-CTX). All plasma and serum samples were stored frozen, initially at -20°C and subsequently at -80°C. Urine was collected into a sterile container at the second void of the day after an overnight fast acidified with concentrated hydrochloric acid and stored at -20°C.

7.5.3 pQCT acquisition

pQCT scans were performed for the non-dominant tibia and radius using an XCT 2000™ (Stratec Medizintecknik, Pforzheim, Germany). Radial length was measured from the olecranon to the distal edge of the ulnar styloid process whereas tibial length was measured from the distal medial malleolus to the tibial plateau. Radial and tibial length were measured with a metal ruler, to the nearest 0.5mm. The scan site was determined as a percentage of the radial/tibia length. On the scout view scan, the reference line was placed on the flattest part of the end plate for the radius or bisecting the medial border of the endplate for the tibia. Measurement sites were 4% and 66% for the radius and 4% and 38% for the tibia. Total and trabecular vBMD and total CSA was defined at the 4% sites using a 280 mg/cm^3 threshold, peel mode 1. Cortical vBMD was measured at the 38% (tibia) and 66% (radius) sites and total CSA were defined using a 710 mg/cm^3 threshold (with separation mode 1). Cortical thickness was calculated using a circular ring model. A phantom was scanned daily for quality assurance (QA). No participants were

repeated for reproducibility assessment for this study therefore it is not possible to state with confidence that the changes in pQCT bone outcomes observed in this study exceeded the least significant change. Previous studies at this center where the women reported in this study had their pQCT scans have reported precision for this center as a co-efficient of variation <1 % (287). Using the precision of 1% as an estimate, the expected least significant change in pQCT bone outcomes for the premenopausal women in this study was calculated as 1 × 2.77, resulting in an LSC of 2.77%. All pQCT scan slices and scout views were qualitatively graded by a single radiographer to assess validity for analysis. Scout view images were graded 0 to 2 based on whether the reference line positioning was (0) perfect, (1) too distal or proximal, or (2) out of range whereas movement artefacts were graded 0 to 3: 0) none, (1) slight, (2) medium streaking and (3) scan unusable. Grade 3 images were excluded. Based on this criteria, some of the 66% radius (n=13) slice scans and 38% tibia slice scans (n=16) were excluded from analysis.

7.5.4 Ethical considerations

Trained research assistants and/or a study nurse explained the study information to participants. Participants gave written informed consent. This study was approved by the Human Research Ethics Committee at the University of Witwatersrand (HREC number: M101525) and the Gauteng Department of Health in South Africa.

7.5.5 Statistical analysis

Statistical analyses were performed using Stata version 16.0 (Stata Corporation Inc., College Station, TX, USA). Data were cleaned and checked for consistency and outliers. Because pQCT measurements were added to the original protocol after recruitment had started, the baseline pQCT scan was defined as the first pQCT scan at either the visit month 0 (enrolment month) or visit month 6. Follow up visit scans were defined as pQCT scans performed at the one year (12 months) and two year (24 months) visits. Primary outcomes were tibial and radial 4% total and 4% trabecular vBMD measured in mg/cm^3 and 4% cross-sectional area (CSA, cm^2). Secondary outcomes were cortical vBMD, CSA, cortical thickness and stress strain index (SSI, mm^3) at the 66% radial and 38% tibial sites. For baseline characteristics, continuous outcomes were presented as means and standard deviations and compared by HIV status using independent-samples t-tests. Categorical data were presented as percentages and compared by HIV status using chi-squared tests. Box plots were used to plot raw data for each of the pQCT bone outcomes, at the 4% sites for radius and tibia, over the 24 months by HIV status and ART

group. We determined differences between WLWH and women without HIV, by comparing means using piecewise linear regression analysis with WLWH as the reference group, such that negative values mean that WLWH have lower values than those with HIV. Piecewise regression analysis using mixed effects linear models were used to generate mean differences and 95% confidence intervals to describe the variation in the mean of each of the pQCT bone outcomes (by HIV status) and in the change of each bone outcome per year within each group (by HIV and ART status) over the 24 months. Piecewise linear regression modelling was chosen because measurement times were not the same for all participants within each visit, participants did not have the same number of measures (missing data) and the time interval between consecutive measures varied (628–630). Piecewise regression modelling assumes that the outcome variable is modeled by one model when the exposure is below a pre-specified threshold value, and then by a different model when the exposure exceeds the threshold value (628–630). The assumptions of piecewise regression modelling are firstly that the threshold value is known (i.e. determined a priori), and secondly that the model type is constant, i.e the model uses linear both below and above the threshold, value (628–630). For this study, the threshold for the piecewise regression analysis was defined as the time at ART initiation. In addition, my exploratory analyses investigated each model as either linear, cubic or quadratic before deciding on a piecewise linear model. The model assumptions were checked by plotting the residuals and, either side of the threshold were normally distributed. We compared an unadjusted model with a model adjusting for a priori confounders; age (years), height (cm) and weight (kgs). Data are presented as mean difference (95% confidence intervals (CI))

7.6 Results

7.6.1 Participant characteristics

A total of 247 (WLWH; n=149, WLWOH; n=98) women were recruited into the study. Out of those 247, 220 (WLWOH; n=84, WLWH; n=136) women had at least one usable pQCT scan measurement (Figure 1). Of those participants with at least 1 pQCT scan, 94 (WLW0H; n=26, WLWH; n=68) had their baseline pQCT scan on visit month 0 and 126 participants (WLW0H; n=57, WLWH; n=69) had their baseline pQCT scan at the 6 month visit (Figure 1). 70% of the 67 WLWH who had their baseline scan at visit month 0 went on to be immediately initiated on ART just after enrolment and 29% of the WLWH who had their baseline scan at 6 months visit. By the 24th month, eighty five, i.e 61% of all the WLWH went on to be initiated on ART after the first visit 56 out of those 85, (40% of the WLWH) were initiated on ART before they had their first pQCT scan (Table 1).

Participants living with HIV who had a baseline scan at 6 months were similar in age, height, weight and BMI to participants living with HIV who had their baseline scan at visit month 0 (Supplementary table 1). Participants without HIV who had their baseline scan at visit month 0 were slightly taller but similar in age, weight and BMI to participants living without HIV who had their baseline scan at visit month 6. There were no differences in hormonal contraception use, smoking status, calcium supplementation use and vitamin D use between the participants with and without HIV who had their baseline pQCT scan at visit month 0 and visit month 6.

Baseline characteristics for all participants included in this study are presented in Table 1. Overall, the participants had a mean age of 33.7 (7.2) years, with WLWH being older than WLWOH. Although weight and height were not significantly different between the groups, WLWOH had a higher BMI. Most of the women were overweight or obese, regardless of their HIV status (66% in WLWOH and 57% in WLWH). Unlike lean mass which was not different between the HIV groups, fat mass was lower in WLWH than in WLWOH. Of all the women included in this analysis, 31% were on hormonal contraception (82% were on depot medroxyprogesterone acetate; DMPA) and there was no difference in hormonal contraceptive use between the groups. There were no differences in vitamin D status as measured by serum 25(OH)D (nmol/l) between groups with most subjects having a serum concentration >50 nmol/l. Serum phosphate, serum calcium and serum total alkaline phosphatase levels were also similar in WLWH and those without HIV (Table 1). Women living without HIV had a higher prevalence of fragility fracture (21% vs 7%: p=0.002) compared with WLWH. Of the WLWH who had at least one pQCT scan, 51% had a preserved CD4 count at enrolment compared with 49% who had a low CD4 count.

Overall, there were 126 and 92 women living with HIV at 12 months and 24 months follow up visits respectively. Reasons for loss to follow-up were that participants could not be contacted, had become pregnant/were lactating during follow-up or, amongst the group living without HIV, became HIV positive.

7.6.2 Baseline pQCT bone outcomes

Table 2 presents results for baseline pQCT bone outcomes for both the radial and tibial trabecular and cortical rich sites. Figure 2 is box plot presentation of raw data for each of the

pQCT bone outcomes, at the 4% sites for radius and tibia, over the 24 months by HIV status and when they were initiated on ART.

7.6.2.1 Radius

At the 4% (distal) site, women living with HIV had lower total and trabecular BMD (g/cm^3) than women living without HIV (Table 2). Adjusting for age, height and weight attenuated the differences but deficits remained especially for trabecular vBMD (-17.57mg/cm^3 (95% CI: -31.48, -3.67; p=0.014). No differences were detected in cross-sectional area between the groups. At the 66% (proximal) site there were no differences between the groups for any of the pQCT measures before or after adjustment. Before adjustment, WLWH had higher predicted bone strength than WLWOH but this was no longer significant when adjusting for age, height and weight.

7.6.2.2 Tibia

At the 4% (distal) tibia WLWH had lower total and trabecular density than WLWOH and this was robust to adjustment. There were no differences in CSA at either the 4% or 38% sites and cortical thickness and SSI at the 38% site were not different between WLWH and WLWOH. However, WLWH appeared to have higher cortical density than their uninfected counterparts both before and after adjustment (Table 2).

7.6.3 Change in pQCT bone outcomes over 24 months

7.6.3.1 Radius

Table 3 presents results for change in pQCT bone outcomes per year for both trabecular and cortical rich sites for the radius and tibia. At the trabecular rich site radius, total density increased by 8.83 mg/cm^3 (CI: 3.45, 14.22; p=0.001) in WLWOH and 7.30 mg/cm^3 (CI: 2.34, 12.25; p=0.004) in WLWH who had been initiated on ART. At the radial trabecular rich site, total cross-sectional area decreased by -7.38 mm^2 (CI: -13.79, -0.98; p=0.024) in WLWOH and by -7.17 mm^2 (CI: -12.91, -1.42; p=0.015) in WLWH who had been initiated on ART. There was no statistical evidence of change in trabecular density in any of the 3 groups of women and there were no significant changes in either of these measures in the WLWH before ART initiation (Table 3).

At the radial cortical rich site, there was no evidence of change in cortical density in WLWOH but in WLWH, before ART initiation, there was a 11.7 mg/cm^3 decrease in cortical density (CI: -23.07, -0.33; p=0.044). Before ART initiation, WLWH showed an increase in total cross-

sectional area at the cortical rich site [9.57 mm^2 (1.01, 18.12); p=0.028] whereas after ART initiation showed a decline in total cross-sectional area [-6.92mm^2 (-10.51, -3.33); p<0.001], after adjustment.

Given that the estimated LSC for this study was 2.77mg/cm3, the changes in total density and total cross-sectional area for the trabecular rich radial site in both WLWOH and WLWH (after ART initiation), exceeded the LSC. Similarly, the changes shown at the cortical rich sites also exceeded the LSC for cortical density and cross-sectional area.

7.6.3.2 Tibia

Changes were only seen at the 4% tibia site in the WLWH after ART initiation where there was a decrease in total BMD by -3.21 mg/cm^3 (CI: -5.61, -0.80; p=0.009), before and after adjusting for age, height and weight. Although total CSA increased in the WLWH after ART initiation this was no longer significant after adjustment. No significant changes were seen at the 38% tibia (Table 7.3).

7.7 Discussion

This is the first prospective study to measure the changes in bone density, geometry and estimated strength in premenopausal African living with and without HIV. In this group of urban, South African women, WLWH were older with lower fat mass than their uninfected counterparts. At baseline, trabecular density (radius & tibia) and total density (tibia) were lower in WLWH than in WLWOH but this was not the case for cortical density at the 38% tibia or 66% radius sites, suggesting that HIV infection may be affecting the trabecular rich sites more than the cortical rich sites. This is contrary to a previous report on DXA based results in this same cohort, which reported no baseline differences in areal BMD (g/cm^2) at any site before or after adjustment for age, BA, weight and height (232). The fact that baseline analysis of DXA measured aBMD did not reveal significant differences between WLWH and those without HIV could be due to the limitations of DXA as a method. DXA does not separate between trabecular and cortical bone and also does not account fully for body size. pQCT provides more bone compartment specific information than DXA and our analysis reports differences in trabecula but not cortical vBMD.

The WLWH who were initiated on ART lost total vBMD (combined trabecular and cortical bone) at the distal tibia but gained total vBMD at the distal radius, over the 24 months. In WLWH, after ART initiation, bone gain at the distal radius averaged 7.3mg/cm^3 whereas bone loss at the

distal tibia averaged 3.2 mg/cm^3 per year. The women in this study were premenopausal and in their early thirties. This is a period where bone loss is not normally expected. In addition, their average vBMD at baseline was lower than that of WLWOH at both the radius and tibia distal sites. Furthermore, WLWH did not show any decline in total or trabecular density before ART initiation, suggesting that the observed 1.1% decline in tibial distal vBMD in WLWH after being initiated on ART is a result of the ART initiation and not the presence of HIV. The BMD loss observed with ART initiation in WLWH is similar in magnitude to the BMD losses sustained during the first 2 years of menopause (566).

An earlier report of DXA measured aBMD in this same cohort of women, observed increases or no change in aBMD over 12 months in both the WLWOH and the WLWH who were not on ART (241). However, aBMD declined over 12 months, and then remained stable, in WLWH who were on ART despite an increase in body weight and improved CD4 count and serum albumin concentration (241). It is possible that the increase in body weight and improved CD4 count and serum albumin concentration, account for the stabilization of the aBMD loss. These findings suggest that the observed bone loss in WLWH after ART initiation was a result of exposure to ART rather than the HIV infection. Most of the women who were initiated on ART in this study were exposed to TDF. We have previously reported lower trabecular vBMD in children living with HIV who are exposed to TDF (631). Lower aBMD with exposure to TDF has also been shown in other studies in women at risk of HIV (557), in WLWH (548,561) and in studies including both men and women living with HIV (553,621).

The increase in distal radius bone density in both WLWH after ART initiation and their uninfected counterparts suggests that the women in this study, who were still in their thirties (mean age 33±3 years), were still accruing bone at the distal radius but not the tibia. It is widely believed that bone accrual occurs throughout childhood, is accelerated during puberty and peaks in early adulthood before plateauing (149,198,632). However, there are authors that have argued that in some populations, bone accrual continues into the thirties (633).

As would be expected in a young adult population, we report no height increases in both groups of women in this study, over the 24 months period. However, there was a decline in distal total cross-sectional area at the radial site in WLWOH and WLWH after ART initiation in this study. This possibly suggests continued bone remodeling at the radius even after longitudinal growth has ceased, where the newly formed bone at the metaphysis is remodeled into the cortical shaft

to create the characteristic long bone shape; a process, which has been described as inwaisting (251,287,634). Alternatively increasing mineral accrual, or reducing bone turnover, may have reduced the partial volume effect on the quantification of bone tissue in the image. This may create an artefactual decrease in bone size, but it is of note, this hasn't been observed in other adult populations.

There were no differences in serum 25(OH)D concentrations, phosphate, calcium and alkaline phosphatase between WLWH and those without, suggesting that HIV and ART may not be associated with compromised vitamin D, calcium or phosphate. However, the mean serum Vitamin D levels in both WLWH and WLWOH were above 50 nmol/l. Although serum Vitamin D levels of 50 nmol/l are regarded as adequate for good skeletal health in healthy adults [24], literature is not exactly clear on the optimum levels of 25(OH)D within the context of HIV and ART or whether or not they should be different or similar to healthy adults.

Few studies have reported pQCT bone outcomes in premenopausal WLWH. A cross-sectional study comparing 151 WLWH to a reference group of 263 WLWOH in USA reported lower cortical vBMD but higher periosteal and endosteal circumference in WLWH than those without HIV (617). We found that the distal radial total and trabecular BMD together with distal trabecular vBMD but not cortical vBMD at both the radius and tibia were declining in WLWH who were initiated on ART. Because vBMD is related to bone strength, the net effect of this decline in vBMD would be to decrease bone strength and therefore increase fracture risk. We did not show any changes in predicted bone strength in WLWH, after ART initiation in this study, possibly due to small sample size and short period of follow up (24 months). It might be possible that WLWH who are on ART may over the years, be at higher risk of fragility fractures at trabecular rich sites. We recommend longitudinal studies with a longer follow up of e.g. 10 years in WLWH to understand the long term consequences of HIV and ART.

In this study, fractures were more common in women living without HIV than in women living with HIV. This contradicts evidence from the literature which supports the fact that fractures are more common in WLWH than those without, as discussed in chapter 6 of this thesis, though most of this evidence is from high income countries. The number of participants living with HIV in this study was almost double that of participants living without HIV. Fracture prevalence has not been studies extensively in people in sub-Saharan Africa, therefore it is not easy to tell if the proportion with fracture amongst both groups is higher or lower than what should be expected.

Since both women living with and without HIV in this study were recruited from the same clinic, it is possible that the selection of this study's sample was biased towards those with health concerns that could have affected their bone outcomes. Questionnaire variables assessing clinical history were self-reported by the participants. It is also possible that recall bias may have affected the results.

7.8 Strengths and Limitations

The longitudinal design and the inclusion of a comparison group of WLWOH are strengths of this study. At the time of this study, no women were pregnant or breastfeeding and this is a strength as it excludes possible confounding by pregnancy and lactation. There was no difference in use of hormonal contraception between WLWH and WLWOH and this means that use of hormonal contraception is unlikely to explain the differences in vBMD at the trabecular rich sites in this study. However, this study is limited by the small numbers and the absence of data on HIV viral load and the duration of HIV infection before enrolment into this study. The limited number of participants who were still in the study by 24 months and the fact that WLWH who were on ART, were not exposed uniformly to ART at the same time and for the same period, are limitations of this study as well. In addition, we had no data on socio-economic status. Women included in this study were from an urban population in Soweto Johannesburg and the data may not be generalizable to other populations. This study was also limited by the fact that some of the women who had their first pQCT measurement at 6 months had already started ART. The majority (70%) of the WLWH who had their baseline scan at month 0 and 29% of the WLWH who had their baseline scan at the 6 month visit were immediately initiated on ART. This difference is by design and the way ART treatment was initiated at the time of the study rather than a true difference in the groups regarding how their ART was started. Data collection for this study was also conducted before the change in guidelines for ART policy in South Africa was made. No participants were repeated for reproducibility assessment for this study therefore an assessment of whether the changes in pQCT bone outcomes observed in this study exceeded the least significant change was based on previous reported %CV at the same center where the women reported in this study had their pQCT scans (287).

7.9 Conclusion

In summary, this study suggests that urban black South African women who are in their thirties have lower vBMD than their uninfected counterparts at both the radial and tibial trabecular rich sites but not at the cortical sites. Our longitudinal results show that WLWH who were initiated on ART lost total vBMD at the distal tibia but gained total vBMD at the distal radius, over the 24

months, which suggests that South African women who are in their thirties may still be gaining bone at the radius while bone accrual at the tibia is complete. Furthermore, that the skeletal response to HIV infection and ART initiation may vary depending on whether it is trabecular or cortical bone and whether it is radial or tibial site. Finally, this also suggests that decline in bone in WLWH is more likely due to ART initiation than due to HIV infection. Given that trabecular rich sites are a common site for fractures e.g. Colles's fracture on the distal radius and that premenopausal women are likely going to be on ART for many years, we recommend further studies to assess the effect of ART on trabecular rich sites over a longer period of time.

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Conflicts of interest

There are no conflicts of interest.

Author contributions

Conception; MMH, LKM, KAW and AP. Design: MMH, KAW, AP and MMH. Data curation: CM-K, AP, KAW, AMR, MO'B. Formal analysis: CM-K, AMR. Interpretation: CM-K, AMR, KW, CLG. Writing- Original draft: CM-K, AMR, KAW and CLG. Writing- Review & editing: CM-K, AMR, MO'B, LKM, JMP, RAF, AP, KAW and CLG. Supervision: AMR, LSC, LKM, RAF, KAW and CLG. Project administration: CM-K, MMH, JMP, AP, KAW and CLG. All authors take responsibility for their contributions as outlined above. All authors contributed to interpretation and the writing of the manuscript.

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Table 7.1 : Comparison of characteristics between women living with and without HIV at baseline

	WLWOH (n=84)	WLWH (n=136)	p-value
Age, yrs, mean(SD)	30.66 (8.18)	33.67 (6.31)	0.002
Age group, %			<0.001
<30yrs	45 (54%)	44 (32%)	
>30yrs	38 (46%)	95 (68%)	
Weight, kg, mean(SD)	71.30 (16.64)	67.11 (15.41)	0.058
Height, cm, mean(SD)	15.81 (0.55)	15.93 (0.57)	0.121
Body mass index, kg/cm²	28.59 (6.73)	26.42 (5.79)	0.012
Body mass index classification, n (%)			0.400
Underweight	4 (5%)	6 (4%)	
Normal weight	24 (29%)	54 (39%)	
Overweight	26 (31%)	43 (31%)	
Obese	29 (35%)	36 (26%)	
Fat mass, kg, mean(SD)	27.14 (11.37)	23.53 (9.88)	0.014
Lean mass, kg, mean(SD)	41.72 (6.08)	40.19 (6.05)	0.070
Percentage fat mass, mean(SD)	38.83 (7.64)	36.11 (8.27)	0.015
Current use of any supplements, %	7 (8%)	67 (49%)	<0.001
Vitamin D supplement use, %	1 (1%)	2 (1%)	0.89
Calcium supplement use, %	3 (4%)	0 (0%)	0.025
Current hormonal contraception use, %	28 (34%)	40 (29%)	0.46
Smoking status, %	4 (5%)	14 (10%)	0.16
History of any fracture, %	22 (27%)	27 (20%)	0.23
History of fragility fracture, %	17 (21%)	9 (7%)	0.002
CD4 count, cells/µl, Mean (SD)		282.27 (150.48)	
CD4 count classification at enrolment, %			
Preserved CD4 count		71 (51%)	
Low CD4 count		68 (49%)	
Art initiation by end of study, %		85 (61%)	
ART initiation before first pQCT scan, %		56 (40%)	
Serum 25(OH)D, nmol/l, Mean(SD)	50.94 (15.99)	52.36 (20.34)	0.59
Serum Phosphate, mmol/l, Mean (SD)	1.19 (0.19)	1.19 (0.20)	0.96
Serum Calciumcorr, mmol/l, Mean (SD)	2.55 (0.11)	2.56 (0.14)	0.95
Serum Total alkaline phosphatase, U/L, Mean (SD)	53.58 (25.95)	52.33 (21.11)	0.70
Urine Phosphate:Cre (mmol/mmol), Mean(SD)	1.19 (0.66)	1.28 (0.83)	0.47
Urine Calcium:Cre (mmol/mmol), Mean(SD)	0.10 (0.10)	0.12 (0.12)	0.35

WLWH; Women living with HIV, WLWOH; Women living without HIV

Table 7.2: Comparison of pQCT bone outcomes in premenopausal women living with and without HIV at baseline

Radius (n=220)	**Unadjusted**		**Adjusted***	
4% site	**MD (95% CI)**	**p-value**	**MD (95% CI)**	**p-value**
4% Total Density, mg/cm³	-16.81 (-34.38 , 0.76)	0.061	-13.89 (-32.46 , 4.67)	0.142
4% Trabecular Density, mg/cm³	-23.29 (-36.60 , -9.97)	0.001	**-17.57 (-31.48 , -3.67)**	**0.014**
4% Total CSA, mg/cm³	3.31 (-12.95 , 19.57)	0.689	-0.04 (-16.71 , 16.62)	0.996
66% site				
66% Cortical Density, mg/cm³	4.21 (-9.15 , 17.58)	0.535	0.18 (-13.79 , 14.15)	0.980
66% Total CSA, mg/cm³	-0.72 (-10.04 , 8.61)	0.880	-0.09 (-9.22 , 9.04)	0.984
66% Cortical thickness, mg/cm³	-0.024 (-0.116 , 0.068)	0.609	-0.045 (-0.140 , 0.051)	0.355
66% SSI, mg/cm³	17.35 (0.89 , 33.81)	0.039	12.50 (-4.63 , 29.64)	0.152
Tibia (n=220)	**Unadjusted**		**Adjusted**	
4% site	**MD (95% CI)**	**p-value**	**MD (95% CI)**	**p-value**
Total Density, mg/cm³	-25.82 (-39.15 , -12.48)	<0.001	**-16.58 (-29.87 , -3.28)**	**0.015**
Trabecular Density, mg/cm³	-21.48 (-32.66 , -10.30)	<0.001	**-12.59 (-23.50 , -1.69)**	**0.024**
4% Total CSA, mg/cm³	36.41 (-0.70 , 73.52)	0.054	25.99 (-10.11 , 62.10)	0.157
38% site				
Cortical Density, mg/cm³	12.15 (5.63 , 18.68)	<0.001	**8.07 (1.36 , 14.78)**	**0.019**
38% Total CSA, mg/cm³	1.01 (-18.29 , 20.30)	0.918	2.87 (-13.61 , 19.36)	0.732
38% Cortical thickness, mg/cm³	0.042 (-0.112 , 0.196)	0.592	0.010 (-0.148 , 0.168)	0.901
38% SSI, mm³	33.10 (-45.69 , 111.88)	0.409	1.76 (-69.75 , 73.26)	0.961

MD (95% CI); Mean differences (95% confidence interval), with WLWH as the reference group, such that negative values mean that WLWH have lower values than those without HIV

****Adjusted for age (years), height (cm) and weight (kgs)***

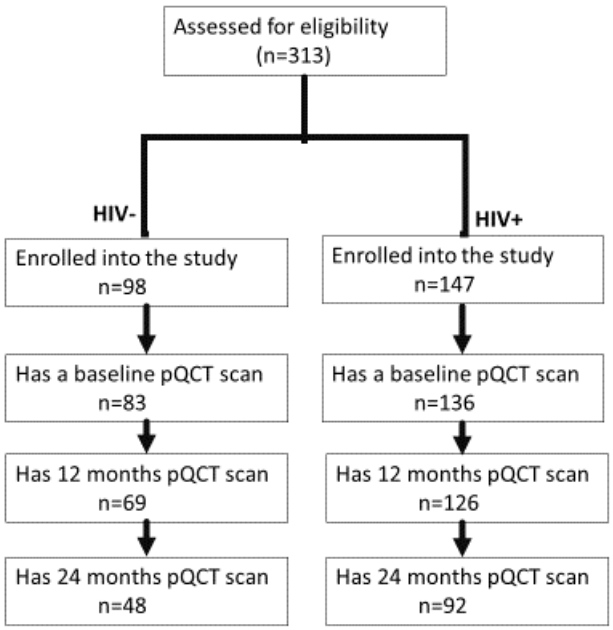
CSA; Cross-sectional area

Table 7.3 : Change in pQCT bone outcomes per year in premenopausal women living with HIV, before and after ART initiation and premenopausal women living without HIV per year

Radius	**Unadjusted**		**Adjusted***	
Women living without HIV	**MD (95% CI)**	**p-value**	**MD (95% CI)**	**p-value**
Δ 4% Total Density, mg/cm³	7.96 (2.81, 13.11)	0.003	**8.83 (3.45, 14.22)**	**0.001**
Δ 4% Trabecular Density, mg/cm³	1.41 (-1.61, 4.42)	0.359	1.79 (-1.41, 4.99)	0.273
Δ 4% Total CSA, mg/cm³	-6.75 (-13.00, -0.49)	0.035	**-7.38 (-13.79, -0.98)**	**0.024**
Δ 38% Cortical Density, mg/cm³	3.17 (-1.65, 7.99)	0.196	3.47 (-1.49, 8.43)	0.170
Δ 38% Total CSA, mg/cm³	-3.46 (-7.48, 0.55)	0.090	**-4.65 (-8.74, -0.57)**	**0.026**
Δ 38% Cortical thickness, mg/cm³	0.025 (-0.010, 0.059)	0.161	0.035 (-0.000, 0.071)	0.053
Δ 38% SSI, mm³	3.61 (-4.34, 11.55)	0.372	3.17 (-4.93, 11.27)	0.442
WLWH, before ART initiation	**MD (95% CI)**	**p-value**	**MD (95% CI)**	**p-value**
Δ 4% Total Density, mg/cm³	3.13 (-9.17, 15.43)	0.617	3.01 (-10.06, 16.09)	0.651
Δ 4% Trabecular Density, mg/cm³	-3.29 (-10.75, 4.18)	0.387	-1.23 (-9.17, 6.72)	0.761
Δ 4% Total CSA, mg/cm³	5.73 (-8.19, 19.65)	0.419	7.28 (-7.25, 21.81)	0.325
Δ 38% Cortical Density, mg/cm³	-7.32 (-18.11, 3.46)	0.183	**-11.70 (-23.07, -0.33)**	**0.044**
Δ 38% Total CSA, mg/cm³	8.38 (-0.04, 16.79)	0.051	**9.57 (1.01, 18.12)**	**0.028**
Δ 38% Cortical thickness, mg/cm³	-0.032 (-0.109, 0.044)	0.407	-0.045 (-0.125, 0.036)	0.275
Δ 38% SSI, mm³	8.77 (-6.87, 24.41)	0.271	9.02 (-7.39, 25.43)	0.280
WLWH, after ART initiation	**MD (95% CI)**	**p-value**	**MD (95% CI)**	**p-value**
Δ 4% Total Density, mg/cm³	6.05 (1.45, 10.64)	0.010	**7.30 (2.34, 12.25)**	**0.004**
Δ 4% Trabecular Density, mg/cm³	1.26 (-1.46, 3.97)	0.363	1.27 (-1.73, 4.27)	0.407
Δ 4% Total CSA, mg/cm³	-5.95 (-11.43, -0.46)	0.034	**-7.17 (-12.91, -1.42)**	**0.015**
Δ 38% Cortical Density, mg/cm³	3.69 (-0.51, 7.90)	0.085	4.09 (-0.37, 8.56)	0.072
Δ 38% Total CSA, mg/cm³	-5.96 (-9.42, -2.50)	0.001	**-6.92 (-10.51, -3.33)**	**<0.001**
Δ 38% Cortical thickness, mg/cm³	0.035 (0.005, 0.065)	0.023	**0.045 (0.013, 0.076)**	**0.006**
Δ 38% SSI, mm³	-3.15 (-9.92, 3.63)	0.361	-3.16 (-10.22, 3.90)	0.379
Tibia	**Unadjusted**		**Adjusted**	
Women living without HIV	**MD (95% CI)**	**p-value**	**MD (95% CI)**	**p-value**
Δ 4% Total Density, mg/cm³	-2.28 (-4.59, 0.03)	0.053	-1.50 (-3.99, 0.99)	0.238
Δ 4% Trabecular Density, mg/cm³	0.71 (-1.69, 3.10)	0.561	1.54 (-0.73, 3.82)	0.183
Δ 4% Total CSA, mg/cm³	9.11 (-1.13, 19.35)	0.081	8.41 (-1.62, 18.44)	0.100
Δ 66% Cortical Density, mg/cm³	1.18 (-0.89, 3.24)	0.263	0.71 (-1.40, 2.81)	0.510
Δ 66% Total CSA, mg/cm³	2.56 (-3.23, 8.36)	0.949	0.19 (-5.79, 6.17)	0.280
Δ 66% Cortical thickness, mg/cm³	-0.005 (-0.048, 0.039)	0.837	-0.003 (-0.044, 0.039)	0.906
Δ 66% SSI, mm³	-17.45 (-43.21, 8.30)	0.183	-20.46 (-47.20, 6.28)	0.133
WLWH, before ART initiation	**MD (95% CI)**	**p-value**	**MD (95% CI)**	**p-value**
Δ 4% Total Density, mg/cm³	0.11 (-5.97, 6.20)	0.970	-0.10 (-6.56, 6.35)	0.975
Δ 4% Trabecular Density, mg/cm³	-1.70 (-7.90, 4.50)	0.591	-1.08 (-7.00, 4.85)	0.721
Δ 4% Total CSA, mg/cm³	-2.51 (-28.17, 23.15)	0.847	-1.38 (-27.02, 24.26)	0.916
Δ 66% Cortical Density, mg/cm³	1.99 (-2.99, 6.97)	0.433	2.03 (-3.21, 7.27)	0.447
Δ 66% Total CSA, mg/cm³	-1.60 (-15.74, 12.55)	0.824	4.01 (-10.32, 18.35)	0.582
Δ 66% Cortical thickness, mg/cm³	0.025 (-0.083, 0.132)	0.655	0.082 (-0.025, 0.188)	0.132
Δ 66% SSI, mm³	50.32 (-11.36, 112.01)	0.109	58.37 (-5.10, 121.83)	0.071
WLWH, after ART initiation	**MD (95% CI)**	**p-value**	**MD (95% CI)**	**p-value**
Δ 4% Total Density, mg/cm³	-3.00 (-5.11, -0.88)	0.006	**-3.21 (-5.61, -0.80)**	**0.009**
Δ 4% Trabecular Density, mg/cm³	0.83 (-1.35, 3.01)	0.456	-0.16 (-2.35, 2.02)	0.884
Δ 4% Total CSA, mg/cm³	11.92 (2.70, 21.14)	0.011	7.96 (-1.46, 17.38)	0.098
Δ 66% Cortical Density, mg/cm³	-0.30 (-2.14, 1.55)	0.752	-0.04 (-1.99, 1.91)	0.966
Δ 66% Total CSA, mg/cm³	-0.86 (-6.06, 4.34)	0.745	-5.39 (-10.84, 0.05)	0.052
Δ 66% Cortical thickness, mg/cm³	-0.025 (-0.064, 0.015)	0.221	-0.008 (-0.047, 0.032)	0.705
Δ 66% SSI, mm³	-18.36 (-41.32, 4.60)	0.117	-17.33 (-41.57, 6.90)	0.160

MD (95% CI); Mean differences (95% confidence interval), with baseline pQCT outcome value as the reference, such that negative values mean lower pQCT outcome values after a year than at baseline
****Adjusted for age, height and weight***
CSA; Cross-sectional area

Figure 7.1: Flow diagram to show participants who had a pQCT scan at each visit



HIV+; living with HIV, HIV-; living without HIV

Figure 7.2: pQCT bone outcome values for premenopausal women living with and without HIV grouped by HIV status and when they were initiated on ART

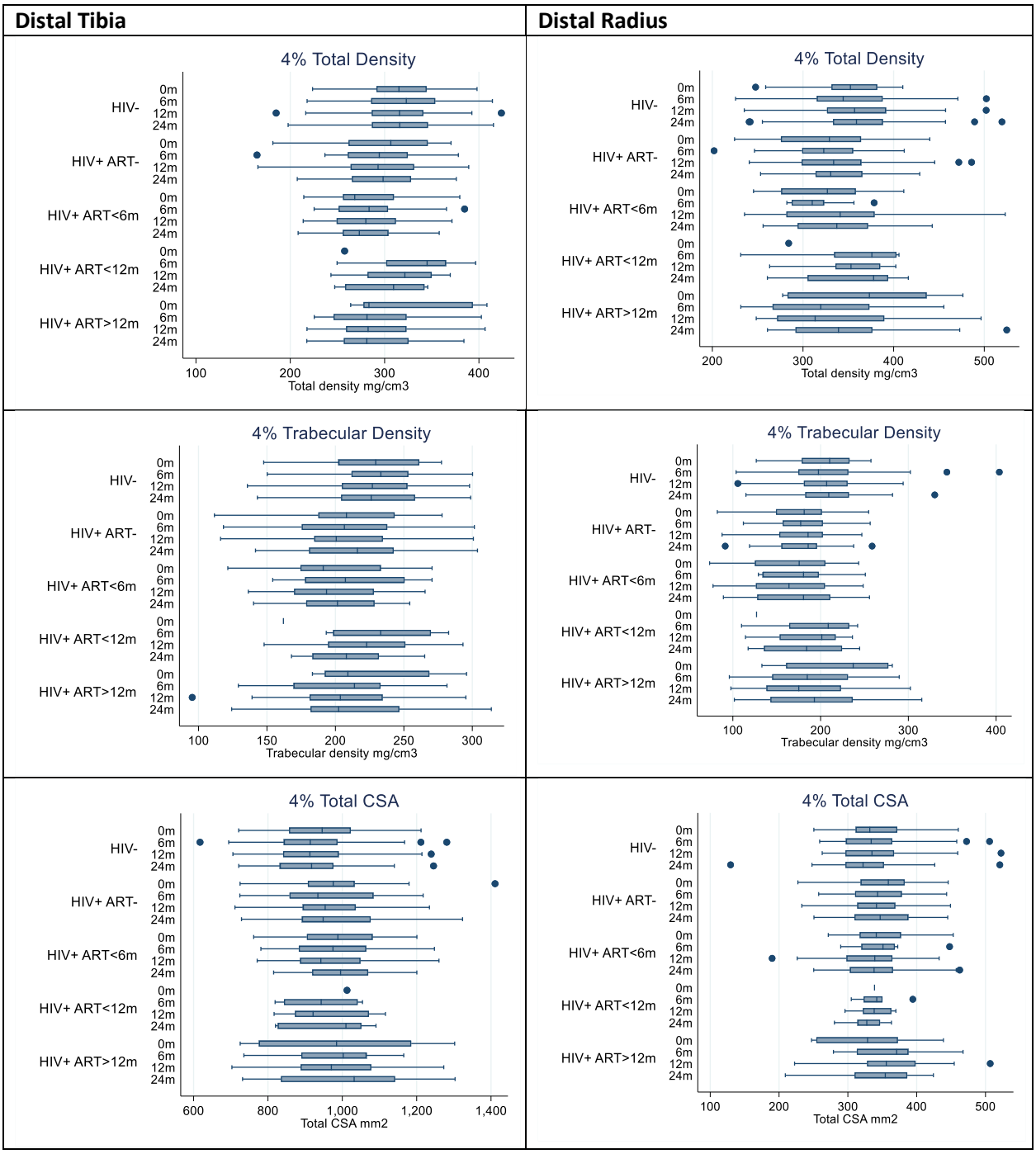


Figure 7.2 shows box plots of raw data for each of the pQCT bone outcomes, at the 4% sites for radius and tibia, over the 24 months by HIV status and when they were initiated on ART.

Supplementary Table 7.1: Comparison of baseline characteristics in women living with and without HIV stratified by when they had their baseline pQCT scan

		WLWOH (n=83)			WLWH (n=136)		
		Baseline scan			Baseline scan		
Baseline Characteristics		**Visit 6 (n=57)**	**Visit 0 (n=26)**	**p-value**	**Visit 6 (n=69)**	**Visit 0 (n=67)**	**p-value**
Continuous Variables		**Mean (SD)**	**Mean (SD)**		**Mean (SD)**	**Mean (SD)**	
Age, years		30.6 (8.4)	31.1 (8.0)	0.778	34.3 (6.6)	33.1 (6.0)	0.249
Height, cm		156.9 (5.2)	160.1 (5.7)	0.007	158.9 (5.9)	160.0 (5.5)	0.288
Weight, kg		71.3 (17.2)	71.4 (15.9)	0.987	69.2 (15.8)	65.6 (15.0)	0.177
Body Mass Index, kg/m²		29.0 (7.0)	27.7 (6.2)	0.435	27.4 (6.1)	25.6 (5.4)	0.069
Fat Mass, kg		28.1(12.0)	25.5 (10.1)	0.355	24.9 (9.6)	22.4 (10.0)	0.139
Lean Mass, kg		42.3 (6.0)	40.4 (6.3)	0.215	42.7 (5.5)	37.8 (5.6)	<0.001
Percentage Fat Mass, %		39.5 (8.1)	37.4 (6.6)	0.243	36.9 (7.7)	35.6 (8.7)	0.363
Categorical Variables		**Frequency (%)**	**Frequency (%)**	**p-value**	**Frequency (%)**	**Frequency (%)**	**p-value**
Hormonal Contraception (%)	No	37 (66.1)	17 (65.4)	0.951	49 (72.1)	47 (70.1)	0.807
	Yes	19 (33.9)	9 (34.6)		19 (27.9)	20 (29.9)	
Smoking status (%)	No	53 (94.6)	26 (100.0)	0.229	61 (89.7)	60 (89.6)	0.977
	Yes	3 (5.4)	0 (0.0)		7 (10.3)	7 (10.4)	
Calcium Supplements (%)	No	55 (98.2)	24 (92.3)	0.185	68 (100.0)	67 (100.0)	N/A
	Yes	1 (1.8)	2 (7.7)		0	0	
Vitamin D use (%)	No	56 (100.0)	24 (96.0)	0.132	68 (100.0)	65 (97.0)	0.151
	Yes	0	1 (4.0)		0	2 (3.0)	

WLWH; Women living with HIV, WLWOH; Women living without HIV

8 CHAPTER 8: Discussion

8.1 Introduction

The main aim of this thesis was to determine the effect of HIV infection on pQCT measured bone architecture. I have reported results from two cohort studies in sub-Saharan Africa, children from Harare, Zimbabwe and premenopausal women from Johannesburg, South Africa, with a focus on outcomes measured by pQCT. In Chapter 4, these pQCT measured bone outcomes in CWH were compared to those in CWOH. One potentially important source of variation was found to be pubertal development, meaning that CWH and CWOH of the same chronological age are not necessarily at the same stage of skeletal development. Chapter 5 contains comparisons of annualized change in pQCT bone outcomes in CWH and CWOH. The extent to which longitudinal growth impairment explains any detrimental effects of HIV on pQCT-assessed bone parameters was also determined. Chapter 7 contains a comparison of change in pQCT bone measures over 24 months in WLWH, before and after initiating ART and WLWOH.

In children, the results demonstrate that CWH show bone deficits at follow up and longitudinal growth impairment mediates the effect of HIV on these bone changes in pQCT bone outcomes, particularly in females. This suggests that CWH were already pre-set on a lower bone accrual trajectory than CWOH (from earlier in childhood). Future studies of bone development in PLWH will require an understanding of the effect of HIV from birth until peak bone mass is attained in PLWH in sub-Saharan Africa. Studies are needed to confirm whether or not children are set at a lower trajectory in early childhood as indicated in the current work, and whether or not they continue to grow and catchup on peak bone accrual, though at older age than their uninfected counterparts. In WLWH, the results demonstrate that urban black South African women living with HIV have lower vBMD than their uninfected counterparts at both the radial and tibial trabecular rich sites. Furthermore, I have demonstrated that the skeletal response to HIV infection and ART initiation may vary depending on whether the site is primarily trabecular or cortical bone, and whether it is the radial or tibial site. Finally, our results also confirm that decline in bone in WLWH is more likely due to ART initiation than due to HIV infection. Given that trabecular rich sites are a common site for fractures e.g. Colles's fracture on the distal radius and that premenopausal women are likely going to be on ART for many years, I recommend further studies to assess the effect of ART on trabecular rich sites, including lateral vertebral assessments, over a longer period of time and whether this is associated with an increase in fracture risk.

To date, much of the available literature on bone in PLWH focuses on DXA measured aBMD. However, DXA utilizes two-dimensional planar imaging and therefore yields an aBMD measure (not vBMD). Bone density is determined by the degree to which a radiation beam is attenuated by a bone. The attenuation of a radiation beam not only depends on the physical density, but also on the size of the bone i.e the length of the path that the beam takes across the bone (27). In estimation of bone density by 2-dimensional imaging e.g. by DXA, small bones will have a lower aBMD than large bones, even if the physical density is the same. So sometimes a low aBMD value can just mean that a bone is small with normal volumetric BMD (27). This is an issue in children, especially if there is stunting. In an attempt to address this, DXA measurements can be size adjusted, yielding LS-BMAD TBLH-BMC^{LBM} (28). As described in chapter 2, pQCT offers an opportunity to better understand the skeletal system by measuring bone size and bone strength in addition to volumetric bone density (255,257–259). pQCT provides a detailed assessment of cortical and trabecular bone compartments and therefore can increase our understanding of how shape and organisation of bone contribute to fracture risk over and above areal BMD measured by DXA. Two major considerations which shaped my PhD thesis were that, firstly, many studies have been performed using DXA. Secondly, most studies have been performed in high income countries were the population is different from that in sub-Saharan Africa, in terms of lifestyle, socio-economics, and diet, yet HIV is a major health burden mostly in sub-Saharan Africa. An explanation for the differences in bone accrual between PLWH and those without is one of the major gaps in the existing literature. For children included in this study, my secondary questions were chosen based on factors I thought would explain the differences in pQCT measured bone outcomes between CWH and CWOH.

8.2 Statistical approach

My statistical analysis was constructed to compare data obtained in people living with and without HIV. To account for missing data, assumed missing at random, I used multiple imputation by chained equations (with 7 imputed datasets), allowing for imputation of categorical and continuous data simultaneously. The method of multiple imputation by chained equations first calculates a regression on a random draw taken from the observed data without missing data for estimating each missing value of a certain variable, which is then replaced by the estimated value plus some random error. It then switches to the next variable. The procedure is repeated m times using different random draws to result in m datasets in which all missing data have been imputed. In this study, the number of datasets, m, was 7.

Only 5.3% (n=33 out of 609) participants had a missing pQCT outcome at baseline, hence the use of multiple imputation and this is the data that is presented in chapter 4. I did not use multiple imputation for the follow up analysis which is presented in chapter 5 as that would have meant imputing 20% of participants (n=123 out of 609) who had a missing pQCT outcome at either baseline or follow up. There are no clear rules or guidelines from literature as to how much missing data can be substituted but other authors have recommended not substituting more than 10% and this was used as the rough guide in this study (635). Multiple imputation by chained equations was used in chapter 4 because the amount of missing data was minimal and less than 10% at baseline as compared to the amount of missing data at follow up which was greater than 10%. Similarly, missing pQCT bone outcome data for the women whose results are presented in chapter 7, also exceeded 10% of the total dataset and therefore I did not use multiple imputation for that reason.

I used multivariable linear regression to test for associations between HIV status and pQCT bone outcomes, stratified by sex and puberty (Tanner 1-2 vs. 3-5), adjusting for age, height, fat mass, physical activity, socio-economics and orphanhood.

Furthermore, I used linear regression to estimate differences in mean and annualised change in (∆) pQCT bone density (trabecular and cortical), size (total cross-sectional area [CSA]) and strength (SSI) between CWH and CWOH; adjusting for socio-economic status (SES) and orphanhood and incorporating an interaction term for baseline pubertal status (Tanner 1-2 [pre/early] vs 3-5 [mid/late]). Instead of using change in pQCT outcomes from baseline to follow up, I reported annualised change in pQCT outcomes which is calculated as the pQCT bone outcomes at follow up minus the pQCT bone outcome at baseline, divided by number of days between the baseline and follow up visits and multiplied by 365.25. I chose this outcome to account for different follow up times because CWH were followed up for longer than CWOH (Table 5.1). This means that I considered only the change in pQCT values which occurred over a follow up of exactly 365 days for both CWH and CWOH. In a sensitivity analysis, I investigated modelling change in bone outcomes adjusted for duration of follow-up, (using bone outcome at visit 1 as the outcome, adjusted for bone outcome at visit 0 and length of the time interval) and my findings were not substantially changed.

I used structural equation modelling to test whether baseline height-for-age-Z-scores (HAZ) mediate the effect of HIV on ∆bone outcomes. This mediation analysis was conducted to

partition the total effect of HIV infection on the pQCT bone measures into a direct effect of HIV infection, not acting through HAZ and an indirect effect acting through height Z-for age scores (mediator). I hypothesized that some proportion of the effect of HIV infection on bone might be explained by effects of stunting experienced by CWH. Mediation analysis in this context requires modeling changes in bone outcomes as a function of HIV infection, repeating this model further adjusting for the mediator, and separately modeling the mediator as a function of HIV infection (636,637). I used coefficients corresponding to the HIV infection effects without and with adjustment for the mediators to estimate, respectively, the total effect and direct effect of HIV infection (637–639). I then presented the coefficients for the direct effects, indirect effects and total effects. Furthermore, I presented results in terms of the proportion mediated, calculated as the ratio between the indirect and the total effect. All statistical models were further adjusted for socio-economic status and orphanhood. Traditional mediation analysis relies on meeting the causal steps criteria as before conducting tests on the indirect. Instead of the traditional approach, I chose a recent structural equation modelling approach by Andrew F. Hayes (449) which demonstrated that it is the test of the indirect effect that matters, not the test on the individual paths in the model. This approach does not draw conclusions about the indirect effect based on testing the total effect but allows for indirect effects to be quantified, with a confidence interval constructed. Using this approach, I have shown that it is possible for HIV to exert an effect on e.g. trabecular vBMD indirectly through a mediator (HAZ) even if one cannot establish through a hypothesis test that the total effect is different from zero. Nevertheless, evidence of mediation was detected for females only and not in male CWH.

In WLWH, I used piecewise regression analysis using mixed effects linear models to compare means and to generate mean differences and 95% confidence intervals in bone parameters (baseline and mean change per year), by HIV and ART status. I compared an unadjusted model with a model adjusting for a priori confounders; age (years), height (cm) and weight (kgs). I chose piecewise linear regression modelling because measurement times were not exactly the same for all participants within each visit, participants did not have the same number of measures (missing data), the time interval between consecutive measures varied and participants who were initiated on ART were all not initiated at the same time. I divided the time period into before and after ART initiation segments and then estimated separate slopes and intercepts in each segment before carrying out statistical tests of before and after ART initiation.

8.3 The IMVASK study cross-sectional analysis

The IMVASK study data offered me the opportunity to explore and report results from the largest study to date to use pQCT in a sample of children and adolescents growing up with HIV infection in SSA. My thesis shows that in Zimbabwe, CWH have deficits in both bone size and strength, compared with children who do not have HIV. At baseline, these deficits included a 6% lower diaphyseal bone size in female CWH in the latter stages of puberty, even after accounting for body size and other confounders. The cortical thickness of male CWH who were in the pre- or early-pubertal stage was larger than that of male CWOH. In females, the cortical thickness was greater in CWH than in CWOH, irrespective of whether or not they had yet reached puberty. It is possible that increased cortical thickness contributed to the higher estimated bone strength that I found. Before or throughout the early stages of puberty, it would appear that female CWH have higher bone strength than CWOH. It is unclear whether this is in part due to the higher cortical density they showed at this pubertal stage.

In spite of the fact that CWH are on average shorter and lighter than CWOH, my findings may indicate that CWH who are in the pre- or early-puberty have sufficient bone strength to compensate for their smaller stature, biasing the SSI measurement. However, as they continue to get taller, the residual impaired bone size leads to deficits in predicted bone strength. As a result, a strength deficit begins to manifest in the middle to late stages of puberty. These findings are concerning since pubertal growth throughout adolescence is an important time of skeletal development, and deficiencies in bone strength that become apparent during later puberty are likely to continue into adulthood, which has repercussions for the risk of future fracture.

In this study, at baseline, CWH were more likely to be in the earlier Tanner stages than CWOH. This is similar to a recent study in Nigeria, of 11 to 19 year old female CWH who were reported to be of earlier Tanner stages than their uninfected counterparts (410). Timing of peak bone accrual is closely related to pubertal development. Delayed puberty is associated with a lower PBM (210). A study conducted comparing growth and puberty in Nigerian CWH and CWOH reported delayed pubertal development (as demonstrated by breast and pubic hair stages of sexual maturation) and increase in mean age at menarche of 1.4 years (from 11.8 years in the HIV uninfected to 13.2 years) among CWH. This is similar to findings from other sub-Saharan African countries. It is also similar to findings in studies assessing growth and puberty form high income countries. In our study is per findings from similar studies from within and outside the African continent (393,410–413). Other studies have suggested that pubertal delay in CWH is

caused by underlying malnutrition, chronic inflammation and opportunistic infections. In South African CWH, the duration and magnitude of the pubertal growth spurt was later than the WHO reference population, with the age at PHV in girls being delayed by about 12 months (PHV=5.9 cm/year), while in boys the age at PHV was delayed by 24 months (PHV=5.7 cm/year) (393). Evidence from high income settings (USA) shows that in addition to delayed pubertal onset, CWH who had an HIV-1 RNA viral load >10,000 copies/mL (vs ≤10,000 copies/mL) or a CD4% <15% (vs ≥15%) had 4-13 months later pubertal onset (411). In an earlier study of CWH in Italy, pubertal onset was delayed by 2 years in females and 1 year in male CWH (413).

This is not the first study to report poorer socioeconomic status and higher orphan status amongst CWH compared with their uninfected counterparts. Similar findings were reported in Nigerian CWH living in Lagos, though the paper assessed growth and pubertal delay only and bone outcomes were not included (410)

To date, only two studies have reported using pQCT in CWH (238,343). Our results are consistent with findings from a smaller cross-sectional pQCT study in South Africa of younger children (7 to 14 years), which suggested CWH have smaller bone size and lower predicted bone strength than CWOH, although this association was not adjusted for fat mass, physical activity or any indicator of SES. Our larger study was able to both stratify by pubertal stage and adjust for a number of relevant confounders and showed, for the first time, how differences in size and strength becomes more pronounced in later puberty. A second longitudinal Canadian study of 9 to 18 year old CWH with diverse ethnic backgrounds, reported no change in pQCT bone outcomes over 2 years (343). However, this study was small (n=31 CWH) across sex and ethnic strata, suggesting insufficient power to detect changes over time. Although notably, as seen in our study, Canadian CWH in early puberty had higher cortical BMD compared to CWOH (343). The authors cited decreased intra-cortical remodeling due to either ART or low physical activity as a possible explanation for higher cortical BMD in CWH (343). I reported lower physical activity levels in CWH than CWOH in this cross-sectional analysis. However, it is unclear whether the lower physical activity levels are resulting in reduced intra-cortical remodeling and therefore an increased cortical BMD in this particular cohort and this needs further investigation.

In addition, it may not be unusual that I have reported higher cortical vBMD in CWH who are in the early stages of puberty as this could have been due to partial volume effects. There is a tendency to underestimate the actual cortical vBMD measurement as a result of the fact that voxels at the periosteal and endocortical borders of the cortex are not completely filled with

bone (the partial volume effect). The extent of this underestimation is greater in thinner cortices such as those at the 4% site or in bones at the 38% site that have grown rapidly but not filled in bone mineral, because they have a higher surface to volume ratio than thicker cortices. Consequently, even if the mass of the mineral per unit volume of the cortical compartment is identical, pQCT measured cortical vBMD increases with cortical thickness (271). This bias that is also accentuated in smaller bones therefore in smaller children, cortical vBMD may seem to increase with cortical thickness despite there being no change in material density (278,279)

This is the first study to use pQCT to examine the association of TDF use on bone architecture in CWH. Our previous DXA findings in the same Zimbabwean IMVASK cohort identified a strong association between TDF use and bone deficits, particularly affecting TBLH-BMC^{LBM}, such that children exposed to TDF for four or more years had on average a 0.5 SD lower TBLH-BMC^{LBM} Z-Score compared with CWH who had not received TDF (229). This effect size is clinically important, as a 0.5 SD reduction in bone density is associated with an increase of 50% in both childhood and, if sustained, future adult fracture risk (229). Even in early life, fetal exposure to TDF used as either part of maternal combination ART or as prophylaxis against mother to child transmission has also been associated with lower bone outcomes (355). The potential mechanisms for adverse bone effects for TDF are not yet clear. Some authors have suggested that TDF directly affects bone formation by affecting osteoblast proliferation and increasing apoptosis (102,103), whereas others suggest that it induces functional vitamin D deficiency (356). In this PhD, after accounting for multiple confounders, I have not demonstrated any negative effect of TDF on cortical bone density, bone size and bone strength. I have however shown that TDF use is particularly associated with lower trabecular bone density in males. Whether this translates to increased fracture risk at trabecular rich skeletal sites, such as the wrist and vertebra, is currently unknown. While ART has allowed CWH to live decades longer than before, evidence of compromised bone health in childhood means that CWH may be at a high risk of fractures later in life, although it is not yet clear if fractures in childhood are rare or not, due to scarcity of studies assessing fractures in CWH.

Optimizing bone health in CWH is crucial to minimize the risk of fractures in later adulthood. Vitamin D is the main regulator of calcium homeostasis and mineralization, which gives bone mass and strength. Though in this PhD I have reported similar dietary intake of vitamin D and calcium in CWH and CWOH other authors have reported that inadequate intake of vitamin D and vitamin D deficiency are highly prevalent in CWH (337,338). In South Africa, a 2-year vitamin D and calcium supplementation randomized placebo controlled trial in 6 to 16 year olds

(n=56) did not report improvements in bone mass despite achieving adequacy in plasma concentrations of 25-OH D among those CWH who were receiving supplementation (349,395). However, their small sample size could have meant the study was not powered enough to detect changes within the 2 years of follow up. Interventions for optimizing bone health in childhood may include ensuring sufficient calcium and vitamin D intake, encouraging weight-bearing exercise, and minimizing modifiable risk factors. Periodically, 25(OH)D concentrations may also be measured and supplemented as necessary, although this is not currently possible in Zimbabwe. Longitudinal research (with longer follow up periods) assessing bone health and novel interventions to minimize bone loss are urgently needed in the population of CWH.

My pQCT results further extend our previous DXA findings in the same Zimbabwean cohort, where we found a higher prevalence of low TBLH-BMC^{LBM} Z-score, a cortical rich region of interest, (10% vs. 6% p=0.066) and LS-BMAD Z-score, a trabecular rich site (14% vs. 6%; p=0.0007) in CWH compared with their uninfected counterparts (229). Further earlier work in a smaller slightly younger (6 to 16 years) Zimbabwean population identified similar prevalence of low LS-BMAD and TBLH-BMC^{LBM} in CWH (15% and 13% respectively, compared to 1% and 3% in those without HIV) (230). While DXA measured areal BMD does not differentiate trabecular and cortical compartments, the lower LS-BMAD previously reported indicates the possibility of a greater deficit in trabecular bone. This aligns with my unadjusted analysis that showed that trabecular vBMD and not cortical density was lower in CWH than in CWOH. However, this is inconsistent with the adjusted analysis in this PhD, as I have report no differences in both trabecular and cortical density in CWH and CWOH after full adjustment.

Pubertal delay is both a common consequence of HIV infection (229) and a risk factor for poor bone growth (439). Hence, as expected, a greater number of CWH were pre-pubertal, than CWOH. Pubertal delay could be the reason for compromised bone accrual in CWH. In our study male CWH in mid/late puberty demonstrated the most adverse skeletal profile. Without stratifying by pre/early vs. mid/late puberty, important interactions between HIV and pubertal stage would have been missed in this PhD analysis. The importance of stratifying by puberty was also demonstrated by a cross-sectional study in the US of 7 to 24 year olds, which similarly reported an HIV*puberty interaction on DXA measured spine and total body bone mineral content and density; the lower bone mass in participants with HIV was more pronounced with advancing puberty (330). However, in this study the differences were more evident in males, whereas in my PhD study, I demonstrated differences in both males and females. It is not clear

to what extent the difference in the source population (*e.g*. US vs. Zimbabwe), study sampling (frequency matched based on Tanner stage not age) and/or the age studied, might account for these different findings (330).

It is not known why CWH who are in the early Tanner stages in this study had higher cortical vBMD and higher cortical thickness than their uninfected counterparts and therefore I can only speculate. It may not be unusual that I have reported higher cortical vBMD in CWH who are in the early stages of puberty as this could have been due to partial volume effects. There is a tendency to underestimate the actual cortical vBMD measurement as a result of the fact that voxels at the periosteal and endocortical borders of the cortex are not completely filled with bone (the partial volume effect). The extent of this underestimation is greater in thinner cortices such as those at the 4% site or in bones at the 38% site that have grown rapidly but not filled in bone mineral, because they have a higher surface to volume ratio than thicker cortices. Consequently, even if the mass of the mineral per unit volume of the cortical compartment is identical, pQCT measured cortical vBMD increases with cortical thickness (271). This bias that is also accentuated in smaller bones therefore in smaller children, cortical vBMD may seem to increase with cortical thickness despite there being no change in material density (278,279)

Since CWH were established on ART, it is also possible that the ART therapy they were receiving resulted in increased cortical thickness compared with their uninfected counterparts, though this may need to be further investigated. Evidence from a Japanese study in 1314 children who were 12 and 18 years of age showed that increases in cortical thickness occurred earlier in girls than in boys. It is possible that both female CWH and CWOH had increased cortical thickness earlier than the males. However, since CWH were shorter in height than CWOH, which implies shorter bones, the cortical thickness was probably adequate in proportion to their height. It is also possible that the increased cortical thickness we reported in CWH who are in the early stages of puberty could have been due to the validity issues surrounding the calculation of cortical thickness using the circular ring model in pQCT. A study assessing the validity of pQCT measured tibial cortical measurements in 15 dry human tibia specimens showed that cortical thickness measures derived using the circular ring model are overestimated, with the overestimation increasing with the thickness of the cortex. Resolution in pQCT is low and possibly results in particle volume averaging which occurs when a single voxel contains tissues of different densities, such as the boundary between bone tissue and soft tissue, and therefore the attenuation coefficient assigned is some middle ground between the 2.

Partial volume averaging is another possible explanation for overestimation of cortical thickness by pQCT though this may not necessarily explain the difference between CWH and CWOH in this study.

Our results suggesting females living with HIV particularly may be entering adulthood with bone size deficits is a concern. Women often experience periods of bone loss, *e.g*., pregnancy (440), lactation (441) and menopause . Lactation which is a known risk factor for bone loss in African women (441,442), with 83% of Zimbabwean women continuing to breastfeed beyond a 1 year post-partum (443). Coupled with HIV infection and ongoing ART, the risk of adult osteoporosis is increased. Recent data from rural South Africa identified a 37% prevalence of femoral neck osteoporosis in women age ≥ 50 years living with HIV (240). Understanding fracture risk in African women living with HIV is an important research priority.

8.4 The IMVASK study follow up analysis

This is the first study to describe longitudinal changes in detailed pQCT measured bone parameters in CWH. These findings suggests that CWH have consistently lower trabecular bone density, bone size and bone strength than CWOH over one year of follow-up. This analysis included 419 participants, out of an initial of 609 participants at baseline. Missing data may have may have resulted in bias as it resulted in a smaller sample size. Our findings suggest that in children aged 8 -16 years, puberty modifies the effect of HIV on bone growth, such that in the later stages of puberty both male and female CWH increase their bone size more than CWOH, although at the end of follow-up, bones size remains smaller in CWH. Furthermore, findings from this PhD have demonstrated that, in females, changes over one year in bone size, density and strength are partly explained by baseline height-for-age, suggesting 3-dimensional bone growth is proportionate to linear growth in females.

There was greater linear growth over the one-year of this study in CWH than CWOH. We have previously reported pubertal delay in the same cohort (229) and other studies have shown height is strongly determined by pubertal development (450). Pubertal transition is classically characterised by a ‘growth spurt’ (*i.e.* peak height velocity). The differences in height change in our cohort could be that more CWH than CWOH, were still undergoing pubertal transition from early to late puberty.

Despite that male CWH who were in the later stages of puberty gained more bone size at the 4% site than CWOH, but still had lower mean bone size than CWOH at the follow up visit. These results could be suggesting that though CWH demonstrate some kind of catch up growth as they transition through puberty, the catch up growth may not be adequate for them to be

comparable to CWOH. These findings are concerning, since deficits in bone accrual at the end of puberty are likely to persist into adulthood.

I have demonstrated that bone size, bone strength and cortical density, especially in females, changes more as a result of deficits in linear growth due to HIV. I have also shown that CWH still had greater bone deficits at follow up despite the greater linear growth over the follow up period. The sum of the proportion mediated may exceed 100% if there are other mediators with a negative proportion mediated (451). The fact that the calculated ratio mediated proportions yielded percentages exceeding 100% suggests that there are other pathways (that operate through other mediators) that affect the 4% trabecular density, 38% tibial CSA and predicted strength in opposite direction from height for age Z-scores.

To date, only one other study has assessed longitudinal changes in pQCT bone outcomes in CWH. In Canadian, 9 to 18 year olds, CWH from diverse ethnic backgrounds (described earlier), no change in pQCT bone outcomes over 2 years was reported (38), probably because the study was underpowered (n=31 CWH). Our findings show no difference in change in annualized pQCT bone outcomes between CWH and CWOH, despite demonstrating lower bone outcomes in CWH than in CWOH at both baseline and follow up. Bone formation occurs at a faster rate before the age of 4 years, and during puberty than in any other time of one's life. Our cohort were enrolled into the study at minimum age of 8 years. Bone studies using pQCT to assess bone within the context of HIV, in the early years (before 8 years), are not available.

ART initiation is one of the strategies meant to avoid or minimise growth impairment in CWH and early ART initiation is one of the measures to prevent growth impairment. All of the CWH in our IMVASK cohort were established on ART for at least 2 years before enrolment. In spite of this, I observed lower bone measures than in CWOH at both baseline and follow up analysis. Some authors have demonstrated that established growth impairment can continue despite the initiation of ART in CWH (410,412). Longitudinal data, over more than 1 year are also not available. This makes it unclear whether or not the lower bone density size, and strength observed in CWH in this PhD study is as a result of factors influencing bone in the early years of life and also makes it unclear if CWH will eventually catchup growth with their uninfected counterparts.

Since more CWH were still in the early Tanner stages compared with CWOH, it means more CWH had not yet entered their pubertal growth spurt. Bone deficits may be magnified during the pubertal years, resulting in a failure to reach optimal peak bone mass and therefore increasing

adult fracture risk. This is important given that fracture rates in CWH have been shown by Mirani et al. to increase with increasing age (420). Bone health concern in CWH is similar to other long-term health concerns for CWH, such as cardiovascular disease, renal disease, and neurocognitive impairment, where childhood clinical problems may not appear until adulthood. This population of perinatally infected CWH needs long-term bone health monitoring.

Childhood HIV infection can manifest as stunting (poor linear growth). An extensive literature has established a higher prevalence of stunting and underweight in CWH than in CWOH (3,4,378–381), which has been confirmed by my cross-sectional results and our earlier studies in Zimbabwean children (3,230). By adjusting for body size however, the analysis in this PhD has shown that at baseline, the lower bone size in female CWH, especially in later puberty, is independent of lower height and weight. At follow up, my PhD findings persistently show a higher prevalence of stunting in CWH than in CWOH for both males [33% (CWH) vs 7% (CWOH)] and females [26% (CWH) vs 10% (CWOH)]. This is similar to several studies that have assessed growth patterns in African countries which have consistently reported high prevalence of stunting as measured by height for age Z-scores in CWH (3,4,378–383). In Zimbabwe CWH have higher odds of stunting; eight times greater in those who have acquired HIV in utero, and four times greater in those who have acquired HIV around the time of birth (384). Improvements in growth have been reported in CWH who have an early HIV diagnosis and less severe HIV symptoms or who start ART early (389). However, in Africa, CWH start ART later in life than other parts of the world, often when the disease is already in a more advanced stage, potentially affecting growth (388). Studies are needed to examine the pattern of growth in later adolescence and timing of the acquisition of peak bone mass in CWH to assess whether deficits are likely to continue through adulthood and increase their risk of fracture. In addition to height and/stunting being associated with BMD, lean mass has been reported to have a positive effect BMD (195). Health interventions are also needed to improve both height and weight outcomes in CWH so as to avoid stunting and underweight. The growing skeleton responds to mechanical loading from e.g. lean muscle mass contraction and physical activity. According to Wolff's law and the 'mechanostat' theory, bone is withdrawn from skeletal places where mechanical stresses are low and added to those where demands are high, since load dictates bone formation and form follows function (197). However, people with the same BMI may have widely diverse body compositions, the effect of fat accumulation on bone throughout growth is not clearly understood.

Putting the results together, I hypothesise that the trajectory of skeletal growth for CWH has been preset to be at a lower trajectory than in CWOH, before the age of 8 years or before 4 years when on average these children start antiretroviral therapy, potentially in the first thousand days of life, (Figure 2). By the time the CWH enter the cohort in this PhD study (at the age of 8 years), they are already at a trajectory of impaired growth such that absolute values of density, size and strength are lower than CWOH. As they transition through puberty, it appears that the development of density and strength runs in parallel to CWOH, meaning they are growing at the same rate but the set point has been predetermined earlier in life. By the time they leave our cohort, there are persistent deficits in some of the parameters of bone. It does look like size may exhibit some evidence of catchup growth, as linear growth accelerates during puberty. Whether or not, that is sufficient to enable CWH to get back on the trajectory for size they would have been, had they not had HIV, is unknown. Moreover, when those children are reaching the end of puberty and peak bone mass is achieved, it is unclear whether children with HIV continue to grow for a longer period of time, to an older age than CWOH, such that they do achieve the same peak bone mass at a later age. Studies are needed to examine the acquisition of peak bone mass in CWH, around the age of 20 to assess whether there has been catchup growth in skeletal development or whether a lower peak bone mass is achieved and that is likely to continue through to adulthood.

8.5 The WBS study longitudinal analysis

To the best of my knowledge, this is the first study to measure radial and tibial vBMD, together with geometric bone outcomes in premenopausal African WLWH. The aim of this study was to determine the effect of HIV and ART on pQCT-measured bone architecture in WLWH compared to WLWOH. In this group of urban, South African women, WLWH were older with lower fat mass than their uninfected counterparts. At baseline, trabecular density (radius & tibia) and total density (tibia) were lower in WLWH than in WLWOH but this was not the case for cortical density at the 38% tibia or 66% radius sites, suggesting that HIV infection may be affecting the trabecular rich sites more than the cortical rich sites. This is contrary to a previous report on DXA based results in this same cohort, which reported no baseline differences in areal BMD (g/cm^2) at any site before or after adjustment for age, BA, weight and height (232). The fact that baseline analysis of DXA measured aBMD did not reveal significant differences between WLWH and those without HIV could be due to the limitations of DXA as a method. DXA does not separate between trabecular and cortical bone and also does not account fully for body size. pQCT provides more bone compartment specific information than DXA and our analysis reports differences in trabecular but not cortical vBMD.

This is the first prospective study comparing pQCT measured bone outcome changes over a period of 24 months in WLWH in Southern Africa. The WLWH who were initiated on ART lost total vBMD (combined trabecular and cortical bone) at the tibia but gained total vBMD at the radius, over the 24 months. In WLWH, after ART initiation, bone gain at the distal radius averaged 7.3mg/cm^3 whereas bone loss at the distal tibia averaged 3.2 mg/cm^3 per year. The women in this study were premenopausal and in their early thirties. This is a period where bone loss is not normally expected. In addition, their average vBMD at baseline was lower than that of WLWOH at both the radius and tibia distal sites. Furthermore, WLWH did not show any decline in total or trabecular density before ART initiation, suggesting that the observed 1.1% decline in distal vBMD in WLWH after being initiated on ART is a result of the ART initiation and not the presence of HIV. This loss is similar in magnitude to the BMD losses sustained during the first 2 years of menopause (566)

An earlier report of DXA measured aBMD in this same cohort of women, observed increases or no change in aBMD over 12 months in both the WLWOH and the WLWH who were not on ART (241). However, aBMD declined over 12 months, and then remained stable, in WLWH who were on ART despite an increase in body weight and improved CD4 count and serum albumin concentration (241). It is possible that the increase in body weight and improved CD4 count and serum albumin concentration, account for the stabilization of the bone loss. These findings suggest that the observed bone loss in WLWH after ART initiation was a result of exposure to ART rather than the HIV infection. Most of the women who were initiated on ART in this study were exposed to TDF. Lower bone density with exposure to TDF has also been shown in other studies in women at risk of HIV (557), in women living with HIV (548,561) and in studies including both men and women living with HIV (553,621).

Both WLWH, after ART initiation and their uninfected counterparts showed evidence of gaining bone at the distal radius. It is widely believed that bone accrual occurs throughout childhood, is accelerated during puberty and peaks in early adulthood before plateauing (149,198,632). However, there are authors that have argued that in some populations, bone accrual continues into the thirties. The mean age at enrolment in this study was 33±3 years. In addition to some of the participants being below 30 years of age, it is possible that the participants in this study were still accruing bone at the distal radius but not the distal tibia. A study which followed up South African children over 10 years demonstrated that black South African young adults continued to have increases in trabecular density at the radius and small decreases in bone cross-sectional area at that site, also that the observations did demonstrate some site

differences between the radius and tibia, which would be consistent with the current study (287). Changes in density and bone area were continuing up to 8 years post peak height velocity.

As would be expected in a young adult population, there were no height increases in both groups of women in this study, over the 24 months period. However, there was a decline in distal total cross-sectional area in WLWOH and WLWH, after ART initiation in this study. This possibly suggests continued bone remodeling even after longitudinal growth has ceased, where the newly formed bone at the metaphysis is remodeled into the cortical shaft to create the characteristic long bone shape; a process, which has been described as inwaisting (251,287,634). Alternatively increasing mineral accrual, or reducing bone turnover, may have reduced the partial volume effect on the quantification of bone tissue in the image. This may create an artefactual decrease in bone size, but it is of note, this hasn't been observed in other adult populations.

There were no differences in serum 25(OH)D concentrations between WLWH and those without, suggesting that HIV and ART may not be associated with compromised vitamin D status. The mean serum 25(OH)D levels in both WLWH and WLWOH were above 50 nmol/l. Although serum 25(OH)D levels of 50 nmol/l are regarded as adequate for good skeletal health in healthy adults [24], literature is not exactly clear on the optimum levels of 25(OH)D within the context of HIV and ART or whether or not they should be different or similar to healthy adults.

The long term precision of pQCT may vary depending on several factors such as the specific pQCT device used, the anatomical site being measured, the population being studied, the duration between scans and the expertise of the person operating the pQCT scanner (289,307,640). The same pQCT scanner was used for all women in WBS study throughout the duration of the study and this improves long term precision of the study. The daily manufacturer recommended quality assurance (QA) was used to ensure regular calibration and maintenance of the pQCT scanner used in this study. This is important for obtaining reliable pQCT results over the course of the study. Studies have shown that pQCT scanning machines have good long-term reproducibility. Ideally, a calculation of coefficient of variation and least significant change indicate better long-term precision. However, no participants were repeated for precision assessment in the WBS study, limiting the interpretation of these results in the context of the precision of pQCT scanner operators.

Few studies have reported pQCT bone outcomes in premenopausal WLWH. A cross-sectional study comparing 151 WLWH to a reference of 263 WLWOH in USA reported lower cortical

vBMD but higher periosteal and endosteal circumference in WLWH than those without (617). Findings from this PhD show that the distal radial total and trabecular BMD together with distal trabecular vBMD but not cortical vBMD at both the radius and tibia were declining in WLWH who were initiated on ART. Because vBMD is related to bone strength, the net effect of this decline in vBMD would be to decrease bone strength and therefore increase fracture risk. I was not able to demonstrate lower predicted bone strength in WLWH, after ART initiation in this study, possibly due to small sample size and short period of follow up (24months). It might be possible that WLWH who are on ART may over the years, be at higher risk of fragility fractures at trabecular rich sites but this needs further investigation. I recommend longitudinal studies with a longer follow up of e.g. 10 years in WLWH to understand the long term consequences of HIV and ART.

8.6 Strengths and limitations

The major strength of this study is the novel use of pQCT in a large population of children and premenopausal women living with HIV from two sub-Saharan African populations. Having both a child and adult population is a strength as it allowed me to assess the effect of HIV in both children who were perinatally infected and in women who were not perinatally infected with HIV. The comparison of CWH with CWOH and the comparison of WLWH with WLWOH is a strength together with the fact that both the child and adult women population were followed up and assessed more than once. The study sample of children from Harare included in this thesis is likely representative of children living in Harare and that is a strength.

For the baseline analysis of children from the IMVASK study, there were several limitations. The analysis is cross-sectional therefore I cannot infer causality.

Further, pQCT is not as widely studied as DXA, is currently being utilised in research and not in clinical practice, and does not have many standard reference databases for healthy populations. This limits my ability to relate my findings to routine clinical practice. I am unable to compare findings from this PhD to normative pQCT reference data as there are no established normative pQCT data for children living in sub-Saharan Africa.

The study was not powered to stratify by pubertal stage, therefore because males appeared to be transitioning through puberty more slowly than females, the study may not have included sufficient males at more advanced stages of puberty.

To stratify by pubertal status with children grouped into 2 groups, i.e Tanner 1 and 2 vs Tanner 3, 4 and 5. Children in Tanner stage 1 are pre-pubertal whereas children in Tanner stage 2 are

in the early stage of puberty. In addition, a Tanner stage 3 child is likely to be in early or peripubertal stage whereas a child in Tanner stage 5 is classified as post-pubertal. For both the baseline and follow up analysis, my ability to test for interaction was limited by small numbers within Tanner stage categories, as I needed to group participants in different pubertal stages based on numbers with each stage, rather than pubertal biology. Further follow-up may be helpful in assessing both the male and female children further.

For the follow up analysis in CWH, participants with missing data were excluded to allow structural equation modelling analysis, thereby limiting the number of participants and potentially reducing power in this study. A potential limitation of this study arises due to confounding, a phenomenon which exists whenever there is a third variable that is associated with two variables and therefore partially explains the relationship between them. Confounding is a threat to the validity of mediation by structural equation modelling as it can bias the mediated effect if any of these relations are confounded and no adjustment is made for the confounders. In this thesis, I found that height impairment mediated the effect of HIV on pQCT bone outcomes in females. Given that height was associated with pQCT bone outcomes, I do infer that the more a participant's height the higher their pQCT bone outcome values. It is possible that there could be another variable, or confounder, that affects both height and pQCT bone outcomes, which was either not measured or adjusted for in this analysis. This means that height and pQCT bone outcomes may appear to be positively related when they are only related because they are both affected by a confounder. Additionally, confounders may affect the relationship between height and HIV and between HIV and pQCT bone outcomes.

Follow-up of longer than one year would provide further important insights into trajectories of bone growth.

Another limitation in this study is that I did not measure, assess or include a group of children who are HIV exposed but uninfected (CHEU). Reassuringly, new data suggest that HEU infants exposed to TDF in utero may not experience the same bone toxicity as their HIV-infected counterparts.

The longitudinal analysis of women from the WBS study in South Africa did not include women who were pregnant and breastfeeding. This was a strength as other authors have demonstrated that pregnancy and breastfeeding affect bone density (132,441). In a Ugandan longitudinal cohort of women who were breastfeeding, WLWH and on ART containing TDF had similar reductions in spine BMD but greater hip BMD loss in the first 3 months of lactation and 2–3%

lower recovery of BMD after they stopped breastfeeding than their uninfected counterparts who recovered fully (441). In a study conducted in women from Zimbabwe, Malawi, South Africa and Uganda, women with no prior TDF exposure were randomised during pregnancy to receive TDF-ART or their infant received nevirapine for prevention of HIV transmission via breastfeeding. Results showed that the mothers who received ART had a 2% and 5.3% (spine and hip, respectively) decline in BMD compared with mothers whose infants received nevirapine (132). My analysis of premenopausal women was also limited by the small numbers and the absence of data on HIV viral load and the duration of HIV infection before enrolment into this study. The limited number of participants who were still in the study by 24 months and the fact that WLWH who were on ART, were not exposed uniformly to ART at the same time and for the same period, are limitations of this study as well. In addition, there was no data on socio-economic status for the premenopausal women included in this PhD. Women included in this study were from an urban population in Soweto, Johannesburg and the data may not be generalizable to other populations. This study was also limited by the fact that some of the women at 6 months had already started ART before their first pQCT measurement. 70% of the 67 WLWH who had their baseline scan at visit month 0 went on to be immediately initiated on ART just after enrolment and 29% of the WLWH who had their baseline scan at 6 months visit. This difference is by design and the way ART treatment was initiated at the time of the study rather than a true difference in the groups. Data collection for this study was also conducted before the change in guidelines for ART policy in South Africa was made. The previous ART guidelines required a person to be initiated on ART when their CD4 count reaches a specific threshold. Current guideline require a person to be initiated on ART earlier, as soon

In any study, when two or more groups are compared, there is always a chance of finding a difference between them just by chance. This is called type 1 error, (alpha error or false-positive error) and occurs when a researcher rejects a null hypothesis that is actually true (641). In this study, the alpha error was set at 5%, to ensure that when there is a difference between the CWH and CWOH, we can be at least 95% confident that this is a true difference and not a chance finding. The 5% limit for alpha, known as the significance level of the study, is set for each single comparison between groups. However, when groups are compared multiple times, the probability of finding a difference just by chance increases depending on the number of times the comparison is made. In this study, we kept reduced the number of pQCT bone outcomes that were reported and did not include other outcomes such as periosteal circumference, endosteal circumference, bone strength index of compression and cross-

sectional moments of inertia in an attempt to reduce the number of outcomes and then reduce possibility of type 1 error.

8.7 Conclusions and recommendations

My results suggest the effect of HIV on bone size and strength in CWH differs by pubertal status. This thesis reported deficits in bone size and strength associated with HIV infection, which are seen more clearly towards the end of puberty in both male and female children. In addition, I have shown an indication of attenuated trabecular bone accrual in those using TDF. I further show that height mediates the effect of HIV on change in pQCT bone outcomes, particularly in females. However, there are deficits in bone size and strength associated with HIV infection over one year follow-up. Results from this study suggests some evidence of catch-up growth in CWH which is not sufficient to address deficits in bone density, size and strength, despite that CWH have seemingly gained more height, more bone size and more bone strength than CWOH. These findings add to information on growth impairment in CWH and therefore have implications for fracture risk in adulthood. The trajectory of skeletal growth seems to have been preset at a lower trajectory than in CWOH before 8 years of age. If no catch up bone accrual occurs, CWH will be at higher risk of fracture in later life. Research is needed to assess the effect of HIV on bone in the early years and to establish whether CWH catchup on bone accrual or not.

In premenopausal women, I have shown that, urban black South African who are in their thirties have lower vBMD than their uninfected counterparts at both the radial and tibial trabecular rich sites. These findings suggest that South African women who are in their thirties may still be gaining bone at the radial distal sites while bone accrual at the tibial distal site is complete. Furthermore, that the skeletal response to HIV infection and ART initiation may vary depending on whether it is trabecular or cortical bone and whether it is radial or tibial site. Finally, this also suggests that decline in bone in WLWH is more likely due to ART initiation than due to HIV infection. Given that trabecular rich sites are a common site for fractures e.g. Colles's fracture on the distal radius and that premenopausal women are likely going to be on ART for many years, I recommend further studies to assess the effect of ART on trabecular rich sites over a longer period of time. Overall, I expect the findings from my PhD to be broadly generalizable across populations of children and premenopausal women living in southern Africa, where HIV prevalence is high. Hence these findings have implications for fracture risk in adulthood. The

study of fracture incidence, in people living with HIV in sub-Saharan Africa, is now a research priority.

8.8 Summary of recommendations for future research and HIV health programmes/policy

Future researches needed to clarify our understanding of how HIV infection is affecting the bone health in both children and adults living with HIV should include the following

1. Longitudinal study of bone in CWH using pQCT in the first 5 years of life
2. Longitudinal follow up of CWH beyond puberty to assess if there is adequate catchup bone accrual
3. Comparison of older men and women living with HIV
4. A pQCT reference database for children living in sub-Saharan Africa

In addition to future research, Practical next steps for policy makers or those responsible for programmes to improve bone health in PLWH are still in the stages where more focus should be put on research and gathering evidence of how the use of non- TDF based ART regimens, physical activity interventions and nutritional supplementation may impact bone health in PLWH. Investigating whether or not vitamin D and calcium supplementation is another viable option to seek to improve bone outcomes in CWH is necessary. Currently, there is the Vitality Trial which is a phase 3 randomised double blinded clinical Trial of vitamin D and calcium supplementation in children living with HIV in Zimbabwe and Zambia (Trial registration number: PACTR20200989766029, Pan African Clinical Trials Registry, registered Sep 2020). The VITALITY study hypothesised that adjuvant treatment with weekly vitamin D3 and daily calcium carbonate given during adolescence will promote bone accrual and mineralization and maximise PBM, which will ultimately reduce the risk of adolescent and adulthood fractures among individuals living with HIV (642). The trial objectives are to investigate the impact of adjuvant treatment with vitamin D3 and calcium carbonate given to CLWH taking ART for 48 weeks on bone density assessed through dual-energy X-ray absorptiometry (DXA), with outcomes to be determined at 48 weeks and participants being followed for a further 48 weeks to investigate the sustainability of the intervention effect. Data collection for the vitality study was from January 2021 to October 2023. The trial has been unblinded but results are yet to be published.

A physical activity intervention targeting only children living with HIV may not be the most feasible. However, physical activity interventions targeting both CWH and CWOH may be possible, though this needs to be proven further through evidence from research. Fortunately, more than 90% of CWH who were recruited in this study were attending school. Evidence from

the literature has shown that school based physical activity interventions improve bone outcomes in children. A physical activity intervention in schools might therefore be a good opportunity to target both CWH and CWOH. Awareness programmes of the importance of bone health within the context of HIV, encouraging e.g. nutrition and physical activity especially in children and adolescents may also be helpful. Awareness programmes of the importance of bone health within the context of HIV, encouraging e.g. nutrition and physical activity especially in children and adolescents may also be helpful.

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Appendix 1: Ethics Approval (LSHTM)

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Observational / Interventions Research Ethics Committee

Mrs Cynthia Kahari
LSHTM

22 May 2019

Dear Cynthia,

Study Title: Understanding the effect of HIV infection and its treatment on trabecular and cortical bone architecture in children, adolescents and premenopausal women.

LSHTM Ethics Ref: 17154

Thank you for responding to the Observational Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Conditions of the favourable opinion

Approval is dependent on local ethical approval having been received, where relevant.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document Type	File Name	Date	Version
Local Approval	WBS Ethics Approval letter_SCCpdf	04/06/2010	4 Jun 2010
Local Approval	WBS Ethics Approval letter	29/10/2010	29 Oct 2010
Local Approval	IMVASK_LSHTM Ethics Approval Letter	16/05/2018	16 May 2018
Consent form	IMVASK English assent Cases 8 to 12yrs 16_8_18	16/08/2018	2.2
Consent form	IMVASK English Blood Storage Consent 16_8_18	16/08/2018	2.2
Consent form	IMVASK English Consent Form Cases 16_8_18	16/03/2019	2.2
Consent form	IMVASK English Consent Controls 16_8_18	16/03/2019	2.2
Consent form	Cynthia Kahari_BRTI_WBS_consent_contract to access and use WBS data	28/03/2019	28 Aug 2018
Investigator CV	Cynthia Kahari_CV_Mar2019	31/03/2019	31Mar2019
Protocol / Proposal	Cynthia Mukwasi-Kahari PHD Proposal_Leosubmission_cmkMar2019	31/03/2019	31 Mar 2019
Consent form	IMVASK consent from PI	31/03/2019	31 Mar 2019
Local Approval	Cynthia Kahari_IMVASK consent from PI	31/03/2019	31 Mar 2019
Local Approval	IMVASK_MRCZ Approval Letter	10/04/2019	10 Apr 2018
Covering Letter	Cynthia Kahari Responce to LSHTM Letter of clarification	21/05/2019	21/05/19
Consent form	IMVASK English assent Controls 8 to 12yrs 16_8_18	16/08/2019	2.2
Local Approval	Cynthia Kahari_BRTI_WBS_consent_contract to access and use WBS data	28/08/2019	28 Aug 2018

After ethical review

The Chief Investigator (CI) or delegate is responsible for informing the ethics committee of any subsequent changes to the application. These must be submitted to the Committee for review using an Amendment form. Amendments must not be initiated before receipt of written favourable opinion from the committee.

The CI or delegate is also required to notify the ethics committee of any protocol violations and/or Suspected Unexpected Serious Adverse Reactions (SUSARs) which occur during the project by submitting a Serious Adverse Event form.

An annual report should be submitted to the committee using an Annual Report form on the anniversary of the approval of the study during the lifetime of the study.

At the end of the study, the CI or delegate must notify the committee using an End of Study form.

All aforementioned forms are available on the ethics online applications website and can only be submitted to the committee via the website at: http://leo.lshtm.ac.uk

Additional information is available at: www.lshtm.ac.uk/ethics

Yours sincerely,

[redacted]

Professor John DH Porter
Chair

ethics@lshtm.ac.uk
http://www.lshtm.ac.uk/ethics/

Improving health worldwide

Appendix 2: Ethics Approval (JREC)

Joint Research Ethics Committee
For The University of Zimbabwe,
College of Health Sciences and
Parirenyatwa Group of Hospitals

University of Zimbabwe
College of Health Sciences

JREC Office No. 4, 5th Floor College of Health Sciences Building
Telephone: +263 4 708149/ 791631 Ext 2261/2242
Email:jrec.office@gmail.com/jrec@medsch.uz.ac.zw, website:www.jrec.uz.ac.zw

Parirenyatwa
Group of Hospitals

APPROVAL LETTER

Date: 28 May 2019 JREC Ref: 123/19

Names of Researcher Cynthia Kahari
Address: Department of Radiology

RE: THE EFFECT OF HIV AND ITS TREATMENT ON TRABECULAR AND CORTICAL BONE ARCHITECTURE IN CHILDREN, ADOLESCENTS AND PREMENOPAUSAL WOMEN.

Thank you for your application for ethical review of the above mentioned research to the Joint Research Ethics Committee. Please be advised that the Joint Research Ethics Committee has reviewed and approved your application to conduct the above named study. You are still required to obtain MRCZ and RCZ approval before you commence the study if required by the nature of your study.

- APPROVAL NUMBER: JREC/123/19
- APPROVAL DATE: 28 May 2019
- EXPIRY DATE: 27 May 2020

This approval is based on the review and approval of the following documents that were submitted to the Joint Ethics Committee:

a) Completed Application Form
b) Full Study Protocol
c) Informed Consent in English and/or appropriate local language

After this date the study may only continue upon renewal. For purposes of renewal please submit a completed renewal form (obtainable from the JREC office) and the following documents before the expiry date:

a. Progress report
b. A Summary of adverse events
c. A DSMB report

OHRP IRB Number: IORG 0000814
PARIRENYATWA GROUP OF HOSPITALS FWA: 00019350

- MODIFICATIONS:

Prior approval is required before implementing any changes in the protocol including changes in the informed consent.

- TERMINATION OF STUDY:

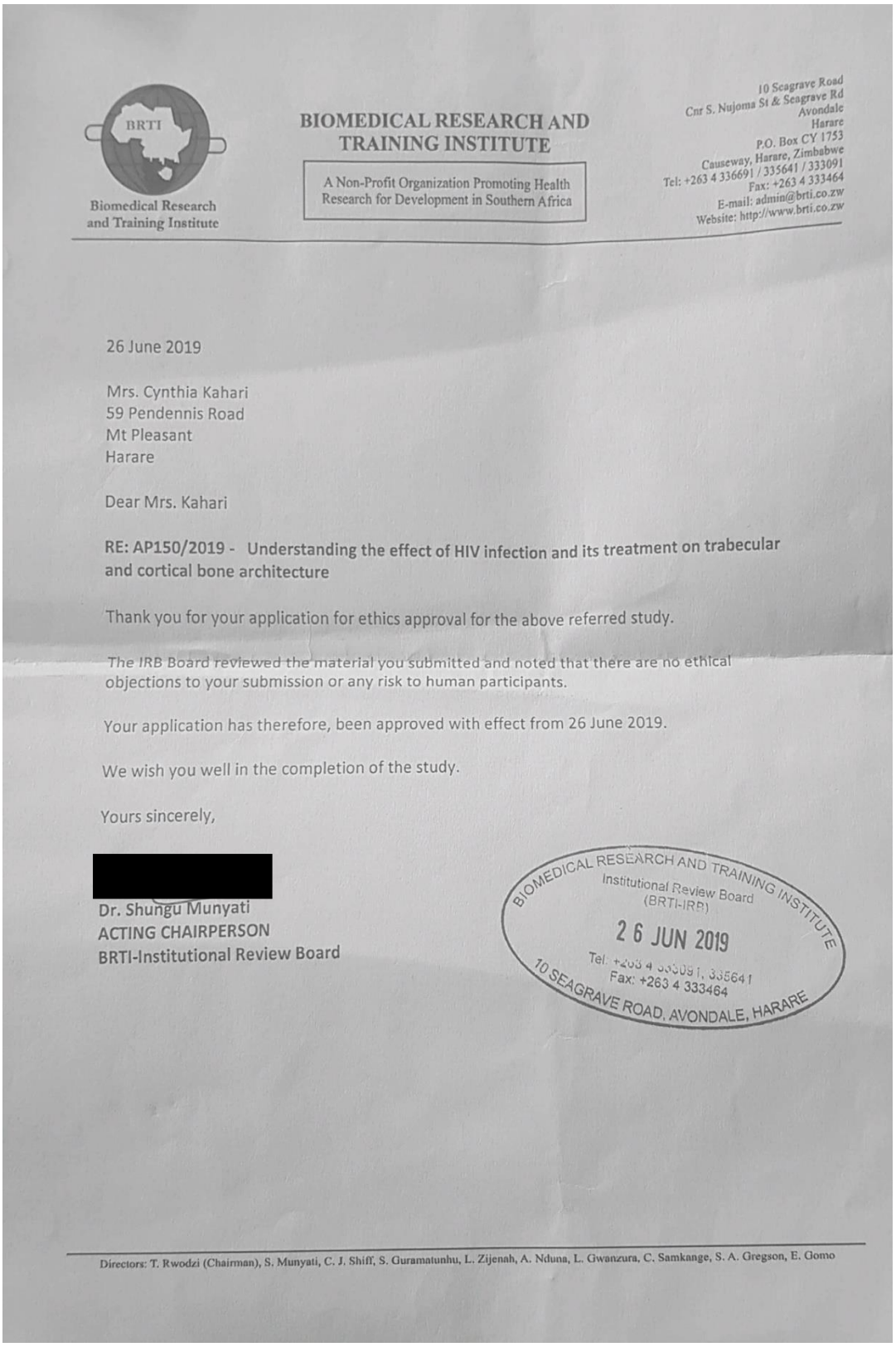
On termination of the study you are required to submit a completed request for termination form and a summary of the research findings/ results.

Yours sincerely,

[redacted]

Professor Rangarirai Masanganise
JREC Chairman
RM/lm/uh

Appendix 3: Ethics Approval (BRTI)



BIOMEDICAL RESEARCH AND TRAINING INSTITUTE

A Non-Profit Organization Promoting Health Research for Development in Southern Africa

Biomedical Research and Training Institute

10 Seagrave Road
Cnr S. Nujoma St & Seagrave Rd
Avondale
Harare
P.O. Box CY 1753
Causeway, Harare, Zimbabwe
Tel: +263 4 336691 / 335641 / 333091
Fax: +263 4 333464
E-mail: admin@brti.co.zw
Website: http://www.brti.co.zw

26 June 2019

Mrs. Cynthia Kahari
59 Pendennis Road
Mt Pleasant
Harare

Dear Mrs. Kahari

RE: AP150/2019 - Understanding the effect of HIV infection and its treatment on trabecular and cortical bone architecture

Thank you for your application for ethics approval for the above referred study.

The IRB Board reviewed the material you submitted and noted that there are no ethical objections to your submission or any risk to human participants.

Your application has therefore, been approved with effect from 26 June 2019.

We wish you well in the completion of the study.

Yours sincerely,

[redacted]

Dr. Shungu Munyati
ACTING CHAIRPERSON
BRTI-Institutional Review Board

BIOMEDICAL RESEARCH AND TRAINING INSTITUTE
Institutional Review Board
(BRTI-IRB)
26 JUN 2019
Tel: +263 4 333091, 335641
Fax: +263 4 333464
10 SEAGRAVE ROAD, AVONDALE, HARARE

Directors: T. Rwodzi (Chairman), S. Munyati, C. J. Shiff, S. Guramatunhu, L. Zijenah, A. Nduna, L. Gwanzura, C. Samkange, S. A. Gregson, E. Gomo

Appendix 4: Ethics Approval (MRCZ)

Telephone: 791792/791193
Telefax: (263) - 4 – 790715
E-mail: mrcz@mrcz.org.zw
Website: http://www.mrcz.org.zw

Medical Research Council of Zimbabwe
Josiah Tongogara / Mazoe Street
P. O. Box CY 573
Causeway
Harare

MRCZ/A/2494

26 August, 2019

Cynthia Kahari
UZCHS- Department of Radiology
P O Box A 178
Avondale
Harare

RE: -The effect of HIV and its treatment on trabecular and cortical bone architecture in children, adolescents and pre-menopausal women

Thank you for the application for review of Research Activity that you submitted to the Medical Research Council of Zimbabwe (MRCZ). Please be advised that the Medical Research Council of Zimbabwe has **reviewed** and **approved** your application to conduct the above titled study.

This approval is based on the review and approval of the following documents that were submitted to MRCZ for review:-

a) Study Protocol version 2, dated 13 August 2019
b) IMVASK English participant consent form_(Cases and Controls), version 2.2, version date 16 Aug 2018
c) IMVASK English assent form_(Cases and Controls), version 2.2, version date 16 Aug 2018
d) Data Collection Tools

APPROVAL NUMBER : MRCZ/A/2494
This number should be used on all correspondence, consent forms and documents as appropriate.

- **TYPE OF MEETING : Full Board**
- **MEETING DATE : 25 July 2019**
- **APPROVAL DATE : 26 August, 2019**
- **EXPIRATION DATE : 25 August, 2020**

After this date, this project may only continue upon renewal. For purposes of renewal, a progress report on a standard form obtainable from the MRCZ Offices should be submitted three months before the expiration date for continuing review.

- **SERIOUS ADVERSE EVENT REPORTING**: All serious problems having to do with subject safety must be reported to the Institutional Ethical Review Committee (IERC) as well as the MRCZ within 3 working days using standard forms obtainable from the MRCZ Offices or website.
- **MODIFICATIONS**: Prior MRCZ and IERC approval using standard forms obtainable from the MRCZ Offices is required before implementing any changes in the Protocol (including changes in the consent documents).
- **TERMINATION OF STUDY**: On termination of a study, a report has to be submitted to the MRCZ using standard forms obtainable from the MRCZ Offices or website.
- **QUESTIONS**: Please contact the MRCZ on Telephone No. (04) 791792, 791193 or by e-mail on mrcz@mrcz.org.zw

Other

- Please be reminded to send in copies of your research results for our records as well as for Health Research Database.
- You're also encouraged to submit electronic copies of your publications in peer-reviewed journals that may emanate from this study.
- In addition to this approval, all clinical trials involving drugs, devices and biologics (including other studies focusing on registered drugs) require approval of Medicines Control Authority of Zimbabwe (MCAZ) before commencement.

Yours Faithfully
[redacted]
MRCZ SECRETARIAT
FOR CHAIRPERSON
MEDICAL RESEARCH COUNCIL OF ZIMBABWE

MEDICAL RESEARCH COUNCIL OF ZIMBABWE
2019 -08- 26
APPROVED
[illegible] HARARE

PROMOTING THE ETHICAL CONDUCT OF HEALTH RESEARCH

Appendix 5: IMVASK Questionnaire

MODULE 1: Demographic Data			
A01	*STUDYNO*	Study No.	**H1**□□□
A02	*DOI*	Date of interview (*Musi wekubvunzurudzwa*) (dd/mm/yyyy)	□□/□□/□□□□
A03	*OID*	Interviewer ID	□□□□
A04	*NATM*	Is the participant's natural mother alive? (*Mai vemwana vapenyu here*)?	Yes □ No □ Don't know
A05	*NATF*	Is the participant's natural father alive? (*Baba vemwana vapenyu here*)?	Yes □ No □ Don't know

MODULE 2: HIV History			
B01	*OIC*	OI clinic No (*nhamba yeku clinic*)	□□□□-□□-□□□□□
B02	*DOD*	What is the participant's date of HIV diagnosis (*HIV yakaonekwa nachiremba rinhi*)? (dd/mm/yyyy). If the exact date of diagnosis unknown, enter the year of diagnosis, with the 15th June as the default date.	□□/□□/□□□□
B03	*TRAN*	What is the participant's mode of HIV acquisition (*Nzira yekutapurirwa kweHIV inozikanwa here)*? 1= mother to child, 2= blood transfusion/other parenteral, 3= sexual transmission 4=don't know. **Refer to patient hand-held records to establish the answer to this question.**	□
B04	*CD4D*	What was the participant's CD4 count at diagnosis? (*CD4 yemwana yanga yakamira papi HIV yemwana payakaonekwa*?). **Refer to patient hand-held records to establish the answer to this question.**	□□□□cells/L Don't know/missing
B05	*LCD4*	What is the lowest CD4 count recorded for the participant? (*CD4 ye pasi pasi*) **Refer to patient hand-held records to establish the answer to this question.**	□□□□cells/L Don't know/mission
B06	*STAGD*	What was the participant's WHO HIV clinical stage at diagnosis? **Refer to patient hand-held records to establish the answer to this question.**	□ Don't know/missing

Please tick the current drugs the participant is taking and give date each drug was commenced:

B07	*ABC*	Abacavir Yes □ No □ □□/□□/□□□□	**B14**	KAL	Kaletra, Aluvia (Lopinavir/ritonavir) Yes □ No □ □□/□□/□□□□
B08	*DDI*	Didanosine Yes □ No □ □□/□□/□□□□	**B15**	TDF	Tenofovir Yes □ No □ □□/□□/□□□□
B09	*DRV*	Darunavir/ritonavir Yes □ No □ □□/□□/□□□□	**B16**	TEN	Tenolam E (Lamivudine, Tenofovir, Efavirenz) Yes □ No □ □□/□□/□□□□
B10	*DTG*	Dolutegravir Yes □ No □ □□/□□/□□□□	**B17**	TLM	Tenolam (Lamivudine, Tenofovir) Yes □ No □ □□/□□/□□□□
B11	*EFV*	Efavirenz Yes □ No □ □□/□□/□□□□	**B18**	TRU	Truvada (Emtricitabine, Tenofovir) Yes □ No □ □□/□□/□□□□
B12	*3TC*	Lamivudine Yes □ No □ □□/□□/□□□□	**B19**	*AZT*	Zidovudine Yes □ No □ □□/□□/□□□□
B13	NVP	Nevirapine Yes □ No □ □□/□□/□□□□	**B20**	OTH	Other Name_____ □□/□□/□□□□

B21	*SEP*	Co-trimoxazole (Septrin) Yes □ No □	□□/□□/□□□□
B22	*ISO*	Isoniazid preventive therapy (IPT) Yes □ No □	□□/□□/□□□□
B23	*PTB*	TB treatment Yes □ No □	□□/□□/□□□□
B24	*FLUC*	Fluconazole prophylaxis Yes □ No □	□□/□□/□□□□

MODULE 3: School attendance			
C01	*ENROL*	Is the participant currently enrolled in school (*Parizvino uri kuenda kuchikoro here*)? 0 = No, 1 = Yes **If NO, go to C06**	□
C02	*SCHNA*	What is the name of the participant's school (*Chikoro chemwana chinonzii*)? _____ 0 = Primary school, 1= Secondary school	□
C03	*GRADE*	What grade/form is the participant in (*Uri mugiredhi kana fomu ripi)?* **Please tick to indicate whether grade or form.**	Grade □ **OR** Form □ □
C04	*DAYS*	In the last month, how many days of school did the participant miss (*Mumwedzi wapfura mazuva mangani ausina kuenda kuchikoro*)?	□□days
C05	*REPT*	Has the participant ever repeated a grade at school (*Wakambodzokorora here imwe yemagiredhi kuchikoro*)? 0 = No, 1 = Yes	□
C06	*EVERAT*	If not currently enrolled, has the participant ever attended school? (Kana mhinduro iri Kwete, parizvino usina kunyoresa kuchikoro, wakamboenda here kuchikoro here?) 0 = No 1 = Yes **If NO, go to C08**.	□
C07	*HGRD*	What was the highest grade/form that the participant completed? (Wakapedza kusvika pachinhanho chipi?) **Please tick to indicate whether grade or form.**	Grade □ **OR** Form □ □
C08	*REAS*	If the participant is not attending school, what is the main reason? (Kana usina kumbobvira wakenda kuchikoro kana kuti usiri kuenda parizvino chikonzero chii?) 1 = Not enough money 2 = Lack of interest to go to school 3 = Lack of school nearby or nearby school not accessible 4 = Illness 5 = Attendance refused by school 6= Negative attitudes of other students or teachers 7= Other, specify: _____	□□

MODULE 4: Socio-economic and household characteristics			
D01	*NOHH*	How many people live in the participant's household? Include all people who regularly live and share meals in the house. Regularly meaning someone who has lived at the house for at least 2 weeks. (Mumba menyu munogara vanhu vangani? Vanhu vanosanganisavese vanodya pamwe chete mumba muno.)	□□
D02	*HAGE*	How old is the household head? (Muriritiri wemhuri ino anemakore mangani?)	□□yrs
D03	*EDM*	For the mother, what is her highest level of education? (Mai vemwana vakadzidza kusvika papi?)1 = Primary, 2 = Secondary, 3 = Training college, 4 = University, 5 = None, 6 = Unknown	□
D04	*EDP*	For the father, what is his highest level of education? (Baba vemwana vakadzidza kusvika papi?) 1 = Primary, 2 = Secondary, 3 = Training college, 4 = University, 5 = None, 6 = Unknown	□
D05	*OWN*	Does the household own the dwelling? (Mhuri yenyu ndiyo muridzi wenzvimbo ino here?) 1 = Own dwelling, 2 = Rent main dwelling, 3 = Rent part of dwelling/lodger, 4 = Use dwelling without paying rent	□
D06	*SAL*	What would you say the regular household income would be per month? (Mari inotambirwa mumba ino pamwedzi ingaita marii pamwedzi?) 1 = Less than USD 100 2 = USD 101-500 3 = USD 201-500 4 = USD 501-900 5 = More than USD 900 6 = Don't know/don't want to say	□

D07	*AST*	Does the household the participant lives in have any of the following (Mhuri yenyu ine zvinhu izvi here) (tick all that apply)?	☐ Electricity ☐ Fridge ☐ Bicycle ☐ Working car/truck ☐ Television ☐ Radio ☐ Tap in house ☐ Tap outside house ☐ Flush toilet ☐ Pit latrine

MODULE 5: Musculoskeletal history

H1	*BRK*	Has the participant ever broken a bone in their lifetime (Mwana akambovhuna mabhonzo muupenyu hwake here)? This includes all fractures, cracks, chips and breaks.	**Yes** ☐ If Yes, complete H2-H8. **No** ☐ If No, continue to next module
H2	*ARM*	**a) Broken bone in arm/shoulder? (akambovhunika ruoko kana pendekete?)**	Yes ☐ No ☐ Don't know ☐
		b) If yes, was this a <u>high impact</u>* injury? Pakavhunika bhonzo yanga iritsaona yakashata here?	Yes ☐ No ☐ Don't know ☐
		c) **How was the broken bone managed (tick all that apply)? (akarapwa sei paakavhunika bhonzo racho)?** X-Ray ☐ Operation ☐ Plaster Cast/Splint ☐ Hospital admission ☐ Bandage only ☐ Nothing ☐ **Examples of high impact injuries include road traffic accidents, a fall of more than 3 metres or being hit by a heavy moving object.*	
H3	*LEG*	**a) Broken bone in leg (akambovhunika gumbo here)?**	Yes ☐ No ☐ Don't know ☐
		b) If yes, was this a high impact* injury? Pakavhunika bhonzo yanga iritsaona yakashata here?	Yes ☐ No ☐ Don't know ☐
		c) **How was the broken bone managed (tick all that apply) (akarapwa sei paakavhunika bhonzo racho)?** X-Ray ☐ Operation ☐ Plaster Cast/Splint ☐ Hospital admission ☐ Bandage only ☐ Nothing ☐	
H4	*WRIST*	**a) Broken wrist (akambovhuna ruoko here)?**	Yes ☐ No ☐ Don't know ☐
		b) If yes, was this a high impact* injury? Pakavhunika bhonzo yanga iritsaona yakashata here?	Yes ☐ No ☐ Don't know ☐
		c) **How was the broken bone managed (tick all that apply) (akarapwa sei paakavhunika bhonzo racho)?** X-Ray ☐ Operation ☐ Plaster Cast/Splint ☐ Hospital admission ☐ Bandage only ☐ Nothing ☐	
H5	*ANK*	**a) Broken ankle (akambovhuna tsoka here)?**	Yes ☐ No ☐ Don't know ☐
		b) If yes, was this a high impact* injury? Pakavhunika bhonzo yanga iritsaona yakashata here?	Yes ☐ No ☐ Don't know ☐
		c) **How was the broken bone managed (tick all that apply) (akarapwa sei paakavhunika bhonzo racho)?** X-Ray ☐ Operation ☐ Plaster Cast/Splint ☐ Hospital admission ☐ Bandage only ☐ Nothing ☐	
H6	*PELV*	**a) Broken pelvis (akambotyoka hudyu here)?**	Yes ☐ No ☐ Don't know ☐
		b) If yes, was this a high impact* injury? Pakavhunika bhonzo yanga iritsaona yakashata here?	Yes ☐ No ☐ Don't know ☐
		c) **How was the broken bone managed (tick all that apply) (akarapwa sei paakavhunika bhonzo racho)?** X-Ray ☐ Operation ☐ Plaster Cast/Splint ☐ Hospital admission ☐ Bandage only ☐ Nothing ☐	
H7	*SPN*	**a) Broken spine (back) (akambotyoka musana here)?**	Yes ☐ No ☐ Don't know ☐
		b) If yes, was this a high impact* injury? Pakavhunika bhonzo yanga iritsaona yakashata here?	Yes ☐ No ☐ Don't know ☐
		c) **How was the broken bone managed (tick all that apply) (akarapwa sei paakavhunika bhonzo racho)?** X-Ray ☐ Operation ☐ Plaster Cast/Splint ☐ Hospital admission ☐ Bandage only ☐ Nothing ☐	
H8	*OTH*	**a) Other broken bones (mamwe mabhonzo)?** Name of bone:	Yes ☐ No ☐ Don't know ☐
		b) If yes, was this a high impact* injury? Pakavhunika bhonzo yanga iritsaona yakashata here?	Yes ☐ No ☐ Don't know ☐
		c) **How was the broken bone managed (tick all that apply) (akarapwa sei paakavhunika bhonzo racho)?** X-Ray ☐ Operation ☐ Plaster Cast/Splint ☐ Hospital admission ☐ Bandage only ☐ Nothing ☐	

MODULE 6: Co-morbidities

F01	*ADM*	How many times has the participant been admitted to hospital in the **last 12 months** (*mwana akambogara muchipatara kangani mugore rapfuura*?	□□
		Has the participant ever been diagnosed with any of the following (check hand held record) (mwana akamboonekwa ane zvirwere izvi here)?:	
F02	*ARTH*	Arthritis or a joint problem (mwana akamboita chirwere chekurwadziwa nemabhonzo)?	Yes □ No □ Don't know □
F03	*EPI*	Epilepsy (tsviyo, chirwere chekuputsika)	Yes □ No □ Don't know □
F04	*KID*	Kidney disease (itsvo)	Yes □ No □ Don't know □
F05	*TB*	Tuberculosis (TB)	Yes □ No □ Don't know □
F06	*ASTH*	Asthma/breathing problems (zviirwere zveekunetseka nekufema)	Yes □ No □ Don't know □
F07	*DIAB*	Diabetes (chirwere cheshuga)	Yes □ No □ Don't know □
F08	*HEART*	Heart problem (*chirwere chemoyo*)	Yes □ No □ Don't know □
F09	*STER*	Are there any other medical problems recorded (mwana akambo batwa nezvimwe zvirwere here)? If Yes, please specify: _____	Yes □ No □ Don't know □
F10	*MED*	Is the participant currently taking any other medications, including inhalers and steroids? Please list drug name and dosage below: (*Mwana anotora mishonga kana ma-inhalers) here? Ndapota nyorai mazita e mishonga nema dheji pasi apa*: Drug_____Dose_____ Drug_____Dose_____	Yes □ No □ Don't know

MODULE 7: Exposures (Smoking, Alcohol and Drugs History)

These questions apply to children 11 years and over. Ask the guardian first then the participant in the absence of the guardian. Please ask the parent/guardian if he/she is comfortable with answering questions related to smoking. Any parents/guardians that are uncomfortable with these questions, can decline to answer.

L01	*SMOK*	Does the participant currently smoke cigarettes (*parizvino mwana wenyu anoputa fodya here*)? 1=Yes, 0 = No, 9 = don't want to answer/don't know/N/A	□
L02	*ALCO*	Does the participant drink alcohol (*mwana wenyu anonwa doro here)*? 1=Yes, 0 = No, 9 = don't want to answer/don't know/N/A	□

MODULE 8: Pubertal development

G01	*MEN*	(Girls) Has the participant started her period (Mwana wako akatanga nguva yake here)?	Yes □ No □ Don't know □
G02	*SAC*	(Girls) How old was the participant when she had her first period (Aive nemakore mangani paakatanga nguva yake)?	□□ years
G03	*LMP*	(Girls) What was the date of the last menstrual period (Zuva rekupedzisira repa mwedzi)? (dd/mm/yyyy)	□□/□□/□□□□
G04	*VOI*	(Boys) How old was the participant when their voice broke (Mwana aive nemakore mangani inzwi rake pakatanga kubhesera)?	□□ years

MODULE 9: Physical activity (IPAQ-Short Form)

We are interested in finding out about the kinds of physical activities that people do as part of their everyday life. The questions will ask the participant about the time they spent being physically active in the last 7 days. Please answer each question even if you do not consider the participant to be an active individual. Please think about the activities done at school, as part of house and yard work, to get from place to place, and in their spare time for recreation, exercise or sport. (*Tinoda kunzwisisa kufamba, kumhanya nekutamba kunoita mwana wenyu zuva nezuva. Mibvunzo iyi ichakubvunzayi kufambe, kumhanya nekutamba kwaitwa ne mwana mumazuva manomwe apfuura. Ndapota pindurayi mibvunzo ese chero musingafungi kuti mwana munhu anosinga nyanyi kufamba, kumhanya kana kutamba. Ndapota chimbofungai kufamba, kumhanya kana kutamba kunoitwa ne mwana panzvimbo dzese sekuchikoro, mumba, panze, pese paanofamba achienda kunedzimwe nzvimbo, uye munguva yake yega yekuzorora, kurovedza muviri kana mutambo*).

Think about all the vigorous activities that you did in the last 7 days. Vigorous physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time. (*Funga pamusoro pekumhanya nekutamba **zvakasimbisisa mumazuva manomwe apfuura**. Pakumhanya nekutamba **zvakanyanyisisa** tinoreva mabasa akaoma emumuviri anotora simba rakawanda uye anoita kuti afemedze zvakanyanya kudarika zvinowanzoitika. Fungai nezvezvinhu zvakaitwa zvichipfura maminitsi gumi panguva imwechete*).

J01	*VPA*	**1. During the last 7 days, on how many days did you (the participant) do vigorous physical activities like heavy lifting, digging, aerobics, or fast bicycling? No vigorous physical activities *Skip to question J03*** *(Mumazuva manomwe apfuura, mwana akapedza mazuva mangani achi mhanya kana kutamba zvakasimbisisa sekusimudza zvinhu zvinirema, kuchera, aerobics, kana kuchovha bhasikoro nekumhanya?* *Kana mwana asina kumboita mazuwa akadai ekumhanya nekutamba **zvakasimbisisa, endayi kumubvunzo J03**)*	□□ days per week
J02	*TVPA*	**2. How much time did you (the participant) usually spend doing vigorous physical activities on one of those days?** (*Mwana akatora nguva yakawandasei achimhanya nekutamba **zvakasimbisisa** pama zuva iwayo)?*	□□hours/day □□minutes/day □ Don't know/not sure

Think about all the moderate activities that your child did in the last 7 days. Moderate activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal. Think only about those physical activities that they did for at least 10 minutes at a time. *(Funga pamusoro pekumhanya nekutamba **kuripakati nepakati mumazuva manomwe apfuura**. Pakumhanya nekutamba **kuripakati nepakati** tinoreva mabasa asina kunyanya kuoma emumuviri anotora simba risina kunyanya kuwanda uye anoita kuti afemedze zvisina kunyanya kudarika zvinowanzoitika. Fungai nezvezvinhu zvakaitwa zvichipfura maminitsi gumi panguva imwechete).*

J03	*DMPA*	**3. During the last 7 days, on how many days did you (the participant) do moderate physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking. If, no moderate physical activities *Skip to question J05*** *(Mumazuva manomwe apfuura, mwana akapedza mazuva mangani achi mhanya kana kutamba **zviripakati nepakati** sekusimudza zvinhu zvisingareme, kuchovha bhasikoro zvirinyore kana kutamba tennis ne mumumwe munhu? Musaverenge kufamba. Kana mwana asina kumboita mazuwa akadai ekumhanya nekutamba **zviripakati nepakati, endayi kumubvunzo J05)***	□□ days per week
J04	*TMPA*	**4. How much time did you (the participant) usually spend doing moderate physical activities on one of those days?** *(Mwana akatora nguva yakawandasei achiita kumhanya kana kutamba **zviripakati nepakati** pazuwa rimwechete ipapo)?*	□□hours/day □□minutes/day □ Don't know/not sure

Think about the time your child spent walking in the last 7 days. This includes at school and at home, walking to travel from place to place, and any other walking that you have done solely for recreation, sport, exercise, or leisure. *Funga pamusoro **pekufamba** kwakaita mwana **mumazuva manomwe apfuura**. Fungai kufamba kwese kwaiita ari kuchikoro nekumba, achifamba kune nzimbo dzakasiyana, achifamba panguwa ye kuzorora kana pane nhabvu.*

J05	*WALK*	**5. During the last 7 days, on how many days did you (the participant) walk for at least 10 minutes at a time? No walking *Skip to question J07*** *(Mumazuva manomwe apfuura, mwana akafamba mazuva mangani kwemaminitsi anopfura gumi nenguva? Kana asina kufamba kupfura izvi **endai kumubvunzo J07)***	□□ days per week
J06	*DWAL*	6**. How much time did you (the participant) usually spend walking on one of those days?** (*Mwana akatora nguva yakawandasei **achifamba** pazuwa rimwe chete ipapo)?*	□□hours/day □□minutes/day □ Don't know/not sure
	TWAL	**The last question is about the time your child spent sitting on weekdays during the last 7 days. Include time spent at school, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading, or sitting or lying down to watch television.** *Mubvunzo wokupedzisira ndewenguva yakapedzwa nemwana **akagara** pasi pakati pevhiki **mumazuva manomwe apfura**. Verengai nguwa yese yaakagara kuchikoro, kumba, achiita basa rekuchikoro kana ari panguva yekuzorora. Izvi zvinogona kusanganisira nguva inoshandiswa kugara patafura, kugara achishanyira shamwari, kuverenga, kana achi gara achiona TV.*	

J07	*SIT*	**7. During the <u>last 7 days</u>, how much time did you (the participant) spend <u>sitting on a week day?</u> (*<u>Mumazuva manomwe apfuura</u>*, *mwana akapedza nguva yakadini akagara pazuwa rimwe chete ipapo)?***	☐☐hours/day ☐☐minutes/day ☐ Don't know/not sure

MODULE 10: Diet and sun exposure

Describe the participant's usual diet in the past month. In the last month, how often has the participant eaten the following foods? (Rondedzera kudya kwemwana kwemazuva ose mumwedzi wapfuura? Anodya zvikafa izvi kangani pamwedzi?)

Code for frequency of eating - write a code in each box:

0=Never, 1=less than 4 times in past month, 2=1-2 times a week, 3=3-5 times a week, 4=Almost every day

Code	Food group	Examples	Frequency code
e001	*LEGUMES*	Beans, peas, nuts, seeds or foods made from any of these e.g peanut butter *(bhinzi, maphizi, nyimo, nzungu, midzi kana zvikafu zvakagadzirwa kubva kune imwe yeiyi e.g. dovi)*	☐
e002	*DAIRY*	Milk, lacto, cheese, cream, milk powder or yogurt *(mukaka, lacto, hodzeko, chizi, mukakaka wehupfu kana yogashi)*	☐
e003	*MEAT*	Pork, beef, goat, mutton, chicken, duck, other birds, liver, kidney, heart or other organ meats *(nyama yenguruve, mombe, hwai, mbudzi, huku, dhadha, dzimwe shiri, chiropa, itsvo, mwoyo kana zvimwe zvemukati memhuka)*	☐
e004	*EGGS*	Eggs (mazai)	box
e005	*FISH*	Fresh or dried fish especially fish with small bones e.g mackerel or kapenta *(hove yakoma (e.g bakayavo kana matemba) neisina kuoma)*	☐
e006	*OIL & MARGARINE*	Cooking oil or margarine (not butter) *(Mafuta okubika kana, margarine, kwete bhata)*	☐
e007	*VITAMINS & MINERALS*	Vitamin tablets, drops or syrups, calcium supplements *(Mapiritsi emavamini, mushonga wekudonhedzera kana ma-syrups, mapiritsi e calcium)*	☐
	Describe the participant's usual exposure to sunlight in the past month (Tsanangura kuti mwana akaswera muzuva kwenguwa yakarebasei mwedzi wapfuura uyu)		
e008	*OUTDOOR*	How much time does the participant usually spend out of doors each day during daylight hours (Mwana wako anovanzo swera aripanze muzuwa kwe nguva yakawanda sei pazuva)? 0 less than 1 hour/day 1 1-2 hours/day 2 > 2 hours/day	0 ☐ 1 ☐ 2 ☐
e009	*SKINEXP*	How much of the participant's skin is usually exposed when she/he is outdoors *(Kana mwana aripanze, nderipi ganda rinenge risina kuvharwa nehembe)?* 0 Just face and hands 1 Face, hands, arms or legs 2 Often no shirt as well as exposing face, hands, arms or legs	0 ☐ 1 ☐ 2 ☐
e010	*HAT*	Does the participant wear a hat to school? (*Mwana anopfeka ngovani kuchikoro here?)* 0 No 1 Yes	0 ☐ 1 ☐

MODULE 11: Disability and functioning (Modified Washington Group Short Question Set on Disability)			
K01	*VISI*	Does the participant have difficulty seeing, even if wearing glasses? *(Kana mwana akapfeka magirazi ake, anoita dambudziko here kuona? Mungati mwana haana dambudziko, ano tambudzika zvishoma, ane dambudziko rakanyanya kana kuti hapana chaanokwanisa kuita zvachose)?*	No - no difficulty ☐ Yes – some difficulty ☐ Yes – a lot of difficulty ☐ Cannot do at all ☐
K02	*AUDI*	Does the participant have difficulty hearing, even if using a hearing aid? *(Mwana anoita dambudziko here rekunzwa, chero achishandisa zvinomubatsira kunzwa? Mungati mwana haana dambudziko, ano tambudzika zvishoma, ane dambudziko rakanyanya kana kuti hapana chaanokwanisa kuita zvachose)?*	No - no difficulty ☐ Yes – some difficulty ☐ Yes – a lot of difficulty ☐ Cannot do at all ☐
K03	*WALK*	Does the participant have difficulty walking or climbing steps? (Mwana anonetseka nekufamba kana kukwira masteps here? *Mungati mwana haana dambudziko, ano tambudzika zvishoma, ane dambudziko rakanyanya kana kuti hapana chaanokwanisa kuita zvachose)?*	No - no difficulty ☐ Yes – some difficulty ☐ Yes – a lot of difficulty ☐ Cannot do at all ☐
K04	*DIST*	It is hard for the participant to walk more than one block (200 metres) (*Zvakaoma kuti mwana afambe kupfuura mamita 200)*	Never ☐ Almost never ☐ Sometimes ☐ Often ☐ Almost always ☐
K05	*RUN*	Does the participant find it hard to run? (*Zvakaoma kuti mwana amhanye)*	Never ☐ Almost never ☐ Sometimes ☐ Often ☐ Almost always ☐
K06	*SPRT*	Does the participant find it hard to do sports activity or exercise? (*Zvakaoma kuti ndite zvemitambo)*	Never ☐ Almost never ☐ Sometimes ☐ Often ☐ Almost always ☐
K07	*ARTH*	Does the participant describe hurting or aching? *(Ndinorwadziwa nemuviri)*	Never ☐ Almost never ☐ Sometimes ☐ Often ☐ Almost always ☐
K08	*MEMO*	Does the participant have difficulty remembering or concentrating? *(Mwana ane dambudziko rekurangarira zvinhu here? Mungati mwana haana dambudziko, ano tambudzika zvishoma, ane dambudziko rakanyanya kana kuti hapana chaanokwanisa kuita zvachose)?*	No - no difficulty ☐ Yes – some difficulty ☐ Yes – a lot of difficulty ☐ Cannot do at all ☐
K09	*SELF*	Does the participant have difficulty with self-care such as washing all over or dressing? *(Mwana anonetseka nekuzvigezesa nekuzvipfekedza here*? *Mungati mwana haana dambudziko, ano tambudzika zvishoma, ane dambudziko rakanyanya kana kuti hapana chaanokwanisa kuita zvachose)?*	No - no difficulty ☐ Yes – some difficulty ☐ Yes – a lot of difficulty ☐ Cannot do at all ☐
K06	*SPEAK*	Using your usual language, does the participant have difficulty communicating, for example understanding or being understood? *(Kanamwana achitaura ane dambudziko rekunzwikwa nevamwe vanhu here? Mungati mwana haana dambudziko, ano tambudzika zvishoma, ane dambudziko rakanyanya kana kuti hapana chaanokwanisa kuita zvachose)?*	No - no difficulty ☐ Yes – some difficulty ☐ Yes – a lot of difficulty ☐ Cannot do at all ☐

Appendix 6: WBS Questionnaire

WOMEN'S BONE STUDY (WBS)

Characteristics and health data collection

WBS number: | | | | | | Month of follow up visit (circle):

6	12	24	36

Date: _____/_____/_____

Group	**Group 1**		**Group 2**		**Group 3**	
1. Repeat HIV test in past 6 weeks Result (0=negative, 1=positive)		Y☐ N☐ 0☐ 1☐		x		x
2. Pregnancy test Negative (if positive inform Dr Hamill)		Y☐ N☐		Y☐ N☐		Y☐ N☐

3. Pregnancy since last visit? (if Yes inform Dr Hamill)		Y☐ N☐		Y☐ N☐		Y☐ N☐
- If currently pregnant: Estimated duration (gestation) in weeks (inform Dr Hamill)						
4. Currently breastfeeding (if Yes inform Dr Hamill)		Y☐ N☐		Y☐ N☐		Y☐ N☐
5. Regular periods		Y☐ N☐		Y☐ N☐		Y☐ N☐
6. Reached menopause (if Yes inform Dr Hamill) - If Y, when was last period (age) _____Yrs_____Mnths - Notes:		Y☐ N☐		Y☐ N☐		Y☐ N☐
7. Using hormonal contraception - If Y, what (e.g. the pill, depo provera):		Y☐ N☐		Y☐ N☐		Y☐ N☐

8. Sterilisation	Y☐ N☐	Y☐ N☐	Y☐ N☐
9. Other family planning e.g. condoms List here:	Y☐ N☐	Y☐ N☐	Y☐ N☐
10. Age in years & months e.g. 37Yrs 6 Mnths			
11. Current smoker	Y☐ N☐	Y☐ N☐	Y☐ N☐
12. Started smoking after last visit?	Y☐ N☐	Y☐ N☐	Y☐ N☐
13. Total month/years of smoking			
14. How many cigarette do you smoke per day			
15. **New** fracture since last visit (if 'No' go to Q. 19)	Y☐ N☐	Y☐ N☐	Y☐ N☐

16. Site of this fracture (e.g. left hip):			
17. Was it a **traumatic** If trauma please list (e.g. car crash, fall from stairs):	Y☐ N☐	Y☐ N☐	Y☐ N☐

OR			
18. Was it **non-traumatic** If non-traumatic please describe (e.g. standing up):	Y☐ N☐	Y☐ N☐	Y☐ N☐
19. Current medication(s) & dose – list (if known). Other than ARV or vitamins 1._____Dose_____ 2._____Dose_____ 3._____Dose_____ 4._____Dose_____			

20. Ask if subject takes: Calcium supplements, if Y List preparations_____ Dose/number of tablets_____	Y☐ N☐	Y☐ N☐	Y☐ N☐
21. Vitamin D supplements, if Y	Y☐ N☐	Y☐ N☐	Y☐ N☐

List preparations_____ Dose/number of tablets_____			
22. Vitamin/mineral supplements (e.g. multivitamins, BCom), if Y List preparations_____ Dose/number of tablets_____	Y☐ N☐	Y☐ N☐	Y☐ N☐

23. History of steroid use since last visit. If Y: List preparations_____ Dose/number of tablets_____ Number of months taken_____		Y☐ N☐		Y☐ N☐		Y☐ N☐		
24. Liopodystrophy (clinician assessed) (If unsure discuss with Dr Hamill)		Y☐ N☐		Y☐ N☐		Y☐ N☐		
25. Current diarrhoea per day (if any), in past 7 days								
25. Current (most recent) CD4 count		x						
26. Lowest CD4 count (if different)		x						

27. Bactrim (PCP) prophylaxis		x		Y☐ N☐		Y☐ N☐		
28. Current (most recent) HIV viral load (if available)		x						
29. Peak viral load, if different from previously recorded (if available)		x						

30. **Current** ARV – list ARVs and start date: 1._____ Start date: 2._____ Start date: 3._____ Start date:					
31. **Previous** ARV regimes – list ARVs and start and stop dates:					

1._____ Start date: Stop date: 2._____ Start date: Stop date: 3._____ Start date: Stop date:						
32. Cumulative duration of ALL ARV (in months)		x				
33. Cumulative duration of NRTI e.g. d4T, 3TC, AZT (in months)		x				
34. Cumulative duration of Tenofovir (TDF) (in months)		x				
35. Cumulative duration of NNRTI e.g. Efavirenz/Nevirapine (in months)		x				
36. Cumulative duration of PI e.g. Kaletra/Alluvia (in months)		x				

37. Major illness since last visit		Y☐ N☐		Y☐ N☐		Y☐ N☐
If major illness please list what & dates 1._____ Start date: Stop date: 2._____ Start date: Stop date: 3._____ Start date: Stop date:						

38. CDC stage of disease e.g. A1		x				

Other comments:

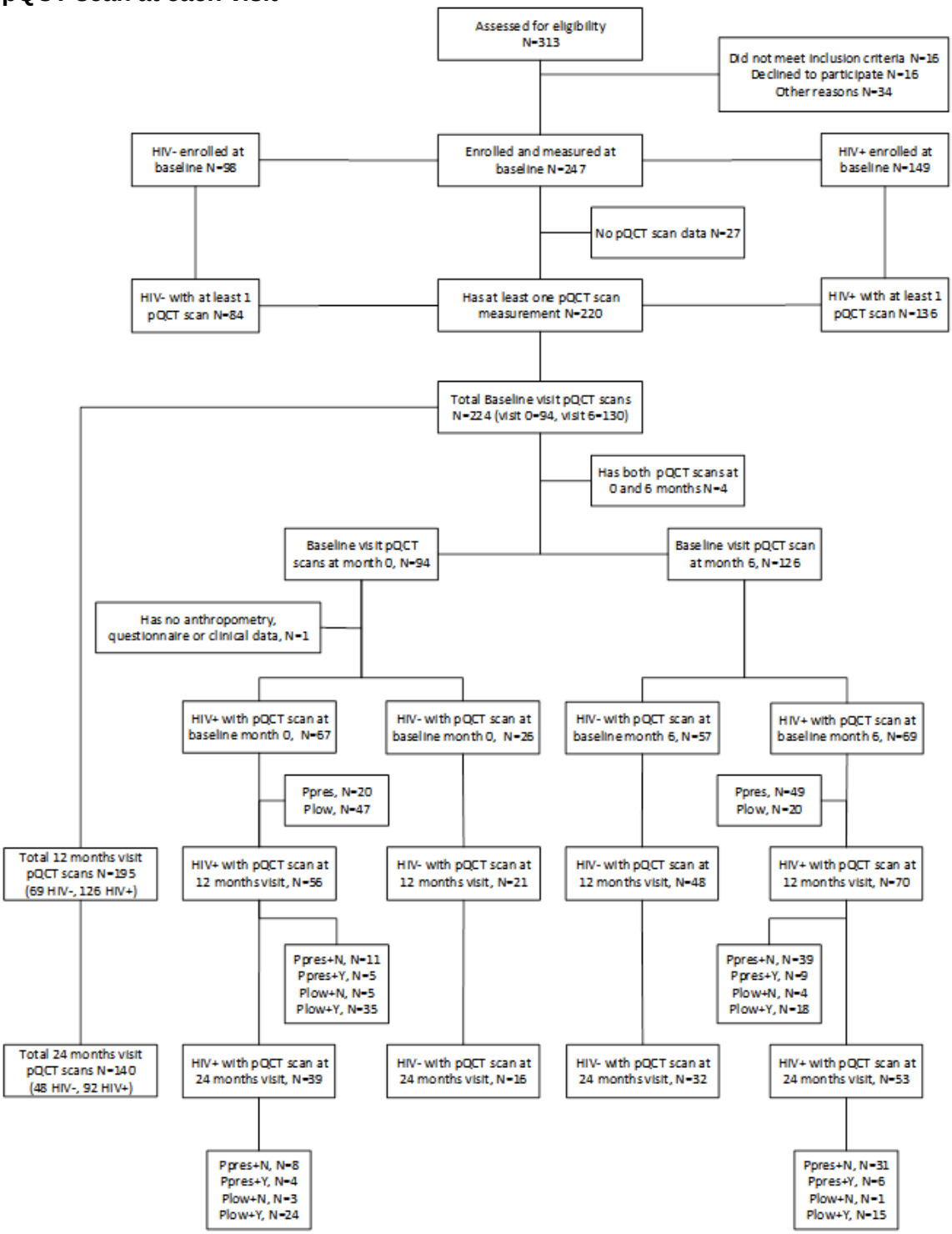
_____

_____

Information collected by:_____ Date:_____

Data captured by:_____ Date:_____

Appendix 7: Supplementary figure 7.1-Flow diagram to show participants who had a pQCT scan at each visit



-HIV+; living with HIV, HIV-; living without HIV
-Ppres; WLWH with a preserved CD4 count at enrolment, Plow; WLWH with a low CD4 count at enrolment
-Ppres+N; WLWH with a preserved CD4 count at enrolment and not yet initiated on ART, Ppres+Y; WLWH with a preserved CD4 count at enrolment and now initiated on ART
-Plow+N; WLWH with a low CD4 count at enrolment and not yet initiated on ART, Ppres+Y; WLWH with a low CD4 count at enrolment and now initiated on ART