University of Southampton

Faculty of Medicine

MRC Lifecourse Epidemiology Centre: Human Development and Health

Skeletal maturation in Zimbabwean children and adolescents and the impact of HIV

Volume 1 of 1

by

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Thesis for the degree of Doctor of Philosophy

October 2025

University of Southampton

Abstract

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HIV remains a significant global health challenge affecting 1.5 million children worldwide with just 57% accessing antiretroviral therapy (ART). HIV infection and its treatment are known to impact growth and development in children. Skeletal maturation is a crucial component of the overall growth and development and is affected by several factors which include genetic ancestry and environmental factors such as, chronic conditions, socio-economic deprivation, and nutrition. In Zimbabwe HIV, malnutrition, and poverty may all impact growth. Understanding factors that influence growth will help inform ways to mitigate poor growth and development, improving outcomes in later life.

Skeletal maturation is assessed as Bone age (BA), with the two most widely used methods being Greulich and Pyle (GP) and Tanner and Whitehouse 3 (TW3), both are based upon assessment of hand wrist radiographs. BA differs from chronological age (CA), which is calculated from the date of birth and does not account for an individual's stage of growth. Until this study, skeletal maturation had not been assessed in Zimbabwean children nor in children living with HIV (CLWH) in Africa. No study had been conducted in an African population to determine the best method to use, nor to determine factors that influence the skeletal maturation process in CLWH, and no study had assessed BA longitudinally in CLWH. The aim of my thesis was to describe skeletal maturation in Zimbabwean children and adolescents living with and without HIV and determine whether HIV and other environmental and lifestyle factors were associated with differences between BA and CA at baseline and after follow-up. I also aimed to determine which method of BA assessment is appropriate for use in Zimbabwean children and adolescents.

I used data from the IMVASK (The Impact of vertical HIV infection on child and adolescent skeletal development) study conducted in Harare, Zimbabwe, which provided a cohort of 609 peripubertal children living with and without HIV aged 8-16 years. Participants were recruited from HIV clinics at Parirenyatwa and Sally Mugabe hospitals and schools within the same catchment area. Hand wrist radiographs were taken at baseline and at one year follow-up. I first assessed BA using the TW3 and GP method. I used Bland and Altman plots to test the agreement between BA by both methods and then with CA. I calculated the inter- and intra-rater reliability and precision. I then determined skeletal maturity deviation (SMD); the difference between BA and CA and compared differences in means by HIV status in males and females at baseline and follow-up. I used linear regression models to determine factors (weight for age z-scores, socioeconomic status, orphanhood, puberty, physical activity, vitamin D and calcium intakes) associated with SMD both at baseline and follow-up.

The TW3 method was the most precise and appropriate for use in Zimbabwean children and adolescents aged 8-16 years, as GP was biased by age. The two methods (TW3 and GP) did not give equal estimates of BA and therefore should not be used interchangeably. At baseline perinatally acquired HIV infection and being underweight were independently associated with delays in skeletal maturation in both males and females. After one-year follow-up female and

male CLWH were still falling behind in skeletal maturation although the association was partially attenuated in males, suggesting that they were not catching up to their fellow HIV negative counterparts. In addition, in males being underweight and in females, lower levels of physical activity were associated with delays in skeletal maturation after one year. In CLWH, delays in initiating ART contributed to delays in skeletal maturation.

In conclusion, the TW3 method of BA assessment should be used in Zimbabwean children and adolescents aged 8-16years.My findings support the “test and treat” policy recommended by WHO programmes where ART is initiated regardless of disease or immunological stage. My results highlight the need for further research to support integration of nutritional and physical activity interventions into routine health care programmes for CLWH, to help improve growth and development.

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Research Thesis: Declaration of Authorship

Print name: Farirayi Nyakoko

Title of thesis: Skeletal maturation in Zimbabwean children and adolescents and the impact of HIV

I declare that this thesis and the work presented in it are my own and has been generated by me as the result of my own original research.

I confirm that:

1. This work was done wholly or mainly while in candidature for a research degree at this University;
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Kowo-Nyakoko F, Gregson CL, Westbury LD, Madanhire T, Offiah AC, Micklesfield LK, et al. The impact of HIV infection on skeletal maturity in peripubertal children in Zimbabwe: a cross-sectional study. BMC Pediatrics. 2024;24(1):480.

Kowo-Nyakoko F, Gregson CL, Madanhire T, Stranix-Chibanda L, Rukuni R, Offiah AC, et al. Evaluation of two methods of bone age assessment in peripubertal children in Zimbabwe. Bone. 2023:116725.

Signature: .....

Date: .....

Acknowledgements

My sincerest gratitude goes to my supervisors, Prof Kate A Ward and Prof Celia Gregson for their mentorship and guidance throughout the course of my PhD. Their expertise, insightful feedback and encouragement made the journey possible. I am forever grateful to Prof Kate A. Ward for holding my hand, supporting me and believing in my abilities even at times when I doubted myself. My sincerest appreciation for her support at the most difficult and challenging time in my journey when baby Mukundi was born. I am grateful for Prof Celia Gregson for introducing me to bone age assessment and affording me the opportunity to learn and grow.

I am deeply thankful to Prof Ann Prentice and the Faculty of Medicine Global Challenges Research Fund for the financial support throughout my PhD. I would like to acknowledge Prof Amaka C. Offiah for sparing her time from a busy schedule to teach me bone age assessment methods. Many thanks to the whole supervisory team Dr Andrea Rehman, Dr Leo Westbury and Dr Linda Stranix-Chibanda and advisors who made this journey possible through their academic support. Special mention goes to Prof Rashida A Ferrand, Dr Ruramayi Rukuni, The Health Research Unit team, Tafadzwa Madanhire and Evangelista Benno for the various roles they played in my journey. Thank you to the IMVASK participants who, without them, the PhD would not have been possible.

To my husband Rodreck Nyakoko, I am forever grateful for your unwavering support of my dreams amid yours. You played the role of a mother to our kids in my absence, stayed up late cheering me on and endured my rantings when things were soar. To my mom and my siblings, I thank you, you were my biggest cheerleaders even though you did not understand the science. You took care of things in my absence.

To my kids, Graeme, Rutendo and Mukundi, this is to show you that your aspirations are worth pursuing. Even the sky cannot limit you.

I dedicate this PhD to my late father, Mr Never Kowo, the echoes of your unwavering support kept me going. This PhD is a fruit you so carefully tendered during my upbringing; I know you are looking down with pride.

Definitions and Abbreviations

ART	Anti-retroviral therapy
BA	Bone age
Bt20	Birth to Twenty
CA	Chronological age
CLWH	Children living with HIV
DXA	Dual-energy X-ray absorptiometry
GH	Growth hormone
GnRH	Gonadotropin-releasing hormone
GP	Greulich and Pyle
HAZ	Height-for-age-z-scores
IGF-I	Insulin-like growth factor 1
IMVASK	The Impact of vertical HIV infection on child and adolescent skeletal development
IOTF	International obesity task force
IPAQ	International Physical Activity Questionnaire
IQR	Inter quartile range
LMIC	Lower middle-income countries
MET	Metabolic rate
NCD	Non communicable diseases
pQCT	Peripheral Quantitative Computed Tomography
SES	Socio-economic status
SMD	Skeletal maturity deviation
TB	Tuberculosis
TDF	Tenofovir disoproxil fumarate
TW3 RUS	Tanner Whitehouse 3 radius ulna and short bones score
TW3	Tanner Whitehouse 3
WAZ	Weight-for-age z-scores
WHO	World Health Organisation

Chapter 1 Bone anatomy, skeletal development and introduction to study setting

1.1 Introduction

Skeletal maturity is a measure of the development of the skeleton incorporating the size, shape and degree of mineralization of the epiphyses and physeal plates of a bone to define their proximity to full maturity (1). The process of skeletal maturation is influenced by several factors which include genetic ancestry, socio-economic status, nutrition, and disease patterns. Risk factors that may influence skeletal maturation adversely including HIV infection and its treatment, and poor micronutrient status and these are particularly relevant to Zimbabwe, the country in which this PhD is based. Studies assessing skeletal maturation in children living with HIV (CLWH) are important to increase knowledge on growth in CLWH and to assess impact of treatment on growth.

Bone age (BA) is the assessment of skeletal maturity; it differs from chronological age (CA) which is calculated from the date of birth and does not necessarily reflect an individual's stage of growth; for example, two eleven-year-olds can differ vastly in their developmental stage, such that they would have different BA's. BA is commonly assessed using hand wrist radiographs. Methods to assess BA should be reliable and practical, and adaptable for use in different populations. The two most widely used methods are the Greulich and Pyle (GP) and the Tanner and Whitehouse 3 (TW3) methods, both based upon assessment of hand wrist radiographs (2, 3). The use of these methods in African populations is questionable given that these methods were developed decades ago. Moreover, there has been a lack of diversity among the populations used to develop atlases on which the two methods are based. In this chapter I will provide a background description of bone anatomy, skeletal development, maturation and factors influencing skeletal maturation in children and adolescents.

1.2 Anatomy of bone

1.2.1 Overview of the human skeleton

The adult human skeleton has 206 bones, divided into 80 bones of the axial skeleton (bones of the head and trunk) and 126 bones of the appendicular skeleton (bones of the limbs). Four categories of bones make up the human skeleton namely the long, short, flat, and irregular

bones which differ in the way they develop and in their anatomy. The skeleton has several functions which includes provision of structural support for the rest of the body, providing levers for the muscles by permitting movement and locomotion and protection of vital internal organs and structures. The skeleton also has an important role in providing maintenance of mineral homeostasis, haematopoiesis and acid base balance.

Long bones are made up of two main regions the diaphysis and the epiphysis (Figure 1.1). The diaphysis is the tubular shaft between the proximal and distal ends of the bone. At the end of each long bone is a wider section called the epiphysis. The metaphysis is where the diaphysis and the epiphysis meet. During growth the epiphysial plate is contained in the metaphysis. The diaphysis is composed primarily of cortical bone, whereas the metaphysis and epiphysis are composed of trabecular meshwork bone surrounded by a relatively thin shell of compact bone (4).

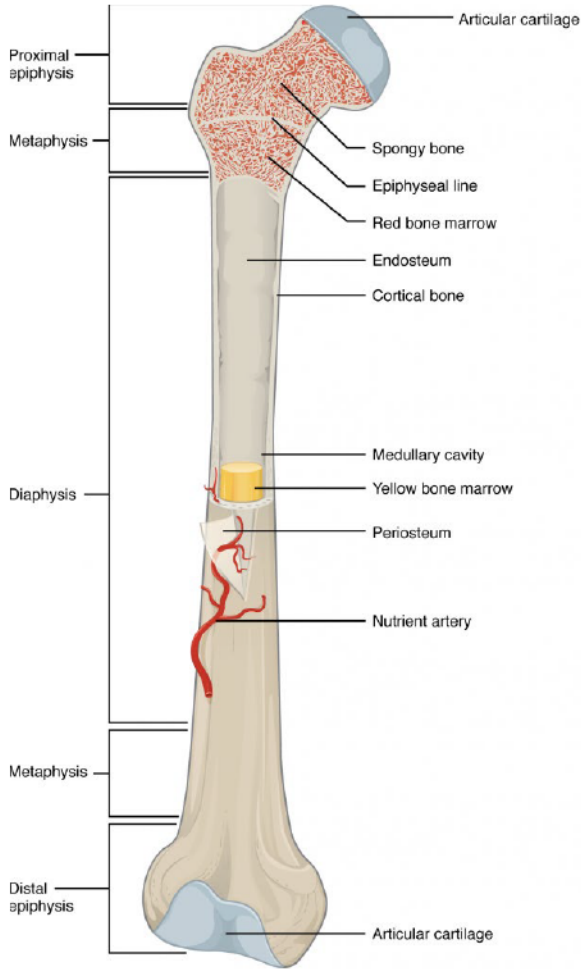

Figure 1.1 Anatomy of a long bone (Anatomy and Physiology http://cnx.org/content/col11496/1.6/) with permission

1.2.2 Gross anatomy of the hand and wrist

The hand and wrist have a total of 27 bones (Figure 1.2). The wrist contains the distal end of the two bones of the forearm, radius and ulna and eight irregularly shaped carpal bones namely the scaphoid, lunate, triquetrum and pisiform on the proximal row and trapezium, trapezoid capitate, and hamate on the distal row. The scaphoid and lunate articulate with the radius to form the radio-carpal joint. All the carpals in the distal row articulate with the metacarpals. The metacarpals articulate distally with the proximal phalanges. Each of the metacarpals is numbered, associated with a digit, and consists of a base shaft and a head. The bones of the fingers are called the phalanges, only the thumb has a proximal and distal phalanx, and the rest of the digits have proximal, middle, and distal phalanges making them fourteen in total. Although the phalanges are small, they are classified as long bones as they contain a shaft, distal head, and proximal base. The radius, ulna and short bones (phalanges and metacarpals) are formed by endochondral ossification and the carpal bones by intramembranous ossification. Maturation occurs earlier in the carpals than the long and short bones.

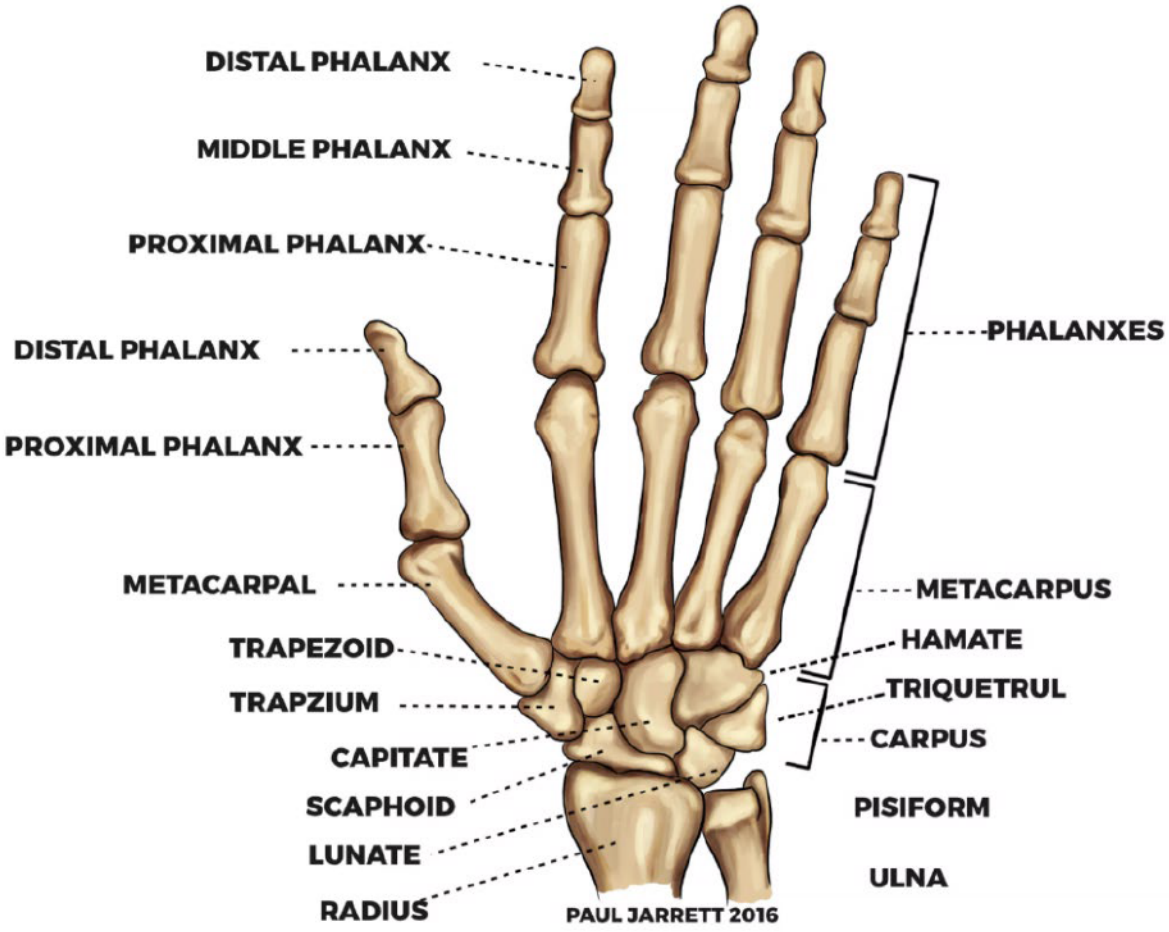

Figure 1.2 Hand and wrist anatomy (https://murdochorthopaedic.com.au/our-surgeons/paul-jarrett/patient-information-guides/hand-wrist-anatomy/) with permission

1.2.3 Growth plate

The growth plate, also known as the physis, is a cartilaginous area of elongation in a long bone that allows for endochondral ossification of the skeleton (Figure 1.3). This is located between the shaft of the bone and the secondary ossification centres at the ends of long bones where longitudinal growth takes place. The growth plate contains one cell type called the chondrocyte which is at different stages of differentiation (5, 6). The chondrocytes pass through three zones resting, proliferative, and hypertrophic zone, in a coordinated stepwise process.

Resting zone: the replication of chondrocytes at this zone is at a slow rate and these act as the stem-like cells that replenish the pool of proliferative chondrocytes (6).

Proliferative zone: replication at this zone is at a high rate and these line up along the long axis of the bone (5).

Hypertrophic zone: at some point the chondrocytes stop replication and differentiate into hypertrophic chondrocytes. During this process the chondrocytes increase in height by 6-10-fold, making an important contribution to longitudinal growth (5, 7). The hypertrophic chondrocytes secrete type X collagen into the cartilaginous matrix and the matrix begins to calcify and produce factors that attract the invading bone cells and blood vessels. Shortly before the blood vessels invade the chondrocyte lacuna hypertrophic chondrocytes undergo apoptosis (7). Parts of the matrix and the dead cells are removed leaving some vertical columns of remaining calcified cartilage.

Proliferation and differentiation of chondrocytes, secretion of extracellular matrix, calcification of the hypertrophic zone, invasion and differentiation of osteoblast, and formation of blood vessel repeat continuously in the growth plate throughout the growth process. During puberty the processes are accelerated and stop at the end of the puberty resulting in completion of longitudinal growth.

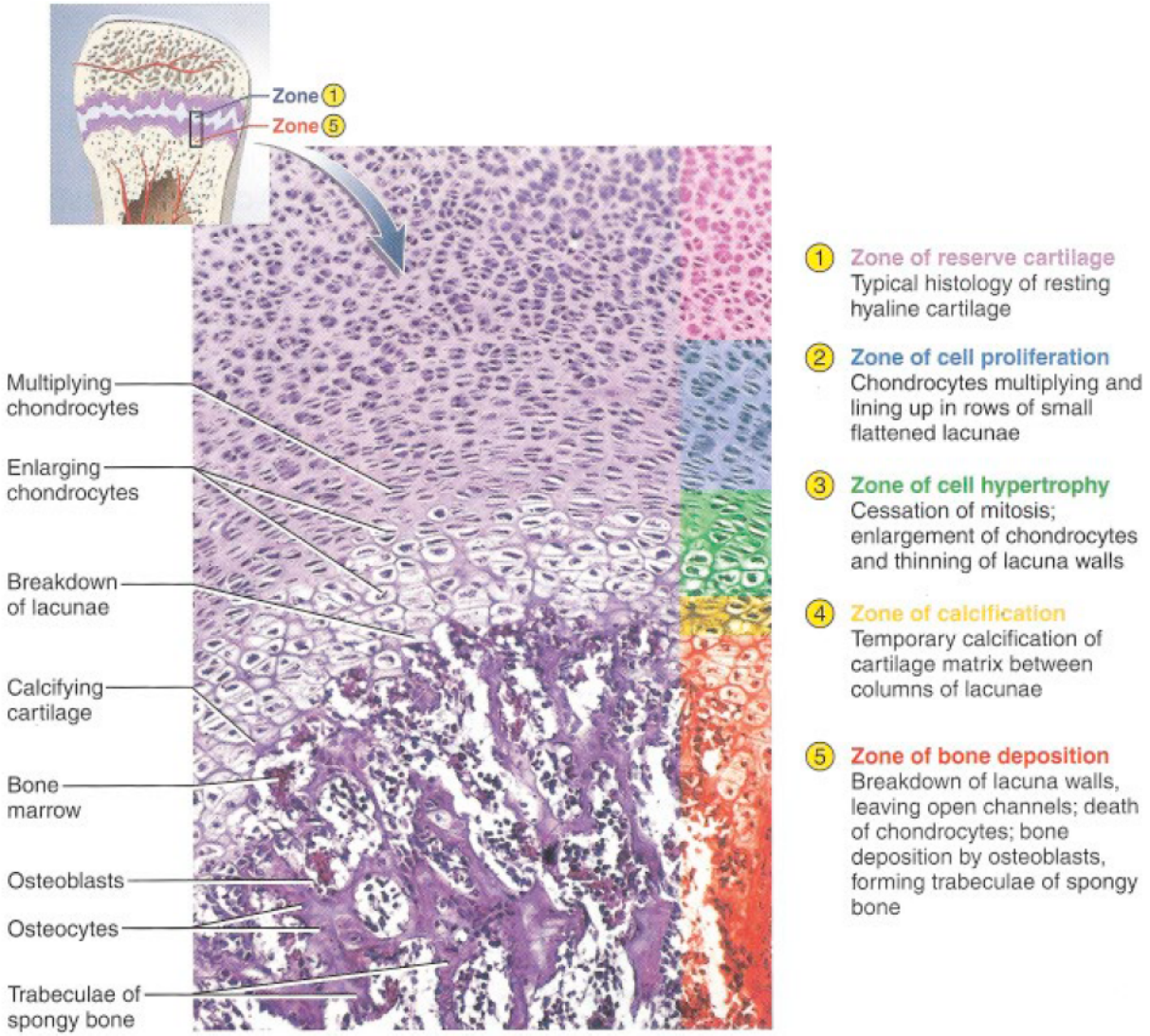

Figure 1.3 Structure and dynamics of an epiphyseal plate in a growing long bone (Saladin et al 2007

1.3 Skeletal development

Skeletal development involves the formation, growth and maturation of bones, a process which begins in utero and continues through adolescence until maturity is reached. The process encompasses several components including increase in size, changes in shape, mineralisation and maturation, beginning in the sixth or seventh week of embryonic life and continuing until about twenty five years of age although there are variations in individuals (8). Bone ossification is by two pathways intramembranous and endochondral ossification and each of these processes begins with a mesenchymal tissue precursor but differs on how the tissue transforms into bone.

1.3.1 Intramembranous ossification

Sheets of mesenchymal connective tissue are replaced with bony tissue during intramembranous ossification and this process produces flat bones of the face, most cranial bones, and the clavicles. Mesenchymal cells in the embryonic skeleton begin to differentiate into specialised bone forming cells, others will differentiate into either capillaries and some into osteoblasts. An ossification centre will be formed by early osteoblasts that appear in a cluster. Osteoblasts secrete osteoid, contributing to the mineralisation process, uncalcified matrix which eventually calcifies as mineral salts are deposited on it thereby entrapping the osteoblast. Entrapped osteoblasts transform into osteocytes, which are crucial for bone growth and renewal and are the most abundant constituting 90 to 95% of all bone cell (9). Trabecular matrix and periosteum are then formed, and compact bone develops superficial to the trabecular bone. The crowded blood vessels condense into red bone marrow.

1.3.2 Endochondral ossification

The development of long and short bones is by endochondral ossification, with prior cartilage formation and this process takes longer than intramembranous ossification. At six to eight weeks in utero mesenchymal cells differentiate into chondroblast (cartilage cells). The cartilage, which is avascular, is a flexible, semi-solid matrix that surrounds and isolates chondroblasts to form chondrocytes. A mixture of cells invade the centre through penetration of capillaries establishing the primary ossification centres (Figure 1.4) (10). Cartilage and chondrocytes continue to grow at the ends of the structure which will eventually become the epiphyses. This increases the structures length, with bone replacing cartilage in the diaphysis whilst remodelling inside the medullary cavity is also happening. Cartilage remains at the epiphyses and the joint surface as articular cartilage by the time the foetal skeleton is fully formed.

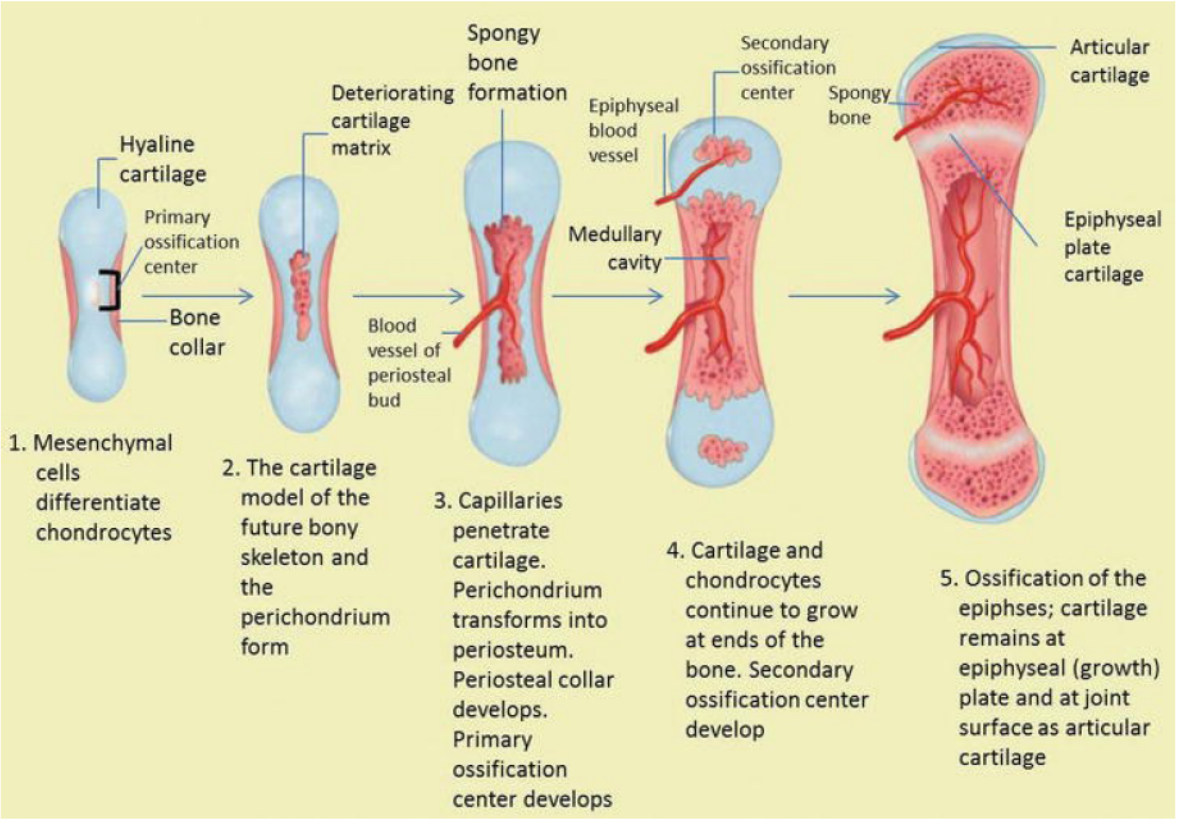

Figure 1.4 Development of endochondral bones Schematic diagram showing the events leading to replacement of an embryonic cartilage model by bone (Setiawati and Rahardjo 2019)

1.3.3 Skeletal maturation and secondary ossification centres

Secondary ossification centres develop at different time points after birth and gradually encroach on the growth plate, such that by the time skeletal maturity is achieved, the cartilage is replaced by bone (except at the articular surfaces) (11). This process completes at the end of puberty, followed by a period of mineral accumulation after which the bones will be fully mature and longitudinal growth stops. In order to allow the complete ossification process, osteoclasts and osteoblasts are directed, by blood vessels, into the new cartilage tissue (12). There are many ossification centres within the skeleton with each individual bone of the hand, foot, elbow, knee, and pelvis having several ossification centres. These are visible on the radiograph when they begin to mineralise and the age of appearance of the individual ossification centres is a useful assessment of the stage of skeletal maturity. In most healthy children, this established sequence of ossification in each bone of the hand, is remarkably constant and the same for both sexes. The objective measure of skeletal maturation is known as BA (i.e., numerical description of the degree of skeletal maturation) assessed from a hand wrist radiograph. The hand and wrist are used to assess skeletal maturation because they contain many small bones (27 bones, radius, ulna, metacarpals, and phalanges), each with its own ossification centres

that passes through the stage of enlargement and shaping of the ossified area, acquiring one or more epiphyses and finally reaches its adult form with epiphyseal fusion which marks the attainment of final adult height (13). The ossification centres of the distal radius appear at one year and that of the ulna may appear at 5-6 years. Variations come in the relative times the appearance of the ossification centres occur and hence skeletal maturity looks at the number of centres present and the stage of development of each. Skeletal maturity differs from skeletal growth in that growth refers to the quantitative increase in size or mass and maturation refers to the sequence of changes that lead to a highly organised specialised and mature state (1).

1.3.4 Stages in the assessment of skeletal maturation

The first centre of ossification to appear is that of the capitate and the hamate of the carpal bones and these appear at two months in girls and about four months in boys (14). The other ossification centres progressively appear. During the pre-pubertal stage assessment of skeletal maturation is primarily based on the size (width) of the epiphysis in relation to the adjacent metaphysis as shown in Figure 1.5A-C (15). This stage of development is characterised by ossification centres for the epiphyses becoming as wide as the metaphysis by increasing in width and thickness. Contours of the epiphyses overlap, or cap, the metaphysis during puberty (Figure 1.5D). The pisiform and the sesamoid become recognisable afterwards (Figure 1.5D-F).

During late puberty, the fusion of the epiphyses to the metaphysis in the long bones of the hand tends to occur in a characteristic pattern: 1) fusion of the distal phalanges, 2) fusion of the metacarpals, 3) fusion of the proximal phalanges, and 4) fusion of the middle phalanges (15). After puberty, all carpals, metacarpals, and phalanges are completely developed, their growth plates are closed, and at that stage the assessment of skeletal maturity is based on the degree of epiphyseal fusion of the ulna and radius (Figure 1.5E and F).

It is important to consider that maturation is not linked to time in a chronological sense. Children may pass through the same chronological time span but at different rates of maturation. The ultimate height potential of an individual may be predicted using the extent of skeletal maturation observed in an individual. The more delayed an individual is in skeletal maturation the longer time they may have before epiphyseal fusion prevent further growth which means an extended window of opportunity to catch up on growth (16).

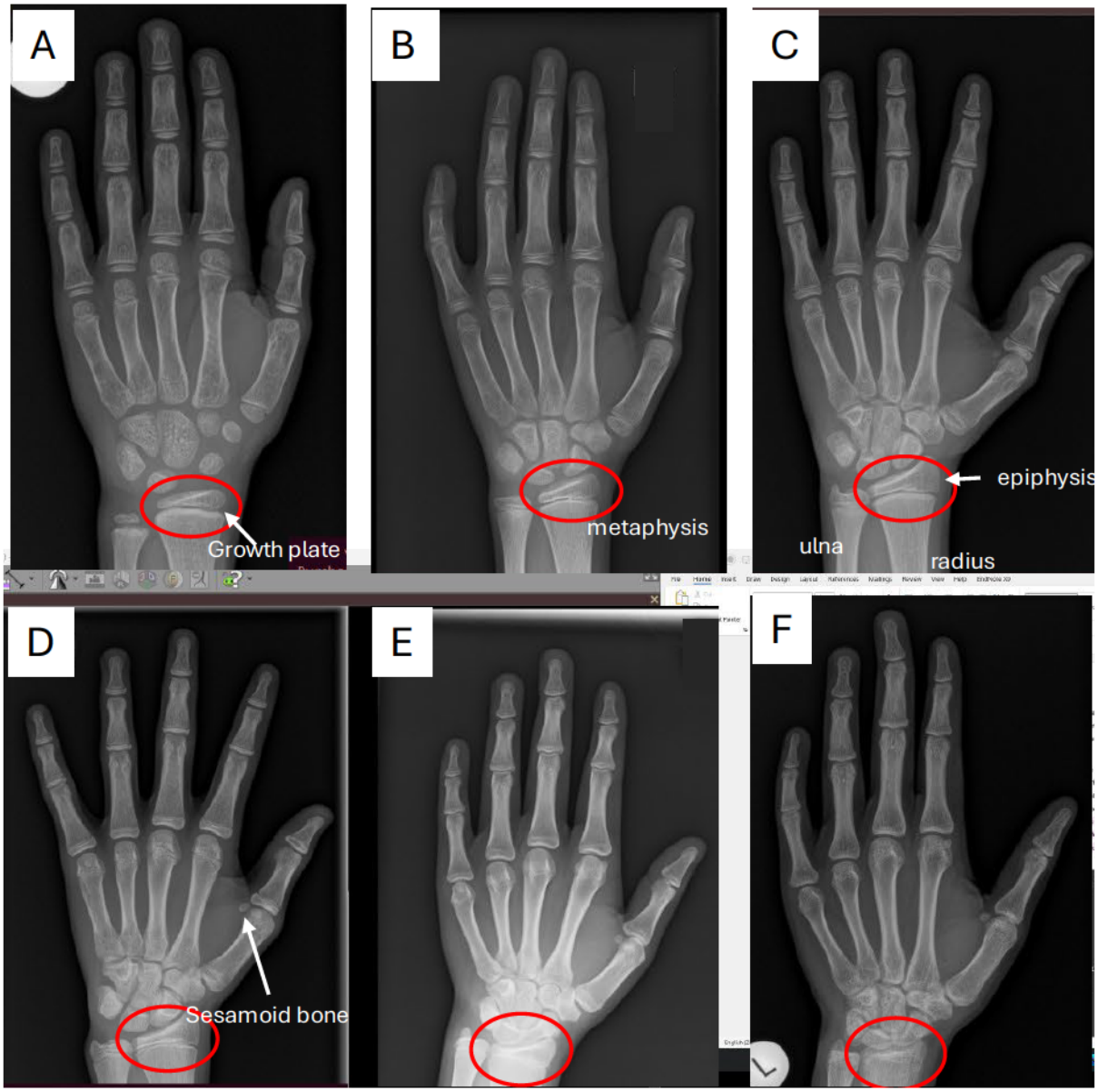

Figure 1.5 Image showing the different stages towards epiphysial fusion of the bones of the hand wrist with particular emphasis on the radius by Farirayi Nyakoko

1.4 Linear growth

Linear or longitudinal growth refers to the increase in height over time and has been recognised as a reliable indicator for a child's general health. This longitudinal growth and development of bone occurs during childhood and adolescence at the growth plates where cartilage proliferates in the epiphyseal and metaphyseal areas of long bones. Linear growth can be divided into four main phases, foetal, infancy, childhood and pubertal, and the mechanism underlying the control of growth in each phase are distinct although each of the phases exist as part of a continuum. There are variations in the pattern of growth during the course of life such that rapid growth is observed during foetal and infancy phases, slows down during childhood until puberty when the growth spurt occurs (17). A child's growth is compared with that of his or

her peers by using relevant growth charts as a reference point like those developed by the World Health Organisation (WHO) (Figure 1.6 and 1.7). The WHO growth standards were developed to harmonise growth assessment tools from widely different ethnic backgrounds and cultural settings. Zimbabwean children aged 0.5 to 4.9 years were reported to have height-for-age z-scores significantly below the WHO child growth standards (18). Growth potential is genetically determined, and growth pattern is influenced by several factors that include endocrine, environmental and nutritional factors (19). Sex-specific patterns in the tempo of growth, the timing of the adolescent growth spurt, overall size, and the age of skeletal maturity are well known with differences evident as early as in utero (20).

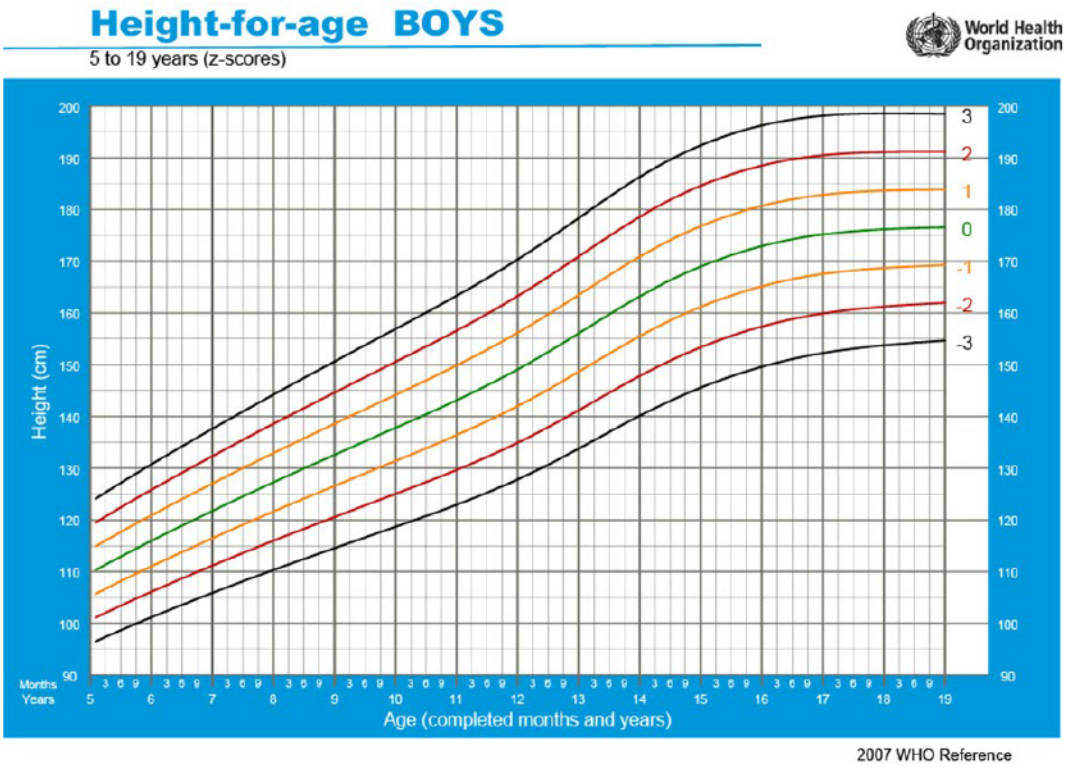

Figure 1.6 Height-for-age z-scores reference for boys aged 5-19 years (World Health Organisation 2007)

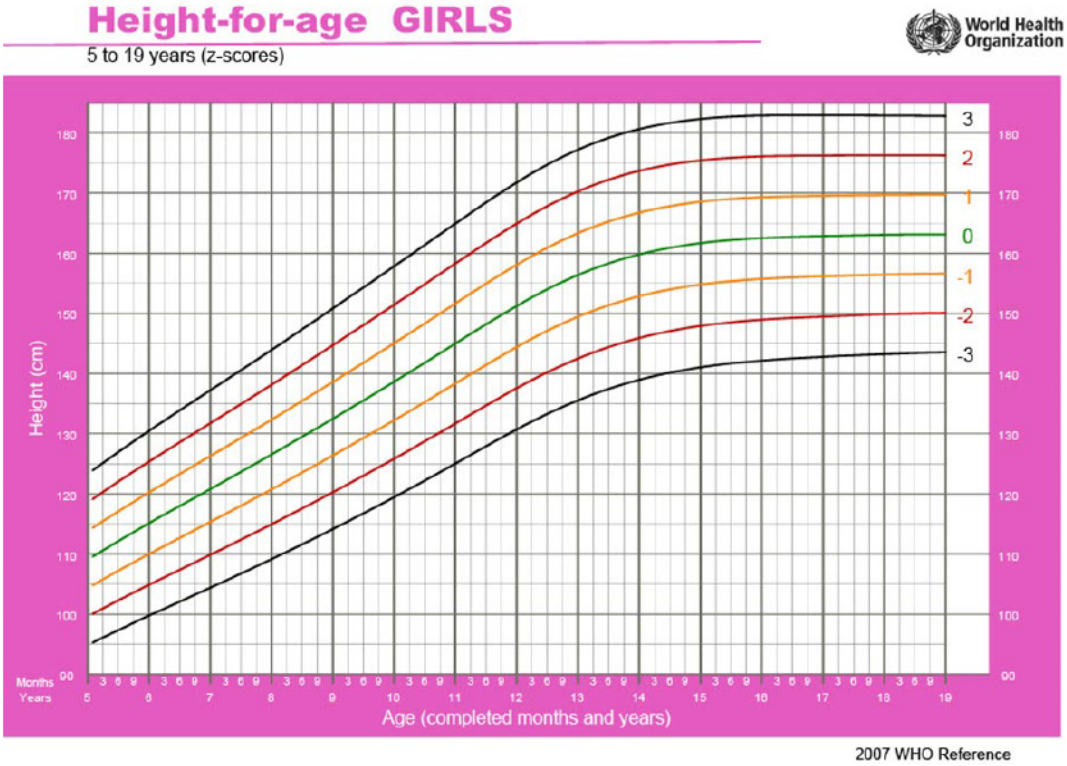

Figure 1.7 Height-for-age z-scores reference for girls aged 5-19 years (World Health Organisation 2007)

1.4.1 Control of linear growth and maturation

1.4.1.1 Endocrine control

There are various regulators that influence chondrocytes in the growth plate that help determine the rate of proliferation and maturation which include GH, insulin-like growth factor I (IGF-1), thyroid hormones, eostrogen and androgen. The GH is produced by the pituitary gland and secreted into the blood stream, when it reaches the liver, it stimulates the hepatic production of IGF-1 which in turn stimulates chondrocyte proliferation and hypertrophy at the growth plate (21). The GH can also stimulate the local production of IGF-1 at the growth plate to stimulate growth in a paracrine/autocrine fashion and may directly stimulate growth at the growth plate by recruiting the resting zone chondrocytes into the proliferative zone. The IGF-I increases bone formation by stimulating the differentiation and activity of osteoblasts involved in altering the length of long bones through endochondral ossification (21, 22).

With the onset of puberty pulses of gonadotropin-releasing hormone (GnRH) are released into circulation which triggers production and release of sex steroid hormones, i.e. oestrogen and testosterone. Increased oestrogen triggers GH and IGF-I production to initiate the growth spurt and stimulating the closure of the growth plate through accelerating the depletion of the

resting chondrocytes in the growth plate a hallmark of skeletal maturation (7, 23, 24). In males, the primary source of oestrogen is through the conversion of testosterone through a process called aromatisation by an adrenal enzyme called aromatase. A deficiency in aromatase results in little or no growth spurt in individuals (25). Testosterone may also directly stimulate bone growth, and unlike the effect of oestrogen, is not associated with an increase in GH. In children with precocious puberty, premature oestrogen exposure therefore leads to premature epiphyseal fusion and decreased final height and, conversely, in individuals with hypogonadism, a lack of oestrogen leads to delayed epiphyseal fusion and tall stature.

The thyroid gland produces thyroid hormones ,5,3'-triiodothyronine (T3) and thyroxine (T4), which are important regulators of metabolism and an important stimulator of normal linear growth. Thyroid hormones support linear growth by promoting the recruitment of resting chondrocytes into a proliferative zone, as well as stimulating chondrocyte hypertrophy (Figure 1.3). Thyroid hormones also indirectly stimulate growth by modulating the production of GH and IGF-I (26). Hypothyroidism delays longitudinal bone growth and endochondral ossification, while thyrotoxicosis, which results in excessive production of thyroid hormones, accelerates both processes. Nonetheless, both hypothyroidism and thyrotoxicosis eventually lead to short stature. Poor growth and skeletal maturity delay is seen in children with GH deficiency and hyperthyroidism (7).

1.4.1.2 Nutritional control

Many macro- and micronutrients are essential for growth and development of the skeleton.

Macronutrients: Inadequate dietary intake of nutrients results in negative consequences on growth and development with protein and energy malnutrition potentially leading to poor linear growth (27). Protein and amino acids are recognized as the main nutrients involved in linear growth. Proteins play a permissive role in growth, since they fulfil the metabolic demand of amino acids, required for tissue growth, and increase levels of hormones, such as insulin and IGF-I, which stimulate endochondral ossification (19). Amino acids are critical for normal growth and matrix formation by chondrocytes.

Micronutrients: Micronutrients are vitamins and minerals required by the body in very small amounts but essential for growth, development and functioning of the body. Deficiencies in micronutrients as a result of inadequate dietary intake, poor absorption or due to some medical conditions may cause abnormalities in nutrient metabolism, which may or may not be reversible especially if they occur during critical periods of growth like childhood and adolescence. Calcium and vitamin D are some of the key micronutrients for bone development

and growth, calcium plays a major role in skeletal mineralisation and 99% of it is found in bone (28). Vitamin D is required for calcium absorption and utilisation and inadequate intake may result in decreased calcium absorption and risk of rickets in children a condition associated with poor growth hence delayed skeletal maturation (29). Because the micronutrients are essential elements it is important that recommended amounts are introduced through diet and the requirements are high during childhood and adolescents for growth and development. The recommended daily calcium intake varies between 700-1200mg/day (30). Vitamin D deficiency is associated with stunting and being underweight and overweight in children (31, 32). Amongst other micronutrients required for growth are zinc, phosphorus, vitamin C, vitamin K, magnesium and iron and these are needed by children and adolescents, deficiencies of which affect skeletal development and growth (33).

Poor absorption or inadequate intake of bone forming minerals like calcium and zinc may contribute to linear growth retardation. Other micronutrients are important for organ and tissue function and integrity and with the exception of vitamin D that can be synthesised in the skin from sunlight most of these micronutrients have to be supplied through diet (27). Vitamin D influences endochondral ossification by stimulating cellular maturation through the vitamin D receptor. Low dietary calcium intake was linked to late puberty, low BMI, and relatively high rates of stunting in The Gambia (28, 34-37). Figure 1.8 summarises the determinants of growth from foetal growth through to puberty.

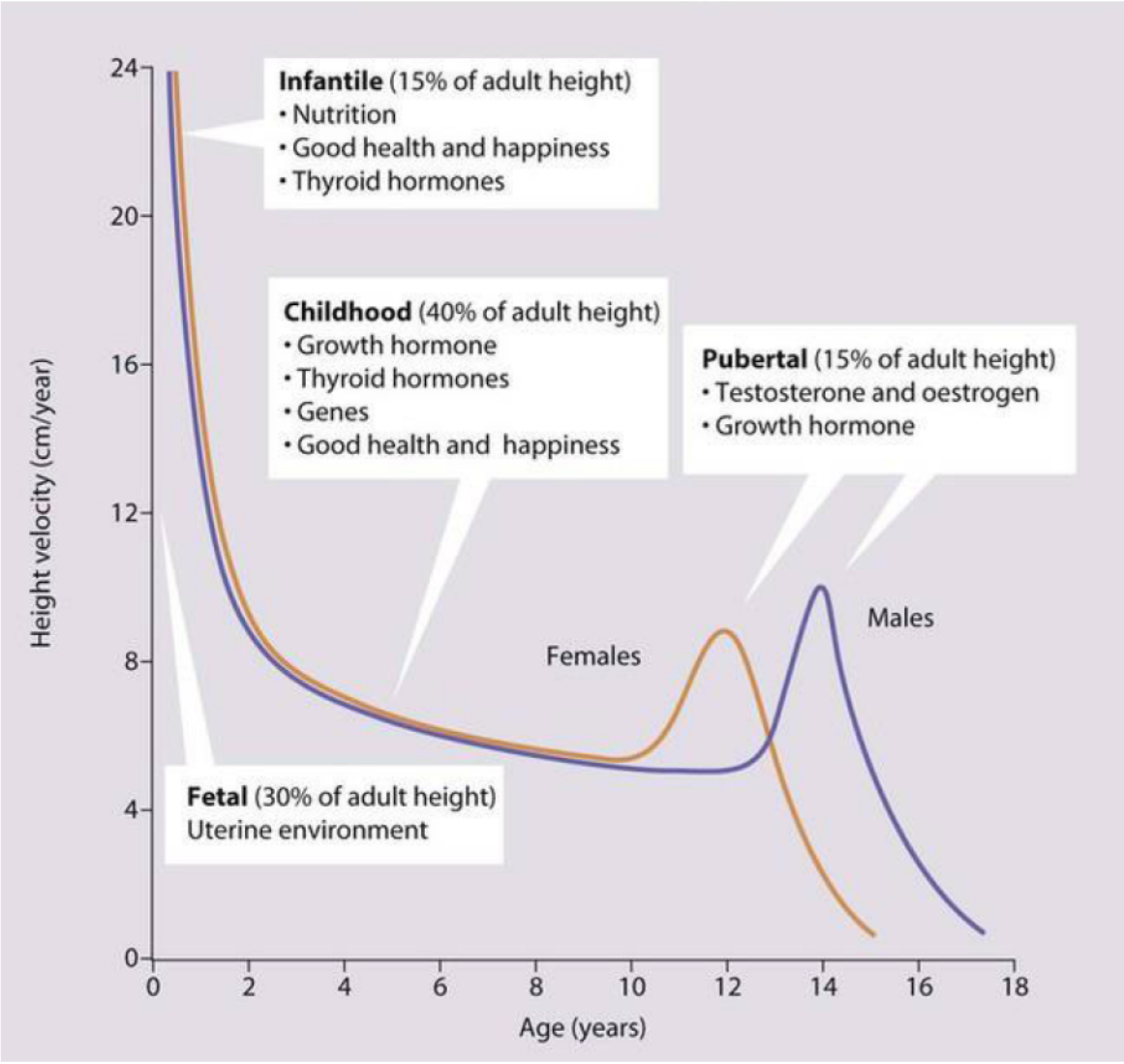

Figure 1.8 Determinants of growth (https://www.slideshare.net/slideshow/l4-endopptx/257614797)

1.4.2 Foetal growth

Major organs are formed during the first trimester due to rapid growth and differentiation and these continue to rapidly grow throughout the second and third trimester with the crown rump velocity of 62cm/year in the second trimester and 48cm/year in the third trimester (38).

Adverse intrauterine conditions such as maternal HIV infection may lead to intrauterine growth retardation through several mechanisms. Poor nutritional status Poor nutritional status as a result of HIV induced wasting is an established risk factor for adverse pregnancy outcomes such as preterm birth, low birth weight, intrauterine growth retardation (39) and will likely increase the risk of mother to child transmission of HIV (40). Additionally, morphological changes in the placenta that contribute to delivery before 37weeks (41) may impair nutrient absorption and oxygen transport critical for the growth and development of the foetus. These adverse intrauterine conditions may have a lasting impression on skeletal growth during the

early years of life, such that the individual's ability to attain full genetic potential may be compromised, although some catch-up growth is possible (42). The population studied in this PhD consists of children with vertically acquired HIV.

1.4.3 Infant growth

Infant growth is the next stage of growth and is the period of fastest growth rate. Infants triple their weight and gain an average of 25cm height gain during the first year of life exhibiting patterns of growth consistent with their genetic backgrounds during this phase (38). Assessing growth and skeletal maturation during this period may help identify underlying growth and development issues and allow for early intervention of the growth process such as nutrition screening.

1.4.4 Childhood growth

This next phase of growth predominates from 3 years of age continuing until the onset of puberty (43). Height velocity during this stage is relatively constant at 4–8 cm/year on average and the lowest is usually in the immediate pre-pubertal period because levels of the GH and IGF are low at this stage and more resources being directed to the development of reproductive organs and other physical changes associated with sexual maturation. Growth assessments should also be conducted during acute care visits, as illnesses can impact growth.

1.4.5 Pubertal growth

This period is characterised by changes which include growth acceleration, alteration in body composition, appearance of secondary sexual characteristics and ultimately deceleration and cessation of skeletal growth (38, 44). Gonadotropin-releasing hormone (GnRH) neurons of the hypothalamus control the initiation of puberty. When the GnRH is released in a pulsatile manner it stimulates the release of FSH and LH from the anterior pituitary gland which in turn acts on the gonads to stimulate the synthesis and release of sex steroid hormones (oestrogen/progesterone and testosterone) and support gametogenesis (formation and development of oocytes/sperm). Sex steroids exert negative feedback on the hypothalamus and pituitary gland to ensure circulating levels remain stable. A rise in FSH stimulates an increase in oestrogen synthesis and oogenesis in females while in males it activates Sertoli cells in the testes, triggering the onset of sperm production. Conversely, a rise in LH stimulates an increase in progesterone production in females while in males, it stimulates Leydig cells in the testes to produce testosterone. Hormonal changes caused by rises in FSH and LH allow for the physical changes of puberty to begin. The first sign of puberty in girls is breast development, and the final

marker of puberty is menarche. In boys, testicular enlargement is the first sign of puberty followed by an increase in growth velocity and subsequent spermarche. Puberty was classified into five stages by Marshall and Tanner in both boys and girls based on the somatic changes in breast, pubic hair, and genital development (Figure 1.9) (45, 46).

The onset and duration of puberty differs between adolescents living in different environments with varying nutrition status and environment (47). The average age of onset of puberty defined as reaching Tanner stage 2 (testicular enlargement in boys and breast development in girls) was reported at 12.5 years and 12.7 years in boys and girls respectively, in the Birth to Twenty cohort in South Africa (48). In The Gambia, later timing of puberty in boys was reported at 16.1 years (37). Additionally, later age at menarche was reported in African populations at 14.90 years in The Gambia (49), 13.2 years in Mozambique (50), and 15.26 years in Nigeria (51). In the studies conducted in Mozambique and Nigeria, a structured questionnaire was used, and the participant was asked to recall the timing of their menarche. In contrast, the study in The Gambia only enquired whether menarche had occurred with a yes/no answer recorded, no specific timing details were asked thereby leading to less precise data. However, menarche was occurring earlier at 12.25 years in the USA, (black girls at 12.0 years and white girls at 12.7years) (52). There are many factors that affect menarche such as body mass index (BMI), family size, socioeconomic status, parental educational level, occupation of parents, loss of parents, child sexual abuse, physical stress, tea consumption, and passive smoking, genetic factors, race, environmental conditions, nutrition, physical activity, geographic location, urban or rural residence, health status, psychological factors, blindness (53-59). In Zimbabwe chronic conditions like HIV have been associated with later age at menarche (60).

Although there are variations between individuals and populations, the pubertal growth spurt occurs on average at age 9-10 years in girls and 11-12 years in boys. Pubertal development usually lasts 3–4 years though as with any stage of growth, the length can be dependent on environmental influences such as chronic illnesses, nutritional intake, physical activity and body composition (49). Additionally, during adolescence 20% of final adult height and 50% of the adult weight is gained on average (47).

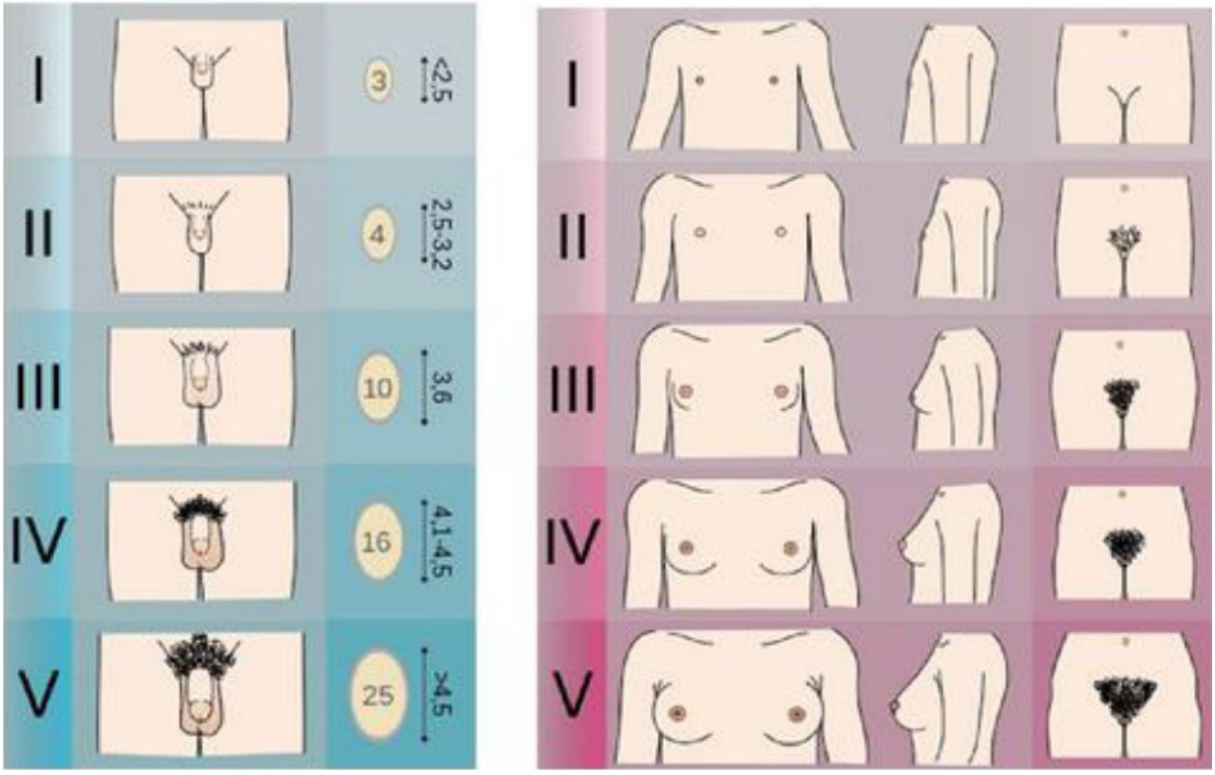

Figure 1.9 Tanner pubertal stages (Tjahynto et al, 2023)

1.4.6 Final adult height and skeletal maturity

When the epiphyseal plates fuse i.e. when skeletal maturation is reached, growth will have been completed and final adult height is reached. Final height in the maximum height an individual may reach and is achieved as a result of a combination of several factors which include the genetic potential, environmental factors, hormonal factors, nutrition and overall health. The genetic potential for height is determined by the genetic make-up inherited from both parents however, whether a person reaches their genetic potential is also affected by other factors like environment, nutrition, and disease patterns. Stunted children are not likely to reach their full genetic potential for height even if they experience catch-up growth (61). About 20% of the variation in adult height in high income countries is due to environmental factors with the proportion likely larger in low-income countries because of socio-economic differences and disease patterns such as HIV and poor nutrition causing delays in growth (62). Studies in Africa have demonstrated high prevalence of low height for age Z-scores, weight for age Z-scores and BMI for age Z-scores and the children may not attain their genetic potential for adult height. People living with HIV had a significantly reduced final adult in America (63, 64). Understanding factors which impact skeletal growth and maturation will provide an opportunity for intervention before skeletal maturity is reached.

1.5 Impaired linear growth

Assessment of growth and maturation is important because it helps healthcare workers monitor a child's development and health to ensure they are progressing within the normal expected range. On the growth standards, children who cross percentiles down wards show impaired linear growth (Figure 1.10) (17). The monitoring will help identify any growth disorders and guide treatment and interventions to promote normal growth. There is growing evidence of the long-term consequences of childhood stunting (poor linear growth) which include short adult height, impaired health and impaired educational and economic performance later in life (65, 66). Long term follow up of 1338 adults studied as children in 1969-1977 was conducted in Guatemala (67). Being stunted at 2 years was associated with less schooling, a lower test performance, a lower household per capita expenditure, and an increased probability of living in poverty. Specifically, stunting was associated with a lower age at first birth and, in women, a higher number of pregnancies and consequently children.

Furthermore, being stunted in childhood may increase the risk of developing cardiovascular disease in adulthood (66, 68-72). Stunting was related to early changes in cardiovascular function where stroke volume, arterial function, and cardiac output were lower in stunted children than in non-stunted children in a South African study of 775 children aged 10-15 years (73).

Given these known associations between poor growth and impaired cognitive development or morbidity and mortality, it is imperative to promote weight and height gain in children with growth failure especially in LMIC's. Whilst evidence on interventions to improve linear growth and the subsequent health outcomes is scarce in LMIC's, faster linear growth at age 2 years was associated with a reduced risk of short adult stature and poor educational attainment (74). Therefore, it is vital to focus on improving childhood nutrition and promote linear growth from an early age as there are lifelong benefits to individuals and their families.

Some chronic diseases and malnutrition can delay skeletal maturation and the onset of puberty. Chronic conditions like HIV are known to impact growth. CLWH have been reported to have poor growth early in life (75). Poor growth in HIV is caused by identifiable illnesses and secondary comorbidities which accompany HIV infection. Severely impaired growth among vertically acquired HIV-infected children in Africa may be partially attributable to the generally later treatment of HIV, often at more advanced disease stages (76).

Clinical diagnosis of poor growth may also be familial short stature or constitutional delay of growth. The two are similar in that the child is healthy and grows normally although below the normal standards for height. In familial short stature the children are born of smaller parents

which may include either one or both parents being short and will eventually attain less height in comparison to children of taller parents. In these children BA will approximate CA as the children will be growing at an appropriate rate during childhood. They also attain sexual maturation and pubertal growth spurt at the ages they are expected to. In constitutional delay of growth there is a delay in the tempo of growth (fast or slow growth) where one requires more time to complete the growth process caused by growth plates not yet fused. The children usually are delayed in reaching adolescence. BA is expected to advance 1 year for each calendar year and for these children, BA tends to progressively deviate from the CA meaning that they fail to reach their maximum height potential (20)

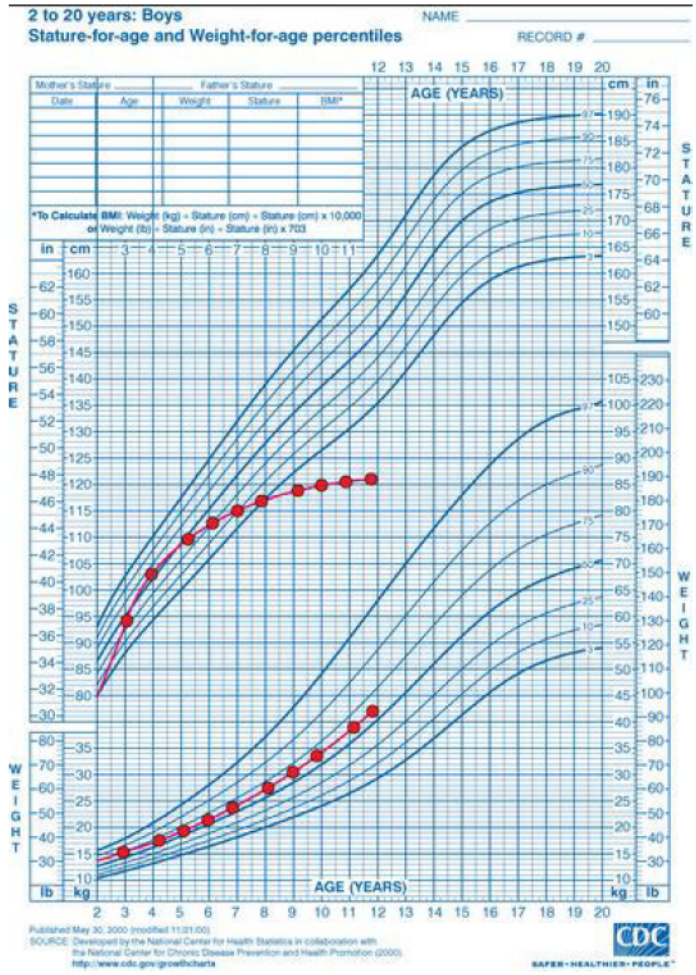

Figure 1.10 Impaired linear growth (//www.scribd.com/doc/172390973/Growth-Chart-Boys-2-to-20)

1.6 Sexual dimorphism, skeletal maturation and puberty

The pattern of bone maturation process is similar in individuals but the rate of maturation differs by sex and ethnic groups attributable to differences in the timing of pubertal onset (20). Skeletal maturation in healthy children begins accelerating earlier in females than in males (2, 77). In females the acceleration of skeletal maturation may occur even before puberty as

estrogen is higher in females than males before puberty(13, 78-80). The onset of puberty usually occurs two years earlier in females than in males (79) corresponding to a BA of approximately 11 years in females and 13 years in males (20), and triggers rapid production of sex hormones which are crucial in the regulation of skeletal maturation and growth (81). Therefore, females are more advanced in skeletal maturation than males from birth and the trend continues throughout childhood and adolescence, plus the process of skeletal maturation lasts longer in the males than in the females by about 2 years (45, 46, 77). These sex differences were first reported in 1925 by Pylor where centres of ossification in the epiphyses of the metacarpals and phalanges appeared in the females during the first year and nearly all by the end of the second year, whilst in the males most appeared late in the second year (82). The same study reported results from 82 radiographs of females and 64 radiographs of males aged 12-22 years showing fusion of the epiphyses and the diaphysis taking place 3 to 4 years sooner in the females than in the males.

A positive correlation between the severity of skeletal advancement (measured as BA-CA) and the severity of pubertal advancement (assessed as the normal age at pubertal onset [12 years] minus the CA at onset of puberty) was reported raising the possibility that skeletal maturation might provide the timing of the onset of puberty (83). This was a study conducted in boys with congenital adrenal hyperplasia, CAH (n=13) and familial male-limited precocious puberty, FMPP (n=22), two conditions that accelerate maturational tempo, and boys with idiopathic short stature, ISS (n=18) in which maturational tempo is sometimes delayed. All the participants were on various treatments for their conditions. In contrast, a study of 30 healthy boys in the USA found no significant correlation between skeletal maturation and pubertal onset (84). The findings differ with typical physiological expectations, and the discrepancy may be compounded by the small sample size hence not adequately powered to identify an association.

1.7 Factors that influence skeletal maturation

There are many factors that influence the rate of skeletal maturation, and these include genetic variation, nutrition, socio-economic deprivation, and certain diseases. These are thought to contribute to the variations in skeletal maturity within and between populations (77). Because of the different genetic and environmental factors, populations from differing geographical locations will differ in mean skeletal maturity at any given age and in the pattern of skeletal maturity increments from one age to the next (77).

1.7.1 Race and ethnicity

The rate of overall growth and skeletal maturation has been shown to vary in different ethnic groups. Black children were taller and grew at a faster rate than their white counterparts in a UK study of approximately 18000 children of different ethnic groups (85). Similarly children of Asian and African ancestry had older BA when compared to European children in a large multiethnic population-based cohort (n=5325) in the Netherlands aimed at assessing the influence of ethnicity on skeletal maturation (86). Although there were more European (83.5%) children than those of African (10.3%) and Asian (6.2%) descent, the differences were still evident. An earlier study conducted in 765 children aged 0-19 years from the USA classified the participants into four ethnic groups i.e., as white, black, Asian, and Hispanic (the Hispanic were Mexican Americans, and the Asian group were from China, Japan, Cambodia, Vietnam and The Philippines) (87). The age groups were categorised as early childhood (0-3 years), middle childhood (4-8 years), late childhood (8.5-13 years), and adolescence (up to 18 years). Greater delays in BA were seen in African children, Hispanic females and in Asian and American males. However, the study focused on recruiting trauma cases and since males are generally more inclined to engage in risky behaviours, this led to a higher number of males than females participating, potentially introducing selection bias. In another study conducted in the USA of 534 White and Black American children of European and African descent aged 0-19 years, variations in skeletal maturation were seen by ethnicity (88). Prepubertal American children of European descent had significantly delayed skeletal maturation when compared with those of African descent; and post pubertal European American males had significantly advanced skeletal maturation when compared with post pubertal African American males. In contrast, Black boys were delayed in skeletal maturation when compared to white boys whilst the girls showed the same pattern and timing of skeletal maturation in a longitudinal study conducted in 607 South African children aged 9-20 years (89). These studies show differences in the pattern of growth by ethnicity although the differences differ by geographic location potential reflecting the influence of socio-economic status (SES). This literature further supports the use of standardised growth charts or development of skeletal maturity standards specific to different ethnic groups to avoid misdiagnosis of growth-related conditions like advanced or delayed growth and development.

1.7.2 Socio-economic status

Some authors have concluded that it is not the ethnic differences but rather the SES of a given population that determines the rate of skeletal maturation (90). Delayed skeletal maturation was reported in low SES status groups in a large Sudanese study of 1683 children aged 3-25 years (91) and high SES status was associated with an advanced skeletal maturation

in a Moroccan population of 623 children aged 6-19 years (92). In contrast, there were no skeletal maturity differences between different SES groups except in the 10–11-year-old boys and girls in a longitudinal growth cohort of 507 children aged 8-16 years followed annually over four years in Portugal (93). The authors could not explain why the differences were observed in that age group, however, that may be consequences of measurement error as BA was assessed manually. There were variations in the measurement of SES in the different studies as there is no standard measure of SES.

1.7.3 Nutrition

Overweight children have accelerated linear growth and undergo accelerated sexual maturation at an earlier age than children with normal weight (94). Hence several studies reported advanced skeletal maturation in overweight children (95-97). The aromatase activity of adipose tissue is thought to increase the total amount of oestrogen in circulation hence the advanced development of overweight children. BA was more advanced in African American children than White children and this was accounted for by their greater adiposity as they had higher BMI SDS (BMI standard deviation score) than the White children in a study of 252 African American and White children aged 5-12 years (98). These findings were also confirmed in a smaller study in Italy that compared skeletal maturation in 25 obese and 25 normal children (mean CA 10 years), obese children were more skeletally advanced than the normal weight children (99). Although a study conducted in China to verify the association between accelerated skeletal maturation and obesity was of much younger children aged 3.1-6.6 years, it was a relatively larger survey of 1330 preschool children (100). The children were classified into four groups according to the International Obesity Task Force (IOTF); underweight, normal, overweight, and obese. The study reported an increased risk of accelerated skeletal maturation in obese participants confirming the association between obesity and accelerated skeletal maturation. Accelerated skeletal maturation results in a decreased final height as it accelerates the early closure of the epiphyseal plate terminating growth prematurely (100, 101). In contrast, a Malawian study reported no relationship between BMI and skeletal delay in a study to determine if skeletal delay was related to nutrition status as measured by BMI (102). Normal BMI was defined as 20 to 25kg/m^2 and only eight children were in the normal range the rest were undernourished (BMI<20kg/m^2). The authors suggested that the negative results were potentially due to the small sample size over a wide age range, or the inaccuracy of the GP method in BA assessment. However, the normal range for BMI (20 to 25kg/m^2) used in this study is not ideal for children and adolescence as according to the IOTF mentioned above, different age groups have different ranges of normal BMI. The fact that BMI is weight adjusted for height and skeletal maturation has a fixed relationship with height (as the process of skeletal

maturation is occurring height is increasing) it would be more ideal to use weight for age Z-scores instead of BMI.

Because malnutrition encompasses both over- and under-nutrition, and poor micronutrient status, it is essential to study the association between under-nutrition (highly prevalent in Africa) and skeletal maturation to assess the growth and development in these children as studies described above focused on over-nutrition.

Few studies have measured BA in populations with micronutrient deficiencies. In Italy, low calcium intake was associated with lower mean BA and delayed pubertal growth in 50 girls aged 11-14 years, selected from the lowest and highest ends of the calcium-intake distribution (103). The participants were asked to record all they consumed on selected days and the mean consumption of food products per day was converted into calcium intake (mg/day) using the National Food Composition Tables. These data were then used to categorise participants into low or high calcium intake groups. Girls with a low calcium intake (n=22) had a lower mean BA of 11.96±0.30 years than those with a high calcium intake (12.98±0.14 years). Again in, Afghanistan, malnourished (defined as having z-scores of <-2 for weight-for-age, height-for-age and BMI) children had delayed skeletal maturity, more so in boys in a study of the effect of vitamin D supplementation and dietary nutritional intake on skeletal maturity and bone health in socio-economically deprived children (29). In this study a total of 565 hand wrist radiographs were analysed. Calorie intake rather than vitamin D influenced skeletal maturity as children with high calorie intake had better height velocity and less delayed skeletal maturity. The authors highlighted the importance of overall calorie intake in the diet rather than micronutrients. However, concluding that vitamin D is not essential for skeletal maturation based on the supplementation overlooks the role of sunlight in vitamin D synthesis. Vitamin D is not solely obtained from dietary intake; a significant amount is synthesised by the body when there is exposure to sunlight. This means that while dietary sources of vitamin D are important, they are not the only means of obtaining this vital nutrient.

Unlike in the above studies, energy intake rather than micronutrients influenced the rate of skeletal maturity in a study that introduced three supplements to a group of infants and toddlers aged 12 -18 months (104). The children received either a high energy and micronutrient supplement, a low energy and micronutrient supplement, or a low energy supplement. After 12 months of supplementation the group receiving the high energy and micronutrient supplement had advanced BA compared to the other two groups. No difference in BA was seen between the group receiving the low energy and micronutrient supplement and the group receiving the low energy supplement alone.

It is essential to understand whether it's micronutrient deficiency, macro-nutrient deficiency or both that are related to skeletal maturity delays, regardless it is likely to be population dependent.

1.7.4 Physical activity

Studies of the effects of physical activity on skeletal maturation are limited therefore, the importance of physical activity in relation to directly influencing growth and biological maturation is unclear. However, it has been established that regular physical activity enhances the accrual of bone mineral content and is linked to positive health outcomes and multidimensional benefits in children and adolescents (105). Conflicting results have been reported on the effects of physical activity on growth. A much smaller study in the USA reported that linear growth in 20 preschool children recovering from malnutrition was greater in children in the active group (practicing more physical activity) than the control group (practising less physical activity) (106). This study had a small sample size therefore not adequately powered to make a sound conclusion. In contrast, prepubertal growth was not adversely affected by sport at competitive level in a much larger study of 184 children aged 9-13 years (107). Rather, stress and intensive physical activity are amongst the environmental factors that could alter the optimal pattern of growth. Skeletal maturation was delayed by 1.3 years in a study of 255 rhythmic gymnasts studied during the 13th European Championships in Greece aged 11-23 years (108).

1.8 Challenges and Disease patterns in Africa

1.8.1 Health care economies

Africa faces one of the most challenging economic environments with the largest number of low income or lower middle-income countries (LMICs) in the world (Figure 1.8). LMICs have more poverty, inequality, adversity and double burden of under and over nutrition. There is poor infrastructure and limited resources compounded with conflict in some countries and poor access to health services. These contribute to high levels of food insecurity and malnutrition. Moreover, the burden of disease in Africa is dominated by infectious diseases e.g. Tuberculosis (TB), malaria, and HIV whilst the region is undergoing epidemiological transition leading to an increased prevalence of non-communicable diseases (NCDs) (109). The malnutrition and infectious disease burden all contribute to poor growth and hence delayed skeletal maturation.

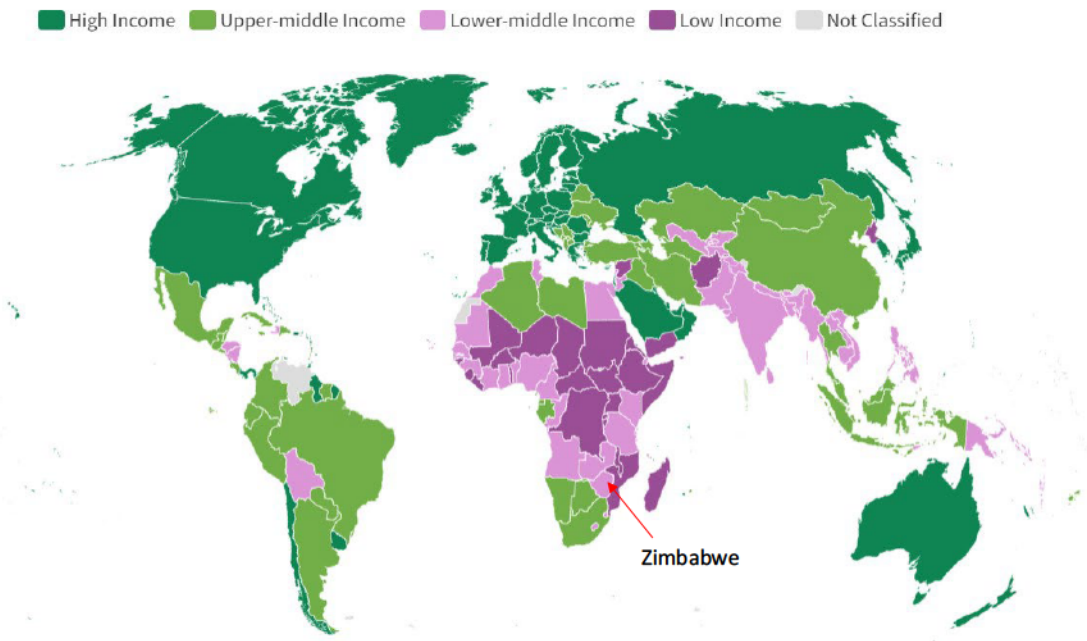

Figure 1.11 World Bank Group classification by income level (29 July 2024, https://blogs.worldbank.org/en/opendata/world-bank-country-classifications-by-income-level-for-2024-2025)

1.8.2 HIV

The Acquired Immune Deficiency Syndrome (AIDS) epidemic has been around for four decades with an estimated 84 million people infected and caused death of 40 million people with developing countries having experienced the greatest morbidity and mortality (29). It was first recognised in 1981 when an increasing number of young homosexual men succumbed to unusual opportunistic infections and rare malignancies later identified as caused by a retrovirus termed Human Immuno-deficiency Virus type 1(HIV-1) (30). HIV-1 is transmitted through body fluids like blood and sexual fluids through sexual, percutaneous and perinatal routes. Following transmission, HIV targets the CD4+T-cells which get depleted progressively because of increased damage and reduced production.

1.8.2.1 Pathophysiology and stages of HIV

HIV replication is characteristic of the retroviridae family marked by single stranded RNA genome and replication through reverse transcription and integration. However, HIV has developed several accessory genes that can influence its replication. Some of these genes seem to confer abilities to establish persistent infection and to control exuberant replication that may more rapidly cause disease and death in the host. The number of copies per ml of plasma HIV-1 RNA defines the viral load. Higher levels of HIV- RNA levels are associated with

more rapid decline of CD4+ T-cells (Figure 1.12). Viral load monitoring is critical to assess the efficacy of antiretroviral therapy (ART). Acute HIV infection causes very high plasma viral loads in the first few months accompanied by a dramatic depletion of CD4+ T-cells. World Health Organization (WHO) definition of virologic failure requires 2 consecutive HIV-1 RNA levels >1000 copies/mL measured 3 months apart after a minimum 6 months of ART, with adherence support in the interim (31).

CD4+T cells are fundamental to the development of a specific immune response to infection particularly intracellular pathogens. As the primary target of HIV, their depletion severely limits the host response capacity. The CD4+ T-cell count is the most significant predictor of disease progression and survival. Previously commencement of treatment was based on CD4 count however that has been abolished with the adoption of the test and treat approach by WHO. Lower CD4 counts are associated with greater risk of disease progression. CD4 counts from 350–500 cells/mm3 are associated with risks of ≤5% across all ages and HIV-RNA strata, while the risk of progression to AIDS increases substantially at CD4 counts <350 cells/mm3, the greatest risk increase occurring as CD4 counts fall below 200 cells/mm3 (32). The stages of HIV infection are shown in Figure 1.13.

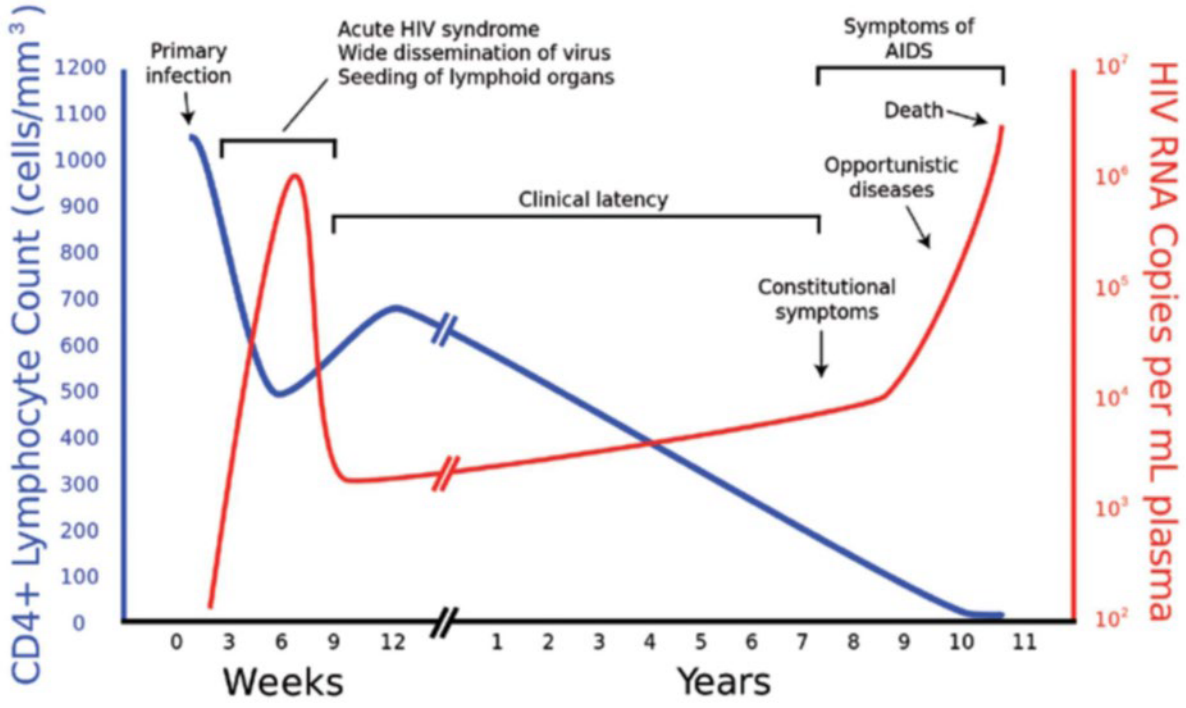

Figure 1.12 Time course of untreated HIV infection. Progression of CD4 T-cell counts during course of the diseases are denoted by the blue line. Progression of viral loads is denoted by the red line

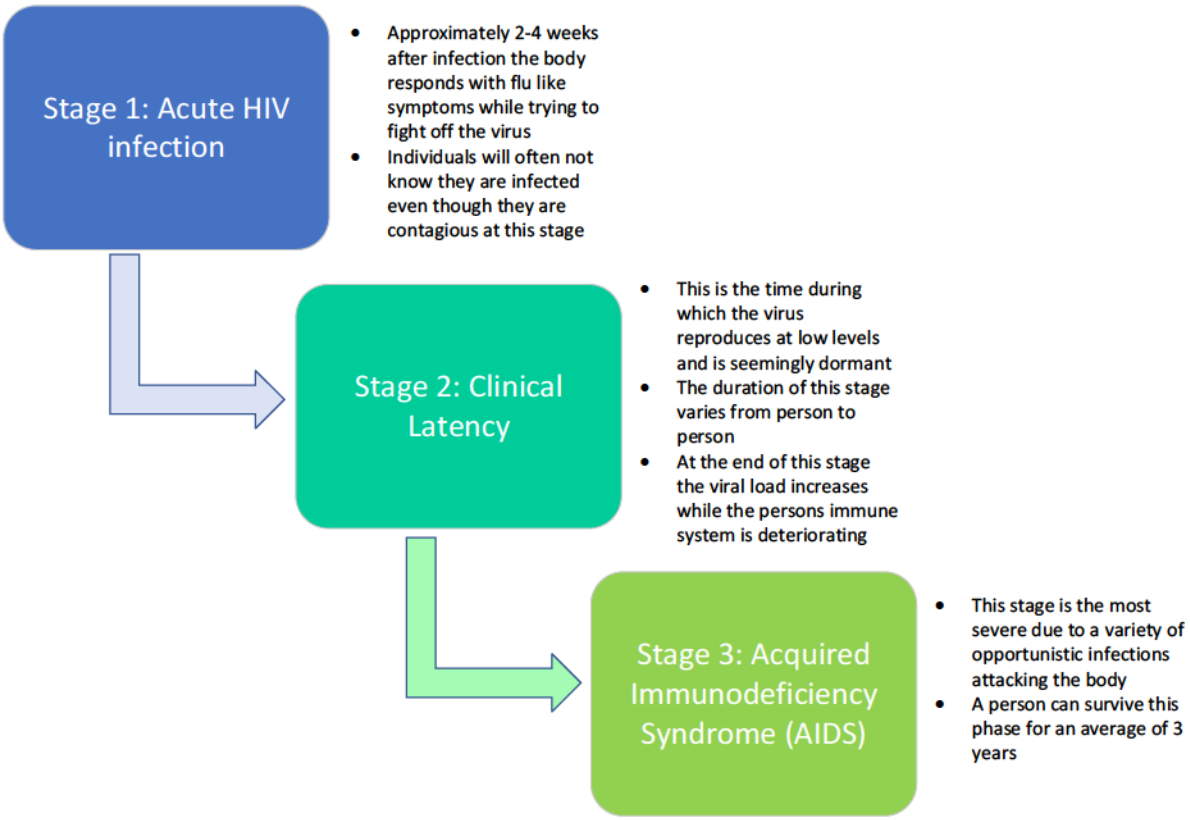

Figure 1.13 Stages of HIV infection (33)

1.8.2.2 Epidemiology of HIV

In 2022, 39 million people were living with HIV worldwide, of these 37.5 million were adults and 1.5 million children (0-14 years) (110). About 29.8 million of all people living with HIV were accessing ART, although coverage of ART substantially lags behind in children: 77% of adults versus 57% of children (110). Eastern and Southern Africa have the highest burden of HIV infection with 20.8 million people currently living with HIV (83% on ART). Evidence in the region is suggesting a stabilisation of the epidemic (110). One of the first manifestations of perinatally-acquired HIV infection to be recognised in children was poor growth (111). The prognosis of HIV infection has changed from premature death to it being a lifelong manageable chronic condition although there is increasing recognition that, despite ART, HIV has adverse effects on multiple organ systems in children resulting in long-term multisystem comorbidities (112, 113). The successful roll-out of ART has dramatically reduced morbidity and mortality in children with perinatal infection. There has been increased numbers of CLWH reaching adulthood and life expectancy of people living with HIV has improved in developing countries and is gradually approaching that of people living without HIV (114-116).

1.8.2.3 HIV and comorbidities

There are other health conditions that often occur alongside HIV in children that may significantly affect their growth and development. HIV increases the risk of active Tuberculosis (TB) as it weakens the immune system making children susceptible to TB infection. Diagnosis of HIV/TB co-infection in children is still challenging, with the overlapping clinical manifestations of the two often leading to missed or late diagnosis (117). HIV alters the pathogenesis of TB increasing the risks of developing active TB. With the depletion of CD4+ T cells in HIV, the risk of TB increases. Both conditions can lead to nutritional deficiencies due to decreased appetite, gastrointestinal symptoms, chronic inflammation increased metabolic demands all of which hinders growth and development in children. Consequently, the risk of developing malignancies is higher in children living with HIV and several factors like immunosuppression and viral coinfection lead to the high rates of cancer. A higher prevalence (4.7%) of HIV related malignancies was reported in Tanzania in a study of 721 children aged 0.17-17 years (118) compared with 0.013 in the general paediatric population in the USA (119).

1.8.2.4 HIV and growth

Children and adolescents have the greatest cumulative negative exposure to the direct and indirect effects of HIV infection and its treatment on skeletal growth and development. In children HIV infection is usually vertical i.e., acquired during the antenatal, perinatal or breastfeeding period. In early childhood the infection contributes to disturbances in linear growth and weight gain with poor growth being reported in 50% of CLWH (111). Impaired growth is one of the sensitive indicators of morbidity and mortality in CLWH (120). Growth disturbances in the context of HIV infection are multifactorial; poor growth is thought to be attributable to secondary infections and complications that accompany HIV infection. A higher prevalence of stunting was reported in Zimbabwean CLWH (35%) when compared with the HIV-negative (5%) children in a cross sectional study of 174 children aged 6-16 years living with and without HIV (114). Similar results were also reported in a much larger study of 609 Zimbabwean children living with and without HIV aged 8-16 years with CLWH more likely to be stunted, underweight and have delayed puberty than the HIV-negative children (121). Children infected intrauterine or intrapartum were six and four times more likely to be stunted than HIV unexposed and uninfected children respectively in a large trial of 14110 infants enrolled in the Zimbabwe Vitamin A for mothers and babies trial conducted before availability of ART (122). The trend is the same even in high income countries with 184 CLWH being significantly shorter and lighter than 1403 HIV-negative children and the growth differences increased with increasing age in a European collaborative study involving eight European countries (123). HIV is therefore associated with poor growth and delayed puberty which has implications on bone mass accrual and achievement of PBM.

A holistic approach that emphasises adequate nutrition and physical activity is essential to optimise growth in CLWH. Deficiencies in macro- and micronutrients can hinder growth and exacerbate the effects of HIV. Additionally, CLWH experience a range of barriers toward engaging in physical activity, such as side effects of antiretroviral therapy, depression, bodily pain, and the presence of opportunistic infections, despite the benefits of an active lifestyle (124).

1.8.2.5 HIV treatment and growth

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. In Zimbabwe ART regimens 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. Tenofovir disoproxil fumarate (TDF) remains the first NRTI to be used in the treatment of HIV-1 infection, though it has been superseded in higher income countries (125). Although TDF use is generally safe and has excellent efficacy for maintaining virological suppression the ART regimen has been associated with greater bone loss in adults and poor bone accrual in children (126, 127). However, some studies have reported that TDF does not impair bone accrual in CLWH (128).

Early or immediate initiation of ART has many benefits which include prevention of poor growth and pubertal delay in children (129). Only recently (2016) guidelines for initiation of ART were changed in Zimbabwe such that immediate ART is now recommended for all regardless of age or disease stage (130). The age at which to start adult dosing in adolescents can be difficult to determine as stunting and wasting are common among adolescents living with HIV. It is recommended that those under the weight of 25 kg should use paediatric dosing guidelines (130). However, many children were initiated on ART in older childhood or adolescence as a result of late HIV diagnosis (131). Although children remained stunted after one year of ART initiation there was evidence that WAZ and BAZ increased after one year in a cohort of 385 newly diagnosed children CLWH aged 6-15 years in Zimbabwe (132). Weight improved in the first year of ART and height improved much more slowly over the five-year period of a South African study of 159 children aged 0-5 years initiated on ART at the beginning of the study (133). Although Initiating ART in later childhood leads to a substantial delay in puberty and menarche, the children benefit from the prolonged window of opportunity for growth provided for by the delayed puberty (134). Although the benefits of ART have been celebrated the long-term effects of HIV infection and prolonged use of ART in growing and developing children is not yet

understood. CLWH are experiencing a range of multisystem morbidities as a result of their infection and/treatment and reportedly HIV infection is challenging skeletal development (111). It is not clear whether use of TDF has long team effects on skeletal maturation and this PhD will explore associations between TDF use and skeletal maturity delays.

1.8.2.6 Skeletal maturation in children living with HIV

There are limited published studies that have reported skeletal maturation in CLWH. Perinatally infected CLWH taking ART had delayed skeletal maturation of over two years relative to CA when compared with the control group whose BA and CA were almost similar at baseline in a Brazilian study conducted (135). This was a 4-year longitudinal study of children aged 5-11 years, 30 CLWH and 30 HIV-negative. Hand wrist radiographs were taken at two time points (baseline and after four years). After four years, the differences in BA and CA were smaller than at baseline. Although the authors attributed the positive change to ART but these children had already been taking ART at the beginning of the study. Hence it may have been a case of children catching up on skeletal maturation. Earlier studies had reported delayed skeletal development even in children established on ART (136). In a cross-sectional study carried out in India, perinatally infected CLWH aged 8 to 14 years had delayed skeletal maturity despite administration of ART (137). Both of these studies were limited by a small sample size of 60 participants (135, 137) and the Indian study lacked a comparator group without HIV (137). None were conducted in SSA. There is a need for further longitudinal studies with a more robust study design, encompassing children of differing maturities who are living with and without HIV, to confirm these findings. There are limited studies in high income countries that have studied skeletal maturation in CLWH. One longitudinal study conducted in USA in 333 children reported a delay in skeletal maturation in adolescents living with HIV who had haemophilia and were on inhibitory antibodies when compared to those not on inhibitors (138).

1.8.3 Malnutrition

Globally, Africa has the highest burden of malnutrition (Figure 1.14), with an estimated 148.1 million preschool children stunted (poor linear growth) in 2022 and 58% of them living in African countries (139). Many studies have reported the effect of under-nutrition on pubertal timing, growth and height-for-age z-score (HAZ) but very few have reported skeletal maturation as the outcome. Adequate nutrition is required for normal growth and development. Malnutrition refers to deficiencies excesses or imbalances in a person's intake of energy and/ nutrients (140). Two broad groups are encompassed in malnutrition which is 1) undernutrition that covers stunting, wasting, and underweight and micronutrient deficiencies and 2) over

nutrition that covers overweight, obesity and diet related non-communicable diseases. Children are said to be stunted, underweight and wasted if their HAZ, weight-for-age z-score (WAZ) and BMI z-score is more than 2 standard deviations below the WHO child growth standard median (141). In adolescents' malnutrition results in delayed puberty. Ensuring good dietary quality and diversity therefore allows children to grow and develop to their full potential.

1.8.4 Demographic background to Zimbabwe

This PhD focusses on a study of children living in Harare, Zimbabwe. Zimbabwe is a land locked country in Southern Africa that shares its boarders with Zambia, Botswana, South Africa, and Mozambique (Figure 1.8). It has a population of 16 million people with 10 provinces and 63 districts. The country is classified as an LMIC by the World Bank and the majority of people in Zimbabwe live below the poverty datum line which is the amount of income of consumption expenditure deemed necessary for a person to meet the basic needs of living (142). The past two decades have seen a decline in the health care system in the country characterised by a mass exodus of health workers, inadequate funding, inadequate infrastructure, economic and political instability (143). The HIV/AIDS pandemic has dramatically reduced the life expectancy and quality of life in Zimbabwe through disease-induced morbidity and mortality (144). The prevalence of HIV in Zimbabwe is at 11% and amongst the 1.3 million people infected, 750 000 are children aged 0-14 years. Furthermore, the prevalence of stunting in Zimbabwe is 23.3% (2019) (145) . Children followed from birth to 24 months had poor growth compared with WHO standards and this was attributed to under-nutrition (122).

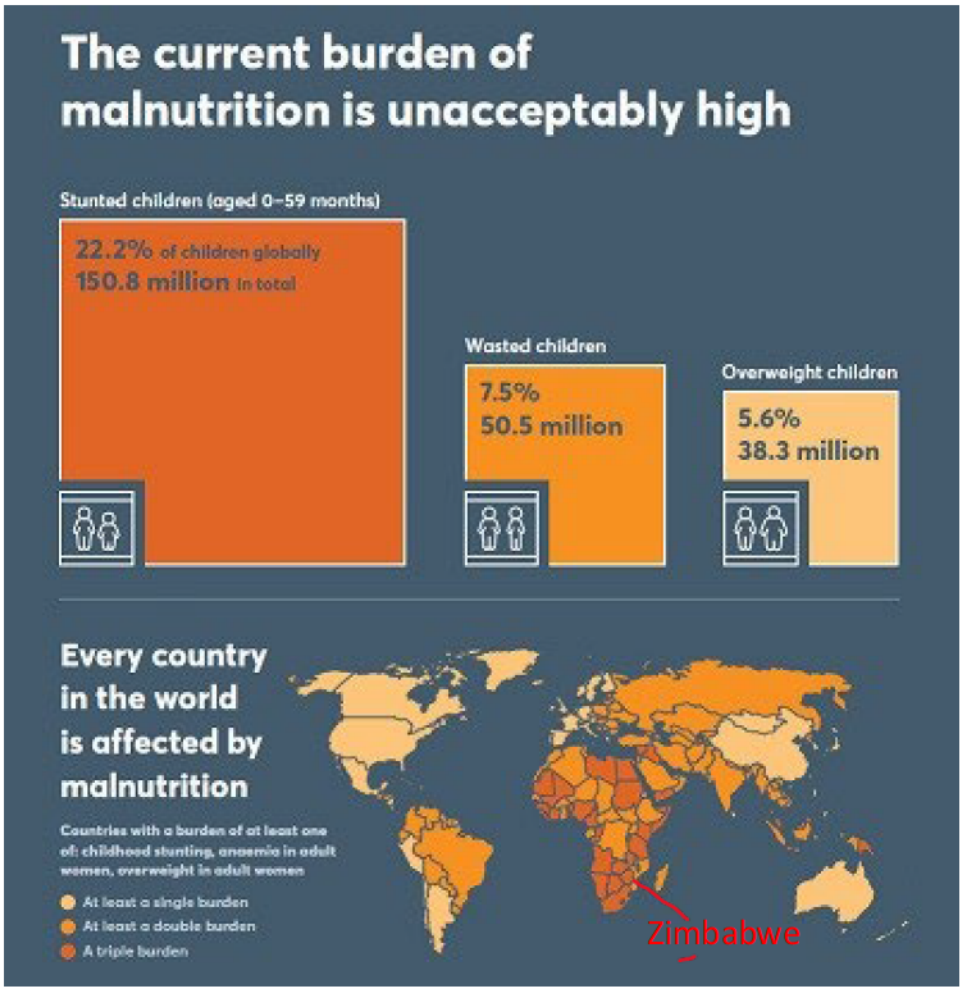

Figure 1.14 The burden of malnutrition in the World (2018 Global nutrition report) The greatest burden of malnutrition is found in Africa

Chapter 2 Literature review of bone age assessment methods and their application to different populations

2.1 Introduction

Bone age (BA) is expressed in years that correspond to the level of maturation of bones i.e. skeletal maturity (see Chapter 1) and is assessed using radiographic techniques. It differs from chronological age (CA) which is calculated from the date of birth of an individual. To date there is no gold standard method of BA assessment although there are two most widely used methods Greulich and Pyle (GP) and Tanner Whitehouse 3 (TW3). GP developed a method for BA assessment based on radiographic features of the hand and wrist over a century ago (2). The Tanner Whitehouse method (TW) was developed two decades later as TW1, modified to TW2 and later TW3 also based on hand wrist radiographs (77). Other methods have been proposed over the years, but none have been used as widely as the GP and TW3 methods. The manual, operator dependent methods of BA assessment are most commonly used however, automated methods such as BoneXpert®(Visiana, Hørsholm, Denmark) have been developed and are mostly utilised in high income countries. The software for the automated methods is expensive, and most African countries would rather channel the resources to more demanding health care programmes. Other imaging modalities like computed tomography, dual energy X-ray absorptiometry, MRI and ultrasound have been investigated in addition to the plain radiographs. An ideal method of BA assessment would be specific to the different populations considering the different ethnicities, SES and disease patterns which affect the process of skeletal maturation. The purpose of this chapter is to explain the choice of method for my thesis.

2.2 Why the hand and wrist?

Although many body areas such as the knee and pelvis (146-149) have been studied over the years to define a standardised and universal method, the bones of the hand remain the most commonly used in BA assessment. BA assessment of the hand and wrist has been available much longer, as early as 1937 (150), than that of other areas such as the knee where reference

data were collected in 1955 (151). In addition, hand wrist radiographs are the most convenient for BA assessment because so many bones are present in such a small area that can be easily radiographed in a single image (Figure 2.1) (15). The changes or features identified on these radiographs are referred to as maturity indicators, which appear in a specific and irreversible sequence as bones progress toward full maturity. Consequently, the number of maturity indicators visible in the hand exceeds those found in other regions used for skeletal maturation assessments, such as the knee joint, which includes the distal femur and proximal tibia. This provides a more comprehensive view of skeletal maturity across various bones (15). Figure 2.2 shows the maturity indicators of the radius as outline by the GP and TW method (The rating A for Tanner Whitehouse is not shown in the diagrams and is only used when there is no sign of secondary ossification present). Hand wrist radiographs were studied to enable the determination of the CA at which the different maturity indicators appear (2) to allow the creation of the atlas by indicating the skeletal age assigned to that maturity indicator.

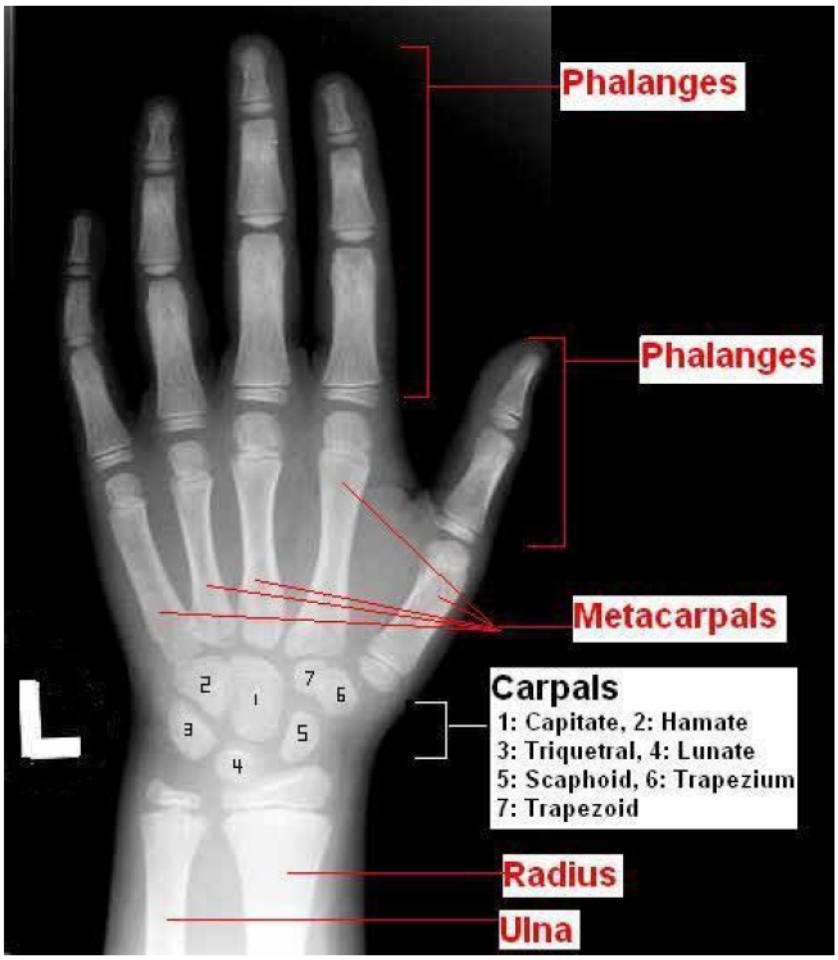

Figure 2.1 Radiographic image showing the anatomy of the hand

The effective radiation dose received by the hand during the procedure is less than 0.00012mSv which is equivalent to less than 20 minutes of the average natural background radiation which typically ranges from 1.5 to 3.5 mSv per year on average worldwide in areas that do not have elevated doses (152, 153). Sources of background radiation include cosmic, terrestrial and exposure to internal radiation. Notwithstanding that dose levels are not constant, they are affected by altitude, geological formations in the rocks and soils where radioisotopes and radionuclides like uranium and thorium are found. Therefore, the health risk of the radiation exposure of hand wrist radiographs for BA assessment is very minimal. Some authors do not favour the use of the hand alone as a measure of skeletal maturity, as they argue it is not a representation of the whole skeleton (15). Nonetheless, the hand and wrist has been widely accepted by clinicians and researchers worldwide to assess skeletal maturity as a pragmatic solution.

The left hand is usually used rather than the right hand as most people are right-handed therefore that side is more is likely to be injured than the left (154). This was specified for physical measurements at the international agreement for the unification of anthropometric measurements to be made on living subjects drawn up at the Monaco and Geneva conferences in the early 1900's (2, 155, 156). However, discrepancies between the two sides for BA assessment were found to be insignificant to constitute a source of error in a study that compared left- and right-hand radiographs of 450 children (157).

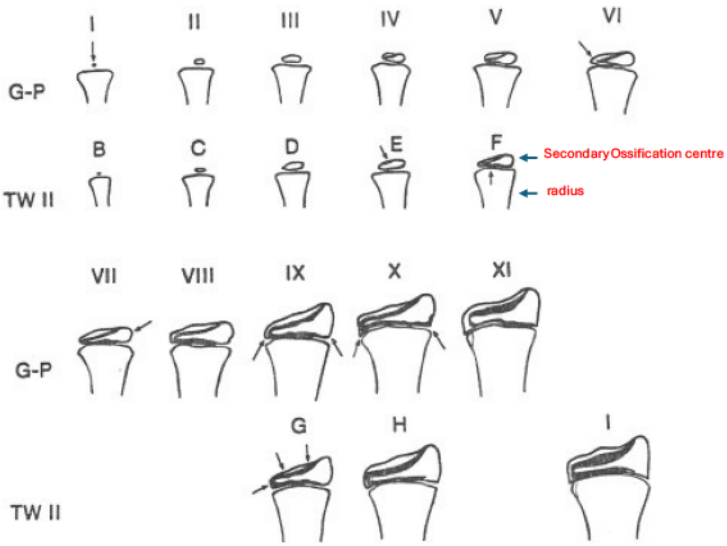

Figure 2.2 Maturity indicators for the radius as defined by Greulich and Pyle and Tanner and Whitehouse II. (Camerron et al 2022)

2.3 Greulich and Pyle

2.3.1 Historical overview

The GP atlas was a continuation of the Brush foundation study under Professor TW Todd in 1929 who began preliminary studies on long term investigation of human growth and development (150). It was later revised by Greulich and Pyle in 1950. The study enrolled three-month-old infants in Cleveland Ohio and thereafter three months to 14-year-old children were added into the program in each successive year until 1942 when the project terminated. All children enrolled were born in the USA, were white and almost all were of northern European ancestry.

Radiographs of the left hand and wrist, shoulder, elbow, hip, knee and foot were taken alongside other measures of height, weight, clinical history at three-month intervals in the first year, every six months from 1 to 5 years, and every year thereafter (2). There was a systematic selection of radiographs for the atlas. From individuals with the same CA, 100 serial radiographs were gathered e.g. 9-year-olds with a maximum deviation of 2% from the defined age. The radiographs were then sorted from the least mature to the most mature. Each standard film was selected if most representative of the central tendency. Only radiographs for age 19 in males and

18 in females were not selected systematically but rather depicted a fully mature hand and wrist (2). The atlas is divided into male and female standards.

2.3.2 Bone age assessment using the Greulich and Pyle method

The atlas consists of 31 and 27 radiographic image templates in males and females respectively which illustrate different phases of bone maturation between 0 to 18 years in females and 19 years in males. For every standard there is a bone specific BA assigned with a short description of the corresponding radiographic features. The characteristics of the maturity indicators are outlined in the appendix of the atlas. A given radiograph is compared with the standard of the nearest CA and same sex in the atlas. The next step is to compare the radiograph to the adjacent standards younger or older than the nearest CA and the radiograph is then assigned a skeletal age equal to that of the nearest age standard that appears to resemble it most closely.

2.3.3 Strengths of the Greulich and Pyle method

The GP method is more commonly used, as it is straightforward and quick therefore ideal to be applied clinically (158). It can be easily taught to new leaners such that they quickly achieve accuracy and inter observer variation that is comparable to that of an experienced reader (159, 160). In a survey conducted 40 years ago in England, 76% of paediatricians used the GP atlas (161). While these observations are now considered outdated, as automated methods of BA assessment have since been adopted in the UK, where paediatricians are no longer trained in BA assessment, these manual methods are still in use in developing countries like Zimbabwe.

2.3.4 Limitations of the Greulich and Pyle method

A standard radiograph is provided in the atlas for each age group and when the standard films are studied serially, the method assumes a fixed appearance with subsequent development of the centres of ossification. The centres appear visibly in a certain order. However, there is evidence of a wide range of normal variation in the pattern of ossification and that the variation is genetically determined, even from studies conducted before the atlas was developed (162). If illness or deprivation affects one maturity indicator to a greater degree than the other, these maturity indicators would translate to different skeletal ages. In addition, relating skeletal age to CA is a problem as maturation is distinct from growth and requires a separate

scale of measurement. Another limitation is that the time interval between two standard films is too long (one year between most standards). Because I have to compare the radiograph to the adjacent standards younger or older than the nearest CA when assessing BA using the GP method it is impossible to be blinded to the CA possibly introducing an observer bias.

Other limitations emanate from the lack of diversity in ethnic groups used in the development of the atlas. The reference population was limited to White Americans and were believed to be children from families of above average in terms of SES as they were recruited upon the request of a paediatrician (2). Consequently, the method was established from children born between 1931 and 1942, hence the method may not be applicable to children nowadays because of evidence of secular trends in growth and maturation. Children are growing and developing faster, with greater average height and accelerated biological maturation (163, 164).

2.4 Tanner Whitehouse 3

2.4.1 Historical overview

As an alternative to the GP method the TW method was formulated, and the method uses a bone specific scoring technique where the appearance of each bone segment is evaluated, scored, and compared to a set of bones at different stages of maturation. The rationale for developing a bone scoring method was based on dissatisfaction with GP method that was based on CA. Therefore, the aim was to develop a method that does not refer directly to age. The first published bone specific method was the Acheson Oxford method in 1957, derived from 500 Oxford pre-school children (165). The hand and knee were used for assessment. Maturation was measured in Oxford maturity scores 1, 2, 3,..., where a unit was awarded to a bone as each distinct shape change made itself manifest. The sum of those units for each bone at any stage of development would be the measure of skeletal maturity. However, combining the individual bone scores to indicate overall maturity of a child raised questions as carpals and epiphyseal ossification do not occur at the same rates in all children. These limitations prompted the works of Tanner and associates in developing the Tanner Whitehouse (TW) method. Tanner and his colleagues noted the long bones were more than the carpals in number and the weighting on the final score may favour long bones from short bones therefore the second and fourth fingers were omitted from the final calculations. The scores were then weighted such that the carpals and

long bones each contributed 50% of the final maturity score. Using the radiographs of 3000 White British healthy boys and girls the technique was used to arrive at population standards which then related to CA. A series of eight maturity indicators were defined for each bone and nine for the radius. Unlike the GP method they evaluated the maturity indicators in relation to their appearance within the full passage of each specific bone from immaturity to maturity and not in relation to chronological age (CA).

The TW method, first known as TW1 (166) was developed in the 1930's and skeletal maturation was evaluated using the "20 bones" score. TW1 was later modified to TW2 (167) in 1983 using additional data collected in the 1950s and 1960s in western Europe. TW 2 was developed such that there were three different ways of BA assessment distinguished: 20 bones score, RUS score (radius, ulna, and short bones only) and Carpal, limited to carpus bones only. A further update was conducted in 2001, namely TW3 (168), when scoring of the radius, ulna and short bones (RUS) was separated from the carpal bones (168). Each bone of the hand is assigned a grade from A to I with each grade having a specific numerical score for males and females separately. Figure 2.3 demonstrates the maturity indicators of the radius from grade B to I as described in the TW3 atlas. The numerical scores are summed up to obtain the overall maturity score. The atlas used by the TW3 method is sex specific. According to the TW3 method, the BA of a child progresses at a rate of one year for every one year of their CA with normal variations ranging from 0 to 2.0 BA years per 1 CA year. This applies at ages 3 to 15 years in girls and 3 to 16 years in boys (168).

2.4.2 Strengths of the Tanner Whitehouse 3 method

The advantage of the TW3 method is that the development of every bone and epiphysis can make its own contribution to each assessment such that small increases of maturity are recorded thereby increasing the precision of the method. Evaluation of a radiograph can be made regardless of the pattern or order in which ossification is occurring.

2.4.3 Limitations of the Tanner Whitehouse 3 method

The TW method is more complex and requires more time for assessment than the GP (7.9 minutes vs 1.4 minutes respectively) (169, 170). The method assesses BA up to 15 years for girls and up to 16.5 years for boys which is taken as full skeletal maturity according to atlases, such that in children who have chronological ages above these may be reported as having delayed BA

when they are not. In legal and forensic cases where BA assessment is used to determine BA the age after CA 16.5 years will always be underestimated.

2.4.4 Comparison of Tanner Whitehouse 3 and Greulich and Pyle method

As indicated in the previous sections of this chapter the GP and TW3 differ in several ways (Table 2.1). The bones of the hand ossify at different rates and the ability of TW3 to assess ossification centres separately makes it ideal for use hence being described as more objective and reproducible compared with GP, which, as explained above, makes a single assessment of the appearance of the whole hand/wrist (171). GP BA is stated in whole or half years whilst the TW3 grades as 0.1, 0.2, 0.3 years etc. Historically, the GP was preferred than the TW3, with only 20% of paediatricians using the TW3 in comparison to 76% using the GP. However, considering the current landscape, it remains to be seen whether these historical trends hold true today in LMIC's where manual methods are still predominantly employed (161).

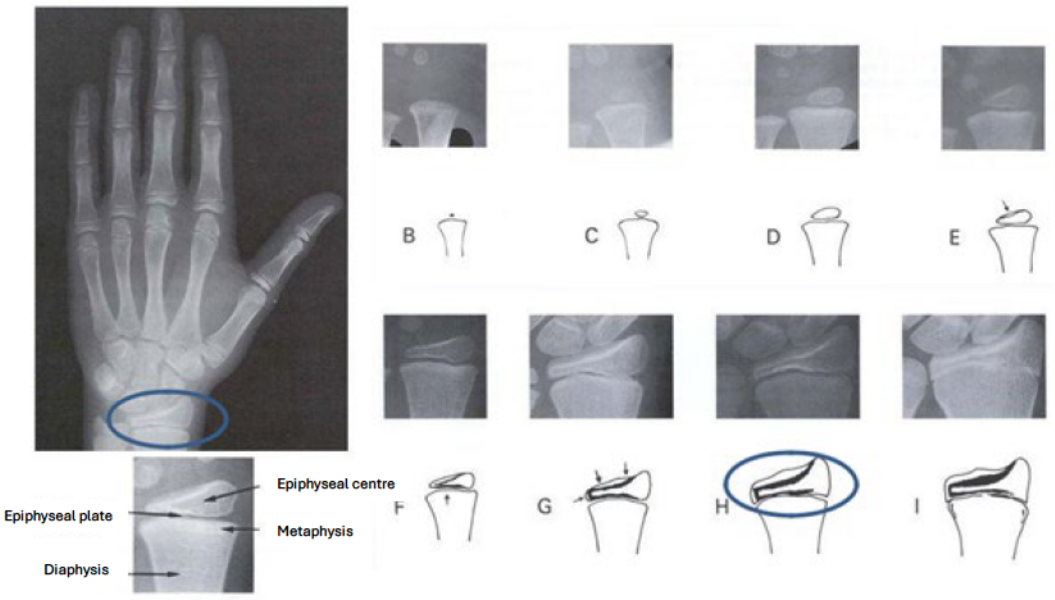

Figure 2.3 An image showing maturity indicators of the radius at different stages for the TW3 method, adapted from Tanner et al 2001.

Table 2.1 A comparison of the Greulich and Pyle and Tanner Whitehouse 3 methods of bone age assessment

	GP	TW3
Age range	Boys 0-19 years	Boys 0-16.5 years
	Girls 0-18 girls	Girls 0-15 years
Original population	USA	British
Time for assessment	Requires less time	Time consuming
Approach	Assesses whole hand	assesses individual ossification centres
Image	Hand wrist radiograph	Hand wrist radiograph
Latest version	1959	2001
Time interval between 2 standards	one year	0.1 years

2.5 Automated methods of bone age assessment

Modern radiology departments especially in high income countries perform BA assessments frequently and often these are done by different people. It becomes increasingly difficult to maintain high standards of BA assessment with an uncertainty on the reliability of the grading. The manual methods of BA assessment are still based on the subjective evaluation of hand wrist radiographs, and the reliability is based on the availability of expert and well-trained individuals to grade the hand wrist radiographs. BonXpert™ is a computerised and automated method of BA assessment introduced to address these challenges. The computerised system makes use of technologies from medical image analysis statistics and machine learning based on the TW3 methodology (172). Using the hand wrist radiographs there is automatic reconstruction of the borders of 15 bones and computation of intrinsic BA from the radius ulna and 13 short bones. The intrinsic BAs are transformed into the GP and TW BA (Figure 2.4). Some studies conducted to compare BonXpert™ and manual GP methods of assessment have reported a high correlation between the two methods (6, 173), although a measure of corelation will only refer to the presence of a relationship and will not show whether there is agreement between the two methods. Even though the studies demonstrated that BoneXpert™ allows for standardised and less variable results (173, 174), hand wrist radiographs with abnormal bone morphology and poor image quality are automatically rejected by the

BoneXpert™ system (175). Despite the advantages of the automated method of BA assessment, the software is too expensive for low resourced settings like Zimbabwe (where a radiographer earns on average $500 USD per month and the software costs between $15000-$20000 USD) hence the manual methods of BA assessment will remain the method of choice.

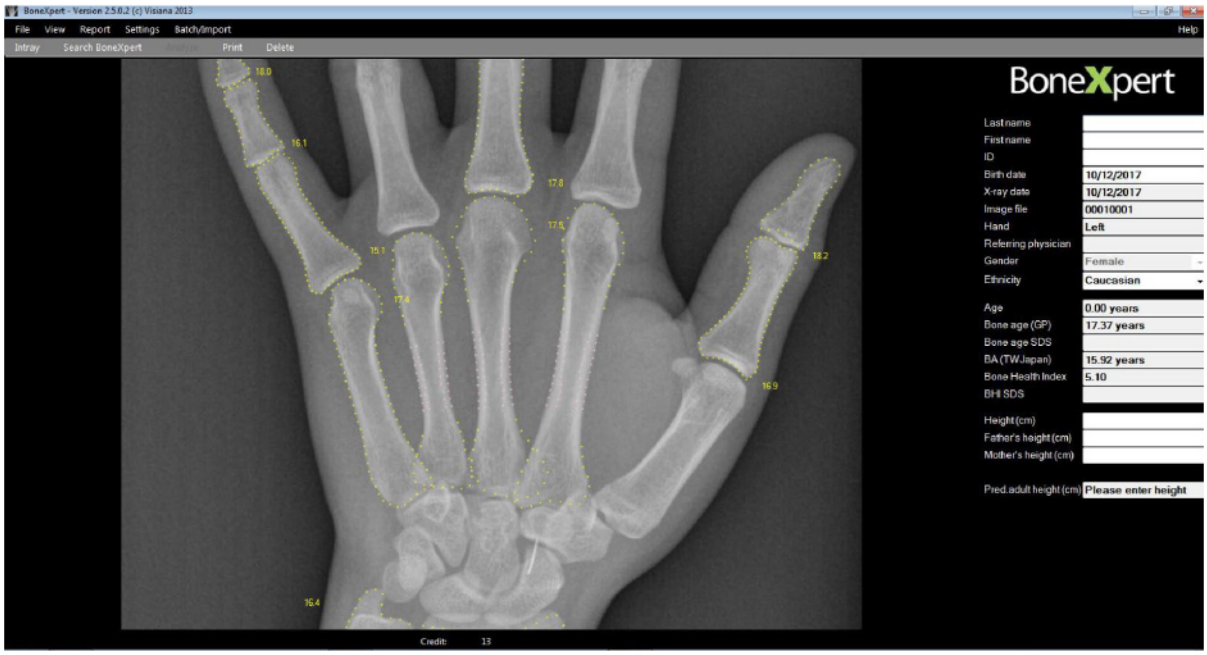

Figure 2.4 BoneXpert automated method of deriving BA (https://thesamson.org/download/58/14.30_Wed_-_Bone_Age_Review_CG.pdf)

2.6 Inter and intra observer variability

It is necessary to quantify observer variability as part of quality control. Interobserver variability is the difference in the measurements between observers whilst intra observer is the difference in repeated measurements by the same observer. In a survey earlier reported in section 2.4, 25% of the Paediatricians conducted BA assessment themselves and in 75% of the centres where the paediatricians work, BA assessment was performed in the radiography departments by radiographers/radiologists (161). Only in 50% and 32% of the centres that used the TW3, and GP method respectively was there one or two members of staff assessing BA. These results indicates that BA assessment is not undertaken by any one experienced individual. BA assessment methods are subject to inter- and intra- observer variability (176); therefore, it is important that BA assessment aims to minimise intra- and inter observer variability. An earlier study compared inter observer variation for TW2 and GP methods on

hand wrist radiographs in the casualty department by two observers who were “relatively inexperienced”. TW2 was found to be more reproducible than GP although they did not quote the magnitude of the difference (177). A later study which also compared inter observer variation with BA assessment performed by three different operators found a significant difference between inter observer variation of 0.96 years (GP) and 0.74 years (TW2) using the two methods (169). Experience on the use of these methods is an important factor as it will reduce the observer variability. Beunen showed a 9% improvement in TW2 intra-observer agreement with increased experience (seven years) (178). In this study the originator of the TW method rated two sets of radiographs with two different observers, one who received no training and had self-taught himself the method and the other who was taught by the originator of the method and had 7 years’ experience. The small differences in the inter observer variation for the two methods resulted in some authors opting for the GP method than the laborious and time-consuming nature of the TW method (177). Furthermore the differences in the observer variability resulted in some authors suggesting against interchangeable use of the methods preferring the TW method instead (3).

2.7 Clinical applications of bone age assessment

BA is used to determine skeletal maturity which reflects developmental stage, growth potential and in some populations is predictive of future adult stature. Endocrinologists and paediatricians usually request BA assessment in children to: 1) evaluate clinical conditions associated with generalized growth abnormalities, 2) monitor response to medical treatment by serial measurements of BA, and 3) determine the growth potential of children by estimating final standing height. Many diseases and disorders, particularly those that cause growth problems show a characteristic relationship (correlation) between CA and BA where BA may be more or less than the CA (Table 2.2). Deficiencies in thyroid hormone or GH cause severe delay in growth indicated by BA. The trend is the same in conditions that impair growth due to metabolic disorder or undernutrition. Other related indications for BA estimation are to manage diseases that affect GH, precocious puberty, adrenal gland disorders, genetic growth disorders such as Turner syndrome, and syndromes that affect the skeleton such as achondroplasia. Growth failure may be a consequence of chronic systemic or organ-specific disease, which BA may help identify and monitor (171).

In monitoring treatment, or the course of a disease, serial measurements of BA can be conducted to show how BA changes with time and/or treatment. Methods to predict adult height have been developed with the most used being that developed by Tanner (168). The child's height, BA and CA are used. Prediction of adult height is often needed in very tall or very short children to determine appropriate interventions that may help address the growth issues. BA is delayed in children with idiopathic short stature by 1.5 to 2 years at 8-11 years of age and in children born small for gestational age by 1-2 years up to the age of 8 years (179, 180). Parameters such as height velocity, menarche, muscle mass and bone mineral density correlate better with BA than with CA (152).

2.7.1 Other uses of bone age

Although the European Society for Paediatric Radiology (ESPR) does not recommend the use of BA assessment (as assessed by GP method) as a tool to determine CA, BA is still being used in forensic and legal investigations when there is need to verify or determine an individual's CA (181). The ESPR agrees with Tim Cole a Professor of Medical Statistics, with extensive expertise in the assessment of growth, who has investigated how appropriate BA is for judging whether an individual is younger than 18: "It turns out that the amount of information it contains depends on the age claimed by the individual (as opposed to their simply being under 18). In some circumstances BA is informative but usually it is not. The issue here is the size of the standard deviation (SD) of the difference between BA and CA, which is 15 months or more. So, the confidence interval around the CA estimated from BA is ±30 months (i.e. ±2 SDs), a range of 5 years. This lack of precision impacts on the value of BA as evidence, and renders it uninformative except in extreme cases" (182). BA is useful when the CA may be in doubt or intentionally falsified, for example, child labour, sexual assault and prostitution. In high income countries many asylum seekers and unaccompanied minors arrive without valid documents to prove their ages (158). Therefore, it is necessary to assign CA so that children receive their rights and adults are not treated as minors hence the use of BA assessment. In some instances, BA will be used to accept or deny international immigration. Countries differ in how they handle asylum seekers, with the US immigration and Customs Enforcement suggesting consideration of CA estimation based on BA but should not be used as complete evidence (183). Some researchers have recommended the use of BA assessment and dental age assessment such that countries like Sweden have introduced a new system that includes dental and skeletal age for CA determination (184-186).

BA assessment is also used to help guide sporting decisions and resources for elite athletes. Some sports prioritise tall stature hence young boys who want to participate competitively have BA assessment done to decide how much time and resources to invest in early sports training (187).

Table 2.2 List of conditions, medications and circumstances in which bone age may be less than chronological age

	Conditions, circumstances and medications that cause delay in bone age
Conditions/disease	Endocrine • Hypothyroidism • Growth hormone deficiency • Panhypopituitarism • Hypogonadism • Cushing disease • Constitutional delay (late bloomer) Non-endocrine • Congenital heart disease • Chronic kidney disease • Juvenile idiopathic arthritis • Inflammatory bowel disease • Liver disease • Celiac disease • Cystic fibrosis • Severe asthma (likely from corticosteroid use) • Immunodeficiency states, including HIV infection • Active tuberculosis • Anorexia
Circumstances	• Malnutrition • Failure to gain weight as a result of disease • Inadequate bone mineralization • Female athlete triad (leading to hypogonadism) • Neglect and abuse

Medications	• Glucocorticoids (including high-dose inhaled corticosteroids and oral budesonide in sensitive • children) • Amphetamine and dextroamphetamine (modest effect) • GnRH analogues (depot leuprolide and histrelin) • Aromatase inhibitors
Genetic	• Trisomy 13, 18, and 21 syndromes • Turner syndrome • Klinefelter syndrome • Russell-Silver syndrome

Adapted from Creo et al 2017

2.8 Clinically defined delayed and advanced bone age

When BA is compared to CA in the clinical setting there are three possible outcomes, delayed BA, BA appropriate to CA or advanced BA (Figure 2.4). Delay and advancement are defined by BA being two standard deviations below or above CA respectively.

2.9 Use of bone age assessment methods in different populations

Methods to assess BA should be reliable and practical, being adaptable for use in populations of different races and ethnicity. Because there was a lack of diversity among the populations used to develop BA methods, their use in different ethnic groups, other than those in which they were derived, is debatable. Data for GP and TW methods were originally obtained from White USA and UK children respectively. Literature documents ethnic variations in growth patterns (188). As discussed in Chapter one, section 1.7, many factors such as genetic ancestry, SES, and disease patterns contribute to the variations in growth and development hence skeletal maturation across diverse populations. Therefore, atlases derived from the original GP and TW3 studies may not accurately represent the BA of individuals from other ethnic groups and/or geographic locations. When applying the GP and TW3 methods to children and adolescents of different ethnic backgrounds, the population specific variations should be considered to have an accurate assessment of BA. There is evidence that many African countries have a high

prevalence of undernutrition and other environmental risk factors which may adversely influence skeletal maturation (189). Therefore, it would be ideal to have population specific reference data for BA assessment. As previously mentioned in section 2.3.2 many clinicians prefer using the GP as it is quick and easy to complete, therefore many more studies have been conducted assessing the applicability of the GP in different populations than the TW3.

For a method to be appropriate for use in a certain population BA should approximate CA i.e., the difference between BA and CA (SMD) should be zero in otherwise healthy children. The method can then be said to be applicable to that population. If the differences are negative, there is underestimation and if positive there is overestimation. However, there is heterogeneity in literature in terms of authors perception of the applicability of methods of BA assessment in different populations around the world. For example Mora et al found an average SMD of -0.01 years for males and 0.11 years for females in 274 participants in a study of African American children aged 0-19 years (88). They felt in their conclusion that the GP method was not applicable in African Americans. Interestingly Zhang et al in another population of 349 participants aged 0-18 years SMD on average was -0.02 years in males in the same direction as Mora et al and 0.03 years in females and the authors concluded that it was applicable in that setting. Similarly, Moradi et al concluded that the GP was applicable in the Iranian population with an average SMD of -0.4 years in boys and 0.04 years in girls. There is no consensus to what applicability is and how much SMD is one allowed to have before one says the population you are studying in inherently different from the refence population from which the GP was first arrived at.

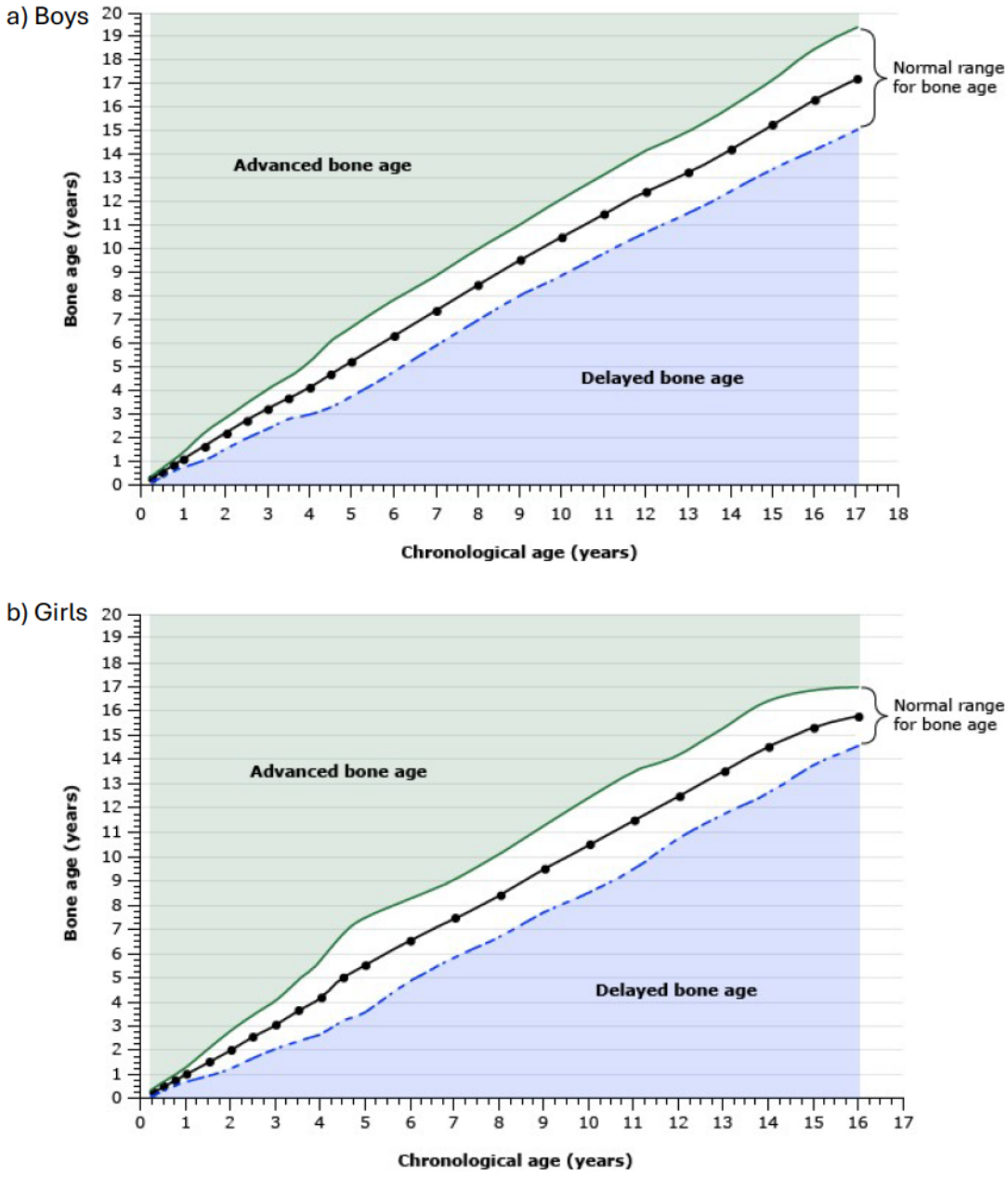

Figure 2.5 The relationship between bone age and chronological age in boys (top) and girls (bottom) as assessed by the Greulich and Pyle method (Gaskin et al 2011)

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years for males and 0.11 years for females in 274 participants in a study of African American children aged 0-19 years (88). They felt in their conclusion that the GP method was not applicable in African Americans. Interestingly Zhang et al in another population of 349 participants aged 0-18 years SMD on average was -0.02 years in males in the same direction as Mora et al and 0.03 years in females and the authors concluded that it was applicable in that setting. Similarly, Moradi et al concluded that the GP was applicable in the Iranian population with an average SMD of -0.4 years in boys and 0.04 years in girls. There is no consensus to what applicability is and how much SMD is one allowed to have before one says the population you are studying in inherently different from the refence population from which the GP was first arrived at.

2.9.1 Greulich and Pyle method in White children

Several studies that have reported the use of the GP method in populations other than those populations in which it was developed (Table 2.3) (190-194). However, some of the studies concluded the method should be used with caution as significant differences between BA and CA were reported either underestimating or overestimating CA in the same population in different age groups (195-199). For example in a study of Turkish males the GP mean BA was delayed in between the ages of 7-13 years and then BA was advanced between 14-17 years hence the conclusion that the GP method should be applied with some modifications (197). In another Turkish study of 333 males and females aged 11-16 years, BA was underestimated in the 11-14 year age groups and overestimated in the 15–16-year-olds (198). Similarly, advanced BA was reported in females from birth until 13 years of age and delayed BA in males from birth until 13 years of age after which point BA was advanced in males between 13 and 17 years of age in a study in 406 Scottish children (199). These studies show that there is variation in the applicability of the same method with underestimation and overestimation of BA in the same population. This demonstrates age-related bias in the use of GP method in different populations which may pose as a limitation in the use of the method for BA assessment. In contrast, earlier studies not reported in Table 2.3 had also reported the applicability of the GP in the girls at all age groups with an age-related bias in the boys (87, 200). In a French population GP overestimated BA in all males and females except boys who are 12 years and girls who are 11 and 18 years old (201). In this study no pattern or explanation can be drawn from the overestimation reported and the results may be a result of measurement error. A few studies concluded that the GP method was not applicable to the White children (88, 200). These results indicate variability in the applicability of the GP method for BA assessment which may be an

indication that modern adolescents are maturing earlier than the cohorts in which these methods were originally derived. Therefore, population specific reference data for BA assessment should ideally be developed to ensure accurate interpretation of growth and development particularly in the context where the findings have significant legal implications.

2.9.2 Greulich Pyle in Asian and Hispanic children

The GP method was reported to be applicable in Korean, Israeli and Iranian children (Table 2.4) (202-204). The Iranian study was of 412 children aged 6-18 years and the GP method reported an SMD of -0.4 years in boys and 0.04 years in girls (203). Similarly, the study in Korea concluded that the method was applicable and the study was conducted in 212 participants aged 7-12 years (202). However, the limited age range of the study population makes it difficult to draw conclusions for the general population as the authors did. In contrast the GP was not applicable in Indian populations with SMD differences of slightly below -0.7 years in males and -0.3 years in females. As discussed in section 2.9 it is unclear how much SMD should be there for the method to be said it is applicable. In some studies applicability is reported in some age groups and not others based on the magnitude of the difference between BA and CA. For example, in a study of Asian- and Hispanic- Americans BA approximated CA in Asian girls from 0 to 13 years, underestimated CA in Asian and Hispanic boys aged 0-13 years and overestimated in boys from 13 to 18 years (87). In a meta-analysis conducted of studies conducted in Asian populations, delays in skeletal maturity were noted in boys during early childhood and middle childhood and during adolescence skeletal maturity was advanced (158). A delay in BA as assessed by the GP method in Middle Eastern males and Iranian males and in Southeast Asian children (Indonesian and Indian) and Asian-American males has been documented (194, 202, 205, 206).

Table 2.3 Summary of studies conducted using the Greulich and Pyle and Tanner Whitehouse 3 in White populations from the year 2000 to date

Author	Year	Country of origin	Sample size	Age range (years)	Sex	Study design	Method of bone age assessment	Mean SMD (years)	
								Males	Females
‡Martrille et al	2023	France	94	0-8 y	M/F	cross sectional	GP	1.27	
†Martinho et al	2021	Portugal	441	10-17 y	F	cross sectional	GP		0.19
Alcina et al	2017	Spain	1150	0-18 y	M/F	cross sectional	GP	0.33	0.01
Maggio et al	2016	Australia	360	0-25 y	M/F	cross sectional	GP	0.24	-0.14
Gungor et al	2015	Turkey	535	10-18 y	M/F	cross sectional	GP	0.64	-0.53
Zabete et al	2015	France	190	10-19y	M/F	cross sectional	GP	-0.19	-0.54
*Mansourvar et al	2014	America	46	10-16 Y	M	cross sectional	GP	0.04	-0.98
Paxton et al	2013	Australia	406	0-18 y	M/F	cross sectional	GP	-0.13	0.31
Hackman et al	2013	Scotland	406	0-21 y	M/F	cross sectional	GP	0.1 Y	0.2 Y
Santoro et al	2012	Italy	535	7-15 y	M/F	cross sectional	GP	-0.1	0.4
Santos et al	2011	Portugal	230	12-10 y		cross sectional	GP	0.12	0.02
Calfee et al	2010	USA		12-18 y		cross sectional	GP	0.98 y	0.66
Zhang et al	2009	America	327	0-18 y		cross sectional	GP`	0.01	-0.15
Buken et al	2007	Turkey	492	11-19y	M/F	Cross sectional	GP	0.13	0.54
Schmidt et al	2006	German	649	1-18 Y	M/F	cross sectional	GP	-0.49y	-0.39
Van Rijn et al	2001	Netherlands	572	5-20 y	M/F	cross sectional	GP	0.28	0.14
Mora et al	2001	American	260	0-19 y	M/F	cross sectional	GP	0.09	-0.14
*kocKoc et al	2001	Turkey	225	7- 17 y	M	cross sectional	GP	-0.2	
Alshamraniet al	2019	UK		2-15 y		cross sectional	Bone expert	GP 0.13 TW3 0.02	GP-0.07 TW3 -0.43
Pinchi et al	2014	Italy	307	6-20 y	M/F	cross sectional	TW3		
Buken et al	2010	Turkey	324	11-16y	M/F	cross sectional	TW3	0.19	0.19
Schimidt et al	2008	German	92	12-16 y	M/F	Cross sectional	TW3		
Freitas et al	2004	Portugal	507	8-18 y		longitudinal	TW2		
Buken et al	2009	Turkey	333	11-16 y	M/F	cross sectional	GP and TW3	GP -0.02 TW3 -0.18	GP 0.66 TW3 -0.21

***SMD** skeletal maturity deviation calculated as bone age minus chronological age, ‡study did not report SMD by sex, †study reported SMD in females only, *study reported SMD in males only*

2.9.3 Applicability of the TW3 method

Fewer studies have reported the applicability of the TW3 method when compared to the GP method (Table 2.3). The TW3 method has been applied in Turkish, Chinese, German and Italian populations with the already mentioned methodological limitations for children above 16 years (202, 207-210). The Turkish study was conducted in 333 children aged 11 to 16 years to determine the accuracy of the TW3 method in BA estimation for forensic age determination (207). The mean differences between BA and CA for both males and females were similar with a delay of 0.19 years. However, sex differences were reported in a study of 392 British children aged 2 to 15 years, BA by TW3 underestimated CA in females by an average of 0.43 years and in the males SMD was not significantly different (211). The study used BoneXpert derived BA when using the TW3 method. Similarly, sex differences were noted in Saudi Arabia, but underestimation of CA by BA was seen in the males (-0.22 years) and not the females. It is difficult to explain differences in the results by use of automated methods of BA assessment because in Saudi Arabia both the automated and manual methods were used and in the males the results from both were conforming. These variations in in the performance of the methods in different populations further illustrate the need to derive population specific methods. An Italian study also reported the TW3 method having the highest number of participants (40%) with BA approximating CA when they compared to GP. The study sought to measure the proficiency of the two methods in predicting the CA. The percentages of participants who were correctly classified, underestimated and overestimated were collected. Correct classification was considered as BA-CA of ± 3 months. A much larger study of 17 401 Chinese children aged 1-20 years, BA was more advanced than CA, in boys aged 6 years and older and girls aged 10 years and older (212).

The limitations in the use of the TW3 method in older children have been demonstrated in an Australian study where the TW3 method was appropriate for the children below 16 years and not in the older children especially to establish the age of majority which is 18 years (209). The study was conducted in 360 participants aged 0 to 24.9 years. The threshold set by the method of a BA of 15 years for females and 16.5 years for males is reached at different CAs by different individuals. Therefore, in populations with delayed maturity the method is limited. Similarly, larger deviation between BA and CA were seen after the age of 15 in an Italian study of 307 children aged 6-20 years further demonstrating the limitations of the method. This literature

further highlights the need to assess the applicability of BA methods in African populations; this work is needed to ascertain the best methods to use as there are ethnic and sexual differences apparent in other settings.

2.9.4 Implications of the different studies on applicability

The results on the applicability of the different methods in African populations are discussed in detail in section 2.10. Given the secular changes in skeletal maturation and the different geo locations and different ethnic groups the use of these two commonly used methods becomes questionable considering there is no gold standard. Some studies have attempted to construct smoothing maturity score curves from the skeletal maturity scores (SMS) of the TW method to set up reference values of converting SMS to BA specific to their populations. In Japan a healthy population of 1457 children aged 3-18 years had TW2 bone ages estimated and the 50th percentile skeletal maturity scores obtained (213). They determined smoothed RUS maturity curves applying the cubic spline function to the 50th-centile scores. The score at each 0.1 year of CA was obtained and allocated as a given RUS skeletal age on the maturity curve. The set of scores and ages were termed the Japanese TW2 RUS maturity standard (TW2-J RUS). The authors compared the TW2-J RUS standards to those of the British, Belgian, Chinese, and Indian populations, and Japanese children rapidly progressed through puberty attaining the adult stage 1-2 years earlier than the other groups of children. In China the smoothed maturity score curves were obtained using the LMS (lambda, median and Sigma) method which provides a way of obtaining normalised growth centiles standards (212). The reference values for Chinese children differed with those of the TW3 standards showing relative advancement of Chinese children to the reference population after the age of 6 years for males and 10 years for females. Before applying these methods in different populations specific references are often needed.

2.10 Studies of bone age in black African populations

To date, studies in populations with African heritage living in high-income countries such as the United States that have been studied have mostly used the GP method (158). The advanced BA was seen only in prepubertal African American children who had advanced BA of 0.09±0.66 years compared to prepubertal European American children who had delayed BA of 0.17±0.67 years in a study of 534 children aged 0-19 years. Furthermore, 10% of prepubertal African American children had BA 2 SD above the reference data in the G&P atlas compared to

8% of European American prepubertal children (88). Larger SMD was of 1.87 years was reported by Mansourvar et al. in a study of 184 children between 8 and 15 years of different ethnicity (194). The authors concluded that the GP was not reliable for assessment of African American children. These studies indicate that African populations living in high-income countries have advanced BA relative to CA whilst delayed BA is reported in African populations living in Africa (discussed fully in the next section 2.11.1). In contrast, only one study reported BA approximating CA (-0.02 years in males and 0.03 years in females) in African American children in a large study of 1390 children aged 0-18 years of different ethnicities.

2.10.1 Cross sectional studies in Black Africans living in Africa

Cross sectional evidence in African populations living in Africa have reported variable SMD. A South African study of 131 African males aged 13-22 years was conducted in adolescents who presented for treatment of trauma and were in reasonably good health (214). BA was assessed using the GP method and the children were divided into two groups, the 13–19-year-olds consistent with the GP age categories with an upper limit of 19 years and 20–22-year-olds. The mean difference between BA and CA for the 13-19 years olds was 0.2 years and for the 20–22-year-olds was 2.1 years. The mean SMD for the 13–19-year-olds may seem small but further analysis reveals that in 57 of the 61 children aged 18 -21 years BA was below CA indicating that skeletal maturation was still ongoing in individuals at ages beyond the stipulated age of maturity of 19 years by the GP method. In 74% of the males aged 13-22 years BA was below CA and 26% had BA above CA (214). The authors concluded that the GP method is not directly applicable to African male South Africans because of the magnitude of the differences between CA and BA. There was another study conducted in Black South African children only that I did not have access to the full article.

Earlier on in Malawi 139 participants aged two to twenty-eight years were studied to describe the differences between BA and CA using the GP method (102). The study excluded participants who were skeletally mature. The mean differences between BA and CA for females was 1.5 years and in males 1.7 years with BA less than CA in 85.6% of the participants. The delays in BA were attributed to nutritional deficits as measured by BMI, although as discussed in chapter one section 1.8.3, the definition of BMI was not ideal for children. The authors recommended use of the GP method with caution, considering the large SMD. Similarly, delayed skeletal maturity in the African populations was found with mean differences between

BA and CA in males and females of 1.56 and 0.97 years respectively in a much larger study of 665 females and 1018 males aged 3-23 years in Sudan, East Africa (91). Delayed BA was consistent across all age groups in the males but in the females aged 13-18years BA was advanced.

These results may be indicating the genetic and environmental differences of the populations under study from the population in which the GP atlas was derived from. This suggests the need to robustly assess any population to be studied and determine the method best suited for that population. They also highlight the importance of a gold standard for BA assessment that is ethnicity specific. The differences between BA and CA noted in the studies reflects on whether this is a problem with the GP as a method or it reflects a true biological phenomenon where the children are truly delayed.

2.10.2 Longitudinal studies of Black Africans living in Africa

To my knowledge there is one longitudinal study conducted in Africa studying skeletal maturation. There are not many recent longitudinal studies of BA assessment, even in non-African ethnic groups, as hand wrist radiographs of study populations were discontinued after the realisation (in the mid 1950's) that medically unnecessary radiographs should be avoided (197). However, with technological advancements and constant improvements in the practice of radiography in relation to radiation protection, the amount of radiation received during a hand wrist radiograph is very minimal, equivalent of 20 minutes of background radiation as mentioned in section 2.2. The South African study aimed to describe the skeletal maturation of 607 urban, male and female, black and white African adolescents aged 9-20 years (89). Singleton children born in 1990 in Soweto Johannesburg were recruited and at 9 years old the children were recruited into the Bone Health sub study to investigate factors affecting the acquisition of peak bone mass. Hand wrist radiographs were taken yearly together DXA scans. The study presented longitudinal data on skeletal maturation utilising the hand wrist radiographs which were assessed using the TW3 RUS. Mean growth curves for RUS bone scores were presented analysing the sex and ethnic differences in patterns of skeletal maturation. Skeletal maturity was on average 1.9 years earlier in girls than in boys, and although the black boys reached skeletal maturity six months later than their white counterparts, the black and white girls reached skeletal maturity at the same age. The growth curves showed that skeletal maturation starts to accelerate later in black girls than in white girls so that they develop faster.

In black boys' skeletal maturation accelerates later but then develop at a similar rate as the white boys hence maturation is achieved at a later CA.

2.11 Comparison of Greulich and Pyle and Tanner Whitehouse 3

Several studies have compared the GP and the TW (either TW2 or TW3) but none have been conducted in African populations. In studies that have compared the two methods variable results have been found. In some studies, the two methods do not give equal estimates of BA with the TW3/TW2 method being reported as the most appropriate method for use. In a historical large and well established study conducted in 1971 in 1009 children aged 7-18 years in Denmark the overall BA estimates were closer to CA when using the TW2 method than the GP method (196). However, the TW2 method was reliable for younger children such that BA estimates in girls older than 13 years and boys older than 15 years showed a larger deviation from CA showing the limitation of the TW as a method which was discussed in section 2.4.2. Again Bull et al in 1999 found similar results in 362 British children aged 2 to 18 years although the study was smaller and did not exactly report the actual differences between BA and CA (3). They did report agreement between the methods using Bland and Altman plots, a method that is preferred in analysing the agreement between two methods. Therefore, in their conclusion the TW2 and GP gave different values for BA which are significant in clinical practice and in essence TW2 would be the preferred method for BA assessment when performing serial measurements on an individual. In addition, a more recent Chinese study of 390 participants aged 3-6 years concluded that BA assessment by the TW3 method gave more reliable results as the BA estimates were closer to CA than GP BA estimates (208). These results indicate that the two methods cannot be used interchangeably.

Table 2.4 Cross-sectional studies of bone age studies in Black African populations living in Africa and high-income countries

Method of bone age assessment	Author	Year	Population	Sample size	Age range	Design	Mean SMD	
Studies of African populations living in Africa								
							Males	**Females**
GP only	Olaotse et al	2023	Botswana	140	5-18 y	cross sectional	0.5	0.64
	Tsehay et al	2017	Ethiopia	108	10-22 y	cross sectional	0.72	0.98
	Elamin et al	2017	Sudan	1638	3-25 Y	cross sectional	I.56	0.97
	*Dembetembe et al	2012	South Africa	131	13-22 y	cross sectional	0.20 (13-19 y) 2.10 (20-21 y)	
	Lewis et al	2002	Malawi	139	1-28 y	cross sectional	1.60	1.80
TW3 only	Hawley et al	2011	South Africa	244	9-10 y	cross sectional	0.66	1.00
Studies of African populations living in high income countries								
	Zhang et al	2009	African American	349	0-18		-0.02	0.03
	Mansourvar	2014	African American	47	8-15 y		1.87	
	Mora	2001	African American	274	0-19 y		-0.01	0.11
	Garamendi	2004	Moroccans	114	13-25		-1.7	
	Ontel	1996	African American					

However, in some studies both methods could be used to estimate CA for their populations as SMD by TW3 method was almost equal to zero and GP BA tended to overestimate CA although the differences were not statistically significant in a study of 307 Italian children aged 6 to 20 years, (215). In this study accuracy was measured (the degree of closeness of measurements of a quantity to that quantity's true value) and found the accuracy similar for both methods. Accuracy was measured as the (true negative + true positive)/N and most studies do not measure the accuracy of the methods of BA assessment. Similarly, both methods could be used in Turkish study of 333 children, aged 11-16 although their population did not have a wider age arrange than the Italian study (198). Interestingly in Saudi Arabia both methods were giving equal estimates of BA in females and not in males in a study of 440 participants aged 1 to 18 years (216). There were other studies that could have contributed to the wider literature on the comparison of GP and TW3 but either the papers were not accessible or only the abstract had English translations (191, 217-219).

2.12 Risk factors for delayed bone age and development

As discussed in Chapter one section 1.7, many factors influence the process of skeletal maturation thereby delaying growth and development in children and adolescents. This delay will be assessed using BA in this thesis. However, there are few data concerning factors associated with a delay or advancement in skeletal maturity in children and adolescents, particularly in LMIC populations where growth is most impacted by early life factors such as nutrition and childhood infections and illnesses. One study in South Africa utilised the longitudinal data from the Birth to Twenty (Bt20) cohort to investigate the biological and environmental factors associated with SMD (difference between BA and CA) at age 9-10 years (220). The analysis was of 244 participants with complete anthropometric and questionnaire data at birth, two, four and 9/10 years was conducted. These time points were selected to represent early-childhood, mid-childhood and early adolescence. The males and females had delayed skeletal maturity of 0.66 years and 1.00 years respectively. Being taller and heavier at 2 years predicted less SMD at age 9/10 years, independent of current size and body composition in males. In females, greater attained height and weight at 2 years predicted more advanced skeletal maturation at 9/10 years although the effect was mediated by current body composition (fat mass, lean mass). Having greater lean mass at 9/10 years and attaining puberty at the time of skeletal maturity assessment was associated with less SMD. In another

study, 196 infants from the Bt20 cohort in South Africa and 467 children from the Fels longitudinal study in the United States of America who were appropriate for gestational age were studied. They found that a greater increase in weight at age 0-2 years was associated with advanced skeletal maturation at age 9 years after accounting for birth weight and BMI at age 9 years (221). Across the two cohorts the magnitude of the effect of greater attained weight was similar with an SDS increase in weight from 0 to 2 years resulting in BA advanced by approximately 0.2 years. There was no indication that the positive effect of infant weight gain on the rate of skeletal maturation differed in boys and girls or by birth weight after testing for interaction. However, this study utilized data from two different countries and geographic locations, which may reflect growth trajectories associated with distinct ethnicities. Comparing these trajectories is challenging due to the significant differences in socioeconomic status (SES) and environmental factors between the two cohorts. Also, the methods of BA assessment were different with the Fels method used in the Fels cohort and TW2 in the Bt20 cohort. It is unclear how these methods compare to each other; do they give equal estimates of BA or not? In essence, these two studies described above indicate the role of early life exposures in determining the rate of skeletal maturation.

In Tasmania a study was conducted to describe factors associated with SMD in children aged 7-17 years (222). Participants were obtained from a case control study investigating the role of growth, bone strength, sports participation, risk taking, and coordination in the aetiology of upper limb fractures. The authors found that body composition, grip strength, diet, ever smoking, and inhaled corticosteroid use may be determinants SMD and thus affect fracture risk in children. Previously in the same population the association between SMD, bone density and upper limb fractures had been investigated (223). SMD was positively associated with measures of bone strength and negatively associated with upper limb fractures. The authors highlighted the importance of SMD and how it may give additional information regarding bone health and fracture risk in children.

In a much larger African study of 1633 males and females in Sudan aged 3-18, poor nutrition and low SES were strongly associated with a delay in skeletal maturation (91).

2.13 Rationale, Hypothesis, Aims and Objectives

2.13.1 Rationale

The study of skeletal maturation provides valuable insights into the growth and development of a child, and clinicians can make predictions of the potential final adult height which is important in identifying and managing growth related issues. Skeletal maturation in the Zimbabwean population has not been studied. It is not known which of the two most used methods of BA assessment (GP and TW3) is more applicable to the Zimbabwean children and adolescents. Studies on BA assessment conducted in Southern Africa looked at GP method of assessment and two studies from the same study population used the TW3 method. These studies conducted in Africa have been limited by a lack of comparison of the two methods to ascertain which is more applicable. Comparison of the two methods has an advantage of better understanding the method of choice when assessing skeletal maturation in an African population. Most clinicians would prefer to use the GP method for age estimation as it is quick but there is need to study how it compares with the TW3 in African populations to choose a method that is more precise in estimating BA. Most studies validating methods of skeletal maturation measurement have been performed in high income countries.

In addition, no study has been conducted in CLWH in African populations whether longitudinal or cross sectional. Although there are other cross sectional studies conducted in HIV negative children in Africa, only one studied skeletal maturity longitudinally in an African population which focused on a younger age highlighting the paucity of skeletal maturity data in Africa (89). Therefore, my study will provide longitudinal analyses of skeletal maturation in children living with and without HIV which has been lacking in Africa. Furthermore, there are limited studies that have looked at factors associated with skeletal maturity delays, such that only one study conducted in South Africa has determined factors associated with skeletal maturation (220, 222). Considering the different factors that influence skeletal maturation discussed in section 1.8, it is imperative to study factors associated with skeletal maturation in the Zimbabwean context to understand the growth and development of children and adolescents in the country. Zimbabwe is challenged by chronic conditions amongst them HIV, low income, multi-morbidity and poor nutrition, all of which influence the process of skeletal maturation. The study focused on children from the IMVASK (The **IM**pact of **V**ertical HIV infection on child and **A**dolescent **Sk**eletal development) study which consists of Zimbabwean

peripubertal children, living with HIV and without HIV, followed up over one year with hand/wrist radiographs taken at baseline and follow-up. The IMVASK study was completed in 2021 in Harare, Zimbabwe.

2.13.2 Hypothesis

The main hypothesis is that HIV infection and treatment, demographic and lifestyle factors (undernutrition, physical activity) predict delayed skeletal maturation and slower progression in skeletal maturation. This hypothesis means the CLWH develop slower than their HIV negative counterparts and may not attain full adult height. The secondary hypothesis is that the TW3 method is more precise and applicable than the GP method when assessing BA in Zimbabwean children.

2.13.3 Study Aim

The overarching aim of my PhD was to describe skeletal maturity and explore the determinants of skeletal maturity delay at two time points in children and adolescents living with and without HIV.

2.13.4 Study objectives

Methodological evaluation (objectives addressed in chapter 3)

1. To determine which method of BA assessment (GP and TW3) is applicable for use in peripubertal children in Zimbabwe.
2. To determine the agreement between the two methods.
3. To determine the inter- and intra-rater reliability and intra- and inter operator precision for each method.

IMVASK cross sectional analyses (objectives addressed in chapter 4)

1. Describe the differences between BA and CA at baseline (i.e., skeletal maturity deviation [SMD])
2. Determine the factors associated with SMD at baseline (HIV status, weight for age z-scores, pubertal stage, orphan status, vitamin D and calcium intake, physical activity)
3. Examine which HIV characteristics are associated with SMD in CLWH in Zimbabwe at baseline (age at ART initiation, CD4 count, viral load, TDF exposure).

IMVASK longitudinal analyses (objectives addressed in chapter 5)

1. Determine the skeletal maturity deviation (SMD) at follow up independent of the SMD at baseline
2. Determine baseline factors (SES, pubertal stage, physical activity, vitamin D and calcium intake), associated with SMD at follow up, independent of SMD at baseline
3. Determine baseline HIV characteristics associated with SMD at follow-up independent of SMD at baseline

2.14 Conclusion

This chapter narrates the literature on the different methods of BA assessment and their use in different populations. Although many studies have been conducted, studies in African populations are limited, and no study has compared the two different methods in SSA. Considering that Africa differs in SES, disease patterns, genetics and ethnicity with the populations in which these methods were extensively studied, it is imperative to investigate patterns of skeletal maturation in SSA. The following chapter will describe the study population and methods

Chapter 3 Study participants and setting

3.1 Introduction

The IMVASK study is one of the largest musculoskeletal cohorts in SSA which began data collection from May 4, 2018, to January 21, 2020 (224). It was a prospective cohort study on the impact of vertical HIV infection on child and adolescent skeletal development in Harare, Zimbabwe. Children and adolescents with and without perinatal HIV infection aged 8-16 years were recruited and followed up after one year. CLWH were recruited from HIV clinics at the two main public sector (tertiary referral) hospitals in Harare (Parirenyatwa and Sally Mugabe Hospital). Both hospitals have paediatric HIV clinics that provide HIV care and treatment to more than 2,000 children (224). Although HIV care is increasingly decentralised to a primary care level across the country, most children in Harare continue to receive care within the HIV clinics in these healthcare facilities. The CLWH were enrolled in the study if they had been established on ART for at least two years. Systematic quota-based sampling was used to recruit children stratified by age and sex. Therefore 50 females and 50 males were recruited into three age groups (8-10 years, 11-13 years, and 14-16 years). Most of the participants were school going children (98%) and the rest were not attending any school. Participants were excluded if they were acutely unwell, not residing in Harare and unaware of their HIV status.

A comparison group of children without HIV were enrolled in three primary schools and three secondary schools randomly selected from the schools in the same catchment area as Parirenyatwa and Sally Mugabe Hospitals. Children aged 8-12 years were selected from primary schools and older children 14-16 years from the secondary schools. Children aged 13 years were sampled from both primary and secondary schools. The number of children selected in each school was proportional to its size giving each child an equal chance of being sampled. Quota-based sampling was used to sample 50 males and 50 females in each of the three age groups. All children underwent HIV testing after enrolment and those in schools who tested positive were considered for enrolment in the HIV cohort by stratified random sampling, using school registers. These children were aged between 8 and 16 years. Total enrolment was 609 (303 CLWH). The IMVASK study protocol, baseline DXA and pQCT (peripheral Quantitative Computed Tomography) results have been published (121, 224, 225). My PhD study included children with baseline and follow-up hand wrist radiographs.

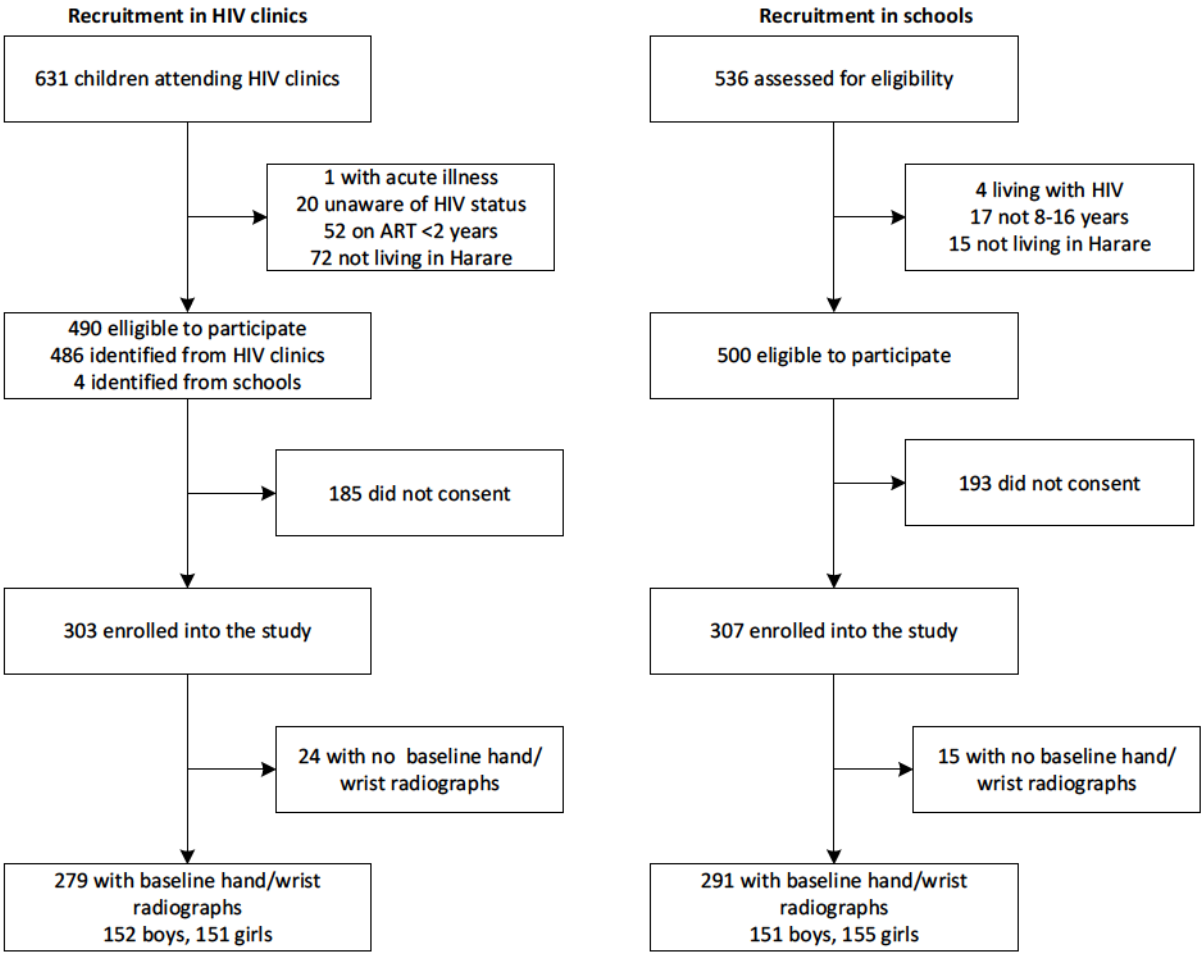


Figure 3.1 Flow diagram to show participants included in the IMVASK bone age study

3.2 Ethics Approval

Study information was explained to participants and guardians by trained research assistants and/or study nurse. Written informed consent was given by guardians for study participation and HIV testing. The children provided age-appropriate written assent. Ethical approval was granted by Parirenyatwa Hospital and Faculty of Medicine and Health Sciences joint research ethics committee (JREC/11/18), Sally Mugabe Hospital ethics committee (ref: 170118/04), the Medical Research Council of Zimbabwe (ref: MRCZ/A/2297), the Ministry of Primary and Secondary education of the Government of Zimbabwe (Harare: reference C/426/Harare). For the purposes of the PhD approval was also sought at the University of Southampton Faculty of Medicine Ethics Committee (ERGO II 62773) retrospectively.

3.3 Methods

3.3.1 IMVASK data collection

Data collection included socio-demographics, anthropometry measurements, lifestyle factors pubertal status and BA measures at baseline and one-year follow up. The list of the measured variables is shown in Table 2.1.

Table 3.1 Measured variables in the IMVASK study

	Instrument used
Bone age assessment	
Bone age	TW3 and GP
Anthropometry	
Height (standing and sitting)	Seca
Weight	Seca
BMI	
Socio-demographics	
Chronological age	Questionnaire
Sex	Questionnaire
School attendance	Questionnaire
Orphanhood/guardianship	Questionnaire
Socio-economic status	Questionnaire
Clinical history	
History of fractures and trauma	Questionnaire
Age at HIV diagnosis	Medical records
WHO HIV disease stage	Medical records
ART regimen and duration	Medical records
Steroid use	Questionnaire
Lifestyle and behaviour	
Physical activity	Questionnaire
Dietary intakes (vitamin D and calcium)	Questionnaire
Smoking, alcohol, and recreational drugs	Questionnaire

Pubertal stage	
Tanner staging	Tanner staging
Laboratory tests	
Viral load	GeneXpert
CD4 count	Alere PIMA CD4 analyser

***WHO**- world Health organisation, **ART**-Anti-Retroviral Therapy*

3.3.2 Chronological age

Baseline CA was calculated from the date of birth to the date the baseline hand wrist radiograph was taken and follow up CA up to the date the follow up hand wrist radiograph was taken.

3.3.3 Imaging of the hand/wrist

Hand wrist radiographs used for BA assessment were taken of the non-dominant side by a trained radiographer. In my study, I utilised the non-dominant hand instead of explicitly using the left hand, as recommended by the GP atlas and the TW3 scoring method. This choice was made because the non-dominant hand is typically used less frequently, making it less susceptible to physical stress and trauma (226), thereby ensuring a more accurate assessment of skeletal maturation. For most individuals, the non-dominant hand is the left hand; however, by using the non-dominant hand, I was also able to accommodate those who are left-handed. A digital radiography (Siemens, Germany) system was used, and images were in DICOM format. The non-dominant hand was positioned with the palm facing downwards in contact with the imaging plate (Figure 2.6). The axis of the middle finger was in direct line with the axis of the forearm. The upper arm and forearm were in the same horizontal plane. The central ray of the X-ray beam was directed on the distal end of the third metacarpal and a tube to film distance of 76 cm was used. A lead apron was used to shield the gonads of the child. The standard exposure parameters for a hand radiograph were used which are 50kv (kilovoltage) and 3mAs (miliAmps second). The radiation dose to the subjects was estimated to be less than 2μSV (micro-Sieverts) equivalent to 20min of the average background as indicated in section 2.2.

Figure 3.2 An image showing a participant positioned for a hand/wrist radiograph during the IMVASK study (with permission)

3.3.4 Bone age assessment and Training

I assessed BA using both TW3 (RUS) and GP methods. I was trained to do BA assessment for the two methods, TW3 and GP at the University of Sheffield, Sheffield, United Kingdom. The training was funded through the UKRI Global Impact Acceleration funding awarded to Professor Celia Gregson for Musculoskeletal knowledge exchange between Zimbabwe and Bristol. The training was given by Professor Amaka C Offiah a Professor of Paediatric Radiology who is an expert in BA assessment. The training was according to the protocol outlined in the GP and TW3 atlas as outlined below. For the purposes of learning the techniques for the two methods I used 100 radiographs available at the time from the IMVASK study, and the steps outlined below.

Firstly, Prof Amaka Offiah took me through the steps of rating radiographs by both methods. I then went through the following steps as a learning procedure

1. I arranged Radiographs in serial order
2. I systematically examined each radiograph, considering one bone at a time in the following order: radius, ulna, metacarpals I, III, V, proximal phalanges I, III, V, middle phalanges I, III, V, and distal phalanges I, III, V., I referred to the provided text for each

bone and the definitive rating criteria to observe how the assessment criteria were applied.

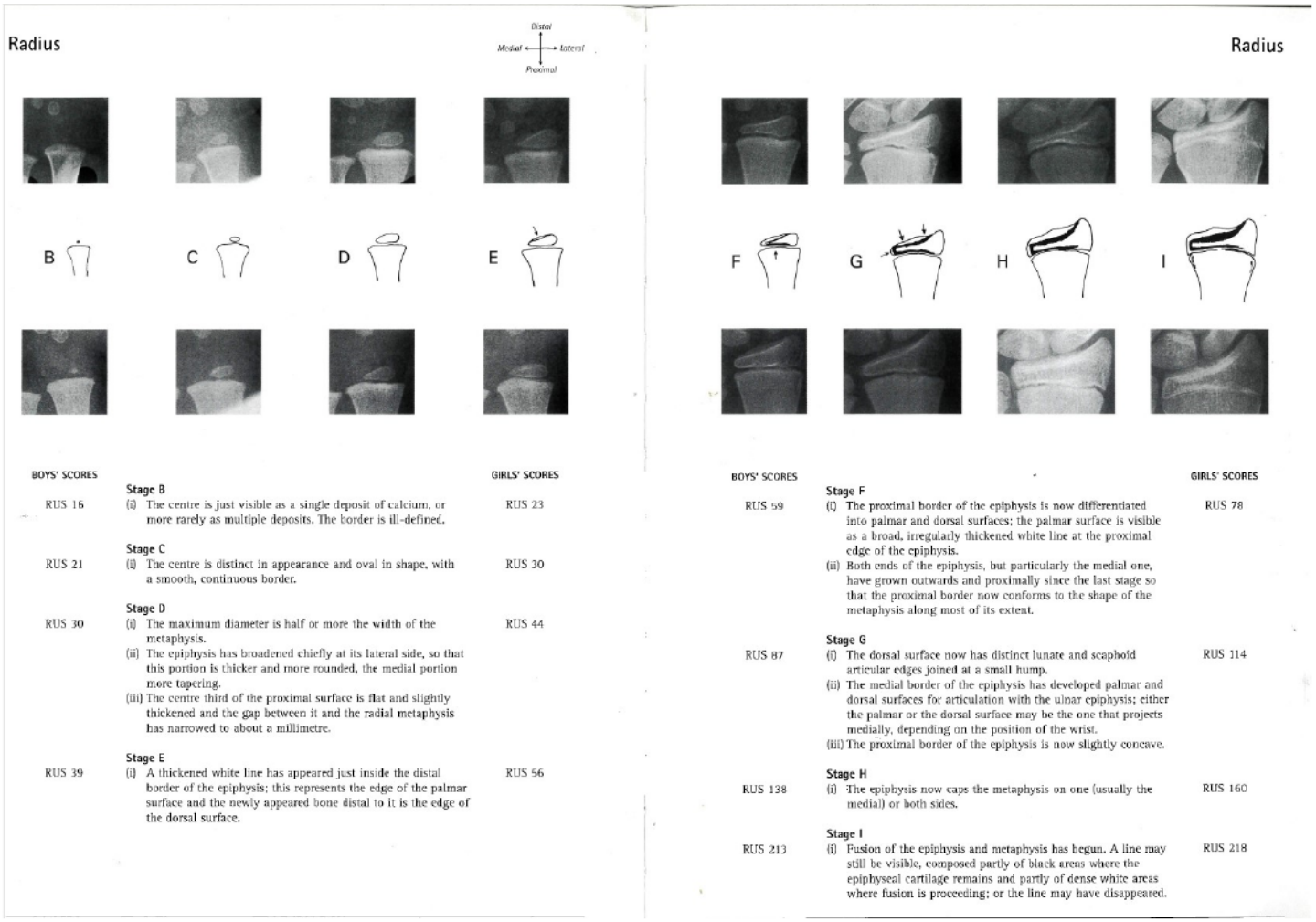
3. I made notes on any problems encountered.
4. I discussed the problems with Prof Amaka Offiah
5. She re rated the radiographs and we made comparisons
6. Differences were then discussed, and some radiographs were re rated
7. The process was repeated using different sets of 30 radiographs

Training was conducted in Feb/Mar 2019. I further disseminated the knowledge gained to other radiographers in Zimbabwe.

However, data for the inter-rater reliability between me and Prof Amaka Offiah got lost when my laptop crushed, therefore no longer available. This is a limitation of my study, as I am unable to present data on the consistency and agreement between the two of us. Therefore, in training the next radiographer there was no benchmark whether the same standards were applied as during the first training of me by Prof Offiah.

3.3.4.1 Bone age assessment using Tanner Whitehouse 3 method

The TW3 (RUS) method of assessment was based on comparison to a sex-matched TW3 atlas to determine the level of maturity for 13 selected regions of interest representing specific bones of the hand and wrist (168). The specific bones included radius, ulna, metacarpals I, III, V; proximal phalanges I, III, V; middle phalanges III, V; distal phalanges I, III, V. Each bone was categorised into specific stages of development labelled from A to I in the atlas (Figure2.7). A numerical score is assigned to each stage of development for each individual bone in the atlas. Using the pictorial and descriptive standards presented in the third addition of the Tanner Whitehouse manual, the above bones were given the alphabetical rating hence the corresponding numerical score. The numerical scores were summed to give a total maturity score ranging from 0 to 1000. Each maturity score gender matches a certain BA via a lookup table provided for by the atlas. The corresponding BA was recorded together with the sex, date of birth and date of examination into an excel file.



Radius

Distal
Medial
Lateral
Proximal

B C D E

BOYS' SCORES		GIRLS' SCORES
	Stage B	
RUS 16	(i) The centre is just visible as a single deposit of calcium, or more rarely as multiple deposits. The border is ill-defined.	RUS 23
	Stage C	
RUS 21	(i) The centre is distinct in appearance and oval in shape, with a smooth, continuous border.	RUS 30
	Stage D	
RUS 30	(i) The maximum diameter is half or more the width of the metaphysis. (ii) The epiphysis has broadened chiefly at its lateral side, so that this portion is thicker and more rounded, the medial portion more tapering. (iii) The centre third of the proximal surface is flat and slightly thickened and the gap between it and the radial metaphysis has narrowed to about a millimetre.	RUS 44
	Stage E	
RUS 39	(i) A thickened white line has appeared just inside the distal border of the epiphysis; this represents the edge of the palmar surface and the newly appeared bone distal to it is the edge of the dorsal surface.	RUS 56

Radius

F G H I

BOYS' SCORES		GIRLS' SCORES
	Stage F	
RUS 59	(i) The proximal border of the epiphysis is now differentiated into palmar and dorsal surfaces; the palmar surface is visible as a broad, irregularly thickened white line at the proximal edge of the epiphysis. (ii) Both ends of the epiphysis, but particularly the medial one, have grown outwards and proximally since the last stage so that the proximal border now conforms to the shape of the metaphysis along most of its extent.	RUS 78
	Stage G	
RUS 87	(i) The dorsal surface now has distinct lunate and scaphoid articular edges joined at a small hump. (ii) The medial border of the epiphysis has developed palmar and dorsal surfaces for articulation with the ulnar epiphysis; either the palmar or the dorsal surface may be the one that projects medially, depending on the position of the wrist. (iii) The proximal border of the epiphysis is now slightly concave.	RUS 114
	Stage H	
RUS 138	(i) The epiphysis now caps the metaphysis on one (usually the medial) or both sides.	RUS 160
	Stage I	
RUS 213	(i) Fusion of the epiphysis and metaphysis has begun. A line may still be visible, composed partly of black areas where the epiphyseal cartilage remains and partly of dense white areas where fusion is proceeding; or the line may have disappeared.	RUS 218

Figure 3.3 An image showing the TW3 standards for the radius for stages B-I (Tanner et al 2001)

3.3.4.2 Bone age assessment using the Greulich and Pyle method

I used the protocol for BA assessment as described in the Greulich and Pyle atlas (2). The atlas has a description of radiographic features that correspond to the assigned BA for each standard. The characteristics of the maturity indicators are outlined in the appendix of the atlas. Firstly, a given radiograph is compared with the standard of the nearest CA and same sex in the atlas. The next step is to compare the radiograph to the adjacent standards, i.e. younger or older than nearest CA. The radiograph is then assigned a BA equal to that of the nearest age standard that appears to resemble it most closely (Figure 2.8), a hand wrist radiograph of a 14-year-old is compared with the standard of the same CA (male standard 25) and an adjacent older standard (male standard 26) of the GP atlas. The hand wrist radiograph most resembles the standard of the same CA (male standard 25).

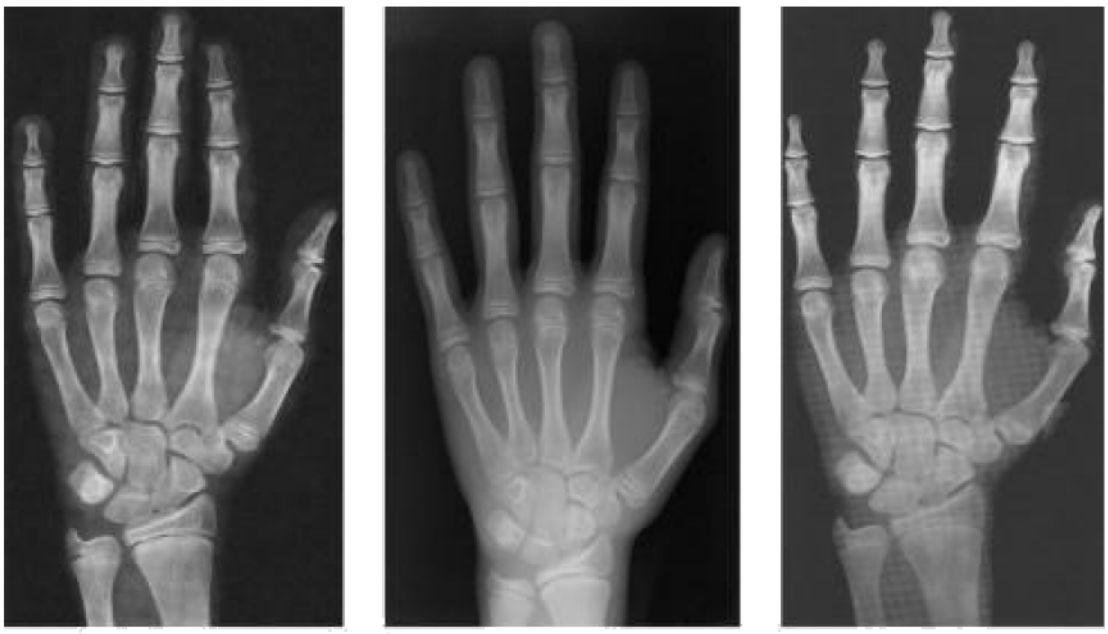


Figure 3.4 An image showing comparison of a hand radiograph for a 14-year-old (middle) using two different GP atlas standards as shown on both sides (Schmidt et al 2008)

To check whether my ratings were reliable, inter- and intra-rater reliability and precision were calculated. I randomly selected and rescored 52 (20%) IMVASK baseline radiographs (of children without HIV) at least three months later than the initial score to calculate the intra-rater reliability and precision (measured as co-efficient of variation). To determine inter-rater reliability and precision, all radiographs (n=252) were re-scored by a second trained assessor (Evangelista Benno). The second assessor was also a fully trained radiographer.

3.4 Procedures

This subsection outlines the baseline participants' characteristics collected in the IMVASK study which were used in this thesis. All data were collected at recruitment (baseline). Socio demographic and clinical data were collected by trained research staff using an interview-administered questionnaire. The data were collected from the children in the company of the parents, and the parents were allowed to answer on behalf of the children. Data such as sex, SES and health behaviours such as smoking status and alcohol and steroid use were collected on android tablets using the Online Data Kit (https://getodk.org/).

3.4.1 Pubertal development

Pubertal status was assessed using the Tanner staging technique for pubertal assessment (45). A nurse and/or doctor carried out Tanner pubertal staging. In the event of discordance in the assignment of the pubertal stage between these categories, testicular and breast development for boys and girls respectively, were used to assign Tanner stage. Grading of penile, testicular and breast growth was from I to V as per Tanner descriptions (45, 46, 227). In boys the following measures were assessed: testicular volume penile size and pubic hair growth. An orchidometer measured testicular volume and for pubic hair growth they measured the quality, distribution and length. In girls the following measures were assessed: breast development, age at menarche and pubic hair growth (228).

3.4.2 Socio-economic status

SES data was derived from the first component from a principal component analysis that combined asset list and household item ownership (229). The asset had details of the head of household age, highest maternal and paternal education levels, monthly household income,

number in the household, household ownership, access to amenities (water, electricity and flush toilet or pit latrine) and household asset ownership (229). Household items considered were fridge, bicycle, car and television or radio. The SES was finally constructed as three tertiles (low, middle, and high) for analysis.

3.4.3 Dietary calcium and vitamin D intake

Dietary calcium and vitamin D intakes were assessed using a dietary diversity questionnaire based on that of the Food and Agriculture Organization of the United Nations (FAO) questionnaire adapted to Zimbabwe and Zambia, and focussing on foods rich in calcium or vitamin D (230, 231). Information collected included intake in the last month of legumes, dairy products, meat, eggs, fish, oil and margarine and vitamin and mineral supplements. Daily calcium dietary intake was classified into three groups: very low (<150mg/day), low (150-299 mg/day), and moderate (300-450 mg/day). Daily dietary vitamin D intake was classified as very low (<4.0 µg/day), low (4.0–5.9 µg/day), and moderate (6.0–8.0 µg/day) (232). The classification was based on external thresholds (121).

3.4.4 Physical activity

For physical activity assessment, the International Physical Activity Questionnaire (IPAQ) which has been validated in multiple countries including South Africa but not Zimbabwe, was used. This assessment was based on multiples of the resting metabolic rate (MET) in MET minutes. Time spent on vigorous activity (e.g. heavy lifting, digging), moderate physical activity (e.g. cycling carrying light objects) walking and sitting was multiplied by the corresponding MET. The amount of physical activity per week was calculated in MET minutes /week and categorised as low (<600 MET minutes/week), moderate (600-3000 MET minutes/week), and high (>3000 MET minutes/week) physical activity according to standard METS from IPAQ (233).

3.4.5 HIV characteristics

In participants with HIV, details collected were age at HIV diagnosis, probable mode of transmission, ART regimen including use of tenofovir disoproxil fumarate (TDF) and duration, age at ART initiation and current CD4 cell count and HIV viral load. Medical records were used to collect the HIV characteristics and CD4 count, and viral load were measured as described in section 3.4.7.

3.4.6 Comorbidities

Details of comorbid conditions that include arthritis, epilepsy, any kidney condition, tuberculosis, asthma, diabetes and cardiovascular conditions were collected in all participants. The data was collected using medical records as well as self-reporting.

3.4.7 Anthropometric measurements

Standing height and weight were measured in duplicate by two independent trained staff members (nurse and research assistant) at the study clinics. If the two measurements differed by more than 0.5 cm or 0.5 kg, a third reading was taken by an additional reader. The mean of the two or three measurements was recorded as the final figure. The equipment for weight and height measurements was calibrated annually over the 3 years of the IMVASK study. The same staff measured anthropometry measures in children with and without HIV.

3.4.7.1 Height

Standing height was measured with the participant barefoot and positioned with the back of the head shoulders buttocks and heels touching the back of the stadiometer where possible. The arms hanged freely on the sides with the heels touching each other. Sitting height was measured with the participant seated on a chair or flat surface with the back and buttocks positioned against a stadiometer with the head in the Frankfort plane. Standing and sitting height measurement was recorded by the two observers separately to the nearest 0.1 cm using a stadiometer (Seca, Hamburg Germany).

3.4.7.2 Weight

Weight was measured with the participant barefoot using a digital Seca (Hamburg, Germany), 875 weight scale. The participants faced forward with arms relaxed on the side and stood with both feet on the scale. The measurement was recorded once the displayed weight had stabilised on the scale. Weight was measured to the nearest 0.1 kg.

3.4.7.3 Body mass index

Body mass index was calculated as weight (kg)/height(m)^2.

3.4.8 Laboratory tests

All children recruited from schools were first tested for HIV to ascertain the HIV negative status. In participants with HIV and those who tested positive from schools, the current viral load and CD4 count were measured. CD4 count was measured using an Alere PIMA CD4 analyser (Waltham, MA, USA). A GeneXpert HIV-1 viral load platform (Cepheid, Sunnyvale, CA, USA) was used to measure HIV viral load. Viral suppression was defined as fewer than 1000 HIV RNA copies per ml according to the World Health Organisation guidelines.

Chapter 4 Evaluation of two methods of bone age assessment in peripubertal children in Zimbabwe

4.1 Aims and objectives

In my literature review in chapter two, I have demonstrated the importance of using the best method for bone age assessment. Therefore, in this chapter I aim to determine which of the two most widely used methods of BA assessment (TW3 and GP) is more applicable in the Zimbabwean population of peripubertal children. I hypothesised that the TW3 method would be more precise and applicable to the Zimbabwean children and adolescents. This hypothesis was informed by the ability of TW3 to assess ossification centres separately giving a score to each maturity indicator, which contributes to an overall score (all bones mature at different rates (234)) than the GP method which assesses BA from the whole hand giving an individual score from the whole hand.

As previously reported in chapter two, the applicability of these methods in different ethnic groups or populations other than those in which they were developed is questionable, given the many factors which influence the process of skeletal maturation. Zimbabwe is a LMIC, with people that have a different ethnicity from the populations which were used to develop the GP and TW3 methods. Zimbabwe is also characterised by a high prevalence of HIV and malnutrition, both of which are risk factors for poor growth. I anticipated that BA would not exceed CA in this study population given local environmental and nutritional challenges mentioned above. Furthermore, variation in the applicability of the methods in Whites, Africans and Asians (section 2.9) indicates a need to evaluate their applicability in the Zimbabwean population (158, 216). Therefore, in this analysis, I aimed to evaluate the two widely used method of BA assessment (TW3 and GP), determine the agreement between the two methods and inter- and intra-rater reliability.

This was a methodological evaluation study, and it was important to have a population of healthy children with no underlying chronic health condition. Therefore, children already known to have HIV, a condition that can constrain growth (75), were excluded in this analysis.

Participants were also excluded if they had CA above 15.0 years in females and 16.5 years in males because TW3 only assesses BA up to these ages.

I performed the BA analysis using both methods for this study. I developed the statistical analysis plan together with the Stata analysis do files and performed all statistical analyses, with support from my supervisors. I produced the tables of results and graphs. I wrote the first draft of the paper that was published and led on all revisions.

4.2 Statistical analyses

To address this question, I evaluated the two methods of BA assessment using hand wrist radiographs taken at baseline in the IMVASK study, determined the agreement between the two methods and determined the inter- and intra-rater reliability and intra- and inter operator precision for each method. I entered the derived BA and reported CA in a Microsoft .csv file and demographic data and clinical variables were in a Microsoft Access database. I merged data files using the study IDs and handled and managed using Stata 17 (StataCorp 2017 Stata Statistical Software: Release 17. College Station, TX: StataCorp LLC) for Windows 10. I cleaned and checked the data files for duplicates of participant IDs, variable completeness, and response consistencies. I performed other consistency checks on age (using date of birth), limit and logic checks were performed on all variables, including checking for biologically implausible values. I assessed categorical variables including HIV status, ART, age group and pubertal status (measured by Tanner stage) for distinctiveness and completeness.

4.2.1 Descriptive statistics

I examined quantitative data for normality using the Shapiro–Wilk test and histograms (visually assess the distribution of quantitative variables). Quantitative data were presented as mean ± standard deviation (SD) and if the data were skewed as median with lower and upper quartiles and categorical data as proportions and frequencies for the descriptive statistics. Chi-squared tests were used to compare categorical variables by sex. Unpaired-student t-tests were used to compare quantitative variables in (1) the whole study population, (2) females and males separately, and (3) in three age bands of 8-10, 11-13 and 14-16 years stratified by sex; this allowed determination of whether the performance of the BA method differed by maturity level. The primary outcome was the difference between BA measured using either of the two

methods, and CA in years, known throughout this thesis as SMD. The mean difference was determined by subtracting CA from BA (BA-CA), presented with 95% confidence intervals. A negative value indicates a BA less than the CA, and a positive value indicates a BA greater than the CA indicating delayed and advanced maturity respectively. Scatter plots for BA by both TW3 and GP methods vs CA were examined with the line of best fit plotted. WAZ, HAZ and body mass index (BMI) for age Z-scores were calculated using the 1990 UK reference data for children (235), with a Z-score of -2.0 or less denoting stunting (HAZ), underweight (WAZ) and wasting (BMI-for-age Z-score).

4.2.2 Bland Altman plots

I generated Bland and Altman plots (236) to compare the agreement between CA and BA by either method, and between GP BA and the TW3 BA. I performed linear regression analysis to determine the direction of the relationship between the mean and the differences between the two methods, compared using Bland and Altman plots, presenting β coefficients and 95% confidence intervals. I used the intraclass correlation coefficient (ICC) to calculate the inter- and intra-rater reliability and the intra- and inter-operator precision I used the coefficient of variation (CV). (CV =sample SD/mean) x 100) (237, 238).

4.3 Results

4.3.1 Participants characteristics

Of the 306 children without HIV enrolled in IMVASK, 54 were excluded; 15 had missing hand/wrist radiographs, 33 females were above 15 years of age, and 6 males were above 16.5 years of age (Figure 4.1). In total 252 (82%) were eligible for the current analyses, with 141 (56%) being males. There was no difference in CA between the males and females; however, mean WAZ and BMI Z-scores were lower in males than females (Table 4.1).

Although there were no sex differences in BA measured using both methods, more males than females had lower BA than CA for GP BA (77% vs 57%, Chi^2 p=0.001), and TW3 BA (65% vs 43%, Chi^2 p<0.001). SES, physical activity, vitamin D and calcium intakes did not differ between the males and females. Nevertheless, 24% of males and 30% of females had a low SES.

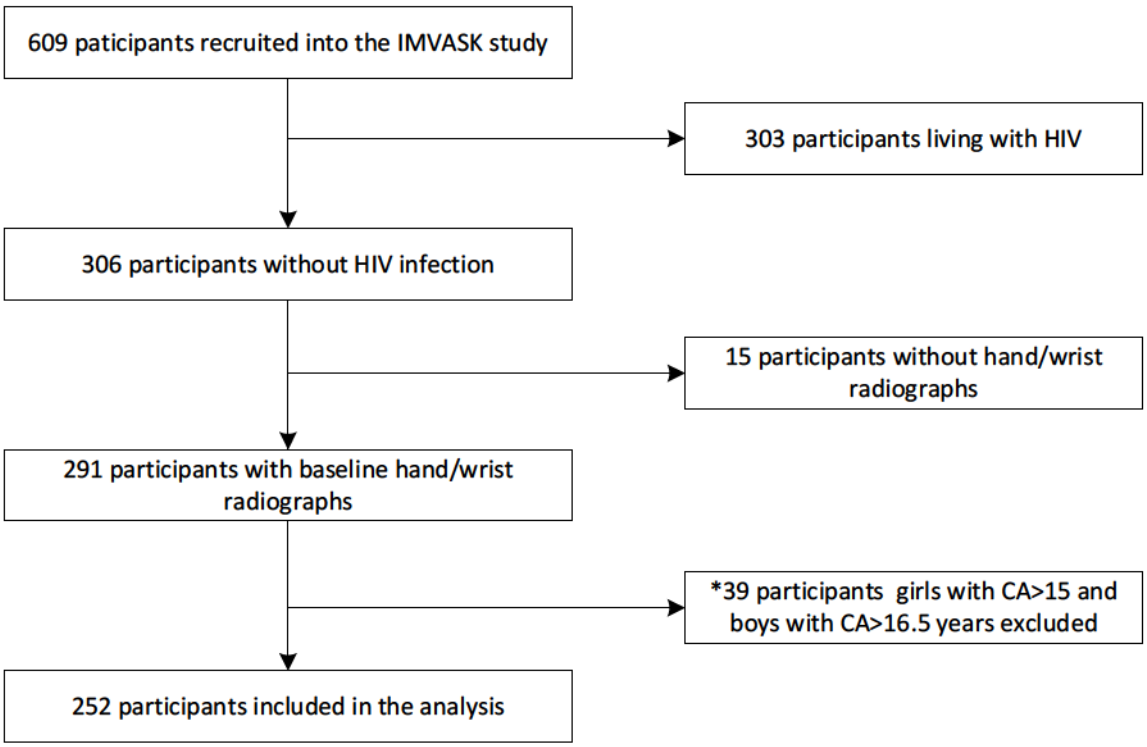


Figure 4.1 Flow chart showing participants included in the analyses presented in this chapter

4.3.2 Comparison of CA and BA by TW3 and GP

There was a positive linear relationship between CA and BA for each method (GP: r=0.93 males, r=0.92 females; TW3: r=0.91 males, r=0.88 females) (Figure 4.2). The slopes for the two methods in both males differed from each other. For every year increase in CA, TW3 BA increased by 0.95 years (95% CI: 0.87, 1.03) and GP BA by 1.09 (95% CI: 1.01, 1.17) years in males. In females for every year increase in CA, TW3 BA increased by 0.92 years (95% CI: 0.81, 1.03) and GP BA by 1.09 years (95% CI: 0.98, 1.20). In the males the TW3 slope (p=0.179) did not differ from the line of identity unlike the GP slope (p=0.030). In the females, both slopes [TW3 (p=0.180) and GP (p=0.110)] did not differ from the line of identity.

Table 4.1 Demographic, lifestyle, anthropometry, and pubertal status characteristics of study participants by sex

	Males (n=141)	Females (n=111)	p-value
Socio-demographics			
Chronological age, mean (SD)	12.2 (2.4)	11.7 (1.9)	0.065
Socio-economic status, n (%)			0.361
Low	34 (24.1)	33 (29.7)	
Middle	52 (36.9)	32 (28.8)	
High	55 (39.0)	46 (41.4)	
Anthropometry			
Height-for-age Z-score, mean (SD)	-0.60 (1.03)	-0.47 (1.16)	0.327
Standing height-for-age Z-score<-2, n (%)	9 (6.4)	8 (7.2)	0.796
Sitting height-for-age Z-score, mean (SD)	-1.31 (1.06)	-1.32 (1.18)	0.920
Sitting height-for-age Z-score<-2, n (%)	31 (22.0)	30 (27.0)	0.354
Weight-for-age Z-score, mean (SD)	-0.69 (1.07)	-0.27 (1.22)	0.004
Weight-for-age Z-score<-2, n (%)	15 (10.6)	7 (6.3)	0.226
BMI-for-age Z-score, mean (SD)	-0.51 (1.05)	-0.07 (1.20)	0.002
BMI-for-age Z-score<-2, n (%)	11 (7.8)	4 (3.6)	0.162
Bone age measures			
GP bone age, mean (SD)	11.5 (2.8)	11.5 (2.4)	0.859
TW3 bone age, mean (SD)	11.8 (2.5)	11.8 (2.1)	0.945
GP bone age < chronological age, n (%)	108 (76.6)	63 (56.8)	0.001
TW3 bone age < chronological age, n (%)	92 (65.2)	48 (43.2)	<0.001
Pubertal status			0.078
Tanner 1	45 (32.1)	23 (20.9)	
Tanner 2	34 (24.3)	30 (27.3)	
Tanner 3	22 (15.7)	28 (25.5)	
Tanner 4	35 (25.0)	22 (20.0)	
Tanner 5	4 (2.9)	7 (6.4)	
Lifestyle factors			
Daily vitamin D intake, n (%)			0.991
Very low, <4.0 mcg/day	17 (12.1)	14 (12.6)	
Low, 4.0-5.9 mcg/day	92 (65.2)	72 (64.9)	
Moderate, 6.0-8.0 mcg/day	32 (22.7)	25 (22.5)	
Daily calcium intake, n (%)			0.914
Very low, <150 mg/day	62 (44.0)	51 (45.9)	
Low, 150-299 mg/day	31 (22.0)	25 (22.5)	
Moderate, 300–450 mg/day	48 (34.0)	35 (31.5)	
Physical activity level, n (%)			0.304
Low, <600 MET mins/week	47 (33.3)	45 (40.5)	
Moderate, 600-3000 MET mins/week	46 (32.6)	27 (24.3)	
High, >3000 MET mins/week	48 (34.0)	39 (35.1)	

***Foot Notes**: student t-tests conducted on continuous variables and chi-squared tests on categorical variable. **GP**-Greulich and Pyle, **TW3**-Tanner Whitehouse 3, **SD**- Standard deviation, MET- multiples of the resting metabolic rate. Z-scores were calculated with 1990 UK reference data*

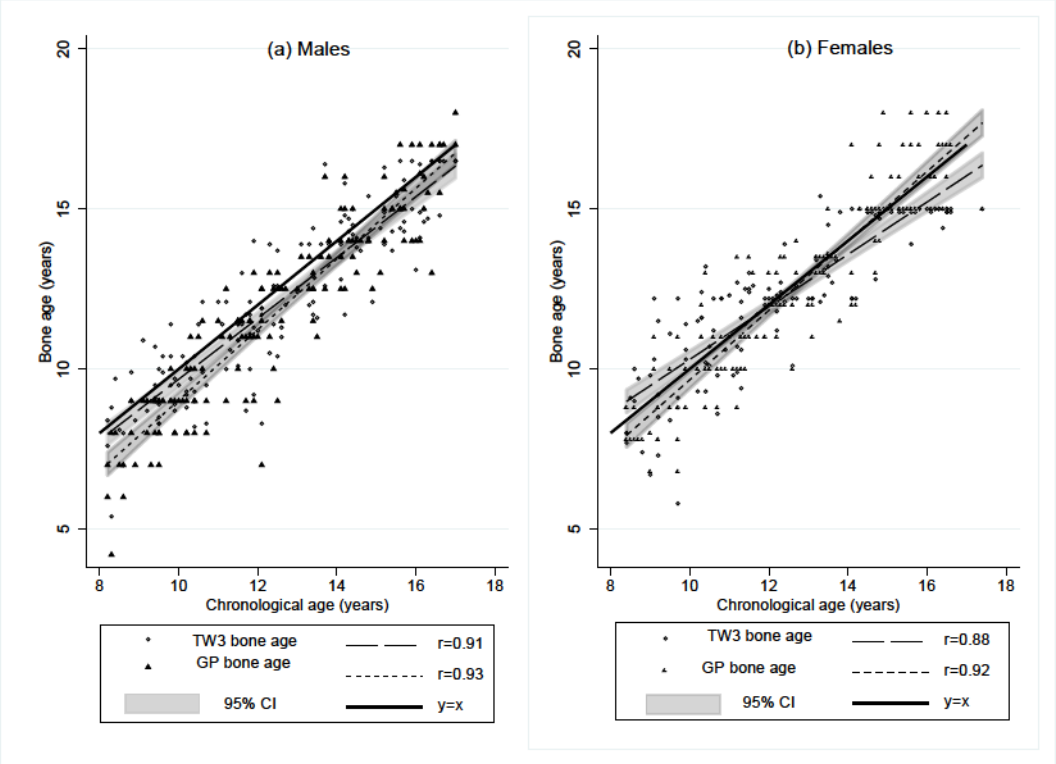


Figure 4.2 Scatter plot showing the relationship between chronological age and bone age by GP (Greulich and Pyle) and TW3 (Tanner Whitehouse 3) (a) males and (b) females.

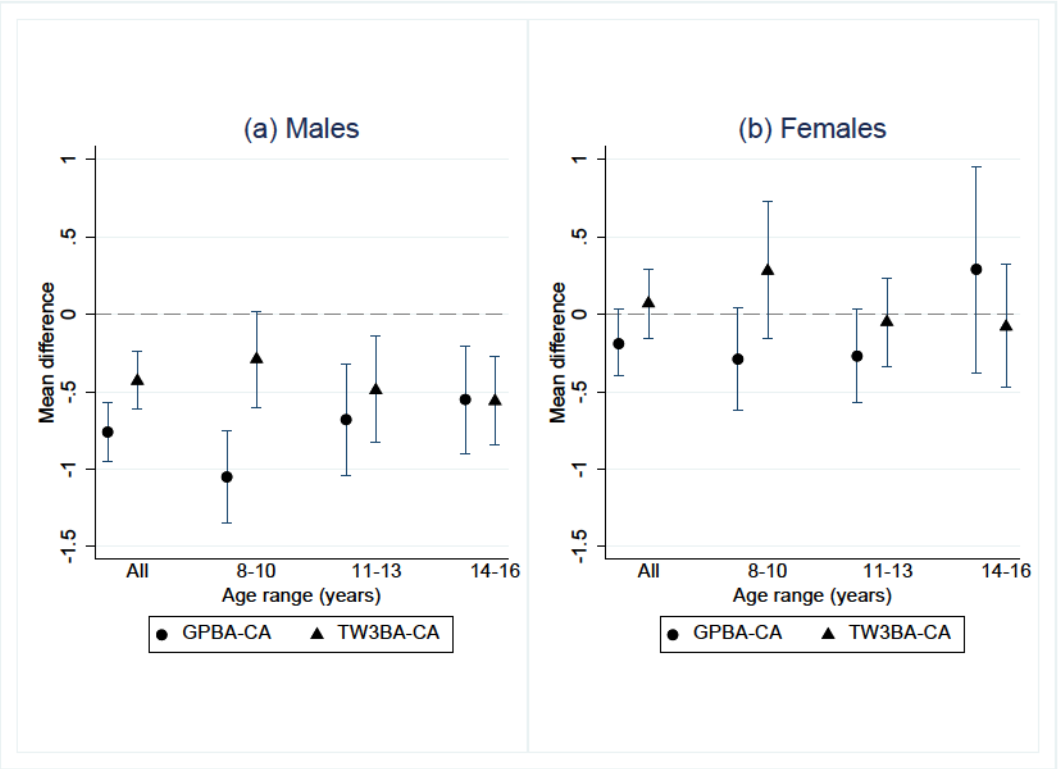


Figure 4.3 Mean differences (95% Confidence Intervals) between bone age and chronological age by GP, and TW3. GP BA-Greulich and Pyle bone age, TW3 BA-Tanner Whitehouse 3 bone age (a): 8-10 (n=49); 11-13 (**n=49**); 14-16 (**n=42**) and (b): 8-10 (**n=44**); 11-13 (**n=48**); 14-16 (**n=20**)

In the whole sample of males, GP BA was lower than CA by 0.76 years (95% CI: 0.57, 0.95) while TW3 BA was lower than CA by 0.43 years (95% CI: 0.24, 0.61). The mean difference between CA and GP BA was a year in those aged 8-10 years, with a difference of half a year in those 11 years and older (Figure 4.3). In contrast, the mean difference between TW3 BA and CA, was less in the younger age group (8-10 years) compared to the older age groups (Figure 4.3 a). Amongst the females, GP BA had a trend towards being lower than CA in the 8-10 and 11-13-year age groups, whereas no differences were seen between TW3 BA and CA (Figure 4.3 b).

There was a negative bias in agreement between CA and BA as measured by GP in males (β=-0.18, $p<0.001$) and females (β=-0.08, $p=0.050$), with GP BA being much lower than CA at younger ages, with less of a difference seen at older ages (Figure 4.4a, b). The beta coefficient represents the differences between BA and CA. For TW3 BA and CA, no systematic differences were observed for either sex (Figure 4.4c, d). The slope for GP Bland and Altman plots was 0.18

in males and 0.16 in females and for TW3 plots was 0.05 in males and -0.08 in females (Figure 4.4 a, b).

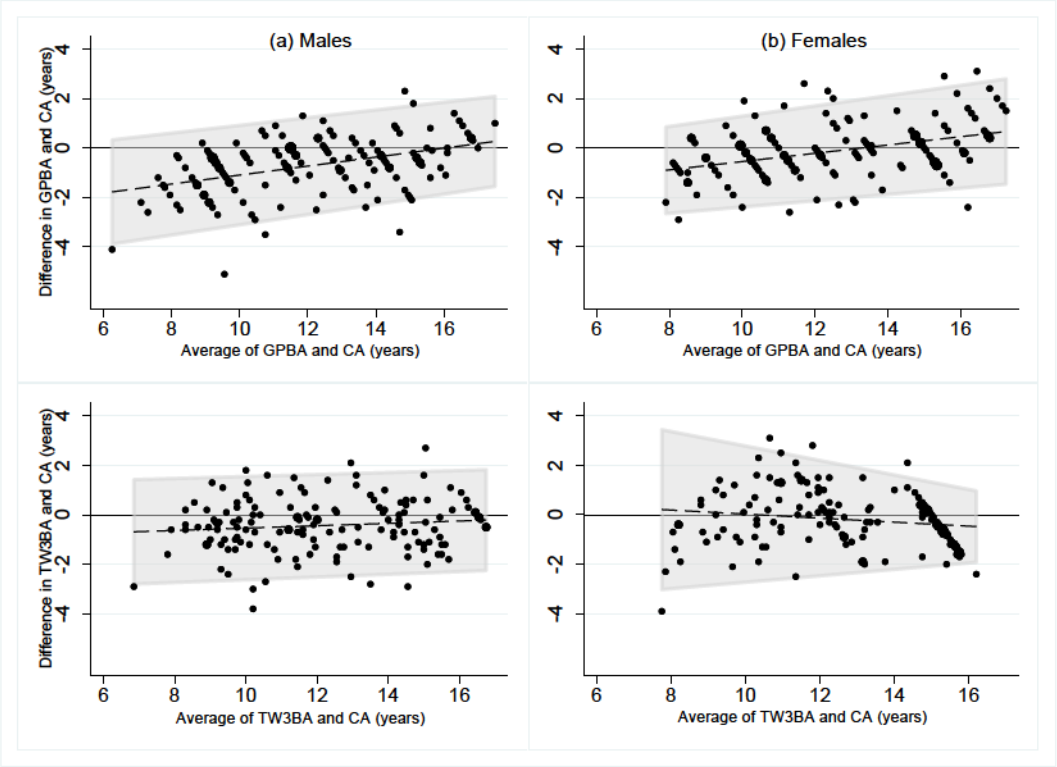


Figure 4.4 Bland and Altman plots showing mean differences between Greulich and Pyle bone age and chronological age (top) Tanner Whitehouse 3 bone age and chronological age (bottom) and for (a) males and (b) females

4.3.3 Comparison of TW3 and GP

Overall, GP BA was lower than TW3 BA by 0.33 years (95% CI: 0.23, 0.88) in the males and 0.25 years (95% CI: 0.07, 0.44) in the females. In both males and females, Bland Altman plots showed differential agreement (agreement differed with age) between the TW3 and GP across all ages (males: β=-1.47 $p<0.001$ and females: β=-1.17 $p<0.001$) (Figure 4.5). Differences between the two methods in younger ages (8-10 years) showed that TW3 BA was higher relative to the GP BA. At the older ages GP BA was higher than TW3 BA.

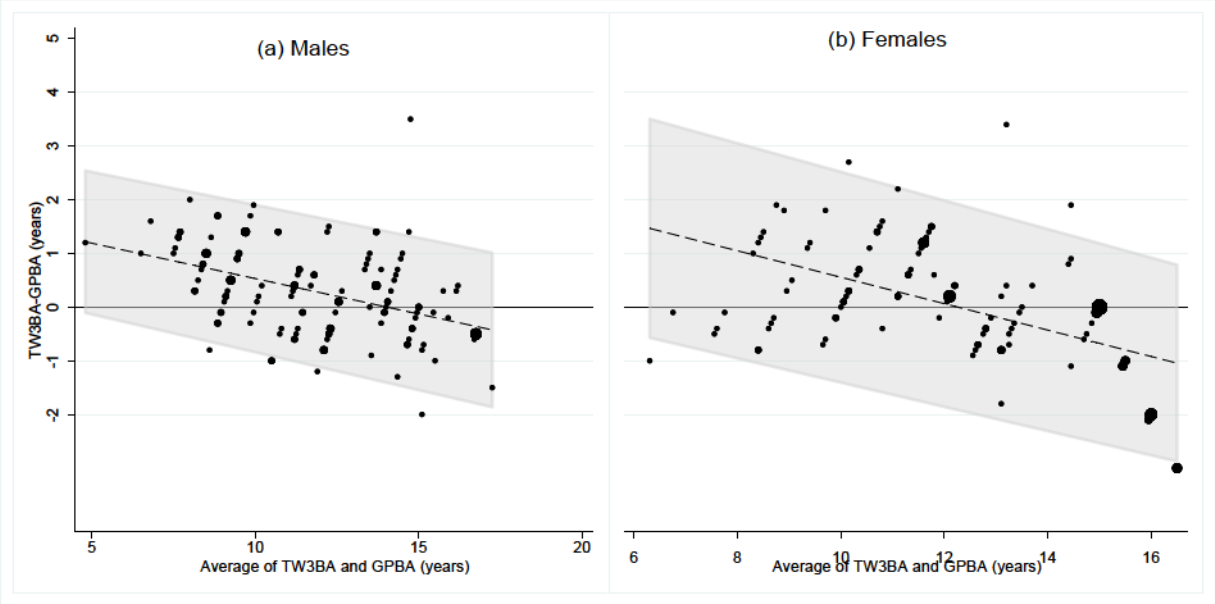


Figure 4.5 Bland Altman plot showing mean differences between GP and TW3 bone age in years for (a) males and (b) females.

4.3.4 Intra- and inter-rater reliability and precision

The intraclass correlation coefficient showed high inter-rater reliability of 0.98 for TW3 and 0.94 for GP (n=252) and high intra-rater reliability of 0.97 for TW3 and 0.98 for GP (n=52). There was good precision for both methods although the TW3 coefficients of variation were better than for GP: intra-operator 1.5% vs 2.4% and inter-operator 1.5% vs 3.7%, respectively.

4.4 Discussion

To my knowledge, this is the first comparison of BA assessment methods in a population of peripubertal children in SSA. These data have demonstrated that TW3 is a more applicable method, than GP, for assessment of skeletal maturity in children and adolescents aged 8-16 years from Zimbabwe. TW3 had better intra- and inter-operator precision, and no age-related bias. The age-related bias using the GP method, which showed greater differences at younger ages than at older age groups, indicates limitations for use in our study population. This may be due to the limitation of the GP method where the time interval between two standard films is too long (one year between most standards) whilst growth is a continuous process. The secondary ossification centres develop at different time points and in a space of a year significant changes in the centres will have occurred. Therefore, the gradual change over time is

captured by the TW3 unlike the GP which is based on distinct age-related standards. Similarly, the age-related bias was reported in studies conducted in Turkey where the GP mean BA underestimated CA in younger ages and overestimated CA in older ages (197, 198). If the GP method is to be used the SMD differences in the different age groups should be taken into consideration. Although there is the age-related bias, the GP method has an advantage of a greater age range (up to 18 and 19 years for females and males respectively) therefore would be ideal for use in older children. This study therefore identified the TW3 as the most appropriate and efficient method for use in the Zimbabwean population thereby guiding clinical practice and research that requires BA assessment.

4.4.1 Comparison of Greulich and Pyle and Tanner Whitehouse 3

My results highlight the importance of comparing two methods to ascertain the best method for use in any population. Upon evaluating the two BA assessment methods, the Bland and Altman analyses demonstrated that the two BA assessment methods (TW3 and GP) do not agree in younger children (8-11 years) but agree in children above 11 years thereby preventing interchangeable use. Similar results were reported in an earlier study in the UK that also used Bland and Altman plots to assess the agreement between GP and TW2 (3). Again, the TW2 method had better precision than GP therefore recommended as the method of choice. Another well-established study conducted in 1971 in 1009 children aged 7-18 years in Denmark reported overall BA estimates by TW2 being closer to CA than BA estimates by GP method although Bland and Altman plots were not used to test agreement between BA and CA (196). In contrast, both methods gave equal estimates and were applicable for use in a Turkish study (198) and in a different study sex differences where noted with both methods giving equal estimates in females and not the males (216).

Many more studies have looked at the applicability of the GP than TW3 method, potentially because TW3 is used less frequently in clinical practice due to time constraints (169, 170). Despite the TW3 taking longer to assess the radiograph (7.9 minutes vs 1.4 minutes) (170), the consistency across age groups and sexes, and good intra- and inter-operator precision, suggest that the extra time is justified. The bones of the hand ossify at different rates and the TW3 method is designed to take this into account, assessing ossification centres separately (rather than the GP) making it an ideal method for use. Because of the agreement in

children aged 11 years and older interchangeable use may be applied although I would not recommend because of the differences in applicable noted in this study.

4.4.2 Reproducibility of the bone age assessment methods

The current study has demonstrated that both methods are reproducible, with the TW3 being more reproducible than the GP method, which is consistent with other studies (3, 88, 190, 215, 239), potentially explained by individual maturity indicator scoring, rather than the GP global grading approach. GP BA units are whole numbers – less precise than the TW3 approach with decimals. Cavallo et al reported the evaluation of each bone segment in TW3 as an advantage minimising inter-rater variability (240). My study also found high inter- and intra-rater agreement supporting the reliability of these two methods in assessing BA, though notwithstanding the limitations attached to the use of GP in this population given the age dependence of the results. One limitation of the TW3 method is that it has a cut off point for skeletal maturity of 15 years for females and 16.5 for boys. In populations that may have later onset, or delayed, puberty, such as in our current study, this limits use, given skeletal maturity is not reached until at least 18 years for females and 19 years for males (2).

In the largest study of African children to date (n=607), TW3 was used to assess and compare BA between Black and White females and males in South Africa. Skeletal maturity was delayed by on average 7 months in black males compared to White males, whereas no ethnic differences in the progression of skeletal maturation were seen in females (89). Similarly, our data shows sex differences in the performance of TW3, suggesting on average 6 months delay in BA in the males compared to CA and no differences in the females, in South Africa. The delay is also seen in the males and not the females when using the GP method in this study and this may be due to the males being more underweight and wasted (WAZ<-2) (Section 4.3.1, Table 1).

Consistent with our findings, previous studies have commonly demonstrated lower GP BA than CA, in South Africa, Saudi Arabia, Turkey and Malawi (102, 198, 214, 241). Our findings are similar to those from a study in Pakistan which advised against using GP method in their population due to variations in BA depending on the age of the children (advanced BA during early childhood and delayed BA during middle and late childhood) (242). Our study extends this work by testing the agreement between GP BA and CA and showing the GP method is dependent upon age, such that one would not be able to accurately determine whether a child is delayed in maturity or whether it is a function of the inaccuracy of using GP. Several other

studies have found the applicability of GP differs by sex, ethnic group and by age (200, 214, 241).

4.4.3 Strengths and limitations

The recruitment of the children from schools in Harare was designed to be representative of the general population in Zimbabwe, as about 98% of the children in the whole country go to school. All schools in Harare were identified and the 6 studied were randomly chosen from this sampling frame (224). I repeated BA assessment to test intra-rater reliability and was repeated by a second assessor further strengthening the method employed. It is well-described that timing of skeletal maturation is affected by environment, nutrition, medication, and socioeconomic deprivation (90, 158, 243). Our population therefore contrasts with the ones in which the GP and TW3 methods were originally developed. The children used to create the GP atlas were from wealthy families living in Ohio, USA and not suffering from any illnesses (2). Generally, HAZ and WAZ were low in our study population, indicating a tendency towards stunting and underweight respectively. Most participants had low or middle SES. Calcium and vitamin D intakes were low, both micronutrients being critical for skeletal development (244). Together these factors may explain why children in Zimbabwe were skeletally immature for their CA.

4.5 Conclusion

Of the two methods assessed, the TW3 is the most precise and appropriate for use in Zimbabwe as GP appears biased by age in those younger than 11 years. The two methods (TW3 and GP) do not give equal estimates of BA and therefore should not be used interchangeably. Hence the TW3 method would be the preferred method to determine skeletal maturity in peripubertal children age between 8 and 16 years, in the region. This study is of relevance for other populations living in Africa. These findings have been published and helped inform the method of choice for BA assessment in the next chapter (245).

Chapter 5 The impact of HIV on skeletal maturation in peripubertal children in Zimbabwe: a cross-sectional study

5.1 Introduction

In chapter four I determined that the TW3 method was the best method of choice for BA assessment in children and adolescents aged 8-16 years thereby informing my use of the TW3 method in the current chapter. As indicated in chapter one, several factors have been shown to influence the process of skeletal maturation (section 1.10). Amongst these factors are demographic, anthropometric and environmental factors like socio-economic deprivation, nutritional factors (e.g., vitamin D and calcium intake), and physical activity (121, 216, 225). However, it is unclear which of those factors influence skeletal maturation in the context of the Zimbabwean population with a high prevalence of HIV and malnutrition. Therefore, I sought to answer two questions in this chapter: 1) what are the differences between BA and CA in peripubertal children living with and without HIV? and 2) what are the factors that influence the process of skeletal maturation in children living with and without HIV? I hypothesised that CLWH will be delayed in skeletal maturation than the HIV negative children. To address these questions, I described SMD in males and females separately, whether living with or without HIV. I also investigated to what extent HIV and other demographic, and lifestyle factors are associated with SMD in peripubertal males and females in Zimbabwe. In a separate analysis of CLWH, I determined the HIV characteristics associated with SMD.

5.2 Methods

This chapter presents results from a cross-sectional analysis of children from the IMVASK study, aged 8-16 years that has been described in detail in chapter two from section 2.13.

5.2.1 Bone age assessment

Digital hand-wrist radiographs of the non-dominant side were taken by a trained Radiographer at baseline. Because the TW3 method assesses BA up to 15 years for girls and 16.5 years for boys, those with CA above these ages were excluded in this study.

5.2.2 Statistical analysis

I analysed data using Stata 17 (StatCorp, TX, USA). Weight for age z-scores (WAZ), Height for age z-scores (HAZ), sitting height z-scores and body mass index (BMI) for age z-scores were calculated using the 1990 UK reference data for children (235), because World Health Organization reference data for WAZ are not available beyond the age of 10 years (246). I used Z-scores of -2.0 or lower for HAZ, WAZ, and BMI to define stunting, underweight and wasting respectively (247). Analyses were stratified by sex because BA assessment and bone development are sex specific. My primary outcome was SMD measured in years calculated as BA minus CA with BA assessed at baseline and CA calculated form date of birth to the day the hand radiograph was taken at baseline. A negative SMD value reflects a delay in skeletal maturity, and a positive value reflects advanced skeletal maturity, relative to CA.

5.2.3 Descriptive statistics

I examined quantitative data for normality using the Shapiro–Wilk test and histograms. I presented normally distributed continuous variables as means ± standard deviations and categorical variables as numbers and proportions of participants in each category. For the non-normally distributed continuous variables, I presented them as medians and interquartile ranges (IQR). I compared the participant characteristics by HIV status, using the student t-test for continuous variables and chi-squared test for categorical variables. I conducted a complete case analysis in this chapter, such that participants with missing variables were excluded from the analysis and a comparison was made of those excluded with those retained in the analysis. The participants I excluded were those with missing variables for Tanner staging, orphan status and BA at baseline.

5.2.4 Analyses in whole study population

I performed sex-stratified univariable linear regression to examine associations between exposure variables (HIV status, WAZ, socio-economic deprivation, orphanhood, physical activity, vitamin D intake, calcium intake, pubertal stage) and SMD. I used WAZ instead of BMI because of the fixed relationship between skeletal maturation and height, which contributes to the BMI calculation. Additionally, for ordinal exposures, I reported p-values from tests for trend. Firstly, I used unadjusted linear regression models to determine the univariable associations between the exposure variables outlined above and SMD. Secondly, I used a multivariable linear regression model that considered HIV and *a priori* factors (WAZ, SES, pubertal stage, orphanhood, and calcium intake) as exposures. I chose *a priori factors* based on previous literature showing that lower WAZ, low SES, orphanhood and low dietary calcium intake are related to other skeletal outcomes (224, 225, 248, 249). In addition, orphanhood was shown to be associated with low bone density in this cohort (121). I included other variables in the multivariable model if there was any evidence of a potential association with SMD in the boys or the girls in the unadjusted analyses (p-value ≤ 0.1).

I did a stratified multiple regression analysis by tanner stage reporting stratum specific beta coefficients and 95% CI. A formal Wald interaction test assessed whether Tanner stage modified the effect of HIV on SMD.

5.2.5 Analyses in children living with HIV

In a sex stratified analysis restricted to CLWH, I used linear regression to assess associations between HIV specific variables (age at ART initiation, CD4 count, viral load and TDF exposure) and SMD. The multivariable linear regression model in CLWH included *a priori* factors outlined above and HIV specific variables with a p-value for trend ≤ 0.1 in the univariable linear regression analysis in either males or females.

Collinearity was assessed using the variance inflation factor with values above 5 indicating collinearity (250). Standard errors for regression coefficients were also assessed to determine the robustness of models. Regression assumptions were assessed to ensure 1) normality of residuals using Q-Q plots, 2) homogenous variance in scatter plot of fitted values versus residuals, and 3) independence of residuals by examining scatter plots of residuals versus exposure variables.

5.3 Results

5.3.1 Participant characteristics

Initially 609 participants (including 306 HIV negative) participants were recruited into the study. Thirty-four participants were excluded based on the BA limits of the TW3 method (7 CLWH and 27 HIV-negative children). More females (n=29) were excluded than the males (n=5). A further 71 participants were excluded because they did not have hand-wrist radiographs or had missing pubertal or orphanhood variables, leaving 504 participants (88% of original sample, 54% males) in the analysis (Figure 5.1).

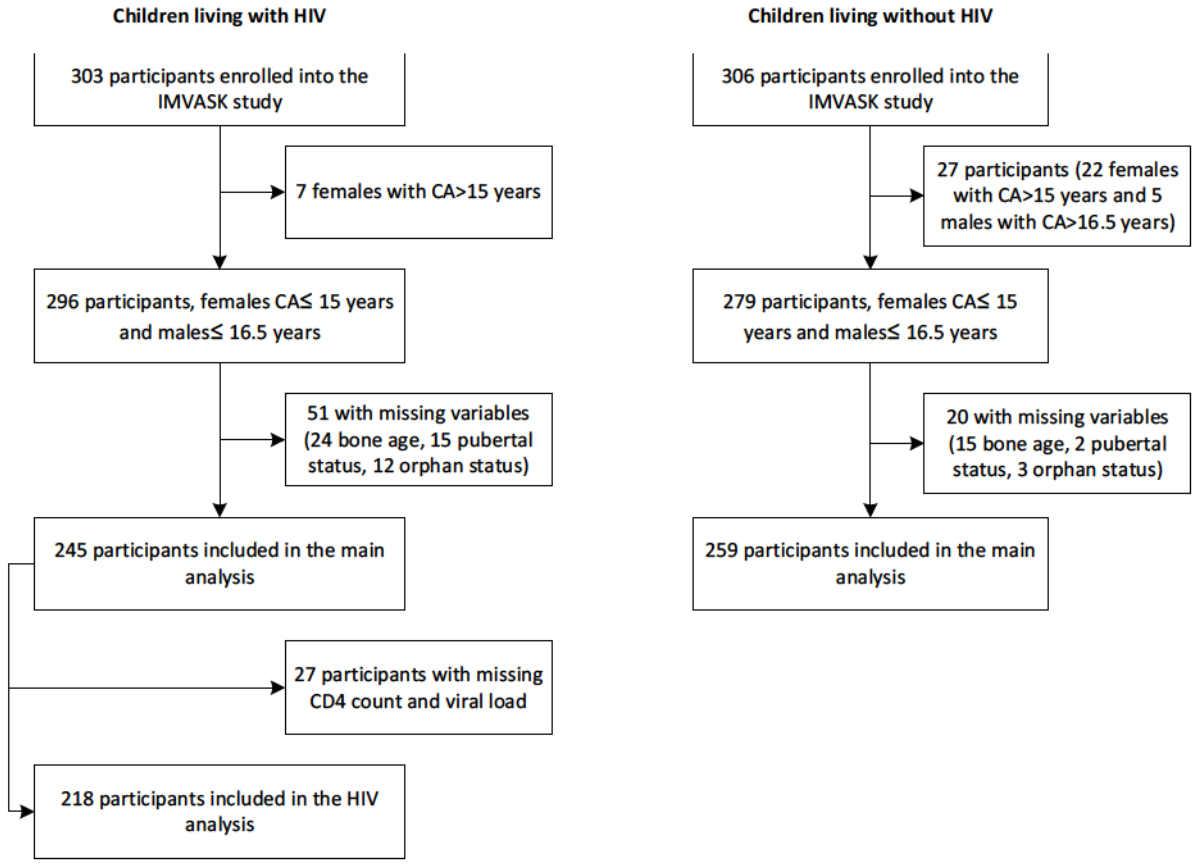


Figure 5.1 Flow diagram showing the participants included in the current analysis. Participants with missing data were excluded from the analysis IMVASK: The Impact of Vertical HIV infection on child and Adolescent Skeletal development, CA-chronological age.

Participants excluded based on age and with missing data were similar to those with complete data in terms of CA, BA, SMD, vitamin D and calcium intake, but were more likely to be living with HIV (both sexes) and have lower HAZ, physical activity and SES (males only) (Table 5.1). Males with missing data had lower HAZ and were more likely to be stunted than those with complete data. In both the males and the females, participants with missing data had lower WAZ and were more likely to be wasted than participants with complete data.

For both males and females, a larger proportion of CLWH had lower SES, were orphaned (one or both parent dead), were stunted, had TB, any cardiovascular condition or were underweight in comparison to HIV-negative participants (Table 5.2, Table 5.3). On average, male CLWH were 7.4 cm shorter and 5.2 kgs lighter than male HIV-negative participants; similarly female CLWH were 7.5 cm shorter and 7.2 kgs lighter than their HIV-negative peers. Female CLWH were more likely to be in earlier Tanner stages (1 and 2) in comparison to those who were HIV-negative, whilst no differences were seen in Tanner stage by HIV status in males. Notably, over 65% of participants had low or very low dietary intakes of calcium, falling far below recommended levels of other populations (e.g., UK recommendations are for calcium intakes between 800-1000mg/day at this age) (251), and there was no difference in intake by HIV status. CLWH reported lower levels of physical activity compared to HIV-negative participants.

CLWH were diagnosed at a median age of 3.0 years (IQR 1.1-5.9) and were established on ART at 3.8 years (IQR 1.9-7.0). Overall, mean ART duration was 7.6 (SD 2.6) years. A high proportion of CLWH had a suppressed viral load and a CD4 count >500 cells per microlitre (79% and 80% respectively) (252).

5.3.2 SMD in children with and without HIV

On average, there was little evidence of SMD in the HIV-negative group, particularly in females; however, SMD was evident in CLWH whether male or female, such that skeletal maturity was delayed by more than a year compared to HIV-negative participants (Table 5.2). When stratified by Tanner stage, BA values were much lower than CA values in older CLWH in earlier Tanner stages (Figure 5.2).

Table 5.1 Demographic, lifestyle, anthropometry, and pubertal status characteristics of participants with and without missing data by sex

	Males Complete data	Missing data	p-value	Females Complete data	Missing data	p-value
	n=269	n=29		n=235	n=41	
Socio-demographics						
Age, mean (SD)	12.3 (2.5)	12.7 (2.3)	0.81	12.1 (2.3)	12.4 (2.7)	0.71
HIV positive	129 (48.0)	23 (79.3)	0.001	116 (49.4)	27 (65.9)	0.062
Socio-economic status, n (%)			0.098			0.60
Group 1: low	79 (29.4)	11 (37.9)		85 (36.2)	12 (29.3)	
Group 2: middle	91 (33.8)	13 (44.8)		75 (31.9)	16 (39.0)	
Group 3: high	99 (36.8)	5 (17.2)		75 (31.9)	13 (31.7)	
Orphan status, one or both parents dead, n (%)	65 (24.2)	6 (30.0)	0.59	58 (24.7)	9 (25.7)	1.00
Anthropometry						
Standing height, mean (SD)	143.7 (14.3)	139.5 (14.0)	0.15	142.6 (12.7)	141.6 (12.3)	0.66
Height for age z-score, mean (SD)	-1.1 (1.2)	-2.0 (1.5)	<0.001	-1.0 (1.2)	-1.2 (1.0)	0.20
Stunting, height for age z-score<-2, n (%)	54 (20.1)	10 (37.0)	0.051	40 (17.0)	8 (19.5)	0.66
Weight, mean (SD)	37.0 (15.4)	37.4 (24.7)	0.92	38.6 (15.7)	35.2 (9.4)	0.29
Weight for age z-score, mean (SD)	-1.1 (1.2)	-1.7 (1.5)	0.029	-0.8 (1.3)	-1.3 (1.0)	0.020
Underweight, weight for age z-score<-2, n (%)	58 (21.6)	7 (25.0)	0.64	31 (13.2)	6 (14.6)	0.80
BMI, mean (SD)	16.8 (1.9)	16.9 (1.6)	0.83	18.0 (3.3)	17.1 (2.5)	0.088
BMI for age z-score, mean (SD)	-0.6 (1.0)	-0.7 (1.1)	0.69	-0.3 (1.1)	-0.7 (1.0)	0.017
Wasting, BMI for age z-score<-2, n (%)	21 (7.8)	6 (23.1)	0.021	11 (4.7)	6 (14.6)	0.026
Pubertal status, n (%)			0.79			0.73
Tanner I	94 (34.9)	8 (44.4)		75 (31.9)	10 (29.4)	
Tanner II	69 (25.7)	4 (22.2)		45 (19.1)	10 (29.4)	
Tanner III	42 (15.6)	4 (22.2)		54 (23.0)	6 (17.6)	
Tanner IV	55 (20.4)	2 (11.1)		48 (20.4)	6 (17.6)	
Tanner V	9 (3.3)	0 (0.0)		13 (5.5)	2 (5.9)	
Lifestyle factors						
Physical activity level, n (%)			0.010			0.42
Low, <600 MET mins/week	102 (37.9)	18 (62.1)		109 (46.4)	18 (43.9)	
Moderate, 600-3000 MET mins/week	75 (27.9)	8 (27.6)		58 (24.7)	14 (34.1)	
High, >3000 MET mins/week	92 (34.2)	3 (10.3)		68 (28.9)	9 (22.0)	
Daily vitamin D intake, n (%)			0.75			0.79
Very low, <4.0 µg/day	13 (4.8)	2 (6.9)		11 (4.7)	1 (2.4)	
Low, 4.0-5.9 µg/day	150 (55.8)	17 (58.6)		136 (57.9)	26 (63.4)	
Moderate, 6.0-8.0 µg/day	106 (39.4)	10 (34.5)		88 (37.4)	14 (34.1)	
Daily calcium intake, n (%)			0.39			0.84
Very low, <150 mg/day	118 (43.9)	15 (51.7)		101 (43.0)	20 (48.8)	
Low, 150-299 mg/day	58 (21.6)	3 (10.3)		52 (22.1)	8 (19.5)	
Moderate, 300–450 mg/day	93 (34.6)	11 (37.9)		82 (34.9)	13 (31.7)	
Bone age measures						

Bone age, mean (SD)	11.6 (2.4)	10.9 (2.0)	0.28	11.6 (2.4)	11.1 (2.6)	0.43
Skeletal maturity deviation, mean (SD)	-0.9 (1.3)	-1.4 (1.6)	0.14	-0.5 (1.4)	-0.8 (1.6)	0.46
Skeletal maturity delay, n (%)	49 (18.2)	7 (24.1)	0.45	30 (12.8)	2 (4.9)	0.19

Student t-tests conducted on continuous variables and chi-squared tests on categorical variable. ***MET*** *– multiples of the resting metabolic rate* ***SD****- Standard deviation. Skeletal maturity deviation-difference between bone age and chronological age. Skeletal maturity delay-skeletal maturity deviation </=-2 year*

Table 5.2 Demographic, lifestyle, anthropometry, and pubertal status characteristics of study participants by sex

	Males			Females		
	HIV- (n=140)	CLWH (n=130)	p-value	HIV- (n=119)	CLWH (n=115)	p-value
Socio-demographics						
Chronological age (years), mean (SD)	12.3 (2.4)	12.6 (2.5)	0.220	12.1 (2.2)	12.3 (2.4)	0.520
Socio-economic status, n (%)			0.180			0.004
Group 1: low	34 (24.3)	45 (34.6)		36 (30.3)	49 (42.6)	
Group 2: middle	52 (37.1)	40 (30.8)		33 (27.7)	41 (35.7)	
Group 3: high	54 (38.6)	45 (34.6)		50 (42.0)	25 (21.7)	
Orphanhood: One or both parents dead, n (%)	11 (7.9)	54 (41.5)	<0.001	7 (5.9)	51 (44.3)	<0.001
Anthropometry						
Height (cm), mean (SD)	147.6(15.2)	140.2(12.5)	<0.001	147.7(11.8)	140.2(13.1)	<0.001
Height for age z-score, mean (SD)	-0.6 (1.0)	-1.7 (1.1)	<0.001	-0.5 (1.1)	-1.5 (1.1)	<0.001
Stunting (height for age z-score < -2), n (%)	8 (5.7)	46 (35.4)	<0.001	9 (7.6)	31 (27.0)	<0.001
Weight (kg), mean (SD)	38.3 (11.5)	33.1(8.0)	<0.001	42.5(12.7)	35.3(10.9)	<0.001
Weight for age z-score, mean (SD)	-0.7 (1.1)	-1.6 (1.2)	<0.001	-0.2 (1.2)	-1.3 (1.2)	<0.001
Underweight (weight for age z-score < -2), n (%)	15 (10.7)	43 (33.1)	<0.001	7 (5.9)	24 (20.9)	<0.001
BMI (kg/m^2), mean (SD)	17.1(2.2)	16.6(1.5)	0.013	19.1(3.6)	17.5(2.7)	<0.001
BMI for age z-score, mean (SD)	-0.5 (1.0)	-0.8 (0.9)	0.026	-0.0 (1.2)	-0.6 (0.9)	<0.001
Wasting (BMI for age z-score < -2), n (%)	11 (7.9)	10 (7.7)	1.000	4 (3.4)	7 (6,1)	0.370
Pubertal status			0.240			<0.001
Tanner I	45 (32.1)	50 (38.5)		23 (19.3)	51 (44.3)	
Tanner II	33 (23.6)	36 (27.7)		29 (24.4)	16 (13.9)	
Tanner III	22 (15.7)	20 (15.4)		28 (23.5)	26 (22.6)	
Tanner IV	36 (25.7)	19 (14.6)		30 (25.2)	18 (15.7)	
Tanner V	4 (2.9)	5 (3.8)		9 (7.6)	4 (3.5)	
Lifestyle factors						
Physical activity level, n (%)			0.043			0.055
Low, <600 MET mins/week	45 (32.1)	58 (44.6)		46 (38.7)	62 (53.9)	
Moderate, 600-3000 MET mins/week	47 (33.6)	28 (21.5)		32 (26.9)	26 (22.6)	
High, >3000 MET mins/week	48 (34.3)	44 (33.8)		41 (34.5)	27 (23.5)	
Daily vitamin D intake, n (%)			0.910			0.350
Very low, <4.0 µg/day	7 (5.0)	6 (4.6)		4 (3.4)	7 (6.1)	
Low, 4.0-5.9 µg/day	76 (54.3)	74 (56.9)		66 (55.5)	70 (60.9)	
Moderate, 6.0-8.0 µg/day	57 (40.7)	50 (38.5)		49 (41.2)	38 (33.0)	
Daily calcium intake, n (%)			0.950			0.990
Very low, <150 mg/day	62 (44.3)	56 (43.1)		52 (43.7)	49 (42.6)	
Low, 150-299 mg/day	31 (22.1)	28 (21.5)		26 (21.8)	25 (21.7)	
Moderate, 300–450 mg/day	47 (33.6)	46 (35.4)		41 (34.5)	41 (35.7)	
Bone age measures						
Bone age (years), mean (SD)	11.8 (2.5)	11.2 (2.3)	0.042	12.1 (2.2)	11.2 (2.5)	0.005
Skeletal maturity deviation	-0.4 (1.1)	-1.4 (1.4)	<0.001	-0.0 (1.2)	-1.1 (1.3)	<0.001
Skeletal maturity delay, n (%)	10 (7.1)	39 (30.0)	<0.001	6 (5.0)	24 (20.9)	<0.001

Student t-tests conducted on continuous variables and chi-squared tests on categorical variable. ***MET*** *- multiples of the resting metabolic rate* ***SD****- Standard deviation. Skeletal maturity deviation-difference between bone age and chronological age. Skeletal maturity delay-skeletal maturity deviation </=-2 year*

Table 5.3 Frequency of comorbidities in males and females whether living with or without HIV

	Males			Females		
	HIV- (n=140	CLWH (n=130)	p-value	HIV- (n=119)	CLWH (n=115)	p-value
Arthritis	0 (0.0)	2 (1.5)	0.230	0 (0)	0 (0)	
Epilepsy	0 (0.0)	2 (1.5)	0.230	1 (0.8)	1 (0.9)	1.000
Kidney	0 (0.0)	2 (1.5)	0.230	0 (0)	0 (0)	
Tuberculosis	1 (0.7)	24 (18.5)	<0.001	0 (0.0)	18 (15.8)	<0.001
Asthma	5 (3.6)	3 (2.3)	0.720	2 (1.7)	6 (5.2)	0.170
Diabetes	0 (0)	0 (0)		0 (0.0)	1 (0.9)	0.490
Cardiovascular disease	0 (0.0)	4 (3.1)	0.052	0 (0.0)	5 (4.3)	0.027

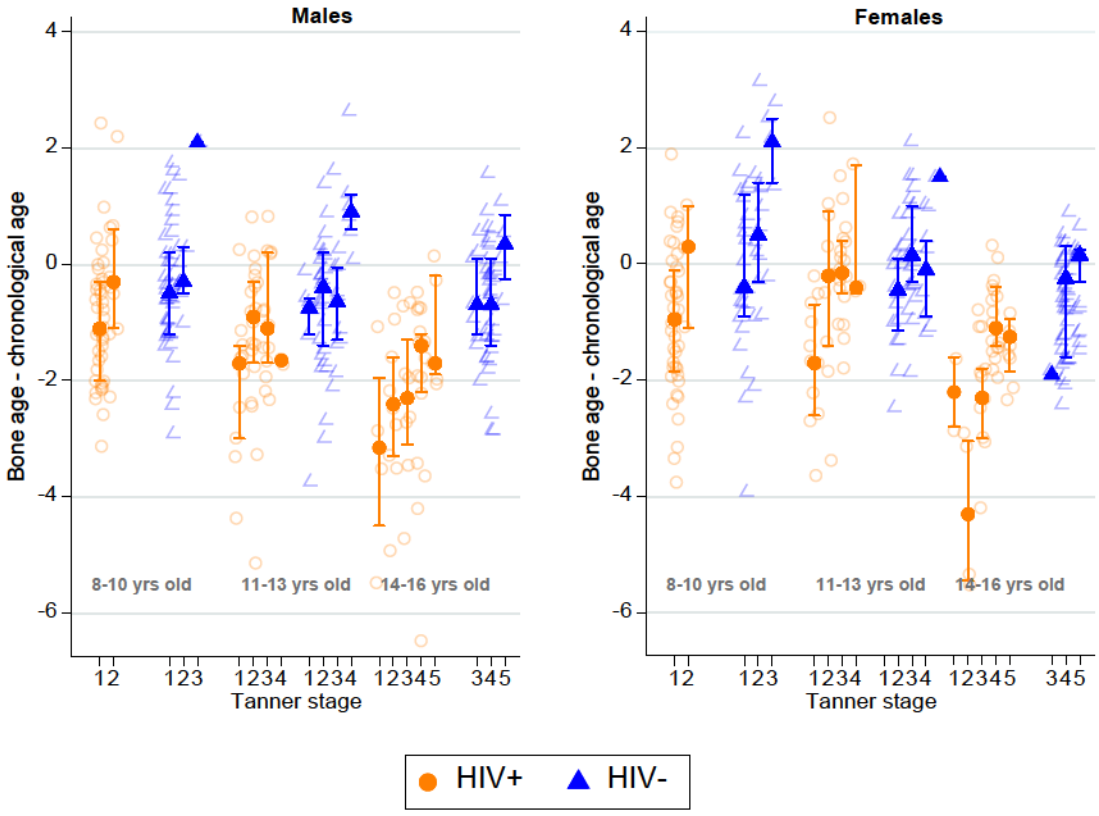


Figure 5.2 Comparison of mean skeletal maturity deviation (difference between bone age and chronological age) in children living with and without HIV by sex, age group and pubertal status. Error bars indicate 95% confidence intervals

5.3.3 Factors associated with SMD at baseline

Among males, living with HIV, being underweight, orphaned, and consuming less dietary calcium, were associated with a more negative SMD (i.e., more delayed skeletal maturation) in univariable analyses (Table 5.3). After adjustment in a multivariable model (which also included SES and pubertal stage), only living with HIV and being underweight remained associated with negative SMD in males. In females, before adjustment, living with HIV, being underweight, orphaned, and to a lesser degree earlier pubertal stage were associated with more negative SMD (Table 5.4). After adjustment in a multivariable model (which also included dietary calcium and SES), again only living with HIV and being underweight remained associated with greater delay in skeletal maturation in females.

Among males the adjusted effect of HIV in those in Tanner stage 1-2 is -0.50 (-0.90, -0.10) and Tanner stage 3-5 is -0.74 (-1.32, -0.26). Among females the adjusted effect of HIV in those in Tanner stage 1-2 is -1.03 (-1.57, -0.49) and Tanner stage 3-5 is -0.92 (-1.41, -0.43). A formal test of interaction in females: 0.10 (-0.54, 0.75) and males: -0.33 (-0.90, 0.23) showed no statistical evidence suggesting Tanner stage modified the effect of HIV on SMD.

Table 5.4 Association between participants characteristics and skeletal maturity deviation in males (unadjusted and adjusted linear regression analysis)

Variable	n	Mean SMD	Unadjusted model β coefficient (95%CI)	p-value	Adjusted model β coefficient (95%CI)	p-value
Males n=270						
HIV status						
HIV negative	140	-0.43	Ref	<0.001	ref	<0.001
HIV positive	130	-1.40	-0.97 (-1.27, -0.67)		-0.60 (-0.91, -0.28)	
Weight for age z-score						
Weight for age z-score>-2	212	-0.59	Ref	<0.001	ref	<0.001
Weight for age z-score<-2	58	-2.03	-1.44 (-1.79, -1.09)		-1.15 (-1.50, -0.80)	
Socio-economic status						
Group 1: High	99	-0.99	Ref	0.728	ref	0.567
Group 2: Middle	92	-0.63	0.36 (-0.02, 074)		0.46 (0.13, 0.79)	
Group 3: Low	79	-1.09	-0.10 (-0.50, 0.29)		0.09 (-0.25, 0.44)	
Pubertal stage						
Tanner 1	95	-0.96	Ref	0.879	ref	0.922
Tanner 2	69	-0.78	0.17 (-0.24, 0.59)		0.14 (-0.22, 0.50)	
Tanner 3	42	-0.98	-0.02 (-0.51, 0.47)		0.03 (-0.39, 0.45)	
Tanner 4&5	64	-0.89	0.07 (-0.36, 0.50)		-0.04 (-0.42, 0.33)	
Orphan status						
Not an orphan	205	-0.72	Ref	<0.001	ref	0.105
One or both parents dead	65	-1.48	-0.76 (-1.12, -0.40)		-0.33 (-0.69, 0.03)	
Physical activity						
High, >3000 MET mins/week	92	-1.05	Ref	0.753		
Moderate,600-3000MET mins/week	75	-0.60	0.45 (0.04, 0.86)			
Low, <600 MET mins/week	103	-0.98	0.08 (-0.29, 0.45)			
Vitamin D intake						
Moderate, 6.0-8.0 µg /day	107	-0.81	Ref	0.231		
Low, 4.0-5.9 µg /day	150	-0.95	-0.14 (-0.47, 0.19)			
Very low, <4.0 µg /day	13	-1.01	-0.20(-0.97, 0.58)			
Calcium intake						
Moderate, 300–450 mg/day	93	-0.78	Ref	0.046	ref	0.166
Low, 150-299 mg/day	59	-0.64	0.14 (-0.30, 0.57)		0.18 (-0.20, 0.55)	
Very low, <150 mg/day	118	-1.13	-0.35 (-0.71, 0.01)		-0.22 (-0.54, 0.10)	

The adjusted model included the following exposures: HIV status, weight for age z-scores, socio-economic status, pubertal stage, orphan status, and calcium intake. P-value for trend shown for variables with more than two categories. CI-confidence interval, SMD-*Skeletal maturity deviation calculated as the difference between bone age and chronological age; negative values reflect lower bone age than chronological age (delay in skeletal maturation)*

Table 5.5 Association between participants characteristics and skeletal maturity deviation in females (unadjusted and adjusted linear regression analysis)

Variable Females n=234	n	Mean SMD	Unadjusted model β coefficient (95%CI)	p-value	Adjusted model β coefficient (95%CI)	p-value
HIV status						
HIV negative	119	-0.02	Ref	<0.001	ref	<0.001
HIV positive	115	-1.08	-1.06 (-1.39, -0.73)		-0.92 (-1.30, -0.54)	
Weight for age z-score						
Weight for age z-score>-2	203	-0.33	Ref	<0.001	ref	<0.001
Weight for age z-score<-2	31	-1.96	-1.63 (-2.11, -1.15)		-1.44 (-1.92, -0.96)	
Socio-economic status						
Group 1: High	75	-0.52	Ref	0.568	ref	0.107
Group 2: Middle	74	-0.46	0.07 (-0.38, 0.51)		0.38 (-0.01, 0.78)	
Group 3: Low	85	-0.64	-0.12 (-0.55, 0.31)		0.38, (-0.01, 0.76)	
Pubertal stage						
Tanner 1	74	-0.85	Ref	0.068	ref	0.907
Tanner 2	45	-0.51	0.33 (-0.18, 0.84)		0.02 (-0.45, 0.49)	
Tanner 3	54	-0.20	0.64 (0.16, 1.13)		0.38 (-0.05, 0.81)	
Tanner 4&5	61	-0.51	0.34 (-0.13, 0.80)		-0.16 (-0.60, 0.28)	
Orphan status						
Not an orphan	176	-0.39	ref	0.003	ref	0.929
One or both parents dead	58	-1.00	-0.61 (-1.01, -0.21)		-0.04 (-0.45, 0.37)	
Physical activity						
High, >3000 MET mins/week	68	-0.39	ref	0.129		
Moderate, 600-3000 MET mins/week	58	-0.45	-0.06 (-0.55, 0.42)			
Low, <600 MET mins/week	108	-0.70	-0.31 (-0.73, 0.11)			
Vitamin D intake						
Moderate, 6.0-8.0 µg /day	87	-0.56	ref	0.564		
Low, 4.0-5.9 µg /day	136	-0.48	0.07 (-0.30, 0.44)			
Very low, <4.0 µg /day	11	-1.25	-0.69 (-1.56, 0.17)			
Calcium intake						
Moderate, 300–450 mg/day	82	-0.49	ref	0.385	ref	0.903
Low, 150-299 mg/day	51	-0.40	0.10 (-0.39, 0.58)		0.20 (-0.23, 0.62)	
Very low, <150 mg/day	101	-0.66	-0.17 (-0.53, 0.23)		0.01 (-0.35, 0.37)	

The adjusted model included the following exposures: HIV status, weight for age z-scores, socio-economic status, pubertal stage, orphan status, and calcium intake. P-value for trend shown for variables with more than two categories. CI-confidence interval, SMD-*Skeletal maturity deviation calculated as the difference between bone age and chronological age; negative values reflect lower bone age than chronological age (delay in skeletal maturation)*

5.3.4 Participant characteristics of children living with HIV

Among CLWH, 27 participants were excluded as they were missing a CD4 count and/or a viral load, meaning 117 males and 101 females were included. There was no difference in physical activity levels, dietary vitamin D or calcium intakes in either males or females, but the mean WAZ and BMI for age z-scores differed with males [n=40 (34%)] more likely to be

underweight than females [n=22 (22%)] (Table 5.5). Females had less negative SMD (i.e., less delay in skeletal maturation) than the males.

5.3.5 Factors associated with SMD in participants living with HIV

In univariable analyses, older age at ART initiation (in both sexes) and greater viral load (in males only) were associated with more negative SMD (Table 5.6 and 5.7)). In males, the negative association between viral load and SMD was partially attenuated after adjustment for WAZ, SES, pubertal stage, orphan status, calcium intake and age at ART initiation. Similarly, after the same adjustment, the trend between age at ART initiation and SMD was no longer evident. No association between viral load and SMD was found in females, but later age at ART initiation was associated with a more negative SMD (Table 5.7). The association between WAZ and SMD was robust to adjustment, and again being underweight was associated with more negative SMD in CLWH in both sexes. Underweight children (z-score < -2) were on average delayed by a year in their skeletal maturity, compared to those who were not underweight.

Table 5.6 Demographic, lifestyle, anthropometry, and pubertal status characteristics in children living with HIV

	Males (n=117)	Females (n=101)	p-value
Socio-demographics			
Chronological age (years), mean (SD)	12.6 (2.5)	12.1 (2.4)	0.16
Socio-economic status, n (%)			0.042
Group 1: low	43 (36.8)	44 (43.6)	
Group 2: middle	33 (28.2)	37 (36.6)	
Group 3: high	41 (35.0)	20 (19.8)	
Orphanhood: One or both parents dead n (%)	46 (39.3)	46 (45.5)	0.41
Anthropometry			
Height (cm), mean (SD)	139.8 (12.3)	138.6 (13.0)	0.49
Height for age z-score, mean (SD)	-1.8 (1.1)	-1.5 (1.1)	0.097
Stunting (height for age z-score<-2), n (%)	43 (36.8)	28 (27.7)	0.19
Weight (kg), mean (SD)	34.0 (12.7)	34.5 (13.9)	0.78
Weight for age z-score, mean (SD)	-1.6 (1.2)	-1.3 (1.1)	0.023
Underweight (weight for age z-score<-2, n (%)	40 (34.2)	22 (21.8)	0.051
BMI (kg/m2), mean (SD)	16.5 (1.4)	17.2 (2.4)	0.010
BMI for age z-score, mean (SD)	-0.8 (0.9)	-0.6 (0.9)	0.040
Wasting (BMI for age z-scores<-2, n (%)	9 (7.7)	5 (5.0)	0.58
Pubertal status			0.19
Tanner I	47 (40.2)	47 (46.5)	
Tanner II	30 (25.6)	13 (12.9)	
Tanner III	19 (16.2)	22 (21.8)	
Tanner IV	17 (14.5)	16 (15.8)	
Tanner V	4 (3.4)	3 (3.0)	
Lifestyle factors			
Physical activity level, n (%)			0.17
Low, <600 MET mins/week	49 (41.9)	53 (52.5)	
Moderate, 600-3000 MET mins/week	26 (22.2)	23 (22.8)	
High, >3000 MET mins/week	42 (35.9)	25 (24.8)	
Daily vitamin D intake, n (%)			0.87
Very low, <4.0 µg/day	5 (4.3)	6 (5.9)	
Low, 4.0-5.9 µg/day	69 (59.0)	60 (59.4)	
Moderate, 6.0-8.0 µg/day	43 (36.8)	35 (34.7)	
Daily calcium intake, n (%)			0.87
Very low, <150 mg/day	51 (43.6)	45 (44.6)	
Low, 150-299 mg/day	24 (20.5)	23 (22.8)	
Moderate, 300–450 mg/day	42 (35.9)	33 (32.7)	
HIV Characteristics			
Age at diagnosis, n (%)			0.70
<4 years exposure	69 (59.0)	63 (62.4)	
4-7.9 years	36 (30.8)	25 (24.8)	
8-12 years	8 (6.8)	10 (9.9)	
>12 years	4 (3.4)	3 (3.0)	
Age at ART initiation, n (%)			0.45
<2 years	30 (25.6)	29 (28.7)	

2-3.9 years	27 (23.1)	27 (26.7)	
4-8 years	42 (35.9)	26 (25.7)	
>8 years	18 (15.4)	19 (18.8)	
ART duration, n (%)			0.38
2-6 years	26 (22.2)	27 (26.7)	
6-10 years	68 (58.1)	61 (60.4)	
>10 years	23 (19.7)	13 (12.9)	
CD4 count, <500 cells per µL	25 (21.4)	18 (17.8)	0.61
Viral load, >1000 RNA copies per ml	24 (20.5)	22 (21.8)	0.87
Tenofovir exposure			0.98
No tenofovir	79 (67.5)	70 (69.3)	
<4 years exposure	24 (20.5)	20 (19.8)	
4 years+ exposure	14 (12.0)	11 (10.9)	
Bone age measures			
Bone age, mean (SD)	11.2 (2.3)	11.1 (2.5)	0.82
Skeletal maturity deviation, n (%)	-1.4 (1.4)	-1.0 (1.3)	0.037
Skeletal maturity delay, n (%)	35 (29.9)	20 (19.8)	0.12

***Foot notes**: Skeletal maturity deviation: difference between bone age and chronological age. Skeletal maturity delay: skeletal maturity deviation ≤-2 years*

Table 5.7 Association between participants characteristics and skeletal maturity deviation in males living with HIV (unadjusted and adjusted linear regression analysis)

Variable	n	Mean SMD	Unadjusted model β coefficient (95%CI)	p-value	Adjusted model β coefficient (95%CI)	p-value
Males n=117						
Weight for age z-score						
Weight for age z-score>-2	77	-1.03	Ref	<0.001	ref	0.001
Weight for age z-score<-2	40	-2.15	-1.12 (-1.62, -0.62)		-0.85 (-1.40, -0.30)	
Socio-economic status						
Group 1: High	41	-1.65	Ref	0.414	ref	0.069
Group 2: Middle	33	-1.32	0.33 (-0.31, 0.98)		0.66 (0.03, 1.29)	
Group 3: Low	43	-1.27	0.38 (-0.23, 0.98)		0.44 (-0.14, 1.03)	
Pubertal stage						
Tanner 1	47	-1.37	Ref	0.351	ref	0.73
Tanner 2	30	-1.13	0.23 (-0.41, 0.88)		0.32 (-0.29, 0.92)	
Tanner 3	19	-1.52	-0.15 (-0.90, 0.60)		-0.02 (-0.78, 0.74)	
Tanner 4&5	21	-2.04	-0.47 (-1.20, 0.25)		0.12 (-0.60, 0.84)	
Orphan status						
Not an orphan	71	-1.21	Ref	0.046	ref	0.298
One or both parents dead	46	-1.73	-0.53 (-1.04, -0.01)		-0.26 (-0.77, 0.25)	
Physical activity						
High, >3000 MET mins/week	42	-1.45	Ref	0.819		
Moderate, 600-3000 MET mins/week	26	-1.26	0.19 (-0.50, 0.89)			
Low, <600 MET mins/week	49	-1.46	-0.01 (-0.58, 0.59)			
Vitamin D intake						
Moderate, 6.0-8.0 µg /day	21	-1.72	Ref	0.571		
Low, 4.0-5.9 µg /day	77	-1.49	-0.37 (-1.05, 0.32)			
Very low, <4.0 µg /day	19	-1.44	-0.31 (-1.19, 0.57)			
Calcium intake						
Moderate, 300–450 mg/day	42	-1.08	Ref	0.017	ref	0.131
Low, 150-299 mg/day	24	-1.12	0.04 (-0.73, 0.65)		-0.11 (-0.78, 0.57)	
Very low, <150 mg/day	51	-1.83	-0.75 (-1.31, -0.19)		-0.59 (-1.18, 0.003)	
Age at ART initiation						
<2 years	30	-0.76	Ref	0.011	ref	0.118
2-3.9 years	27	-1.41	-0.66 (-1.37, 0.05)		-0.61 (-1.31, 0.08)	
4-8 years	42	-1.64	-0.74 (-1.40, -0.25)		-0.73 (-1.39, -0.08)	
>8 years	18	-1.99	-1.23 (-2.03, -0.44		-0.83 (-1.67, 0.002)	
CD4 count						
>/=500 cells per µL	92	-1.33	Ref	0.233		
<500 cells per µL	25	-1.71	-0.38 (-1.00, 0.25)			
Viral load						
<1000 RNA copies per ml	93	-1.24	Ref	0.009	ref	0.073
>1000 RNA copies per ml	24	-2.08	-0.84 (-1.45, -0.22)		-0.56 (-1.19, 0.08)	
TDF years of exposure						
No exposure	79	-1.32	Ref	0.534		
<4 years	24	-1.60	-0.28 (-0.93, 0.36)			
≥4 years	14	-1.66	-0.35 (-1.15, 0.46)			

The adjusted model included the following exposures: pubertal status, weight for age z-scores, socio-economic status, orphan status, calcium intake, age at ART initiation and viral load. ART-Anti-retroviral therapy, TDF- tenofovir disoproxil fumarate. CI-confidence interval SMD: Skeletal maturity deviation calculated as the difference between bone age and chronological age; negative values reflect lower bone age than chronological age (delay in skeletal maturation)

Table 5.8 Association between participants characteristics and skeletal maturity deviation in females living with HIV (unadjusted and adjusted linear regression analysis)

Variable	n	Mean SMD	Unadjusted model β coefficient (95%CI)	p-value	Adjusted model β coefficient (95%CI)	p-value
Female n=101						
Weight for age z-score>-2	79	-0.80	ref	0.001	ref	0.001
Weight for age z-score<-2	22	-1.86	-1.06 (-1.66, -0.47)		-01.00 (-1.60, -0.41)	
Socio-economic status						
Group 1: High	20	-1.18	ref	0.801	ref	0.706
Group 2: Middle	37	-0.79	0.39 (-0.34, 1.11)		0.39 (-0.26, 1.04)	
Group 3: Low	44	-1.15	0.03 (-0.68, 0.73)		0.09(-0.56, 0.74)	
Pubertal stage						
Tanner 1	47	-1.20	ref	0.226	ref	0.011
Tanner 2	13	-1.02	0.18 (-0.64, 1.01)		0.69 (-0.16, 1.50)	
Tanner 3	22	-0.81	0.38 (-0.30, 1.06)		0.73 (0.09, 1.39)	
Tanner 4	19	-0.86	0.34 (-0.38, 1.06)		0.66 (-0.03, 1.35)	
Orphan status						
Not an Orphan	55	-1.01	ref	0.920	ref	0.583
One or both parents dead	49	-1.04	-0.03 (-0.55, 0.50)		0.05 (-0.45, 0.55)	
Physical activity						
High, >3000 MET mins/week	26	-0.90	ref	0.630		
Moderate, 600-3000 MET mins/week	26	-1.07	-0.17 (-0.93, 0.59)			
Low, <600 MET mins/week	55	-1.07	-0.17 (-0.81, 0.47)			
Vitamin D intake						
Moderate, 6.0-8.0 µg /day	33	-0.85	ref	0.908		
Low, 4.0-5.9 µg /day	23	-1.23	0.12 (-0.44, 0.68)			
Very low, <4.0 µg /day	45	-1.06	-0.39 (-1.55, 0.77)			
Calcium intake						
Moderate, 300–450 mg/day	33	-0.85	ref	0.525	ref	0.732
Low, 150-299 mg/day	26	-1.25	-0.38 (-1.09, 0.33)		-0.17 (-0.82, 0.55)	
Very low, <150 mg/day	48	-1.06	0.21 (-0.81, 0.39)		-0.02 (-0.62, 0.57)	
Age at ART initiation						
<2 years	29	-0.53	ref	0.006	ref	<0.001
2-3.9 years	27	-0.99	-0.47 (-1.15, 0.22)		-0.59 (-1.25, 0.08)	
4-8 years	26	-1.27	-0.74 (-1.43, -0.05)		-1.14 (-1.84, -0.43)	
>8 years	19	-1.51	-0.98 (-1.74, -0.23)		-1.47 (-2.30, -0.65)	
CD4 count						
>/=500 cells per µL	83	-0.98	ref	0.408		
<500 cells per µL	18	-1.26	-0.28 (-0.40, 0.97)			
Viral load						
<1000 RNA copies per ml	79	-1.12	ref	0.196	ref	0.176
>1000 RNA copies per ml	22	-0.70	0.41 (-0.21, 1.04)		0.47 (-0.13, 1.06)	
TDF exposure						
No exposure	70	-0.96	ref	0.567		
<4 years	20	-1.26	-0.30 (-0.96, 0.37)			
4 years +	11	-1.05	-0.10 (-0.95, 0.76)			

The adjusted model included the following exposures: pubertal status, weight for age z-scores, socio-economic status, orphan status, calcium intake, age at ART initiation and viral load. ART-Anti-retroviral therapy, TDF- tenofovir disoproxil fumarate. CI-confidence interval SMD: Skeletal maturity deviation calculated as the difference between bone age and chronological age; negative values reflect lower bone age than chronological age (delay in skeletal maturation)

5.3.6 Extra analysis

I repeated the analysis using multiple imputation. All the results were consistent other than the association between age at ART association and SMD in the boys because the sample size increased hence the statistical power also increased. I also saw this association in males which mirrored the association in females (see appendix C, page 190, Table 4 for imputed analysis)

5.4 Discussion

I report results from the first study to my knowledge to describe skeletal maturation in CLWH in Southern Africa. Delayed skeletal maturation was common among CLWH whether male or female and the delay in skeletal maturity was more marked in the older children who were in earlier Tanner stages. Therefore, having HIV and being underweight were strongly associated with delayed skeletal maturation. In CLWH, later age at ART initiation was associated with greater delay in skeletal maturation in females.

5.4.1 Delayed skeletal maturation in CLWH

My study has shown that CLWH have delayed skeletal maturation whether male or female when compared with their HIV-negative peers. These findings are consistent with two studies from Brazil and India where children aged 5-11 and 8-14 years were studied respectively (137, 253). However, the Brazilian and Indian studies did not account for potential confounders unlike in my study where linear regression analysis assessed the independent effect of HIV on skeletal maturation after accounting for weight-for-age z-scores, socio-economic status, pubertal stage, orphan status, physical activity and vitamin D and calcium intake. In addition, the Brazilian and Indian studies had small sample sizes of 60 participants and did not include older adolescents, and in the case of the Indian study, a comparator group of HIV-negative children. Females living with HIV had less negative SMD than males which could be a result of females having better adherence to ART as reported in several studies (254-256).

Similar to this study, delays in skeletal maturation have been reported in other studies in black African HIV-negative children and adolescents living in Africa although the magnitude of delay differed ranging from 0.5 years to 1.6 years (102, 214, 257). In a comparison of black and white children in South Africa, black males matured later than white males by 6 months (89). In contrast, a systematic review of studies of black children, age 0-18 years, living in high income countries showed that they have advanced BA relative to CA (158). The differences in skeletal

maturation between black children in Africa and those in high income countries may be explained by the optimal environmental conditions in high income countries in terms of better access to healthcare and nutrition which are likely to contribute to maximising genetic potential, all of which play a key role in the skeletal development of children. Longitudinal data in much larger Africa populations are required to determine whether delays in skeletal maturation in CLWH are transient or persist throughout adolescence until the end of growth. In chapter five I will describe skeletal maturation after one year in this cohort of children.

I observed that there was no evidence that Tanner stage was modifying the effect of HIV on SMD suggesting that factors other than developmental stage may be more critical in understanding skeletal growth in children living with HIV. However, I would need longer prospective data on growth to see whether there is catch-up growth or not in CLWH. Furthermore, my study was not adequately powered to test an interaction.

5.4.2 Factors associated with delayed skeletal maturation

My findings add onto the literature that HIV infection can adversely impact normal growth and development as living with HIV was independently associated with delays in skeletal maturation in this population. The delay in skeletal maturation leads to impaired linear growth resulting in CLWH being shorter than their peers. Whether they catch up to their peers requires follow up studies until the end of growth. I also showed that chronologically older CLWH were more likely to be in earlier Tanner stages, with greater delays in skeletal maturation than children in later tanner stages of the same age. It is possible that the delayed older CLWH in earlier tanner stages may experience a period of accelerated skeletal maturation to 'catch-up' in skeletal maturation on initiation of puberty, and the rate of skeletal maturation may be more rapid in duration compared to their fellow counterparts who were more advanced in maturation at the same age.

Age at ART initiation was associated with SMD only in females highlighting the importance of early initiation of ART; similar trends were observed in males although this did not reach significance at the $p<0.05$ level. Similarly, a longitudinal analysis of skeletal maturation in a 4-year follow-up study of Brazilian children reported reduced SMD in CLWH starting ART early (253) . Starting ART early has several benefits which include lower mortality, better virologic control and improved growth in children (258-260). Mortality was substantially reduced in infants with immediate ART initiation in the trial on Children with HIV Early Antiretroviral Therapy (CHER), such that ART following diagnosis was recommended for all infants with HIV (259, 261). Furthermore, the risk of severe illness was reduced with early ART initiation in the START and

TEMPRANO trials (262, 263). Therefore, with better control of disease growth is significantly improved because children are not unwell and less prone to repeated infections. These findings support WHO recommendations to start ART upon HIV diagnosis regardless of CD4 count aligning with the Zimbabwean government implementing the "test and treat" approach in the 2016 national policy and guidelines (262, 263). Delayed ART initiation is still a reality with only 80% of Zimbabwean children (0-14 years) having access to ART (110). My study did not show an association between TDF use and SMD, even though TDF has been associated with bone deficits in density and strength (i.e. bone accrual) in the same population (224, 225), potentially reflecting a direct effect of TDF on bone accrual rather than on puberty. It is still not clear how TDF causes bone loss although different authors have proposed several mechanisms, which include alteration of gene expression in osteoclasts, altered renal phosphate handling and functional vitamin D deficiency (264-266). Viral load monitoring is critical to assess the efficacy of ART and in my study, I found a weak association between viral load and SMD in the males. This may be due to low compliance or drug resistance, however, of the two it is most likely attributed to the male gender being associated with less adherence to ART. (254, 256). To confirm this, I would need to conduct a study to explore associations between viral load and adherence.

Underweight children, living with or without HIV, were delayed in BA by over a year compared to those not underweight, consistent with a study in India where BA was measured in 100 underweight children (267). In contrast, there was no relationship found between BMI and skeletal maturation in Iranian and Malawian children aged 6-15 years and 2-28 years respectively (102, 268). The Malawian study used Greulich Pyle to assess BA which I have found to be less biased by age and less precise than the TW3 method as I reported in Chapter 3 (245). In the South African Birth to Twenty's (Bt20) Bone Health Study (220), being heavier and taller at age two years in males, and having greater lean mass and having entered puberty earlier in females were associated with more advanced skeletal maturation at age 9-10 years, which is in general agreement with the current findings. The Bt20 study showed the importance of a healthy weight in early childhood and its implications on later growth and development. It is not possible to determine early life environmental exposures and growth measures in the current study.

The association between being underweight and SMD may be explained by the fact that underweight is an indicator of malnutrition. Therefore, the insufficient intake of nutrients such as proteins, minerals and vitamins which are essential for the endochondral ossification at the growth plate would have a negative impact on skeletal maturation. Additionally, underweight

children often have delayed onset of puberty meaning sex hormones like oestrogen that trigger GH and IGF-I production to initiate the growth spurt and stimulate the closure of the growth plate are secreted later thereby delaying skeletal maturation. Although nutrient supplementation can be a vital intervention for underweight children, linear growth would be improved if the intervention substantially improves socioeconomic status and living conditions (47). However, a double-blind, randomized controlled trial in Israel involving 200 short and lean healthy prepubertal children showed that supplementation with a formula providing 25% of the recommended dietary reference intake for calories, high in whey protein (28% of calories), 25% fat (from canola oil), and 47% carbohydrates, along with vitamins and minerals, positively impacted their growth (269).

In contrast to my study, an association between low SES and SMD was reported in a study conducted in Sudan (91), and this may be attributed to the different measurements of SES in the two studies. The Sudanese study defined low SES as having insurance (insurance in Sudan is available only for families on low income as defined by their department of social affairs), whilst in my study, SES data was derived from the first component from a principal component analysis that combined asset list and household item ownership. The limitation for Sudanese method of determining SES is that it may not capture the full spectrum of socioeconomic conditions such that some individuals who may not have insurance for other reasons maybe misclassified. On the other hand, the choice of household items and assets chosen in my study can subjectively influence the SES measure and the value and significance of the assets may vary potentially skewing the results. Therefore, use of a broader definition of SES and a method that is validated within the population under study may provide a more comprehensive understanding of SES and yield better results.

I did not find an association between PA and SMD and studies on the association between PA and SMD are limited. It is possible that the self-reported method of measuring physical activity in my study may be subject to recall bias and the children could not accurately remember past activities and may struggle to estimate the duration or intensity of their activities. Therefore, a less subjective way of assessing physical activity would be more ideal.

5.4.3 Strengths and limitations of the study

One of the strengths of this study is that it is the first to describe skeletal maturation in Zimbabwean children and adolescents. Another strength is that the study included a comparator group of HIV negative children. The study sample that was a likely representation of children living in Harare as CLWH were recruited from HIV clinics in the only two tertiary

hospitals in Harare and HIV negative children from schools in the same catchment area of the tertiary hospitals. Using the TW3 method instead of the GP method was also a strength as it was shown to be less valid in chapter three (245). The study had enough numbers hence powered enough to determine factors associated with SMD.

However, there are limitations to this study. For example, the cross-sectional nature of our study meant the exposure and the outcome were measured at the same point in time therefore minimally informative for causal inference. In addition, there is risk of reverse causality where the outcome SMD may cause the exposures. Another limitation was the exclusion of older children because of the method of choice for BA assessment (which only grades up to 15 years in girls and 16.5 years in boys). As a result of this exclusion, the associations of SMD with age at ART initiation and viral load in boys with HIV, and pubertal status in girls with HIV may have been inadequately powered to show these associations because of the relatively small sample size in CLWH in the later Tanner stages.

There is a possibility of measurement error when assessing BA and to determine the consistency around the BA measurements a second person graded the radiographs. Lack of local anthropometric reference data necessitated the use of data from the UK to define malnutrition potentially resulting in misclassification. Although diet data were collected using a standardised and validated tool, evidence has shown that frequency and quantity of food portions consumed are often poorly estimated due to recall bias thus leading to inaccuracies in determining dietary vitamin D and calcium intake.

In this analysis I used a complete case analysis approach where participants with missing variables (tanner stage, orphan status, CD4 and viral load) were excluded. Although the statistical power of the study was reduced because of the reduced sample size the participants with and without missing data had similar characteristics such that performing a complete case analysis would not introduce any bias. Again, the chances of detecting a significant association if it exists were increased. However, in the follow up analysis I accounted for missing data by using multiple imputation by chained equations. Another limitation of my study was the inability to explore observed associations by CD4 count because too few participants had lower CD4 count (males :27 and females: 18) although a much larger study has reported an association between lower CD4 count and poor growth (270). My study was also limited in that children with comorbidities like TB were not excluded from the study as both TB and HIV can significantly impact growth and development through nutritional deficiencies and chronic inflammation. It is possible that delayed growth and development may be worsened in children because of the combined effect of the two conditions and may not reflect the impact of one condition.

5.5 Conclusion

In conclusion, perinatally acquired HIV infection and being underweight were independently associated with delays in skeletal maturation in both males and females. The implications of delays in skeletal maturation include impaired linear growth leading to CLWH being shorter than their HIV negative peers or their expected final adult height impacting the children cognitively, socially and psychologically. In CLWH, delays in initiating ART contributed to the delays in skeletal maturation. Our findings support the test and treat programmes where ART is initiated regardless of disease or immunological stage. Given the consequences of delayed development on final height and subsequent outcomes, longitudinal studies are needed to determine the implications of a delayed development on later health in children living with and without HIV. Therefore, in the next chapter I will look at longitudinal data collected after one year.

Chapter 6 Skeletal maturation after one year in peripubertal children living with and without HIV

6.1 Introduction

In chapter 5 my cross-sectional analysis found that living with HIV and being underweight were associated with delays in skeletal maturity in both males and females. CLWH were delayed in skeletal maturation than the HIV negative children. In this current chapter I want to determine if the delays in skeletal maturity are transient, or whether they persist after one year. Therefore, I aimed to determine change in SMD after one year and determine factors associated with the change.

The cumulative negative exposure to the direct and indirect effects of HIV infection and its treatment on skeletal growth and development is seen more in children and adolescents than adults as they are still going through the growth process. Therefore, tracking their growth and development would allow observation of growth patterns and trajectories especially in the context of HIV. Longitudinal studies would allow identification of critical periods during growth and development and assessment of long-term consequences of delayed skeletal maturation, and whether catch-up growth occurs as the children mature. HIV negatively impacts growth with several studies having shown that CLWH often present with lower HAZ and WAZ, indicating stunting and underweight conditions (122, 271) . Before the advent of ART, CLWH had the most profound growth failure compared to HIV negative children with a sharp decline in HAZ in the first 6 weeks of life followed by a progressive decline up to 21 months (122). However, the initiation of ART has been shown to improve HAZ and WAZ, particularly when started early, demonstrating a potential for growth recovery with effective treatment (271-273). Despite improvements with ART, many children may still not reach the growth parameters of their uninfected peers, highlighting the need for continued monitoring and nutritional support (274).

Longitudinal studies assessing skeletal maturation in CLWH are lacking in sub-Saharan Africa, where 90% of CLWH live (110). The two studies that have investigated skeletal maturation in CLWH have reported delayed skeletal maturation (137, 253) which corresponds

to a lower BA than CA and of these two, only one study in Brazil looked at longitudinal data. Although not tested, the magnitude of the SMD in the Brazilian study was higher at baseline than at follow-up, males (1.59 vs 0.58 years) and females (2.11 vs 1.29 years). However, the Brazilian study only used t-tests in their statistical analysis whilst I used linear regression analysis that allowed for the examination of the relationship between SMD and other variables and control for confounding variables not just a comparison of the means. In the previous chapter I have demonstrated HIV infection and being underweight as important factors associated with delayed skeletal maturation with CLWH being more delayed in skeletal maturation than the HIV-negative children. The delays were greater in older children in lower pubertal stages suggesting potential lasting effects of HIV on growth and development into early adulthood. It is therefore unclear whether the CLWH do "catch-up" in skeletal maturation, and hence achieve their genetic growth potential, with their uninfected counterparts as they accelerate through puberty.

Therefore, the aim of this study was to understand whether the differences in skeletal maturation in CLWH and HIV negative children were sustained after one year. To assess change, BA assessment was repeated to determine the SMD at follow up, calculated as BA at follow up minus CA at follow up, independent of the SMD at baseline to generate an indirect measure of change. I also determined baseline factors (SES, pubertal stage, physical activity, vitamin D and calcium intake), associated with SMD at follow up, independent of SMD at baseline. This analysis was based on 12 months of longitudinal data from children living with and without HIV (8-16 years). I hypothesised that after one-year CLWH would remain delayed in skeletal maturation than HIV negative children.

6.2 Methods

6.2.1 Study design and participants

As previously described in chapter three, this was a cohort study (IMVASK), of children aged 8-16 years, with and without HIV with two visits 12 months apart. The IMVASK study was conducted as per published protocol and is explained in detail in chapter three (224).

6.2.2 Bone age assessment

BA was assessed from hand wrist radiographs taken of the non-dominant side. As the IMVASK population at follow up were now aged 9-17 years, if the same approach as in chapter four and five were used, it meant exclusion of more participants in the follow up study. Therefore, I decided to use a combination of the two BA methods, after testing the appropriateness of this decision (see 6.2.3). The TW3 Radius, Ulna and Short bones (TW3 RUS) (77) method was used on participants aged below 15 and 16.5 years and in girls above 15 years and boys above 16.5 years the GP atlas was used (2) as the upper age limit of GP is 19 years for males and 18 years for females.

6.2.3 Appropriateness of using two methods of BA assessment

To test for the appropriateness of using two methods of BA assessment, scatter plots for BA against CA were generated and the slope of the regression lines calculated. The slopes were calculated with and without the GP bone ages for the older children and no differences were seen in the slopes (Figure 6.1). Figure 6.1a shows males 8-16.5 years: TW3 method used and above 16.5 years the GP method used; regression coefficients with GP 0.87, without GP 0.88. Figure 5.1b shows females 8-15 years: TW3 method used and above 15 years the GP method used; regression coefficients with GP 0.86, without GP 0.86.

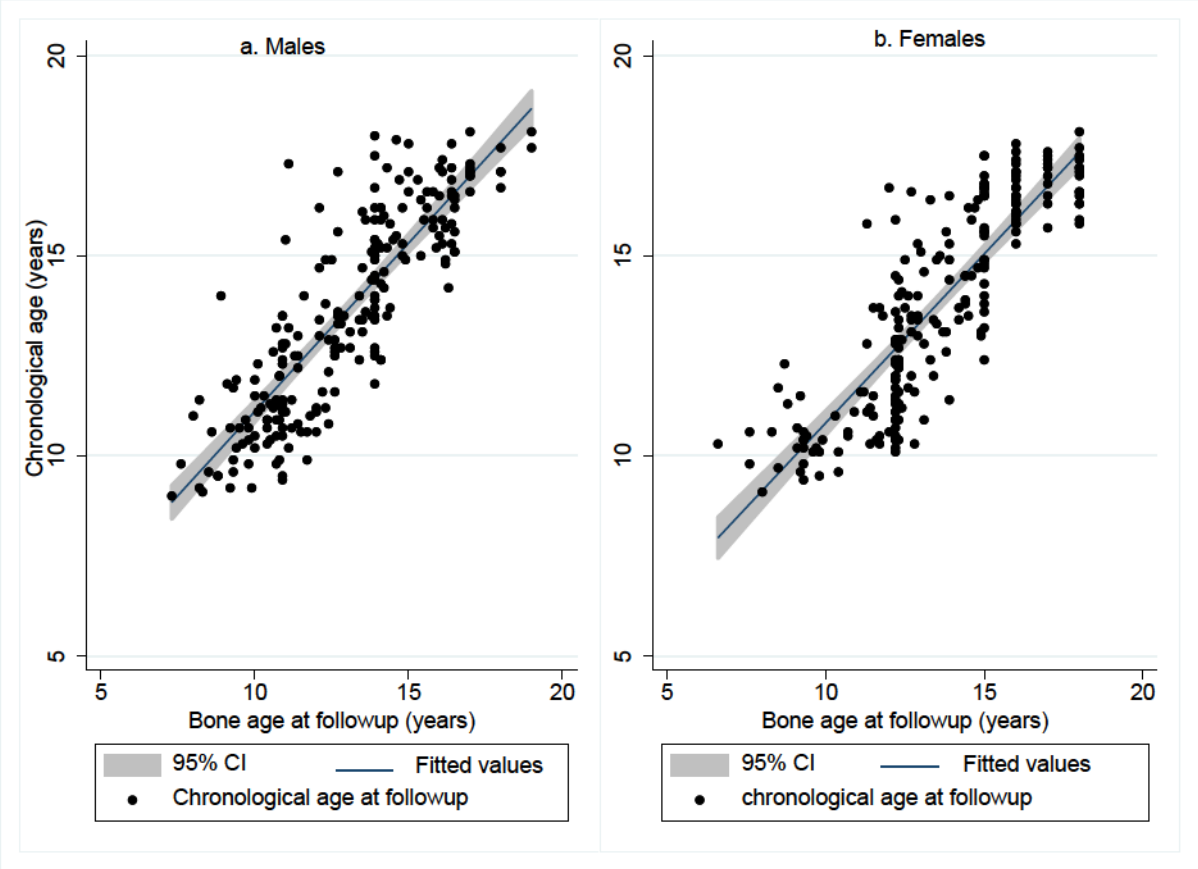


Figure 6.1 Relationship between bone age and chronological age a) Tanner Whitehouse 3 method used and above 16.5 years the Greulich and Pyle method used, b) TW3 method used and above 15 years the GP method used

6.2.4 Statistical analyses

I performed the statistical analyses using Stata 17 (StatCorp, Texas, USA). A negative SMD value reflects a delay in skeletal maturity, and a positive value reflects advanced skeletal maturity relative to CA. I cleaned the data and checked for consistency and outliers. I used the Student t-tests for continuous variables and chi-squared tests for categorical variables to compare within each sex and between children with and without HIV and to compare participants remaining in the study and those lost to follow up. Normally distributed continuous variables were presented as means ± standard deviations and categorical variables as numbers and proportions of participants in each category. Non-normally distributed continuous variables were presented as medians and interquartile ranges (IQR). I derived histograms to show the distribution of SMD at baseline and at follow up by HIV status, WAZ and levels of physical activity.

6.2.4.1 Determining the best method of change in SMD

I needed to determine the most appropriate way to assess change in SMD. There were three possible methods of calculating the outcome of interest:

1. SMD at follow-up (BA at follow-up - CA at follow-up) in years, adjusted for SMD at baseline and follow-up period,
2. Conditional change in SMD characterised by residuals obtained after estimating sex-specific linear regression models for SMD at follow-up on SMD at baseline with adjustment for individual follow-up duration; this measure of change is independent of baseline level
3. The absolute change in SMD calculated as SMD at follow-up minus SMD at baseline.

Each method has advantages and disadvantages. Method 3 which is the direct method (SMD at follow up minus SMD at baseline) is a difficult concept to understand because it is the longitudinal change of a difference in BA and CA therefore difficult to interpret. Although the measure of change is independent of the baseline SMD in method 2 which is the residual approach, it is difficult to explain because it requires a deeper statistical reasoning behind the function of residuals in a linear regression model. The interpretation of residuals is more challenging as they are measuring how much greater some quantity is from what we would expect from the baseline level; they can artificially reduce the estimated standard errors (as the residuals are used in the conditional change models as if they have no uncertainty associated with them, when actually the residuals themselves have been estimated from a previous model) and they can lead to biased parameter estimates. Again, because the residuals are ascertained from a linear regression where the characteristic at follow-up is estimated from the same characteristic at baseline, smaller numbers of observations will result in a less robust derivation of residuals as they have been estimated from a regression which is based on fewer observations (275). Therefore, it is not a reliable method. Whilst method 1 is not strictly a longitudinal change in SMD, it is an indirect measure of change as it looks at SMD at one time point whilst adjusting for SMD at an earlier time point. I therefore chose method one as the output is easier to interpret and accounts for SMD at baseline.

6.2.4.2 Multiple imputation

To account for missing data on pubertal status, CD4 count and viral load, I decided to impute missing data to increase the power to be able to answer my research questions. I first identified auxiliary variables (HIV status, SES, sex, CA, physical activity, vitamin D and calcium intake). I added these to the imputation model to support the plausibility of the assumption of missing at random. These auxiliary variables were identified: 1) as *a priori* factors; 2) after considering their strength of association with the variables containing missing values (if continuous, correlation coefficient $r>0.4$ and if categorical, chi squared test $p<0.05$). Using *imputation by chained equations command* in Stata, I used a binary distribution to impute dichotomised CD4 count and viral load whilst an ordinal distribution was used to impute Tanner staging.

6.2.4.3 Minimally adjusted models

I examined univariable associations between each baseline exposure variable in turn (HIV status, WAZ, SES, physical activity, vitamin D intake, calcium intake, pubertal stage) and the outcome SMD at follow-up after accounting for SMD at baseline and the follow-up period (minimally adjusted models). P-values from tests for trend were reported for ordinal variables.

6.2.4.4 Multivariable model

A fully adjusted linear regression model (including the variables baseline SMD, follow-up period, HIV status and *a priori* factors WAZ, SES, pubertal stage, physical activity, and calcium intake as exposures) was also fitted. Other variables were included in the fully adjusted model if there was evidence of an association (p-value for trend ≤ 0.1) in the minimally adjusted analysis, in either the males or the females.

6.2.4.5 Factors attenuating the association between HIV and SMD

To examine which factors attenuated the association between HIV status and SMD at follow-up when included as adjustments, I fitted an initial model with SMD at follow-up as the outcome and SMD at baseline, follow-up period and HIV status as exposures. The baseline exposures WAZ, SES, pubertal status, physical activity, and calcium intake were then added individually to this model and estimates of association between HIV status and SMD at follow-up were reported.

6.2.4.6 Factors associated with SMD in children living with HIV

In an analysis restricted to CLWH, minimally adjusted linear regression models were used to examine associations between baseline exposure variables (including the HIV specific variables, age at ART initiation, CD4 count, viral load and TDF exposure) and SMD at follow-up with adjustment for baseline SMD and follow-up period. The fully adjusted linear regression model in CLWH included baseline SMD, follow-up period, HIV status and *a priori* factors WAZ, SES, pubertal stage, physical activity, calcium intake and HIV specific variables with a p-value for trend ≤ 0.1 in the minimally adjusted linear regression models in either the males or females.

6.3 Results

6.3.1 Participant characteristics

Of the 609 participants included in the IMVASK study, 160 were lost to follow up. By design, CA was similar in both males and females whether living with or without HIV. Female participants with HIV were more likely to be in the earlier Tanner stages than those living without HIV whilst no differences in pubertal stage were seen in the boys between those living with or without HIV (Table 6.1). No differences were seen in SES, daily dietary vitamin D and calcium intake in children living with or without HIV whether males or females. Males and females living with HIV were more likely to be orphaned and have lower levels of physical activity than those living without HIV. Male CLWH were 4.02 kg (95% CI: -0.03, 8.08) lighter and 7.04cm (95% CI: 3.55, 10.52) shorter and female CLWH were 6.65 kg (95% CI: 2.49, 10.81) lighter and 6.67 cm (95% CI: 3.34, 10.00) cm shorter than their counterparts living without HIV. Therefore, CLWH were more likely to be underweight and stunted than HIV-negative children whether males or females. On average SMD at follow up was more negative (greater delay in skeletal maturity) in CLWH in both males (-1.0 vs -0.2 years) and females (-0.8 vs 0.2 years) than those living without HIV. There were differences in the follow-up period between children living with and without HIV in both males and females with a slightly longer follow up period seen in CLWH. Participants who were lost to follow up were similar in CA, HIV status, SES, orphan status, pubertal status, WAZ, HAZ, vitamin D intake and physical activity (Table 6.2). Differences were seen in the daily calcium intake, females who were lost to follow-

up were more likely to take very low calcium intake than those who remained in the study.

6.3.2 HIV characteristics in children living with HIV

Children with HIV were initiated on ART at a median age of 3.8 (IQR 2.1-6.8) years in males and 4.0 (IQR 2.0-7.6) in females. Nearly 20% of both males and females had a CD4 count of less than 500 cells per micro-litre. My results show that 20% of males and 26% of females had a viral load of more than 1000 RNA copies per ml and 30% of the males and 33% of females had been prescribed TDF (Table 6.3).

6.3.3 Distribution of SMD by HIV status

The purpose of this analysis was to explore whether there was a change in distribution of SMD from baseline to follow up allowing for a visual comparison of how SMD differs at these two time points. There was a small shift in the distribution of SMD between baseline and follow up in males; this was the case whether they were living with or without HIV (Figure 5.2 a and b). A significant shift was seen in females without HIV with no difference in the distribution of SMD between baseline and follow up in females with HIV (Figure 6.2 c and d).

Table 6.1 Baseline sociodemographic and anthropometric characteristics for participants who attended follow-up by HIV status in male and females

	Males n=228				Females n=221			
	n	HIV- n=115	CLWH n=113	p-value	n	HIV- n=116	CLWH n=105	p-value
Socio-demographics								
Chronological age follow-up, mean (SD)	228	13.4 (2.5)	13.6 (2.5)	0.39	221	13.8 (2.5)	13.7 (2.5)	0.88
Chronological age baseline, mean (SD)	228	12.4 (2.5)	12.6 (2.5)	0.61	221	12.7 (2.5)	12.5 (2.5)	0.69
Pubertal stage, n (%)	220			0.23	214			<0.001
Tanner I		33 (28.7)	43 (41.0)			20 (17.5)	37 (37.0)	
Tanner II		31 (27.0)	28 (26.7)			25 (21.9)	17 (17.0)	
Tanner III		18 (15.7)	15 (14.3)			17 (14.9)	24 (24.0)	
Tanner IV		30 (26.1)	16 (15.2)			38 (33.3)	17 (17.0)	
Tanner V		3 (2.6)	3 (2.9)			14 (12.3)	5 (5.0)	
Socio-economic status, n (%)	228			0.23	221			0.095
Low		29 (25.2)	40 (35.4)			42 (36.2)	44 (41.9)	
Middle		40 (34.8)	36 (31.9)			32 (27.6)	37 (35.2)	
High		46 (40.0)	37 (32.7)			42 (36.2)	24 (22.9)	
Orphanhood: one/both parents dead, n (%)	221	8 (7.0)	46 (43.0)	<0.001	216	6 (5.3)	46 (45.1)	<0.001
Daily vitamin D intake, n (%)	228			0.25	221			0.54
Moderate, 6.0-8.0 µg /day		13 (11.3)	20 (17.7)			17 (14.7)	10 (9.5)	
Low, 4.0-5.9 µg /day		76 (66.1)	75 (66.4)			74 (63.8)	71 (67.6)	
Very low, <4.0 µg /day		26 (22.6)	18 (15.9)			25 (21.6)	24 (22.9)	
Physical activity level, n (%)	228			0.007	221			0.026
Low, <600 MET mins/week, n (%)		37 (32.2)	53 (46.9)			43 (37.1)	57 (54.3)	
Moderate, 600-3000 MET mins/week, n (%)		43 (37.4)	22 (19.5)			32 (27.6)	25 (23.8)	
High, >3000 MET mins/week, n (%)		35 (30.4)	38 (33.6)			41 (35.3)	23 (21.9)	
Daily calcium intake, n (%)	228			0.86	221			0.57
Very low, <150 mg/day, n (%)		52 (45.2)	47 (41.6)			50 (43.1)	38 (36.2)	
Low, 150-299 mg/day, n (%)		23 (20.0)	23 (20.4)			25 (21.6)	25 (23.8)	
Moderate, 300–450 mg/day, n (%)		40 (34.8)	43 (38.1)			41 (35.3)	42 (40.0)	
Anthropometry								
Weight, mean (SD)	228	39.1 (15.1)	34.9 (16.0)	0.041	221	43.2 (16.8)	36.8 (14.3)	0.003
Weight for age z-score, mean (SD)	228	-0.7 (1.0)	-1.6 (1.2)	<0.001	221	-0.3 (1.2)	-1.2 (1.1)	<0.001
Underweight (weight for age z-score<-2), n (%)	228	12 (10.4)	33 (29.2)	<0.001	221	8 (7.0)	18 (17.1)	0.022
Mean standing height	228	147.5 (14.9)	140.1 (11.6)	<0.001	221	147.2 (12.2)	141.1 (13.0)	<0.001
Height for age z-score, mean (SD)	228	-0.6 (0.9)	-1.7 (1.1)	<0.001	221	-0.6 (1.1)	-1.4 (1.1)	<0.001
Stunting (height for age z-score<-2), n (%)	228	8 (7.0)	36 (31.9)	<0.001	221	11 (9.5)	27 (25.7)	0.002
BMI	228	17.0 (2.1)	16.5 (1.4)	0.035	221	19.0 (3.6)	17.6 (2.8)	0.002
BMI for age z-score, mean (SD)	228	-0.6 (1.0)	-0.8 (0.9)	0.048	221	-0.0 (1.1)	-0.5 (0.9)	<0.001
Wasting (BMI for age z-score<-2), n (%)	228	10 (8.7)	11 (9.7)	0.82	221	2 (1.7)	6 (5.7)	0.16
Bone age measures								
Bone age follow-up, mean (SD)	228	13.3 (2.5)	12.7 (2.2)	0.053	221	14.0 (1.9)	12.9 (2.1)	0.001

Bone age at baseline, mean (SD)	228	11.9 (2.5)	11.2 (2.3)	0.031	221	12.5 (2.3)	11.5 (2.5)	0.002
Skeletal maturity deviation (SMD) follow-up	228	-0.2 (1.0)	-1.0 (1.5)	<0.001	221	0.2 (1.2)	-0.8 (1.3)	<0.001
Skeletal maturity deviation baseline	228	-0.5 (1.0)	-1.3 (1.4)	<0.001	221	-0.2 (1.2)	-1.0 (1.3)	<0.001
Follow-up period	228	1.0 (0.2)	1.2 (0.2)	<0.001	221	1.1 (0.3)	1.2 (0.3)	0.023

Student t-tests conducted on continuous variables and chi-squared tests on categorical variables. ***SD****-standard deviation,* ***MET***- multiples of the resting metabolic rate, ***SMD****-skeletal maturity deviation*

Table 6.2 Comparison of participant characteristics between those with complete data and participants lost to follow up

	Boys Complete data n=228	**lost to follow up n=75**	**p-value**	**Girls Complete data n=221**	**lost to follow up n=85**	**p-value**
Chronological age at follow up	13.5 (2.5)			13.7 (2.5)		
HIV status			0.59			0.45
HIV negative	116 (50.9)	35 (46.7)		115 (52.0)	40 (47.1)	
CLWH	112 (49.1)	40 (53.3)		106 (48.0)	45 (52.9)	
Socio-economic status, n (%)			0.43			0.23
Low	69 (30.3)	23 (30.7)		86 (38.9)	25 (29.4)	
Middle	75 (32.9)	30 (40.0)		70 (31.7)	28 (32.9)	
High	84 (36.8)	22 (29.3)		65 (29.4)	32 (37.6)	
Orphan status, one or both parents dead,n (%)	54 (24.4)	17 (23.3)	1.00	52 (24.1)	20 (24.1)	1.00
Weight for age_Z-score, mean (SD)	-1.2 (1.2)	-1.2 (1.4)	0.69	-0.7 (1.2)	-1.0 (1.3)	0.088
Underweight, Weight for age_Z-score<-2, n(%)	45 (19.8)	20 (26.7)	0.26	26 (11.8)	12 (14.1)	0.57
Height for age_Z-score, mean (SD)	-1.2 (1.1)	-1.4 (1.4)	0.083	-1.0 (1.2)	-1.1 (1.2)	0.23
Stunting, Height for age_Z-score<-2, n(%)	45 (19.7)	20 (27.4)	0.19	37 (16.7)	15 (17.6)	0.87
BMI for age_Z-score, mean (SD)	-0.7 (1.0)	-0.5 (1.1)	0.15	-0.3 (1.0)	-0.5 (1.1)	0.12
Wasting, BMI for age_Z-score<-2, n(%)	21 (9.3)	6 (8.2)	1.00	8 (3.6)	9 (10.6)	0.025
Pubertal stage, n (%)			0.68			0.48
1	75 (34.2)	27 (37.0)		58 (27.0)	27 (32.1)	
2	59 (26.9)	14 (19.2)		42 (19.5)	13 (15.5)	
3	33 (15.1)	13 (17.8)		41 (19.1)	21 (25.0)	
4	46 (21.0)	16 (21.9)		55 (25.6)	19 (22.6)	
5	6 (2.7)	3 (4.1)		19 (8.8)	4 (4.8)	
Daily vitamin D intake, n (%)			0.52			0.79
Very low, <4.0 µg/day	33 (14.5)	9 (12.0)		27 (12.2)	8 (9.4)	
Low, 4.0-5.9 µg/day	151 (66.2)	47 (62.7)		145 (65.6)	59 (69.4)	
Moderate, 6.0-8.0 µg/day	44 (19.3)	19 (25.3)		49 (22.2)	18 (21.2)	
Physical activity level, n (%)			0.90			0.87
Low, <600 MET mins/week	90 (39.5)	32 (42.7)		100 (45.2)	40 (47.1)	
Moderate, 600-3000 MET mins/week	65 (28.5)	20 (26.7)		57 (25.8)	23 (27.1)	
High, >3000 MET mins/week	73 (32.0)	23 (30.7)		64 (29.0)	22 (25.9)	
Daily calcium intake, n (%)			0.54			0.027
Very low, <150 mg/day	100 (43.9)	36 (48.0)		87 (39.4)	48 (56.5)	
Low, 150-299 mg/day	45 (19.7)	17 (22.7)		51 (23.1)	15 (17.6)	
Moderate, 300–450 mg/day	83 (36.4)	22 (29.3)		83 (37.6)	22 (25.9)	

Table 6.3 Baseline HIV characteristics for males and females living with HIV

	Males n=113		Females n=105	
HIV characteristics				
Age at ART initiation, years	113	3.8 (2.1-6.8)	105	4.0 (2.0-7.6)
Age at HIV diagnosis, years	113	3.0 (1.5-5.8)	105	3.2 (1.2-6.2)
Age at ART initiation, years n (%)	113		105	
<2		27 (23.9)		27 (25.7)
2-3.9		30 (26.5)		26 (24.8)
4-8		39 (34.5)		30 (28.6)
>8		17 (15.0)		22 (21.0)
ART duration (years)	113		105	
2-6		25 (22.1)		28 (26.7)
6-10		65 (57.5)		58 (55.2)
>10		23 (20.4)		19 (18.1)
CD4 count, <500 cells per µL, n (%)	111	22 (19.8)	96	18 (19)
Viral load, >1000 RNA copies/ml, n (%)	108	22 (20.4)	100	26 (26.0)
Years of Tenofovir exposure	113		105	
No tenofovir		79 (69.9)		70 (66.7)
<4 years exposure		20 (17.7)		23 (21.9)
≥4 years exposure		14 (12.4)		12 (11.4)

Student t-tests conducted on continuous variables and chi-squared tests on categorical variables. ***ART****- anti retroviral therapy*

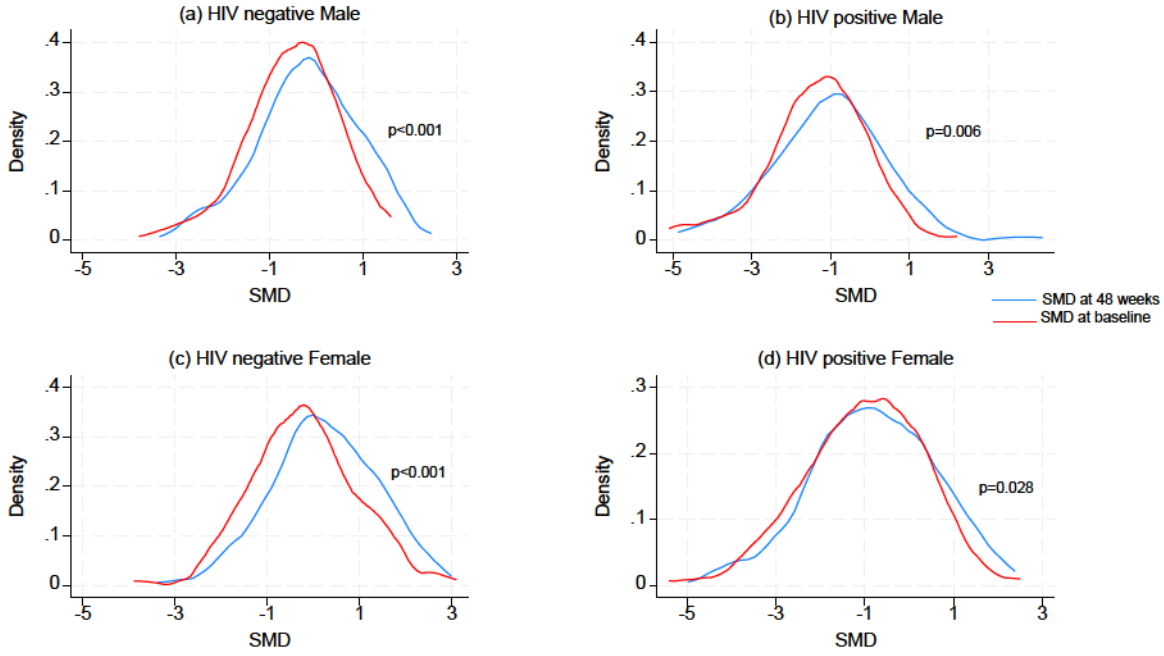


Figure 6.2 The distribution of SMD at baseline and follow up in males and females by HIV status. SMD- skeletal maturity deviation calculated as bone age minus chronological age in years.

6.3.4 Distribution of SMD by weight for age z-scores

A greater shift in distribution of SMD in the positive direction at baseline and follow up was seen in the males and females who were of normal weight with little or no shift in both the underweight males and females (Figure 6.3).

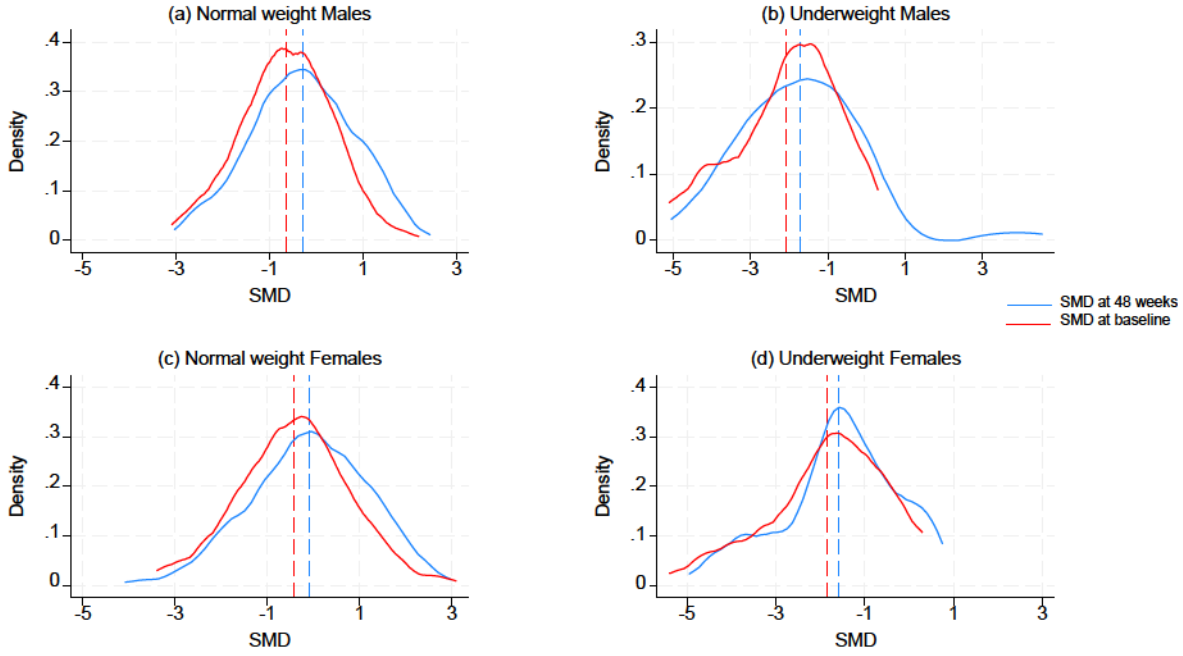


Figure 6.3 The distribution of SMD at baseline and follow up in males and females by weight for age z-scores. SMD- skeletal maturity deviation calculated as bone age minus chronological age in years. Underweight was having weight for age z-scores<-2.

6.3.5 Distribution of SMD by physical activity

The shift in distribution of SMD at baseline and follow up was observed largely in females with moderate and high physical activity and in males with moderate physical activity (Figure 6.4). Little or no shift in distribution was seen in males and females with low physical activity and males with high physical activity.

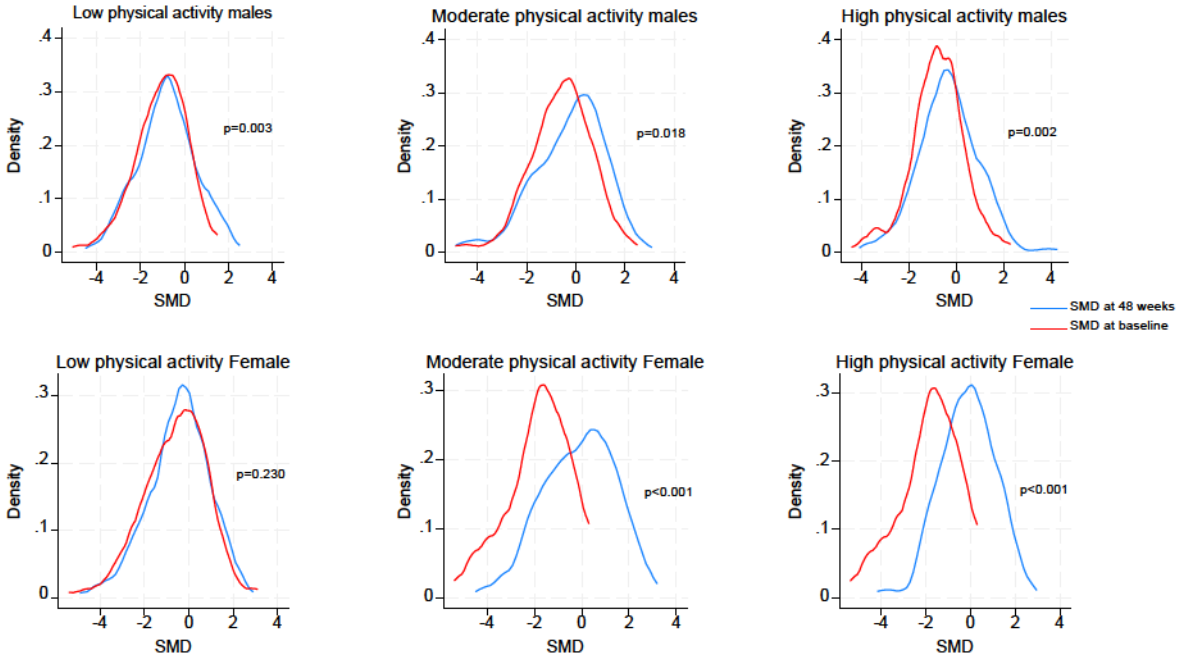


Figure 6.4 The distribution of SMD at baseline and follow up in males and females by levels of physical activity. SMD- skeletal maturity deviation calculated as bone age minus chronological age in years Physical activity was categorised into low, moderate and high

6.3.6 Association between baseline HIV status and SMD at follow-up after accounting for baseline SMD

In the minimally adjusted analyses, skeletal maturity at follow-up was delayed among children with HIV by 0.36 (95% CI: 0.09, 0.63) years and 0.37 (0.11, 0.64) years in males and females respectively compared to their counterparts living without HIV (Figure 6.5) after accounting for SMD at baseline. However, after full adjustment for SMD at baseline, follow-up period, WAZ, SES, pubertal status, physical activity, and dietary calcium intake, the evidence that the males living with HIV had delayed skeletal maturation at follow up was partially attenuated. WAZ and to a lesser extent pubertal stage in the males, and physical activity in the females resulted in the greatest attenuation of the association between HIV status and SMD at follow-up when included as adjustments as evidenced by the reduced point estimates (Table 6.3). SES and physical activity in males and calcium intake in females did not explain the association between HIV and SMD at follow up.

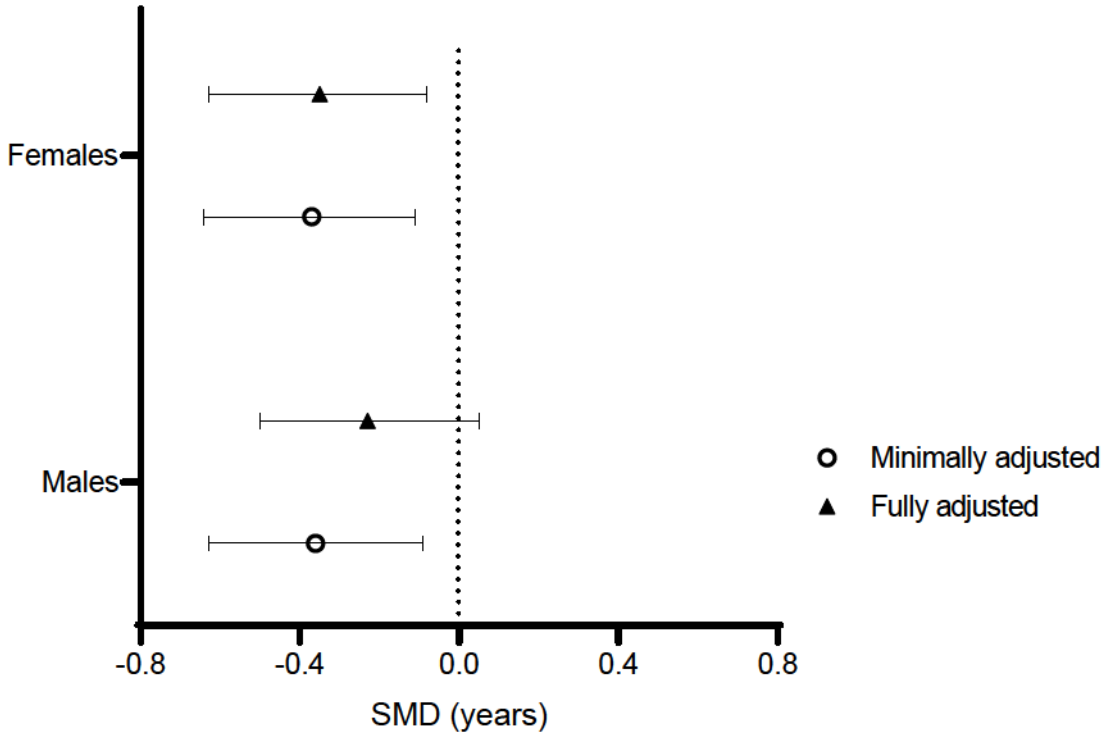


Figure 6.5 Mean differences and confidence intervals in SMD at follow up in children living with HIV compared to those without HIV after accounting for baseline SMD and follow-up period. ***SMD****-skeletal maturity deviation*. *The minimally adjusted model included the following exposures: baseline SMD, follow-up period and HIV status*. The *fully adjusted model included the following exposures: baseline SMD, follow-up period, HIV status, weight for age z-scores, socio-status, pubertal stage, physical activity, and calcium intake*

6.3.7 Factors associated with SMD at follow-up after accounting for SMD at baseline

In the minimally adjusted analysis, living with HIV and lower WAZ were associated with greater delay in skeletal maturity at follow up after accounting for baseline SMD and follow-up period in both males and females (Table 6.4). In addition, less physical activity (in females only) was associated with greater delay in skeletal maturity at follow-up after accounting for baseline SMD and follow-up period in the minimally adjusted model. After full adjustment, lower WAZ remained associated with greater delay in skeletal maturity at follow-up in both males and females. HIV status was robust in the fully adjusted analysis in females. There was partial attenuation of the association between physical activity and SMD in the females.

Table 6.4 Difference in skeletal maturity deviation at follow-up (years) for children living with HIV compared to HIV negative children, depending on predictor variables included

Sample	Predictor variables included	Estimate (95% CI)
Males	Set 1	-0.36 (-0.63, -0.09)
	Set 1, WAZ	-0.28 (-0.54, -0.02)
	Set 1, SES	-0.36 (-0.63, -0.09)
	Set 1, Pubertal stage	-0.34 (-0.62, -0.07)
	Set 1, Physical activity	-0.36 (-0.64, -0.09)
	Set 1, Calcium intake	-0.37 (-0.64, -0.10)
Females	Set 1	-0.37 (-0.64, -0.11)
	Set 1, WAZ	-0.38 (-0.64, -0.13)
	Set 1, SES	-0.38 (-0.65, -0.11)
	Set 1, Pubertal stage	-0.38 (-0.66, -0.10)
	Set 1, Physical activity	-0.33 (-0.61, -0.06)
	Set 1, Calcium intake	-0.37 (-0.64, -0.10)

Skeletal maturity deviation was calculated as bone age (years) – chronological age (years); more negative values reflect greater delay in skeletal maturity. ***Set 1*** *predictors included HIV status, baseline skeletal maturity deviation and follow-up time. WAZ-weight for age z-score, SES- socio-economic status, CI-confidence interval*

6.3.8 Factors associated with SMD at follow-up in CLWH

In the analysis restricted to CLWH, lower WAZ, older age at ART initiation and TDF exposure were associated with greater delay in skeletal maturity at follow-up in the minimally adjusted analysis in the males (Table 6.5). The association between SMD and TDF exposure duration was partially attenuated (p=0.064) in the fully adjusted model. In females, lower self-reported physical activity, and at least four years of TDF exposure were associated with greater delay in skeletal maturity at follow-up in the minimally adjusted analysis. Only physical activity remained associated with SMD after adjustment; TDF exposure duration was partially attenuated (p=0.084) after full adjustment.

Table 6.5 Associations between baseline characteristics and skeletal maturity deviation (years) at one year follow up after adjustment for baseline skeletal maturity deviation and follow-up period in males and females

	Males n=220				Females n=214			
Baseline characteristic	**Minimally adjusted model**		**Fully adjusted model**		**Minimally adjusted model**		**Fully adjusted model**	
	β coefficient (95%CI)	**p -value**	**β coefficient (95%CI)**	**p-value**	**β coefficient (95%CI)**	**p-value**	**β coefficient (95%CI)**	**p-value**
HIV status								
HIV negative	ref	0.066	ref	0.172	ref	0.001	ref	0.005
HIV positive	-0.27 (-0.56, 0.02)		-0.20 (-0.49, 0.09)		-0.43 (-0.67, -0.18)		-0.37 (-0.62, -0.11)	
Weight for age z-score								
Weight for age z score>-2	ref	0.008	ref	0.005	ref	0.040	ref	0.030
Weight for age z score<-2	-0.51 (-0.87, -0.14)		-0.55 (-0.92, -0.17)		-0.41 (-0.80, -0.02)		-0.43 (-0.82, -0.04)	
Socio-economic status								
High	ref	0.858	ref	0.794	ref	0.291	ref	0.479
Middle	0.34 (0.03, 0.64)		0.38 (0.07, 0.69)		-0.08 (-0.38, 0.22)		0.05 (-0.25, 0.35)	
Low	-0.06 (-0.38, 0.26)		-0.08 (-0.40, 0.25)		-0.15 (-0.43, 0.13)		-0.09, -0.37, 0.19)	
Pubertal stage								
Tanner 1-2	ref	0.972	ref	0.977	ref	0.365	ref	0.993
Tanner 3-5	-0.005 (-0.27, 0.26)		0.004 (-0.26, 0.27)		0.11 (-0.12, 0.34)		-0.001 (-0.23, 0.23)	
Physical activity								
High, >3000 MET mins/week	ref	0.323	ref	0.546	ref	0.023	ref	0.077
Moderate, 600-3000 MET mins/week	-0.08 (-0.42, 0.26)		-0.08 (-0.42, 0.26)		-0.002 (-0.31, 0.32)		0.06 (-0.24, 0.37)	
Low, <600 MET mins/week	-0.17 (-0.48, 0.15)		-0.10 (-0.42, 0.22)		-0.30 (-0.57, -0.02)		-0.23 (-0.51, 0.05)	
Vitamin D intake								
Moderate, 6.0-8.0 µg /day	ref	0.875			ref	0.424		
Low, 4.0-5.9 µg /day	0.10 (-0.18, 0.37)				-0.09 (-0.34, 0.15)			
Very low, <4.0 µg /day	-0.21 (-0.86, 0.44)				-0.14 (-0.69, 0.40)			
Calcium intake								

Moderate, 300–450 mg/day	ref	0.286	ref	0.279	ref	0.552	ref	0.588
Low, 150-299 mg/day	0.19 (-0.17, 0.55)		0.18 (-0.17, 0.54)		0.07 (-0.24, 0.38)		0.05 (-0.25, 0.36)	
Very low, <150 mg/day	0.16 (-0.13, 0.46)		0.17 (-0.14, 0.47)		0.08 (-0.18, 0.34)		0.07 (-0.19, 0.33)	

Skeletal maturity deviation (SMD) was calculated as bone age (years) – chronological age (years); more negative values reflect greater delay in skeletal maturity. Minimally adjusted model: baseline SMD and follow-up period as adjustments. Fully adjusted model: baseline SMD, follow-up period, HIV status, weight for age z-score, socio-economic status, pubertal stage, physical activity, and calcium intake exposure as predictors. WAZ-weight for age z-scores; SES-socio-economic status; PA-physical activity

Table 6.6 Associations between baseline characteristics and skeletal maturity deviation (years) at one year follow up after adjustment for baseline skeletal maturity deviation and follow-up period (analyses restricted to males and females living with HIV)

	Males n=113				Females n=105			
Variable	**Minimally adjusted model β coefficient (95%CI)**	**p - value**	**Fully adjusted model β coefficient (95%CI)**	**p-value**	**Minimally adjusted model β coefficient (95%CI)**	**p-value**	**Fully adjusted model β coefficient (95%CI)**	**p-value**
Weight for age z-score								
Weight for age z score>-2	ref	0.004	Ref	0.001	**ref**	0.175	ref	0.144
Weight for age z score<-2	-0.82 (-1.37, -0.27)		-0.92 (-1.47, -0.36)		-0.32 (-0.78, 0.14)		-0.36 (-0.84, 0.12)	
Socio-economic status								
Tertile 3: High	ref	0.346	Ref	0.316	ref	0.898	ref	0.871
Tertile 2: Middle	0.47 (-0.08, 1.01)		0.61 (0.09, 1.13)		0.31 (-0.15, 0.76)		0.26 (-0.22, 0.74)	
Tertile 1: Low	-0.23 (-0.76, 0.29)		-0.28 (-0.80, 0.24)		0.08 (-0.35, 0.52)		0.08 (-0.38, 0.53)	
Pubertal stage								
Tanner 1	ref	0.046	Ref	0.349	ref	0.157	ref	0.495
Tanner 2	-0.22 (-0.77, 0.34)		-0.05 (-0.57, 0.48)		-0.64 (-1.13, -0.15)		-0.60 (-1.15, -0.06)	
Tanner 3	-0.69 (-1.38, -0.004)		-0.26 (-0.97, 0.45)		-0.38 (-0.82, 0.06)		-0.42 (-0.92, -0.08)	
Tanner 4&5	-0.47 (-1.10, 0.15)		-0.25 (-0.85, 0.36)		-0.32 (-0.78, 0.12)		-0.17 (-0.73, 0.38)	
Physical activity								
High, >3000 MET mins/week	ref	0.203	Ref	0.475	ref	0.043	ref	0.043
Moderate, 600-3000 MET mins/week	-0.55 (-1.18, 0.07)		-0.55 (-1.17, 0.08)		-0.31 (-0.80, 0.18)		-0.20 (-0.71, 0.30)	
Low, <600 MET mins/week	-0.35 (-0.85, 0.15)		-0.23 (-0.74, 0.27)		-0.44 (-0.86, -0.02)		-0.45 (-0.89, -0.001	
Vitamin D intake								
Moderate, 6.0-8.0 µg /day	ref	0.901			ref	0.479		
Low, 4.0-5.9 µg /day	0.12 (-0.36, 0.60)				-0.003 (-0.36, 0.35)			
Very low, <4.0 µg /day	-0.42 (-1.45, 0.62)				-0.51 (-1.32, 0.30)			
Calcium intake								

Moderate, 300–450 mg/day	ref	0.446	Ref	0.773	ref	0.655	ref	0.615
Low, 150-299 mg/day	0.59 (-0.02, 1.19)		0.24 (-0.33, 0.81)		0.22 (-0.21, 0.66)		0.13 (-0.33, 0.59)	
Very low, <150 mg/day	0.18 (-0.33, 0.69)		0.06 (-0.50, 0.61)		0.08 (-0.30, 0.47)		0.10 (-0.29, 0.49)	
Age at ART initiation								
<2 years	ref	0.006	Ref	0.574	ref	0.095	ref	0.764
2-3.9 years	-0.30 (-0.92, 0.31)		-0.20 (-0.77, 0.38)		-0.13 (-0.61, 0.34)		-0.12 (-0.56, 0.32)	
4-8 years	-0.34 (-0.94, 0.25)		-0.01 (-0.62, 0.60)		-0.21 (-0.67, 0.26)		-0.08 (-0.63, 0.47)	
>8 years	-1.03 (-1.79, -0.27)		-0.34 (-1.11, 0.44)		-0.44 (-0.96, 0.08)		-0.10 (-0.70, 0.49)	
CD4 count								
>/=500 cells per µL	ref	0.696			ref	0.413		
<500 cells per µL	0.11 (-0.46, 0.68)				-0.18 (-0.61, 0.25)			
Viral load								
<1000 RNA copies per ml	ref	0.372			ref	0.312		
>1000 RNA copies per ml	0.26 (-0.31, 0.82)				-0.21 (-0.61, 0.20)			
TDF exposure								
No exposure	ref	0.047	Ref	0.064	ref	0.03	ref	0.084
<4 years	-0.72 (-1.31, -0.14)		-0.77 (-1.38, -0.16)		-0.14 (-0.54, 0.26)		-0.12 (-0.56, 0.32)	
4 years +	-0.76 (-1.44, -0.09)		-0.46 (-1.15, 0.22)		-0.61 (-1.13, -0.08)		-0.55 (-1.13, 0.03)	

Skeletal maturity deviation (SMD) was calculated as bone age (years) – chronological age (years); more negative values reflect greater delay in skeletal maturity. Minimally adjusted model: baseline SMD and follow-up period as adjustments. Fully adjusted model: baseline SMD, follow-up period, HIV status, weight for age z-score, socio-economic status, pubertal stage, physical activity, calcium intake, age at ART initiation and tenofovir exposure as predictors. WAZ-weight for age z-scores; SES-socio-economic status; PA-physical activity; ART-anti retroviral therapy, TDF-Tenofovir Disoproxil Fumarate

Table 6.7 Height gain (cm) from baseline to follow up over one year in male and females living with and without HIV

Group	Sex	n	Mean change in height, CI (cm)	p-value
HIV-	males	115	4.90 (4.45, 5.34)	<0.001
	females	107	3.84 (3.17, 4.51)	<0.001
HIV+	males	112	6.33 (5.47, 7.18)	<0.001
	females	106	4.74 (4.17, 5.31)	<0.001

CI: confidence interval

Table 6.8 Height gain over one year from baseline to follow up in male and females living with and without HIV by tanner staging

Group	Sex	n	HIV- Mean change in height, CI (cm)	p-value	n	HIV+ Mean change in height, CI (cm)	p-value
Tanner stage 1/2	males	64	4.80 (4.44, 5.17)	<0.001	70	5.49 (4.92, 6.07)	<0.001
Tanner stage 1/2	females	42	6.21 (5.68, 6.74)	<0.001	55	5.75 (5.31, 6.20)	<0.001
Tanner stage 3/4/5	males	50	4.48 (3.66, 5.31)	<0.001	34	6.11 (5.14, 7.07)	<0.001
Tanner stage 3/4/5	females	63	1.51 (0.96, 2.07)	<0.001	45	2.19 (1.54, 2.85)	<0.001

CI: confidence interval

Table 6.9 Mean difference in change in height over one year between children living with and without HIV by Tanner stage

Group	Mean difference in change in height between HIV+ and HIV- groups, cm	p-value
Males in Tanner stage 1/2	-0.69 (-1.38, -0.001)	0.05
Females in Tanner stage 1/2	0.46 (-0.23, 1.14)	0.19
Males in Tanner stage 3/4/5	-1.62 (-2.88, -0.36)	0.01
Females in Tanner stage 3/4/5	-0.68 (-1.53, 0.16)	0.11

6.3.9 Change in height over one year

There is a significant increase in height in both males and females whether living with or without HIV, although the greatest increase was in the males living with HIV [6.33 (5.47, 7.18) cm] (Table 6.7). Both males and females in earlier Tanner stages whether living with or without HIV had a greater increase in height than those in later Tanner stages except in males living with HIV in Tanner stage 3/4/5 [7.18 (5.96, 8.40) cm]. There were statistically significant differences between the change in height amongst the males living with and without HIV whether in Tanner stage 1/2 or 3/4/5 (Table 6.9). No significant differences were seen in the females.

6.4 Discussion

My study, to my knowledge is the first to report longitudinal measurement of skeletal maturation in CLWH in African populations giving valuable insight into the growth and development amongst these children and adolescents. I have shown that delay in skeletal maturation was on average greater at baseline compared to at follow up, whether living with or without HIV although the skeletal maturity delay continued to be greater in CLWH. In addition, females with HIV have consistently delayed BA than their HIV-negative counterparts after accounting for SMD at baseline and follow up period which was observed in the males although the association did not reach the $p<0.05$ significance. My findings suggest that being underweight in males and living with HIV and less physical activity in females were predictors of delayed skeletal maturation. Again, in CLWH being underweight in boys and less physically active in females were associated with greater delay in skeletal maturation.

My study showed that males and females living with HIV remained less mature than those living without HIV after one year follow-up after accounting for potential confounders although in the males the association was partially attenuated. In the only longitudinal study to date to report data on skeletal maturation in CLWH conducted in Brazil that had a longer follow up period of four years, both males and females living with HIV were consistently delayed in skeletal maturation after the four years (253). However, unlike in our study, the Brazilian study did not calculate change in SMD, did not control for potential confounders, and the sample size was small (60 participants) rather, only Student's t-tests were performed at baseline and follow up. The lack of evidence in the delay in skeletal maturation in the males at follow up in my study may indicate a sudden growth spurt (period of rapid increase in height) or acceleration through puberty, or nutrition as the greatest attenuation is seen with WAZ. This is supported by a study in The Gambia where the boys who took calcium grew faster and stopped growing earlier than boys who did not take calcium (276). There was greater increase in height over one year in male

CLWH as shown in Table 6.7 an indication that males living with HIV had a greater growth potential during this period than their female counterparts. Although these results may suggest some degree of 'catch-up' in skeletal maturation in male CLWH as they transition through puberty, it remains to be seen if that is enough for them to reach full potential for adult height.

6.4.1 Factors associated with SMD at follow up

Importantly, I have highlighted the importance of adequate childhood and adolescent nutrition on skeletal maturation as being underweight at baseline in both males and females whether living with or without HIV was associated with SMD at follow up. Similarly, in the only other longitudinal study that has looked at determinants of SMD in South Africa reported that greater attained weight at two years of age predicted more advanced skeletal maturation (220). Therefore, my results are consistent with the wider literature in Southern Africa although the time of assessment of WAZ in the populations are different. The South African study showed the influence of early growth on skeletal maturation something that could possibly be explored in future studies on skeletal maturation in CLWH.

My study highlights an intriguing area for potential research within paediatric health in CLWH as lower physical activity in female CLWH was associated with greater delays in skeletal maturation. In HIV negative children, some studies show conflicting results as Increased physical activity did not have an effect on skeletal maturation in a longitudinal study of 64 Belgian children aged 13-18 years (277). The benefits of physical activity on growth rates whether direct or indirect are well documented (278). However, evidence of interventional studies that show whether increasing physical activity will improve skeletal maturation is scarce. The association of physical activity and delays in skeletal maturation I found in my study raises questions on the type, frequency and intensity of activities that may influence growth and development in CLWH. I also showed that most females in the study only did low to moderate physical activity which are extreme levels where other factors beyond physical activity may be having the direct effect on better growth. Similar results have been reported in a study conducted in Brazil of 91 CLWH aged 10-19 years where 74% of the participants were not involved in any physical activity and a higher proportion of them were girls (80%) (279). In essence, girls experience more noticeably changes in their body during adolescence e.g. increased body fat, breast development which may lead to decreased confidence and self-esteem hence less desire to participate in physical activity. Additionally, in CLWH, some authors have suggested deficits in muscle power and function and a predisposition to sedentary lifestyle due to the nature of their condition (280). There are no data from other studies that

shows this observation in Zimbabwean females may be directly linked to physical activity. Therefore, despite the findings of my study it is important to note that these do not imply causality as this is an observational study. To establish a causal relationship, an interventional trial adequately powered to assess whether a physical activity intervention improves growth in CLWH would be required. Notwithstanding that this observation may be confounded by other factors such as SES which was determined pragmatically using a proxy measure. Therefore, to substantiate these results, a controlled trial of a physical activity intervention is needed, accounting for all factors that could influence the outcomes, to determine if there is a causal link between physical activity and skeletal maturation. However, there is no evidence from other studies that shows this observation in Zimbabwean females may be directly linked to physical activity.

If physical activity is increased in children who are still growing there is evidence that bones growth in width and put more mineral (281, 282). The bones respond to physical activity by changing their shape and distribution which is potentially driven through changes in length and width to cope with the lever arms and stronger muscles. It is plausible that people with lower physical activity do not need to growth their bones as much therefore will not need to adapt because there is no extra loading on the bone. If physical activity is increased the bones will need to adapt to the extra load (283). However, this observation relating to physical activity may be related to other factors that are causing the delay such as being underweight, SES and HIV.

Although there is a weak association between TDF exposure and SMD after full adjustment in male CLWH (the association was statistically significant in the minimally adjusted model), my results in part support the hypothesis that TDF has detrimental effects on growth in children. The weak association was also noted between age at ART initiation and SMD, we observed that there was a trend towards a greater delay in skeletal maturation with a greater delay in taking ART. Hence older age at ART initiation does not only potentially delays growth as shown in this study but also predicted low bone mineral density (a marker for osteoporosis) in perinatally infected children highlighting the importance of starting ART early to improve overall health in CLWH (114). While the government of Zimbabwe has implemented the “test and treat” approach where ART is initiated upon diagnosis as recommended by the World Health Organisation (WHO), there are likely some children who may be missed by such programmes preventing improvements in their health.

6.4.2 Strengths and limitations

The strength of my study is that it is the first longitudinal study of an African population describing skeletal maturation in CLWH using a large sample size. There are no specific studies of CLWH in Africa whether longitudinal or cross sectional. There has only been one other longitudinal study in an African population focused on a younger age group in HIV-negative children in South Africa (89). In addition, this is the second study in Africa to examine factors associated with SMD. The other strength is the inclusion of a comparator group and the longitudinal follow up that allows for identifying change over time. The longitudinal study also meant that I could examine the baseline exposures in relation to outcomes recorded after a follow up period of one year and this reduces the possibility of reverse causation. In a cross-sectional analysis, the exposure and outcome are measured at the same time with a risk of the outcome causing the exposure (reverse causality) and this is less likely in this longitudinal study. The sampling method was robust thereby making the participants a likely representation of children living in Harare. The use of two methods of BA assessment and multiple imputation models preserved the sample size and statistical power though the study was not designed to test the assessment of risk factors so may be underpowered for that part of the work.

A limitation of my study is that the follow up period was only one year so it was impossible to demonstrate whether the males and the females living with and without HIV reached skeletal maturation at the same time or there were sex differences as in the longitudinal study in South Africa where females matured earlier than boys by 1.9 years (89). My study only had skeletal maturation measured at two time points hence difficult to establish the pattern of skeletal maturation in the adolescents. There was a loss to follow up of 26% of the participants who were more likely to take low calcium than those who remained in the study possibly contributing to the lack of association between calcium intake and SMD.

6.5 Conclusion

Results from this study show evidence of female and male CLWH still falling behind or delayed in development in skeletal maturation over one year follow up, albeit attenuated somewhat in males. This suggests that they are not catching up to the females who are HIV negative and failure to attain full potential adult height has negative consequences on the cognitive, social and psychological being of an individual. My findings indicate that physical activity should be promoted in females whether living with or without HIV as it contributes to growth and development. Further research is required to examine the effect of HIV in early life

and follow up the children until the end of growth to determine whether complete fusion of the epiphysis eventually occurs at the same time or later than HIV negative children and assess the outcomes in later life as a result thereof.

Chapter 7 Discussion

7.1 Main findings of my thesis

- TW3 is the method of choice for use in Zimbabwean children and adolescents aged 8-16 years as the method was more precise and not biased by age. There was greater inter and intra- rater variability of the TW3 method when compared to the GP method.
- CLWH were delayed in skeletal maturation compared to children without HIV.
- At baseline living with HIV and being underweight were associated with greater delays in skeletal maturation in both the males and females.
- In CLWH later age at ART initiation was associated with greater delays in skeletal maturation
- After one year there was evidence of females living with HIV being delayed in skeletal maturity than those living without HIV.
- Underweight males had greater delay in skeletal maturation after one year than those who were not underweight

7.2 Introduction

The IMVASK study offered me an opportunity to explore, and report results from the largest study conducted to date to assess skeletal maturity in a sample of children and adolescents living with HIV in Africa. Five major considerations that shaped my PhD were that firstly, skeletal maturation had not been previously studied in Zimbabwean children and adolescents, with limited studies have been performed in African countries. Most studies have been conducted in Western, Asian and European countries where populations are different in terms of SES, lifestyle, diet, disease patterns and genetics. Secondly, no study has compared the two methods of BA assessment in an African population, most studies in Africa have used the GP method only and only one study to my knowledge has used the TW3 method. Therefore, it is unclear which method is more applicable to the Zimbabwean setting that may translate to the African setting. Thirdly, studies of skeletal maturation in CLWH are limited and none have been conducted in African populations where the majority of CLWH reside. The fourth consideration is that only one study conducted in South Africa has reported factors associated with skeletal maturity delays in an African population, and these were early life, rather than

adolescent lifestyle factors. My study becomes the second. Lastly, there are no studies reporting longitudinal data on skeletal maturation in CLWH in African populations. The major gaps existing in literature are that of a description of patterns of skeletal maturation in Zimbabwean children and explanations for the differences in skeletal maturation between children living with and without HIV. In determining factors associated with delays in skeletal maturation I considered *a priori* factors I thought would explain the differences in skeletal maturation between CLWH and HIV negative children.

The overarching aim of my thesis was therefore to describe skeletal maturation in peripubertal children in Zimbabwe and determine the impact of HIV on skeletal maturity. The outcome in my study was SMD (BA-CA). I have reported results from a cohort of children aged 8-16 years from Harare living with and without HIV. To begin with, in chapter four I performed a methodological evaluation study which aimed to compare the two most widely used methods of BA assessment, TW3 and GP to ascertain which method is best suited for Zimbabwean children and adolescents. I assessed all the radiographs by both methodologies and trained another operator to do the assessment. Until now, BA assessment has not been conducted in Zimbabwe therefore it was not clear which method is most suitable for use. Secondly, in chapter five, I described the skeletal maturation of Zimbabwean children and adolescents and factors associated with differences in BA and CA. It is important to understand growth and development in Zimbabwean children considering that the country has a high prevalence of HIV and malnutrition, conditions known to constrain growth. In chapter six I determined skeletal maturation after one year and factors associated with SMD, accounting for SMD at baseline, and the follow up period.

My results in chapter four show that the TW3 is the method of choice for use in Zimbabwean children and adolescents aged 8-16 years. Although both methods showed a good inter and intra- rater reliability, the GP method showed an age-related bias where BA estimates were lower than CA in younger children and higher as the children aged hence the choice of the TW3 method. In chapter five I demonstrated that CLWH had greater deviation between BA and CA compared to children without HIV. This suggests that CLWH were delayed in skeletal maturation when compared to HIV negative children. Future studies of skeletal maturation may require determining the effect of HIV from birth until skeletal maturation is reached in African children. These studies are needed to confirm whether the CLWH are pre-set on a slower rate of growth with delayed puberty, as indicated in my current work, and whether they continue to grow and ultimately catch up in development (indicated by skeletal maturation), attaining full adult height, potentially later than their HIV negative counterparts. My study confirmed the

differences in growth and development between males and females, as females were more skeletally mature than the males at any given CA. Furthermore, I have also demonstrated that living with HIV and being underweight were associated with greater delays in skeletal maturation in both the males and females. After one year of follow-up, females and males living with HIV continued to be delayed in skeletal maturity compared to those living without HIV. In addition, being underweight was associated with SMD, observed again after follow-up, with underweight males having greater delay in skeletal maturation than those who were not underweight. My results also confirm that starting ART at a later age may have consequences on growth and development of CLWH, as an early start of ART had a positive effect on skeletal maturation. I have also shown that less physical activity in the females was associated with delayed skeletal maturation at follow up.

7.3 Implications of my thesis

7.3.1 Assessment of growth and development

Findings in my thesis have wider implications for the assessment of growth and development in Zimbabwean children and adolescents. My results demonstrate that the TW3 method is the most appropriate method when assessing BA in healthy children aged 8-16 years. It was vital to include children with no underlying chronic condition that may influence the results by showing either advanced or delayed skeletal maturation. The exclusion of CLWH in this analysis was therefore an essential part of my study design to make the results more robust given that HIV is a chronic condition known to affect growth. However, in studies or in situations where BA assessment is required for older children above 15 years, the GP method would be the only method available for use, hence it's use in older children in chapter six.

As the children got older and more mature, differences between BA and CA reduced when BA was assessed by the GP method which was not observed with the TW3 method. Although this age-related bias was seen when using the GP method, BA being closer to CA may be viewed as an indication of better performance of the GP method in older children. On the other hand, there are no reference data for skeletal maturation for African children, more so Zimbabwean children. Therefore, it is not clear whether BA estimated by the GP method shows flaws in the method or the results are reflecting the pattern of growth and skeletal maturation of Zimbabwean children where the children may be delayed at younger ages and appear to catch up as they grow older. This highlights the disadvantage of a lack of a gold standard measure of BA assessment particularly in the context of HIV, a condition that constrains growth and the

need to create reference data for skeletal maturation in African populations. Considering two cohorts in Africa have used the TW3 method (current cohort and the Birth to Twenty cohort in South Africa), and that TW3 showed better precision and agreement with CA in chapter four, reference values of BA specific to Southern Africa could be derived given the data are from the same region with similar disease patterns and lifestyle.

My study informs that in younger children aged 8-11 years the two methods cannot be used interchangeably as they do not give equal estimates of BA. However, in children above 11 years there was agreement between the two methods. Therefore, interchangeably use of the methods of BA assessment will depend on the age group of the participants. The evidence of interchangeable use in older children coupled with methodological limitations that came with the use of the TW3 method in older children at follow up who were now aged 9-17 years informed my choice of using the GP method in those age 15 years and older. The GP method has an advantage of having a wider age range for BA assessment of up to 19 years for boys and 18 years for girls.

My study validates the use of TW3 method for BA assessment by clinicians in Zimbabwean children aged 8-16 years and in cases of legal action where the age of a child may be required to be estimated. However, the limitation may be that the children will appear younger than their CA. Although the European Society of Paediatric Radiologists does not recommend use of BA in estimating CA it is a widely accepted practice by clinicians and responsible authorities. In the absence of an alternative method I would recommend the use of TW3 method for estimating CA in children aged 8-16 years in Zimbabwe.

7.3.2 Factors associated with skeletal maturity delay

My work has shown that living with HIV impacts skeletal maturation and therefore the growth and development of adolescents. The lag in skeletal maturation, indicating pubertal delay seen in CLWH may result in failure to attain full adult height potential resulting in short adult stature. The stunting has been negatively associated with cognitive function linked to poor academic performance (284). In addition, there is stigma associated with stunting and HIV in children which arises from a complex interplay of social, cultural, and economic factors. In children the stigma stems from negative treatment from peers leading to low self-esteem (285). Research has shown that society perceives stunting to be a result of parental neglect or inadequate care such that mothers or families are blamed for their child's growth deficiency (286). As a result, some parents may shy away from seeking medical help delaying any interventions that may support the child's growth. HIV related stigma and discrimination are

also major challenges to HIV care as adolescents living with HIV experience enacted stigma in the form of being stereotyped excluded or discriminated against due to their HIV status and all of which contributes significantly to poor medication adherence and retention in care amongst the adolescent potentially negatively impacting growth and development (287). Many adolescents living with HIV may be orphans, and may be managing concerns about food security, livelihood and household issues in addition to their health. More targeted interventions are needed, such as specific HIV education at individual, family, healthcare, and societal levels to mitigate stigma associated with stunting in HIV to improve on care.

Nonetheless, HIV programs are the most funded healthcare programs in Zimbabwe and children are managing to access ART resulting in suppressed viral loads (as evidenced in this study) hence good HIV care is accessible, yet CLWH are more likely to be underweight than HIV negative children as evidenced in this study. Likewise, Zimbabwean adults have good viral suppression observed but have untreated or not well managed chronic conditions like hypertension an indication that HIV care is very siloed in Zimbabwe. Research has indicated that, with the exception of HIV most chronic conditions were either undiagnosed or untreated amongst health care workers in Zimbabwe (288). In addition, there are limited funds available for NCDs care such that ART sites have limited capacity to screen for NCD's in adults living with HIV as well as unavailability of the medication with patients resorting to medication rationing, sharing with others, using home remedies or taking multiple doses to compensate for missed doses (289).

The result of my work supports World Food Program (WFP) effort to ensure the integration of a comprehensive food and nutrition component in HIV treatment support package to ensure better health outcomes for CLWH. The WFP focuses on addressing the unmet needs of food-insecure and malnourished CLWH and mitigating the impacts of HIV at the household level thereby ensuring that CLWH growing up through adolescence who are malnourished have health overall and not just viral suppression. However, WFP assistance to urban communities reduced in 2023 with 33,000 individuals assisted, a significant drop from 326,000 in 2021, in spite of the high number of people requiring food assistance in Zimbabwe (290). This derails the efforts by the WFP to ensure good nutrition hence better health outcomes in CLWH

My results show how malnutrition can potentially have a negative effect is on adolescent growth and development notwithstanding that interventional studies on nutritional supplementation would be required to infer causality. The underweight children were less skeletal mature than children with normal weight. Being underweight is often a direct consequence of malnutrition, particularly undernutrition. Insufficient nutrient intake can lead to

inadequate weight gain. There is evidence that poor macronutrient intake especially protein has negative consequences on growth by affecting the growth plate chondrocytes. Poor intake of proteins results in lower levels of hormones like insulin and IGF-I (21) which stimulate endochondral ossification responsible for linear growth potentially explaining the delayed skeletal maturation in underweight children in my study. Whether supplementing children with multi micronutrient and dietary interventions would help, requires clinical trials to be conducted but there is a need to ensure a balanced and nutrient rich diet where children consume adequate calories, proteins, vitamins and minerals. There is currently a drought in Zimbabwe with an estimated 6 million people expected to be food insecure during the 2024-2025 lean season (291), with climate change, droughts may become common in future and crop failure will likely increase. In addition, Zimbabwe is experiencing hyperinflation that is not likely to end soon and half of the population is living by $1.2 a day, the World bank sets the international poverty line at $2.15 per person per day, below which is defined as extreme poverty (292). This means half of the children in Zimbabwe are at risk of malnutrition. As a country if half of the children are at risk of impaired adolescent development because of malnutrition, evidence has shown that has implications for their development, not just skeletally, but cognitively, socially, and educationally (293). The WFP will have to increase their efforts to curb the effects of malnutrition on growth and development to ensure a healthy population. It is not known what the adult manifestation of delayed skeletal maturation would be hence clinical trials which follow children up to end of growth may be necessary. There is strong evidence of the impact of poor nutrition during childhood and adolescence on health outcomes which include risk of cardiovascular disease, physical and mental health (47, 294, 295) and further research is required to study the impact on fertility, mental health, educational attainment and national productivity.

My study has shown an association between later age at ART initiation and delayed skeletal maturation, supporting the adoption of “Treat ALL” recommendation by WHO, where, all individuals with confirmed HIV diagnosis are eligible for ART irrespective of WHO clinical stage or CD4 count (130). The Zimbabwe guidelines on HIV state that children are a priority for HIV treatment and should be started on ART on the same day of diagnosis if possible (130). In adolescents the age at which to start adult dosing can be difficult to determine hence It is recommended that those under the weight of 25 kg should use paediatric dosing guidelines. These HIV guidelines do not only potentially reduce the negative impact of HIV on growth and development but improve the health of children and adolescents overall.

The VITALITY clinical trial was recently conducted in Zimbabwe and Zambia where CLWH received calcium and vitamin D supplementation (PACTR202009897660297) (296, 297).

Although calcium and vitamin D are important for the growth and mineralisation of bone, it would be important to know how the supplementation impacts skeletal maturation and hence growth trajectories. Calcium and vitamin D are usually given to improve bone health, hence a protein, calorie and multi micronutrient supplementation study may be more ideal to show if the growth and development of CLWH may improve.

The results of my study indicate that physical activity may be important for growth and development, particularly in girls living with HIV. However, further research is required to explore physical activity interventions in CLWH to establish causality as this cannot be inferred solely from observational studies. needs to be conducted to determine the impact of physical activity on growth. Like in my study, physical activity levels amongst children and adolescents in Zimbabwe were reported to be lower than recommended despite the Government of Zimbabwe mandating the instruction and examination of physical education from elementary to high school sessions in its schools with dedicated time slots throughout the week (298). However, evaluation of the proportion of schools where the time slots are adhered to and the activities being implemented is difficult (298). In addition, an article in the Herald (A Zimbabwean newspaper) published in 2015 reported the mushrooming of “private” home schools located in people’s backyards in the high-density areas and most likely with none or limited sports facilities to promote physical activity (299). With the shortage of government schools these “private home schools” are coming in to bridge the gap albeit with some compromises. I have observed that the mushrooming private schools are attended to by those socially deprived as they are cheaper, and one is able to negotiate fees payment. Whether the observations of my study are due to physical activity, or an indicator of SES that was not detected in the adjustments included in my model, can’t be confirmed currently. However, my results may help inform policies and programs to promote physical activity in children and adolescents as the health benefits overall are well documented (300).

The wider implications are that Zimbabwe would benefit in children attaining full adult height potential. There is evidence that being taller is associated with reduced morbidity and mortality, higher income, reduced levels of education, better health and less illness (61). A healthier Zimbabwean population may likely result in a healthier workforce hence more productivity and resources being channelled more efficiently towards other social and economic priorities. Whether children catch up in development and eventually attain their full adult height will need following up until the children have transitioned through puberty and reach skeletal maturation. If the patterns of skeletal maturation after one year continue to the end of growth, I expect the CLWH whether male or female will reach skeletal maturation one

year later than their HIV negative counterparts. In HIV negative children the females may reach skeletal maturation 0.7 years earlier than the males. The delay may possibly result in short adult height in CLWH.

7.4 Strengths

The major strengths of my thesis emanate from the study being the first to describe skeletal maturation in Zimbabwean children, the first to compare two methods of BA assessment in an African population living in Africa, the first to study skeletal maturation in CLWH in an African population and the first to present longitudinal data in CLWH. Having a comparator group of HIV negative children was a strength as it allowed comparison of CLWH and HIV negative children, and the children being followed up and assessed for skeletal maturation twice. The IMVASK study population was sampled from two tertiary hospitals and their catchment areas and that is a strength as the children are a likely representation of children living in Harare.

The other strength is that BA was assessed by a second assessor and twice by me providing a measure of inter and intra rater reliability which ensures consistency and reproducibility of the assessment. The use of Bland and Altman plots was a strength as they are widely recommended to test agreement between two methods.

Another strength of this study is that I have studied adolescents, a particularly challenging population to study, especially in a highly deprived setting. While there was loss to follow up that may have reduced the sample size, I used multiple imputation models on missing variables to preserve the sample size and maximise statistical power. The primary outcome for the IMVASK study was bone mineral density measured by DXA and there was a time when the DXA machine broke down for a considerable amount of time. Managing to retain the adolescents in the study in such a challenging environment is a strength. High rate of loss to follow up amongst adolescents has been previously reported particularly because it is phase of rapid physical and psychosocial development (301). The major reasons for loss to follow up were not formally recorded although these included phone number not reachable, participants having moved out of Harare and death. However, there was no electronic case report form to capture the reasons for loss to follow up, a limitation to the design of the study.

7.5 Limitations

There were several limitations in my study. The baseline analysis was cross sectional therefore I could not infer causality. The complete case analysis I used in the cross-sectional analysis limited the number of participants thereby reducing the statistical power.

Another limitation is that I have conducted this study in only one cohort. Although I have tested which method is best for use and factors associated with any delays in skeletal maturation in children aged 8-16 years, there are early exposures like birth weight, gestational age, that potentially impact skeletal maturation that were not possible to measure because of the age group of my participants which could be potentially explored in future studies.

Another limitation is that there were fewer older males in later Tanner stages in the study to adequately explore associations between exposures and skeletal maturation at these stages in males. From this, I may infer that males were transitioning through puberty later than the females hence the need for follow up studies until the end of growth to show the trajectories of growth by sex though my results suggest that females mature earlier than males. My results agree with wider literature that females are more skeletally mature than boys from birth and reach skeletal maturity on average 2 years earlier than males (77). In SA females reached skeletal maturity 1.9 years earlier than males (89).

7.5.1 Selection bias and generalisability

By design, my study focused on children living in Harare therefore shows the pattern of growth in urban communities. However, 61.4% of the Zimbabwean population live in the rural areas (302). There are factors that influence growth that may differ between the urban and rural areas such as access to health, nutrition, socio-economic status and physical activity such that rural-urban disparities in mean child health outcomes in developing countries have been reported, with stunting rates higher in rural than urban populations (303). Ideally studies should include rural and urban population. However, given that urban poverty and malnutrition have increased, particularly in the last decade, some authors have argued that the rural-urban gap in child malnutrition has declined because of the worsening urban health levels and this may be applicable to Zimbabwe (304). With this in mind, my results may be generalisable to the whole Zimbabwean population.

On the other hand, selection bias could have been introduced by the selection of CLWH from HIV clinics in central hospitals in Harare and HIV negative children from schools in the

same catchment area as the hospitals. Skeletal maturity is a proxy for height, and I hypothesise that stunted children are more likely to go to the central hospitals and taller children to school. Therefore, selection bias may have been introduced because I may have preferentially selected CLWH who happen to be shorter and HIV negative children who happen to be taller. In addition, HIV care is decentralised in Zimbabwe with patients seen at polyclinics and central hospitals; such that patients managed at polyclinics are healthier with better viral loads than those kept at central hospitals. Hence the CLWH recruited into the study may possibly not be representative of CLWH across the country. For future studies I would consider recruitment of CLWH from the polyclinics as well. At the time the study was conducted school enrolment was high at 98% and all HIV negative children were enrolled in schools, with the 2% non-school going children likely to represent CLWH, who are deprived or have other chronic conditions and not characteristic of those that have been recruited. However, things have changed massively in Zimbabwe economically since the study was conducted and now children are asked to pay extra fees that are unofficial because of the economic crisis making it highly likely that formal school enrolment may have dropped. In addition, IMVASK study participants were similar to participants in other African studies in terms of age, WAZ and HAZ (305, 306).

7.5.2 Misclassification of WAZ, HAZ and BMI Z-scores

As discussed in chapter five misclassification may have occurred because of using UK reference data and that might also apply to results in chapter six. Therefore, there is need to develop reference data for anthropometric measurements specific to the Zimbabwean population for use in future studies to avoid misclassification of participants. The UK and WHO growth standards were designed to represent optimum child growth patterns and will likely classify children living in countries such as Zimbabwe as stunted when they are of normal height (18). Zimbabwe is classified as an LMIC (Figure 1.7). LMIC's are characterised with poor dietary diversity and environmental constraints that inhibit growth (307).

7.5.3 Confounding

There were other confounding factors like protein and calorie intake, and deprivation that were not included in the models because data on these factors were not collected such that there could be residual confounding that was uncontrolled for (Figure 6.1). Whilst I had a measure of SES it probably did not capture deprivation adequately. The SES measure had some questions on education, household item list, and asset list which are crude measures and do not capture the nuance of how people live. The SES was measured at one time point and does

not reflect what children's lives have been like for the preceding 8 or 16 years which has been determining their development at the point that I measured the variables. Deprivation influences food insecurity, food diversity and protein and calorie intake and will likely influence HIV status, age at ART initiation, and weight.

I saw an association between weight and SMD and weight is a crude measure of nutrition. It is not clear how much weight consists of bone, muscle or fat. The mechanism by which low weight is associated with SMD is not clear, is it a lot of fat or muscle or both? Therefore, for future research, body composition measures such as lean mass and fat mass should be incorporated into the models. Although height and weight are the most used indicators of nutrition status, anthropometry is a non-specific indicator of multiple past and current processes in older children. Proper interpretation requires the use of additional data related to food and diet, socio - economic status, the prevalence of infections, the result of poor care and the presence of adverse environmental circumstances (308).

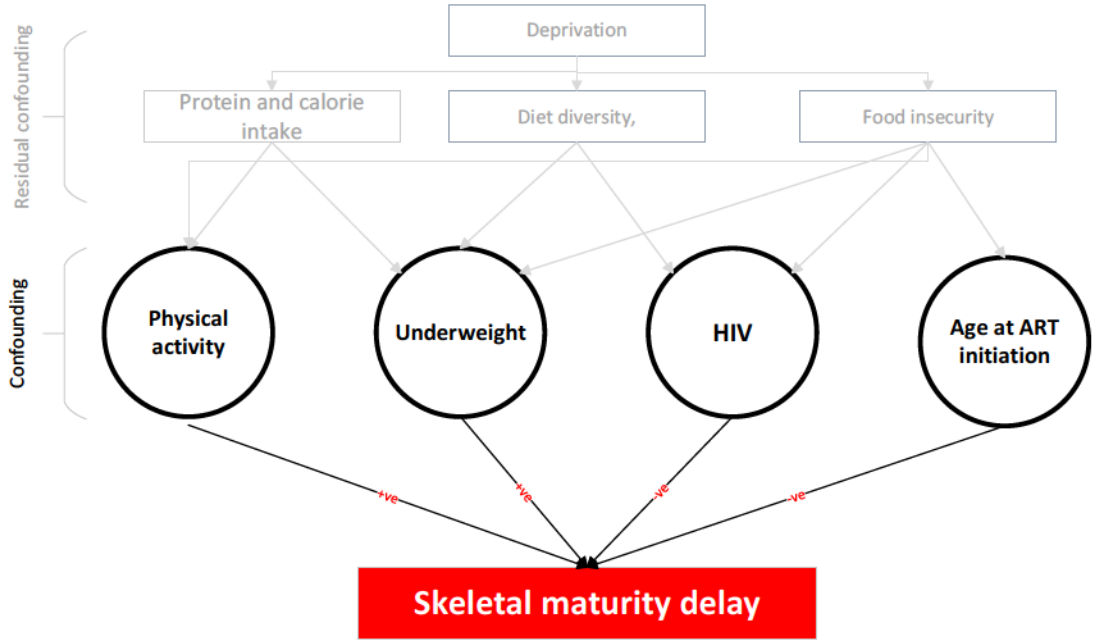


Figure 7.1 Summary of thesis findings denoting potential residual confounding in grey boxes

7.5.4 Observer bias and unblinding of CA

There may have been observer bias when assessing BA at baseline as I was not blinded to CA because it was visible on the radiograph and that I needed the CA to guide the decision on the GP atlas (a disadvantage of the GP method). However, by the time I assessed the follow up

radiographs I was blinded to the CA. If I were to do a BA study again, I would be blinded to the CA throughout.

7.5.5 Chance

Of the eleven variables studied four variables were associate with the outcome and that could have been by chance. However, there is biological plausibility for the association between HIV status, weight, age at ART initiation and physical activity with delays in skeletal maturation.

7.6 Final conclusions

My results suggest that TW3 is the method of choice for children aged 8-16 years in Zimbabwe and for children above those ages the GP method can be used. The findings of my thesis showed that HIV infection, being underweight and being less physically active was associated with delays in skeletal maturity. These results add to the literature to understand growth impairment in CLWH. I have also shown that starting ART later in childhood predisposes CLWH to delays in skeletal maturation. My results suggest that male CLWH have an extended window of opportunity to catch up on growth compared with females; however, the children need to be followed up for longer to see if they eventually catch up. If the growth and development continue at the same rate as observed after one year, I estimate that male CLWH reach skeletal maturity at 19.9 years and HIV negative males at 19.1. the female CLWH will reach skeletal maturation at 18.8 years and HIV negative female at 17.8 years. In addition, the trajectory of skeletal maturation was already lower before the age of 8 years. If CLWH fail to catch up, they will be at risk of not attaining their full adult height potential which has implications in later health. I would recommend studies in the younger age groups and follow up until the end of skeletal maturation. Overall, I expect my results to be generalisable across populations of children in Southern Africa where HIV prevalence is high.

7.7 Future work and recommendations

Future research needed to understand how HIV infection is affecting skeletal maturation in CLWH should include:

1. Longitudinal studies of BA assessment in the first five years of life
2. Longitudinal follow up of CLWH until the end of growth to assess if there is adequate catch up and whether delayed skeletal maturation has poorer outcomes at the end of growth

3. Bone age reference data for children in Zimbabwe that may be applied to similar populations in Southern Africa. The children used to develop the reference data would be healthy children with no condition known to constrain growth with a wider age range. Standardised growth charts may be used to identify children who fall within the normal range of HAZ and WAZ. The population would be recruited from different regions of the country, socio-economic backgrounds and ethnic groups so that data is collected from a diverse sample which is a true representation of the Zimbabwean population.
4. An intervention on nutritional supplementation has been conducted in two Southern African countries PACTR20200989766029 (Zimbabwe and Zambia) (297). Therefore, future work involves determining if nutritional interventions in CLWH may have an impact on skeletal maturation.
5. Delays in skeletal maturation were seen not only in CLWH but also in those who are HIV negative. It is therefore essential to see if nutritional supplementation impacts skeletal maturation in HIV negative children. A nutritional supplementation trial was conducted in The Gambia where mothers received calcium supplementation at 24 weeks to term. Their offspring were followed up until the end of growth and future studies involve assessing skeletal maturation in these children and determine the impact of maternal calcium supplementation of skeletal maturation.

Policy makers and clinicians should consider:

1. Diet and multi micro-nutrient interventions in CLWH
2. Awareness programmes on the importance of normal growth in children encouraging balanced diet and physical activity in children and adolescents

7.8 Research outputs

7.8.1 Awards

- I was awarded a £500 Travel grant by the International Conference on Children's Bone Health (ICCBH) (9 February 2022)
- I applied for and was successful in becoming part of the American Society for Bone and Mineral Research, student cohort program, 2022
- I was awarded the New Investigator Award (£50) at the BRS 2020 online annual meeting

7.8.2 Publications

- **Kowo-Nyakoko F**, Gregson CL, Westbury LD, Madanhire T, Offiah AC, Micklesfield LK, et al. The impact of HIV infection on skeletal maturity in peripubertal children in Zimbabwe: a cross-sectional study. BMC Pediatrics. 2024;24(1):480.
- **Kowo-Nyakoko F**, Gregson CL, Madanhire T, Stranix-Chibanda L, Rukuni R, Offiah AC, et al. Evaluation of two methods of bone age assessment in peripubertal children in Zimbabwe. Bone. 2023:116725.
- Rukuni R, Rehman AM, Mukwasi-Kahari C, Madanhire T, **Kowo F**, McHugh G, et al. Effect of HIV infection on growth and bone density in peripubertal children in the era of antiretroviral therapy: a cross-sectional study in Zimbabwe. The Lancet Child & Adolescent Health. 2021;5(8):569-81.
- Rukuni R, Gregson C, Kahari C, **Kowo F**, McHugh G, Munyati S, et al. The IMpact of Vertical HIV infection on child and Adolescent SKeletal development in Harare, Zimbabwe (IMVASK Study): a protocol for a prospective cohort study. BMJ open. 2020;10(2):e031792.
- Madanhire T, Breasail MÓ, Mukwasi-Kahari C, **Kowo-Nyakoko F**, Ebeling PR, Ferrand RA, et al. Prevalence of HIV-associated osteoporosis and fracture risk in mid-life women: a cross-sectional study in Zimbabwe. Journal of Bone and Mineral Research. 2024:zjae138.
- 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. 2022:10.1097.

7.8.3 Abstracts

Summarised below are the conference abstracts which I presented during my PhD, the full version of each abstract is available in appendix B

- **Bone Research Society Annual Meeting 2022 (oral presentation):** Multiple factors predict skeletal maturity deviation in Zimbabwean children and adolescents living with HIV.
- **International Conference on Children's Bone Health 2022 (poster presentations):**
    1. Multiple factors predict skeletal maturity deviation in Zimbabwean children and adolescents living with HIV.
    2. Evaluation of two methods of bone age assessment in peripubertal children in Zimbabwe

Appendix A Faculty of Medicine Ethics Committee ERGO approval

11 May 2022

Mrs Farirayi Nyakoko and Prof Kate Ward,
MRC Lifecourse Epidemiology, Southampton General Hospital,
Tremona Road, Southampton, SO16 6YD

Dear Farirayi,

Re: **ERGO 62773 - Comparing the skeletal maturity of children and adolescents from two sub–Saharan African countries (Zimbabwe and The Gambia) and assessing the impact of HIV and maternal calcium supplementation on growth**

Thank you for submitting your revisions to the ethics application for the above project. I am pleased to inform you that full approval has now been granted by the Faculty of Medicine Ethics Committee.

Approval is valid from today until 30 September 2024, the date specified in your application.

Please note the following points:

- the above ethics approval number must be quoted in all correspondence relating to your research, including emails;
- if you wish to make any substantive changes to your project you must inform the Faculty of Medicine Ethics Committee as soon as possible.

We wish you success with your project.

Yours sincerely,

Dr Anja Timm, Vice-chair, **Faculty of Medicine Ethics Committee**

Appendix B Conference abstracts

B.1 ICCBH 2022 abstract

Applicability and agreement of two methods of bone age assessment in Zimbabwean Children

[1,2,3] **Farirayi Nyakoko*,** [4,5] Celia L Gregson, [2]Tafadzwa Madanhire, [6] Lynda Stranix-Chibanda [2,7] Ruramayi Rukuni, [8] Amaka C Offiah, [5] Lisa Micklesfield, [1] Cyrus Cooper, [2,7] Rashida A Ferrand, [9] Andrea M Rehman, [1,5] Kate A Ward

Affiliation

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Objectives

This study aimed to determine applicability and agreement between two methods of bone age (BA) assessment, Greulich & Pyle (GP) and Tanner Whitehouse 3 (TW3), in Zimbabwean children and adolescents.

Methods

We conducted a cross-sectional study of children, aged 8-16 years, who tested negative for HIV. Boys and girls were recruited by stratified random sampling from schools in Harare. Non-dominant hand-wrist radiographs were taken, and BA assessed using both GP and TW3. Paired sample student t-tests were used to compare the mean differences between BA (GP, TW3) and chronological age (CA) in boys and girls separately, and in each sex split by 2-year age band; data are presented as mean difference (95% CI). Bland-Altman plots assessed agreement between CA and BA as determined by both methods, and between GP and TW3 BA. All radiographs were graded by a second rater to assess inter-rater reliability and 10% were randomly selected and re graded by first observer to assess intra-rater reliability.

Results

We recruited 252 children (44% females). Boys and girls were of similar CA (12.2±2.4, 11.7±1.9 years) and BA (GP: 11.5 ±2.8, 11.5± 2.1 years; TW3 :11.8±2.5, 11.8±2.1 years). The difference in boys between CA and GP BA was most overt in the younger children with a mean difference of 1.05 years (CI:0.75,1.35). TW3 BA agreed well with CA in the young boys, but by age14-16 a difference of 0.59 years (CI:0.27,0.91) was observed. No differences were seen in the girls using either method. In boys and girls, the agreement between CA and TW3 was good across the age ranges whereas between CA and GP the agreement changed with age. In both boys and girls, agreement between TW3 and GP methods was inconsistent across the age ranges. The intra- and inter-rater coefficient of variation was (GP 2.4%; TW3 1.5%) and (GP 3.7% TW3 1.5%) respectively.

Conclusion

In general boys were less skeletally mature than girls. TW3 method was in closer agreement with CA and showed no systematic bias by age and is therefore more applicable to the Zimbabwean population. The TW3 and GP methods do not agree in younger children and therefore cannot be used interchangeably.

B.2 BRS Annual meeting 2022

Multiple factors predict skeletal maturity deviation in Zimbabwean children and adolescents living with HIV

[1,2,3] **Farirayi Nyakoko*,** [4,5] Celia L Gregson, [2]Tafadzwa Madanhire, [6] Lynda Stranix-Chibanda [2,7] Ruramayi Rukuni, [8] Amaka C Offiah, [5] Lisa Micklesfield, [1] Cyrus Cooper, [2,7] Rashida A Ferrand, [9] Andrea M Rehman, [1,5] Kate A Ward

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Introduction

Skeletal maturity (SM) is measured as bone age (BA) on hand radiographs. Chronological age (CA) exceeding BA may indicate growth impairment and is common in HIV infection. We aimed to describe skeletal maturity deviation (SMD), defined as the offset between CA and BA, and determine its risk factors in Zimbabwean children/adolescents.

Methods

A cross-sectional study of children aged 8-16 years living with and without HIV was conducted. Children with HIV were recruited from HIV clinics at Parirenyatwa Hospital in Harare and children without HIV from schools in the same catchment area. Hand-wrist radiographs of the

non-dominant hand were independently assessed by two observers, using the Tanner Whitehouse3 BA method. Paired sample student t-tests for continuous data and chi squared tests for categorical data compared participants' characteristics. Multivariate linear regression was used to examine associations between risk factors and SMD, stratified by HIV and sex.

Results

CA for boys with HIV (n=145) was 12.6±2.5years and for girls (n=134) was 12.5±2.5years, and for boys without HIV (n=147) was 12.4±2.5years and 12.7±2.5years for girls without HIV (n=144). Mean SMD±SD in children with HIV was 1.4±1.4years in boys and 1.1±1.3years in girls, and in those without HIV was 0.4±1.1years in boys and 0.2±1.2years in girls. BA was less than CA in 72% of participants.

In multivariate analyses, underweight was associated with SMD in children with HIV and boys without HIV, compared to those with normal BMI. Amongst girls with HIV, shorter durations of anti-retroviral therapy (ART) (<2 years) were associated with SMD 1.10 years (95%CI:0.14,2.05), as was unsuppressed viral load (>1000 RNA copies/ml), SMD 0.59 (95%CI:0.01,1.17). Whilst amongst boys, older age at ART initiation was associated with SMD; those starting ART at age 8+ years had mean 1.22 years [95%CI:0.33,2.10) SMD. Furthermore, low socio-economic status was associated with SMD 0.59 (95%CI:0.14,1.04) compared to being in middle socio-economic position. In both boys with HIV and girls without HIV, very low dietary calcium intake (<150 mg/day) was associated with SMD of 0.67(95%CI:0.08,1.25) and 0.53 (95%CI:0.01,1.04) respectively, compared against those with low intake (150-299 mg/day).

Conclusion

In children with HIV, low calcium intake, delayed ART initiation, shorter ART duration, underweight and unsuppressed viral load were all independently associated with SMD, which may be indicative of delayed skeletal maturity. Underweight, low calcium intake and socio-economic deprivation similarly predict SMD in HIV negative children.

Appendix C Published manuscripts

Bone 170 (2023) 116725

Contents lists available at ScienceDirect

Bone

journal homepage: www.elsevier.com/locate/bone

Full Length Article

Evaluation of two methods of bone age assessment in peripubertal children in Zimbabwe

Farirayi Kowo-Nyakoko [a,b,c,*], Celia L. Gregson [d,e], Tafadzwa Madanhire [b], Lynda Stranix-Chibanda [f], Ruramayi Rukuni [b,g], Amaka C. Offiah [h], Lisa K. Micklesfield [e], Cyrus Cooper [a], Rashida A. Ferrand [b,g], Andrea M. Rehman [i], Kate A. Ward [a,e]

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ARTICLE INFO

Keywords:
Age
Radiograph
Skeletal maturity
Africa
Children
Growth

ABSTRACT

Objectives: Bone age (BA) measurement in children is used to evaluate skeletal maturity and helps in the diagnosis of growth disorders in children. The two most used methods are Greulich and Pyle (GP), and Tanner and Whitehouse 3 (TW3), both based upon assessment of a hand-wrist radiograph. To our knowledge no study has compared and validated the two methods in sub-Saharan Africa (SSA), and only a few have determined BA despite it being a region where skeletal maturity is often impaired for example by HIV and malnutrition. This study aimed to compare BA as measured by two methods (GP and TW3) against chronological age (CA) and determine which method is most applicable in peripubertal children in Zimbabwe.

Methods: We conducted a cross-sectional study of boys and girls who tested negative for HIV. Children and adolescents were recruited by stratified random sampling from six schools in Harare, Zimbabwe. Non-dominant hand-wrist radiographs were taken, and BA assessed manually using both GP and TW3. Paired sample Student *t*-tests were used to calculate the mean differences between BA and chronological age (CA) in boys and girls. Bland-Altman plots compared CA to BA as determined by both methods, and agreement between GP and TW3 BA. All radiographs were graded by a second radiographer and 20 % of participants of each sex were randomly selected and re-graded by the first observer. Intraclass correlation coefficient assessed intra- and inter-rater reliability and coefficient of variation assessed precision.

Results: We recruited 252 children (111 [44 %] girls) aged 8.0–16.5 years. The boys and girls were of similar mean ± SD CA (12.2 ± 2.4 and 11.7 ± 1.9 years) and BA whether assessed by GP (11.5 ± 2.8 and 11.5 ± 2.1 years) or TW3 (11.8 ± 2.5 and 11.8 ± 2.1 years). In boys BA was lower than CA by 0.76 years (95 % CI: −0.95, −0.57) when using GP, and by 0.43 years (95 % CI: −0.61, −0.24) when using TW3. Among the girls there was no difference between BA and CA by either GP [−0.19 years (95 % CI: −0.40, 0.03)] or TW3 [0.07 years (95 % CI: −0.16, 0.29)]. In both boys and girls, there were no systematic differences between CA and TW3 BA across age groups whereas agreement improved between CA and GP BA as children got older. Inter-operator precision was 1.5 % for TW3 and 3.7 % for GP ($n = 252$) and intra-operator precision was 1.5 % for TW3 and 2.4 % for GP ($n = 52$).

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https://doi.org/10.1016/j.bone.2023.116725
Received 27 October 2022; Received in revised form 27 January 2023; Accepted 28 February 2023
Available online 3 March 2023

Conclusion: The TW3 BA method had better precision than GP and did not systematically differ from CA, meaning that TW3 is the preferred method of assessment of skeletal maturity in Zimbabwean children and adolescents. TW3 and GP methods do not agree for estimates of BA and therefore cannot be used interchangeably. The systematic differences in GP BA assessments over age means it is not appropriate for use in all age groups or stages of maturity in this population.

1. Introduction

Bone age (BA) is a measure of the development of the skeleton incorporating the size, shape and degree of mineralization of the epiphyses and physeal plates of a bone to define their proximity to full maturity [1]. BA differs from chronological age (CA), which is calculated from the date of birth and does not necessarily reflect an individual's stage of puberty. For example, two eleven-year-olds can differ vastly in their developmental stage but CA is the same. Methods to assess BA should be reliable and practical, being adaptable for use in different populations. The two most widely used methods are the Greulich and Pyle (GP) and the Tanner and Whitehouse 3 (TW3) methods, both based upon assessment of hand/wrist radiographs [2,3]. The GP uses a technique which matches a given radiograph with an atlas of standards representing different sexes and ages and the radiograph is then assigned a skeletal age equal to that of the nearest age. In contrast, TW3 uses an individual bone specific scoring technique where the appearance of each bone is evaluated and compared to a set of bones at different stages of maturation. The GP method is more commonly used, as it is straightforward and quick, though it was founded on the assumption that the skeleton matures in a uniform way, assigning a global score to the hand and wrist [4]. GP was developed in 1959 based upon the left hand radiographs of a reference population of white North American children collected in the 1930's [3]. The TW method, first known as TW1 was also developed in the 1930's using radiographs of white European children, it was later modified to TW2 in 1983 using additional data collected in the 1950s and 1960s in western Europe. The TW1 and TW2 methods originally scored 20 bones (radius, ulna, short bones and carpal bones) of the hand before a further update in 2001, namely TW3, when scoring of the radius, ulna and short bones (RUS) was separated from the carpal bones [2]. The TW method is more complex and requires more time for assessment than GP (7.9 min and 1.4 min respectively) [5,6]. The bones of the hand ossify at different rates and the ability of TW3 to assess ossification centres separately makes it ideal for use hence being described as more objective and reproducible than GP [7].

The applicability of these methods in varying environments, populations with varying genetic ancestry, socio-economic status, and disease patterns is questionable as all these factors have an influence on the process of skeletal maturation. Sub-Saharan Africa (SSA) has a high prevalence of HIV and malnutrition, leading to delayed puberty. Given that these BA methods were developed decades ago in European and American populations, it is not known how applicable they are to African populations and previous studies are limited. None have compared the performance of the two methods, despite the fact that SSA is a region where skeletal development is commonly impaired [8,9]. Therefore, this study aimed to determine whether GP or TW3 is most applicable for use in peripubertal children in Zimbabwe. The agreement between the two methods, inter- and intra-rater reliability and intra- and inter-operator precision for each was determined.

2. Methods

This cross-sectional study used hand/wrist radiographs collected from IMVASK (The IMpact of Vertical HIV infection on child and Adolescent SKeletal development), a prospective cohort study conducted in Harare, Zimbabwe [10]. Children without HIV were recruited by stratified random sampling within three age and sex strata from six government schools within the same catchment area as the main public-sector hospitals in Harare (Parirenyatwa and Sally Mugabe hospitals) from where children with HIV were recruited. For the purposes of this methodological evaluation manuscript, participants were restricted to those testing negative for HIV. Participants were also excluded if they had CA above 15.0 years in girls and 16.5 years in boys because TW3 only assesses BA up to these ages.

Sociodemographic and clinical data were collected using a questionnaire administered by trained research staff. Anthropometric measurements included height (sitting and standing) and weight, taken in duplicate by two independent staff members. If the two measurements differed by >0.5 kg or 0.5 cm, a third reading was taken by an additional reader. The mean of the two or three measurements was recorded as the final figure. Weight was measured to the nearest 0.1 kg using a Seca 875 weight scale, and height to the nearest 0.1 cm using a stadiometer (Seca, Hamburg Germany). The equipment was calibrated annually over the 3 years of IMVASK.

A nurse and/or doctor carried out Tanner pubertal staging using testicular volume (assessed using an orchidometer) penile size and pubic hair growth (quality, distribution, and length) in boys and breast development (size and contour), age of menarche and pubic hair growth in girls for assessment [11]. Grading of penile, testicular and breast growth was from I to V as per tanner descriptions. In the event of disagreement of the pubertal stage testicular and breast development for boys and girls respectively were used to assign tanner stage. Socioeconomic status was determined from indicators in a principal component analysis that included an asset list which showed the head of household age, highest maternal and paternal education levels, monthly household income, number in the household, house ownership, access to amenities and household item ownership list [12]. Socio-economic status was then split into three groups (low, middle, and high). Dietary calcium and vitamin D intake were assessed using a tool developed for the Zimbabwean context based on the validated dietary diversity and food frequency tool from India and Malawi, and international guidelines applicable to SSA [13,14]. The dietary intake was classified into three groups: daily dietary calcium intake as very low (<150 mg/d), low (150–299 mg/d), and moderate (300–450 mg/d) and daily dietary vitamin D intake as very low (<4.0 μg/d), low (4.0–5.9 μg/d), and moderate (6.0–8.0 μg/d). Physical activity was self-reported using the International physical activity questionnaire which has been validated in multiple countries. The amount of physical activity was assessed as multiples of the resting metabolic rate (MET) in MET minutes/week and categorised to low (MET minutes <600/week), moderate (MET minutes = 600 to 3000/week), and vigorous (MET minutes>3000/week) [15,16]. The same thresholds of physical activity were used in this study regardless of age.

Radiographs were taken of the non-dominant hand/wrist by a trained radiographer. A digital radiography (Siemens, Germany) system was used with images stored in DICOM format. The non-dominant hand was positioned with the palm facing downwards in contact with the imaging plate. The axis of the middle finger aligned with the axis of the forearm. The upper arm and forearm were in the same horizontal plane. The central ray of the X-ray beam was directed on the distal end of the third metacarpal and a tube to film distance of 76 cm was used. A lead apron shielded the gonads. Standard exposure parameters for a hand radiograph were used. Radiation dose to the participant was estimated to be less than 2 μSV (micro-Sieverts).

BA was assessed using both GP and TW3 (RUS) methods by a single radiographer. The TW3 (RUS) was based on comparison to a sex-

matched TW3 atlas to determine the level of maturity for 13 selected regions of interest representing specific bones of the hand and wrist [2]. The specific bones included radius, ulna, metacarpals I, III, V; proximal phalanges I, III, V; middle phalanges III, V; distal phalanges I, III, V. The atlas categorises each bone into specific stages of development labelled from A to I. A numerical score is assigned to each stage of development for each individual bone in the atlas. The numerical scores were summed to give a total maturity score ranging from 0 to 1000. Each maturity score matches a certain skeletal age via a lookup table. The GP method of assessment involves comparison of the radiographs to the nearest matching sex-specific reference radiograph provided in the GP atlas [3]. The assessor was aware of the sex but not the CA of the children. Estimated BA was compared to CA, calculated from the date of birth to the date of examination of the radiograph.

To calculate intra-rater reliability and precision (co-efficient of variation), 52 (20 %) radiographs were randomly selected and rescored by the first assessor at least three months later than the initial score. All radiographs ($n = 252$) were re-scored by a second trained radiographer to determine inter-rater reliability and precision.

Ethics approval was granted by the Medical Research Council of Zimbabwe (Harare; reference MRCZ/A/2297) and the University of Southampton, UK (ERGO II 62773). Written informed consent was provided by the parents/guardian, and the children provided written assent.

2.1. Statistical analysis

Statistical analyses were conducted using Stata 16 (StatCorp, Texas, USA). Weight for age Z-scores (WAZ), height for age Z-scores (HAZ) and body mass index (BMI) Z-scores were calculated using the 1990 UK reference data for children [17] with a Z-score of −2.0 denoting stunting (HAZ) and underweight (WAZ).

The primary outcome was the difference between BA measured using two methods, and CA in years. Quantitative data were examined for normality using the Shapiro Wilkes test and histograms. Quantitative data are presented as mean ± standard deviation (SD) and categorical data as proportions and frequencies. Chi-squared tests were used to compare categorical variables by sex. Paired-student *t*-tests were used to compare quantitative variables in (1) the whole study population, (2) girls and boys separately, and (3) stratified by sex and age (in bands of 8–10, 11–13 and 14–16 years); this allowed determination of whether the performance of the BA method differed by maturity level. The mean difference was determined by subtracting CA from BA (BA-CA), presented with 95 % confidence intervals. A negative value indicates BA less than CA, and a positive value indicates BA greater than CA indicating delayed and advanced maturity respectively. Scatter plots for BA by both TW3 and GP method vs CA were examined with the line of best fit plotted.

Bland-Altman plots [18] were generated to assess agreement between CA and BA for each method, and between GP BA and the TW3 BA. Linear regression analysis was performed to determine the direction of the relationship between the average and differences in the methods, β coefficient and 95 % confidence intervals are presented. Inter- and intra-rater reliability were assessed using the intraclass correlation coefficient. The intra- and inter-operator coefficient of variation assessed BA reproducibility, i.e., precision. The formula used to calculate CV was (sample SD / mean) × 100 [19,20].

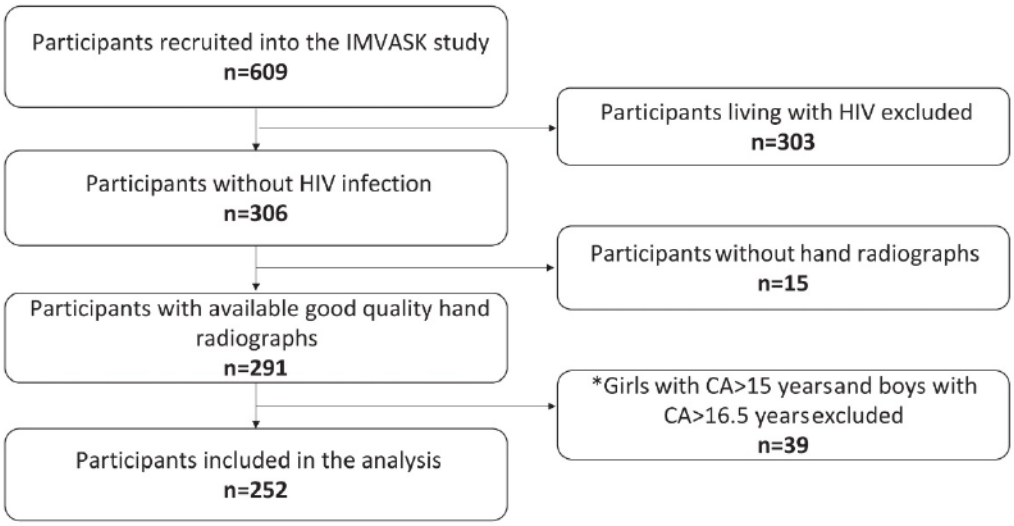
3. Results

3.1. Participant characteristics

Of the 306 children without HIV enrolled in IMVASK, 54 were excluded; 15 had missing hand radiographs, 33 girls were above 15 years of age, and 6 boys were above 16.5 years of age (Fig. 1).

In total 252 (82 %) were eligible for these analyses, with 141 (56 %) being boys. There was no difference in chronological age between the boys and girls, however mean WAZ and BMI-z scores was lower in boys than girls (Table 1).

Although there were no sex differences in BA measured using both methods more boys than girls had lower BA than CA for GP BA (77 % vs 57 %, Chi^2 $p = 0.001$), and TW3 BA (65 % vs 43 %, Chi^2 $p < 0.001$).



Footnotes: *IMVASK: TheIMpact of Vertical HIV infection on child and Adolescent SKeletal development in Harare, Zimbabwe, CA: chronological age. *Threshold for TW3 analysis is at 15 years for girls and 16.5 years for boys*

Fig. 1. Flow chart showing participants included in the analysis.

Table 1
Demographic, lifestyle, anthropometry, and pubertal status characteristics of study participants by sex.

	Boys (n = 141)	Girls (n = 111)	p-Value
Socio-demographics			
Chronological age, mean (SD)	12.2 (2.4)	11.7 (1.9)	0.065
Socio economic status, n (%)			0.361
Low: group 1	34 (24.1)	33 (29.7)	
Middle: group 2	52 (36.9)	32 (28.8)	
High: group 3	55 (39.0)	46 (41.4)	
Anthropometry			
Height-for-age Z-score, mean (SD)	−0.60 (1.03)	−0.47 (1.16)	0.327
Standing height-for-age Z-score < −2, n (%)	9 (6.4)	8 (7.2)	0.796
Sitting height for age Z score, mean (SD)	−1.31 (1.06)	−1.32 (1.18)	0.920
Sitting height-for-age Z-score < −2, n (%)	31 (22.0)	30 (27.0)	0.354
Weight-for-age Z-score, mean (SD)	−0.69 (1.07)	−0.27 (1.22)	0.004
Weight-for-age Z-score < −2, n (%)	15 (10.6)	7 (6.3)	0.226
BMI Z score, mean (SD)	−0.51 (1.05)	−0.07 (1.20)	0.002
BMI Z score < −2, n (%)	11 (7.8)	4 (3.6)	0.162
Bone age measures			
GP bone age, mean (SD)	11.5 (2.8)	11.5 (2.4)	0.859
TW3 bone age, mean (SD)	11.8 (2.5)	11.8 (2.1)	0.945
GP bone age < chronological age, n (%)	108 (76.6)	63 (56.8)	0.001
TW3 bone age < chronological age, n (%)	92 (65.2)	48 (43.2)	<0.001
Pubertal status			0.078
Tanner 1	45 (32.1)	23 (20.9)	
Tanner 2	34 (24.3)	30 (27.3)	
Tanner 3	22 (15.7)	28 (25.5)	
Tanner 4	35 (25.0)	22 (20.0)	
Tanner 5	4 (2.9)	7 (6.4)	
Lifestyle factors			
Daily vitamin D intake, n (%)			0.991
Very low, <4.0 μg/day	17 (12.1)	14 (12.6)	
Low, 4.0–5.9 μg/day	92 (65.2)	72 (64.9)	
Moderate, 6.0–8.0 μg/day	32 (22.7)	25 (22.5)	
Daily calcium intake, n (%)			0.914
Very low, <150 mg/day	62 (44.0)	51 (45.9)	
Low, 150–299 mg/day	31 (22.0)	25 (22.5)	
Moderate, 300–450 mg/day	48 (34.0)	35 (31.5)	
Physical activity level, n (%)			0.304
Low, <600 MET min/week	47 (33.3)	45 (40.5)	
Moderate, 600–3000 MET min/week	46 (32.6)	27 (24.3)	
High, >3000 MET min/week	48 (34.0)	39 (35.1)	

Foot notes: Student t-tests conducted on continuous variables and chi-squared tests on categorical variable. GP-Greulich and Pyle, TW3-Tanner Whitehouse 3, SD-Standard deviation.

Socio-economic status, physical activity, vitamin D and calcium intake did not differ between the boys and girls. Nevertheless, 24 % of boys and 30 % of girls were in the low socio-economic status.

3.2. Comparison of CA and BA by TW3 and GP

There was a positive linear relationship between CA and BA for each method (GP: $r = 0.93$ boys, $r = 0.92$ girls; TW3: $r = 0.91$ boys, $r = 0.88$ girls) (Fig. 2). The slopes for the two methods in both boys [TW3 β 0.95 (95 % CI: 0.87), 1.03; GP β 1.09 (95 % CI: 1.01, 1.17)] and girls [TW3 β 0.92 (95 % CI: 0.81, 1.03); GP β 1.09 (95 % CI: 0.98, 1.20)] differed from each other. In the boys the TW3 slope ($p = 0.179$) does not differ from the line of identity unlike the GP slope ($p = 0.030$). In the girls both slopes [TW3 ($p = 0.180$) and GP ($p = 0.110$)] do not differ from the line of identity.

In the whole sample of boys GP BA was lower than CA by 0.76 years (95 % CI: −0.95, −0.57) while TW3 BA was lower than CA by 0.43 years (95 % CI: −0.61, −0.24) (Fig. 3). The mean difference between CA and GP BA was a year in those aged 8–10 years, with an offset of half a year earlier in the older group. In contrast, the mean difference between TW3 BA and CA, was less in the younger age group compared to the older age groups (Fig. 4a). Among the girls, GP BA had a trend to be lower than CA in the 8–10- and 11–13-year age groups, whereas no differences were seen between TW3 BA and CA (Fig. 4b).

There was a positive bias in agreement between CA and BA as measured by GP in boys ($\beta = 0.18$, $p < 0.001$) and girls ($\beta = -0.08$, $p = 0.050$), with GP BA being much lower than CA at younger ages and less of a difference at older ages (Fig. 3a, b). For TW3 BA and CA, no systematic differences were observed for either sex. The slope for GP Bland-Altman plots was 0.18 in boys and 0.16 in girls and for TW3 plots was 0.05 in boys and −0.08 in girls.

3.3. Comparison of GP and TW3

Overall, GP BA was lower than TW3 BA by 0.33 years (95 % CI: −0.88, −0.23) in the boys and 0.25 years (95 % CI: −0.44, −0.07) in the girls. In both boys and girls, Bland-Altman plots show differential agreement between the TW3 and GP across all ages (boys: $\beta = -1.47$ $p < 0.001$ and girls: $\beta = -1.17$ $p < 0.001$) (Fig. 5). Differences between the two methods in younger ages (8–10 years) show that TW3 BA was higher relative to the GP BA. At the older ages GP BA was higher than TW3 BA.

3.4. Intra- and inter-rater reliability

The intraclass correlation coefficient showed high inter-rater reliability of 0.98 for TW3 and 0.94 for GP ($n = 252$) and high intra-rater reliability of 0.97 for TW3 and 0.98 for GP ($n = 52$). There was good precision for both methods although the TW3 mean percentage coefficient of variation were better than for GP at (intra-operator 1.5 % vs2.4 %) and (inter-operator 1.5 % vs 3.7 %) respectively.

4. Discussion

To our knowledge, this is the first comparison of bone age assessment methods in a population of peripubertal children in sub-Saharan Africa. These data show that TW3 is a more applicable method, than GP, for assessment of skeletal maturity in children and adolescents from Zimbabwe. TW3 has better intra- and inter-operator precision, and no age-related bias. In contrast the age-related bias using the GP BA method, with greater differences at younger ages, indicates limitations for use in our study population. TW3 and GP methods did not agree in younger children and cannot be used interchangeably.

On evaluating the two bone age assessment methods, Bland-Altman analyses show that the two assessments differ especially in the young children, preventing interchangeable use. Similar results were found in a study in the UK where GP and TW2 were compared [21], and TW2 was recommended as the preferred method as it had better precision than GP. Otherwise, few studies have compared both methods [22–25]. Many more studies have looked at the applicability of the GP than TW3 method, potentially because TW3 is used less frequently in clinical practice due to time constraints [5,6]. Despite the TW3 taking longer to assess (7.9 min vs 1.4 min) [6], the consistency across age groups and sexes, and good intra- and inter-rater reliability, suggest that the extra time is justified. The bones of the hand ossify at different rates and the ability of the TW3 method to assess ossification centres separately makes it an ideal method for use.

The current study has demonstrated that both methods are reproducible with the TW3 being more reproducible than the GP method which is consistent with other studies [21,25–28], potentially explained by individual maturity indicator scoring, rather than the GP global grading approach. GP BA units are whole numbers – less precise than the TW3 approach with decimals. Cavallo et al. reported the evaluation of each bone segment in TW3 as an advantage minimising inter-rater variability [29]. Our study also found high inter- and intra-rater agreement supporting the reliability of these two methods in assessing

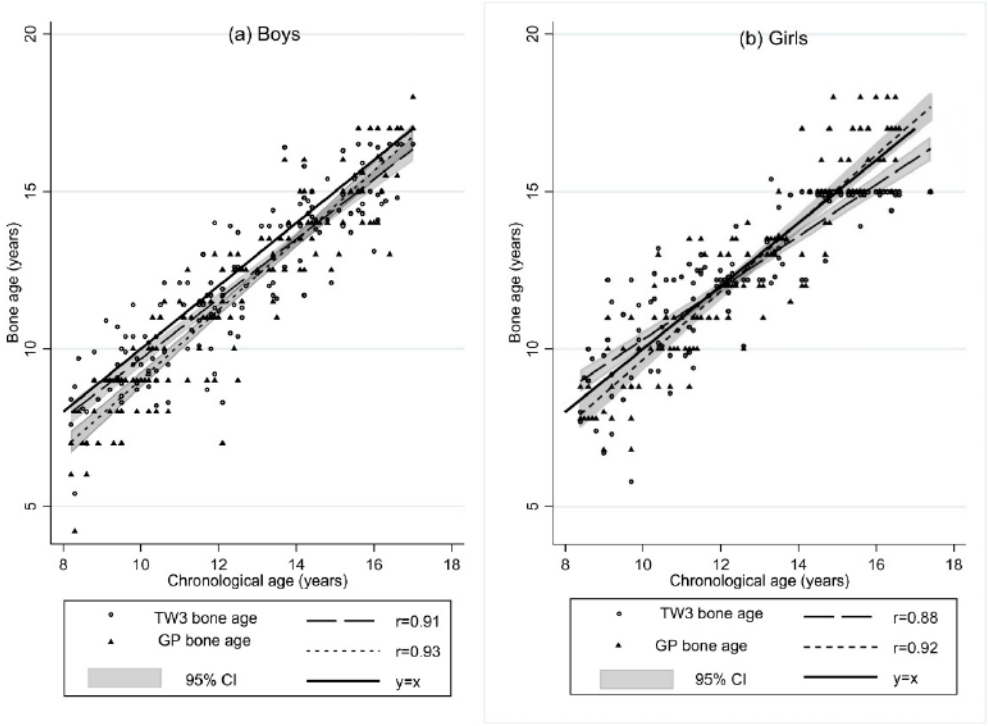


Fig. 2. Scatter plot showing the relationship between Chronological age and bone age by GP (Greulich and Pyle) and TW3 (Tanner Whitehouse 3) for (a) boys and (b) girls.

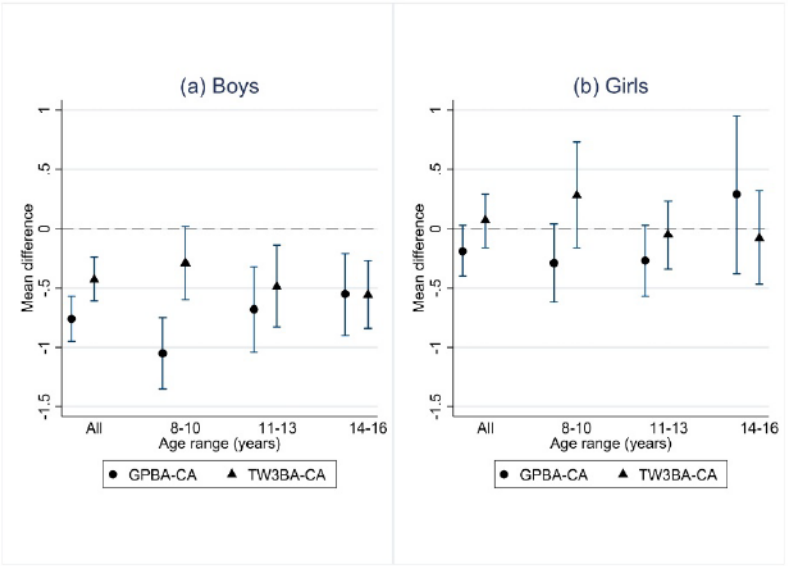


Fig. 3. Forest plot showing the mean differences (95 % Confidence Intervals) between CA and BA assessed by GP, and TW3. Foot notes: Student paired t-tests conducted on all continuous variables, CA-chronological age, GPBA-Greulich and Pyle bone age, TW3BA-Tanner Whitehouse 3 bone age. A negative value means delayed relative to the CA and a positive value means advanced relative to the CA. Sample size for age groups is Boys: 8–10 (n = 49); 11–13 (n = 49); 14–16 (n = 42) and girls: 8–10 (n = 44); 11–13 (n = 48); 14–16 (n = 20).

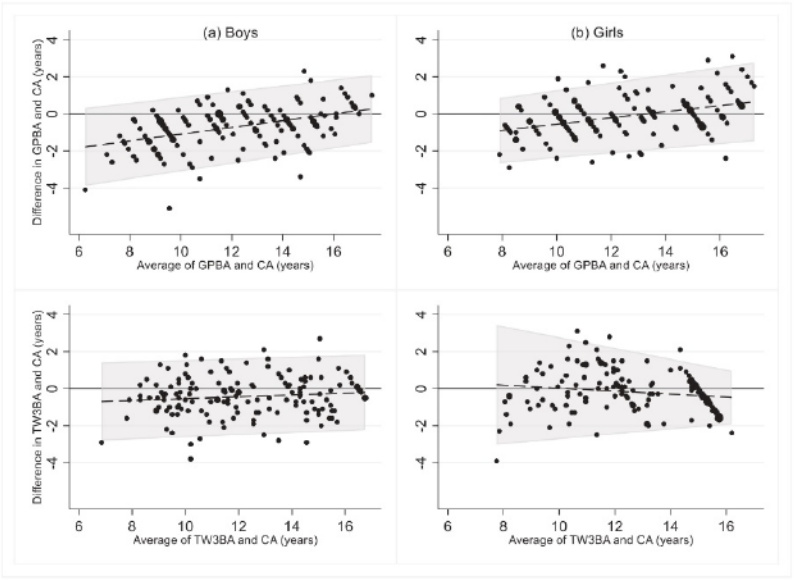


Fig. 4. Bland Altman plots showing mean differences between GPBA and CA (top) TW3BA and CA (bottom) and for (a) boys and (b) girls. Variance of the difference in measures was: top left 1.32; bottom left 1.24; top right 1.31 and bottom right 1.35.

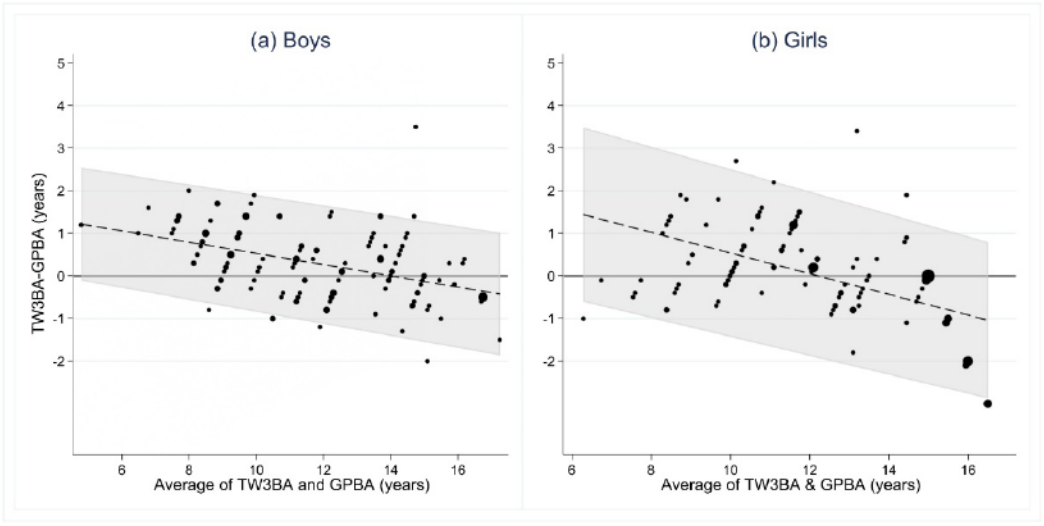


Fig. 5. Bland Altman plot showing mean differences between GP and TW3 bone age in years for (a) boys and (b) girls.

bone age, though notwithstanding the limitations attached to the use of GP in our population given the age dependence of the results.

One limitation of the TW3 method is that it has a cut off point for skeletal maturity of 15 years for girls and 16.5 for boys. In populations that may have later onset, or delayed, puberty, such as in our current study, this limits use, given skeletal maturity is not reached until at least 18 years for girls and 19 years for boys [3].

In the largest study of African children to date ($n = 607$), TW3 was used to assess and compare BA between black and white girls and boys in South Africa. Skeletal maturity was delayed by on average 7 months in black boys compared to white boys, whereas no ethnic differences in the progression of skeletal maturation were seen in girls [30]. Similarly, our data shows sex differences in the performance of TW3, suggesting on average 6 months delay in BA in the boys compared to CA and no differences in the girls.

Consistent with our findings, previous studies have commonly demonstrated lower GP BA than CA, in South Africa, Saudi Arabia, Turkey and Malawi [22,24,31,32]. Our findings are similar to those

from a study in Pakistan which advised against using GP method in their population due to variations in BA depending on the age of the children (advanced BA during early childhood and delayed BA during middle and late childhood) [33]. Our study extends this work by testing the agreement between GPBA and CA and showing the GP method was dependent upon age, such that one would not be able to accurately determine whether a child is delayed in maturity using GP. Several other studies have found the applicability of GP differing by sex, ethnic group or by age [22,31,34].

The recruitment of the children from schools in Harare was designed to be representative of the general population in Zimbabwe, as about 98 % of the children in the whole country go to school. All schools in Harare were identified and the 6 studied were randomly chosen from this sampling frame [10]. Bone age assessment was repeated by a second assessor further strengthening the method employed. It is well-described that timing of skeletal maturation is affected by environment, nutrition, medication, and socioeconomic status [4,35,36]. Our population therefore contrasts with the ones in which the GP and TW3 methods were originally developed. The children used to create the GP atlas were from wealthy families and not suffering from any illnesses [3]. Generally, HAZ and WAZ were low in our study population, indicating a tendency towards stunting and underweight respectively. Most participants had low or middle socio-economic status. Calcium and vitamin D intakes were low, both micronutrients being critical for skeletal development [37]. Together these factors may explain why children in Zimbabwe were skeletally immature for their chronological age.

5. Conclusion

Of the two methods assessed, the TW3 is the most precise and appropriate for use in Zimbabwe as GP appears biased by age. The two methods (TW3 and GP) do not give equal estimates of bone age and therefore should not be used interchangeably. Hence the TW3 method would be the preferred method for use in determining skeletal maturity in peripubertal children in the region. This study is of relevance for low-income African populations in SSA who are faced with a high burden of communicable and non-communicable diseases and delays in skeletal maturation.

Funding

PhD funding support from the University of Southampton Global Challenges Research Fund and Faculty of Medicine, and part-funding from UK Medical Research Council Program U105960371. RAF is funded by the Wellcome Trust (206316_Z_17_Z). The IMVASK study was funded by the Wellcome Trust through a fellowship to RR (206764/Z/17/Z). For the purpose of open access, the author has applied a CC BY public copyright licence (where permitted by UKRI, 'Open Government Licence' or 'CC BY-ND public copyright licence'

CRediT authorship contribution statement

FK-N KAW CLG: conceptualisation, methodology, interpreting data, writing original draft, FK-N Investigation ACO LS-C RR RAF LKM AMR Writing-review and editing, CC KAW CLG AMR LS-C supervision, validation, AMR FK-N KAW CLG TM: formal analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

Professor Ann Prentice for her support in the development of this work.

References

[1] J.M. Tanner, Growth at Adolescence, 1962.
[2] J.M. Tanner, R. Whitehouse, N. Cameron, W. Marshall, M. Healy, H. Goldstein, Assessment of skeletal maturity and prediction of adult height (TW2 method): Saunders London, 2001.
[3] W.W. Greulich, S.I. Pyle, Radiographic Atlas of Skeletal Development of the Hand and Wrist, Stanford University Press, 1959.
[4] K. Alshamrani, F. Messina, A.C. Offiah, Is the greulich and pyle atlas applicable to all ethnicities? A systematic review and meta-analysis, Eur. Radiol. 29 (6) (2019) 2910–2923.
[5] D. King, D. Steventon, M. O'sullivan, A. Cook, V. Hornsby, I. Jefferson, et al., Reproducibility of bone ages when performed by radiology registrars: an audit of Tanner and Whitehouse II versus Greulich and Pyle methods, Br. J. Radiol. 67 (801) (1994) 848–851.
[6] A.M. Mughal, N. Hassan, A. Ahmed, Bone age assessment methods: a critical review, Pakistan J. Med. Sci. 30 (1) (2014) 211.
[7] K. Khan, A.S. Elayappen, Bone growth estimation using radiology (Greulich–Pyle and Tanner–Whitehouse methods), in: Handbook of Growth and Growth Monitoring in Health and Disease, Springer, 2012, pp. 2937–2953.
[8] S.M. Arpadi, Growth failure in children with HIV infection, J. Acquir. Immune Defic. Syndr. 2000 (25) (1999) S37–S42.
[9] M. De Onis, M. Blössner, E. Borghi, Prevalence and trends of stunting among pre-school children, 1990–2020, Public Health Nutr. 15 (1) (2012) 142–148.
[10] R. Rukuni, C. Gregson, C. Kahari, F. Kowo, G. McHugh, S. Munyati, et al., The IMpact of vertical HIV infection on child and adolescent SKeletal development in Harare, Zimbabwe (IMVASK Study): a protocol for a prospective cohort study, BMJ Open 10 (2) (2020).
[11] P.M. Duke, I.F. Litt, R.T. Gross, Adolescents' self-assessment of sexual maturation, Pediatrics 66 (6) (1980) 918–920.
[12] S. Vyas, L. Kumaranayake, Constructing socio-economic status indices: how to use principal components analysis, Health Policy Plan. 21 (6) (2006) 459–468.
[13] S. Filteau, A.M. Rehman, A. Yousafzai, R. Clugh, M. Kaur, H. Sachdev, et al., Associations of vitamin D status, bone health and anthropometry, with gross motor development and performance of school-aged Indian children who were born at term with low birth weight, BMJ Open 6 (1) (2016), e009268.
[14] Infant WGo, Indicators YCF, Developing and Validating Simple Indicators of Dietary Quality and Energy Intake of Infants and Young Children in Developing Countries: Summary of Findings From Analysis of 10 Data Sets, FANTA, Project Washington, DC, USA, 2006.
[15] M. Hagströmer, P. Oja, M. Sjöström, The international physical activity questionnaire (IPAQ): a study of concurrent and construct validity, Public Health Nutr. 9 (6) (2006) 755–762.
[16] C.L. Craig, A.L. Marshall, M. Sjöström, A.E. Bauman, M.L. Booth, B.E. Ainsworth, et al., International physical activity questionnaire: 12-country reliability and validity, Med. Sci. Sports Exerc. 35 (8) (2003) 1381–1395.
[17] T.J. Cole, J.V. Freeman, M.A. Preece, British 1990 growth reference centiles for weight, height, body mass index and head circumference fitted by maximum penalized likelihood, Stat. Med. 17 (4) (1998) 407–429.
[18] D. Giavarina, Understanding bland altman analysis, Biochem. Med. 25 (2) (2015) 141–151.
[19] O. Shechtman, The coefficient of variation as a measure of sincerity of effort of grip strength, part II: sensitivity and specificity, J. Hand Ther. 14 (3) (2001) 188–194.
[20] C.-C. Glüer, G. Blake, Y. Lu, B. Blunt, M. Jergas, H. Genant, Accurate assessment of precision errors: how to measure the reproducibility of bone densitometry techniques, Osteoporos. Int. 5 (4) (1995) 262–270.
[21] R. Bull, P. Edwards, P. Kemp, S. Fry, I. Hughes, Bone age assessment: a large scale comparison of the Greulich and Pyle, and Tanner and Whitehouse (TW2) methods, Arch. Dis. Child. 81 (2) (1999) 172–173.
[22] K. Alshamrani, A. Hewitt, A. Offiah, Applicability of two bone age assessment methods to children from Saudi Arabia, Clin. Radiol. 75 (2) (2020) 156, e1–e9.
[23] K. Alshamrani, A.C. Offiah, Applicability of two commonly used bone age assessment methods to twenty-first century UK children, Eur. Radiol. 30 (1) (2020) 504–513.
[24] B. Büken, Ö.U. Erzengin, E. Büken, A.A. Şafak, B. Yazıcı, Z. Erkol, Comparison of the three age estimation methods: which is more reliable for Turkish children? Forensic Sci. Int. 183 (1–3) (2009) 103, e1–e7.
[25] V. Pinchi, F. De Luca, F. Ricciardi, M. Focardi, V. Piredda, E. Mazzeo, et al., Skeletal age estimation for forensic purposes: a comparison of GP, TW2 and TW3 methods on an Italian sample, Forensic Sci. Int. 238 (2014) 83–90.
[26] S. Mora, M.I. Boechat, E. Pietka, H. Huang, V. Gilsanz, Skeletal age determinations in children of European and African descent: applicability of the Greulich and Pyle standards, Pediatr. Res. 50 (5) (2001) 624–628.
[27] R.R. van Rijn, M.H. Lequin, S.G. Robben, W.C. Hop, C. van Kuijk, Is the Greulich and Pyle atlas still valid for dutch Caucasian children today? Pediatr. Radiol. 31 (10) (2001) 748–752.
[28] A. Maggio, A. Flavel, R. Hart, D. Franklin, Assessment of the accuracy of the greulich and pyle hand wrist atlas for age estimation in a contemporary Australian population, Aust. J. Forens. Sci. 50 (4) (2018) 385–395.

Appendix C

[29] F. Cavallo, A. Mohn, F. Chiarelli, C. Giannini, Evaluation of bone age in children: a mini-review, Front. Pediatr. 9 (2021) 21.
[30] T.J. Cole, E.K. Rousham, N.L. Hawley, N. Cameron, S.A. Norris, J.M. Pettifor, Ethnic and sex differences in skeletal maturation among the birth to twenty cohort in South Africa, Arch. Dis. Child. 100 (2) (2015) 138–143.
[31] K.A. Dembetembe, A.G. Morris, Is greulich pyle age estimation applicable for determining maturation in male Africans? S. Afr. J. Sci. 108 (9) (2012) 1–6.
[32] C. Lewis, C. Lavy, W. Harrison, Delay in skeletal maturity in Malawian children, J. Bone Jt. Surg. Br. Vol. 84 (5) (2002) 732–734.
[33] A.M. Zafar, N. Nadeem, Y. Husen, M.N. Ahmad, An appraisal of Greulich Pyle Atlas for skeletal age assessment in Pakistan, JPMA J. Pak. Med. Assoc. 60 (7) (2010) 552.
[34] R.T. Loder, D.T. Estle, K. Morrison, D. Eggleston, D.N. Fish, M.L. Greenfield, et al., Applicability of the greulich and pyle skeletal age standards to black and white children of today, Am. J. Dis. Child. 147 (12) (1993) 1329–1333.
[35] A. Schmeling, W. Reisinger, D. Loreck, K. Vendura, W. Markus, G. Geserick, Effects of ethnicity on skeletal maturation: consequences for forensic age estimations, Int. J. Legal Med. 113 (5) (2000) 253–258.
[36] H.A. Delemarre-van de Waal, Environmental factors influencing growth and pubertal development, Environ. Health Perspect. 101 (suppl 2) (1993) 39–44.
[37] W. Koo, N. Walyat, Vitamin D and skeletal growth and development, Curr. Osteoporos. Rep. 11 (3) (2013) 188–193.

Kowo-Nyakoko *et al. BMC Pediatrics* (2024) 24:480
https://doi.org/10.1186/s12887-024-04965-y

BMC Pediatrics

RESEARCH Open Access

The impact of HIV infection on skeletal maturity in peripubertal children in Zimbabwe: a cross-sectional study

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Abstract

Introduction HIV infection and its treatment compromises skeletal development (growth and maturation). Skeletal maturity is assessed as bone age (BA) on hand and wrist radiographs. BA younger than chronological age (CA) indicates delayed development. We conducted a cross-sectional study to determine differences between BA and CA (i.e., skeletal maturity deviation [SMD]), and risk factors associated with SMD in peripubertal children with and without HIV established on antiretroviral therapy (ART) including use of tenofovir disoproxil fumarate (TDF).

Methods Children with HIV taking ART for at least two years and a comparison group of HIV-negative children, aged 8–16 years and frequency-matched by age and sex, were recruited from HIV clinics and local schools in the same catchment area, in Harare, Zimbabwe. BA was assessed from non-dominant hand-wrist radiographs using the Tanner Whitehouse 3 method. Negative SMD values correspond to delayed development, i.e., BA younger than CA. Multivariable linear regression models determined factors associated with SMD overall, and in children with HIV.

Results In total, 534 participants (54% males) were included; by design CA was similar in males and females, whether living with or without HIV. Mean (SD) SMD was more negative in CWH than in HIV-negative children in both males [-1.4(1.4) vs. -0.4(1.1) years] and females [-1.1(1.3) vs. -0.0(1.2) years]. HIV infection and weight-for-age Z-score<-2 were associated with more negative SMD in both males and females after adjusting for socio-economic status, orphanhood, pubertal stage, and calcium intake. Age at ART initiation was associated with SMD in both males and females with those starting ART later more delayed: starting ART aged 4–8 years 1.14 (-1.84, -0.43), or over 8 years 1.47 (-2.30, -0.65) (p-value for trend < 0.001). Similar non-significant trends were seen in males. TDF exposure TDF exposure whether < 4years or ≥ 4 years was not associated with delayed development.

Conclusion Perinatally-acquired HIV infection and being underweight were independently associated with delayed skeletal maturation in both males and females. Starting ART later was independently associated with skeletal maturation delay in CWH. Given the known effects of delayed development on later health, it is important to find interventions to ensure healthy weight gain through early years and in CWH to initiate ART as early as possible.

Keywords Adolescence, Africa, Bone age, Children, HIV, Puberty

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Introduction

In 2022, 39 million people were living with HIV worldwide; two-thirds in sub-Saharan Africa [1]. The roll-out of antiretroviral therapy (ART) has resulted in a remarkable increase in life-expectancy, but coverage of treatment substantially lags in children: in 2022, 76% of adults versus 57% of children (aged up to 14 years) were accessing ART.

While increasing numbers of children with HIV (CWH) are reaching adulthood because of ART, long-standing HIV infection and/or treatment is associated with an increased risk of multisystem morbidities, including an adverse effect on development, i.e. growth and maturation [2, 3]. One of the first recognised manifestations of perinatally-acquired HIV infection was poor linear growth [2]. Additionally, HIV infection has been associated with low bone mineral density and impaired bone architecture [3–6]. Delays in development can have consequences on an individual's health in later life, as well as intergenerational effects. On average, during adolescence, individuals gain 20% of their final height and 50% of their body mass with considerable remodelling of the skeleton during adolescence making it a critical period for health [7].

Skeletal maturity is a measure of development incorporating the size, shape, and degree of mineralisation of the epiphyses and physeal plates of bone to define their proximity to full maturity. Maturation is a sequence of changes through growth and puberty which culminates in the development of secondary sexual characteristics and cessation of linear growth [8]. Bone age (BA) is an objective measure of skeletal maturation and is assessed by hand-wrist radiographs. The bones of the hand and wrist mature sequentially, and the stages of this process can be compared against a reference standard. A lag of BA behind chronological age (CA) (calculated from the date of birth) indicates impaired skeletal development in children. Skeletal maturation is influenced by several factors such as genetic ancestry and environmental factors like socio-economic deprivation, nutritional status (e.g., vitamin D and calcium intake), physical activity and co-morbid disease [4, 6, 9]. Studies have suggested that CWH may have delayed skeletal maturation despite ART [10, 11]; however, none have been conducted in sub-Saharan Africa where the majority of CWH live. Given the impact of maturation on achieving genetic potential and consequently future health, it is important to understand how skeletal development progresses through puberty in males and females from sub-Saharan Africa. This study in peripubertal males and females from Zimbabwe, therefore, aimed to describe the differences between BA and CA (i.e., skeletal maturity deviation [SMD]), determine to what extent HIV and other demographic and lifestyle factors predict SMD, and examine which HIV characteristics are associated with SMD in children living with HIV in Zimbabwe.

Methods

Study design and participants

A cross-sectional study of children aged 8–16 years, with and without HIV was conducted in Harare, Zimbabwe, nested within the IMpact of Vertical HIV infection on child and Adolescent Skeletal development (IMVASK) study; the protocol has previously been published [3]. Zimbabwe has experienced an early inset, sustained generalised HIV epidemic with an adult HIV prevalence of 11% in 2022. Of the 1.3 million people living with HIV, 75 000 are children [12]. CWH were recruited from HIV clinics at the two main public sector (tertiary referral) hospitals in Harare (Parirenyatwa and Sally Mugabe Hospital) between May 4, 2018, and Jan 21, 2020. Both hospitals have paediatric HIV clinics that provide HIV care and treatment to more than 2,000 children [3]. Although HIV care is increasingly decentralised to a primary care level across the country, most children in Harare continue to receive care within the HIV clinics in these healthcare facilities. Individuals were eligible if they had been taking ART for at least two years, were not acutely unwell, were residing in Harare and aware of their HIV status. Systematic quota-based sampling was used to recruit children stratified by sex into three age groups (8–10, 11–13 and 14–16 years). HIV-negative children were recruited from six public-sector schools within the same catchment area as the hospitals, again by stratified random sampling using school registers [3].

Boys and girls with CA above 16.5 and 15 years respectively were excluded for this analysis as these are above the cut off ages for Tanner Whitehouse 3 method of BA assessment.

Procedures

Sociodemographic and clinical data were collected by trained research staff using an interview-administered questionnaire. Data were collected on android tablets using the Online Data Kit (https://getodk.org/). A nurse and/or doctor carried out Tanner pubertal staging using testicular volume (assessed using an orchidometer), penile size and pubic hair growth (quality distribution and length) in boys and breast development (size and contour), age at menarche and pubic hair growth in girls for assessment [13]. Grading of penile, testicular and breast growth was from I to V as per Tanner descriptions [14–16]. In the event of discordance in the assignment of the pubertal stage between these categories, testicular and breast development for boys and girls respectively, were used to assign Tanner stage. Socio-economic status (SES) was constructed as three groups (low, middle, and high) using the first component from a principal

component analysis that included the head of household age, highest maternal and paternal education levels, monthly household income, number in the household, household ownership, access to amenities and household asset ownership [17]. Dietary calcium and vitamin D intake were quantified using a validated dietary diversity and food frequency tool from India and Malawi, adapted to the Zimbabwean context [18]. Daily calcium dietary intake was classified into three groups: very low (<150 mg/day), low (150–299 mg/day), and moderate (300–450 mg/day). Daily dietary vitamin D intake was classified as very low (<4.0 µg/day), low (4.0–5.9 µg/day), and moderate (6.0–8.0 µg/day) [19]. These thresholds were determined based on the distribution of intakes across the population [4]. Physical activity was self-reported using the International Physical Activity Questionnaire which has been validated in multiple countries; this assessment was based on multiples of the resting metabolic rate in MET minutes/week and categorised as low (<600 MET minutes/week), moderate (600–3000 MET minutes/week), and high (>3000 MET minutes/week) physical activity [20].

For participants with HIV, ART regimen including use of tenofovir disoproxil fumarate (TDF) was recorded; CD4 count was measured using an Alere PIMA CD4 analyser (Waltham, MA, USA). A GeneXpert HIV-1 viral load platform (Cepheid, Sunnyvale, CA, USA) was used to measure HIV viral load.

Anthropometric measurements

Standing height and weight were measured in duplicate by two independent trained staff members (nurse and research assistant). If the two measurements differed by more than 0.5 kg–0.5 cm, a third reading was taken by an additional reader. The mean of the two or three measurements was recorded as the final figure. Weight was measured to the nearest 0.1 kg using a Seca 875 weight scale, and height to the nearest 0.1 cm using a stadiometer (Seca, Hamburg Germany).

Bone age assessment

Digital hand-wrist radiographs of the non-dominant side were taken by a trained radiographer. Details of the method of obtaining the radiographs have previously been published [21]. BA was assessed using the Tanner Whitehouse 3 Radius, Ulna and Short bones method which has been found to be precise and, in contrast to the Greulich-Pyle method, not biased by age in this population of peripubertal adolescents [21, 22].

Statistical analysis

Data were analysed using Stata 17 (StatCorp, TX, USA). Weight for age z-scores (WAZ), Height for age z-scores (HAZ) and body mass index (BMI) for age z-scores were calculated using the 1990 UK reference data for children [23], because World Health Organization reference data for WAZ are not available beyond the age of 10 years [24]. Z-scores of -2.0 or lower for HAZ, WAZ and BMI z-score were used to define stunting, underweight and wasting respectively [25]. Analyses were stratified by sex because BA assessment and bone development are sex specific. The primary outcome was SMD in years. A negative SMD value reflects a delay in skeletal maturity, and a positive value reflects advanced skeletal maturity relative to CA.

Quantitative data were examined for normality using the Shapiro–Wilk test and histograms. Normally distributed continuous variables were presented as means±standard deviations and categorical variables as numbers and proportions of participants in each category. Non-normally distributed continuous variables were presented as medians and interquartile ranges (IQR). Participant characteristics were compared by HIV status, using the student t-test for continuous variables and chi-squared test for categorical variables. To account for missing data on pubertal status, CD4 count and viral load, we first identified auxiliary variables (HIV status, socio-economic status, sex, chronological age, physical activity, vitamin D and calcium intake). These were added to the imputation model to increase power and to support the plausibility of the assumption of missing at random. These auxiliary variables were identified: (1) as an a priori factor; (2) after considering their strength of association with the variables containing missing values (if continuous, correlation coefficient $r>0.4$ and if categorical, chi squared test $p<0.05$). Using imputation by chained equations in Stata a binary distribution was used to impute dichotomised CD4 count and viral load whilst an ordinal distribution was used to impute Tanner staging.

Linear regression was performed to examine associations between exposure variables (HIV status, WAZ, socio-economic deprivation, orphanhood, physical activity, vitamin D intake, calcium intake, pubertal stage) and SMD. WAZ was used instead of BMI because of the fixed relationship between skeletal maturation and height, which contributes to the BMI calculation. For ordinal exposures, p-values from tests for trend were additionally reported. Firstly, unadjusted linear regression models were used to determine the univariable associations between the exposure variables outlined above and SMD. Secondly, a multivariable linear regression model considered HIV and a priori factors (WAZ, SES, pubertal stage, orphanhood, and calcium intake) as exposures. *A priori factors* were chosen based on previous literature showing that lower WAZ, low SES, orphanhood and low dietary calcium intake are related to other skeletal outcomes [3, 6, 26, 27]. In addition, orphanhood was shown

to be associated with low bone density in this cohort [4]. Other variables were included in the multivariable model if there was any evidence of a potential association with SMD in the boys or the girls in the unadjusted analyses (p-value ≤ 0.1).

In an analysis restricted to CWH, linear regression was used to assess associations between HIV specific variables (age at ART initiation, CD4 count, viral load and TDF exposure) and SMD. The multivariable linear regression model in CWH included a priori factors outlined above and HIV specific variables with a p-value for trend ≤ 0.1 in the univariable linear regression analysis.

Collinearity was assessed using the variance inflation factor with values above 5 indicating collinearity [28]. Standard errors for regression coefficients were also assessed to determine the robustness of models. Regression assumptions were assessed to ensure (1) normality of residuals using Q-Q plots, (2) homogenous variance in scatter plot of fitted values versus residuals, and (3) independence of residuals by examining scatter plots of residuals versus exposure variables.

Results

Participant characteristics

We initially recruited 609 (303 HIV negative) participants into the study, of whom 74 were excluded (35 were above 15 and 16.5 years for females and males respectively and 39 did not have hand-wrist radiographs) with 535 participants (88% of original sample, 54% males) included in the analysis (Fig. 1).

For both males and females, a larger proportion of CWH had lower SES, were orphaned (one or both parent dead), stunted, or underweight in comparison to HIV-negative participants (Table 1). On average, male CWH were 7.1 cm shorter and 3.3 kg lighter than male HIV-negative participants; similarly female CWH were 6.0 cm shorter and 6.4 kg lighter than their HIV-negative peers. Female CWH were more likely to be in earlier Tanner stages (1 and 2) in comparison to those who were HIV-negative, whilst no differences were seen in Tanner stage by HIV status in males. Notably, over 65% of participants had low or very low intakes of calcium, falling far below recommended rates of other populations (e.g., UK recommendations are for calcium intakes between 800 and 1000 mg/day at this age) [29], and there was no difference in intake by HIV status. CWH reported lower levels of physical activity compared to HIV-negative participants.

CWH were diagnosed at a median age of 3.0 years (IQR 1.1–5.9) and were established on ART at 3.8 years (IQR 1.9-7.0). Overall, mean ART duration was 7.6 (SD 2.6) years, and 87 participants were on a TDF regimen with a median duration of 3.0 years (IQR 1.4–5.5). A high proportion of CWH had a suppressed viral load and a CD4 count > 500 cells per microlitre (79% and 80% respectively) [30] (Supplementary Table 1).

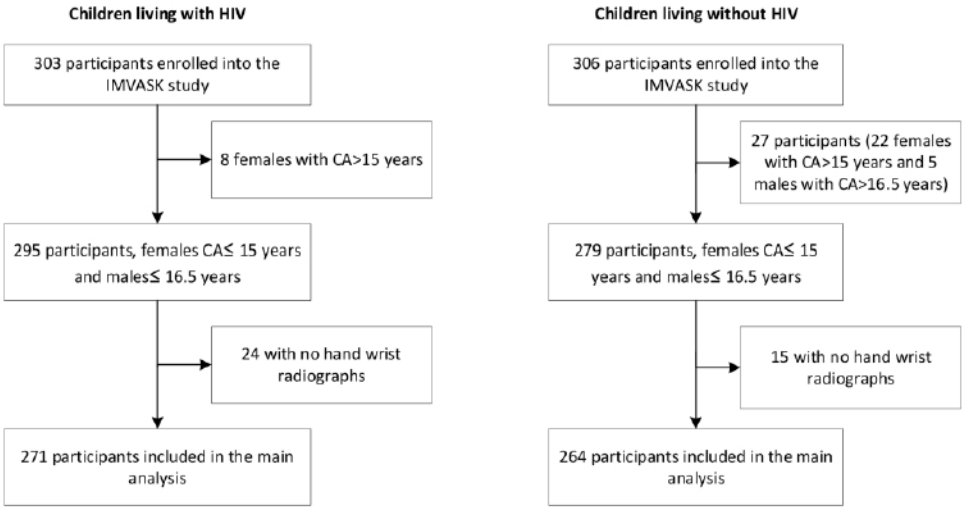

Fig. 1 Flow diagram showing the participants included in the analysis. IMVASK: The **Im**pact of **V**ertical HIV infection on child and **A**dolescent **Sk**eletal development, **CA**-chronological age

Table 1 Characteristics of study participants by HIV status in males and females

	Males			Females		
	HIV- (*n* = 141)	HIV+ (*n* = 146)	*p*-value	HIV- (*n* = 123)	HIV+ (*n* = 125)	*p*-value
Socio-demographics						
Chronological age (years), mean (SD)	12.3 (2.4)	12.6 (2.5)	0.220	12.1 (2.2)	12.3 (2.4)	0.520
Socio-economic status, n (%)			0.180			0.008
Group 1: low	54 (38.3)	46 (31.5)		51 (41.5)	29 (23.2)	
Group 2: middle	53 (37.6)	49 (33.6)		35 (28.5)	43 (34.4)	
Group 3: high	34 (24.1)	51 (34.9)		37 (30.1)	53 (42.4)	
Orphanhood: One or both parents dead, n (%)	11 (7.9)	59 (42.4)	< 0.001	7 (5.8)	52 (43.0)	< 0.001
Anthropometry						
Height (cm), mean (SD)	146.9 (15.1)	139.8 (12.3)	< 0.001	145.4 (11.9)	139.4 (12.9)	< 0.001
Height for age z-score, mean (SD)	-0.6 (0.9)	-1.7 (1.1)	< 0.001	-0.5 (1.1)	-1.5 (1.1)	< 0.001
Stunting (height for age z-score < -2), n (%)	8 (5.7)	52 (35.6)	< 0.001	9 (7.6)	31 (27.0)	< 0.001
Weight (kg), mean (SD)	38.7 (14.5)	35.4 (17.4)	< 0.001	41.6 (16.7)	35.2 (13.5)	< 0.001
Weight for age z-score, mean (SD)	-0.7 (1.1)	-1.6 (1.2)	< 0.001	-0.3 (1.2)	-1.3 (1.2)	< 0.001
Underweight (weight for age z-score < -2), n (%)	15 (10.6)	47 (32.2)	< 0.001	7 (5.7)	27 (21.6)	< 0.001
BMI (kg/m^2), mean (SD)	17.1(2.2)	16.6(1.5)	0.013	19.1(3.6)	17.5(2.7)	< 0.001
BMI for age z-score, mean (SD)	-0.5 (1.0)	-0.8 (0.9)	0.026	-0.1 (1.2)	-0.6 (0.9)	< 0.001
Wasting (BMI for age z-score < -2), n (%)	11 (7.8)	13 (8.9)	1.000	4 (3.3)	8 (6.4)	0.370
Pubertal status			0.240			< 0.001
Tanner I	45 (31.9)	55 (40.1)		23 (19.0)	52 (43.7)	
Tanner II	34 (24.1)	37 (27.0)		30 (24.8)	18 (15.1)	
Tanner III	22 (15.6)	21 (15.3)		28 (23.1)	27 (22.7)	
Tanner IV	36 (25.5)	19 (13.9)		31 (25.6)	18 (15.1)	
Tanner V	4 (2.8)	5 (3.6)		9 (7.4)	4 (3.4)	
Lifestyle factors						
Physical activity level, n (%)			0.043			0.055
Low, < 600 MET mins/week	48 (34.0)	46 (31.5)		44 (35.8)	29 (23.2)	
Moderate, 600–3000 MET mins/week	47 (33.3)	33 (22.6)		32 (26.0)	30 (24.0)	
High, > 3000 MET mins/week	46 (32.6)	67 (45.9)		47 (38.2)	66 (52.8)	
Daily vitamin D intake, n (%)			0.910			0.350
Very low, < 4.0 µg/day	32 (22.7)	27 (18.5)		29 (23.6)	22 (17.6)	
Low, 4.0–5.9 µg/day	92 (65.2)	96 (65.8)		79 (64.2)	89 (71.2)	
Moderate, 6.0–8.0 µg/day	17 (12.1)	23 (15.8)		15 (12.2)	14 (11.2)	
Daily calcium intake, n (%)			0.950			0.990
Very low, < 150 mg/day	48 (34.0)	52 (35.6)		42 (34.1)	44 (35.2)	
Low, 150–299 mg/day	31 (22.0)	30 (20.5)		27 (22.0)	27 (21.6)	
Moderate, 300–450 mg/day	62 (44.0)	64 (43.8)		54 (43.9)	54 (43.2)	
Bone age measures						
Bone age (years), mean (SD)	11.8 (2.5)	11.2 (2.3)	0.042	12.0 (2.2)	11.2 (2.4)	0.005
Skeletal maturity deviation	-0.4 (1.1)	-1.4 (1.4)	< 0.001	-0.0 (1.2)	-1.1 (1.4)	< 0.001
Skeletal maturity delay, n (%)	11 (7.8)	45 (30.8)	< 0.001	6 (4.9)	26 (20.8)	< 0.001

Student t-tests conducted on continuous variables and chi-squared tests on categorical variable. MET – multiples of the resting metabolic rate SD- Standard deviation. Skeletal maturity deviation-difference between bone age and chronological age. Skeletal maturity delay-skeletal maturity deviation </=-2 year

SMD in children with and without HIV

On average, there was little evidence of SMD in the HIV-negative group, particularly in females; however, SMD was evident in CWH whether male or female, such that skeletal maturity was delayed by more than a year compared to HIV-negative participants (Table 1). When stratified by Tanner stage, BA values were much lower than CA values in older children in earlier Tanner stages (Fig. 2).

Among males, living with HIV, being underweight, being orphaned, and consuming less dietary calcium, were associated with a more marked SMD (i.e., more delayed skeletal maturation) in univariable analyses. After adjustment in a multivariable model (which also included SES and pubertal stage), only living with HIV and being underweight remained associated with negative SMD in males (Table 2). In females, before adjustment, living with HIV, being underweight, orphaned, and to a lesser

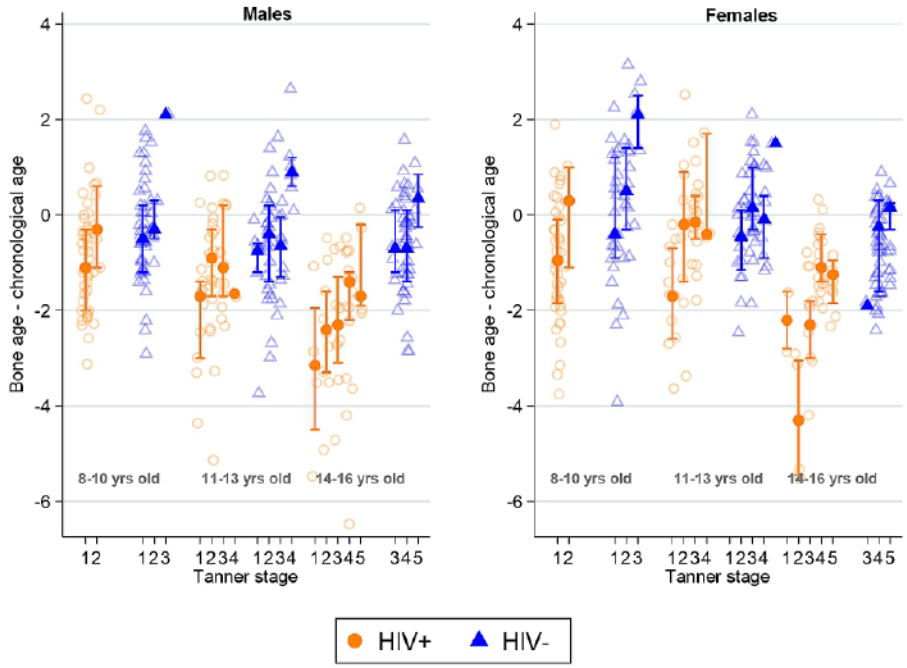

Fig. 2 Comparison of mean skeletal maturity deviation (difference between bone age and chronological age) in children living with and without HIV by sex, age group and pubertal status. Error bars indicate 95% confidence intervals

degree earlier pubertal stage was associated with more negative SMD. After adjustment in a multivariable model (which also included dietary calcium and SES), again only living with HIV and being underweight remained associated with greater delay in SMD in females (Table 3).

Factors associated with SMD in children with HIV

There was no difference in physical activity levels, and dietary vitamin D and calcium intake by sex but the mean WAZ and BMI for age z-scores differed with males [n=40 (34%)] more likely to be underweight than females [n=22 (22%)] (Supplementary Table 2). Females had less negative SMD (i.e., less delay in skeletal maturation) than the males.

In univariable analyses, older age at ART initiation (in both sexes) and greater viral load (in males only) were associated with more negative SMD (Tables 4 and 5). After adjustment for WAZ, SES, pubertal stage, orphan status, viral load, and years of TDF exposure, there was negative association between age at ART initiation and SMD and calcium intake and SMD in males and the negative association between viral load and SMD was partially attenuated. No association between viral load and SMD was found in females, but later age at ART initiation was still associated with more negative SMD (Table 5). The association between WAZ and SMD was robust to adjustment, and being underweight was still associated with more negative SMD in CWH in both sexes. Underweight children (z-score < -2) were on average delayed by a year in their skeletal maturity, compared to those who were not underweight (Tables 4 and 5).

Discussion

We report results from the first study to our knowledge to determine skeletal maturation in the children with HIV in Southern Africa. Underweight and having HIV were strongly associated with delayed skeletal maturation. The delay in skeletal maturity was more marked in the older children who were at earlier Tanner stages. Living with HIV infection and being underweight were strong predictors of skeletal maturity deviation in both males and females. In CWH, later age at ART initiation predicted greater delay in skeletal maturation in females [31].

Similar to this study, a delay in skeletal maturation has been reported in other studies in black African

Table 2 Associations between participant characteristics and skeletal maturity deviation (SMD) in males (unadjusted and adjusted linear regression analysis)

Variable	Males $n=287$			
	Unadjusted model	*p*-value	Adjusted model	*p*-value
	β coefficient (95%CI)		β coefficient (95%CI)	
HIV status				
HIV negative	ref	<0.001	ref	<0.001
HIV positive	-0.96 (-1.25, -0.66)		-0.59 (-0.90, -0.28)	
Weight for age z-score				
Weight for age z-score>-2	ref	<0.001	ref	<0.001
Weight for age z-score<-2	-1.41 (-1.83, -1.15)		-1.18 (-1.52, -0.84)	
Socio-economic status				
Group 1: High	ref	0.742	ref	0.440
Group 2: Middle	0.29 (-0.10, 0.68)		0.33 (-0.01, 0.67)	
Group 3: Low	-0.08 (-0.31, 0.47)		-0.11 (-0.44, 0.22)	
Pubertal stage				
Tanner 1	ref	0.990	ref	0.693
Tanner 2	0.07 (-0.34, 0.48)		0.12 (-0.23, 0.47)	
Tanner 3	-0.05 (-0.53, 0.43)		0.02 (-0.39, 0.43)	
Tanner 4&5	0.02 (-0.40, 0.45)		-0.06 (-0.43, 0.30)	
Orphan status				
Not an orphan	Ref	<0.001	ref	0.105
One or both parents dead	-0.76 (-1.11, -0.41)		-0.32 (-0.68, 0.02)	
Physical activity				
High, > 3000 MET mins/week	Ref	0.977		
Moderate,600-3000MET mins/week	0.37 (-0.02, 0.76)			
Low, < 600 MET mins/week	-0.03 (-0.39, 0.34)			
Vitamin D intake				
Moderate, 6.0–8.0 µg /day	Ref	0.297		
Low, 4.0–5.9 µg /day	-0.14 (-0.61, 0.32)			
Very low, < 4.0 µg /day	0.22 (-0.32, 0.76)			
Calcium intake				
Moderate, 300–450 mg/day	Ref	0.046	ref	0.114
Low, 150–299 mg/day	0.18 (-0.25, 0.61)		0.16 (-0.20, 0.53)	
Very low, < 150 mg/day	-0.34 (-0.70, 0.01)		-0.24(-0.55 0.07)	

The adjusted model included the following exposures: HIV status, weight for age z-scores, socio-economic status, pubertal stage, orphan status, and calcium intake. P-value for trend shown for variables with more than one category. CI-confidence interval, SMD-Skeletal maturity deviation calculated as the difference between bone age and chronological age; negative values reflect lower bone age than chronological age (delay in skeletal maturation)

HIV-negative children and adolescents [31–33]. In one, a slightly older cohort of black South African males aged 13–21 years, BA was on average 0.5 years younger than CA [33]. In another Malawian study, CA ranged between 2 and 28 years and there was a high negative average SMD of approximately 1.6 years for both males and females [31]. In a comparison of black and white children in South Africa, black males matured later than white males by 6 months [34]. In contrast, a systematic review of studies of black children, age 0–18 years, living in high income countries showed they have advanced BA relative to CA [35]. The differences in skeletal maturation between black children in Africa and those in high income countries may be explained by the optimal environmental conditions in terms of better access to healthcare and nutrition which are likely to contribute to achieving better genetic potential, all of which play a key role in the skeletal development of children.

Importantly living with HIV was a key predictor of SMD independent of other factors in this cohort such that CWH were less skeletally mature by over 6 months on average, compared to HIV-negative children, despite being established on ART. Older CWH were more likely to be in earlier Tanner stages, with chronologically older CWH in earlier Tanner stages having the more negative SMD. It is possible these children will experience some 'catch-up' skeletal maturation on initiation of puberty, which may be more rapid in duration than individuals who are more advanced in maturation at the same age. The 6-month delay is less than that seen in Brazilian and Indian CWH on ART, aged 5–11 and 8–14 years respectively, who had an SMD of over one year, though these

Table 3 Associations between participant characteristics and skeletal maturity deviation (SMD) in females (unadjusted and adjusted linear regression analysis)

Variable	Females $n=246$			
	Unadjusted model	*p*-value	Adjusted model	*p*-value
	β coefficient (95%CI)		β coefficient (95%CI)	
HIV status				
HIV negative	Ref	< 0.001	ref	< 0.001
HIV positive	-1.03 (-1.35, -0.71)		-0.91 (-1.28, -0.55)	
Weight for age z-score				
Weight for age z-score>-2	Ref	< 0.001	ref	< 0.001
Weight for age z-score<-2	-1.64 (-2.10, -1.19)		-1.40 (-1.86, -0.93)	
Socio-economic status				
Group 1: High	Ref	0.512	ref	0.054
Group 2: Middle	0.23 (-0.20, 0.65)		-0.004 (-0.38, 0.37)	
Group 3: Low	0.13 (-0.29, 0.55)		-0.38, (-0.76, -0.003)	
Pubertal stage				
Tanner 1	Ref	0.053	ref	0.898
Tanner 2	0.42 (-0.09, 0.92)		0.01 (-0.45, 0.47)	
Tanner 3	0.68 (0.21, 1.16)		0.38 (-0.05, 0.80)	
Tanner 4&5	0.37 (-0.09, 0.83)		-0.17 (-0.60, 0.27)	
Orphan status				
Not an orphan	ref	0.003	ref	0.914
One or both parents dead	-0.60 (-0.99, -0.20)		-0.04 (-0.45, 0.36)	
Physical activity				
High, > 3000 MET mins/week	ref	0.214		
Moderate,600-3000MET mins/week	0.17 (-0.26, 0.60)			
Low, < 600 MET mins/week	0.25 (-0.16, 0.66)			
Vitamin D intake				
Moderate, 6.0–8.0 µg /day	ref	0.509		
Low, 4.0–5.9 µg /day	0.12 (-0.43, 0.67)			
Very low, < 4.0 µg /day	0.21 (-0.42, 0.85)			
Calcium intake				
Moderate, 300–450 mg/day	ref	0.292	ref	0.881
Low, 150–299 mg/day	0.11 (-0.36, 0.59)		0.19 (-0.23, 0.61)	
Very low, < 150 mg/day	-0.20 (-0.60, 0.19)		-0.01 (-0.37, 0.34)	

The adjusted model included the following exposures: HIV status, weight for age z-scores, socio-economic status, pubertal stage, orphan status, and calcium intake. P-value for trend shown for variables with more than one category. CI-confidence interval, SMD-Skeletal maturity deviation calculated as the difference between bone age and chronological age; negative values reflect lower bone age than chronological age (delay in skeletal maturation)

studies were smaller and did not include older adolescents, nor in India a comparator group [10, 11].

Age at ART initiation was a predictor of SMD in both males and females highlighting the importance of early initiation of ART. Similarly, a longitudinal analysis of skeletal maturation in a 4-year follow-up study of Brazilian children [11] reported reduced SMD in CWH starting ART early. These findings support WHO recommendations to start ART upon HIV diagnosis regardless of CD4 count [36, 37]. As 80% of Zimbabwean children (0–14 years) have access to ART [1], delayed ART initiation is still a reality [1]. Our study did not show an association between TDF use and SMD, even though TDF has been associated with bone deficits in density and strength (i.e. bone accrual) in the same population [3, 6], potentially reflecting a direct effect of TDF on bone accrual rather than on puberty, or an underpowered analysis [38, 39].

Underweight children, living with or without HIV, were delayed in BA by over a year compared to those not underweight, consistent with a study in India where BA was measured in 100 underweight children. Low calcium intake was associated with delays in skeletal maturation in male CWH highlighting the importance of nutrition in growth and development. In contrast, there was no relationship found between BMI and skeletal maturation in Iranian and Malawian children aged 6–15 years and 2–28 years respectively [31, 40]. The Malawian study used Greulich Pyle to assess BA which we have found to be less biased by age and less precise than Tanner Whitehouse 3 method [21]. In the South African Birth to Twenty Bone Health Study [41], being heavier and taller at age two years in males and having greater lean mass and having entered puberty in females were associated with more advanced development at age 9–10 years,

Table 4 Associations between participant characteristics and skeletal maturity deviation (SMD) in males living with HIV (unadjusted and adjusted linear regression analysis)

Variable	Unadjusted model	*p*-value	Adjusted model	*p*-value
	β coefficient (95%CI)		β coefficient (95%CI)	
Males *n* = 146				
Weight for age z-score				
Weight for age z-score>-2	ref	< 0.001	ref	0.001
Weight for age z-score<-2	-1.22 (-1.67, -0.77)		-0.92 (-1.41, -0.44)	
Socio-economic status				
Group 1: High	ref	0.187	ref	0.101
Group 2: Middle	-0.06 (-0.62, 0.49)		0.21 (-0.35, 0.78)	
Group 3: Low	-0.38 (-0.94, 0.18)		-0.45 (-0.98, 0.08)	
Pubertal stage				
Tanner 1	ref	0.115	ref	0.941
Tanner 2	0.04 (-0.53, 0.62)		0.26 (-0.30, 0.82)	
Tanner 3	-0.17 (-0.86, 0.52)		-0.08 (-0.76, 0.61)	
Tanner 4&5	-0.54 (-1.19, 0.12)		0.09 (-0.59, 0.76)	
Orphan status				
Not an orphan	ref	0.046	ref	0.298
One or both parents dead	-0.52 (-0.98, -0.06)		-0.27 (-0.73, 0.19)	
Physical activity				
High, > 3000 MET mins/week	ref	0.765		
Moderate, 600–3000 MET mins/week	0.11 (-0.48, 0.70)			
Low, < 600 MET mins/week	-0.09 (-0.63, 0.44)			
Vitamin D intake				
Moderate, 6.0–8.0 µg/day	ref	0.187		
Low, 4.0–5.9 µg/day	-0.08 (-0.72, 0.56)			
Very low, < 4.0 µg/day	0.49 (-0.29, 1.27)			
Calcium intake				
Moderate, 300–450 mg/day	ref	0.010	ref	0.019
Low, 150–299 mg/day	0.12 (-0.50, 0.74)		-0.06 (-0.66, 0.54)	
Very low, < 150 mg/day	-0.65 (-1.15, -0.15)		-0.62 (-1.13, -0.10)	
Age at ART initiation				
< 2 years	ref	< 0.001	ref	0.026
2–3.9 years	-0.63 (-1.23, -0.02)		-0.50 (-1.11, 0.11)	
4–8 years	-0.83 (-1.40, -0.25)		-0.73 (-1.33, -0.12)	
> 8 years	-1.28 (-2.01, -0.54)		-0.75 (-1.54, 0.04)	
CD4 count				
>/=500 cells per µL	ref	0.191		
< 500 cells per µL	-0.36 (-0.91, 0.18)			
Viral load				
< 1000 RNA copies per ml	ref	0.004	ref	0.052
> 1000 RNA copies per ml	-0.85 (-1.43, -0.28)		-0.58 (-1.17, 0.01)	
TDF years of exposure				
No exposure	ref	0.052	ref	0.977
< 4 years	-0.43 (-1.01, 0.15)		-0.04 (-0.63, 0.56)	
≥ 4 years	-0.56 (-1.24, 0.11)		0.01 (-0.72, 0.73)	

The adjusted model included the following exposures: pubertal status, weight for age z-scores, socio-economic status, orphan status, calcium intake, age at ART initiation and viral load. ART-Anti-retroviral therapy, TDF- tenofovir disoproxil fumarate. CI-confidence interval SMD: Skeletal maturity deviation calculated as the difference between bone age and chronological age; negative values reflect lower bone age than chronological age (delay in skeletal maturation) *mean SMD for each category

which is in general agreement with the current findings. Early life environmental exposures and growth measures were not measured in the current study but the importance of a healthy weight and association with less delay in development suggests a lifelong effect of weight on development, consistent with previous observations.

The strengths of this study are having a comparator group of HIV negative children, and the study sample

Table 5 Associations between participant characteristics and skeletal maturity deviation (SMD) in females living with HIV (unadjusted and adjusted linear regression analysis)

Variable	Unadjusted model	*p*-value	Adjusted model	*p*-value
	β coefficient (95%CI)		β coefficient (95%CI)	
Females *n* = 125				
Weight for age z-score				
Weight for age z-score>-2	ref	< 0.001	ref	0.001
Weight for age z-score<-2	-1.27 (-1.82, -0.72)		-0.99 (-1.58, -0.40)	
Socio-economic status				
Group 1: High	ref	0.878	ref	0.460
Group 2: Middle	0.37 (-0.19, 0.93)		0.22 (-0.33, 0.76)	
Group 3: Low	-0.04 (-0.66, 0.58)		-0.17 (-0.77, 0.44)	
Pubertal stage				
Tanner 1	ref	0.221	ref	0.023
Tanner 2	0.10 (-0.65, 0.85)		0.55 (-0.22, 1.32)	
Tanner 3	0.35 (-0.28, 0.99)		0.69 (0.06, 1.32)	
Tanner 4	0.33 (-0.35, 1.01)		0.69 (-0.04, 1.42)	
Orphan status				
Not an Orphan	ref	0.920	ref	0.920
One or both parents dead	-0.02 (-0.51, 0.46)		0.09 (-0.41, 0.58)	
Physical activity				
High, > 3000 MET mins/week	ref	0.490		
Moderate, 600–3000 MET mins/week	-0.01 (-0.61, 0.60)			
Low, < 600 MET mins/week	0.23 (-0.37, 0.84)			
Vitamin D intake				
Moderate, 6.0–8.0 µg/day	ref	0.427		
Low, 4.0–5.9 µg/day	0.49 (-0.29, 1.27)			
Very low, < 4.0 µg/day	0.45 (-0.48, 1.38)			
Calcium intake				
Moderate, 300–450 mg/day	ref	0.133	ref	0.998
Low, 150–299 mg/day	-0.34 (-1.00, 0.33)		-0.19 (-0.81, 0.43)	
Very low, < 150 mg/day	-0.42 (-0.97, 0.13)		-0.04 (-0.56, 0.49)	
Age at ART initiation				
< 2 years	ref	0.001	ref	< 0.001
2–3.9 years	-0.35 (-0.99, 0.30)		-0.48 (-1.10, 0.15)	
4–8 years	-0.83 (-1.46, -0.19)		-1.21 (-1.88, -0.54)	
> 8 years	-1.02 (-1.69, -0.34)		-1.59 (-2.33, -0.84)	
CD4 count				
>/=500 cells per µL	ref	0.660		
< 500 cells per µL	-0.15 (-0.82, 0.52)			
Viral load				
< 1000 RNA copies per ml	ref	0.196	ref	0.176
> 1000 RNA copies per ml	0.41 (-0.19, 1.02)		0.51 (-0.06, 1.08)	
TDF exposure				
No exposure	ref	0.865	ref	0.831
< 4 years	-0.13 (-0.75, 0.49)		-0.13 (-0.74, 0.48)	
4 years +	0.01 (-0.83, 0.86)		0.25 (-0.59, 1.08)	

The adjusted model included the following exposures: pubertal status, weight for age z-scores, socio-economic status, orphan status, calcium intake, age at ART initiation and viral load. ART-Anti-retroviral therapy, TDF- tenofovir disoproxil fumarate. CI-confidence interval SMD: Skeletal maturity deviation calculated as the difference between bone age and chronological age; negative values reflect lower bone age than chronological age (delay in skeletal maturation) *mean SMD for each category

was a likely representation of children living in Harare. Another strength is the use of the TW3 method instead of the GP method which was shown to be less valid in our previous work [21]. The cross-sectional nature of our study means causality cannot be implied. There were indications that SMD was associated with age at ART initiation and viral load in boys with HIV, and pubertal status in girls with HIV; however, the relatively small sample

size in CWH and in the later Tanner stages may explain this.

Conclusion

In conclusion perinatally acquired HIV infection and being underweight were independently associated with a delay in skeletal maturation in both the boys and the girls. Given the consequences of delayed development on final height and subsequent outcomes, longitudinal studies are needed to determine the implications of a delayed development on later health in children living with and without HIV.

Abbreviations

ART	Anti-retroviral therapy
BA	Bone age
CA	Chronological age
CWH	Children living with HIV
SMD	Skeletal maturity deviation

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12887-024-04965-y.

Supplementary Material 1

Acknowledgements

We are grateful to Professor Ann Prentice, Dr Ruramayi Rukuni, Mr Joseph Chipanga, Dr Lynda Stranix Chibanda and Prof Cyrus Cooper for their support in the development of this work.

Author contributions

FK-N KAW CLG LDW: conceptualisation, methodology, interpreting data and writing original draft; FK-N: Investigation; ACO RAF LKM AMR: writing-review and editing; KAW CLG LDW AMR RAF: supervision, validation; AMR FK-N KAW CLG TM LDW: formal analysis. All authors reviewed the manuscript.

Funding

PhD funding support from the University of Southampton Global Challenges Research Fund and Faculty of Medicine, and part-funding from UK Medical Research Council Program U105960371. RAF is funded by the Wellcome Trust (206316_Z_17_Z). The IMWASK study was funded by the Wellcome Trust through a fellowship to RR (206764/Z/17/Z).

Data availability

Anonymised research data will be made available for sharing on the London School of Hygiene & Tropical Medicine (LSHTM) open access data repository (LSHTM Data Compass). Email: Tsitsi.Bandason@lshtm.ac.uk.

Declarations

Ethics approval and consent to participate

Study information was explained to participants and guardians by trained research assistants and/or study nurse. Written informed consent and age-appropriate written assent was given by guardians and participants respectively. Ethical approval was granted for this study by Parirenyatwa Hospital and Faculty of Medicine and Health Sciences joint research ethics committee (JREC/11/18), Sally Mugabe Hospital ethics committee (ref: 170118/04), the Medical Research Council of Zimbabwe (ref: MRCZ/A/2297).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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Received: 1 December 2023 / Accepted: 23 July 2024
Published online: 27 July 2024

References

1. UNAIDS. Global HIV statistics fact sheet 2023. 2023. https://www.unaids.org/sites/default/files/media_asset/UNAIDS_FactSheet_en.pdf
2. Arpadi SM. Growth failure in children with HIV infection. Journal of acquired immune deficiency syndromes (1999). 2000;25:S37-42.
3. Rukuni R, Gregson C, Kahari C, Kowo F, McHugh G, Munyati S, et al. The IMpact of vertical HIV infection on child and adolescent SKeletal development in Harare, Zimbabwe (IMWASK study): a protocol for a prospective cohort study. BMJ open. 2020;10(2):e031792.
4. Rukuni R, Rehman AM, Mukwasi-Kahari C, Madanhire T, Kowo-Nyakoko F, McHugh G, et al. Effect of HIV infection on growth and bone density in peripubertal children in the era of antiretroviral therapy: a cross-sectional study in Zimbabwe. Lancet Child Adolesc Health. 2021;5(8):569–81.
5. Ahmad AN, Ahmad SN, Ahmad N. HIV infection and bone abnormalities. Open Orthop J. 2017;11:777.
6. Mukwasi-Kahari C, Rehman AM, Breasail Ó, Rukuni M, Madanhire R, Chipanga TJ. Impaired bone architecture in peripubertal children with HIV, despite treatment with antiretroviral therapy: a cross-sectional study from Zimbabwe. J Bone Miner Res. 2022.
7. Norris SA, Frongillo EA, Black MM, Dong Y, Fall C, Lampl M, et al. Nutrition in adolescent growth and development. Lancet. 2022;399(10320):172–84.
8. Mora S, Gilsanz V. Pubertal growth of the male skeleton. Osteoporosis in Men: Elsevier; 2010. pp. 95–103.
9. Alshamrani K, Hewitt A, Offiah AC. Applicability of two bone age assessment methods to children from Saudi Arabia. Clin Radiol. 2020;75(2):156. e1-. e9.
10. Kumar V, Patil K, Munoli KB. Comparative evaluation of dental age, bone age, and chronological age in the human immunodeficiency virus positive children. J Pharm Bioallied Sci. 2014;6(Suppl 1):S90.
11. Holderbaum RM, Veeck EB, Oliveira HW, Silva CL, Fernandes Â. Comparison among dental, skeletal and chronological development in HIV-positive children: a radiographic study. Brazilian Oral Res. 2005;19:209–15.
12. UNAIDS. HIV and AIDS estimates 2022. https://www.unaids.org/en/regionscountries/countries/zimbabwe
13. Duke PM, Litt IF, Gross RT. Adolescents' self-assessment of sexual maturation. Pediatrics. 1980;66(6):918–20.
14. Marshall WA, Tanner JM. Variations in pattern of pubertal changes in girls. Arch Dis Child. 1969;44(235):291.
15. Marshall WA, Tanner JM. Variations in the pattern of pubertal changes in boys. Arch Dis Child. 1970;45(239):13–23.

16. Baird J, Walker I, Smith C, Inskip H. Review of methods for determining pubertal status and age of onset of puberty in cohort and longitudinal studies. London, UK: CLOSER; 2017.
17. Vyas S, Kumaranayake L. Constructing socio-economic status indices: how to use principal components analysis. Health Policy Plann. 2006;21(6):459–68.
18. Filteau S, Rehman AM, Yousafzai A, Chugh R, Kaur M, Sachdev H, et al. Associations of vitamin D status, bone health and anthropometry, with gross motor development and performance of school-aged Indian children who were born at term with low birth weight. BMJ open. 2016;6(1):e009268.
19. Infant WGo, Indicators YCF. Developing and validating simple indicators of dietary quality and energy intake of infants and young children in developing countries: summary of findings from analysis of 10 data sets. (FANTA) Project Washington, DC, USA; 2006.
20. Craig CL, Marshall AL, Sjöström M, Bauman AE, Booth ML, Ainsworth BE, et al. International physical activity questionnaire: 12-country reliability and validity. Med Sci Sports Exerc. 2003;35(8):1381–95.
21. Kowo-Nyakoko F, Gregson CL, Madanhire T, Strani x-Chibanda L, Rukuni R, Offiah AC et al. Evaluation of two methods of bone age assessment in peripubertal children in Zimbabwe. Bone. 2023:116725.
22. JM T, RH W, Marshall W. Assessment of skeletal maturity and prediction of adult height. London UK Academic; 1975.
23. Cole TJ, Freeman JV, Preece MA. British 1990 growth reference centiles for weight, height, body mass index and head circumference fitted by maximum penalized likelihood. Stat Med. 1998;17(4):407–29.
24. Organisation WH. WHO growth reference data, weight-for-age (5-10 years). https://www.who.int/tools/growth-reference-data-for-5to19-years/indicators/weight-for-age-5to10-years
25. Caulfield LE, Richard SA, Rivera JA, Musgrove P, Black RE. Stunting, wasting, and micronutrient deficiency disorders. Disease Control Priorities in Developing Countries 2nd edition. 2006.
26. Shlisky J, Mandlik R, Askari S, Abrams S, Belizan JM, Bourassa MW, et al. Calcium deficiency worldwide: prevalence of inadequate intakes and associated health outcomes. Wiley Online Library; 2022:0077–8923. Report No.
27. Low W. Relation between socio-economic status and skeletal maturation of Chinese children. Z für Morphologie und Anthropologie. 1972(H 1):1–11.
28. Shrestha N. Detecting multicollinearity in regression analysis. Am J Appl Math Stat. 2020;8(2):39–42.
29. SACN Report. Vitamin D and Health 2016 https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/537616/SACN_Vitamin_D_and_Health_report.pdf
30. Garcia SAB, Guzman N. Acquired immune deficiency syndrome CD4+count. StatPearls: StatPearls Publishing; 2021.
31. Lewis C, Lavy C, Harrison W. Delay in skeletal maturity in Malawian children. J bone Joint Surg Br Volume. 2002;84(5):732–4.
32. Govender D, Goodier M. Bone of contention: the applicability of the greulich–pyle method for skeletal age assessment in South Africa. SA J Radiol. 2018;22(1):1–6.
33. Dembetembe KA, Morris AG. Is Greulich-Pyle age estimation applicable for determining maturation in male Africans? South Afr J Sci. 2012;108(9):1–6.
34. Cole TJ, Rousham EK, Hawley NL, Cameron N, Norris SA, Pettifor JM. Ethnic and sex differences in skeletal maturation among the birth to twenty cohort in South Africa. Arch Dis Child. 2015;100(2):138–43.
35. Alshamrani K, Messina F, Offiah AC. Is the Greulich and Pyle atlas applicable to all ethnicities? A systematic review and meta-analysis. Eur Radiol. 2019;29(6):2910–23.
36. Group TAS. A trial of early antiretrovirals and isoniazid preventive therapy in Africa. N Engl J Med. 2015;373(9):808–22.
37. Group ISS. Initiation of antiretroviral therapy in early asymptomatic HIV infection. N Engl J Med. 2015;373(9):795–807.
38. Baranek B, Wang S, Cheung AM, Mishra S, Tan DH. The effect of tenofovir disoproxil fumarate on bone mineral density: a systematic review and meta-analysis. Antivir Ther. 2020;25(1):21–32.
39. Grant PM, Cotter AG. Tenofovir and bone health. Curr Opin HIV AIDS. 2016;11(3):326.
40. Hedayati Z, Khalafinejad F. Relationship between body mass index, skeletal maturation and dental development in 6-to 15-year old orthodontic patients in a sample of Iranian population. J Dent. 2014;15(4):180.
41. Hawley NL, Rousham EK, Johnson W, Norris SA, Pettifor JM, Cameron N. Determinants of relative skeletal maturity in South African children. Bone. 2012;50(1):259–64.

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Appendix D Supplementary Table

Table 7.1 Supplementary Table showing associations between baseline characteristics and skeletal maturity deviation (years) at one year follow up after adjustment for baseline skeletal maturity deviation and follow-up period in males and females of imputed data

	Males n=228					Females n=221				
Variable	**n**	**Minimally adjusted model β coefficient (95%CI)**	**p - value**	**Fully adjusted model β coefficient (95%CI)**	**p-value**	**n**	**Minimally adjusted model β coefficient (95%CI)**	**p-value**	**Fully adjusted model β coefficient (95%CI)**	**p-value**
HIV status										
HIV negative	115	ref	0.027	ref	0.09	116	ref	0.001	ref	**0.002**
HIV positive	113	-0.32 (-0.59, -0.04)		-0.25 (-0.54, 0.04)		105	-0.44 (-0.69, -0.19)		-0.41 (-0.66, -0.15)	
Weight for age z-score										
Weight for age z score>-2	183	ref	0.006	ref	0.006	194	ref	0.028	ref	0.082
Weight for age z score<-2	45	-0.51 (-0.88, -0.15)		-0.54 (-0.91, -0.16)		26	-0.42 (-0.80, -0.05)		-0.33 (-0.71, 0.04)	
Socio-economic status										
Tertile 3: High	83	ref	0.764	ref	0.714	66	ref	0.628	ref	0.506
Tertile 2: Middle	76	0.30 (-0.01, 0.61)		0.34 (0.03, 0.65)		69	0.03 (-0.28, 0.34)		0.10 (-0.18, 0.39)	
Tertile 1: Low	69	-0.07 (-0.39, 0.24)		-0.09 (-0.41, 0.24)		86	-0.07 (-0.36, 0.23)		-0.08, -0.35, 0.20)	
Pubertal stage										
Tanner 1	76	ref	0.989	ref	0.972	84	ref	0.418	ref	0.540
Tanner 2	59	0.04 (-0.30, 0.37)		0.07 (-0.26, 0.40)		70	-0.41 (-0.79, -0.02)		-0.51 (-0.84, -0.17)	
Tanner 3	33	-0.21 (-0.62, 0.20)		-0.14 (-0.54, 0.27)		63	-0.27 (-0.67, 0.12)		-0.34 (-0.67, -0.001)	
Tanner 4&5	52	0.07 (-0.28, 0.42)		0.06 (-0.29, 0.40)		130	0.07 (-0.25, 0.39)		-0.16 (-0.46, 0.14)	
Physical activity										
High, >3000 MET mins/week	73	ref	0.204	ref	0.347	64	ref	0.008	ref	0.037
Moderate, 600-3000 MET mins/week	65	-0.12 (-0.46, 0.22)		-0.13 (-0.46, 0.21)		57	-0.02 (-0.35, 0.30)		0.04 (-0.25, 0.34)	
Low, <600 MET mins/week	90	-0.20 (-0.51, 0.11)		-0.15 (-0.47, 0.16)		100	-0.36 (-0.64, -0.08)		-0.27 (-0.54, 0.00)	
Vitamin D intake										

Moderate, 6.0-8.0 mcg/day	88	ref	0.858			79	ref	0.891		
Low, 4.0-5.9 mcg/day	129	0.07 (-0.20, 0.34)				130	-0.03 (-0.29, 0.22)			
Very low, <4.0 mcg/day	11	-0.30 (-0.92, 0.33)				12	0.02 (-0.53, 0.57)			
Calcium intake										
Moderate, 300–450 mg/day	83	ref	0.354	ref	0.362	83	ref	0.743	ref	0.701
Low, 150-299 mg/day	46	0.22 (-0.14, 0.57)		0.20 (-0.15, 0.55)		50	0.06 (-0.26, 0.38)		-0.01 (-0.30, 0.29)	
Very low, <150 mg/day	99	0.14 (-0.15, 0.43)		0.14 (-0.16, 0.44)		88	0.05 (-0.23, 0.32)		0.05 (-0.20, 0.30)	

Skeletal maturity deviation (SMD) was calculated as bone age (years) – chronological age (years); more negative values reflect greater delay in skeletal maturity. Minimally adjusted model: baseline SMD and follow-up period as adjustments. Fully adjusted model: baseline SMD, follow-up period, HIV status, weight for age z-score, socio-economic status, pubertal stage, physical activity, and calcium intake exposure as predictors. WAZ-weight for age z-scores; SES-socio-economic status; PA-physical activity

Appendix E IMVASK Questionnaire

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 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 ☐**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 last 7 days, how much time did you (the participant) spend sitting on a week day? (*Mumazuva manomwe apfuura*, 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 ☐

Bibliography

1. Mora S, Gilsanz V. Pubertal growth of the male skeleton. Osteoporosis in Men: Elsevier; 2010. p. 95-103.
2. Greulich WW, Pyle SI. Radiographic atlas of skeletal development of the hand and wrist: Stanford university press; 1959.
3. Bull R, Edwards P, Kemp P, Fry S, Hughes I. Bone age assessment: a large scale comparison of the Greulich and Pyle, and Tanner and Whitehouse (TW2) methods. Archives of disease in childhood. 1999;81(2):172-3.
4. Clarke B. Normal bone anatomy and physiology. Clinical journal of the American Society of Nephrology: CJASN. 2008;3(Suppl 3):S131.
5. Kember N. Cell population kinetics of bone growth: the first ten years of autoradiographic studies with tritiated thymidine. Clinical Orthopaedics and Related Research (1976-2007). 1971;76:213-4.
6. Abad V, Meyers JL, Weise M, Gafni RI, Barnes KM, Nilsson O, et al. The role of the resting zone in growth plate chondrogenesis. Endocrinology. 2002;143(5):1851-7.
7. Nilsson O, Marino R, De Luca F, Phillip M, Baron J. Endocrine regulation of the growth plate. Hormone research. 2005;64(4):157-65.
8. Breeland G, Sinkler MA, Menezes RG. Embryology, bone ossification. StatPearls [Internet]: StatPearls Publishing; 2023.
9. Bonewald LF, Johnson ML. Osteocytes, mechanosensing and Wnt signaling. Bone. 2008;42(4):606-15.
10. Mackie E, Ahmed Y, Tatarczuch L, Chen K-S, Mirams M. Endochondral ossification: how cartilage is converted into bone in the developing skeleton. The international journal of biochemistry & cell biology. 2008;40(1):46-62.
11. Xie M, Chagin AS. The epiphyseal secondary ossification center: Evolution, development and function. Bone. 2021;142:115701.
12. Kontulainen SA, Hughes JM, Macdonald HM, Johnston JD. The biomechanical basis of bone strength development during growth. Optimizing bone mass and strength. 2007;51:13-32.
13. Cech DJ, Martin ST. Functional movement development across the life span: Elsevier Health Sciences; 2011.
14. Garn SM, Rohmann CG. Variability in the order of ossification of the bony centers of the hand and wrist. American Journal of Physical Anthropology. 1960;18(3):219-30.
15. Cavallo F, Mohn A, Chiarelli F, Giannini C. Evaluation of bone age in children: A mini-review. Frontiers in Pediatrics. 2021;9:580314.
16. Cooke DW, Divall S, Radovick S. Normal and aberrant growth in children. Williams textbook of endocrinology: Elsevier; 2015. p. 964-1073.
17. Benyi E, Sävendahl L. The physiology of childhood growth: hormonal regulation. Hormone research in paediatrics. 2017;88(1):6-14.
18. Marume A, Moherndran A, Tinarwo P, Mahomed S. Development of a Zimbabwean child growth curve and its comparison with the World Health Organization child growth standards. African Journal of primary health care & family medicine. 2022;14(1):1-9.
19. Inzaghi E, Pampanini V, Deodati A, Cianfarani S. The effects of nutrition on linear growth. Nutrients. 2022;14(9):1752.
20. Rogol AD, Clark PA, Roemmich JN. Growth and pubertal development in children and adolescents: effects of diet and physical activity. The American journal of clinical nutrition. 2000;72(2):521S-8S.
21. Lui JC. Nutritional regulation of the growth plate. The biology of the first 1,000 days: CRC Press; 2017. p. 237-52.

22. Lui JC, Baron J. Mechanisms limiting body growth in mammals. Endocrine reviews. 2011;32(3):422-40.
23. Ağırdil Y. The growth plate: a physiologic overview. EFORT Open Reviews. 2020;5(8):498-507.
24. Phillip M, Maor G, Assa S, Silbergeld A, Segev Y. Testosterone stimulates growth of tibial epiphyseal growth plate and insulin-like growth factor-1 receptor abundance in hypophysectomized and castrated rats. Endocrine. 2001;16:1-6.
25. Blanchard O, Tsagris L, Rappaport R, Duval-Beaupere G, Corvol M. Age-dependent responsiveness of rabbit and human cartilage cells to sex steroids in vitro. The Journal of Steroid Biochemistry and Molecular Biology. 1991;40(4-6):711-6.
26. Miura M, Tanaka K, Komatsu Y, Suda M, Yasoda A, Sakuma Y, et al. Thyroid hormones promote chondrocyte differentiation in mouse ATDC5 cells and stimulate endochondral ossification in fetal mouse tibias through iodothyronine deiodinases in the growth plate. Journal of Bone and Mineral Research. 2002;17(3):443-54.
27. Prentice A, Schoenmakers I, Laskey MA, De Bono S, Ginty F, Goldberg GR. Symposium on 'Nutrition and health in children and adolescents' Session 1: Nutrition in growth and development Nutrition and bone growth and development: A meeting of the Nutrition Society hosted by the Irish Section was held on 14–16 June 2006 at University College Cork, Cork, Republic of Ireland. Proceedings of the Nutrition Society. 2006;65(4):348-60.
28. Flynn A. The role of dietary calcium in bone health. Proceedings of the Nutrition Society. 2003;62(4):851-8.
29. Uday S, Manaseki-Holland S, Bowie J, Mughal MZ, Crowe F, Högler W. The effect of vitamin D supplementation and nutritional intake on skeletal maturity and bone health in socio-economically deprived children. European journal of nutrition. 2021;60(6):3343-53.
30. Vannucci L, Fossi C, Quattrini S, Guasti L, Pampaloni B, Gronchi G, et al. Calcium intake in bone health: a focus on calcium-rich mineral waters. Nutrients. 2018;10(12):1930.
31. Walli NZ, Munubhi EK, Aboud S, Manji KP. Vitamin D levels in malnourished children under 5 years in a tertiary care center at Muhimbili National Hospital, Dar es Salaam, Tanzania—A cross-sectional study. Journal of tropical pediatrics. 2017;63(3):203-9.
32. Mokhtar RR, Holick MF, Sempértegui F, Griffiths JK, Estrella B, Moore LL, et al. Vitamin D status is associated with underweight and stunting in children aged 6–36 months residing in the Ecuadorian Andes. Public Health Nutrition. 2018;21(11):1974-85.
33. Akseer N, Al-Gashm S, Mehta S, Mokdad A, Bhutta ZA. Global and regional trends in the nutritional status of young people: a critical and neglected age group. Annals of the New York Academy of Sciences. 2017;1393(1):3-20.
34. Goldberg GR, Jarjou LM, Cole TJ, Prentice A. Randomized, placebo-controlled, calcium supplementation trial in pregnant Gambian women accustomed to a low calcium intake: effects on maternal blood pressure and infant growth. The American journal of clinical nutrition. 2013;98(4):972-82.
35. Prentice A, Dibba B, Sawo Y, Cole TJ. The effect of prepubertal calcium carbonate supplementation on the age of peak height velocity in Gambian adolescents. The American journal of clinical nutrition. 2012;96(5):1042-50.
36. Prentice AM, Ward KA, Goldberg GR, Jarjou LM, Moore SE, Fulford AJ, et al. Critical windows for nutritional interventions against stunting. The American of Clinical Nutrition. 2013;97(5):911-8.
37. Ward K, Cole T, Laskey M, Ceesay M, Mendy M, Sawo Y, et al. The effect of prepubertal calcium carbonate supplementation on skeletal development in Gambian boys—a 12-year follow-up study. The Journal of Clinical Endocrinology & Metabolism. 2014;99(9):3169-76.
38. Murray PG, Clayton PE, editors. Endocrine control of growth. American Journal of Medical Genetics Part C: Seminars in Medical Genetics; 2013: Wiley Online Library.

39. Mehta S, Manji KP, Young AM, Brown ER, Chasela C, Taha TE, et al. Nutritional indicators of adverse pregnancy outcomes and mother-to-child transmission of HIV among HIV-infected women. The American journal of clinical nutrition. 2008;87(6):1639-49.
40. Villamor E, Saathoff E, Msamanga G, O'Brien ME, Manji K, Fawzi WW. Wasting during pregnancy increases the risk of mother-to-child HIV-1 transmission. JAIDS Journal of Acquired Immune Deficiency Syndromes. 2005;38(5):622-6.
41. Obimbo MM, Zhou Y, McMaster MT, Cohen CR, Qureshi Z, Ong'ech J, et al. Placental structure in preterm birth among HIV-positive versus HIV-negative women in Kenya. JAIDS Journal of Acquired Immune Deficiency Syndromes. 2019;80(1):94-102.
42. Weaver CM, Fuchs RK. Skeletal growth and development. Basic and applied bone biology: Elsevier; 2014. p. 245-60.
43. Karlberg J. On the modelling of human growth. Statistics in medicine. 1987;6(2):185-92.
44. Soliman A, De Sanctis V, Elalaily R, Bedair S. Advances in pubertal growth and factors influencing it: Can we increase pubertal growth? Indian journal of endocrinology and metabolism. 2014;18(Suppl 1):S53.
45. Marshall WA, Tanner JM. Variations in pattern of pubertal changes in girls. Archives of disease in childhood. 1969;44(235):291.
46. Marshall WA, Tanner JM. Variations in the pattern of pubertal changes in boys. Archives of disease in childhood. 1970;45(239):13-23.
47. Norris SA, Frongillo EA, Black MM, Dong Y, Fall C, Lampl M, et al. Nutrition in adolescent growth and development. The Lancet. 2022;399(10320):172-84.
48. Kowalski AJ, Addo OY, Kramer MR, Martorell R, Norris SA, Waford RN, et al. Longitudinal associations of pubertal timing and tempo with adolescent mental health and risk behavior initiation in urban South Africa. Journal of Adolescent Health. 2021;69(1):64-73.
49. Prentice S, Fulford AJ, Jarjou LM, Goldberg GR, Prentice A. Evidence for a downward secular trend in age of menarche in a rural Gambian population. Annals of Human Biology. 2010;37(5):717-21.
50. Padez C. Age at menarche of schoolgirls in Maputo, Mozambique. Annals of human biology. 2003;30(4):487-95.
51. Tunau K, Adamu A, Hassan M, Ahmed Y, Ekele B. Age at menarche among school girls in Sokoto, Northern Nigeria. Annals of African medicine. 2012;11(2):103-7.
52. Biro FM, Pajak A, Wolff MS, Pinney SM, Windham GC, Galvez MP, et al. Age of menarche in a longitudinal US cohort. Journal of pediatric and adolescent gynecology. 2018;31(4):339-45.
53. Wiley AS. Milk intake and total dairy consumption: associations with early menarche in NHANES 1999-2004. PloS one. 2011;6(2):e14685.
54. Goon DT, Toriola AL, Uever J, Wuam S, Toriola OM. Growth status and menarcheal age among adolescent school girls in Wannune, Benue State, Nigeria. BMC pediatrics. 2010;10:1-6.
55. Gaudineau A, Ehlinger V, Vayssiere C, Jouret B, Arnaud C, Godeau E. Factors associated with early menarche: results from the French Health Behaviour in School-aged Children (HBSC) study. BMC public health. 2010;10:1-7.
56. Wronka I. Association between BMI and age at menarche in girls from different socio-economic groups. Anthropologischer Anzeiger. 2010:43-52.
57. Günther AL, Karaolis-Danckert N, Kroke A, Remer T, Buyken AE. Dietary protein intake throughout childhood is associated with the timing of puberty. The Journal of nutrition. 2010;140(3):565-71.
58. Merzenich H, Boeing H, Wahrendorf J. Dietary fat and sports activity as determinants for age at menarche. American journal of epidemiology. 1993;138(4):217-24.
59. Mollaei E, Hosseinpour F, Fasihi S, Ziaee T. Age of Menarche and its Relationship with Some Factors in the Schoolgirls of Gorgan in 2006. J Gorgan Bouyeh Fac Nurs Midwifery. 2006;7(1):48-54.
60. Guzha BT, Mateveke B, Mubata H, Chapupu T, Dondo V, Chirehwa M, et al. Assessment of the impact of HIV infection on the hypothalamic-pituitary-ovarian axis and pubertal development

among adolescent girls at a tertiary centre in Zimbabwe: a cross-sectional study. BMC Endocrine Disorders. 2025;25(1):16.
61. Perkins JM, Subramanian SV, Davey Smith G, Özaltin E. Adult height, nutrition, and population health. Nutrition reviews. 2016;74(3):149-65.
62. Silventoinen K. Determinants of variation in adult body height. Journal of biosocial science. 2003;35(2):263-85.
63. Stagi S, Galli L, Cecchi C, Chiappini E, Losi S, Gattinara C, et al. Final height in patients perinatally infected with the human immunodeficiency virus. Hormone Research in Paediatrics. 2010;74(3):165-71.
64. Kessler M, Kaul A, Santos-Malavé C, Borkowsky W, Kessler J, Shah B. Growth patterns in pubertal HIV-infected adolescents and their correlation with cytokines, IGF-1, IGFBP-1, and IGFBP-3. Journal of Pediatric Endocrinology and Metabolism. 2013;26(7-8):639-44.
65. Dewey KG, Begum K. Long-term consequences of stunting in early life. Maternal & child nutrition. 2011;7:5-18.
66. Asiki G, Newton R, Marions L, Kamali A, Smedman L. The effect of childhood stunting and wasting on adolescent cardiovascular diseases risk and educational achievement in rural Uganda: a retrospective cohort study. Global Health Action. 2019;12(1):1626184.
67. Hoddinott J, Behrman JR, Maluccio JA, Melgar P, Quisumbing AR, Ramirez-Zea M, et al. Adult consequences of growth failure in early childhood. The American journal of clinical nutrition. 2013;98(5):1170-8.
68. Febba A, Sesso R, Barreto GP, Liboni CS, Franco MC, Casarini DE. Stunting growth: association of the blood pressure levels and ACE activity in early childhood. Pediatric nephrology. 2009;24:379-86.
69. Cohen MS. Fetal and childhood onset of adult cardiovascular diseases. Pediatric Clinics. 2004;51(6):1697-719.
70. Martin RM, McCarthy A, Smith GD, Davies DP, Ben-Shlomo Y. Infant nutrition and blood pressure in early adulthood: the Barry Caerphilly Growth study. The American journal of clinical nutrition. 2003;77(6):1489-97.
71. Victora CG, Adair L, Fall C, Hallal PC, Martorell R, Richter L, et al. Maternal and child undernutrition: consequences for adult health and human capital. The lancet. 2008;371(9609):340-57.
72. De Lucia Rolfe E, de Franca GVA, Vianna CA, Gigante DP, Miranda JJ, Yudkin JS, et al. Associations of stunting in early childhood with cardiometabolic risk factors in adulthood. PloS one. 2018;13(4):e0192196.
73. van Rooyen JM, Kruger HS, Huisman HW, Schutte AE, Malan NT, Schutte R. Early cardiovascular changes in 10-to 15-year-old stunted children: the Transition and Health during Urbanization in South Africa in Children study. Nutrition. 2005;21(7-8):808-14.
74. Adair LS, Fall CH, Osmond C, Stein AD, Martorell R, Ramirez-Zea M, et al. Associations of linear growth and relative weight gain during early life with adult health and human capital in countries of low and middle income: findings from five birth cohort studies. The Lancet. 2013;382(9891):525-34.
75. Arpadi SM. Growth failure in HIV-infected children. WHO Consultation on Nutrition and HIV/AIDS in Africa: Evidence, Lessons and Recommendations for Action, Durban, South Africa. 2005:10-3.
76. Williams PL, Jesson J. Growth and pubertal development in HIV-infected adolescents. Current Opinion in HIV and AIDS. 2018;13(3):179-86.
77. J. M. Tanner MJRH, H. Goldstein and N. Cameron. . Assessment of skeletal maturity and prediction of adult height (TW3 method). London: W. B. Saunders; 2001.
78. Cutler Jr G. The role of estrogen in bone growth and maturation during childhood and adolescence. The Journal of steroid biochemistry and molecular biology. 1997;61(3-6):141-4.
79. Satoh M, Hasegawa Y. Factors affecting prepubertal and pubertal bone age progression. Frontiers in Endocrinology. 2022;13:967711.

80. Igarashi M, Ayabe T, Yamamoto-Hanada K, Matsubara K, Sasaki H, Saito-Abe M, et al. Female-dominant estrogen production in healthy children before adrenarche. Endocrine connections. 2021;10(10):1221-6.
81. Wells JC. Sexual dimorphism of body composition. Best practice & research Clinical endocrinology & metabolism. 2007;21(3):415-30.
82. Pryor JW. Time of ossification of the bones of the hand of the male and female and union of epiphyses with the diaphyses. American Journal of Physical Anthropology. 1925;8(4):401-10.
83. Flor-Cisneros A, Leschek EW, Merke DP, Barnes KM, Coco M, Cutler Jr GB, et al. In boys with abnormal developmental tempo, maturation of the skeleton and the hypothalamic-pituitary-gonadal axis remains synchronous. The Journal of Clinical Endocrinology & Metabolism. 2004;89(1):236-41.
84. Flor-Cisneros A, Roemmich JN, Rogol AD, Baron J. Bone age and onset of puberty in normal boys. Molecular and cellular endocrinology. 2006;254:202-6.
85. Lu Y, Pearce A, Li L. OP38 Ethnic differences in childhood height trajectories and the role of early life factors: evidence from the uk millennium cohort study. BMJ Publishing Group Ltd; 2017.
86. Grgic O, Shevroja E, Dhamo B, Uitterlinden AG, Wolvius EB, Rivadeneira F, et al. Skeletal maturation in relation to ethnic background in children of school age: the Generation R Study. Bone. 2020;132:115180.
87. Ontell F, Ivanovic M, Ablin DS, Barlow T. Bone age in children of diverse ethnicity. AJR American journal of roentgenology. 1996;167(6):1395-8.
88. Mora S, Boechat MI, Pietka E, Huang H, Gilsanz V. Skeletal age determinations in children of European and African descent: applicability of the Greulich and Pyle standards. Pediatric research. 2001;50(5):624-8.
89. Cole TJ, Rousham EK, Hawley NL, Cameron N, Norris SA, Pettifor JM. Ethnic and sex differences in skeletal maturation among the Birth to Twenty cohort in South Africa. Archives of disease in childhood. 2015;100(2):138-43.
90. Schmeling A, Reisinger W, Loreck D, Vendura K, Markus W, Geserick G. Effects of ethnicity on skeletal maturation: consequences for forensic age estimations. International journal of legal medicine. 2000;113(5):253-8.
91. Elamin F, Abdelazeem N, Elamin A, Saif D, Liversidge HM. Skeletal maturity of the hand in an East African group from Sudan. American Journal of Physical Anthropology. 2017;163(4):816-23.
92. Chaumoitre K, Lamtali S, Baali A, Saliba-Serre B, Lahmam A, Aboussad A, et al. Influence of socioeconomic status and body mass index on bone age. Hormone research in paediatrics. 2010;74(2):129-35.
93. Freitas D, Maia J, Beunen G, Lefevre J, Claessens A, Marques A, et al. Skeletal maturity and socio-economic status in Portuguese children and youths: the Madeira growth study. Annals of Human biology. 2004;31(4):408-20.
94. Sargent JD, Blanchflower DG. Obesity and stature in adolescence and earnings in young adulthood: analysis of a British birth cohort. Archives of pediatrics & adolescent medicine. 1994;148(7):681-7.
95. Klein KO, Larmore KA, de Lancey E, Brown JM, Considine RV, Hassink SG. Effect of obesity on estradiol level, and its relationship to leptin, bone maturation, and bone mineral density in children. The Journal of Clinical Endocrinology & Metabolism. 1998;83(10):3469-75.
96. Van Lenthe FJ, Kemper C, van Mechelen W. Rapid maturation in adolescence results in greater obesity in adulthood: the Amsterdam Growth and Health Study. The American journal of clinical nutrition. 1996;64(1):18-24.
97. De Simone M, Farello G, Palumbo M, Gentile T, Ciuffreda M, Olioso P, et al. Growth charts, growth velocity and bone development in childhood obesity. International journal of obesity and related metabolic disorders: journal of the International Association for the Study of Obesity. 1995;19(12):851-7.

98. Russell DL, Keil MF, Bonat SH, Uwaifo GI, Nicholson JC, McDuffie JR, et al. The relation between skeletal maturation and adiposity in African American and Caucasian children. The Journal of pediatrics. 2001;139(6):844-8.
99. Giuca MR, Pasini M, Tecco S, Marchetti E, Giannotti L, Marzo G. Skeletal maturation in obese patients. American journal of orthodontics and dentofacial orthopedics. 2012;142(6):774-9.
100. Ke D, Lu D, Cai G, Zhang J, Wang X, Suzuki K. Accelerated skeletal maturation is associated with overweight and obesity as early as preschool age: a cross-sectional study. BMC pediatrics. 2020;20(1):1-12.
101. Fahmy J, Kaminsky C, Kaufman F, Nelson M, Parisi M. The radiological approach to precocious puberty. The British Journal of Radiology. 2000;73(869):560-7.
102. Lewis C, Lavy C, Harrison W. Delay in skeletal maturity in Malawian children. The Journal of bone and joint surgery British volume. 2002;84(5):732-4.
103. Bonofiglio D, Garofalo C, Catalano S, Marsico S, Aquila S, Andò S. Low calcium intake is associated with decreased adrenal androgens and reduced bone age in premenarcheal girls in the last pubertal stages. Journal of bone and mineral metabolism. 2004;22(1):64-70.
104. Jahari A, Haas J, Husaini M, Pollitt E. Effects of an energy and micronutrient supplement on skeletal maturation in undernourished children in Indonesia. European Journal of Clinical Nutrition. 2000;54(2):S74-S9.
105. Sherar L, Cumming S. Physical activity and growth. Human Growth and Development: Elsevier; 2022. p. 469-90.
106. Torun B, Viteri F. Influence of exercise on linear growth. European journal of clinical nutrition. 1994;48(1):186-9.
107. Damsgaard R, Bencke J, Matthiesen G, Petersen JH, MÜLLER J. Is prepubertal growth adversely affected by sport? Medicine & Science in Sports & Exercise. 2000;32(10):1698-703.
108. Georgopoulos N, Markou K, Theodoropoulou A, Paraskevopoulou P, Varaki L, Kazantzi Z, et al. Growth and pubertal development in elite female rhythmic gymnasts. The Journal of Clinical Endocrinology & Metabolism. 1999;84(12):4525-30.
109. Gouda HN, Charlson F, Sorsdahl K, Ahmadzada S, Ferrari AJ, Erskine H, et al. Burden of non-communicable diseases in sub-Saharan Africa, 1990–2017: results from the Global Burden of Disease Study 2017. The Lancet Global Health. 2019;7(10):e1375-e87.
110. UNAIDS Factsheet. Global HIV statistics Fact Sheet 2023 (cited 20 Feb 2023) [Available from: https://www.unaids.org/sites/default/files/media_asset/UNAIDS_FactSheet_en.pdf.
111. Arpadi SM. Growth failure in children with HIV infection. Journal of acquired immune deficiency syndromes (1999). 2000;25:S37-42.
112. Banks LM, Zuurmond M, Ferrand R, Kuper H. The relationship between HIV and prevalence of disabilities in sub-Saharan Africa: systematic review (FA). Tropical medicine & international health. 2015;20(4):411-29.
113. Hanass-Hancock J, Nixon SA. The fields of HIV and disability: past, present and future. Journal of the International AIDS Society. 2009;12(1):28.
114. Gregson CL, Hartley A, Majonga E, McHugh G, Crabtree N, Rukuni R, et al. Older age at initiation of antiretroviral therapy predicts low bone mineral density in children with perinatally-infected HIV in Zimbabwe. Bone. 2019;125:96-102.
115. Lowenthal ED, Bakeera-Kitaka S, Marukutira T, Chapman J, Goldrath K, Ferrand RA. Perinatally acquired HIV infection in adolescents from sub-Saharan Africa: a review of emerging challenges. The Lancet infectious diseases. 2014;14(7):627-39.
116. Nakagawa F, Lodwick RK, Smith CJ, Smith R, Cambiano V, Lundgren JD, et al. Projected life expectancy of people with HIV according to timing of diagnosis. Aids. 2012;26(3):335-43.
117. Venturini E, Turkova A, Chiappini E, Galli L, de Martino M, Thorne C. Tuberculosis and HIV co-infection in children. BMC infectious diseases. 2014;14:1-10.
118. Irira M, Ngocho JS, Youze J, Shayo I, Komba V, Minja L, et al. Prevalence and outcome of HIV-associated malignancies among HIV-infected children enrolled into care at Kilimanjaro

Christian Medical Center 2006 to 2014: a hospital-based retrospective analytical study. Journal of pediatric hematology/oncology. 2020;42(1):69-73.
119. Stefan DC. Malignancies in children with HIV infection. Cancers in People with HIV and AIDS: Progress and Challenges: Springer; 2014. p. 349-57.
120. Isanaka S, Duggan C, Fawzi WW. Patterns of postnatal growth in HIV-infected and HIV-exposed children. Nutrition reviews. 2009;67(6):343-59.
121. Rukuni R, Rehman AM, Mukwasi-Kahari C, Madanhire T, Kowo-Nyakoko F, McHugh G, et al. Effect of HIV infection on growth and bone density in peripubertal children in the era of antiretroviral therapy: a cross-sectional study in Zimbabwe. The Lancet Child & Adolescent Health. 2021;5(8):569-81.
122. Omoni AO, Ntozini R, Evans C, Prendergast AJ, Moulton LH, Christian PS, et al. Child growth according to maternal and child HIV status in Zimbabwe. The Pediatric Infectious Disease Journal. 2017;36(9):869.
123. Study EC. Height, weight, and growth in children born to mothers with HIV-1 infection in Europe. Pediatrics. 2003;111(1):e52-e60.
124. Vancampfort D, Mugisha J, De Hert M, Probst M, Firth J, Gorczynski P, et al. Global physical activity levels among people living with HIV: a systematic review and meta-analysis. Disability and Rehabilitation. 2018;40(4):388-97.
125. Gallant JE, Staszewski S, Pozniak AL, DeJesus E, Suleiman JM, Miller MD, et al. Efficacy and safety of tenofovir DF vs stavudine in combination therapy in antiretroviral-naive patients: a 3-year randomized trial. Jama. 2004;292(2):191-201.
126. Han WM, Wattanachanya L, Apornpong T, Jantrapakde J, Avihingsanon A, Kerr SJ, et al. Bone mineral density changes among people living with HIV who have started with TDF-containing regimen: A five-year prospective study. PloS one. 2020;15(3):e0230368.
127. Okonkwo RI, Weidmann AE, Effa EE. Renal and bone adverse effects of a tenofovir-based regimen in the treatment of HIV-infected children: a systematic review. Drug safety. 2016;39:209-18.
128. Giacomet V, Maruca K, Ambrosi A, Zuccotti GV, Mora S. A 10–year follow– up of bone mineral density in HIV– infected youths receiving tenofovir disoproxil fumarate. International journal of antimicrobial agents. 2017;50(3):365-70.
129. Barlow-Mosha L, Musiime V, Davies MA, Prendergast AJ, Musoke P, Siberry G, et al. Universal antiretroviral therapy for HIV-infected children: a review of the benefits and risks to consider during implementation. African Journal of Reproduction and Gynaecological Endoscopy. 2017;20(1).
130. NMTPAC & THE AIDS and TB Directorate MHCC. Guidelines for HIV Prevention, Testing and Treatment of HIV in Zimbabwe August 2022 [

131. Simms V, Dauya E, Dakshina S, Bandason T, McHugh G, Munyati S, et al. Community burden of undiagnosed HIV infection among adolescents in Zimbabwe following primary healthcare-based provider-initiated HIV testing and counselling: a cross-sectional survey. PLoS medicine. 2017;14(7):e1002360.
132. Simms V, McHugh G, Dauya E, Bandason T, Mujuru H, Nathoo K, et al. Growth improvement following antiretroviral therapy initiation in children with perinatally-acquired HIV diagnosed in older childhood in Zimbabwe: a prospective cohort study. BMC pediatrics. 2022;22(1):446.
133. Feucht UD, Van Bruwaene L, Becker PJ, Kruger M. Growth in HIV-infected children on long-term antiretroviral therapy. Tropical Medicine & International Health. 2016;21(5):619-29.
134. Szubert AJ, Musiime V, Bwakura-Dangarembizi M, Nahirya-Ntege P, Kekitiinwa A, Gibb DM, et al. Pubertal development in HIV-infected African children on first-line antiretroviral therapy. Aids. 2015;29(5):609-18.
135. Holderbaum RM, Veeck EB, Oliveira HW, Silva CL, Fernandes Â. Comparison among dental, skeletal and chronological development in HIV-positive children: a radiographic study. Brazilian oral research. 2005;19(3):209-15.

136. Saavedra JM, Henderson RA, Perman JA, Hutton N, Livingston RA, Yolken RH. Longitudinal assessment of growth in children born to mothers with human immunodeficiency virus infection. Archives of pediatrics & adolescent medicine. 1995;149(5):497-502.
137. Kumar V, Patil K, Munoli KB. Comparative evaluation of dental age, bone age, and chronological age in the human immunodeficiency virus positive children. Journal of Pharmacy & Bioallied Sciences. 2014;6(Suppl 1):S90.
138. Donfield SM, Lynn HS, Lail AE, Hoots WK, Berntorp E, Gomperts ED. Delays in maturation among adolescents with hemophilia and a history of inhibitors. Blood, The Journal of the American Society of Hematology. 2007;110(10):3656-61.
139. De Onis M, Blössner M, Borghi E. Prevalence and trends of stunting among pre-school children, 1990–2020. Public health nutrition. 2012;15(1):142-8.
140. WHO_Factsheet. Manutrition 2024 [cited 2024 23 April]. Available from: www.who.int/news-room/fact-sheets/detail/malnutrition?gad_source.
141. World Health Organisation. Double burden of nutrition [Available from: https://www.who.int/nutrition/double-burden-malnutrition/en/. (cited 25 Jun 2024)
142. Phiri M, Maruta EP, Chazireni E. Poverty in Zimbabwe: A Critical Review. European Journal of Social Sciences Studies. 2020;5(5).
143. Kidia KK. The future of health in Zimbabwe. Global health action. 2018;11(1):1496888.
144. Chingwaru W, Vidmar J. Culture, myths and panic: Three decades and beyond with an HIV/AIDS epidemic in Zimbabwe. Global public health. 2018;13(2):249-64.
145. The world bank data (cited 19 Aug 2024) [Available from: https://data.worldbank.org/indicator/SH.STA.STNT.ZS?locations=GM.
146. Aly SM, Shrestha B, Hong DJ, Omran A, Wang W. Identification of age and sex based on knee radiography. Forensic science international. 2016;267:231. e1-. e7.
147. Ebeye OA, Eboh DE, Onyia NS. Radiological assessment of age from epiphyseal fusion at the knee joint. Anatomy. 2016;10(1):1-7.
148. Sullivan S, Flavel A, Franklin D. Age estimation in a sub-adult Western Australian population based on the analysis of the pelvic girdle and proximal femur. Forensic science international. 2017;281:185. e1-. e10.
149. Li Y, Huang Z, Dong X, Liang W, Xue H, Zhang L, et al. Forensic age estimation for pelvic X-ray images using deep learning. European radiology. 2019;29:2322-9.
150. Todd TW. Atlas of skeletal maturation (hand). St Louis: CV Mosby. 1937:303.
151. Pyle SI, Hoerr NL. Radiographic atlas of skeletal development of the knee: a standard of reference: Charles C. Thomas; 1955.
152. Martin DD, Wit JM, Hochberg Ze, Sävendahl L, Van Rijn RR, Fricke O, et al. The use of bone age in clinical practice–part 1. Hormone research in paediatrics. 2011;76(1):1-9.
153. World Nuclear Association. What is background radiation [Available from: https://world-nuclear.org/images/articles/4_Background_Radiation(1).pdf.
154. Mackenzie K, Peters M. Handedness, hand roles, and hand injuries at work. Journal of safety research. 2000;31(4):221-7.
155. Duckworth W. The international agreement for the unification of anthropometric measurements to be made on the living subject. Report of the commission appointed by the XIVth International Congress of prehistoric anthropology and archaeology at Geneva (1912), to supplement the work commenced by the XIIIth Congress in the session at Monaco (1906). English translation of the official version. American Journal of Physical Anthropology. 1919;2(1):61-7.
156. Satoh M. Bone age: assessment methods and clinical applications. Clinical Pediatric Endocrinology. 2015;24(4):143-52.
157. Dreizen S, Snodgrasse RM, Webb-Peploe H, Parker GS, Spies TD. Bilateral symmetry of skeletal maturation in the human hand and wrist. AMA Journal of Diseases of Children. 1957;93(2):122-7.
158. Alshamrani K, Messina F, Offiah AC. Is the Greulich and Pyle atlas applicable to all ethnicities? A systematic review and meta-analysis. European radiology. 2019;29(6):2910-23.

159. Roche A, Rohmann CG, French NY, Dávila GH. Effect of training on replicability of assessments of skeletal maturity (Greulich-Pyle). American Journal of Roentgenology. 1970;108(3):511-5.
160. Creo AL, Schwenk WF. Bone age: a handy tool for pediatric providers. Pediatrics. 2017;140(6).
161. Buckler J. How to make the most of bone ages. Archives of disease in childhood. 1983;58(10):761.
162. Reynolds EL, Sontag LW. Seasonal variations in weight, height, and appearance of ossification centers. Journal of Pediatrics. 1944;24:524-35.
163. Eichelberger DA, Chaouch A, Rousson V, Kakebeeke TH, Caflisch J, Wehrle FM, et al. Secular trends in physical growth, biological maturation, and intelligence in children and adolescents born between 1978 and 1993. Frontiers in Public Health. 2024;12:1216164.
164. Centre ICE. Growth and puberty secular trends, environmental and genetic factors. 2007.
165. Acheson RM. 3 The Oxford Method of Assessing Skeletal Maturity. Clinical Orthopaedics and Related Research®. 1957;10:19-39.
166. Tanner JM. A new system for estimating skeletal maturity from the hand and wrist, with standards derived from a study of 2600 healthy British children. Part II: the scoring system. 1959.
167. Tanner JM. Assessement of Skeletal Maturity and Predicting of Adult Height (TW2 Method). Prediction of adult height. 1983:22-37.
168. Tanner JM, Whitehouse R, Cameron N, Marshall W, Healy M, Goldstein H. Assessment of skeletal maturity and prediction of adult height (TW2 method): Saunders London; 2001.
169. King D, Steventon D, O'sullivan M, Cook A, Hornsby V, Jefferson I, et al. Reproducibility of bone ages when performed by radiology registrars: an audit of Tanner and Whitehouse II versus Greulich and Pyle methods. The British journal of radiology. 1994;67(801):848-51.
170. Mughal AM, Hassan N, Ahmed A. Bone age assessment methods: a critical review. Pakistan journal of medical sciences. 2014;30(1):211.
171. Khan K, Elayappen AS. Bone growth estimation using radiology (Greulich–Pyle and Tanner–Whitehouse methods). Handbook of growth and growth monitoring in health and disease: Springer; 2012. p. 2937-53.
172. Thodberg HH, Kreiborg S, Juul A, Pedersen KD. The BoneXpert method for automated determination of skeletal maturity. IEEE transactions on medical imaging. 2008;28(1):52-66.
173. Lepe GP, Villacrés F, Silva F C, Guiloff S. Correlation in radiological bone age determination using the Greulich and Pyle method versus automated evaluation using BoneXpert software. 2018.
174. Artioli TO, Alvares MA, Carvalho Macedo VS, Silva TS, Avritchir R, Kochi C, et al. Bone age determination in eutrophic, overweight and obese Brazilian children and adolescents: a comparison between computerized BoneXpert and Greulich-Pyle methods. Pediatric radiology. 2019;49:1185-91.
175. Bhandari P, Parihar AS, Gupta A, Sharma G. A Bone Age Assessment–A Critical Review. Journal of Advanced Medical and Dental Sciences Research. 2019;7(10):37-40.
176. Cox LA. Tanner-Whitehouse method of assessing skeletal maturity: problems and common errors. Hormone Research in Paediatrics. 1996;45(Suppl. 2):53-5.
177. Cole AL, Webb L, Cole T. Bone age estimation: a comparison of methods. The British journal of radiology. 1988;61(728):683-6.
178. Beunen G, Cameron N. The reproducibility of TW2 skeletal age assessments by a self-taught assessor. Annals of Human Biology. 1980;7(2):155-62.
179. Wit J, Rekers-Mombarg L, Dutch Growth Hormone Advisory Group. Final height gain by GH therapy in children with idiopathic short stature is dose dependent. The Journal of Clinical Endocrinology & Metabolism. 2002;87(2):604-11.
180. Arends N, Boonstra V, Mulder P, Odink R, Stokvis-Brantsma W, Rongen-Westerlaken C, et al. GH treatment and its effect on bone mineral density, bone maturation and growth in short

children born small for gestational age: 3-year results of a randomized, controlled GH trial. Clinical Endocrinology. 2003;59(6):779-87.
181. Ording Müller L-S, Offiah A, Adamsbaum C, Barber I, Di Paolo PL, Humphries P, et al. Bone age for chronological age determination—statement of the European Society of Paediatric Radiology musculoskeletal task force group. Pediatric radiology. 2019;49:979-82.
182. Cole T. The evidential value of developmental age imaging for assessing age of majority. Annals of human biology. 2015;42(4):379-88.
183. Cerda VX. Office of Detention and Removal Operations, US Department of Homeland Security. Age Determination Procedures for Custody Decisions. 2004.
184. Garamendi P, Landa M, Ballesteros J, Solano M. Reliability of the methods applied to assess age minority in living subjects around 18 years old: a survey on a Moroccan origin population. Forensic science international. 2005;154(1):3-12.
185. Patel PS, Chaudhary AR, Dudhia BB, Bhatia PV, Soni NC, Jani YV. Accuracy of two dental and one skeletal age estimation methods in 6-16 year old Gujarati children. Journal of forensic dental sciences. 2015;7(1):18.
186. Dorling K. Asylum seeker age assessments. Children & Young People Now. 2017;2017(8):45-.
187. Ostojic S. Prediction of adult height by Tanner–Whitehouse method in young Caucasian male athletes. QJM: An International Journal of Medicine. 2013;106(4):341-5.
188. Zhang A, Sayre JW, Vachon L, Liu BJ, Huang H. Racial differences in growth patterns of children assessed on the basis of bone age. Radiology. 2009;250(1):228-35.
189. Christian AK, Dake FA. Profiling household double and triple burden of malnutrition in sub-Saharan Africa: prevalence and influencing household factors. Public Health Nutrition. 2022;25(6):1563-76.
190. van Rijn RR, Lequin MH, Robben SG, Hop WC, van Kuijk C. Is the Greulich and Pyle atlas still valid for Dutch Caucasian children today? Pediatric radiology. 2001;31(10):748-52.
191. Roche A, Davila G, Eyman S. A comparison between Greulich-Pyle and Tanner-Whitehouse assessments of skeletal maturity. Radiology. 1971;98(2):273-80.
192. Paxton ML, Lamont AC, Stillwell AP. The reliability of the G reulich–P yle method in bone age determination among A ustralian children. Journal of medical imaging and radiation oncology. 2013;57(1):21-4.
193. Kullman L. Accuracy of two dental and one skeletal age estimation method in Swedish adolescents. Forensic science international. 1995;75(2-3):225-36.
194. Mansourvar M, Ismail MA, Raj RG, Kareem SA, Aik S, Gunalan R, et al. The applicability of Greulich and Pyle atlas to assess skeletal age for four ethnic groups. Journal of forensic and legal medicine. 2014;22:26-9.
195. JOHNSTON FE. Skeletal age and its prediction in Philadelphia children. Human biology. 1963;35(2):192-202.
196. Andersen E. Comparison of Tanner-Whitehouse and Greulich-Pyle methods in a large scale Danish survey. American journal of physical anthropology. 1971;35(3):373-6.
197. Koc A, Karaoglanoglu M, Erdogan M, Kosecik M, Cesur Y. Assessment of bone ages: is the Greulich-Pyle method sufficient for Turkish boys? Pediatrics international. 2001;43(6):662-5.
198. Büken B, Erzengin ÖU, Büken E, Şafak AA, Yazıcı B, Erkol Z. Comparison of the three age estimation methods: which is more reliable for Turkish children? Forensic science international. 2009;183(1-3):103. e1-. e7.
199. Hackman L, Black S. The Reliability of the G reulich and P yle Atlas When Applied to a Modern S cottish Population. Journal of forensic sciences. 2013;58(1):114-9.
200. Loder RT, Estle DT, Morrison K, Eggleston D, Fish DN, Greenfield ML, et al. Applicability of the Greulich and Pyle skeletal age standards to black and white children of today. American Journal of Diseases of Children. 1993;147(12):1329-33.

201. Zabet D, Rérolle C, Pucheux J, Telmon N, Saint-Martin P. Can the Greulich and Pyle method be used on French contemporary individuals? International journal of legal medicine. 2015;129:171-7.
202. Kim JR, Lee YS, Yu J. Assessment of bone age in prepubertal healthy Korean children: comparison among the Korean standard bone age chart, Greulich-Pyle method, and Tanner-Whitehouse method. Korean journal of radiology. 2015;16(1):201-5.
203. Moradi M, Sirous M, Morovatti P. The reliability of skeletal age determination in an Iranian sample using Greulich and Pyle method. Forensic science international. 2012;223(1-3):372. e1-. e4.
204. Soudack M, Ben-Shlush A, Jacobson J, Raviv-Zilka L, Eshed I, Hamiel O. Bone age in the 21st century: is Greulich and Pyle's atlas accurate for Israeli children? Pediatric radiology. 2012;42:343-8.
205. Patil ST, Parchand M, Meshram M, Kamdi N. Applicability of Greulich and Pyle skeletal age standards to Indian children. Forensic science international. 2012;216(1-3):200. e1-. e4.
206. Awais M, Nadeem N, Husen Y, Rehman A, Beg M, Khattak YJ. Comparison between Greulich-Pyle and Girdany-Golden methods for estimating skeletal age of children in Pakistan. Journal of the College of Physicians and Surgeons Pakistan. 2014;24(12):889.
207. Büken B, Şafak AA, Büken E, Yazici B, Erkol Z, ERZENGİN ÖU. Is the Tanner–Whitehouse (TW3) method sufficiently reliable for forensic age determination of Turkish children? Turkish Journal of Medical Sciences. 2010;40(5):797-805.
208. Gao C, Qian Q, Li Y, Xing X, He X, Lin M, et al. A comparative study of three bone age assessment methods on Chinese preschool-aged children. Frontiers in Pediatrics. 2022;10:976565.
209. Maggio A, Flavel A, Hart R, Franklin D. Skeletal age estimation in a contemporary Western Australian population using the Tanner–Whitehouse method. Forensic science international. 2016;263:e1-e8.
210. Schmidt S, Nitz I, Schulz R, Schmeling A. Applicability of the skeletal age determination method of Tanner and Whitehouse for forensic age diagnostics. International journal of legal medicine. 2008;122:309-14.
211. Alshamrani K, Offiah AC. Applicability of two commonly used bone age assessment methods to twenty-first century UK children. European radiology. 2020;30(1):504-13.
212. Zhang S-Y, Liu L-J, Wu Z-L, Liu G, Ma Z-G, Shen X-Z, et al. Standards of TW3 skeletal maturity for Chinese children. Annals of human biology. 2008;35(3):349-54.
213. Ashizawa K, Asami T, Anzo M, Matsuo N, Matsuoka H, Murata M, et al. Standard RUS skeletal maturation of Tokyo children. Annals of human biology. 1996;23(6):457-69.
214. Dembetembe KA, Morris AG. Is Greulich-Pyle age estimation applicable for determining maturation in male Africans? South African Journal of Science. 2012;108(9):1-6.
215. Pinchi V, De Luca F, Ricciardi F, Focardi M, Piredda V, Mazzeo E, et al. Skeletal age estimation for forensic purposes: A comparison of GP, TW2 and TW3 methods on an Italian sample. Forensic science international. 2014;238:83-90.
216. Alshamrani K, Hewitt A, Offiah AC. Applicability of two bone age assessment methods to children from Saudi Arabia. Clinical radiology. 2020;75(2):156. e1-. e9.
217. Milner G, Levick R, Kay R. Assessment of bone age: a comparison of the Greulich and Pyle, and the Tanner and Whitehouse methods. Clinical radiology. 1986;37(2):119-21.
218. Kim SY, Yang SW. Assessment of Bone Age: A comparison of the Greulich Pyle Method to the Tanner Whitehouse Method. Journal of Korean Endocrine Society. 2001;13(2):198-204.
219. Kim SY, Oh YJ, Shin JY, Rhie YJ, Lee KH. Comparison of the Greulich-Pyle and Tanner Whitehouse (TW3) Methods in Bone Age Assessment. Journal of Korean Society of Pediatric Endocrinology. 2008;13(1):50-5.
220. Hawley NL, Rousham EK, Johnson W, Norris SA, Pettifor JM, Cameron N. Determinants of relative skeletal maturity in South African children. Bone. 2012;50(1):259-64.

221. Demerath EW, Jones LL, Hawley NL, Norris SA, Pettifor JM, Duren D, et al. Rapid infant weight gain and advanced skeletal maturation in childhood. The Journal of pediatrics. 2009;155(3):355-61.
222. Powell S, Ma D, Jones G. Determinants of skeletal age deviation in a cross-sectional study. The Journal of Clinical Endocrinology & Metabolism. 2008;93(2):521-6.
223. Jones G, Ma D. Skeletal age deviation assessed by the Tanner–Whitehouse 2 method is associated with bone mass and fracture risk in children. Bone. 2005;36(2):352-7.
224. Rukuni R, Gregson C, Kahari C, Kowo F, McHugh G, Munyati S, et al. The IMpact of Vertical HIV infection on child and Adolescent SKeletal development in Harare, Zimbabwe (IMVASK Study): a protocol for a prospective cohort study. BMJ open. 2020;10(2):e031792.
225. Mukwasi-Kahari C, Rehman AM, Ó Breasail M, Rukuni R, Madanhire T, Chipanga J, et al. Impaired Bone Architecture in Peripubertal Children With HIV, Despite Treatment With Antiretroviral Therapy: A Cross-Sectional Study From Zimbabwe. Journal of Bone and Mineral Research. 2022.
226. Peng C-T, Chan Y-K, Yu S-S. Data Imbalance Immunity Bone Age Assessment System Using Independent Autoencoders. Applied Sciences. 2022;12(16):7974.
227. Baird J, Walker I, Smith C, Inskip H. Review of methods for determining pubertal status and age of onset of puberty in cohort and longitudinal studies. London, UK: CLOSER. 2017.
228. Duke PM, Litt IF, Gross RT. Adolescents' self-assessment of sexual maturation. Pediatrics. 1980;66(6):918-20.
229. Vyas S, Kumaranayake L. Constructing socio-economic status indices: how to use principal components analysis. Health policy and planning. 2006;21(6):459-68.
230. Filteau S, Rehman AM, Yousafzai A, Chugh R, Kaur M, Sachdev H, et al. Associations of vitamin D status, bone health and anthropometry, with gross motor development and performance of school-aged Indian children who were born at term with low birth weight. BMJ open. 2016;6(1):e009268.
231. USAID Working Group on Infant and Young Child Feeding Indicators. Developing and validating simple indicators of dietary quality and energy intake of infants and young children in developing countries: summary of findings from analysis of 10 data sets.: Food and Nutrition Technical Assistance Project (FANTA). 2006 [Available from: https://www.fantaproject.org/publications/HIVguide.shtml?page=15. (cited 25 Jun 2024)
232. Infant WGo, Indicators YCF. Developing and validating simple indicators of dietary quality and energy intake of infants and young children in developing countries: summary of findings from analysis of 10 data sets. (FANTA) Project Washington, DC, USA; 2006.
233. Craig CL, Marshall AL, Sjöström M, Bauman AE, Booth ML, Ainsworth BE, et al. International physical activity questionnaire: 12-country reliability and validity. Medicine and science in sports and exercise. 2003;35(8):1381-95.
234. Roche AF. Bone growth and maturation. Postnatal Growth Neurobiology: Springer; 1986. p. 25-60.
235. Cole TJ, Freeman JV, Preece MA. British 1990 growth reference centiles for weight, height, body mass index and head circumference fitted by maximum penalized likelihood. Statistics in medicine. 1998;17(4):407-29.
236. Giavarina D. Understanding bland altman analysis. Biochemia medica. 2015;25(2):141-51.
237. Shechtman O. The coefficient of variation as a measure of sincerity of effort of grip strength, Part II: sensitivity and specificity. Journal of Hand Therapy. 2001;14(3):188-94.
238. Glüer C-C, Blake G, Lu Y, Blunt B, Jergas M, Genant H. Accurate assessment of precision errors: how to measure the reproducibility of bone densitometry techniques. Osteoporosis international. 1995;5(4):262-70.
239. Maggio A, Flavel A, Hart R, Franklin D. Assessment of the accuracy of the Greulich and Pyle hand-wrist atlas for age estimation in a contemporary Australian population. Australian Journal of Forensic Sciences. 2018;50(4):385-95.

240. Cavallo F, Mohn A, Chiarelli F, Giannini C. Evaluation of Bone Age in Children: A Mini-Review. Frontiers in Pediatrics. 2021;9:21.
241. Alshamrani K, Hewitt A, Offiah A. Applicability of two bone age assessment methods to children from Saudi Arabia. Clinical Radiology. 2020;75(2):156. e1-. e9.
242. Zafar AM, Nadeem N, Husen Y, Ahmad MN. An appraisal of Greulich-Pyle Atlas for skeletal age assessment in Pakistan. JPMA The Journal of the Pakistan Medical Association. 2010;60(7):552.
243. Delemarre-van de Waal HA. Environmental factors influencing growth and pubertal development. Environmental health perspectives. 1993;101(suppl 2):39-44.
244. Koo W, Walyat N. Vitamin D and skeletal growth and development. Current osteoporosis reports. 2013;11(3):188-93.
245. Kowo-Nyakoko F, Gregson CL, Madanhire T, Stranix-Chibanda L, Rukuni R, Offiah AC, et al. Evaluation of two methods of bone age assessment in peripubertal children in Zimbabwe. Bone. 2023:116725.
246. Organisation WH. WHO Growth reference data, weight-for-age (5-10years) [Available from: https://www.who.int/tools/growth-reference-data-for-5to19-years/indicators/weight-for-age-5to10-years. (cited 25 Jun 2024)
247. Caulfield LE, Richard SA, Rivera JA, Musgrove P, Black RE. Stunting, wasting, and micronutrient deficiency disorders. Disease Control Priorities in Developing Countries 2nd edition. 2006.
248. Shlisky J, Mandlik R, Askari S, Abrams S, Belizan JM, Bourassa MW, et al. Calcium deficiency worldwide: Prevalence of inadequate intakes and associated health outcomes. Wiley Online Library; 2022. Report No.: 0077-8923.
249. Low W. Relation between socio-economic status and skeletal maturation of Chinese children. Zeitschrift für Morphologie und Anthropologie. 1972(H. 1):1-11.
250. Shrestha N. Detecting multicollinearity in regression analysis. American Journal of Applied Mathematics and Statistics. 2020;8(2):39-42.
251. SACN Report. Vitamin D and Health 2016 [Available from: https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/537616/SACN_Vitamin_D_and_Health_report.pdf. (cited 26 Jun 2024)
252. Garcia SAB, Guzman N. Acquired Immune Deficiency Syndrome CD4+ Count. StatPearls [Internet]: StatPearls Publishing; 2021.
253. Holderbaum RM, Veeck EB, Oliveira HW, Silva CL, Fernandes Â. Comparison among dental, skeletal and chronological development in HIV-positive children: a radiographic study. Brazilian Oral Research. 2005;19:209-15.
254. Weiser SD, Palar K, Frongillo EA, Tsai AC, Kumbakumba E, Depee S, et al. Longitudinal assessment of associations between food insecurity, antiretroviral adherence and HIV treatment outcomes in rural Uganda. Aids. 2014;28(1):115-20.
255. Negash T, Ehlers V. Personal factors influencing patients' adherence to ART in Addis Ababa, Ethiopia. Journal of the Association of Nurses in AIDS Care. 2013;24(6):530-8.
256. Uzochukwu B, Onwujekwe O, Onoka A, Okoli C, Uguru N, Chukwuogo O. Determinants of non-adherence to subsidized anti-retroviral treatment in southeast Nigeria. Health policy and planning. 2009;24(3):189-96.
257. Govender D, Goodier M. Bone of contention: The applicability of the Greulich–Pyle method for skeletal age assessment in South Africa. SA Journal of Radiology. 2018;22(1):1-6.
258. Schomaker M, Leroy V, Wolfs T, Technau K-G, Renner L, Judd A, et al. Optimal timing of antiretroviral treatment initiation in HIV-positive children and adolescents: a multiregional analysis from Southern Africa, West Africa and Europe. International journal of epidemiology. 2017;46(2):453-65.
259. Violari A, Cotton MF, Gibb DM, Babiker AG, Steyn J, Madhi SA, et al. Early antiretroviral therapy and mortality among HIV-infected infants. New England Journal of Medicine. 2008;359(21):2233-44.

260. Shiau S, Strehlau R, Technau K-G, Patel F, Arpadi SM, Coovadia A, et al. Early age at start of antiretroviral therapy associated with better virologic control after initial suppression in HIV-infected infants. Aids. 2017;31(3):355-64.
261. Organization WH. Consolidated guidelines on HIV prevention, testing, treatment, service delivery and monitoring: recommendations for a public health approach: World Health Organization; 2021.
262. Insight Start Study Group. Initiation of antiretroviral therapy in early asymptomatic HIV infection. New England Journal of Medicine. 2015;373(9):795-807.
263. Group TAS. A trial of early antiretrovirals and isoniazid preventive therapy in Africa. New England Journal of Medicine. 2015;373(9):808-22.
264. Baranek B, Wang S, Cheung AM, Mishra S, Tan DH. The effect of tenofovir disoproxil fumarate on bone mineral density: a systematic review and meta-analysis. Antiviral therapy. 2020;25(1):21-32.
265. Grant PM, Cotter AG. Tenofovir and bone health. Current Opinion in HIV and AIDS. 2016;11(3):326.
266. Grigsby IF, Pham L, Mansky LM, Gopalakrishnan R, Mansky KC. Tenofovir-associated bone density loss. Therapeutics and Clinical Risk Management. 2010:41-7.
267. Kumar V, Venkataraghavan K, Krishnan R, Patil K, Munoli K, Karthik S. The relationship between dental age, bone age and chronological age in underweight children. Journal of pharmacy & bioallied sciences. 2013;5(Suppl 1):S73.
268. Hedayati Z, Khalafinejad F. Relationship between body mass index, skeletal maturation and dental development in 6-to 15-year old orthodontic patients in a sample of Iranian population. Journal of Dentistry. 2014;15(4):180.
269. Lebenthal Y, Yackobovitch-Gavan M, Lazar L, Shalitin S, Tenenbaum A, Shamir R, et al. Effect of a nutritional supplement on growth in short and lean prepubertal children: a prospective, randomized, double-blind, placebo-controlled study. The Journal of Pediatrics. 2014;165(6):1190-3. e1.
270. Collaborative Initiative for Paediatric HIV Education Research Global Cohort Collaboration, Jesson J, Crichton S, Quartagno M, Yotebieng M, Abrams EJ, et al. Growth and CD4 patterns of adolescents living with perinatally acquired HIV worldwide, a CIPHER cohort collaboration analysis. Journal of the International AIDS Society. 2022;25(3):e25871.
271. Sutcliffe CG, van Dijk JH, Munsanje B, Hamangaba F, Sinywimaanzi P, Thuma PE, et al. Weight and height z-scores improve after initiating ART among HIV-infected children in rural Zambia: a cohort study. BMC infectious diseases. 2011;11:1-7.
272. Weigel R, Phiri S, Chiputula F, Gumulira J, Brinkhof M, Gsponer T, et al. Growth response to antiretroviral treatment in HIV-infected children: a cohort study from Lilongwe, Malawi. Tropical medicine & international health. 2010;15(8):934-44.
273. Naidoo R, Rennert W, Lung A, Naidoo K, McKerrow N. The influence of nutritional status on the response to HAART in HIV-infected children in South Africa. The Pediatric infectious disease journal. 2010;29(6):511-3.
274. Madanhire T, Macdougall A, Kasonka L, Mabuda HB, Chisenga M, Mujuru H, et al. Patterns of linear growth among children and adolescents living with HIV on antiretroviral therapy in Zimbabwe and Zambia. BMC Infectious Diseases. 2025;25(1):269.
275. Freckleton RP. On the misuse of residuals in ecology: regression of residuals vs. multiple regression. Journal of Animal Ecology. 2002:542-5.
276. Ward KA, Jarjou L, Prentice A. Long-term effects of maternal calcium supplementation on childhood growth differ between males and females in a population accustomed to a low calcium intake. Bone. 2017;103:31-8.
277. Beunen GP, Malina RM, Renson R, Simons J, Ostyn M, Lefevre J. Physical activity and growth, maturation and performance: a longitudinal study. Medicine and science in sports and exercise. 1992;24(5):576-85.

278. Hallal PC, Victora CG, Azevedo MR, Wells JC. Adolescent physical activity and health: a systematic review. Sports medicine. 2006;36(12):1019-30.
279. Tanaka LF, Latorre MdRDdO, Silva AM, Konstantyner TCRdO, Peres SV, Marques HHdS. High prevalence of physical inactivity among adolescents living with HIV/AIDS. Revista Paulista de Pediatria. 2015;33:326-31.
280. Somarriba G, Lopez-Mitnik G, Ludwig DA, Neri D, Schaefer N, Lipshultz SE, et al. Physical fitness in children infected with the human immunodeficiency virus: associations with highly active antiretroviral therapy. AIDS research and human retroviruses. 2013;29(1):112-20.
281. Burt LA, Greene DA, Ducher G, Naughton GA. Skeletal adaptations associated with pre-pubertal gymnastics participation as determined by DXA and pQCT: a systematic review and meta-analysis. Journal of Science and Medicine in Sport. 2013;16(3):231-9.
282. Jürimäe J, Gruodyte-Raciene R, Baxter-Jones AD. Effects of gymnastics activities on bone accrual during growth: A systematic review. Journal of sports science & medicine. 2018;17(2):245.
283. Vainionpää A, Korpelainen R, Sievänen H, Vihriälä E, Leppäluoto J, Jämsä T. Effect of impact exercise and its intensity on bone geometry at weight-bearing tibia and femur. Bone. 2007;40(3):604-11.
284. Beckmann J, Lang C, du Randt R, Gresse A, Long KZ, Ludyga S, et al. Prevalence of stunting and relationship between stunting and associated risk factors with academic achievement and cognitive function: A cross-sectional study with South African primary school children. International journal of environmental research and public health. 2021;18(8):4218.
285. Widiastuti A, Ulkhasanah ME, Sani FN, Kartikasari AY, editors. DESCRIPTION OF STIGMA FOR STUNTING CHILDREN. Proceeding of International Conference on Science, Health, And Technology; 2022.
286. Anita S, Novita N, Vasra E. Factors Associated with Stunting Incidents in Toddlers. Journal of Maternal and Child Health Sciences (JMCHS). 2023;3(1):49-56.
287. Kip EC, Udedi M, Kulisewa K, Go VF, Gaynes BN. Stigma and mental health challenges among adolescents living with HIV in selected adolescent-specific antiretroviral therapy clinics in Zomba District, Malawi. BMC pediatrics. 2022;22(1):253.
288. Calderwood CJ, Marambire E, Nzvere FP, Larsson LS, Chingono RM, Kavenga F, et al. Prevalence of chronic conditions and multimorbidity among healthcare workers in Zimbabwe: Results from a screening intervention. PLOS Global Public Health. 2024;4(1):e0002630.
289. Gonah L, Moodley I, Hlongwana K. Capacity of antiretroviral therapy sites for managing NCDs in people living with HIV in Zimbabwe. Southern African Journal of HIV Medicine. 2020;21(1).
290. World Food Programme. Zimbabwe Annual Country Report 2023-Country Strategic Plan 2022-2026 2023 [Available from: https://reliefweb.int/report/zimbabwe/zimbabwe-annual-country-report-2023-country-strategic-plan-2022-2026. (cited 21 Aug 2024)
291. World Food Programme. Zimbabwe 2024 [Available from: https://www.wfp.org/search?search_api_fulltext=zimbabwe. (cited 19 Aug 2024)
292. World Bank. Measuring poverty 2023 [Available from: https://www.worldbank.org/en/topic/measuringpoverty. (cited 19 Aug 2024)
293. Amoadu M, Abraham SA, Adams AK, Akoto-Buabeng W, Obeng P, Hagan Jr JE. Risk Factors of Malnutrition among In-School Children and Adolescents in Developing Countries: A Scoping Review. Children. 2024;11(4):476.
294. Alves JGB, Alves LV. Early-life nutrition and adult-life outcomes. Jornal de Pediatria. 2024;100:S4-S9.
295. Forsdahl A. Are poor living conditions in childhood and adolescence an important risk factor for arteriosclerotic heart disease? Journal of Epidemiology & Community Health. 1977;31(2):91-5.
296. Dzavakwa NV, Chisenga M, McHugh G, Filteau S, Gregson CL, Kasonka L, et al. Vitamin D3 and calcium carbonate supplementation for adolescents with HIV to reduce musculoskeletal

morbidity and immunopathology (VITALITY trial): study protocol for a randomised placebo-controlled trial. Trials. 2022;23(1):78.
297. Dzavakwa NV, Chisenga M, McHugh G, Filteau S, Gregson CL, Kasonka L, et al. Update: Vitamin D3 and calcium carbonate supplementation for adolescents with HIV to reduce musculoskeletal morbidity and immunopathology (VITALITY trial): study protocol for a randomised placebo-controlled trial. Trials. 2024;25(1):499.
298. Manyanga T, Makaza D, Munambah NE, Mahachi C, Mavingire C, Mlalazi TF, et al. Indicators of physical activity and nutritional status among children and adolescents in Zimbabwe: Findings from three global matrix initiatives. Journal of Exercise Science & Fitness. 2023;21(2):202-9.
299. Gerede K. Backyard colleges compromise education. 2015.
300. Loprinzi PD, Cardinal BJ, Loprinzi KL, Lee H. Benefits and environmental determinants of physical activity in children and adolescents. Obesity facts. 2012;5(4):597-610.
301. Kranzer K, Bradley J, Musaazi J, Nyathi M, Gunguwo H, Ndebele W, et al. Loss to follow-up among children and adolescents growing up with HIV infection: age really matters. African Journal of Reproduction and Gynaecological Endoscopy. 2017;20(1).
302. UNICEF. Country Office Annual Report 2022 2022 [Available from: https://www.unicef.org/media/136821/file/Zimbabwe-2022-COAR.pdf. (cited 23 Aug 2024)
303. Van de Poel E, O'Donnell O, Van Doorslaer E. Are urban children really healthier? Evidence from 47 developing countries. Social science & medicine. 2007;65(10):1986-2003.
304. Fotso J-C. Urban–rural differentials in child malnutrition: trends and socioeconomic correlates in sub-Saharan Africa. Health & place. 2007;13(1):205-23.
305. Mwambenu B, Ramoloko V, Laubscher R, Feucht U. Growth and the pubertal growth spurt in South African adolescents living with perinatally-acquired HIV infection. Plos one. 2022;17(1):e0262816.
306. Bakeera-Kitaka S, McKellar M, Snider C, Kekitiinwa A, Piloya T, Musoke P, et al. Antiretroviral therapy for HIV-1 infected adolescents in Uganda: assessing the impact on growth and sexual maturation. Journal of Pediatric Infectious Diseases. 2008;3(02):097-104.
307. George CM, Coglianese N, Bauler S, Perin J, Kuhl J, Williams C, et al. Low dietary diversity is associated with linear growth faltering and subsequent adverse child developmental outcomes in rural Democratic Republic of the Congo (REDUCE program). Maternal & Child Nutrition. 2022;18(3):e13340.
308. Shetty P. Measures of nutritional status from anthropometric survey data. 2003.