Skeletal and Muscle Health Among Rural South Indian Older Population

by

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Abstract

India is at early stages of demographic transition to an aging society, but little is known about musculoskeletal health. Mobility and Independent Living among Elders' Study (MILES), a rural south Indian older adults' cohort, provides an opportunity to expand our musculoskeletal health understanding. This dissertation goal was to compare peripheral quantitative computed tomography (pQCT) parameters of Indian population with other ethnic groups and, assess its associations with mortality and physical performance.

We observed Indian men compared to US Caucasians had significantly lower trabecular volumetric bone mineral density (vBMD), cortical thickness and higher endosteal circumference, suggesting higher risk of osteoporotic fractures.

Over an average follow up of 64.2 months, among 499 MILES participants, 123 died (73 men; 50 women). Among men, trabecular vBMD (radius and tibia), cortical vBMD (radius and tibia), cortical thickness (radius and tibia), polar strength strain index (SSIp) of tibia and muscle density were inversely associated and, endosteal circumference (radius and tibia) were positively associated with mortality. Among women cortical vBMD (radius and tibia), cortical thickness (radius and tibia), SSIp (radius and tibia) were inversely associated, and endosteal circumference (radius and tibia) were positively associated with mortality. Gait speed among men mediated the muscle density and mortality association.

On cross sectional analysis; among men, muscle density was associated with grip strength; cortical vBMD (radius), trabecular vBMD (tibia), cortical thickness (tibia), endosteal circumference (tibia) and muscle density were associated with short physical performance

battery (SPPB); cortical thickness (tibia) was associated with 400-meter walk. Among women, trabecular vBMD (radius and tibia), cortical thickness (radius and tibia), SSIp (radius) and muscle density were associated with grip strength; cortical vBMD and SSIp of tibia and muscle density were associated with SPPB and; trabecular vBMD (tibia) was associated with 400-meter walk.

These dissertation findings have public health importance; as it suggests Indian older population have lower bone strength indices. It presents for the first time mortality and physical performance associations with bone and muscle measures. These findings indicate the vulnerability of older population in India, and calls attention of policy makers for musculoskeletal health research and inclusion in national programs.

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1. Aging

1.1 Global Aging

The global demographic transition is occurring and we expect most people across the world to live beyond 60 years [1]. Globally the population aged 60 years and more increased more than two-fold from 382 million in 1980 to 962 million in 2017 and is expected to double again to 2.1 billion by 2050 [2, 3]. The older population in developing regions are growing faster compared to the developed regions. In 2017, more than two thirds of the older population lived in developing nations [2, 4]. Estimates suggest that by 2050, 70% of the older population will be residing in developing countries [2]. Among the various regions of the world, in 2017 Asia accounted for 57% of the global population aged 60 years and over [2].

The three main demographic factors contributing to the increased older population are: declining fertility, declining mortality and increased survival. There has been non uniform and varied reduction of mortality across various age groups which improved life expectancy. Considerable improvements in childhood mortality and mortality related to infectious diseases have also propelled life expectancy, especially in the developing regions [3, 5, 6].

The oldest old, population aged 80 years and over is also increasing globally. The proportion of the oldest old rose from 9% in 1980 to 14% in 2015 and is estimated to remain stable till 2030; after 2030 till 2050 it is projected to increase to more than 20% [7].

1.2 Aging in India

There is an accelerated population aging in developing nations [8]. In Asian populations, the proportion of elderly (60 years and over) is estimated to increase from 10.5% to 22.4% during 2012-2050. While in India, this share of elderly is expected to rise from 8% in 2015 to 19% in 2050, but the absolute numbers will be large, i.e. around 323 million by 2050 [9, 10]. The acceleration of the elderly (60 years and over) will be faster during the second

half of the 21ˢᵗ century and India would have an estimated 34% of the world's elderly population share by 2100 [9]. With the population of India growing, the rate of growth among the elderly (60 years and over) is 3 fold higher than the other age groups. The growth rate of the younger population is already decreasing; this puts India in a unique position of a rapid transition from a young nation to a rapidly aging nation [9].

With the decreased fertility, mortality and increased life expectancy which are fuelling the world's aging population, there are significant interstate disparities in terms of levels and growth of the elderly population in India. Overall the southern states (Telangana, Andhra Pradesh, Karnataka, Tamil Nadu and Kerala) along with Maharastra, Himachal Pradesh, Odisha and Punjab are front runners in aging population (60 + years) compared to Uttar Pradesh, Rajasthan, Madhya Pradesh, Bihar, Jharkhand, Chhattisgarh and Uttarakhand which have much lower proportions.

Some key challenges India faces because of the aging epidemic include: (i) the current context of sociocultural aspect, there is a feminization of aging; i.e. the sex ratio has increased from 938 women for 1000 men aged 60 or over in 1971, to 1033 in 2011 and is estimated to go up to 1060 in 2026 (ii) according to the census of 2011, around 71% of the elderly (60+ years) live in rural areas and (iii) migration of the younger age groups from rural areas can also impact the elderly population [9].

The oldest old (80 years and over) is projected to increase at a very rapid pace in India at the rate of 700% from 2000-2050. In absolute numbers, the 80+ years population is estimated to increase from 6 million in 2000 to 48 million by 2050 and is expected to rise thereafter. This group of population is predominantly widowed and women who would be highly dependent on younger generations [9].

2. Health in Aging

2.1 Global Scenario

Globally most people today can live through their 60s and beyond; in the less developed nations, this is predominantly due to lower mortality in their younger lives [3]. However, the increased life expectancy over the past few decades has altered the mortality patterns. Globally ischemic heart diseases, stroke and Chronic Obstructive Pulmonary Disease (COPD) are the three major causes of deaths and the burden of this is higher among the less developed nations compared to developed nations [11]. Evidence suggests that older populations are not experiencing better health conditions especially in the context of mild to moderate disabilities [12]. The burden of these disabilities is higher among the less developed nations.

Aging puts a person at risk of having more than one diseases or multiple conditions at the same time. Though there is a lack of clarity in the classification of diseases as a multi-morbidity definition; the occurrence of multiple diseases puts the elderly at a disadvantage. There is a complex underlying interaction that happens with multiple diseases such as, different diseases treatment recommendations, and drugs for different diseases. This may affect the quality of life, morbidity, and mortality due to a single disease in the multi-morbidity setting [13]. In less developed nations, this multi-morbidity is further escalated due to the interface of communicable and non-communicable diseases [14]. Many times this pattern or complex of multiple diseases and their interactions is referred to as the geriatric syndrome [12].

Musculoskeletal health is important in older populations to maintain physical, social and functional life style. It is globally acknowledged that diminished musculoskeletal health has an adverse impact on morbidity and mortality [12]. Older adults with musculoskeletal disorders like osteoarthritis, having sustained an osteoporotic fracture or having decreased physical capability levels have higher mortality[15, 16]. Most of the studies related to musculoskeletal disorders among older populations have been carried out in developed regions.

In developing regions there has been a 60% growth of musculoskeletal conditions from 1999 to 2010 and it is estimated that there will be a significant increase of these conditions among older populations in the coming decades [17].

2.2 Aging, Health, and India

The Indian older adult populations are highly vulnerable due certain factors including lower socio economic status (SES), where around 50% of the elderly are of lower socio-economic strata [18], half of them are dependents either due to widowhood or separated and within this majority (70%) of them are older women [19]. There is a scarcity of large scale studies among older adults in India. Various small scale studies point towards a high prevalence of tobacco use [20] and physical inactivity [21]. It is estimated that the elders in India face NCD related morbidity and disability burden which will contribute to disability at the national level [22].

The Longitudinal Aging Study of India (LASI) is a nationally representative longitudinal study to examine aging and retirement among India's population aged 45 years and over. It is aimed at collecting a plethora of information on (i) individual health (morbidity in terms of chronic conditions, endemic infectious diseases, injury, falls, eye sight, etc.; mental health, functional health, healthcare utilization, women's health, health behaviours); (ii) Biomarkers e.g. Blood pressure, anthropometry, grip strength, gait speed, balance tests, lung functions, vision test, C Reactive Protein (CRP), haemoglobin, Haemoglobin A1C, Cystatin C, and Vitamin D. LASI also includes a genetic repository;(iii) Household information including housing and environment, household consumption, assets and debts, household income; and (iv) Individual information like work, retirement and pension, some experimental modules (addressing topics beyond the main study) and family, social networks and social welfare. LASI aims to recruit a sample size of 60,250 participants aged 45 years and over. The first two

waves are planned between 2016-2020 [23]. As a part of the LASI pilot study conducted during 2010 among 1679 adults aged 45 years and over, the prevalence of at least difficulty of one of the activities of daily living (ADL) was 16.6% in men aged 60-75 years, 25.7% in men aged 75 +; 18.3% women aged 60-75 and 36.4% women aged 75+. The prevalence of low grip strength (cut-offs used were: 21 kg for women and 37 kg for men) was 78.7% in men aged 60-75 years, 80.1% in men aged 75+ years, 69.9% in women aged 60-75 years and 70.1% in women aged 75+ years [24]. It was reported that around 58.4% of the participants aged 45 years and over were physically inactive (moderate level) and 73% of the participants were physically inactive (vigorous level). It also reported that smoking and physical inactivity were significantly related to self-rated health [25]. These high prevalence of difficulty in ADL, low grip strength and physical inactivity suggests that the older population in India are at high risk of disability.

Gaps in knowledge:

There are few studies of the older populations in India. Most relied on subjective (self-reported) data rather than objective measures. This is primarily due to various surveys and census data which do not capture morbidity and disability patterns and their progression longitudinally. Cohort studies among elder adults are rare in India and it is imperative for longitudinal studies to collect information on the occurrence of various morbidities and disabilities among the elderly in India [26]. LASI is one such initiative which is trying to address gaps in knowledge about Indian elder adults, but there is very limited musculoskeletal data in LASI.

The Mobility and Independent Living Among Elders Study (MILES) is another cohort study in the southern part of India. This is a random sample of 562 men and women aged 60 years and over followed longitudinally. This study has collected information related to

musculoskeletal measures, like the Peripheral quantitative computed tomography (pQCT) of radius and tibia, grip strength, Short Physical Performance Battery (SPPB), and the 400-meter walk along with other biomarkers and questionnaires related to health [27]. This study provides a unique opportunity to describe the musculoskeletal phenotypes in older Indian adult population with objective and standard measures for comparability internationally.

3. Musculoskeletal System

The musculoskeletal system is an important and complex system with the bone and skeletal muscle being its two predominant components. The other components of the system are tendons, ligaments, cartilages, vascular and nervous tissues. Traditionally these components have been focused and studied separately [28, 29]. With the advancement of age, there are significant changes in musculoskeletal system impacting physiological functions, mobility and functional ability. [30].

3.1 Bone

Bone is a complex intricately designed flexible tissue interwoven with minerals and bound with specialized cells, thus making it a unique design to keep it light weight yet hard and strong. Around 90% of the bone matrix is made up of elastic collagen fiber which provides flexibility thus supplementing resistance to fractures. The hard part of the bone is made up of minerals especially calcium which forms the bone mineral matrix providing the rigidity to the structure. The bone structure and composition is actively remodelled throughout life by bone cells – osteocytes, osteoblasts, and osteoclasts.

Human bones are of two types flat bones and long bones. The long bones comprise of thick and dense layer of calcified tissue also called cortex or the compact bone which encloses the medullary cavity. The calcified tissues of cortical bone progressively become weak towards the periphery and in turn is compensated by a network of fine calcified trabeculae forming the trabecular bone. This divides long bones into cortical (compact) and trabecular (spongy) bone (Figure 3.1). Periosteum covers the bone externally and has an outer fibrous layer and inner cell layer. Endosteum covers the bone internally and separates it from the marrow. The cylindrical portion is called diaphysis and is mostly made of cortical bone, epiphysis is at the end of the bone and is mostly made of trabecular bone.

Metaphysis is also called as growth plate and is located between the diaphysis and the epiphysis where the bone growth occurs.
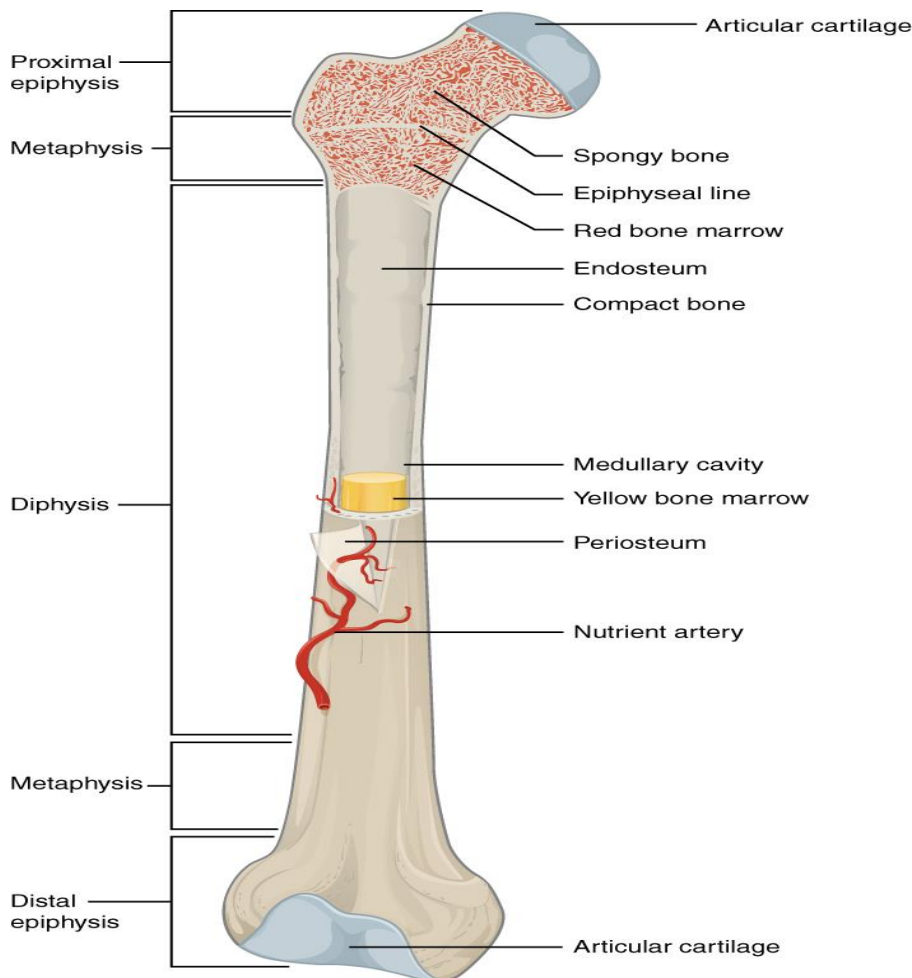
Figure 3.1 Gross and microarchitecture of a typical long bone

"Anatomy of long bone" from Wikimedia commons, the free media repository. URL: https://upload.wikimedia.org/wikipedia/commons/2/23/603_Anatomy_of_Long_Bone.jpg Copyright: OpenStax College [CC BY 3.0 (https://creativecommons.org/licenses/by/3.0)]

Though cortical and trabecular bone are made up of similar cells and elements, they are structurally and functionally different. The cortical bone has 80-90% volume calcified, whereas the trabecular bone has only 15-25% volume calcified with blood vessels, connective tissue, and bone marrow. Based on these differences, cortical bone predominantly provides mechanical / protective function and the trabecular bone provides

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predominantly metabolic function. It is important to note that trabecular bones of the vertebrae predominantly provide mechanical strength and protection [31].

Based on the mechanostat hypothesis, muscle forces in the body put bones through three different strains: modeling, remodeling and microdamage. The response of the bone to these strains follow three rules: bone responds to dynamic loads; a short duration of load is enough to trigger a response; and routine loads lead to maintenance as the bone cells get accustomed to this and become less responsive to these loads [32, 33]

3.2 Bone Growth

There is a longitudinal and radial growth of the bones during childhood and adolescence. The longitudinal growth occurs at the growth plates with subsequent mineralization to form new bones[34] [35].

3.3 Bone Modeling

The longitudinal bone growth continues until the end of second decade of life. Skeletal growth and maintenance occurs by the process of modeling. Modeling occurs throughout the skeleton resulting in alterations of skeletal size and macro architecture often occurring at sites where resorption has not previously occurred. As per the word modeling suggests, bones change their form in response to the various physiological and mechanical influences and gradually adjusts to them. The bones may broaden or alter the axis through either removal or addition of bone due to biomechanical influences. With aging, there is widening of bones due to adding of new bone mineralization to the periosteum and resorption of old bone from the endosteum [35].

3.4 Bone Remodeling

Remodeling is a distinct process through which the skeleton is maintained by repairing damaged bone, replacing old bone, facilitating vital functions like calcium homeostasis, and preserving the mechanical integrity of the skeleton. This involves a continuous elimination of old bone and substitution of new bone with ensuing mineralization. This process occurs to prevent the accrual of microdamage to the bone tissue. Remodeling is a lifelong process happening at all stages of life due to the biomechanical stress and microdamage that occurs due to it. Remodeling is carried out by a unit of osteocytes, osteoclasts, and osteoblasts.

Basic multicellular units (BMU) comprising of osteoclasts, endothelial cells, connective tissue, osteocytes and osteoblasts are key for remodeling. The remodeling occurs through resorption of the microdamage and followed by bone formation. Resorption happens over three weeks and bone formation takes three to four months. Remodeling is different in cortical and trabecular bones.

Microdamage or injury initiate the remodeling signal. Once there is a damage or a site identified for remodeling, a bone remodeling compartment (BRC) by lining cells is created. The BRC consists of the BMUs and other immune cells. The hematopoietic stem cells (HSC) also enter the BRC through the capillaries and differentiate into osteoclasts and cause bone resorption. Later on, they differentiate into osteoblasts to form new bone.

The osteocyte senses mechanical loading and responds to fluid shear stress translating strains into biochemical signals, thus modulating bone remodelling – this is called as mechanosensation. They regulate osteoblast and osteoclast activity and regulate bone remodelling. They also regulate phosphate and calcium homeostasis. Thus osteocytes play a key role in bone remodeling [36].

During bone resorption, osteoclasts are located in the cavities on the bone surface. Once the osteoclasts come in contact with the bone matrix they form a sealing zone with ruffled

borders to tightly bind with the underlying bone matrix. The ruffled border is rich in material necessary for resorption.

Macrophage colony-stimulating factor (M-CSF) plays a key role for fusion of osteoclast progenitor cells to form multinucleated, active osteoclasts. Receptor activator of nuclear factor kappa-B (RANK) ligand, a member of the tumor necrosis factor (TNF) family, is present on the surface of osteocytes and osteoblasts. In a process involving cell-cell interactions, RANK ligand binds to the RANK receptor on osteoclast progenitors, stimulating osteoclast differentiation and activation. Alternatively, a soluble decoy receptor, referred to as osteoprotegerin (OPG), can bind RANK ligand and inhibit osteoclast differentiation. Interleukins 1, 6, and 11; TNF; and interferon γ modulate osteoclast differentiation and function. The hormones that influence osteoclast function do not target these cells directly but instead target cells of the osteoblast lineage to increase production of M-CSF and RANK. Both Parathyroid hormone (PTH) and $1,25(OH)_2D$ (Vitamin D) increase osteoclast number and activity by this indirect mechanism. Calcitonin, in contrast, binds to its receptor on the basal surface of osteoclasts and directly inhibits osteoclast function. Estradiol has multiple cellular targets in bone, including osteoclasts, immune cells, and osteoblasts; actions on all these cells serve to decrease osteoclast number and decreased bone resorption. Various hormonal influences on the bone remodeling are shown in figure 3.2 [37, 38]
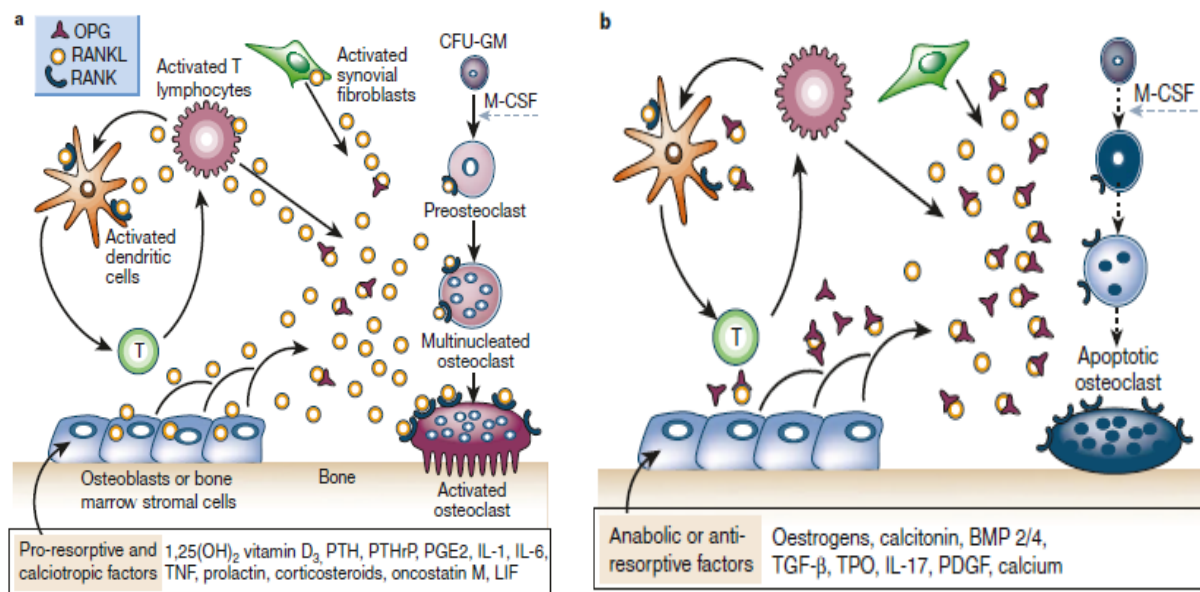
Figure 3.2 Hormonal control of bone resorption
A. Pro-resorptive and calciotropic factors. B. Anabolic and antiosteoclastic factors. RANKL expression is induced in osteoblasts, activated T cells, synovial fibroblasts, and bone marrow stromal cells. It binds to membrane-bound receptor RANK to promote osteoclast differentiation, activation, and survival. Conversely, osteoprotegerin (OPG) expression is induced by factors that block bone catabolism and promote anabolic effects. OPG binds and neutralizes RANKL, leading to a block in osteoclastogenesis and decreased survival of preexisting osteoclasts. CFU-GM, colony-forming units, granulocyte macrophage; M-CSF, macrophage colony-stimulating factor; RANKL, receptor activator of nuclear factor NFκB; PTH, parathyroid hormone; PGE_2, prostaglandin E_2; TNF, tumor necrosis factor; LIF, leukemia inhibitory factor; TPO, thrombospondin; PDGF, platelet-derived growth factor; OPG-L, osteoprotegerin-ligand; IL, interleukin; TGF-β, transforming growth factor β. (Reproduced from WJ Boyle et al: Nature 423: 337, 2003. [38])

Osteoclasts carry out resorption of old bone and osteoblasts form new bone. The remodeling of bone accelerates during the perimenopausal phase and continues to be faster among post menopause women and it is thought to increase at a slower pace among men in aging [34] [35]. As per Wolffe's law – bone remodeling throughout the lifespan adapts to the biomechanical stress on them [39].

Cortical bone / compact bone accounts for about 80% of the total adult human skeleton and is predominantly made up of long bones and appendicular skeleton. The remodeling of cortical bone is usually increased volume (new bone formation) on the periosteum and resorption (old bone) in the endosteum. The new bone resorption generally

exceeds the formation of bone in aging. This process increases the diameter of the cortical bone and increases the porosity of the bone, thus making it more fragile. The cortical bone loss starts at the perimenopausal age and increases 5-10 years after menopause. The increased cortical bone loss leading to fragile bones are a major risk factor for hip and wrist fractures [40].

The trabecular bone accounts for about 20% of the skeleton. The loss of trabecular bone occurs even before the cortical bone, sometimes as early as 30 years of age. Trabecular bone loss is compounded by not only thinning of the bone plates but also with the disintegration of the trabeculae. As trabecular bones are important for the mechanical strength of the spine, the trabecular bone loss is a major risk factor for fragility fractures of spine [40]. Trabecular bone loss among women is predominantly due to increased resorption with loss of trabeculae, whereas in men it is mostly trabecular thinning[41]. Although trabecular bone remodels more frequently than cortical bone, it is also extremely vulnerable to local or systemic factors that can cause significant imbalances in bone turnover.

3.5 Epidemiology of Osteoporosis and Osteoporotic Fractures

Osteoporosis:

Based on the NIH consensus statement 2000, osteoporosis is a skeletal condition where the bone strength is weakened with a consequent increase in risk of fractures. Bone strength reflects the integration of two features: (i) bone density and (ii) bone quality. Bone density of an individual is determined based on the peak bone mass and quantity of bone loss. Bone density is expressed in grams of mineral per area / volume. The bone quality refers to architecture, turnover, damage accumulation (e.g., microfractures) and mineralization [42]. The criteria of clinical definition of osteoporosis depends on presence of low-trauma fracture, i.e. a fracture from a fall at standing height excluding the face, skull or digits [43, 44]. The compromised bone strength and increased bone fragility in osteoporosis is due to defects of - trabecular microarchitecture, material properties of bone and repairs of microdamage and excessive bone remodeling rates [43].

The NIH consensus statement 2000 suggests that diagnosis of osteoporosis should be based on risk-based assessment rather than just the T-score assessment alone [42]. The T-score based assessment or the diagnostic criteria for osteoporosis is based on BMD measurement by the dual-energy x-ray absorptiometry (DXA) scan of hip or spine. If the BMD T score is between -1.0 to -2.5 SD it is defined as low bone mass, if the T score is lower than -2.5 SD then it is osteoporosis and if the T score is lower than -2.5 SD and presence of at least one fragility fracture then it is severe osteoporosis [45]. The T score is calculated in relation to the mean of young female adult mean, i.e. Third National Health and Nutrition Examination Survey (NHANES III) reference database for femoral neck measurements in white women aged 20–29 years [46].

Burden of osteoporosis global scenario:

The incidence of hip fractures increase significantly with aging [47] and as the global population is aging, this demographic change alone is estimated to lead to increases in the numbers of hip fractures from 1.3 million in 1990 to 2.6 million by 2025 and 4.5 million by 2050 [48]. Considering that the age specific hip fracture incidence rates stay stable in Europe and North America but increase at the rate of 3% per year in other regions, hip fractures may total 4.7 million annually by 2020 itself [49]. There is a huge variation in the incidence rates of hip fractures among women and men across 50 countries [50]. The age standardised hip fracture rates have more than 140-fold variation in some countries comparing Nigeria and Norway. The geographic variability is lower among men when compared to women; but countries with higher rates of hip fracture among women have higher rates of hip fracture in men [50].

Vertebral fractures prevalence using a similar morphometric definition (a quantitative method to identify osteoporotic vertebral fractures based on the measurement of vertebral heights) across many geographical regions was similar among men and women except in Japanese where it was higher among women compared to men. The incidence of a morphometric vertebral fracture is higher than hip fracture. The radiological vertebral fractures incidence was higher among Thai men and women compared to the Rotterdam study. There are only six countries with clinical vertebral fracture incidence rates data and these rates are lower than the radiological vertebral fracture incidence[50]. Based on several large population based cohort studies using spine radiographs, the age specific prevalence of vertebral fractures is higher in the Caucasian countries and lower in Latin America and Asia [51]. There is less variation of vertebral fractures prevalence in Europe and North America; on the contrary higher variation of prevalence is seen in Asia and Latin America [52]. It is also important to note that approximately two thirds of vertebral fractures do not come to clinical attention [53].

Information on the burden of forearm fractures is available only in few countries and is not comparable due to different definitions across the studies. Age standardised distal forearm fractures were lower in Japan and higher in Hungary compared to the United States [50].

The economic burden of osteoporotic fractures as per Disability Adjusted Life Years (DALYs) in 2000 was 5.8 million and more than half (58%) of this DALYs were for fractures that occurred in Europe and America. DALYs were greater in women than men. Hip fractures were 41% of the total DALYs of osteoporotic fractures [54].

Mortality post hip fractures have been extensively studied. The relative hazards (RH) for mortality was highest in the first 3 months post hip fracture, RH 5.75 in women and RH 7.95 in men. The mortality decreased over time but was still higher even 10 years post hip fracture, RH 1.96 in women and RH 1.79 in men. Men seem to have higher mortality rate in the first year post hip fracture. The excess mortality post hip fracture was associated with age, and higher among men till age of 80 years after which the gender difference decreases [55, 56]. Though most of these mortality studies were conducted in Europe, North and South America, and Australia, with one study from Singapore, the mortality risk did not seem to be different based on geography [55].

The Dubbo Osteoporosis Epidemiology Study (DOES), examined mortality after different types of fractures. It was observed that mortality increased following all types of fragility fractures with mortality risk being high in the first five years after the fracture. Mortality risk increased 3 to 4-fold when a subsequent fracture occurs within the first five years of the first fracture. Mortality post vertebral fracture was lower than the hip fractures [15].

Osteoporotic fractures represent a growing public health problem in both developed and less developed nations, with severe consequences for both the patient and the health care system. The burden of fractures relates to the costs as well as the morbidity and associated mortality [57]. There is evidence from the developed countries that at the population level,

fracture incidence increases with advancing age, leading to significant healthcare costs. The estimates for the United states fracture-related economic costs is around $17 billion for 2005, and it is predicted to rise by almost 50% by 2025 [58].

Risk factors of osteoporosis:

(i)   Bone Density: Osteoporosis is a progressive musculoskeletal disease characterized by reduced bone density and degradation of the bone microarchitecture increasing bone fragility making a person prone to fractures [59]; low bone density is a key risk factor for osteoporosis. Based on a meta-analysis of 11 studies and 90,000 person years of follow up with around 2000 fractures, every standard deviation (SD) decrease of areal bone mineral density (BMD) at spine and hip, increases the risk of fracture two-fold [60]. Based on the recent analysis of Study of Osteoporotic Fractures (SOF), BMD was a strong predictor of non-vertebral fractures over a period of 20 years and hip fracture over a period of 25 years [61]. In the SOF study half of the women (54%) aged 65 years or over who had a hip fracture were not osteoporotic at hip as per the clinical definition. i.e. the BMD at hip among these women was not lower than -2.5 SD below the young normal reference range. However lower BMD was associated with higher risk of fracture [62]. Bone density has low sensitivity i.e. it can identify people who are at risk of future fracture, but it cannot predict an individual who will develop future fracture [60].

(ii)   Clinical risk factors: These risk factors can influence the occurrence of a fracture either by reduction in bone density or other mechanisms. Risk factors which have been shown to be independent risk factors for fractures include: older age, previous history of fragility fractures, genetic or hereditary, glucocorticoid therapy, smoking, excess alcohol intake, rheumatoid arthritis, lower BMI, falls, Caucasian ethnicity and geographic location farther from equator. Other risk factors include: hypogonadism,

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premature menopause, malabsorption, hyperthyroidism, chronic liver diseases, chronic renal disease, immobility and drugs like – androgen deprivation therapy and aromatase inhibitors [63].

A prospective 8.6 mean years' follow-up of the Osteoporotic fractures in men study (MrOS) study in United States adjusting for competing mortality risk observed that: higher age (>75 years), lower femoral neck BMD, lower protein intake, any previous history of fracture after 50 years of age, greater height, greater height loss since age of 25 years, use of long acting benzodiazepines, use of tricyclic antidepressants, history of myocardial infraction or angina, and lower executive function were all associated with incident hip fractures. If the femoral neck BMD is in the osteoporotic range and if a man had more than 4 risk factors, the incidence rate of hip fracture was 50 times higher compared to men with no risk factors and normal femoral neck BMD [64].

A similar study carried out in older women aged 65 years and over enrolled in the SOF study followed for incident hip fractures for a mean 10.1 years of follow up. The risk factors identified were: older age, greater height at age 25, maternal history of hip fracture, women with a history of previous fractures, low or poor self-rated health, hyperthyroidism, long-acting benzodiazepines or anticonvulsant drugs use, impaired cognition, high caffeine intake, low physical activity, low walking speed and lower BMD [65, 66].

Analysis of 960 women and 689 men in Australia with a median follow up of 12 years of the DOES observed that, for each SD reduction in femoral neck BMD, women had 3.6 and men had a 3.4-fold increase in hip fracture. History of fall over the last 12 months and history of previous fractures were independent risk factors. Based on the population attributable risk fraction (PARF), 47% of hip fractures in women and

28% in men were attributed to osteoporosis, advancing age, 12 months' prior history of fall and history of fracture [67]. At the individual level, history of a fracture had a 2 to 3.5 fold increase risk of a subsequent fracture [57] and increase in 5-year mortality [15].

3.6 Osteoporosis in India

The International Osteoporosis Federation (IOF) estimated that in 2003 osteoporosis was prevalent among 26 million Indian people and would increase to 36 million by 2013 [68]; however the 2013 estimates suggest that 50 million people in India are either osteoporotic or have low bone mass [69, 70].

A community based cross sectional study among low socio economic status women aged 30-60 years of age from Hyderabad in southern part of India was conducted. DXA was performed among these women and it was observed that 29% of women had osteoporosis and 52% had low bone mass at the femoral neck; 43% osteoporosis and 43% low bone mass at the lumbar spine [71]. Another large cross sectional study conducted among 792 men and 808 women in New Delhi, aged 50 years and above, reported osteoporosis prevalence of 24.6% in men and 42.5% among women. Low bone mass was found among 54.3% men and 44.9% women [72]. Based on the US NHANES III data, the prevalence of osteoporosis based on the BMD among women aged 50 years and over was 13-18% and among men, 3-6%[73]. This suggests that prevalence estimates of osteoporosis among Indian older population are much high.

The Indian council of Medical Research (ICMR) conducted a large multicentre study to generate India specific reference ranges for peak bone mineral density (BMD). This study showed that Indians have lower BMD compared to Caucasians [74]. Most studies in India used the DXA manufacturers white Caucasian reference database. A study with 4427 men and women aged 50 years and over, conducted at a southern Indian tertiary care centre, compared

the Hologic DXA 4500 series database (Caucasian database) and the ICMR database. It was observed that the Hologic database identified more subjects with osteoporosis (42.7% at spine and 11.4% hip), compared to the ICMR database (27.7% at spine and 8.3% at hip) [75]. Another study from northern India included 2304 women aged 18-85 years recruited from four different residential localities. This study showed that using Caucasian Lunar BMD database identified more women with osteoporosis at spine but the prevalence was similar at femoral neck[76]. Another community based cross sectional study from western India measured BMD among 1137 men and women, 25 to 35 years' using Hologic 4500 DXA. It was observed that peak BMD was lower among Indians compared to Caucasians [77].

The BMD cut-offs based on the Indian ICMR database suggests that the peak BMD among Indians is lower compared to the peak BMD of Caucasians. This lower BMD among Indians is probably due to genetic variation, smaller skeletal size and nutritional differences. It also suggests the increased vulnerability of Indians for osteoporosis and fragility fractures.

Hip fractures data in India is sparse and most of the data is from hospital based studies (Table 3.1). Based on a study done in Northern India in one district, the age specific incidence rates were 159 per 100,000 in women and 109 per 100,000 in men above the age of 50 years. With increasing age, the incidence rate increased to 962 (95% CI: 198-2812) per 100,000 in women aged 90-94 years and 638 (234-1388) in men per 100,000 at the age of 85-89 years (Figure 3.4) [78].
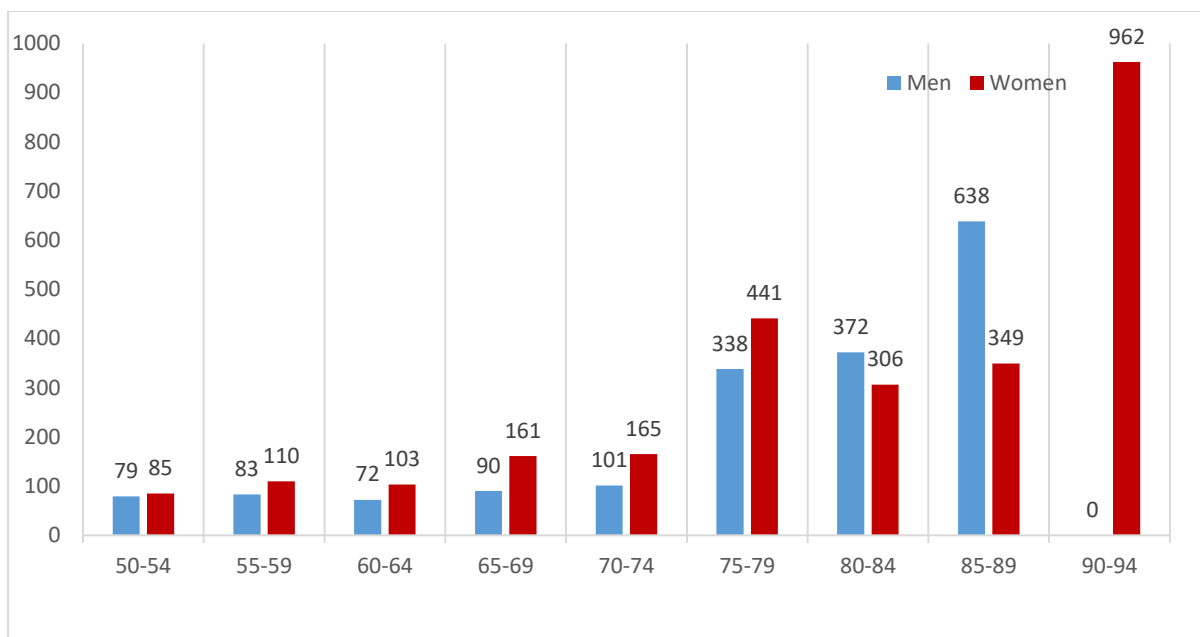
Figure 3.3 Age and sex specific hip fracture incidence rates per 100,000 during 2009 in Rohtak district, North India

A Singapore based study examined the age standardised incidence rate of hip fractures among Indians living in Singapore, and observed that the rate among Indians was 128 per 100,000 for men and 361 per 100,000 for women. These rates among Indians were lower than the Chinese living in Singapore [79]. Indian women seem to have a lower rate of hip fracture incidence compared to the western populations [80]. Ratio of hip fracture occurrence in women to men was 2:1 for Caucasians, compared to 3:2 among Indians [78]. Post hip fracture the first year mortality among Indians was as high as 30% in public hospital settings [78]. It is projected that there could be around 792,334 hip fractures by 2050 in India and could cost around 612 million USD [81]. But these projections seem to be far underestimating the burden and economic costs because these estimates have been based on a single study.

To our knowledge, there has been one study of vertebral fractures in India called the Delhi Vertebral Osteoporosis Study (DeVOS). This study enrolled 808 healthy men and women in New Delhi from three residential localities aged 50 years and more. All subjects underwent lateral lumbar and thoracic spine X-rays. The prevalence of vertebral fractures was 18.8% among men and 17.1% among women. The prevalence of vertebral fractures increased

with age among women from 14.5% in 50-59 years' age to 22% in more than 70 years' age. This increased prevalence of vertebral fractures with age was not observed among men [82]. The prevalence of vertebral fractures in the North American region among women aged ≥ 50 years was 20-24% and men aged ≥ 50 years was 22-35% [52], which are higher compared to Indian population aged ≥ 50 years.

Table 3.1 Studies on hip and vertebral fracture rates among Indians

Author, year [ref]	Type of study	Location	Sample size	Hip fracture rates
Dhanwal et.al, 2013[78]	Hospital based cross sectional study in 2009	Rohtak district, North India	543 patients	With increasing age, hip fracture incidence increased in both men and women with highest incidence rate of 962 per 100,000 in women of 90-94 years and 638 per 100,000 in men of 85-90 years. The crude hip fracture rates were 159 for women and 105 for men per 100,000 above the age of 50 years. The hip fracture rates in women were higher compared to those in men but this was not statistically significant.
Koh et.al., 2001[79]	Population based study from Singapore; medical records and claims processing system; all patients aged 50 years and over, who had a diagnosis of hip fracture from 1985 to 1998	Singapore	9406 hip fracture events	Age adjusted hip fracture incidence rates among Indians living in Singapore over the age of 50 years were: 128 for men and 361 for women per 100,000. Incidence rates increased with age and were highest at 70 years and over age group with men having 340 and women 882 per 100,000.
Marwaha et.al., 2012[82]	Community based cross sectional study; participants aged 50 years and over were recruited for a radiological evaluation	Delhi	345 males and 415 females	Radiological evaluation of thoracic and lumbar spine. The prevalence of vertebral fractures was 18.8% among men and 17.1% among women. The prevalence of vertebral fractures increased with age among women from 14.5% in 50-59 years' age to 22% in more than 70 years' age. This increase in vertebral fractures with age was not seen among men

Risk factors of osteoporosis – Indian setting:

Based on a community based cross sectional study of 200 peri and postmenopausal women in northern India, increasing age, low exercise, menopause, and low calcium diet were associated with low BMD [83]. Another large cross sectional study of 1400 slum dwelling women aged 25-50 years in western India (Mumbai) also observed older age, menopause, low physical exercise, low calcium, and poor or inadequate diet were associated with low BMD [84]. In another cross sectional study of 1137 young (25-35 years) healthy volunteers in western India (Mumbai), height, weight, and BMI were positively correlated with BMD among men and women. Vitamin D was positively correlated with BMD in men and serum intact parathyroid hormone (iPTH) levels had negative correlation among men and women with BMD. This study also observed that Indian subjects had lower peak BMD compared to Caucasians. Vitamin D deficiency which is higher among the Indian populations may contribute to their lower peak BMD putting them at greater risk for osteoporosis [77].

In a hospital based study of hip fractures, 90 hip fracture patients were age and sex matched to controls: >75% of hip fracture patients had Vitamin D deficiency (serum 25-OHD of <20 ng/dl) and >60% of them had secondary hyperparathyroidism (defined as higher serum iPTH i.e. >54 pg/dl) [85]. Another hospital based study of 43 non traumatic hip fracture patients reported that 97% of fragility hip fracture patients had Vitamin D deficiency (serum 25-OHD of <20 ng/dl) [86]. A cross sectional study of 943 urban and 205 rural healthy subjects in southern India observed that, Indians consume lower amounts of calcium (average intake men: urban - 323 mg/dl, rural- 271 mg/dl; women: urban – 306 mg/dl, rural – 262 mg/dl) compared to recommended dietary allowance (RDA) of 400 mg/d for adults. Vitamin D levels were lower in urban areas (men – 18.54 ng/dl; women- 15.5 ng/dl) compared to rural areas (men – 23.7 ng/dl; women- 19 ng/dl). The Vitamin D deficiency (serum 25-OHD of <20 ng/dl) was observed among 44% men and 70% women in rural areas compared to 62% in men and 75%

women in urban areas [87]. But in both the studies, Vitamin D levels were still lower than the normal range.

A hospital based case control study of 100 radiologically confirmed hip fracture patients and controls who were age and sex matched in New Delhi; physical activity levels were defined as 1 = bedridden, 2 = walks with the help of a stick or walker, 3 = walks without any support but leads a self-defined sedentary lifestyle, 4 = walks without any support and leads a self-defined active lifestyle and physical activity level >2 was defined as an active person; It was observed that hip fracture risk was reduced among active persons (OR 0.024; 95% CI 0.006-0.10), increased BMI (0.81; 0.68-0.97) and subjects who take calcium supplements (0.076; 0.017-0.340), and high hip fracture risk was observed among subjects who had difficulty in getting up from a chair (14.53; 3.86-54.23) [88].

Clinical and epidemiological data from India on osteoporosis and osteoporotic fractures are sparse [89]. However, based on the existing data which is mostly cross sectional and hospital based studies suggest that; Vitamin D deficiency exists in the population and is related to osteoporosis and hip fractures among Indians; lower physical activity or physical performance is related with lower BMD and hip fractures.

Osteoporosis is not a national health priority in India. Vitamin D deficiency is not formally acknowledged under any of the nation's health program, but is an important public health issue. There are national programs on providing vitamins and calcium supplements for school children and pregnant women. There is a need for priority action on Vitamin D food fortification with emphasis on attainment of peak bone mass through good nutrition and life style measures.

India currently needs large scale studies on the current burden of osteoporosis and fragility fractures. There is lack of data on incident hip fractures and its treatment and care

which points to the need to establish hip fracture registries which can improve the care overall in the country.

There is a need for large multi center studies on fragility fractures incidence, physical performance measures and their relationship to incident fractures. There is a need for studies to identify various factors for osteoporosis or low BMD and how these risk factors prospectively impact disability or fractures among older populations in India. It is also important to focus on studies which measure the bone quality and geometry and how these can be early predictors of fractures, disability, and mortality. It is important to establish these studies with standardised protocols for comparisons across countries. Generating evidence through these studies may help the government to put osteoporosis as one of its national health priority and start proactively addressing it for the health of older populations.

3.7 Muscle

Skeletal Muscle structure:

Half of our body composition is muscle – 40% skeletal muscle and 10% smooth and cardiac muscle. The skeletal muscle structure is hierarchical and consists of (Figure 3.5) muscle, muscle fasciculus, muscle fiber, and myofibrils. Each muscle fiber is supplied with one nerve ending. At the molecular level, each myofibril has about 1500 myosin and 3000 actin filaments which are key to muscle contraction. The actin filaments are lighter in color and are called I bands. The dark color structures are called A Bands and consist of myosin filaments and the ends of the actin filaments. There are cross bridges which are small protrusions on the myosin filaments except at their center. These cross bridges interact with the actin filaments and cause the contractions. Z disk passes through the myofibril aligning them across the muscle fiber and is connected with the actin filaments. H band is the mid part of the myosin filament which is not covered by the actin filament. When the muscle contracts the I band and H band becomes smaller as actin and myosin filaments interlock with each other for contracting the muscle [90].
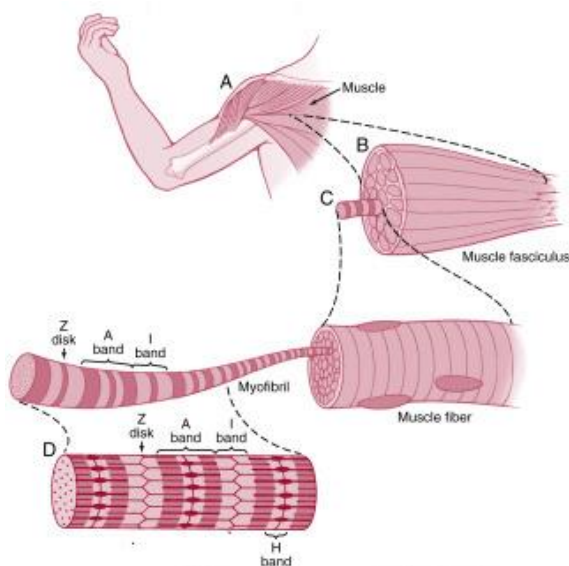
Figure 3.4 Skeletal Muscle structure
Hierarchal structure of skeletal muscle (Adapted from: Hall, J.E. 2016, Guyton and Hall textbook of medical Physiology, 13th Edition, Elsevier, Philadelphia, PA. [90])

Human skeletal muscle fibers are of two types, type 1 (slow twitch) and type II (fast twitch. Type II fibers are further classified as type IIa and type IIx. Type I muscle fibers are surrounded by more capillaries, have capacity for aerobic metabolism and are fatigue resistant. Type I fibers produce less force, are slower and produce more tension, thus they are key for stabilization and postural control. Type II fibers have lesser capillaries, have capacity of anaerobic metabolism, are faster and quicker to become fatigued compared to Type I fibers. Type IIx fibers produce the most force and rely on anaerobic metabolism. Type IIa fibers are also known as intermediate fibers and are a mix of Type I and Type IIx fibers, thus they use both aerobic and anaerobic metabolism. Adult muscle fiber size is achieved by the ages of 12 to 15 years. All these fibers, type I and type II (a and x) muscle fibers are larger in men compared to women, however in men type II muscle fibers are larger than type I and among women type I fibers are larger than type II [91].

3.8 Muscle and Aging

Aging affects all species and substantially influences the scope of our activities and quality of life. With aging, muscle mass decreases by 30-50% from age 40 to 80 years of age. [92-96]. With increasing age along with the decrease in muscle mass there is decrease in muscle strength [97, 98]. Among older adults, loss of muscle mass is associated with the decrease in muscle strength, however, decline in muscle strength is more compared to loss in muscle mass and maintaining or gaining muscle mass does not prevent age related muscle strength [98]. Muscle mass is a function of muscle area and the muscle fiber length, and the number of fibers. The number of muscle fibers do not increase during the adult life [99]. The muscle mass decreases with aging perhaps due to both the change in the area of the muscle fibers and reduction in muscle fibers. The reduction in muscle mass or atrophy first occurs after 40 years and for almost all by 50 years. The earlier loss is mostly associated with sedentary lifestyles

[94]. The age related muscle size decrease is due to the decrease in fibre number. Between 24-50 years there is only 5% decrease in muscle fibers but it declines by 35% between 52-77 years i.e. approximately there is 1% decrease per year in the muscle mass after 50 years. This decline in muscle mass is mainly explained by loss in muscle fibre numbers, but along with this, there is also atrophy at the muscle fibers [100]. One major cause of the decrease in the number of muscle fibers is the loss of motor units [94].

Reduced muscle strength has been reported to be more than just loss of muscle mass among elderly [101, 102]. There may be other factors of muscle or muscle quality which play a role in the age related decline in muscle function and performance.

The muscle aerobic capacity (capacity of the muscle to meet the demand of oxygen) is under higher energy demands during physical activity. This particular feature is observed to reduce at an accelerated pace after the age of 50 years for each successive age decade independent of the physical activity levels [103].

Myosteatosis is an excess deposit of fat in the skeletal muscles both at intramuscular and intermuscular levels. With aging there is a change in the total and regional fat distribution and myosteatosis [104, 105]. Myosteatosis among older adults has been associated with decreased muscular function and physical performance [101, 106, 107].

With age the neuromuscular system also undergoes changes. As discussed above the there is a motor nerve which connects each motor unit made of group of muscle fibers. These motor units undergo changes throughout the life span and these units reduce in number with age [108]. These reductions in motor unit numbers and its association with decreased muscle strength is observed to be true for men aged 80 years and over compared to young men (25 years) [109]. In parallel to this during aging, there is conversion of type II muscle fibers to type I, this change may be due to the stress on the muscles to conduct a movement slowly and with decreased intensity[110]. These together indicate changes in the neuromuscular system with

age and can impact the functional performance among elderly. These changes in the skeletal muscle with aging play a major role in the functional performance of elderly.

As a part of aging, other physiological mechanisms like the reduced protein turnover, altered endocrine factors (testosterone, growth hormone, insulin like growth factor (IGF)-1), autocrine factors (mechanogrowth factor – MGF) and inflammation (interlukins and TNF) play a role on the muscle fiber, mass and function with age [100].

3.9 Bone and Muscle Cross Talk

Bone muscle cross talk has been a focus of recent research. It is theorized that both skeletal muscles and bones grow and are maintained to fit the mechanical and metabolic needs of the body, however they also deteriorate with non-use, disease, and age. Age related musculoskeletal deterioration is associated with increased disability, risk of falls and fractures [111]. Not only mechanical but several biochemical signals are important in the bone muscle cross talk apart from the mechanostat hypothesis. The bone muscle cross talk is a synergistic combination of mechanical loading and biochemical signals between the bone and muscle [112]. Various mechanisms have been proposed on this bone muscle cross talk as below:

i.   Biomechanical relationship of bone and muscle: Based on the mechanostat theory, forces of muscle produce mechanical loading on the bone and stimuli to which bone is sensitive [113].

ii. Biochemical relationship of bone and muscle: It appears that there are endocrine factors apart from the mechanical stimuli through myokines, adipokines and osteokines, which play a role in the bone muscle cross talk. [111].

iii. Factors secreted by muscle effect bone: Muscle acts as an endocrine and paracrine organ by secreting certain factors during a range of muscle activity, disuse or damage. These factors include myostatin, irisin, several types of growth factors, inflammatory

cytokines and peptides, which have possible effects on bone cells, anabolic and catabolic processes of bone thus altering the bone metabolism[112, 114].

iv. Factors secreted by bone effect muscle: Recently it is recognised that osteocytes function as a secretory endocrine organ and regulates phosphate thorough the fibroblast growth factor 23 (FGF23) which in turn maintains the normal bone mineral content. The Wnt1 and Wnt3 which are expressed by osteocytes due to mechanical loading and support myogenesis [112]. Bone also secretes certain factors when it undergoes stress like the prostaglandin E2 and osteocalcin which play a role in muscle growth and function[115, 116].

v. Familiar associations of bone and muscle: There is good evidence of possible genetic influence associated with bone and muscle function, growth and development[117]. There is evidence suggesting an association between osteoporosis, sarcopenia and vitamin D. There is evidence of estrogen and testosterone association to bone and muscle function. There is evidence suggesting that glucocorticoids affect the musculoskeletal unit [111].

vi. Other indirect associations: The physical interaction of muscle and bones with structures like tendons, ligaments, cartilages and connective tissues affect the cross talk between bone and muscle [111].

vii. Association of nervous system: The nervous system plays a role in the bone and muscle cross talk. There are several neural systems and pathways implicated in this. For instance, sympathetic nervous system is observed to regulate bone mass and play a role in improved skeletal muscle growth and regeneration; it can also negatively affect skeletal remodeling in older populations. Central and somatic systems stimulate muscle fibers[111].

3.10 Disability

The Nagi framework of disability suggests that a pathology gives rise to impairments, which in turn results in functional limitations and thus finally resulting in disability. Of the many impairments epidemiologically associated with disability, musculoskeletal disease is one of the most important one apart from cardiovascular and neurological diseases. Theoretically, disability is the difficulty in fulfilling ones' social roles and function due to decreased physical and cognitive capabilities. There are multiple frameworks of disability but there are certain functional domains which are especially important among older populations, which are physical, cognitive, psychological, social and sensory. Within the physical domain personal role, social role and physical capacity are very important. [118].

There are multiple ways of assessing disability especially physical functioning among the elderly including both self-reported measures and objective measures (Table 3.2).

Table 3.2 Assessment of disability methods

Assessment of disability	Interpretation of disability
Self-reported measures	
Activities of Daily Living (ADL): Five tasks assessed: • Eating • Dressing • Bathing • Transferring from bed to chair • Using the toilet	Disability present if difficulty in one or more tasks.
Instrumental Activities of Daily Living include the assessment of: include the following tasks: • Preparing meals • Shopping • Housekeeping • Managing money • Taking medications • Using the telephone.	Disability present if difficulty in one or more tasks.
Objective measures	
Short Physical Performance Battery (SPPB) which consist of the following: • Side-by-side, semi-tandem and tandem stands, each held for 10 seconds • Four-meter walk at usual pace • Five timed chair stands as quickly as possible, if the first stand is successful	A total score of 12, maximum of 4 for each test is assigned. Based on the scores: • Score <=6 is severe disability • Score of 6-9 is disability • Score 10-12 is normal score
Gait speed used independently either for 4 meters of 6 meters	A gait speed of <1 m/s is considered disability A gait speed <0.8 m/s predicts more severe disability
400 meter self-paced walk	Disability if not able to complete the walk or complete the walk within 15 minutes.
Grip strength	Cut offs: 37kg for men and 21 kg for women [24]

Please note: the cut-offs derived are mostly from US, European and Australian studies

As life expectancy increases, the extended length of life comes at a cost, like quality of life and the impact on disability. Estimates of disability vary due to various ways of measuring disability. Disability is one of the leading health conditions among the older populations. In 2014, almost 22% of population older than 65 years in United states have any form of disability

(vision, hearing, mobility, communication, cognition and self-care). Mobility disability (walking or climbing stairs) was the commonest disability reported at 14.2%. Prevalence of any form of disability among the oldest old (85 years and over) was 42% compared to 17% among people aged 65-75 years. Of the people 65 years and over enrolled in Medicare, 44% reported limitations in ADL or IADL or were living in long term care. Of these 12% people had limitations on one or more IADL only and did not have any ADL difficulty. From 1996 to 2013, the age-adjusted proportion of people aged 65 years and over, with ADL or IADL disability did not change [119].

The Survey of Health, Ageing and Retirement in Europe (SHARE), is a multidisciplinary and cross-national panel database of micro data on health, socio-economic status and social and family networks of about 140,000 individuals aged 50 or older. The fourth wave of this study was conducted in 2010-2011. The WHO Study on Global Ageing and adult Health (SAGE), is a longitudinal study (serial cross sections with follow up of some earlier wave participants) collecting data on adults aged 50 years and older, plus a smaller comparison sample of adults aged 18–49 years, from nationally representative samples in China, Ghana, India, Mexico, Russian Federation and South Africa. The first wave of SAGE was done in 2007-2008. SAGE Wave 1 used multistage cluster sampling with a larger sample of persons aged 50 years and older, and a smaller comparative sample of persons aged 18–49 years. Proxy respondents were used for respondents who were unable to respond for themselves. The sample size for this wave was a total of 42487 (China – 14813, Ghana – 5110, India – 11230, Mexico – 2756, Russian Federation – 4355 and South Africa – 4223). In the SHARE wave 4 and SAGE wave 1, information related to ADL disability and grip strength were collected. In SAGE wave 1 additionally gait speed (timed walk test for 4 meters) was also measured.

Based on the analysis of both these studies (SHARE and SAGE), there were marked differences in the prevalence of ADL disability across the world, ADL disability was defined

as difficulty in any one or more of the five activities - eating, bathing, dressing, getting in and out of bed, and using the toilet (Figure 3.6). As observed, the prevalence of any ADL difficulty was 15% of people aged 75 years and over in Switzerland compared to 60% in Mexico, 68% in Ghana, 78% in India and 80% in Russian federation. The prevalence of ADL disability among 75 years and over was higher compared to 65-74 years across all the countries. The prevalence of ADL limitation in India was almost 2-3 fold higher than China. These differences of ADL disability in India may be due to various factors which needs to be understood further. The capacity of health systems in India and health priorities to address disability as a key priority is lacking in India which could further widen the gap of ADL disabilities compared to other developed countries [120].
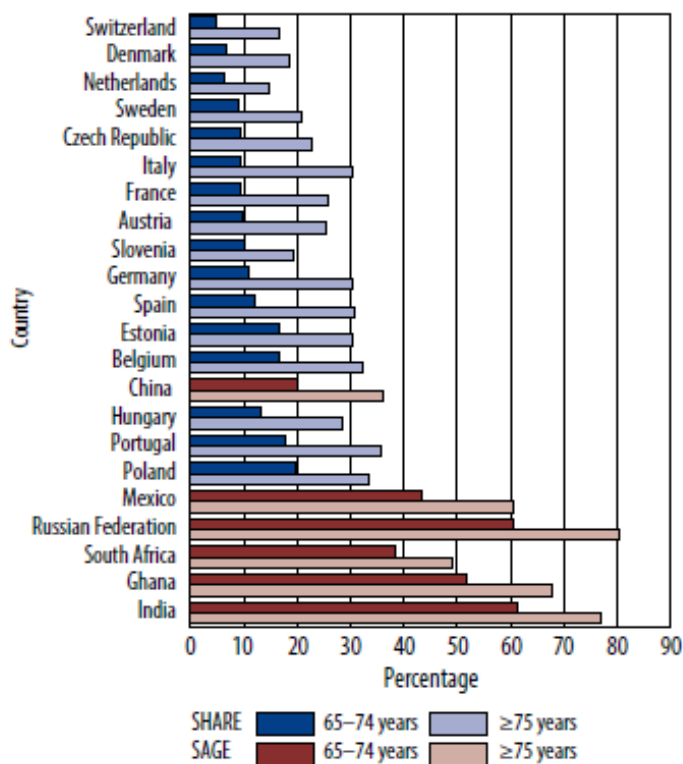
Figure 3.4 Percentage of population aged 65 years and over with at least difficulty with one activity of daily living (ADL)
Five ADL items included were eating, bathing, dressing, getting in and out of bed, and using the toilet. Adapted from World Report on Aging and Health, WHO 2015 [120].

Grip strength at various age groups (starting from 50 years) from the SHARE fourth wave and SAGE first wave observed (Figure 3.7) in all the countries that, women tend to have lower grip strength compared to men; grip strength declines with age in both men and women. Mean grip strength at age 50 years was lowest among Indians (men – around 28 Kg and women – around 26 Kg) and Mexicans (men – around 31 Kg and women – around 26 Kg) compared to men at age 50 (more than 45 kg mean grip strength) of Australia, Germany, South Africa and Netherlands and women at age 50 (more than 35 kg) of South Africa, Netherlands and Australia. The mean grip strength when plotted on a line graphs across ages of 50 to ≥80 years (Figure 7), it was observed that across the ages Indian and Mexican men and women had the lowest grip strength across all the ages. The cross-sectional decline in grip strength with age among Indian men (28 kg at 50 years to 20 kg to ≥80 years) was higher compared to women (26 kg at 50 years to 23 kg to ≥80 years). These differences may be due to genetic makeup, nutrition and lifestyle factors [120].
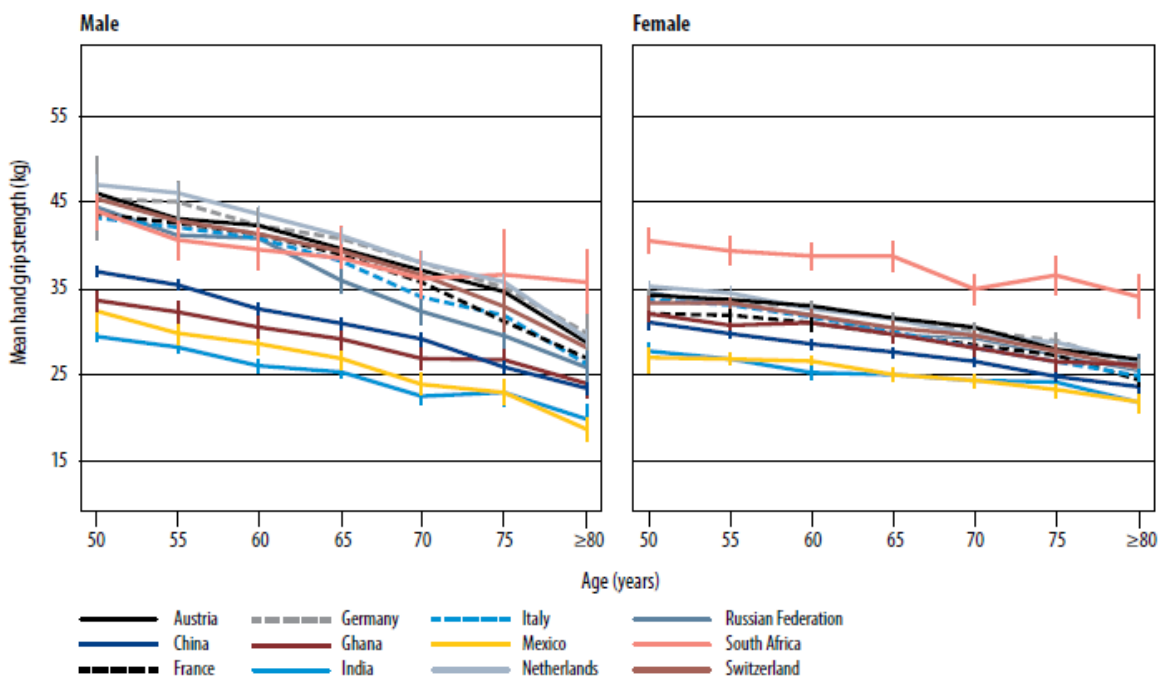
Figure 3.5 Hand grip strength of men and women aged 50 years and over
Data from the fourth wave of SHARE (2010-2011) and first wave of SAGE (2007-2008), data is not adjusted for height and weight. Adapted from World Report on Aging and Health, WHO 2015[120]

Gait speed, time taken to walk 4 meters measured in the six countries of SAGE study (Figure 3.8) observed that time to complete increased with age across all countries for both men and women. The age distribution of time to complete 4 meters among Indian men and women from age 50 years to 84 years can be seen as 3 slopes. The first slope was between 50-64 years where it increased at a steady pace (men 0.7 secs to 0.8 secs; women at 0.9 secs), the second slope was from 65 years to 74 years where it increased (men 0.8 secs to 1 secs; women at 0.9 sec to 1.2 secs), this further increased beyond 75 years in both men and women at a higher rate (men 1 secs to 1.1 secs; women at 1.2 secs to 1.4 secs). Indian men started with faster gait speed at 0.7 secs compared to women at 0.9 secs and continued to have faster gait speed than women. The gap between men and women increased after the age of 80 years (men 1.1 secs and women 1.4secs) [120].
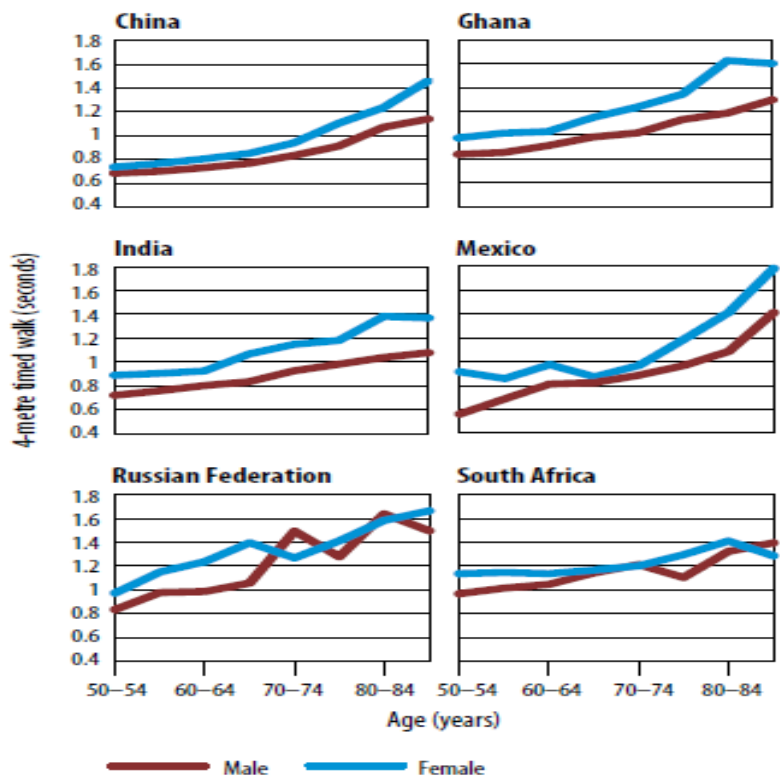
Figure 3.6 Gait speed (seconds to walk 4 meters), by age, sex and country – WHO SAGE
Adapted from World Report on Aging and Health, WHO 2015 [120].

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3.11 Disability in India

The second round of the India Human Development Survey (IHDS-II) was conducted in 2011–2012, with a nationally representative survey of 42,152 households and 53,582 individuals across India. The analysis of 21,925 subjects aged ≥ 60 years (men =10,523 and women= 11402) who were asked questions of ADL difficulty observed that 17.9% men and 26.2% women had ADL disability [121]. The LASI pilot observed a prevalence of 12% ADL disability[24]. A cross sectional study was conducted on 836 men and women aged 60 years and more in the northern part of India to assess ADL disability. It was observed that inability to perform at least one or more of the ADL was prevalent among 17.6% [122]. Another cross sectional study of 495 men and women aged ≥ 60 years in western India (Kolkata) observed that ADL disability was present in 16% [123]. Among 974 subjects aged ≥ 60 years in southern India, ADL disability was observed in 22%[124].

Based on the LASI pilot, among 712 subjects ≥ 45 years using the cut off of grip strength (37 Kg for men and 21 Kg for women), 87% of men and 77% of women had lower grip strength [24]. Based on the SAGE wave 1, the average grip strength (adults 50 + years) age and height adjusted were 28.4 Kg in men and 19.2 Kg in women from India. States like Karnataka (a southern state) and West Bengal (eastern state) the mean grip strength was higher than the national average compared to other states. This variation may be due to development status (in terms of social economic status, Infant mortality rate etc.) which is higher in Karnataka and West Bengal. The states which have lower grip strength compared to national average are predominantly rural and also have lower development indicators. This variation across the different states of India may suggest differences in overall health status[125](Figure 3.9). Based on an analysis of men and women aged 50 years and over enrolled in the Health and Retirement study (HRS), the average grip strength for men (50+ years) was 37.7 Kg and

for women (50+ years) was 23.07. This suggests that US population (50 + years) had higher average grip strength values compared to Indian counterparts. [126].

Another cross sectional study of 206 women aged ≥60 years observed that 53% of the women had lower grip strength using the criteria of: 17 kg for BMI =23; 17.3 kg for BMI 23-26; 18 kg for BMI 26-29 and 21 kg for BMI >29 [127]. In a cross sectional study among 723 health men and women ≥ 60 years of the geriatrics department of a large tertiary hospital (AIIMS, New Delhi), observed that the grip strength decreased with age and varied with BMI. A value of 25th percentile cut off for age was 20, 15 and 15 for age group 60–65, 66–70 and above 70 years in men; among women it was 8, 6 and 6 for the age groups respectively. Similarly, when the cut offs were examined based on the 25th percentile BMI category – among men was 14, 18, 20 and 15; 7, 8, 7 and 7 for women; for the BMI categories <18.5, 18.5–24.9, 25–29.9 and ≥30 respectively [128].
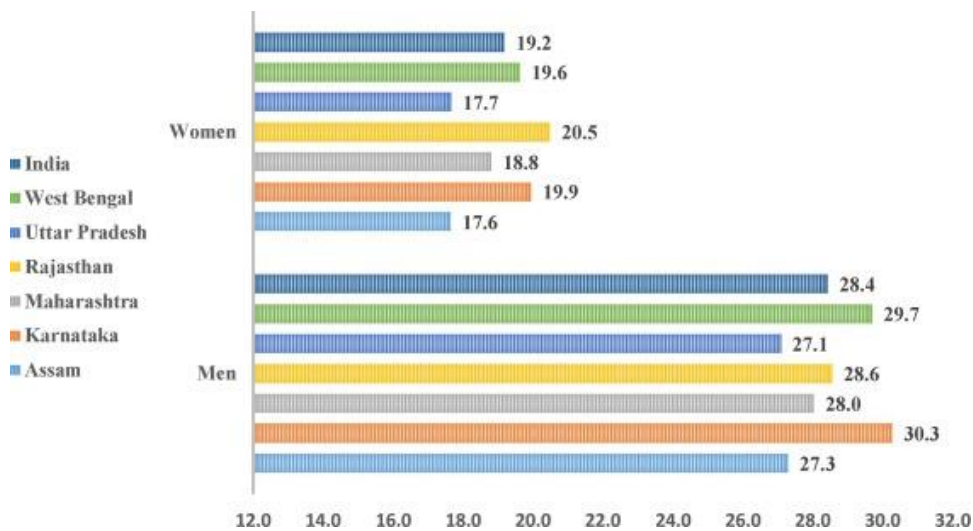
Figure 3.7 Age and height adjusted grip strength (kg) in India and selected States, WHO-SAGE Wave 1
Adopted from Age Socioeconomic patterns and regional variations in grip strength among older adults (50+) in India: Evidence from WHOs Study of Global Ageing and adult Health (SAGE), Archives of Gerontology and Geriatrics, Arokiasmy et.al.,2018 [125]

3.12 Measuring Bone and Muscle – pQCT Perspective

Bone measurement:

Bone densitometry is a quantitative assessment of the skeleton and Bone Mineral Density (BMD) is the most widely accepted surrogate index of bone mass and strength. The most frequently used densitometer parameter is areal bone mineral density (aBMD) and this is also used for clinical diagnosis of osteoporosis. aBMD is measured using a Dual Energy X-ray Absorptiometry (DXA). DXA allows a rapid measurement of both axial and appendicular skeletal sites. In contrast to DXA measures of aBMD, peripheral quantitative computed tomography (pQCT) provides a three-dimensional measure of volumetric that is not confounded by differences in bone size. In addition, pQCT has the ability to distinguish trabecular and cortical bone. Compared with DXA, pQCT is more sensitive to detecting bone loss because it selectively measures changes in the more metabolically active trabecular bone compartment.

pQCT provides a volumetric model of the object using several cross sectional images of multiple X-rays. The object in pQCT is generally the appendicular skeleton (radius and tibia). These images are processed using a computerised algorithm. The pQCT is fully automated system used for determination of bone parameters in the peripheral skeletal sites. pQCT provides an actual description of the cross-sectional geometry and bone composition (both trabecular and cortical). pQCT provides various measures of bone density, bone structure, geometry and estimates of biomechanical strength parameters. Based on animal studies, pQCT has good precision and accuracy in skeletal characterisation and additionally provides several parameters of bone property [129]. Comparative assessment among juvenile and older dogs also suggest that DXA derived aBMD adjusted for femur length has similar variability to pQCT vBMD but it is more sensitive [130].

Precision of pQCT measures of total, trabecular and cortical vBMD, cortical area and cortical content were precise (≤5%) across measures of radius and tibia [131].

For this dissertation the following pQCT bone parameters will be studied:

i.   Trabecular vBMD at the 4% radius and 4% tibia sites.

ii. Cortical vBMD at 33% radius and 33% tibia sites

iii. Cortical thickness at 33% radius and 33% tibia sites

iv. Endosteal circumference at 33% radius and 33% tibia sites

v.   Polar Strength strain index (SSIp) at 33% radius and 33% tibia sites.

These parameters were chosen because, vBMD is an indicator for bone matrix mineralisation and represents the mechanical quality of the solid bone tissue both at the trabecular and cortical sites. Endosteal circumference and cortical thickness were chosen as they represent bone geometry and strength. SSIp was chosen as it predicts the failure load [132, 133] and also has been shown to be a good predictor of long bone bending [133]. All these parameters also have age-related changes due to adaption of stress, strain, and load on the bone and fractures [134, 135].

Muscle measurement:

Lean and fat mass derived using a DXA are the most commonly utilized parameters in body composition research. Apart from the aBMD measurement, DXA separates the mineral free soft tissue (lean mass) from fat mass. The lean and fat mass measured using DXA have been observed to vary with body type [136, 137]. DXA can underestimate fat mass when compared to a computed tomography among obese adults and this increases with the heavier participants [137]. But DXA has the succinct advantages of ability to quantify the whole body fat and lean mass. On the contrary it cannot provide tissue specific lean and fat mass (subcutaneous, visceral etc.) or measure the muscle density (myosteatosis).

The pQCT is a QCT scan of a periphery and collects axial slices around a limb (radius or tibia). The pQCT is calibrated to European forearm phantom and quantifies all tissues in terms of vBMD. pQCT is capable of imaging fat, muscle, and bone to determine tissue content, density, and area. However, this is limited to only the limbs. It cannot provide enough clarity to differentiate individual muscles and the resolution limits the measurement of thin structures due to partial volume effects [138]. The pQCT derived muscle parameters provide a global measure of appendicular muscle. pQCT can separate appendicular subcutaneous adipose tissue (SAT) from muscle and provide measures of muscle size, density, SAT area and Intrermuscular adipose tissue (IMAT).

For this dissertation the following pQCT muscle parameters will be studied:

i. Muscle density

This particular parameter was chosen because;

Muscle density (MD) crudely is the density of the entire muscle group and it contains more mass per unit volume than fat. This can serve as a proxy for inter and intra muscular fat infiltration [139]. MD also reflects the compactness of muscle fibers, protein within the muscle and other soft tissues and as observed; it can be termed as a measure of muscle quality. MD is inversely related to lipid content and 50 % variance of MD can be explained by the inter and intra muscular adipose tissue[140]. Both Health ABC study and InCHIANTI study have observed that MD were risk factors for disability in healthy older adults [141, 142]. It was observed that one unit decrease in MD led to 17% increase in the odds of fall [143].

4. Dissertation Goals

With the increased aging in India and the growing number of older adults living in rural parts of India, their musculoskeletal health which impacts their day to day activities has not been well studied. There is sparse epidemiological data on the effects of lower bone density and muscle weakness among older adults. This dissertation will address the lack of (i) comparisons of bone parameters with international data, (ii) how the bone and muscle parameters impact mortality and (iii) how the physical performance measures impact the bone and muscle parameters.

The Mobility and Independent Living in Elders Study (MILES) was established to determine the prevalence of chronic disease and disability and to identify their risk factors in older rural Indians. As a part of this study bone and muscle parameters were collected using the pQCT and various physical performance measures based on international epidemiological study protocols.

The main goals for this dissertation are to utilise the data from MILES study among rural south Indian population, to compare the pQCT derived bone parameters with other ethnic populations, assess the pQCT derived bone and muscle parameters as predictors for mortality and to explore the association of physical performance measures on the pQCT derived bone and muscle measures.

Paper1: To compare the pQCT derived bone parameters of rural south Indian with US Caucasian and Afro Caribbean older men.

Aim: The main aim of the paper is to compare Volumetric Bone Mineral Density (vBMD), bone structure and structural geometry among rural south Indian, US Caucasian and Afro-Caribbean older men

Hypothesis: We hypothesize that, the vBMD bone structure and structural geometry measures of rural south Indian older men will be lower than US Caucasian and Afro Caribbean older men.

Paper 2: To assess pQCT derived bone and muscle parameters as predictors of mortality among rural south Indian older population

Aim: The main aim of the paper is to assess vBMD, bone structure, bone geometry measures and muscle density as predictors of all-cause mortality among rural south Indian older population

Hypothesis: We hypothesize that, vBMD, bone structure, bone geometry measures and muscle density will have an inverse relationship with all-cause mortality among rural south Indian older population (men and women).

Paper 3: The main aim of the paper is to analyse association of physical performance measures on the pQCT derived bone and muscle measures among rural south Indian older population

Aim: The main aim of the paper is to evaluate whether physical performance measures are associated with pQCT bone and muscle quality measures among rural south Indian older population

Hypothesis: We hypothesize that, physical performance measures (Grip Strength, SPPB score and 400 meter walk) will be positively related to vBMD, bone structure, bone geometry measures and muscle density.

5.   Paper 1: Volumetric Bone Mineral Density (vBMD), Bone Structure and Structural Geometry Among Rural South Indian, United States Caucasian and Afro-Caribbean Older Men

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5.1 Abstract

Purpose: Geographical and racial comparisons of bone mineral density (BMD) have largely focused on DXA measures of areal BMD. In contrast, peripheral quantitative computed tomography (pQCT) measures volumetric BMD (vBMD), bone structural geometry and provides estimates of biomechanical strength. To further understand potential geographical and racial differences in skeletal health, we compared pQCT measures among US Caucasian, Afro-Caribbean and rural south Indian men.

Methods: We studied men aged ≥ 60 years enrolled in the Mobility and Independent Living among Elders Study (MILES) in rural south India (N=245); Osteoporotic Fractures in Men Study (MrOS) in the US (N=1148) and the Tobago Bone Health Study (N=828).

Results: The BMI (kg/m^2) of rural south Indian men (21.6) was significantly lower compared to the US Caucasians (28) and Afro-Caribbean men (26.9). Adjusting for age, height, body weight and grip strength; rural south Indian men compared to US Caucasians had significantly lower trabecular vBMD [-1.3 to -1.5 Standard Deviation (SD)], cortical thickness [-0.8 to -1.2 SD]; significantly higher endosteal circumference [0.5 to 0.8 SD]; but similar cortical vBMD. Afro-Caribbean men compared to US Caucasians had similar trabecular vBMD but significantly higher cortical vBMD [0.9 to 1.2 SD], SSIp [0.2 to 1.4 SD] and tibial endosteal circumference [1 SD],

Conclusions: In comparison to US Caucasians, rural south Indian men have reduced bone strength (lower trabecular vBMD) and Afro-Caribbean men have greater bone strength (higher cortical vBMD). These results suggest an underlying higher risk of osteoporotic fractures and greater future fracture burden among rural south Indian men.

5.2 Introduction

Global demographic patterns are changing with accelerated population aging fueled by declining fertility and increased longevity[145]. As per 2010 estimates, 8% of the world's population (524 million) was 65 years and older and is expected to triple by 2050[145]. Even though the developed countries have the oldest populations, the majority and rapidly aging populations are from less developed countries[145]. During 2010, about 65% of those aged 60 years and older lived in less developed countries, this is projected to increase to 80% by 2050[146]. This demographic transition has important social, economic, and public health implications[147]. Even with increasing attention to the aging population throughout the globe, research related to aging has been primarily conducted in developed countries[148].

India is the second most populous country in the world and its older population segment is increasing dramatically. For example, approximately 8% of India's population was aged 60 years and older in 2010 (93 million) and this population segment is projected to increase to 19% by 2050 (323 million)[10]. Of importance, more than two-thirds of India's elders live in rural areas[10]. The United States population aged 65 years and older between 2012 and 2050 is experiencing growth and will almost double[149]. The 18 % of the 2010 population of the US aged 60 and over is estimated to increase to 27% by 2050 [150]. Trinidad and Tobago is also experiencing rapid aging. The 60 and over population in 2010 was 11% and is estimated to increase up to 32% by 2050[150]. Trinidad and Tobago is among the top ten countries with the largest percentage increases in the share of the 60 and over population[150].

These increases in the older populations around the world will lead to large increases in the prevalence of many chronic conditions and degenerative diseases including osteoporosis[145]. A significant consequence of osteoporosis is fracture, which occurs due to imbalance of bone

strength and force on the bone[151]. Osteoporosis has major and continued impact on the morbidity, quality of life and mortality [152]. The International Osteoporosis Foundation estimates that one third of women and one fifth of men aged 50 years and over experience osteoporotic fractures [153].

Hip fractures increase with age and age standardized rates for men vary >140 fold[154] across the world's population. The factors contributing to this geographic variability in hip fracture rates are unknown, but may reflect differences in bone strength. A few studies have compared areal bone mineral density (aBMD) among different race and ethnic populations. For example, African-American and Afro-Caribbean men have higher aBMD at the hip and lumbar spine compared to US Caucasian and Hispanic men [41, 155-157]. US Asians, Hong Kong Chinese and South Koreans have lower aBMD at lumbar spine compared to US Caucasian[157]. aBMD of Indian women aged 20-60 years, when compared with the US NHANES III data was about 27% lower[158]. However, aBMD is a two dimensional imaging technique that integrates cortical and trabecular BMD. Most of the evidence of geographical / racial comparisons has been limited to aBMD.

Peripheral Quantitative Computed Tomography (pQCT) is an alternative technology developed for quantitative determination of bone density, structure and structural geometry. To further understand potential geographical and racial differences, we compared pQCT measures of volumetric BMD (vBMD), bone structure and structural geometry which are the indices of biomechanical strength among older men from three distinct race/ethnic populations: rural south Indian, US Caucasians and Afro-Caribbean.

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5.3 Materials and Methods

Study subjects:

The current cross-sectional analysis compared pQCT measures from three cohort studies. The Mobility and Independent Living in Elders Study (MILES) was established in 2012 to estimate the prevalence of age related diseases and risk factors for disability among Indians residing in rural south India[159]. A random sample of 562 men and women 60 years and over, were enrolled from Medchal Region of Telangana state of southern India. The response rate for men in MILES was 74%. Of the 495 men and women who underwent pQCT, all 245 men were included in this analysis.

The Osteoporotic Fractures in Men (MrOS) study is a prospective study designed to identify risk factors for fracture among older men (65 years and more); 5994 men were recruited in 2000-2002 at 6 different geographic regions of the US. Key recruitment methods included mailings using community and provider contact lists; regional and senior newspaper advertisements; and presentations targeted to seniors. Sites used a centrally developed recruitment brochure. Response to mass mailings at some sites surpassed 10–15% and appointment show rates averaged above 85%. The final number enrolled in MrOS was 5% more than the original recruitment goal of 5700 [160, 161]. The current analysis included men from the Minneapolis and Pittsburgh sites which obtained pQCT measures during the second visit of the study between 2005 and 2006. Of the 1180 participants who completed the second visit at these sites, 1148 (97%) US Caucasians were included in this analysis.

The Tobago Bone Health study was initiated as part of population based prostate cancer screening cohort study between 1997 and 2003 among men older than 40 years of age [162]. These men were recruited by word of mouth, poster, flyers, public health announcements and health care

workers and represent about 50% of the men age 40-79 residing on the island of Tobago [163]. Between 2004 and 2007, men in the cohort were invited to return for a repeat examination which included pQCT [164]. Of the 2153 men who underwent pQCT, 828 men with all 4 grandparents of African ancestry and aged 60 years and over, were included in this analysis.

pQCT and Calibration:

pQCT scans on the radius and tibia were performed using the Stratec XCT-2000 (Stratec Medizintechnik, Pforzheim, Germany) in MILES, Tobago and the Pittsburgh site of MrOS study. The Minneapolis site of MrOS performed the scans using Stratec XCT-3000 scanner. Technicians followed a standardized protocol for positioning and scanning of each subject. First, a coronal scout view of a 40-mm section encompassing the distal end of the radius or tibia was obtained. Second, the flat portion of the radio-carpal joint or tibia endplate was marked in the scout view and the scanner gantry moved a fixed distance proximal and along the subject's arm or leg from the marked position. The length of tibia was measured from the medial malleolus to the medial condyle of the tibia. Radius length was measured from olecranon to ulna styloid process. Scans were taken at 4% and 33% of the length of radius and at 4%, 33% and 66% of the length of tibia. Subject scans were repeated if artefacts due to motion or beam hardening were present. To monitor the stability of the pQCT scanners, a manufacturer supplied cylindrical Quality Assurance (QA) phantom was scanned daily before subject scans were acquired. The phantom is 5 cm in diameter with a hydroxy-apatite core manufactured to have a uniform absorption value comparable to trabecular bone of moderate density. The absorption and cross-sectional area measurements recorded for the phantom are automatically stored in a phantom QA log file generated by the scanner software. This QA log file was checked periodically to ensure that the scanner calibration did not drift. In addition, a European Forearm Phantom (EFP) was scanned at the beginning and

end of each study to ensure that the scanner calibration did not drift from factory settings. The EFP has four distinct density zones that mimic trabecular and cortical bone typical for a distal and proximal radius. Three repeat scans were taken and analyzed for comparing the density. Fit coefficients were derived and correction factor for MILES scanner density measurement was applied (MILES= 1.02 (MrOS) + 1.9). The Tobago study scanner did not have any difference when compared with the MrOS scanner. All pQCT scans were analyzed by a single investigator using the manufacturer software package version 6.00 for the XCT scanners. This software provides a suite of segmentation options to quantify total, trabecular and cortical bone properties from each pQCT image. Before each image was analyzed it was checked for artefacts due to motion or beam hardening. Scans with artefacts were not analyzed. All 4% radius and tibia scans were analyzed using the CALCBD option with an automatic gradient search (contour mode 2) applied to segment bone from the soft tissue background and concentric peeling (peelmode 1, 45%) to segment trabecular and cortical bone. Proximal scans acquired at the 33% and 66% limb locations were segments using a fixed threshold of 710 mg/cm3(Cortmode1). Coefficients of variation (CVs) were determined for pQCT scans by replicating measurements on 15 subjects (CV $\leq$ 2.1%). Though there are differences in the XCT 2000 and 3000 scanners, these machines were calibrated at the factory to the European Forearm Phantom. Even with the slight differences in technical parameters such as effective X-ray beam energy position, the EFP calibration step ensured that volumetric density derived on each scanner are directly comparable. None of the scanners had a calibration drift due to service issues during the study.

pQCT parameters:

For this analysis we focused on the following pQCT parameters that are physiologically important in skeletal aging: at the 4% site of radius and tibia - trabecular vBMD and Strength Strain Index (SSIp); at the 33% sites of radius and tibia - cortical vBMD, cortical thickness, endosteal circumference and SSIp. vBMD was chosen as it is an indicator of bone matrix mineralization or mechanical quality of the solid bone tissue. Endosteal circumference and cortical thickness were chosen as they represent bone geometry and strength. SSIp was chosen as it predicts the failure load [132] and also has been shown to be a good predictor of long bone bending [133]. All these parameters also have age related changes due to adaption of stress, strain and load on the bone [134, 135].

Other measures:

Information on demographics, lifestyle factors, self-reported health status and direct measures of body weight and height were obtained. Body Mass Index (BMI) was calculated as body weight in kilograms divided by height in meters squared. Fracture history, among the US Caucasians was based on self-report of fractures at baseline after age 50 years and incident fractures from the baseline visit to the second visit. Among rural south Indians, fracture history was based on the participants' recall of a fracture in the last 5 years; and among the Afro-Caribbean men, this was based on health history of fracture event ever. Self-reported history of falls in the past 12 months was collected in all three studies. Information on diabetes was based on glucose levels ≥126mg/dL (after a minimum of an 8-hour fast), or self-report of diabetes or insulin or hypoglycemic medications among rural south Indians and Afro-Caribbean's whereas among US Caucasians it was self-reported. Hypertension among the Afro-Caribbean and rural south Indian populations was based on self-report, or medication inventory or blood pressure assessment.

Hypertension among US Caucasians was self-reported. Grip strength was measured using hand held dynamometers among all the three studies. Ever smoking status was based on self-reported current and past smoking status in all the three populations. Drinking alcohol among US Caucasians was having at least 12 drinks in the past 12 months. Among Afro-Caribbean population it was based on the question of how many drinks in a typical week for the past 12 months. The rural south Indian population it was based on current consumption of alcohol.

Statistical analysis:

Characteristics of the three groups are expressed as percentages or mean ± standard deviation (SD), confidence intervals and were compared by ANOVA or chi square. Any pQCT parameter with a value of mean ± 3 SD was identified within each study and removed from analysis. pQCT parameters were compared across the three groups using general linear models (GLM). Comparisons were performed adjusting for age, height, weight and grip strength. Percentage and standard deviation differences in the mean pQCT parameters were also performed keeping US Caucasians as the referent group. Standard deviation differences presented are the difference in mean pQCT parameters in terms of number of SDs based on the US Caucasians. Results were considered statistically significant when a p-value was less than 0.001 with Bonferroni correction for multiple comparisons. Statistical analyses were performed using SAS (version 9.3; SAS Institute, Cary, NC, USA).

5.4 Results

Characteristics of the populations

When compared to US Caucasians (77.2 ± 5.2), rural south Indian (68.2 ± 6.6) and Afro-Caribbean (68.8 ± 6.8) men were significantly younger by more than 8 years (Table 5.1). Rural

south Indian men had significantly lower body weight (55.9 ± 11.5), height (160.6 ± 5.6) and BMI (21.6 ± 3.9) compared to US Caucasians and Afro-Caribbean men. Rural south Indian men had significantly lower grip strength (20 ± 8.1 kg) compared to US Caucasians (37.8 ± 7.7kg) and Afro-Caribbeans (35.9 ± 10.8 kg). Afro-Caribbeans had lower prevalence of smoking (past and current), whereas alcohol consumption was significantly higher in rural south Indian men compared to US Caucasians and Afro-Caribbeans. Rural south Indian men were less likely to report a past history of fall (9%) and fractures (4.9%) compared to US Caucasians and Afro-Caribbean men. The prevalence of hypertension among the older men was similar among rural south Indian and US Caucasians whereas Afro-Caribbean men had significantly higher rates of hypertension with two thirds being hypertensive. Afro-Caribbean men were significantly more likely to be diabetic (29.7%) compared to rural south Indians (18.8%) and US Caucasians (15.6%). Based on self-report of current health status, significantly fewer rural south Indian men opined their health status as good (46.1%) compared to 86% of US Caucasians and Afro-Caribbean.

vBMD, bone structure and structural geometry:

Rural south Indian men had significantly lower trabecular vBMD at the radius (-28.4%) and tibia (-24.8%) compared to US Caucasians (Table 5.2). Cortical thickness at the radius and tibia (-13.4% and -16.6%) were significantly lower when compared with US Caucasians. Endosteal circumference was significantly higher at the radius (9.4%) and tibia (12.1%) in rural south Indians compared to US Caucasian men. Cortical vBMD at the radius (-0.7%) and tibia (-0.1%) were similar to US Caucasians. Except for SSIp at 4% tibia (-20.7%) which was significantly lower, SSIp at 4% radius, 33% radius and 33% tibia were similar to US Caucasians. These differences were independent of age, height, weight and grip strength.

Afro-Caribbean men had similar trabecular vBMD compared to US Caucasians. All the other assessed pQCT parameters were significantly higher among Afro Caribbean men except tibial cortical thickness (-4.1%), which was significantly lower and endosteal circumference of radius which was not different compared with US Caucasians (Table 5.2).

Standard deviation differences in the skeletal parameters, independent of age, height, weight and grip strength were compared to US Caucasians (Figure 5.1). Rural south Indian men compared to US Caucasians had lower (-1.3 to -1.5 SD) trabecular vBMD, cortical thickness (-0.8 to -1.2 SD) and SSIp at 4% tibia (-0.7 SD); and higher endosteal circumference (0.5 to 0.8 SD). Among the Afro-Caribbean men, cortical vBMD, tibial endosteal circumference, radial cortical thickness and SSIp were 0.2 to 1.4 SD higher than US Caucasian men. Tibial cortical thickness was -0.3 SD lower compared to US Caucasians.

5.5. Discussion

Rural south Indian men compared to US Caucasians had lower trabecular vBMD, cortical thickness and SSIp at 4% tibia; higher endosteal circumference; but similar cortical vBMD, radial SSIp and SSIp a 33% tibia. The European Male Aging Study (EMAS) which compared south Asians and whites living in Manchester, United Kingdom, had comparable observations of lower tibial trabecular vBMD, tibial and radial cortical thickness; similar radial cortical vBMD; but on the contrary radial trabecular vBMD was similar and tibial cortical vBMD was lower [165]. In the current study, Afro Caribbean men compared with US Caucasians had higher cortical vBMD, tibial endosteal circumference, SSIp and radial cortical thickness; lower cortical thickness at the tibia; and similar trabecular vBMD. These differences suggest geographic / racial differences in measures of bone structure, geometry and strength.

With increasing age, cortices get thinner, the cortical envelope becomes more porous, and trabecular bone mass declines, all of which contribute to increased bone fragility among older adults [134, 135, 166, 167]. The rural south Indian older men in our study had lower trabecular vBMD and cortical thickness in both the upper and lower limbs. This in combination with lower 4% tibial SSIp suggests increased susceptibility to fractures and future morbidity among rural south Indian men. Cortical vBMD is a determinant of intrinsic stiffness of bone tissue. The rate of loss of cortical vBMD is steady or slower from 50 years till 75 years of age, compared to trabecular vBMD [168, 169]. Though the US Caucasians were older, rural south Indian men had similar cortical vBMD and significantly lower trabecular vBMD.

Our results suggest that the patterns of pQCT parameter differences across different geographic / race groups were similar to the DXA based aBMD comparisons published earlier [157, 170]. The aBMD at the femoral neck, total hip and lumbar spine were significantly higher among Afro-Caribbean compared to US Caucasian men. The aBMD of US Asians (significant for total hip), Hong Kong Chinese (significant for femoral neck, total hip and lumbar spine) and South Koreans (significant for total hip and lumbar spine) was lower compared to US Caucasians [157]. To our knowledge, there is lack of data on aBMD comparisons among older Indian men. Healthy Indian men aged 20-29 had significantly lower aBMD at the hip, forearm and lumbar spine when compared to the third US National Health and Nutrition Examination Survey (NHANES III, 1988-94)[171]. In contrast, a study of UK white and Indian men aged 20-40 years showed similar aBMD [172]. These observations suggest conflicting directionality of aBMD and pQCT differences among different racial / geographic groups. Among the US MrOS cohort, several pQCT parameters were strongly associated with non-vertebral fractures among older men and these associations were independent of aBMD [173]. Considering this, it is important to focus on vBMD,

bone structure and structural geometry in furthering our understanding of geographical differences in hip fractures.

Our rural south Indian population compared to US Caucasians had lower trabecular vBMD, lower cortical thickness, lower 4% tibial SSIp and higher endosteal circumference suggesting an increased fragility of radius and tibial bones among the Indian population. Conversely, self-reported history of fracture was lower among the rural south Indian men. This may reflect recall bias and needs to be interpreted with caution. In addition, the time frame for fracture history recall was shorter among rural south Indian men (past 5 years), than the US Caucasian men (fracture since age 50) or Afro-Caribbean men (ever had fracture). However, age standardized incidence of hip fractures for Indian men is around 122 per 100,000 and for US men is 155 per 100,000 [11], which suggests a lower fracture burden among Indian menThese rates were based on a single study in one district (Rohtak) of northern India in 2009 from four orthopedic hospitals and hence may not be representative of hip fracture rates across the diverse country of India. [78]. National population based data are needed for rates of hip fractures in India.

Our results suggest that the rate of hip fracture at least among rural Indians should be higher than US white men. The lower fracture prevalence among rural south Indian men is contrary to our expectations but could reflect the following. First, the fractures were self-reported and not adjudicated. Second, Indians have lower life expectancy at birth (68 years) compared to US (79 years) and Trinidad & Tobago (70 years) [85]. In 2012, life expectancy at age 60 years for Indian and Trinidad & Tobago men was 16 years compared to 21 years for US men [88]. This lower life expectancy at birth and at age 60 may lead to fewer fractures due to competing mortality. Third, Indians have a lower hip axis length [174] which could impact fracture risk.

The Afro Caribbean population had significantly higher cortical vBMD, radial cortical thickness, endosteal circumference, SSIp and similar trabecular vBMD compared with US Caucasians. This suggests that the skeleton of this population is less fragile in comparison to US Caucasians. These observations are consistent with the lower fracture rates among men of Afro-Caribbean ancestry [170].

It has been estimated that around 50 million people in India are osteoporotic [175] and this is likely due to combination of multiple factors including genetic, nutritional, lifestyle and smaller skeletal size [77]. The increasing aging population in India coupled with increased osteoporosis will impact the number of fractures observed among Indian elderly. The current analysis adds to the literature and presents a scenario of fragile bones among rural south Indian older men.

There are several potential limitations to the current analysis. We used a cross-sectional design and hence cannot infer causation. The sample size of the Indian population was lower and was restricted to one specific rural southern region in India; thus generalization of these finding to the larger Indian population needs to be done with caution. The geographical differences between the populations may also reflect other factors including genetic, lifestyle, chronic diseases, concurrent medications, and physical activity. Different ascertainment methods of the covariates limited harmonization of the data and we did not include them in the models. In this analysis we adjusted the covariates age, height, weight and grip strength, which were collected similarly in all the three studies. Another possible limitation was the partial volume effect, which may underestimate cortical vBMD due to thinner cortices. However, the current analysis also has several notable strengths. First, to our knowledge this is the first description of pQCT parameters among older men from rural south India and the first comparison to a large well characterized

population of older US Caucasian men and Afro-Caribbean men. Second, we performed cross calibration of each pQCT machine using a European forearm phantom.

In conclusion, compared to US Caucasians, rural south Indian men had reduced bone strength primarily because of lower trabecular vBMD and Afro-Caribbean men had greater bone strength primarily because of higher cortical vBMD. These findings suggest an underlying higher risk of osteoporotic fractures among rural Indian men that may translate to a greater future fracture burden. Though there have been estimations of hip fracture rates among Indians[69, 175], the relationship between the pQCT measures studied and fracture risk has not been established in India.

5.6 Tables and Figures

Table 5.1 Characteristics of the study populations: Rural south Indian, US Caucasian and Afro-Caribbean older men

Characteristics	US Caucasian (N=1148)		Rural South Indian (N=245)		Afro-Caribbean (N=828)	
Age in years [Mean ± SD (95% CI)]	77.2 ± 5.2	(76.9,77.6)	68.2 ± 6.6*	(67.5,68.9)	68.8 ± 6.8*	(68.4,69.3)
Weight (kg) [Mean ± SD (95% CI)]	84.1 ± 13.4	(83.3,84.9)	55.9 ± 11.5*	(54.2,57.6)	81 ± 15.2 *	(80.2,82.1)
Height (cm) [Mean ± SD (95% CI)]	173.1 ± 6.8	(172.7,173.5)	160.6 ±5.6*	(159.8,161.5)	173.2 ± 9.2	(172.9,173.8)
BMI (kg/m2) [Mean ± SD (95% CI)]	28 ± 4	(27.7,28.2)	21.6 ±3.9*	(21.1,22.1)	26.9 ± 4.7 *	(26.7,27.3)
Grip Strength (kg) [Mean ± SD (95% CI)]	37.8 ± 7.7	(37.3,38.2)	20 ±8.1*	(19.2,21.3)	35.9 ± 10.8 *	(36.2,37.4)
Ever smoked [% (95% CI)]	63.9	(61.2, 66.7)	75.1*	(69.7, 80.5)	31.9*	(28.7, 35.1)
Drinks Alcohol [% (95% CI)]	61.1	(58.3, 63.9)	71.4*	(65.8, 77.1)	50.7*	(47.4, 54.3)
History of fracture [% (95% CI)]	24.3	(21.8, 26.8)	4.9*	(2.2, 7.6)	14.8*	(12.4, 17.2)
History of fall [% (95% CI)]	25.7	(23.2, 28.2)	9*	(5.4, 12.6)	16*	(13.5, 18.5)
Hypertension [% (95% CI)]	52.7	(49.8, 55.6)	52.7	(46.4, 58.9)	66.9*	(63.7, 70.1)
Diabetes [% (95% CI)]	15.6	(13.5, 17.7)	18.8	(13.9, 23.7)	29.7*	(27.2, 33.4)
Health status opinion – Good / Excellent [% (95% CI)]	86.2	(84.2, 88.1)	46.1*	(39,9, 52.4)	85.9	(84.3, 88.9)

Please see the "Materials and methods" section for ascertainment methods
Means expressed in the table are Least Squares means (LS-Means)
*p value <0.05 when compared with the US Caucasian population.

Table 5.2 Mean and Percent difference in the pQCT parameters of rural south Indian and Afro-Caribbean older men compared with US Caucasian (age, height, weight and grip strength adjusted)

pQCT Parameters	US Caucasian Mean (95% CI)	Rural South Indian Mean (95% CI) (p value)	% difference [a]	Afro-Caribbean men Mean (95% CI) (p value)	% difference [a]
Trabecular vBMD – Radius 4%	197.3 (193.9, 200.7)	141.2 (132.8, 149.6) (<0.0001) *	-28.4	201.7 (198.2, 205.2) (0.308)	2.2
Trabecular vBMD – Tibia 4%	229.9 (226.9, 232.8)	172.9 (165.6, 180.2) (<0.0001) *	-24.8	227.5 (224.4, 230.5) (0.9341)	-1.0
Cortical vBMD – Radius 33%	1164.5 (1162.3, 1166.8)	1156.5 (1151,1162.1) (0.0591)	-0.7	1201.6 (1199.3, 1203.9) (<0.0001) *	3.2
Cortical vBMD – Tibia 33%	1140.7 (1138.5, 1142.9)	1139 (1133.5, 1144.5) (1.00)	-0.1	1168.3 (1165.9, 1170.6) (<0.0001) *	2.4
Cortical Thickness – Radius 33%	3.29 (3.26, 3.34)	2.85 (2.76, 2.94) (<0.0001) *	-13.4	3.49 (3.46, 3.53) (<0.0001) *	6.1
Cortical Thickness – Tibia 33%	5.59 (5.53, 5.64)	4.66 (4.53, 4.79) (<0.0001) *	-16.6	5.36 (5.30, 5.42) (<0.0001) *	-4.1
Endosteal Circumference – Radius 33%	21.2 (21, 21.4)	23.2 (22.5, 24) (<0.0001) *	9.4	21.7 (21.4, 22) (0.0531)	2.4
Endosteal Circumference – Tibia 33%	39.7 (39.2, 40.1)	44.5 (43.3, 45.6) (<0.0001) *	12.1	46 (45.5, 46.5) (<0.0001) *	15.9
SSI p – Radius 4%	490.3 (481.3, 499.2)	459 (436.9, 481.1)	-6.4	575.1 (565.9, 584.2)	17.3

Table 5.2 Continued

pQCT Parameters	US Caucasian Mean (95% CI)	Rural South Indian Mean (95% CI) (p value)	% difference [a]	Afro-Caribbean men Mean (95% CI) (p value)	% difference [a]
		(0.0657)		(<0.0001) *	
SSI p – Tibia 4%	2363.5 (2318.3, 2408.7)	1874.7 (1763.6, 1985.9) (<0.0001) *	-20.7	2530.3 (2483.3, 2577.4) (<0.0001) *	7.1
SSI p – Radius 33%	347.3 (342.3, 352.3)	346.7 (334.5, 359) (1.00)	-0.2	420.9 (415.8, 426) (<0.0001) *	21.2
SSI p – Tibia 33%	1999 (1974.9, 2023.1)	1883.5 (1824, 1943) (0.0051)	-5.8	2460.5 (2435.3, 2485.6) (<0.0001) *	23.1

a: Percent difference compared to US Caucasian

* : Difference compared to US Caucasians is significant (p<0.001)

Difference compared to US Caucasians significant (p<0.001); Error bars represent 95% confidence intervals of the SD difference.

Figure 5.1 Standard deviation difference of mean pQCT parameters among rural south Indian and Afro-Caribbean compared to US Caucasian older men (age, height, weight and grip strength adjusted)
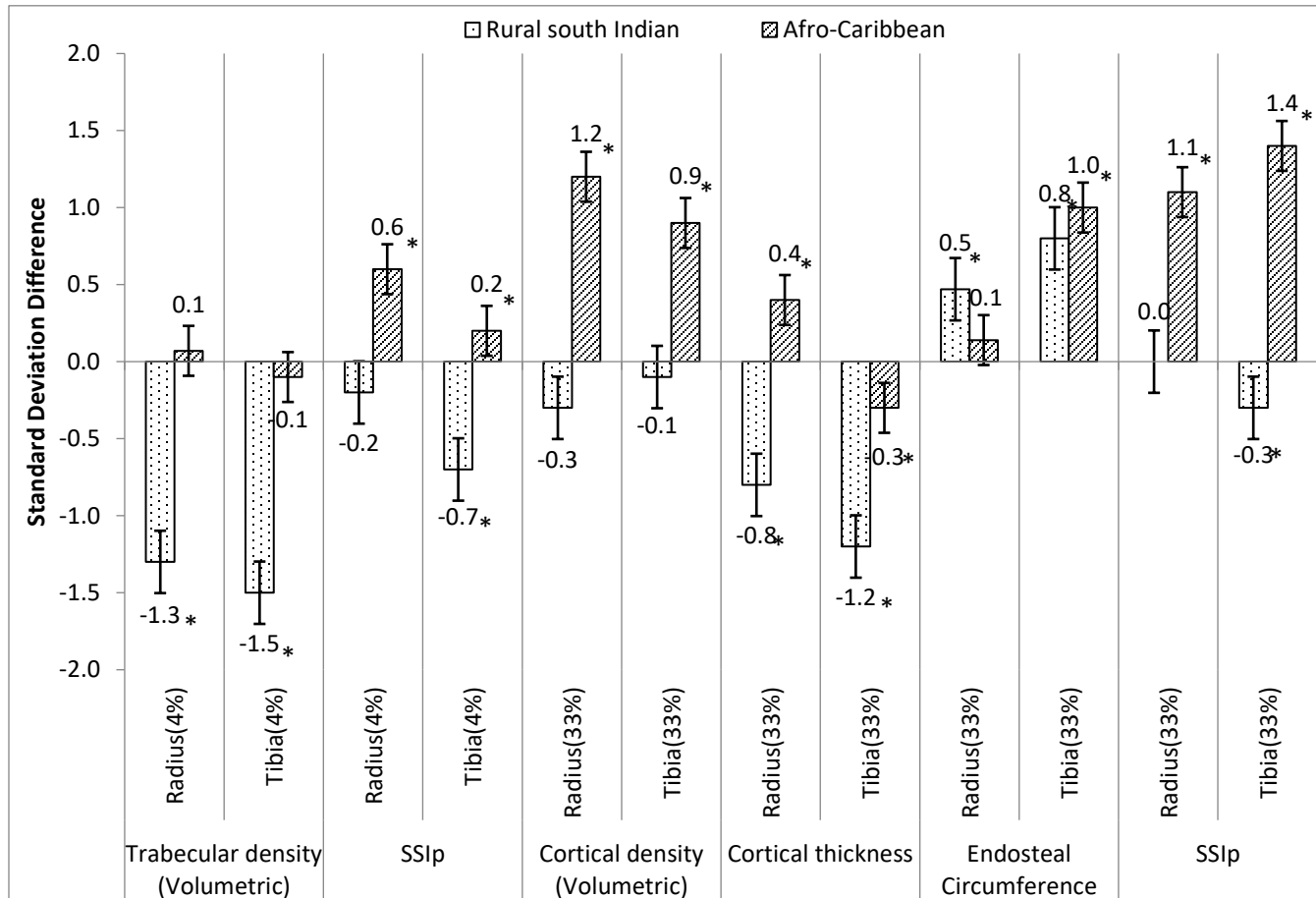
6. pQCT Derived Bone Parameters and Muscle Density as Predictors of Mortality

6.1 Association of pQCT Derived Bone Measures and Mortality

DXA has been the reference standard for the measurement of BMD and also the clinical definition of osteoporosis. There have been multiple studies which have confirmed the relationship of BMD measured by DXA and mortality[176-181]. Based on a meta-analysis conducted on 10 prospective studies with 46182 participants from 5 countries (US, Netherlands, Sweden, Australia, and Brazil) with a median 7 years of follow up. BMD was measured using DXA in these studies. The pooled results of the meta analysis observed per SD decrease in BMD, the all-cause mortality risk was 1.2 fold (Hazards Ratio (HR) 1.20; 95%CI 1.09–1.30). [182]. Most of the evidence on BMD and mortality comes from the US, Europe or other developed countries and there is a lack of evidence of BMD and mortality among Indians.

However, DXA does not assess trabecular or cortical BMD or the bone geometry separately. This is relevant as there is an accelerated turnover of trabecular bone compared to the cortical bone with increasing age and trabecular bone turnover is linked with adverse health outcomes[183]. But this does not mean that the cortical bone loss is not important, in fact the cortical bone loss plays an important role in the bone fragility. Focus on the aBMD from DXA neglects this particular aspect of the bone content, but it also ignores the bone structure and geometrical aspects which may play a role in mortality. Very few studies have looked at the vBMD (trabecular and cortical), bone structural and geometrical measures association with mortality.

Age, Gene/Environment Susceptibility (AGES) - Reykjavik Study, is a single-center prospective population study of 30,795 Icelandic older men and women born in 1907–1935 and living in Reykjavik in 1967. A total of 4654 participants aged 66 years and over were involved in the analysis of a Quantitative Computed Tomography measured vBMD (trabecular and cortical)

at proximal femur. After 9.4 years of follow up, it was observed that subjects with the lowest quartile of trabecular vBMD had 1.21-fold higher risk of all-cause mortality compared to other three quartiles (Q2-Q4) after adjusting for age, sex and hip size. When including other risk factors baseline history of diabetes, chronic lung disease, coronary artery calcium score, creatinine, cognitive status, self-reported health status, 25OHD, smoking status, physical activity level, weight change from age 50, and self-reported history of previous fracture, the risk for death was reduced but was still significantly higher (HR 1.12, 95% CI 1.01–1.25) and independent of cortical vBMD. Fracture status as a time varying covariate attenuated the association further to non-significant status (HR 1.07, 95% CI 0.96–1.19). The subjects with the lowest quartile of cortical vBMD compared with other quartiles (Q2-Q4) in fully adjusted models without fracture as a time varying covariate (HR 1.08, 95% CI 0.97-1.20) and adjusting for fracture as a time varying covariate (HR 1.05; 95% CI 0.94-1.16) was not associated with all-cause mortality. This study also looked at a subset of the 2653 participants with information on the rate of bone loss. The greater loss of the vBMD for both trabecular and cortical (Q1) was significantly associated with higher mortality compared to the other quartiles (Q2-Q4) of vBMD loss[184].

Another study, African American-Diabetes Heart Study (AA-DHS) among 675 subjects with an average age of 56 years, who were diagnosed with type 2 diabetes underwent Quantitative Computed Tomography of the chest and abdomen for vBMD of thoracic and lumbar vertebrae. These participants were followed up for 7.6 mean years and all-cause mortality was the primary outcome. It was observed that, among men vBMD was inversely associated with all-cause mortality at lumbar vBMD [HR per SD increase = 0.70 (95% CI 0.52–0.95, p = 0.02)] and thoracic vBMD [HR per SD increase = 0.71 (95% CI 0.54–0.92, p = 0.01)] whereas among women vBMD was not associated with all-cause mortality. These associations were adjusted for age, sex, BMI,

smoking, alcohol consumption, hypertension, prior cardiovascular disease, coronary artery calcified plaque score, hormone replacement therapy (women), African ancestry proportion and eGFR [185].

6.2. Gaps in Literature

pQCT has gained importance in musculoskeletal research as it provides greater insights compared to DXA into the structure, geometry, and strength of the bone and also has muscle parameters. The two studies mentioned above had studied the QCT measures which may provide better insights than a pQCT. To our knowledge, no studies have reported association of pQCT and mortality. The presence of various parameters representing different dimensions of bone, its strength and geometry may be an early predictor of fractures which in turn are related to mortality among older populations. Considering this, the various bone related measures may be early predictors of mortality among older populations. As there are multiple parameters which are derived by pQCT, it is needed to explore if any of these parameters are sensitive and predict mortality earlier than the other parameters.

Indian population based on various studies related to DXA have observed lower bone mass and density. Considering this, pQCT measures which are sensitive and provide information beyond the mass and strength have not been studied in this population. There is no data on pQCT parameters among Indian older population. There are no mortality studies which have observed the relationship of bone mass or density with mortality among older Indians. Considering Indians have weaker bones, lower life expectancy than other developed nations and increasing older population, it is important to explore whether pQCT derived bone measures predict mortality among Indian older population.

6.3 Association of pQCT Derived Muscle Density and Mortality:

Fat infiltration into the skeletal muscle, both the inter and intra muscular regions is termed "myosteatosis". Myosteatosis is emerging as an important factor underpinning muscle quality and as a predictor of muscle function and metabolic status [186]. Increased myosteatosis has been observed to be associated with aging [187], reduced muscle strength [188], increased hip fractures / fragility fractures[189, 190] and increased mortality[191]. Intermuscular fat has been observed to be associated with poorer muscle function and strength [101, 106, 188, 192, 193]. Muscle strength compared to muscle mass was highly correlated with muscle mass and has been found to be associated with mortality among Health ABC study [194]. Muscle strength measured using grip strength has been extensively studied and based on a meta-analysis of 42 studies with 3,002,203 participants, including one study from India, observed that for every 5 kg decrease in grip strength mortality risk was 1.16 fold (95% CI 1.12-1.20) [195].

Among the older population, muscle mass alone cannot fully explain the loss of physical function and muscle strength. The rate of decline of muscle strength is faster than muscle mass among older adults. Maintenance of muscle mass does not prevent the loss of muscle strength among older adults. Considering the inconsistency of evidence between changes in muscle mass and muscle strength among older adults suggests there are other factors of muscle quality which may contribute to age related decline in muscle function and performance [109].

The muscle density as measured by a pQCT or QCT is widely used in large population studies as a measure of both the inter and intra muscular fat infiltration and thus represents the muscle quality rather than just the muscle mass. There have been few studies which have looked at its association with mortality (refer to Table 6.1).

Among 935 participants (men and women) of the inCHIANTI study in Italy with a six year follow up, pQCT derived muscle density was associated with all-cause mortality (per SD increase HR 0.78; 95% CI 0.69-0.88) on univariate analysis but on adjustment with other covariates this association was attenuated and was not significant (per SD increase HR 0.94; 0.80- 1.11) [196]. On the contrary, among 1063 male subjects of MrOS with a mean follow up of 7.2 years, muscle density (pQCT derived) was significantly associated with all-cause mortality on univariate analysis (per SD increase HR 1.39; CI 1.25–1.55) and on adjustment (1.18; 1.05 - 1.33) with age, race, study site, height, weight change since baseline, smoking, drinking, sedentary lifestyle, Physical Activity Scale for the Elderly score, health status, frailty status, diabetes, hypertension, myocardial infarction, stroke, cancer, renal disease, antihypertensive drugs use, lipid-lowering drugs, antidiabetic drugs use, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, calf muscle cross sectional area, DXA whole body fat (%) and DXA % trunk fat. This study also observed similar associations of muscle density with cardiovascular mortality. [197]. Another study among 1652 Afro Caribbean men aged 40 years and over followed for a period of 5.9 years, observed that per SD decrease in muscle density (pQCT derived) was significantly associated with all-cause mortality when adjusted for age (1.36; 1.11-1.67) and when adjusted (1.4; 1.11 – 1.78) for age, height, smoking status, alcohol consumption, physical activity, TV viewing time, health status, cancer, Type 2 diabetes, renal disease, stroke, myocardial infarction, hypertension, BMI and muscle area. In a sub analysis in this study, subjects ≥ 65 years showed a significant association with all-cause mortality when adjusted for age (1.36; 1.03 – 1.53) and adjusted for other covariates as mentioned above (1.40; 1.11 – 1.78) [191].

The Walking Leg Circulation Study II (WALCS II) with subjects (59 years and older at baseline) having peripheral arterial disease (PAD) in Chicago were followed up for 47.6 months. QCT of lower calf muscle was used to derive muscle density measure. Among the subjects with PAD, muscle density lowest tertile (1.80; 1.07- 3.03) and second tertile (0.91; 0.51- 1.62) were significantly associated with all-cause mortality [198]. Other clinical studies have also found that the CT measured muscle density is a predictor for mortality among patients who have had abdominal aortic aneurysm repair [199], mechanically ventilated critically ill patients [200] and transcatheter aortic valve replacement surgery [201]. These suggest that muscle density can be a marker for aging and survival.

6.4 Gaps in Literature

Muscle density as a measure for muscle quality or myosteatosis is gaining importance in both epidemiological and clinical studies as a marker for mortality. The few studies which have examined this association (as described above) have been conducted among Caucasian and African ancestry ethnic groups. There is a lack of information on other ethnic groups and the survival relationship of muscle density.

Very little data is available on pQCT or CT derived muscle measures among older populations of India. Considering the increase in older population, but lower life expectancy compared to developed nations and higher disability rates in India, it is important to study this population to understand the muscle density and its impact on mortality.

Table 6.1 Muscle density and its association with all-cause mortality

Author and year [ref]	Study design and participants	Muscle density measuring method	Results	Remarks
Cesari et.al., 2009 [196]	Aging in the Chianti area, InCHIANTI study, a population based cohort of Italy. 934 participants aged 65-102 followed for 6 years	Right leg pQCT using XCT 2000; Stratec, Pforzheim, Germany. BonAlyse software version 3.1 was used to ascertain muscle density.	Muscle density unadjusted was significantly associated with all-cause mortality (per SD increase - HR 0.78, 95% CI 0.69 – 0.88). But on adjusting for various covariates (height, weight, age and gender, site, education, Mini-Mental State Examination score, Center for Epidemiological Studies-Depression scale score, physical activity, congestive heart failure, coronary artery disease, hypertension, peripheral artery disease, respiratory disease, osteoarthritis, stroke, interleukin-6 (log value), C-reactive protein (log value), and tumor necrosis factor- a (log value)) the significance was lost.	Unadjusted muscle area and fat area were also associated with mortality but they also lost the significance when adjusted with the various covariates. Walking speed was significantly associated with mortality even after adjusting for the various covariates.
McDermott et.al., 2012 [198]	Walking and Leg Circulation Study II (WALCS II), a prospective, observational study of 434 participants with Peripheral arterial disease (PAD) in Chicago, with a mean follow up of 47.6 months among the 75	Computed Tomography (CT) scanner (Light Speed, General Electric Medical Systems Waukesha, WI, USA), Bon Alyse Software was used to ascertain muscle density at 66.7% tibia length.	Lower calf muscle density was associated with higher all-cause mortality, adjusting for age, sex, and race(p=0.016). This association did not attenuate further additional adjustment for comorbidities, smoking, BMI, physical activity, and the Ankial Brachial Index (ABI) (p=0.020). On tertile analysis, lowest tertile of muscle density was associated with higher all-cause mortality, HR = 1.80 (-1.07-3.03), 2nd tertile-HR=0.91(-0.51-1.62) and highest density tertile (HR=1.00), P trend was =0.020)	Muscle density was also associated with cardio-vascular mortality. Poor plantar flexion strength, lower baseline leg power and poor handgrip were significantly associated with higher all-cause mortality.

Table 6.1 Continued

Author and year [ref]	Study design and participants	Muscle density measuring method	Results	Remarks
	years mean age of participants			
Miljkovic et.al., 2015 [197]	The Osteoporotic Fractures in Men (MrOS) Study in United States, men aged 65 years and over at Minneapolis and Pittsburgh sites were included in this cohort study. A total of 1063 participants we included in the analysis. Mean follow up of 7.2 years.	pQCT using Stratec XCT-2000 and Stratec XCT-3000, calibrated using the European forearm phantom. Muscle measures were taken at tibia 66%. Muscle density was ascertained by image processing using stratec analysis software (Version 5.5) by a single investigator blinded to outcome status.	Per SD increase in muscle density was significantly associated with all-cause mortality: a. unadjusted (HR 1.39 (1.25–1.55) b. adjusted for age, race, study site (HR - 1.20 (1.07–1.35) c. adjusted for b+, height, weight change since baseline, smoking, drinking, sedentary lifestyle, Physical Activity Scale for the Elderly score, health status, frailty status, diabetes, hypertension, myocardial infarction, stroke, cancer, renal disease, antihypertensive drugs use, lipid-lowering drugs, antidiabetic drugs use, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, and calf muscle cross sectional area (HR - 1.18 (1.06, 1.33) d. adjusted for c+ DXA whole body fat (%) (HR - 1.24 (1.09, 1.41) e. adjusted for c+ DXA % trunk fat (HR- 1.18 (1.05, 1.33)	Cardio-vascular mortality was also significantly associated with muscle density; but non cardio-vascular mortality was not statistically significantly associated. Measures of adiposity and intermuscular fat were not associated with mortality.
Zhao et.al., 2016 [191]	1652 Afro Caribbean men aged 40 years and	pQCT scan of the calf at 66% tibia,	Per SD decrease in muscle density was associated significantly with all-cause mortality: a. Age adjusted - HR 1.36 (1.11–1.67)	In all men (age 40–91 years), all-cause mortality was associated with per SD

Table 6.1 Continued

Author and year [ref]	Study design and participants	Muscle density measuring method	Results	Remarks
	over enrolled in Tobago Health Study	performed using Stratec XCT-2000 scanner. Muscle density was ascertained by image analysis with stratec software version 5.5D by a trained investigator unaware of the participant's disease status	b. Adjusted for age, height, smoking status, alcohol consumption, physical activity, TV viewing time, health status, cancer, T2D, renal disease, stroke, myocardial infarction and hypertension – HR 1.30 (1.03–1.63) c. Adjusted for b+ BMI and muscle area – HR 1.37 (1.08–1.75) Among participants aged 65 years and over, per SD decrease in muscle density was associated significantly with all-cause mortality: a. Age adjusted – HR 1.26 (1.03–1.53) a. Adjusted for age, height, smoking status, alcohol consumption, physical activity, TV viewing time, health status, cancer, T2D, renal disease, stroke, myocardial infarction, hypertension, BMI and calf muscle cross-sectional area – HR 1.40 (1.11–1.78)	greater Inter muscular fat (IMAT) adjusting for all the variables. Similarly, older men (aged ≥65 years) with per SD greater IMAT was associated with greater all-cause mortality adjusting for all the variables.

7. Paper 2: pQCT Derived Bone Parameters and Muscle Density as Predictors of Mortality Among a Rural South Indian Older Population

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7.1 Abstract

Peripheral quantitative computed tomography (pQCT) bone measures and their association with mortality have not been studied; however, muscle density and mortality has been studied among Caucasian and Afro Caribbean ethnic groups. These associations have not been explored among older Indian populations. We examined pQCT derived bone measures and muscle density association with all-cause mortality, and tested whether grip strength and gait speed mediate these associations. We analysed 499 (245 men and 254 women) pQCT scans performed on community dwelling older men and women ($\geq$ 60 years) recruited in the Mobility and Independent Living Among Elders Study (MILES) in rural south India, with an average follow up of 64.2 months (5.3 years). Among men, trabecular volumetric bone mineral density (vBMD) of radius (hazard ratio per SD increase in parameter = 0.59 [95% CI = 0.43, 0.81]) and tibia (0.60[0.45, 0.81]), cortical vBMD of radius (0.61, [0.47, 0.79]) and tibia (0.62, [0.49, 0.79]), cortical thickness of radius (0.55, [0.42, 0.7]) and tibia (0.60, [0.47, 0.77]), polar strength strain index (SSIp) of tibia (0.73 [0.54, 0.98]), endosteal circumference of radius (1.63, [1.25, 2.12]) and tibia (1.54, [1.19, 1.98]) and muscle density (0.67, [0.51, 0.87]) were associated with all-cause mortality. Among women cortical vBMD of radius (0.62, [0.46, 0.84]) and tibia (0.61 [0.46, 0.81]), cortical thickness of radius (0.52, [0.35, 0.77]) and tibia (0.42, [0.30, 0.60]), SSIp of radius (0.68 [0.47,0.98]) and tibia (0.59 [0.38, 0.91]) and endosteal circumference of radius (1.37 [0.99, 1.89]) and tibia (1.83, [1.37, 2.45]) were associated with all-cause mortality. Among men, gait speed mediated the association of muscle density and mortality. This study presents unreported independent associations of pQCT bone measures and muscle density with all-cause mortality among rural south Indian population. Further research is needed to study mediation of some key performance measures on this association.

7.2 Introduction

Multiple studies have observed the relationship of bone mineral density (BMD) measured by Dual energy X-ray absorptiometry (DXA) and mortality[176-181]. A meta-analysis conducted on 10 prospective studies with 46182 participants from 5 countries (US, Netherlands, Sweden, Australia, and Brazil) with median follow up of 7 years showed, an increased all-cause mortality of 1.2 fold (Hazard Ratio (HR) 1.20; 95%CI 1.09–1.30) per one standard deviation (SD) decrease in BMD (measured using DXA) [182]. However, areal BMD (aBMD) measured by DXA is an integrated measure of trabecular and cortical bone and does not measure the geometry of bone. Peripheral Quantitative Computed Tomography (pQCT) has gained importance in musculoskeletal research as it provides greater insights compared to DXA on the bone structure, geometry and strength. To our knowledge there have been no studies examining the pQCT derived bone measures and their associations with mortality.

Among the older population, muscle mass alone cannot fully explain the loss of physical function and muscle strength. This suggests that there are other factors of muscle quality which may contribute to age related declines in muscle function and performance [109]. Myosteatosis is an excess deposit of fat in the skeletal muscles both at intramuscular and intermuscular levels [104, 105] and among older adults it has been associated with decreased muscular function and physical performance [101, 106, 107]. Increased myosteatosis has been associated with aging [187], reduced muscle strength [188], increased hip fractures / fragility fractures[189, 190] and increased mortality[191]. Intermuscular fat has been associated with poorer muscle function and strength [101, 106, 188, 192, 193]. Muscle strength though highly correlated with muscle mass, has been independently associated with mortality in the Health ABC study [194]. Muscle strength measured using grip strength has been extensively studied and based on a meta-analysis of 42 studies with 3,002,203 participants, including one study from India, showed mortality risk of 1.16 fold (95% CI 1.12-1.20) for every 5 kg

decrease in grip strength [195]. Gait speed a measure of lower limb strength and function has been studied extensively for its association with mortality. A pooled analysis of 34,485 community dwelling older adults followed for 6 to 21 years showed that pooled hazards risk for every 0.1 m/s increase of gait speed improved the survival by 12 % (95% CI 10-13%) [202].

Muscle composition analysis using pQCT suggests that muscle density is a surrogate marker of fat infiltration within the muscle. Muscle density is gaining importance in both epidemiological and clinical studies as a marker for muscle quality, as a predictor of muscle function, metabolic status and mortality [186]. However, most studies have examined the association of muscle density and mortality among Caucasian and African ancestry ethnic groups.

Little is known about pQCT derived bone and muscle measures among older populations of India. The current demographic trends in India predict an increase in the older population, despite a lower life expectancy compared to developed nations and with subsequent higher disability rates. To our knowledge there have been no studies on the relationship among pQCT bone measures and muscle density with mortality among Indian older population.

In this analysis we examined volumetric BMD (vBMD), bone structure, bone geometry measures and muscle density as predictors of all-cause mortality among rural south Indian older men and women. We tested the hypothesis that lower bone phenotypes and muscle density will be associated with increased mortality independent of confounding factors. We will test the hypothesis that these relationships may be mediated by grip strength and gait speed.

7.3 Materials and Methods

Study population:

The Mobility and Independent Living Among Elders Study (MILES) is a prospective study which enrolled 562 community dwelling men and women aged 60 years and over, all recruited in 2012 from Medchal region of Telangana state of southern India. This study was designed to determine the prevalence of age-related chronic diseases, disability and to examine the extent of clinical and subclinical disease[27]. The study was approved by institutional review board at the participating institutions and all subjects provided written informed consent.

At baseline, two visits for data collection were conducted. During the first visit (February 2012 – November 2012), participants completed questionnaires including information on health status, smoking, alcohol consumption etc., physical performance tests, fasting glucose tests and blood pressure measurements. At the second visit of the baseline (June 2012 – June 2013), pQCT scans were conducted. Of the total 562 participants recruited, 17 died (11 men, 6 women) between the baseline visit 1 and visit 2; 26 participants (14 men and 12 women) were not able to come for pQCT measurement; one older man was physically disabled for the test; 3 women vacated their house and moved out; and 4 participants (3 men and 1 women) refused to continue in the study. The scans were conducted on 511 participants and this analysis includes the 499 scans (245 men and 254 women) .

pQCT and calibration:

pQCT scans on the radius and tibia were performed using the Stratec XCT-2000 (Stratec Medizintechnik, Pforzheim, Germany). Technicians followed a standardized protocol for positioning and scanning of each subject. Scans were taken at 4% and 33% of the length of radius and at 4%, 33% and 66% of the length of tibia. Subject scans were repeated if artefacts due to motion or beam hardening were present. To monitor the stability

of the pQCT scanners, a manufacturer supplied cylindrical Quality Assurance (QA) phantom was scanned daily before subject scans were acquired. All pQCT scans were analysed by a single investigator using the manufacturer software package version 6.00 for the XCT scanners. This software provides a suite of segmentation options to quantify total, trabecular and cortical bone properties from each pQCT image. Before each image was analysed it was checked for artifacts due to motion or beam hardening. Scans with artefacts were not analysed. All 4% radius and tibia scans were analysed using the CALCBD option with an automatic gradient search (contour mode 2) applied to segment bone from the soft tissue background and concentric peeling (peelmode 1, 45%) to segment trabecular and cortical bone. Proximal scans acquired at the 33% and 66% limb locations were segments using a fixed threshold of 710 mg/cm3(Cortmode1). Coefficients of variation (CVs) were determined for pQCT scans by replicating measurements on 15 subjects (CV $\leq$ 2.1%).

pQCT parameters:

For this analysis we focused on the following pQCT parameters that are physiologically important in skeletal aging: at the 4% site of radius and tibia - trabecular vBMD; at the 33% sites of radius and tibia - cortical vBMD, cortical thickness, endosteal circumference and polar strength strain indiex (SSIp).; at 66% tibia – muscle density. These parameters were chosen because, vBMD is an indicator for bone matrix mineralisation and represents the mechanical quality of the solid bone tissue both at the trabecular and cortical sites. Endosteal circumference and cortical thickness represent bone geometry and strength. SSIp predicts the failure load [132, 133] and also has been shown to be a good predictor of long bone bending [133]. All these parameters also have age-related changes due to adaption of stress, strain, and load on the bone and fractures [134, 135]. Muscle density serves as a surrogate marker of fat infiltration within the muscle [139]. It reflects the compactness of

muscle fibers, protein within the muscle and other soft tissues and can be viewed as a proxy measure of muscle quality.

Mortality assessment:

As a part of the MILES cohort data collection, each participant is followed up for death through community health volunteers at the village level. Based on the information on death from the community health volunteers, MILES research staff visits the participants' home to ascertain the event of death and administers a verbal autopsy tool.

All deaths until 31st March 2019 were considered in this analysis. There were total of 123 deaths (men 73/245; women 50/254); the maximum time to death was 76.7 months since the baseline pQCT measurement. The average follow-up was 64.2 months (5.3 years).

Covariates:

Information on the covariates was collected through interviewer administered questionnaires. Self-reported health status was categorised as good/excellent and fair/poor/very poor. Smoking status was categorised as current smoker and not a current smoker. Alcohol consumption was categorised as consumes alcohol and does not consume alcohol. Self-report of the history of stroke was recorded. Direct measures of weight using SECCA® scale was conducted and recorded. Height was measured using a SECCA® stadiometer. Guilick II tape measure was used to measure waist circumference on the bare skin at the umbilicus and hip circumference at the maximum circumference over the buttocks. Two readings were taken and if the difference between the two readings was ≥ 0.5 cms then two more reading were taken. Average of the two readings of height was done. Body Mass Index (BMI) was calculated as body weight in kilograms divided by height in meters squared. Diabetes was categorised as present if glucose levels were ≥126mg/dL (after a minimum of an 8-hour fast), self-report of diabetes or used insulin or hypoglycemic medications. Hypertension was considered present if participant self-reported hypertension, reported use of an anti-hypertensive medication or

blood pressure assessment ($\geq$ 140/90 mm of Hg). Activities of daily living (ADL) was assessed using the standard tasks of eating, dressing, bathing, transferring from bed to chair and using the toilet. ADL was categorised as ADL disability if the participant reported difficulty in any one of the tasks. Past five years' history of fractures (hip, arm, wrist shoulder, spine and any other bones) was asked and categorised as having a fracture if any one of the fractures were reported by the respondent. Serum 25-hydroxy vitamin D levels (ng/ml) were measured using the high performance liquid chromatography (HPLC) method. Grip strength was directly measured using hand held dynamometer. Reading were taken twice for each hand. In this analysis we used the average of the two reading of grip strength of the participants' dominant hand. Short Physical Performance Battery (SPPB) is a group of measures used as a tool to predict disability among older populations [203]. This combines gait speed, balance tests and chair stands with a total score ranging from 0 (worst performance) to 12 (best performance). The 4-meter timed walk was performed twice for each participant as a part of the Short physical performance battery (SPPB). Gait speed was calculated as the meters per second (4 meters / time taken by participant). Average of the two walks was taken as the gait speed of the participant for this analysis. A 400-meter walk test was performed and the data was categorized as participants who completed the test and who did not attempt or not complete the test. All the baseline information of the covariates was collected during the first visit and pQCT scans were performed after a gap of 4-6 months during visit 2 of the baseline.

Statistical analysis:

There were significant differences in the pQCT parameters (Table 7.2) and some covariates between men and women; hence sex specific analyses were undertaken. The values of pQCT parameters with $\pm$ 3 SD were identified and removed from analysis. Serum 25-hydroxy vitamin D values were not normally distributed and were log transformed. We described the participant's characteristics at the baseline visit using means $\pm$ SD or prevalence.

Two-sample t-tests or Wilcoxon rank sum tests (continuous variables) or chi-square tests (categorical variables) were used to compare characteristics between survivors and deceased participants separately in men and women. The pQCT parameters were categorized as quartiles and Kaplan-Meier survival curves with log rank test were conducted. The pQCT parameters which are continuous variables were standardised for standard deviation difference among the participants. Cox proportional hazards models were used to assess the association between the pQCT measures and all-cause mortality; hazards ratio and 95% confidence intervals were calculated. The covariates of interest were age, height, weight, current smoker, consumes alcohol, self-reported health status, hypertension, diabetes, stroke, 25-hydroxy vitamin D level and ADL disability. Gait speed and grip strength were considered as mediators for this analysis. Minimally adjusted model (model 1) – adjusted for age, height and weight and fully adjusted mode (model 2) - model 1 + smoking, alcohol, health status opinion, hypertension, diabetes, stroke, log 25-hydroxy vitamin D and ADL disability. The mediator models were model 3 = model 2 + grip strength and; model 4 = model 2 + gait speed. All the models with muscle density were also adjusted for muscle cross sectional area (CSA). The proportional hazards assumption was confirmed by Supremum test.

As grip strength and gait speed are associated with mortality and these are in the causal pathway of bone / muscle and mortality; a causal mediation analysis was conducted. Mediation was considered present if there was attenuation of hazards ratio of more than 10% in the models with and without the mediator variables (models 3 and 4 compared to model 2). The pQCT parameters in the mediator models which had significant association with all-cause mortality were considered for the causal mediation analysis. The overall proportion of mediation was considered in the causal mediation analysis. Results were considered statistically significant when a p-value was less than 0.05.

Based on the power analysis, 73 deaths among 245 men and 50 deaths among 254 women with a SD of 1 (for assessing standard deviation difference) and alpha error of 0.05 and 80% power, the current sample will be able to detect a HR of 0.72 and less among men and HR of 0.67 and less among women. All statistical analysis was carried out using Stata/IC 13.1 and SAS 9.4 software.

7.4 Results

Baseline characteristics:

The baseline characteristics comparing men and women, and comparing men and women who survived versus deceased are presented in the table 7.1.

Men compared to women were significantly taller, heavier, had lower BMI, higher waist circumference, lower hip circumference, higher grip strength, faster gait speed, higher SPPB score, higher prevalence of completion of 400-meter walk test, currently smoking and alcohol consumption. The mean age of the men (68.2 ± 6.62) was similar to women (67.2 ± 6.21).

There were a total of 123 deaths (25%) [73 men (30%) and 50 (20%) women] occurring among 499 (245 men and 254 women) over a period of 81 months of follow up. Men who were alive were significantly younger compared to the deceased which was similar among women.

Men who died were taller, had lower grip strength, slower gait speed, lower SPPB score and were less able to complete the 400-mtr walk. Women who died were lighter (less weight), had lower BMI, lower waist circumference, higher 25-hydroxy vitamin D levels, slower gait speed, lower grip strength, lower SPPB score and, reported lower health status and a higher prevalence of stroke.

pQCT parameters and all-cause mortality:

Based on the unadjusted log rank test (table 7.2) and Kaplan Meir's curves (Fig 7.1 and 7.2), men had significant decreasing risk of all-cause mortality with increasing quartiles of trabecular vBMD of radius and tibia, cortical vBMD of radius, cortical thickness of radius and tibia, and muscle density. All -cause mortality significantly increased with increasing quartiles of endosteal circumference of radius and tibia. Mortality did not vary across quartiles of SSIp of radius or tibia (Fig 7.1).

Among women, all-cause mortality risk significantly decreased with increasing quartiles of cortical vBMD of radius and tibia, cortical thickness of radius and tibia, and SSIp of radius and tibia. The mortality risk increased with the increasing quartiles of endosteal circumference of radius and tibia. All-cause mortality did not vary across quartiles of trabecular vBMD of radius or tibia, and muscle density (Fig 7.2).

Among men in the minimally adjusted (age, height and weight) cox proportional hazards models (table 7.3) (hazard ratio per SD increase in parameter [95% CI]), trabecular vBMD at 4% radius (0.63 [0.47, 0.84]) and tibia (0.64[0.48, 0.85]), cortical vBMD at 33% radius (0.63, [0.49, 0.81]) and tibia (0.64, [0.51, 0.80]), cortical thickness at 33% radius (0.56, [0.43, 0.73]) and tibia (0.61, [0.48, 0.77]), SSIp at 33% tibia (0.74 [0.55, 0.99]) and muscle density (0.63, [0.49, 0.81]) were all significantly inversely associated with all-cause mortality. Endosteal circumference at 33% radius (1.56, [1.21, 2.00]) and tibia (1.54, [1.20, 1.96]) were positively and significantly associated with all-cause mortality. After adjusting for other covariates (fully adjusted models – model 2), all the pQCT parameters which were associated in minimally adjusted models, remained statistically significant.

Among women (table 7.4), in the minimally adjusted models, all-cause mortality was significantly inversely associated with cortical vBMD of radius (0.58, [0.42, 0.79]) and tibia (0.64 [0.48, 0.87]), cortical thickness of radius (0.49, [0.32, 0.73]) and tibia (0.43, [0.29, 0.61]),

and SSIp of tibia (0.59 [0.38, 0.93]). The endosteal circumference at radius (1.39 [1.01, 1.93]) and tibia (1.79, [1.34, 2.34]) were positively associated with all-cause mortality. In the fully adjusted models (model 2), all the minimally adjusted associations were significant except for endosteal circumference of radius which attenuated. However, SSIp at radius (0.68 [0.47, 0.98]) was significantly associated with mortality.

Mediation analysis:

The pQCT parameters which were significant in model 2 were compared with the mediator models (models 3 and 4) for identifying attenuation of hazards ratio of more than 10%. Among men, muscle density showed more than 10% attenuation with the gait speed. Based on the mediation analysis (not shown) gait speed mediates the association between muscle density and all-cause mortality significantly to the extent of 10% (95% CI: 0.2%, 19.7%).

Among women, attenuation of more than 10% in the grip strength model was observed for cortical vBMD at radius and tibia, cortical thickness at tibia, and SSIp at radius and tibia. In the gait speed model attenuation of more than 10% was observed for cortical vBMD at radius and tibia, cortical thickness at radius and tibia and SSIP at tibia. The mediation analysis of these pQCT parameters observed no significant mediation by gait speed or grip strength on all-cause mortality among women.

7.5 Discussion

The current study showed that trabecular vBMD (radius and tibia) cortical vBMD (radius and tibia), cortical thickness (radius and tibia), endosteal circumference (radius and tibia), SSIp (tibia) and muscle density were independent predictors of all-cause mortality among rural south Indian older men. It was further observed that, cortical vBMD (radius and tibia), cortical thickness (radius and tibia), SSIp (radius and tibia) and endosteal circumference

(tibia) were independent predictors of all-cause mortality among rural south Indian older women. Gait speed significantly mediated the association of mortality and muscle density among men. However, among women no significant mediation by gait speed or grip strength was observed with any of the pQCT parameters.

To our knowledge there have been no studies testing the hypothesis that pQCT derived bone measures association with mortality. In the Age, Gene/Environment Susceptibility (AGES) - Reykjavik Study among men and women, QCT trabecular vBMD at proximal femur was inversely associated with all-cause mortality independent of covariates including gender (lowest quartile vs highest three quartiles (1.12 [1.01, 1.25])); but cortical vBMD was not associated with mortality [184]. In the African American-Diabetes Heart Study (AA-DHS) QCT was measured at chest and abdomen for thoracic and lumbar spine vBMD. Among men thoracic and lumbar spine vBMD was inversely associated with all-cause mortality at lumbar vBMD [HR per SD increase = 0.70 (95% CI 0.52–0.95, p = 0.02)] and thoracic vBMD [HR per SD increase = 0.71 (95% CI 0.54–0.92, p = 0.01)], but no association was observed among women [185]. These findings were similar to our study wherein trabecular vBMD was associated with mortality among men but not among women.

It has been well established that fractures are associated with increased mortality [56]. Results from the (AGES) - Reykjavik Study observed that history of fracture before the bone assessment did not alter the vBMD and mortality association, whereas incident fracture post the bone assessment attenuated the mortality association with trabecular vBMD [184]. This suggests that fractures may explain the association between lower BMD and mortality. We did not have information on incident fractures but adjusting for history of fracture in the past five years, had little effect on the association of trabecular vBMD and mortality among men and women; however, adjustment for fracture history attenuated the SSIp of radius association with mortality among women. This should be interpreted in the light of the limitations of our study,

and further studies are needed to understand the association and the role of incident fractures among older Indian population and mortality.

In our previous analysis comparing south Indian rural men with US Caucasian men, we observed that the trabecular vBMD among men was very low (-1.3 SD for radius, -1.5 SD for tibia), cortical vBMD was low (-0.3 SD for radius, -0.1 SD for tibia), lower cortical thickness (-0.8 SD for radius, -1.2 SD for tibia) and higher endosteal circumferences (0.5 SD for radius, 0.8SD for tibia) [144]. This suggests that the Indian older population have lower bone density and strength measures which could influence earlier mortality either through fractures or other mechanisms.

Trabecular bone loss is more pronounced at earlier ages before 50 years (women 37%, men 42%) compared to cortical loss which is minimal [168]. Throughout adulthood periosteal apposition counterbalances endosteal bone loss by reconfiguring the available bone mass to maintain biomechanical properties. However, with increasing age the bone loss shifts more to the cortical compartment of the bone leading to cortical thinning and increased cortical porosity which leads to loss of biomechanical strength and increased risk for fracture [204]. Bone loss in aging is the net result of periosteal bone formation and endosteal bone resorption [205], however in aging the bone formation is less compared to bone resorption which leads to bone loss in the endosteal region and increase in the endosteal circumference. Endosteal resorption also determines the cortical porosity. The lower levels of cortical vBMD, lower cortical thickness and higher endosteal circumference in our study among men and women, suggest increased cortical thinning and porosity which may be impacting mortality among the Indian older population. The lack of association of trabecular vBMD with mortality among women needs to be explored further.

The association between lower BMD and mortality has not been consistent and suggests that there may be other common pathways or risk factors in this association. There has been

some attention towards the association of cardiovascular disease (CVD) deaths and bone density, but the results of these studies have been inconclusive. A large analysis of NHANES III observed no association of low BMD and CVD mortality among men. However, among women soon after menopause, low BMD was associated with mortality from cardiovascular disease [206]. BMD was associated with mortality independent of coronary artery calcium score and chronic lung disease [184]. In a meta-analysis of 28 studies, low BMD was associated with an increased risk of developing coronary artery disease, cerebrovascular conditions, and CVD-associated death [207]. This may suggest that the CVD or pulmonary disorders may share similar pathways in the link between BMD and mortality and needs further exploration.

Shared risk factors for low BMD and increased mortality may contribute to an association between the two factors. Increased low grade inflammation has been linked to higher mortality [208, 209] and lower BMD [210, 211] and fractures [212, 213]. Endogenous sex hormones are associated with mortality [214, 215], lower BMD [216, 217] and fractures [218, 219]. Age at which the women attains menopause is negatively associated with BMD [220] increased fractures[221, 222] and mortality[221] and it has been observed that Indian women have an earlier menopause [223, 224]. Nevertheless, we have no information on these factors and their influence on these bone-mortality relationships cannot be ruled out.

The mechanostat hypothesis and the concept of bone and muscle crosstalk suggest there may be association of bone and physical performance measures. The pQCT bone measures have been associated with grip strength [225-233] and gait speed [230, 232, 233]. Grip strength [195] and gait speed [202] also predict mortality. Considering this, grip strength and gait speed may mediate the association between the pQCT bone measures and mortality. However, in our analysis we observed no mediation between the pQCT bone parameters and mortality.

Over a six year follow up of the inCHIANTI study in Italy, pQCT derived muscle density was associated with all-cause mortality (per SD increase, 0.78 [0.69-0.88]) in models

adjusted for height and weight. This association was attenuated and was not significant (per SD increase, 0.94 [0.80- 1.11]) in fully adjusted models [196]. These results are similar to our findings among women. Among older men in the MrOS with a mean follow up of 7.2 years, muscle density (pQCT derived) was significantly inversely associated with all-cause mortality on univariate analysis and on adjustment with various covariates [197]. Among Afro Caribbean men aged 40 years in the Tobago health study, muscle density was also significantly inversely associated with all-cause mortality when adjusted for age and other covariates [191]. Our study also observed similar inverse relationship of muscle density and mortality among men but not among women. The average distribution of muscle density among men and women was not different, suggesting a potential gender influence on the adverse effects of fat infiltration into muscle which has to be further explored.

Muscle density is a proxy measure of myosteatosis. Insulin resistance and oxidative stress are considered to be factors that influence myosteatosis and mortality. In our study, among men the association of muscle density and mortality persisted even after adjusting for diabetes status. This was similarly observed in other studies[191, 197] suggesting that there may be other pathways which influence the muscle density and mortality association.

Gait speed is associated with muscle density [141, 196, 234] and is associated with mortality [202] . The mediation of gait speed between the muscle density and mortality was significant among men in our study, suggesting that gait speed may be in the pathway of myosteatosis and mortality. Other physical performance measures did not have any mediation effect of muscle density and mortality, but needs further exploration considering the limitations of our study.

The current analysis has several potential limitations. This was an observational study and thus causality cannot be determined. The study sample was small and was limited to a rural south Indian region. Considering the diversity of India, the external validity of the findings

needs to be interpreted with caution. Though grip strength and gait speed can mediate the association between pQCT measures and mortality, the power of the study may be a limited for assessing causal mediation. As some of the key indicators of shared pathways were not measured, there could be residual confounding in these relationships. We were unable to look at cause specific deaths. As this was an exploratory and hypothesis generating study, we did not adjust for multiple comparisons.

However, our study has some important strengths. To our knowledge this was the first study describing pQCT measures and mortality association among older Indian populations. We used a community based random sample and was a longitudinal follow up for a considerable duration. We adjusted for many important potential confounding variables and also conducted mediation analysis.

In conclusion, this study presents unreported independent association of all-cause mortality with trabecular vBMD, cortical vBMD, cortical thickness, endosteal circumference, SSIp and muscle density among rural south Indian older men; and cortical vBMD, cortical thickness, endosteal circumference and SSIp among rural south Indian older women. Grip strength and gait speed did not mediate the association of bone and muscle among women, however significant mediation was observed by gait speed on muscle density and mortality among men. Further research is needed to confirm our findings in larger Indian older populations and to study the role of mediation of some of the key performance measures on this association.

7.6 Tables and Figures

Table 7.1 Participants characteristics among men and women of MILES

Baseline characteristics	Total participants			Men			Women		
	Men (N=245)	Women (N=254)	p value (Men Vs Women)	Alive (N=172)	Deceased (N=73)	p value (alive vs deceased)	Alive (204)	Deceased (N=50)	p value (alive vs deceased)
Age (years)	68.2 ± 6.62	67.2 ± 6.21	0.0749	67.3 ± 6.47	70.32 ± 6.54	0.0003*	66.59 ± 5.68	69.68 ± 7.59	0.01*
Height (cm)	160.62 ± 5.6	147.01 ± 5.95	<0.0001*	160.06 ± 5.3	161.92± 6.09	0.0209*	147.37 ± 5.59	145.54 ± 7.12	0.18
Weight (kg)	55.87 ± 11.51	49.96 ± 12.21	<0.0001*	55.98 ± 11.34	55.62± 11.97	0.682	50.81 ± 12.1	46.53 ± 12.12	0.02*
BMI (kg/m^2)	21.58 ± 3.92	22.98 ± 4.8	0.0045*	21.79 ± 3.97	21.09 ± 3.78	0.204	23.28 ± 4.85	21.76 ± 4.48	0.047*
Waist circumference (cm)	82.75 ± 12.06	78.57 ± 13.57	<0.0001*	82.73 ± 12. 29	82.76± 11.56	0.96	79.53 ± 13.49	74.62 ± 13.29	0.02*
Hip circumference (cm)	88.27 ± 7.39	91.81 ± 10.59	0.0008*	88.56 ± 7.29	87.56 ± 7.61	0.44	92.42 ± 10.53	89.33 ± 10.56	0.05
Vitamin D (ng/ml)	30.62± 16.15	29.35 ± 17.65	0.1752	30.73 ± 16.21	30.36± 16.11	0.69	28.58±18.36	32.76±13.68	0.026*
Average Gait speed (m/s)	0.69 ± 0.18	0.58 ± 0.16	<0.0001*	0.72 ± 0.18	0.62 ± 0.18	<0.0001*	0.61 ± 0.15	0.46 ± 0.17	<0.0001*
Average Grip strength (kg)	20 ± 6.04	12.45 ± 4.74	<0.0001*	20.99 ± 5.91	17.68 ± 5.76	0.0003*	12.86 ± 4.52	10.76 ± 5.29	0.01*
SPPB score	8.78 ± 2.75	7.22 ± 2.82	<0.0001*	9.39 ± 2.44	7.33 ± 2.89	<0.0001*	7.74 ± 2.59	5.14 ± 2.78	<0.0001*
Follow up time (years)	5.22 ± 1.66	5.47 ± 1.5	0.0603	6.12 ± 0.33	3.12 ± 1.64	<0.0001*	6.12 ± 0.32	2.84 ± 1.56	<0.0001*
Health status (Good)	113 (46.1)	105 (41.3)	0.2814	85 (49.4)	28 (38.3)	0.112	93 (45.5)	12 (24)	0.006*
ADL difficulty (at least one activity)	197 (80.4)	199 (78.4)	0.5694	135 (78.5)	62 (85)	0.245	155 (76)	44 (88)	0.06
400 meter walk completed	179 (73)	147 (57.8)	0.0004*	139 (81)	40 (55)	<0.0001*	124 (61)	23 (46)	0.0578
Current smokers	107 (43.7)	1 (0.4)	<0.0001*	70 (41)	37 (51)	0.149	1 (0.5)	0 (0)	0.62
Consumes alcohol	175 (71.4)	146 (57.5)	0.0011*	123 (71)	52 (71)	0.964	118 (57.8)	28 (56)	0.81
Hypertension	149 (60.8)	157 (61.8)	0.8196	98 (57)	51 (70)	0.059	120 (58.8)	37 (74)	0.05
Diabetes	41 (16.7)	57 (22.4)	0.1087	29 (17)	12 (16,4)	0.935	42 (21)	15 (30)	0.15
Stroke	17 (6.9)	9 (3.5)	0.0880	10 (6)	7 (10)	0.285	4 (2)	5 (10)	0.01*
History of fracture	13 (5.3)	16 (6.3)	0.58	9 (5.2)	4 (5.5)	0.93	11 (5.3)	5 (10)	0.32

* represents statistically significant p <0.05

Table 7.2 Unadjusted Log Rank test p-value of the pQCT bone and muscle density quartiles

Variable	Log rank test p value	
	Men	Women
Trabecular V BMD (Radius 4%)	0.0001*	0.3866
Cortical vBMD (Radius 33%)	0.0141*	<0.0001*
Cortical Thickness (Radius 33%)	<0.0001*	<0.0001*
Endosteal circumference (Radius 33 %)	0.0001*	0.0114*
SSI p (Radius 33%)	0.7188	0.0155*
Trabecular V BMD (Tibia 4%)	0.0008*	0.2604
Cortical vBMD (Tibia 33%)	0.0557	0.0319*
Cortical Thickness (Tibia 33%)	0.0001*	<0.0001*
Endosteal circumference (Tibia 33 %)	<0.0001*	0.0002*
SSI p (Tibia 33%)	0.6066	<0.0001*
Muscle Density	0.0002*	0.5247

* represents statistically significant p <0.05

Table 7.3 Hazard Ratios (95% CI) for Mortality Per SD Increase in the pQCT measures among 245 men of MILES

	Model 1		Model 2		Model 3		Model 4	
	HR	P value	HR	P value	HR	P value	HR	P value
Trabecular V BMD (Radius 4%)	0.63(0.47, 0.8)	0.0018*	0.59(0.43, 0.81)	0.0011*	0.60(0.44, 0.81)	0.0009	0.59(0.42, 0.82)	0.0016
Cortical vBMD (Radius 33%)	0.63(0.49, 0.81)	0.0003*	0.61(0.47, 0.79)	0.0003*	0.62(0.47, 0.80)	0.0003	0.60(0.46, 0.80)	0.0004
Cortical Thickness (Radius 33%)	0.56(0.43, 0.73)	<0.0001*	0.55(0.42, 0.72)	<0.0001*	0.58(0.44, 0.77)	0.0001	0.52(0.39, 0.69)	<0.0001
Endosteal circumference (Radius 33 %)	1.56(1.21, 2.00)	0.0005*	1.63(1.25, 2.12)	0.0003*	1.56(1.20, 2.02)	0.0010	1.72(1.32, 2.25)	<0.0001
SSI p (Radius 33%)	0.85(0.65, 1.11)	0.2303	0.85(0.64, 1.12)	0.2531	0.88(0.66, 1.16)	0.3576	0.86(0.65, 1.15)	0.863
Trabecular V BMD (Tibia 4%)	0.64(0.48, 0.853)	0.0024*	0.60(0.45, 0.81)	0.0010*	0.64(0.48, 0.86)	0.003	0.62(0.45, 0.86)	0.0036
Cortical vBMD (Tibia 33%)	0.64(0.51, 0.80)	0.0001*	0.62(0.49, 0.79)	0.0001*	0.60(0.47, 0.77)	<0.0001	0.63(0.49, 0.80)	0.0002
Cortical Thickness (Tibia 33%)	0.61(0.48, 0.77)	<0.0001*	0.60(0.47, 0.77)	<0.0001*	0.65(0.51, 0.84)	0.001	0.63(0.49, 0.81)	0.0004
Endosteal circumference (Tibia 33 %)	1.54(1.20, 1.96)	0.0006*	1.54(1.19, 1.98)	0.0009*	1.5(1.15, 1.95)	0.0027	1.45(1.14, 1.94)	0.0038
SSI p (Tibia 33%)	0.74(0.55, 0.99)	0.0392*	0.73(0.54, 0.98)	0.037*	0.77(0.57, 1.03)	0.085	0.75(0.56, 1.01)	0.058
Muscle Density[a]	0.63(0.49, 0.81)	0.0003*	0.67(0.51, 0.87)	0.0031*	0.73(0.55, 0.96)	0.025	0.78(0.58, 1.05)	0.095$

Model 1 = Age, height and weight adjusted; Model 2 = Model 1 + current smoker, alcohol consumption, opinion of health status, ADL, log 25-hydroxy vitamin D, Hypertension, diabetes and stroke; Mediator models: Model 3 = Model 2 + grip strength; Model 4 = Model 2+Gait speed.

a = in models with muscle density as a predictor muscle cross sectional area was also included

* represents statistically significant P <0.05

$ represents attenuation of >10% comparing model 2 and model 3

& represents attenuation of >10% comparing model 2 and model 4

Table 7.4 Hazard Ratios (95% CI) for Mortality Per SD Increase in the pQCT bone measures and muscle density among 254 women of MILES

	Model 1		Model 2		Model 3		Model 4	
	HR	P value	HR	P value	HR	P value	HR	P value
Trabecular V BMD (Radius 4%)	0.97(0.69,1.34)	0.8443	0.83(0.58,1.20)	0.324	1.07(0.71,1.61)	0.7575	0.94(0.65,1.35)	0.718
Cortical vBMD (Radius 33%)	0.58(0.42,0.79)	0.0006*	0.62(0.46,0.84)	0.0020*	0.68(0.49,0.95)	0.0219[&]	0.73(0.54,0.99)	0.0411[$]
Cortical Thickness (Radius 33%)	0.49(0.32,0.72)	0.0004*	0.52(0.35,0.77)	0.0011*	0.49(0.30,0.78)	0.0028	0.57(0.39,0.85)	0.0055[$]
Endosteal circumference (Radius 33 %)	1.39(1.01,1.93)	0.041*	1.37(0.99,1.89)	0.0570	1.49(1.02,2.18)	0.0383	1.35(0.95,1.93)	0.1538
SSI p (Radius 33%)	0.71(0.49,1.01)	0.0614	0.68(0.47,0.98)	0.0394*	0.75(0.49,1.13)	0.1711[&]	0.67(0.46,0.97)	0.0326
Trabecular V BMD (Tibia 4%)	0.95(0.69,1.30)	0.7397	0.89(0.63,1.24)	0.4846	1.08(0.74,1.58)	0.6754	0.98(0.71,1.35)	0.9014
Cortical vBMD (Tibia 33%)	0.64(0.48,0.87)	0.0036*	0.61(0.46,0.81)	0.0006*	0.69(0.51,0.93)	0.015[&]	0.68(0.51,0.90)	0.0043[$]
Cortical Thickness (Tibia 33%)	0.43(0.29,0.61)	<0.0001*	0.42(0.30,0.60)	<0.0001*	0.47(0.32,0.67)	<0.0001[&]	0.49(0.35,0.70)	<0.0001[$]
Endosteal circumference (Tibia 33 %)	1.79(1.34,2.39)	<0.0001*	1.83(1.37,2.45)	<0.0001*	1.82(1.34,2.48)	0.0001	1.77(1.32,2.37)	0.0001[$]
SSI p (Tibia 33%)	0.59(0.38,0.93)	0.0219*	0.59(0.38,0.91)	0.0163*	0.79(0.50,1.25)	0.3175[&]	0.70(0.46,1.07)	0.0986
Muscle Density[a]	0.81(0.59,1.10)	0.1839	0.86(0.63,1.18)	0.3495	0.78(0.57,1.23)	0.1941	0.99(0.70,1.41)	0.9585

Model 1 = Age, height and weight adjusted; Model 2 = Model 1 + current smoker, alcohol consumption, opinion of health status, ADL, log 25-hydroxy vitamin D, Hypertension, diabetes and stroke; Mediator models: Model 3 = Model 2 + grip strength; Model 4 = Model 2+Gait speed.

a = in models with muscle density as a predictor, further adjusted for muscle cross sectional area

* represents statistically significant P <0.05

$ represents attenuation of >10% comparing model 2 and model 3

& represents attenuation of >10% comparing model 2 and model 4
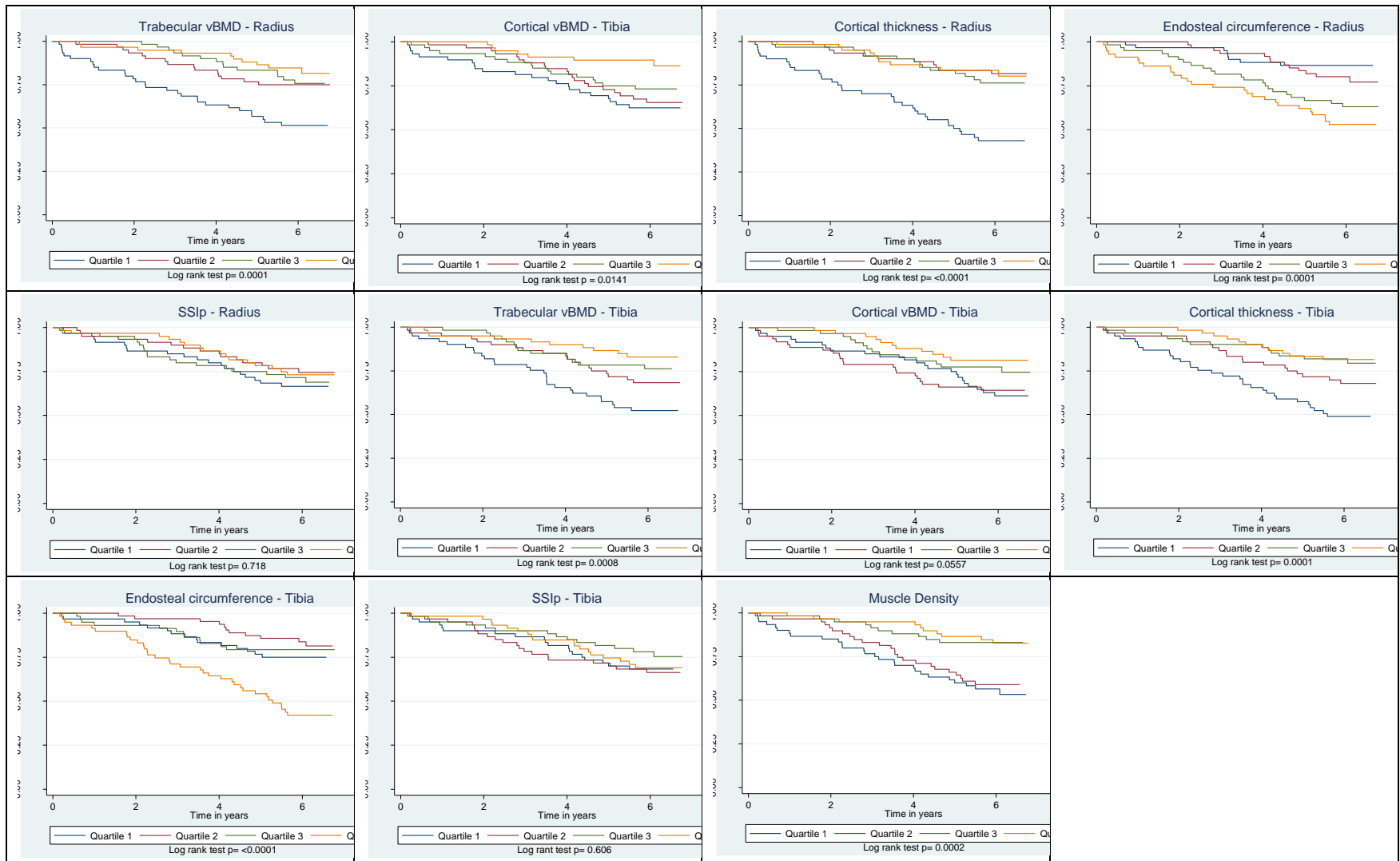
Figure 7.1 Kaplan meir curves of quartiles of pQCT parameters and log rank test among men
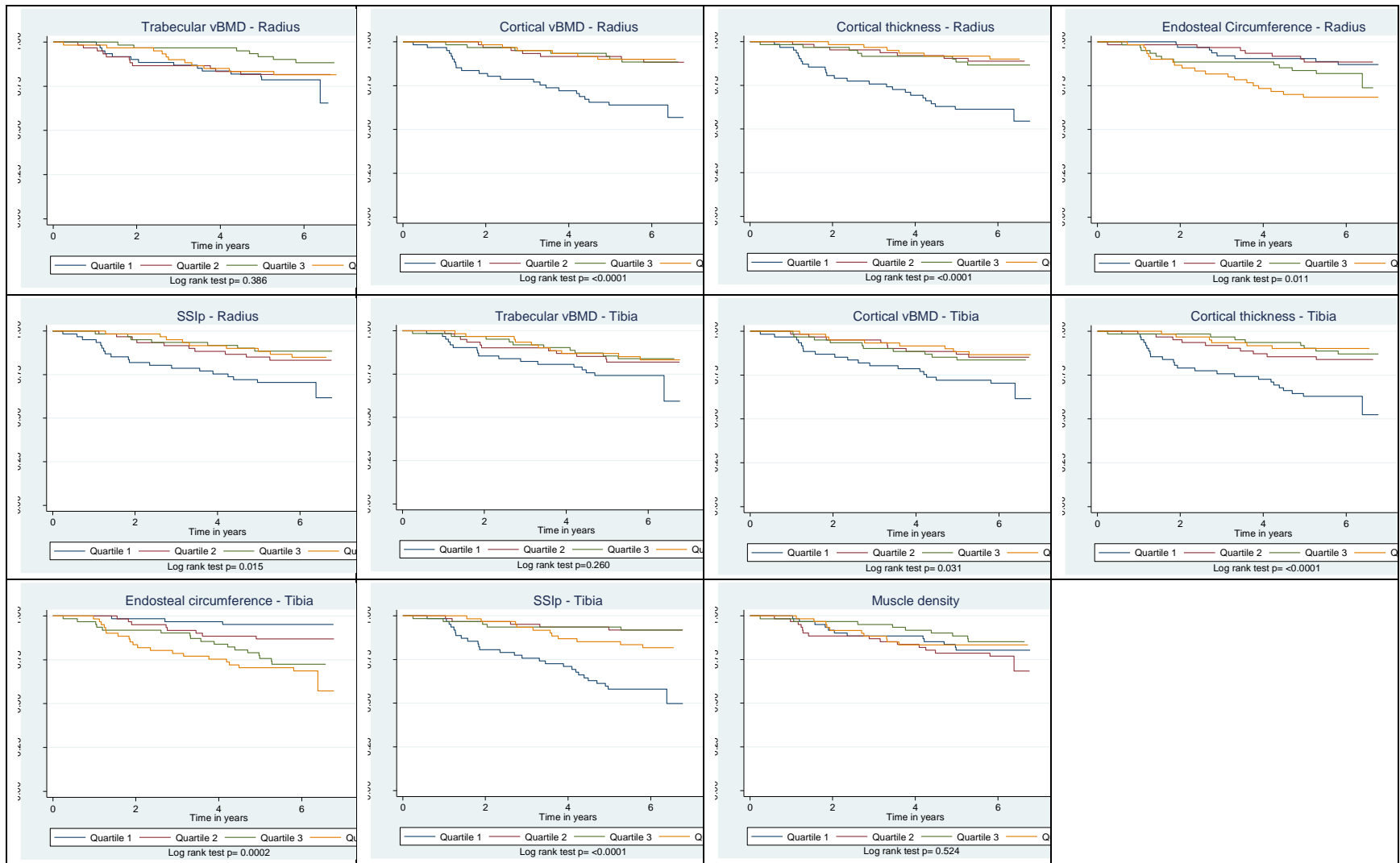
Figure 7.2 Kaplan meir curves of quartiles of pQCT parameters and log rank test among women

8. Physical Performance Measures Association with pQCT Bone and Muscle Quality Measures

8.1 Physical Performance Measures (Grip Strength, SPPB, and 400-meter Walk) and their Association with pQCT Derived vBMD, Bone Structure, and Geometrical Properties

Based on the mechanostat hypothesis, bones adapt to mechanical loading. Mechanical loading can be physiological, which is generated on the bone during muscular usage, muscle contraction, etc. or non-physiological due to trauma. This mechanical loading is suggested to have a direct effect on bone structure and strength [235]. Physical performance is one such physiological mechanical loading that impacts bones. Considering this, physical performance measures could have a direct influence on bone strength and structural parameters. Objective measures of physical performance like grip strength, SPPB, 400-meter walk have been well validated in the literature as a marker of disability with aging [118, 203, 236-240].

pQCT, as discussed earlier is a 3D technique assessing bone structure. In particular, it has the ability to measure vBMD of trabecular and cortical compartments apart from other parameters of bone structure, geometry and strength measures.

A systematic review of various studies which have used pQCT assessed the effect of exercise on bone mass and geometry among postmenopausal women and observed that; exercise has more influence on cortical than trabecular bone; high loading exercises have a greater impact on mass and geometry of bone; exercise positively influences bone mass and geometry [241]. Though exercise is not the same as physical performance the review findings indicate that higher physical performance may have positive influence on bone mass and geometry.

There have been studies in the literature which have examined physical performance measures and their association with pQCT derived bone measures among older populations (Refer

to table 8.1).Most of the studies have looked at grip strength and bone measures associations [225-233], few studies have considered gait speed[230, 232, 233] and fewer considered chair stands [230], but no studies have looked at the association of 400 meter walk and pQCT bone measures .

Among 129 community dwelling women age xx in Belgium, grip strength was an independent predictor of pQCT derived total vBMD (β 0.003; p- 0.007) and cortical vBMD (β 0.002; p- 0.002) but not trabecular vBMD at 4% radius when adjusted for BMI, vitamin D levels or intake, age, years since menopause, calcium intake, PTH levels, Sex hormone binding globulin (SHBG) and insulin like growth factor (IGF-1)[225] . A cross sectional study among 63 men (21-78 years) and 101 women (18-80) in Japan observed that grip strength predicted bone strength SSIp at distal radius (β – 0.199; p 0.0003) when adjusted for age and sex. In a model with grip strength as an outcome variable age, sex, SSIp at distal radius (β – 0.309; p 0.0002) and, vBMD at distal radius (β -0.204; p 0.006) were significantly associated [226]. The South Dakota Rural Bone Health Study (SDRBHS) a population based cross sectional study enrolled 1189 individuals (20-66 years) and examined the relationship of pQCT derived bone measures association with grip strength. Grip strength was a significant predictor of cortical BMC, cortical area, periosteal and endosteal circumferences, and SSIp at the 20% distal radius and periosteal circumference, cortical area, and cortical thickness at the 4% site [227]. Among Japanese men and women aged 26 years and over, grip strength was correlated (unadjusted) with total vBMD (men – r 0.19, p <0.001; women – r 0.41, p<0.001), cortical vBMD (men – r 0.17, p 0.02; women – r 0.26, p<0.001), trabecular vBMD (men – r 0.16, p 0.03; women – r 0.32, p<0.001) and SSIp (men – r 0.31, p <0.001; women – r 0.45, p<0.001) These correlations were higher among women than men [228]. The MrOS analysis of 1172 men aged 65 years and over observed that quartiles of grip strength (highest vs lowest) were associated with cortical vBMD (p <0.001) and SSIp (p<0.01). At the

radius, 1 SD increase in grip strength was associated with 0.3% higher cortical vBMD, and at tibia,, 0.2% higher cortical vBMD [229, 230]. The Hertfordshire cohort study among 642 men (mean age 69.2 years) and women (mean age 69.5 years) ,showed that t grip strength was associated with radial total bone area for both men (β 9.66) and women (β 5.97) and radial periosteal circumference for men (β 0.5) [232]. Another analysis from MrOS with 2857 central QCT and 786 pQCT bone measures, it was observed that grip strength was not related to the QCT measures of femoral neck and spine. The percent difference between the Q1 and Q4 of pQCT derived radius SSIx (7.5%), (7.5%), CSA (4.8%) and periosteal circumference (PC) (2.5%) were significantly associated grip strength adjusted for site, age, race, height, weight, appendicular lean mass (ALM) and leg power [231]. In one of the few longitudinal analysis, change in grip strength was significantly associated with change in radius 4% total mass (β 0.09) and change in tibia 4% trabecular vBMD (β 0.06) among men [233].

To summarise, the association of trabecular vBMD and grip strength has been inconclusive with one study observing the association crossectionally among men and women [228] and longitudinally, change in grip strength was associated with change in trabecular vBMD of tibia among men [233], however many studies did not observe any association [225, 227, 229, 230, 232]. Cortical vBMD and its association with grip strength has also been inconclusive, with some studies showing association [228-230], but not in others s [227]. Grip strength was consistently positively associated with the SSI [226-229, 231] on cross sectional analysis.

In MrOS , gait speed was associated with cortical vBMD among older men (1SD increase in gait speed, 0.4% higher radius cortical vBMD & 1SD increase in gait speed, 0.3% higher tibia cortical vBMD) [230]. Among the Hertfordshire cohort, endosteal circumference among women (β -0.57) was negatively associated with grip strength [232], SSI among men ((β 37.1) was

significantly associated with grip strength [232] and longitudinal change in gait speed was not associated with any change in pQCT bone measures[233].

Analysis of men aged ≥ 65 years enrolled in MrOS, observed that one SD increase in time taken for chair stands was negatively associated with radius cortical vBMD (-0.2%) and tibia cortical vBMD (-0.3%). Using the support of arm of the chair to get up from chair was negatively associated with radius cortical vBMD (-0.7%) and tibia cortical vBMD (-0.8%) compared to the subject who does not use arms for raising from chair. [230].

8.2 Gaps in Literature

Studies on the association of pQCT derived bone measures with physical performance measures have been largely skewed towards grip strength and gait speed but the results have been inconclusive. Most of these studies have been limited to the US, Europe or Japan. These associations have been studied in cross sectionally except for a single study which looked at the change in bone measures in association with change in grip strength and gait speed. To our knowledge, 400-meter walk test which is a well-established test for mobility disability and SPPB (short physical performance battery) which includes gait speed and chair stands along with balance test, have not been studied in the context of their association with pQCT bone measures.

There is a need to explore the relationship of several key pQCT measures like trabecular vBMD, cortical vBMD, cortical thickness, endosteal circumference and SSI, which provide an insight into the quality of bone in terms of its content, structure geometry and strength with physical performance measures which include grip strength, SPPB and 400-meter walk. This is critical to improve our understanding of the influence of physical performance on bone

crossectionally and longitudinally so that necessary interventions to strengthen bone quality can be included in the care of older populations and reduce morbidity and mortality due to fractures.

There is a lack of information on pQCT derived bone measures and physical performance measures in India. With the increasing older populations and higher disability in the country, there is a need for epidemiological studies to explore the associations of bone and physical performance measures for generating evidence to fill the existing gap.

Table 8.1 Association of Grip strength, SPPB and 400-meter walk with pQCT bone measures

	Author, year (reference)	Study Design	Study population	Physical performance measure	pQCT details	Results	Variables adjusted for
1	Boonen et al 1997 [225]	Cross sectional design	129 community-based women, aged 70-87 years in Belgium	Grip strength	XCT 900, distal radius (4%). Total, cortical and trabecular density.	Grip strength was an individual predictor of total bone density (β 0.003; p 0.007) and cortical density (β 0.002; p 0.002), but not trabecular density (β 0.257; p 0.31).	Adjusted for BMI, Vitamin D, Age, Years since menopause, calcium intake, Parathyroid hormone levels (PTH), Sex hormone binding globulin (SHBG) and Insulin like growth factor 1 (IGF 1).
2	Hasegawa et al. 2001 [226]	Cross sectional design	63 males (21-78 years) and 101 females (18-80 years)	Grip strength	XCT 2000 on non-dominant forearm (4% and 66%). vBMD, BMC, Area, SSIp and CSMI	Grip strength predicted bone strength (SSI: β – 0.199; p 0.0003). With grip strength as an outcome, R^2 / 64.7% was explained by age, sex, SSIp (β 0.309; p 0.0002) and, vBMD (β -0.204; p 0.006)	Age and Sex

Table 8.1 Continued

	Author, year (reference)	Study Design	Study population	Physical performance measure	pQCT details	Results	Variables adjusted for
3	Specker et al. 2004 [227]	Cross sectional design	South Dakota Rural Bone Health Study (SDRBHS) 1189 individuals: 504 rural Hutterites (188 men), 349 rural individuals, 184 men), and 336 non rural individuals aged 20 to 66 years.	Grip Strength	XCT 2000 on forearm (4% and 20%). vBMD, BMC, Area, SSIp and CSMI	It was observed that grip strength was a significant predictor of cortical BMC, cortical area, periosteal and endosteal circumferences, and SSIp at the 20% distal radius and periosteal circumference, cortical area, and cortical thickness at the 4% site.	Age, weight, height, percent body fat and estrogen status
4	Kaji et al. 2005 [228]	Cross sectional design	250 women and 230 men aged 26 years and above who visited Kuno hospital in Japan	Grip strength	XCT – 960 on non-dominant forearm (4% and 20%). Total, trabecular & cortical vBMD, Cortical area, Thickness and SSI	Grip strength was correlated with, Total vBMD (Men – r 0.19, p <0.001; Women – r 0.41, p<0.001) , Cortical vBMD (Men – r 0.17, p 0.02; Women – r 0.26, p<0.001) , Trabecular vBMD (Men – r 0.16, p 0.03; Women – r 0.32, p<0.001) and SSIp (Men – r 0.31, p <0.001; Women – r 0.45, p<0.001)	No adjustment done

Table 8.1 Continued

	Author, year (reference)	Study Design	Study population	Physical performance measure	pQCT details	Results	Variables adjusted for
5	Cousins et al. 2010 [229]	Cohort study – Cross sectional analysis	MrOS. 1172 Men 65 years and older from Pittsburgh and Minnesota	Grip strength	XCT 2000 on tibia (4% and 66%) and non-dominant fore arm (4% and 33%). vBMD, BMC, Area, CSMI and SSI measured.	Quartiles of grip strength compared between highest and lowest quartiles, is not associated with (p trend) - Total vBMD (p 0.507), Trabecular vBMD (p0.592) but associated with Cortical vBMD (p <0.001) and SSIp (p<0.01).	Age, clinic site, race, radius length and weight.
6	Barbour et al. 2010 [230]	Cohort study – Cross sectional analysis	MrOS. 1172 Men 65 years and older from Pittsburgh and Minnesota	Grip strength, Gait speed - 6m walk, time to complete 5 chair stands, ability to stand from a chair without using arms	XCT 2000 on tibia (4% and 66%) and non-dominant fore arm (4% and 33%). vBMD trabecular and cortical	At radius 1 SD increase in – (i) grip strength had 0.3% higher cortical vBMD, (ii) gait speed had 0.4% higher cortical vBMD (iii) chair stands time had -0.2% lower cortical vBMD (iv) using arm for a chair to stand had -0.7% lower cortical vBMD At tibia 1 SD increase in - (i) grip strength had 0.2% higher cortical vBMD, (ii) gait speed had 0.3% higher cortical vBMD (iii) chair	Age and weight

Table 8.1 Continued

	Author, year (reference)	Study Design	Study population	Physical performance measure	pQCT details	Results	Variables adjusted for
						stands time had -0.3% lower cortical vBMD (iv) using arm for a chair to stand had -0.8% lower cortical vBMD. On the other hand, men who stand using arms of the chair had -6.9% lower trabecular vBMD at tibia.	
7	Edwards et al. 2013 [232]	Cohort study – Cross sectional analysis	Hertfordshire Cohort Study (HCS) in the United Kingdom. 322 Men and 320 women who were born between 1931 and 1939	Grip strength and gait speed for 3 m walk	XCT 2000 – forearm (4% and 66%) tibia (4%, 38% and 66%). vBMD, BMC, Area, SSI and periosteal circumference.	Significant association was observed for - grip strength and radial total bone area for both men (β 9.66) and women (β 5.97); radial periosteal circumference for men (β 0.5) Gait speed was associated with Endosteal circumference in women (β -0.57) and SSI of men (β 37.1)	Age, height, weight adjusted-for-height, limb-length-adjusted-for-height, social class, smoking status, alcohol consumption, calcium intake, physical activity, diabetes mellitus, and in women, years since menopause and estrogen

Table 8.1 Continued

	Author, year (reference)	Study Design	Study population	Physical performance measure	pQCT details	Results	Variables adjusted for
							replacement therapy,
8	Patel et.al., 2018 [233]	Longitudinal analysis – change of bone measures with change of physical performance measures	Hertfordshire cohort study 443 (184 men and 166 women) born between 1931 and 1938 in UK. The participants who had the measures of pQCT and physical performance done twice, once in 2004-05 and 2011-12	Grip strength and gait speed for 3 m walk	Radius 4% & tibia 4% - Total mass, trabecular vBMD Radius 66% - Total area, cortical area, cortical vBMD Tibia 14% - Total area, cortical area, cortical vBMD	The change between the two-time points of measures among men and women were significantly different for – radius: total area, cortical area and cortical vBMD; Tibia: total mass and cortical area. The change in grip strength and gait speed were not significant. Grip strength univariate analysis had significant association only in men for radius total mass and tibia trabecular vBMD. But these changes were attenuated on adjustment of demographic and lifestyle factors. Women had no association with grips strength. Gait speed did not have any	Age, BMI, social class, smoker status, alcohol consumption, physical activity, dietary calcium intake and years since menopause and HRT use in women

Table 8.1 Continued

	Author, year (reference)	Study Design	Study population	Physical performance measure	pQCT details	Results	Variables adjusted for
						association with change in bone measures.	
9	Chalhoub et.al., 2018[231]	Cross sectional analysis	MrOS participants from US, 2857 who had a QCT and 786 who had pQCT; all subjects were men and aged ≥65 years	Grip strength	QCT of femoral head with the measures of CSA, cortical thickness, trabecular and cortical vBMD. QCT at lumbar spines measured total vBMD, CSA. pQCT at 4, 33 and 66% of tibia and 33% of radius and measures were SSIx, Polar moment of Inertia (PMI) cortical thickness, periosteal circumference (PC), CSMI and CSA	The QCT measures of femoral neck and lumbar spine were not associated with grip strength. pQCT measures: The percent difference between the Q1 and Q4 of pQCT measure and adjusted for the variables showed radius; SSIx (7.5%), CSA (4.8%) and PC (2.5%) were significantly associated grip strength. The tibial pQCT measures were not associated with grip strength.	Site, age, race, height, weight, appendicular lean mass (ALM) and leg power

8.3 Physical Performance (Grip Strength, SPPB, and 400-meter Walk) and their Association with pQCT Derived Muscle Density Measure

Muscle composition deteriorates with age, there is increased ectopic deposition of fat in the muscle known as myosteatosis [242]. The fat deposits within the skeletal muscle measured as the intermuscular adipose tissue (IMAT) have been associated with mobility limitations [243-245], impaired physical performance [193, 244], decreased muscle strength and power [193] in older adults.

Muscle composition analysis using pQCT suggests that muscle density is a measure or an estimate of both inter and intra muscular adipose tissue or fat deposit. Muscle density thus can be a better marker of myosteatosis compared to IMAT if available.

Studies describing the association of muscle density with grip strength, SPPB and 400-meter walk are summarised in the table 8.2.

The studies on the association of grip strength with muscle density have been inconclusive where some studies showed a positive association [234, 246] while others showed no association [141, 247, 248]. It is also important to note that most of the tests for these associations were unadjusted except for two studies which adjusted for age, sex and appendicular lean mass / BMI, moderate/vigorous physical activity, fasting glucose, visceral adipose tissue and C-Reactive Protein (CRP) [246, 247].

Most of the studies showed that there was an association of gait speed with muscle density [141, 196, 234] except for one study which showed a positive association [246].

Two previous studies have reported positive associations of chair stands per second, with muscle density [141, 234]. Only one study looked at the SPPB and found that it was positively

associated with muscle density adjusting for age, sex, moderate/vigorous physical activity, fasting glucose, visceral adipose tissue and systemic inflammation (CRP) [247].

Based on a cross sectional analysis, the InCHIANTI study observed that diabetics had significantly lower muscle density; however, after adjusting for BMI this association was attenuated and no longer significant. Muscle density attenuated the association of 4 meter and 400-meter walk times with diabetes by around 11% and 13% respectively [249]. This probably suggests that diabetes confounds the relationship between 400-meter walk and muscle density.

8.4 Gaps in the Literature

Muscle density, which represents fat accumulation in the inter and intra muscular regions, has been less studied for its association with physical performance. Most of the evidence is largely limited to Caucasian populations and developed nations. Effects of muscle density on physical performance grip strength, gait speed, chair stands and SPPB have been studied, but 400-meter walk has not been explored for its association with muscle density.

To our knowledge, pQCT derived lower leg muscle density and its association with physical performance measures, such as grip strength, SPPB and 400-meter walk, have not been described among the older Indian population. Considering the higher disability rates and increased aging in India, it is important to explore the association of physical performance measures with muscle density as this may indicate higher fat infiltration in the muscle or poor muscle quality may be associated with poorer physical performance. It is important to understand the how physical performance influences muscle density, and whether it can act as an early predictor for poor muscle quality so that necessary interventions to strengthen muscle quality among older populations can be included.

Table 8.2 Muscle density association with Grip strength, SPPB and 400-meter walk

	Author, year (reference)	Study Design	Study population	Physical performance measure	Muscle density measurement	Results	Variables adjusted for
1	Cesari et.al., 2009 [196]	Longitudinal design for mortality but muscle density and gait speed cross sectional at baseline	934 participants 65 years and older of the Invecchiare inChianti study in Italy	Gait speed - 7 meter walk test	pQCT measure at tibia 66% using XCT 2000	Gait speed significantly correlated with muscle density (r = .268)	Not adjusted
2	Cawthon et.al., 2009 [234]	Cohort study	Health ABC study, 3011 adults aged 70-80 years	Grip strength. Gait speed – 6 meter walk. Time for repeated chair stands	Computed tomography (CT) scans of the thigh for muscle density	Lower quartile of muscle density had significantly lower – grip strength, gait speed and chair stands time	Not adjusted
3	Cawthon et.al., 2011[141]	Cohort study	Health ABC study, 1263 women and 1221 men aged 70-80 years	Grip strength. Gait speed – 6-meter walk.	Computed tomography (CT) scans of the	Muscle density correlations were as follows:	Not adjusted

Table 8.2 Continued

	Author, year (reference)	Study Design	Study population	Physical performance measure	Muscle density measurement	Results	Variables adjusted for
				Time for repeated chair stands	thigh for muscle density	Grip strength: Men (r-0.05) not significant; women (r-0.03) not significant Gait speed - Men (r-0.17) significant; women (r-0.24) significant Chair stands time - Men (r-0.15) significant; women (r-0.20) significant. Based on the factor analysis, muscle density was more closely related to adiposity than physical performance.	
4	Scott et.al., 2015 [246]	Cross sectional study	50 community dwelling older adults (65 years and over) conducted in	Grip strength. Gait speed using GAITrite electronic walkway	pQCT assessed muscle density using XCT 3000	Grip strength (β 0.507 (0.055, 0.960) was significantly associated with muscle density.	Adjusted for age, sex and appendicular lean mass / BMI

Table 8.2 Continued

	Author, year (reference)	Study Design	Study population	Physical performance measure	Muscle density measurement	Results	Variables adjusted for
			Melbourne, Australia	system ® 10m walk		Gait speed (β 0.016 (-0.009, 0.040) was not significantly associated with muscle density	
5	Weeks et.al., 2016 [248]	Cross sectional study	52 healthy adults (26 men, 26 women) between the ages of 18 and 64 years in Australia	Grip strength	pQCT XCT 3000 was used and muscle density of forearm, leg and thigh	Among the older population (upper tertile, mean age 48.3 ±9.4 years) participants; Muscle density was not significantly associated with grip strength (R^2 0.03; p 0.198)	Not adjusted
6	Scott et.al., 2018 [247]	Cross sectional	85 community dwelling adults aged 50 years who were overweight or obese (BMI≥25) and more of Melbourne, Australia	Grip strength and SPPB	pQCT derived muscle density using XCT 3000 at 66% tibia	Participants with a SPPB score ≤9 (41%; indicating poor physical performance) had significantly lower calf muscle density,	Age, sex, moderate/vigorous physical Activity, fasting glucose, visceral adipose tissue and CRP.

Table 8.2 Continued

	Author, year (reference)	Study Design	Study population	Physical performance measure	Muscle density measurement	Results	Variables adjusted for
						Calf muscle density was positively correlated with SPPB (r0.322 (p 0.003). Muscle density was significantly associated with SPPB on multivariable regression (β 0.118 (0.007, 0.228). Grip strength was not associated with muscle density.	

9. Paper 3: Association of pQCT Derived Bone and Muscle Density Measures with Physical Performance Measures Among a Rural South Indian Older Population

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9.1 Abstract

Physical performance measures and their association with peripheral quantitative computed tomography (pQCT) derived bone measures have been inconclusive. Muscle density has been less studied for its association with physical performance. We examined the association of pQCT derived bone measures and muscle density with physical performance measures among rural south Indian older population. The pQCT scans of 499 (men and women) of the Mobility and Independent Living among Elders' Study (MILES) were analysed for their association with grip strength, Short Physical Performance Battery (SPPB) score and 400 meter walk completion independently. Among older men, muscle density (β = 0.03, [95% CI = 0.002, 0.05]) was independently and positively associated with grip strength; cortical volumetric bone mineral density (vBMD) of radius (0.1, [0.03, 0.17]), trabecular vBMD of tibia (0.07, [0.01, 0.14]), cortical thickness of tibia (0.1, [0.03, 0/17]) and muscle density (0.13, [0.07, 0.2]) were positively associated and endosteal circumference of tibia (-0.08, [-0.15, -0.01]) was negatively associated with SPPB score; cortical thickness of tibia (0.65, [0.19, 1.11]) was positively associated with 400-meter walk completion. Among older women, trabecular vBMD of radius (0.05, [0.01, 0.09]) and tibia (0.04, [0.002, 0.08]), cortical thickness of radius (0.04, [0.01, 0.07]) and tibia (0.03, [0.001, 0.07]), polar Strength strain Index (SSIp) of radius (0.04, [0.002, 0.08]) and muscle density (0.05, [0.01, 009]) were positively associated with grip strength; cortical vBMD (0.08, [0.01, 0.15]), SSIp tibia (0.1, [0.04, 0.16]) and muscle density (0.09, [0.01, 0.17]) were associated with SPPB and; trabecular vBMD of tibia (0.5, [0.07, 0.93]) was positively associated with 400-meter walk completion. These observations suggest that physical performance measures may impact skeletal strength and muscle density. Interventions to improve physical performance could have positive impact on bone and muscle quality.

9.2 Introduction

Bone adapts to mechanical loading generated physiologically due to muscular usage, muscle contraction, and non-physiologically due to trauma. This mechanical loading has a direct role on bone structure and strength [235]. Physical performance is one such physiological mechanical loading that may impact the skeleton and could have a direct influence on bone strength and structural parameters. Objective measures of physical performance like grip strength, SPPB, 400-meter walk have been well validated in the literature as a marker of disability in aging [118, 203, 236-240].

Peripheral quantitative computed tomography (pQCT) is a 3D technique to assess bone structure and geometry. In particular, it has the ability to measure the volumetric bone mineral density (vBMD) along with other geometric and strength measures.

Exercise positively influences bone mass and geometry [241]. Studies on physical performance measures and their association with pQCT derived bone measures have primarily examined grip strength [225-233], few studies have considered gait speed [230, 232, 233] and fewer considered chair stands [230], however, these associations have been inconclusive. Most of these studies have been carried out in the US, Europe or Japan. To our knowledge, the 400-meter walk test which is a well-established test for mobility disability and short physical performance battery (SPPB) a composite index which includes gait speed and chair stands along with the balance test, have not been studied in this context. There is also limited data on pQCT derived bone measures and physical performance measures among older population in India.

Muscle composition deteriorates with age, and there is ectopic deposition of fat in the muscle with aging known as myosteatosis [242]. The fat deposits within the skeletal muscle have

been associated with mobility limitations in older adults [243-245], impaired physical performance [193, 244], decreased muscle strength and power [193].

Muscle composition analysis using pQCT provides a muscle density measure, which is representative of fat infiltration within the muscle and is also considered as an indicator of muscle quality. Muscle density has been less studied for its association with physical performance. Most of the studies have been carried out among on Caucasian populations. Effects of muscle density on physical performance measures like, grip strength, gait speed, chair stands and SPPB have been studied, but 400-meter walk test has not been explored for its association with muscle density. In addition, these associations have not been described among Indian older populations.

As India is a rapidly aging nation and aging is related to disability and poorer musculoskeletal measures, it is important to explore the association of pQCT derived bone measures and muscle density with physical performance measures. We performed a cross sectional analysis to assess the association of pQCT derived bone measures and muscle density with physical performance measures among rural south Indian older population. We hypothesized that pQCT derived volumetric BMD (vBMD), bone structure, bone geometry measures, and muscle density will be positively related to physical performance measures (grip strength, SPPB score and 400-meter walk). We also hypothesized that these associations would be similar in men and women.

9.3 Materials and Methods

Study population:

Healthy older individuals ($\geq$ 60 years of age) residing in the Medchal region of Telangana state in India were recruited for the Mobility and Independent Living Among Elders Study

(MILES). At baseline the study enrolled 562 men and women in 2012 and collected information on physical performance, life style, physical activity, blood pressure and fasting blood sugar [27]. A second baseline visit was conducted in 2012-2013 with a 6-8 month gap between the two visits for performing pQCT scans on these individuals. A total of 499 scans (245 men and 254 women) were available for analysis.

pQCT and calibration:

Stratec XCT-2000 (Stratec Medizintechnik, Pforzheim, Germany) was used for pQCT scans. A standard protocol for positioning and scanning was used by the technicians. Manufacturer provided cylindrical Quality Assurance (QA) phantom was used for stability and quality of the scanner. All the pQCT scans were read and analysed by a single investigator using the manufacturer software package version 6.00 for the XCT scanners.

pQCT parameters (dependant variables)

pQCT parameters were considered as the dependant variables in this analysis and the measures included were, trabecular vBMD at 4% of radius and tibia, cortical vBMD at 33% radius and tibia, cortical thickness at 33% radius and tibia, endosteal circumference at 33% radius and tibia, polar strength strain index (SSIp) at 33% radius and tibia, and muscle density at 66% tibia.

Independent variables:

Grip strength was directly measured using a hand held dynamometer. Readings were taken twice for each hand. In this analysis we have used the average of the two readings of the participants' dominant hand.

SPPB included the 4-meter gait speed test, balance test and chair stands. A total of 12 points were possible for the SPPB [237]. The total SPPB score was used in this analysis.

A 400-meter timed walk was performed on all the participants. Participants were categorised as those who were able to complete the 400-meter walk and those who did not complete or attempt the test.

Covariates:

Information on various covariates were collected. Information on self-reported health status was categorised as good/excellent and fair/poor/very poor. Categorical variables of smoking categorised as current smoker and not a current smoker; alcohol consumption categorised as consumes alcohol and does not consume alcohol. Weight was measured using SECCA® scale. Height was measured using a SECCA® stadiometer, and average of two reading was taken; if the difference between two readings was ≥ 0.5 cms then two more reading were taken and the average of these readings was recorded. Body Mass Index (BMI) was calculated by dividing body weight in kilograms with height in meters squared. Participants with glucose levels of ≥126mg/dL (after a minimum of an 8-hour fast), or self-report of diabetes or using insulin or hypoglycemic medications were categorised as diabetics. Participants with blood pressure (≥ 140/90 mm of Hg), self-reported hypertensive, or on anti-hypertensive medication were categorised as having hypertension. Self-reported history of stroke was recorded. Activities of daily living (ADL) was assessed using the standard tasks of eating, dressing, bathing, transferring from bed to chair and using the toilet, and was categorised as ADL disability if the participant reported difficulty in any one of the tasks. Self-reported time of continuously walking to get to and from places in a week was considered as physical activity and was categorized as ≤ 30 mins and > 30 mins per week.

Statistical analysis:

The pQCT parameters were significantly different among men and women (Table 2) and hence, sex specific analyses were performed. Outliers of the pQCT parameters were considered if the values were more than ± 3 SD and were removed from the analysis. The pQCT parameters were standardised (SD from mean) for this analysis. Participants characteristics were described as means (SD) or prevalence, (n (%)). Two-sample t-tests / Wilcoxon rank sum test (continuous variables) or chi-square tests (categorical variables) were used to compare characteristics between men and women. Linear regression models were used to assess the association of pQCT measures with physical performance (Grip Strength, SPPB Score and 400-meter walk completion). Separate and increasingly adjusted models were performed for grip strength, SPPB score and 400-meter walk completion as independent variables for each of the pQCT parameters as following: model 1 = unadjusted; model 2 = age, height and weight; model 3= model 2 + smoking, alcohol, health status, hypertension, diabetes, stroke, ADL disability and physical activity. All the muscle density models were adjusted for muscle cross sectional area (CSA) in the fully adjusted models (model 3). Multicollinearity was assessed using the variance inflation factor (VIF). Results were considered statistically significant when a p-value was less than 0.05. The power analysis suggested the current sample has the ability to identify a very small effect size ≥0.032 (Cohen's F^2) in both men and women.

9.4 Results

The characteristics of men and women are presented in table 9.1. Age of men and women was similar, but men were significantly taller and heavier compared to women. However, the BMI of men (21.6±3.9) was significantly lower compared to women (23±4.8). Grip strength of men

(20±6.04) was significantly higher compared to women (12.5±4.74). The SPPB score was also higher among men (8.8±2.75) compared to women (7.22±2.82). The 400-meter walk completion was higher among men (73%) compared to women (58%). Current smoking was higher among men at 44% and alcohol consumption was also higher among men at 71% compared to women at 1% and 58% respectively. The prevalence of ADL disability, hypertension, diabetes and stroke were similar among men and women. Men were more physically active (>30 mins of continuous walking in a week) at 34% compared to women (13%).

The pQCT parameters distributions among men and women are presented in table 9.2. The trabecular vBMD, cortical vBMD, cortical thickness and SSIp of radius and tibia were significantly different between men and women, with men having higher measures compared to women. The endosteal circumference of radius and tibia, and muscle density were similar among men and women.

The associations of the pQCT measures with physical parameters for men is presented in table 9.3.

Grip strength (men):

In the unadjusted model, grip strength was significantly positively associated with one standard deviation change in, radial trabecular vBMD (β = 0.03, [95% CI limits = 0.01, 0.05]) and tibial trabecular vBMD (0.05, [0.03, 0.07]), radial cortical vBMD (0.04, [0.02, 0.06]), cortical thickness at radius (0.05, [0.03, 0.07]) and tibia (0.05, [0.03, 0.07]), SSIp of radius (0.04, [0.02, 0.06]) and tibia (0.04, [0.02, 0.06]) and muscle density (0.03, [0.01, 0.05]); and negatively associated with endosteal circumference at radius (-0.03, [-0.05, -0.01]) and tibia (-0.01, [-0.04, -0.01]). When adjusted for age, height and weight, associations with radial trabecular vBMD, SSIp of radius and tibia were attenuated and lost significance. However, cortical vBMD of tibia which

was not significant in the unadjusted model was significant in model adjusted for age, height and weight. In the fully adjusted models, only muscle density was significantly associated with grip strength (0.03, [0.002, 0.05]).

SPPB score (men):

In the unadjusted model, SPPB score was positively associated with cortical vBMD of radius (0.09, 0.04, 0.13]) and tibia (0.08, [0.04, 0.13]), cortical thickness of radius (0.07, [0.02, 0.11]) and tibia (0.09, [0.05, 0.14]), trabecular vBMD of tibia (0.06, [0.01, 0.10]) and muscle density (0.14, [0.1, 0.18]) and; inversely associated with tibial endosteal circumference (-0.07, [-0.11, -0.02]). Adjusting for age, height and weight all these pQCT parameters remained significantly associated with gait speed. When adjusting for all the covariates (age, height, weight, opinion on health status, current smoking, alcohol consumption, ADL disability, hypertension, diabetes, stroke and physically activity), cortical vBMD of radius (0.1, [0.03, 0.17]). trabecular vBMD of tibia (0.07, [0.01, 0.14]), cortical thickness of tibia (0.1, [0.03, 0.17]) and muscle density (0.13, [007, 0.2]) were positively associated and endosteal circumference of tibia (-0.08, [-0.15, -0.01]) was negatively associated with SPPB score.

400-meter walk completion (men):

In the unadjusted and, age, height and weight adjusted models, trabecular vBMD of radius and tibia, cortical thickness of tibia and muscle density were significantly associated with 400 meter walk completion. However, in fully adjusted models only cortical thickness of tibia (0.65, [0.19, 1.11]) remained significantly positively associated with 400 meter walk completion.

The association of pQCT parameters with physical performance measures among women is presented in the table 9.4.

Grip strength (women):

In unadjusted models and in models adjusting for age, height and weight, trabecular vBMD of radius and tibia, cortical vBMD of radius and tibia, cortical thickness of radius and tibia, SSIp of radius and tibia and muscle density were positively associated with grip strength. In the fully adjusted models, trabecular vBMD of radius (0.05, [0.01, 0.09]) and tibia (0.04, [0.002, 0.08]), cortical thickness of radius (0.04, 0.01, 0.07]) and tibia (0.03, [0.001, 0.07]), SSIp of radius (0.04, [0.002, 0.08]) and muscle density (0.05, [0.01, 0.09]) remained statistically significant.

SPPB score (women):

In the unadjusted models, cortical vBMD of radius (0.09, [0.05, 0.14]) and tibia (0.09, [0.05, 0.14]), cortical thickness of radius (0.11, [0.07, 0.15]) and tibia (0.13, [0.09, 0.18]), SSIp of radius and tibia (0.05, [0.01, 0.10]) and tibia (0.1, [0.06, 0.15]), trabecular vBMD of tibia0.07, [0.03, 0.11]) and muscle density (0.09, [0.05, 0.14]) were positively associated and endosteal circumference of radius (-0.07, [-0.11, -0.02]) and tibia (-0.09, [-0.13, -0.05]) were negatively associated with grip strength. On adjusting for age, height and weight SSIp radius attenuated and no longer significant. Further adjusting for other covariates, cortical thickness at tibia (008, [0.01, 0.15]), SSIp tibia (0.1, [0.04, 0.16]) and muscle density)0.09, [0.01, 0.17]) were significantly positively associated with grip strength.

400-meter walk completion (women):

In the unadjusted model and model adjusting for age, height and weight, trabecular vBMD of tibia, cortical vBMD of radius and tibia, cortical thickness of tibia, SSIp of tibia and muscle density were positively associated and endosteal circumference of tibia was negatively associated with 400 meter walk completion. However, on adjusting for other covariates in fully adjusted

model, trabecular vBMD of tibia (0.05, [0.07, 0.93]) only remained significantly associated with 400 meter walk completion.

9.5 Discussion

The current cross sectional analysis of MILES among men observed independent associations of muscle density with grip strength; cortical vBMD of radius, trabecular vBMD of tibia, cortical thickness of tibia, endosteal circumference and muscle density with SPPB score; cortical thickness of tibia with 400-meter walk completion; among older women, trabecular vBMD of radius and tibia, cortical thickness of radius and tibia, SSIp of radius and muscle density with grip strength; cortical vBMD, SSIp tibia and muscle density with SPPB score and; trabecular vBMD of tibia with 400-meter walk completion.

Among community dwelling women in Belgium, grip strength was an independent predictor of pQCT derived cortical vBMD (β 0.002; p 0.002) but not trabecular vBMD at 4% radius when adjusted for BMI, Serum vitamin D, age, years since menopause, calcium intake, PTH levels, Sex hormone binding globulin (SHBG) and insulin like growth factor (IGF-1)[225] . These findings conflict with our results for cortical vBMD which was not independently associated, but trabecular vBMD both at radius and tibia were independently associated with grip strength.

A cross sectional study among 63 men (21-78 years) and 101 women (18-80) in Japan observed that grip strength was positively associated with SSIp at distal radius (β – 0.199; p 0.0003) when adjusted for age and sex [226], however in our study, SSIp at radius had a significant independent association predictor among women but not in men.. The South Dakota Rural Bone Health Study (SDRBHS) a population based cross sectional study observed that grip strength was a significantly negatively associated with endosteal circumferences and positively associated with SSIp (at 20% radius) and cortical thickness (at 4% site) [227]. Similar unadjusted associations

were observed for cortical thickness of radius and SSIp of radius in both men and women, whereas endosteal circumference was not associated among women in unadjusted models. The MrOS study analysis of 1172 men aged 65 years and over observed that quartiles of grip strength (highest vs lowest) were associated cortical vBMD (p <0.001) and SSIp (p<0.01) [229]. In another MrOS analysis, 1SD increase in grip strength had 0.3% higher cortical vBMD at radius and 0.2% higher cortical vBMD at tibia [230]. These observations were similar in our study for the minimally adjusted models (age, height and weight) but in contrast our analysis observed, these associations were no longer significant in fully adjusted models.

Overall, the association between trabecular vBMD and grip strength has been inconclusive with our analysis observing association only in unadjusted models among men but independently associated among women. Several studies observed unadjusted associations [225, 227, 229, 230, 232] but contrastingly one study observed a relationship among men and women [228]. Cortical vBMD and its association with grip strength also has been inconclusive, in some studies among men and women it was associated [228-230], but was not associated in another study. [227]. Grip strength was consistently associated with the SSI [226-229, 231] on cross sectional analysis, however, in our study it was independently associated among women but not in men.

To our knowledge SPPB and 400-meter walk have not been studied for their association with pQCT bone measures although gait speed and chair stands have been studied separately. In a MrOS analysis, gait speed was associated with cortical vBMD among older men [230] and in Hertfordshire cohort, endosteal circumference among women (β -0.57) was negatively associated with grip strength [232], SSI among men ((β 37.1) was significantly associated with grip strength [232]. In MrOS, chair stand time was negatively associated with radius cortical vBMD (-0.2%) and tibia cortical vBMD (-0.3%), also using the support of arm of the chair to get up from chair

was negatively associated with radius cortical vBMD (-0.7%) and tibia cortical vBMD (-0.8%) compared to the subject who does not use arms for raising from chair. [230]. Considering these findings of the tests which are a part of SPPB, our study observed, radius cortical vBMD, tibia endosteal circumference were associated among men, and cortical vBMD and SSIp among women. In addition, our study observed; grip strength was only associated with muscle density among men but among women it was associated with trabecular vBMD of radius and tibia, cortical thickness of radius and tibia, SSIp of radius and muscle density; SPPB among men was associated with cortical vBMD of radius, trabecular vBMD of tibia, cortical thickness of tibia, endosteal circumference and muscle density, however among women it was associated with cortical vBMD, SSIp tibia and muscle density; the 400-meter walk completion was associated with cortical thickness of tibia whereas among women it was associated with trabecular vBMD of tibia. These observations point towards gender differences in the association of physical performance measures and pQCT derived bone measures.

Association of bone and physical performance measures can be due to bone muscle cross talk. The bone muscle cross talk has been a focus of recent research. It is theorized that both skeletal muscles and bones grow and are maintained to fit the mechanical and metabolic needs of the body, however they also deteriorate with non-use, disease, and age [111]. Not only mechanical but several biochemical signals play a role in the bone muscle cross talk apart from the mechanostat hypothesis. The bone muscle cross talk is a synergistic combination of mechanical loading and biochemical signals between the bone and muscle [112]. Various mechanisms have been proposed like – (i) Biomechanical relationship of bone and muscle [113], (ii) biochemical relationship of bone and muscle through endocrine factors apart from the mechanical stimuli through myokines, adipokines and osteokines, which play a role in the bone muscle cross talk. [111], (iii) factors

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secreted by muscle which effect bone like the myostatin, irisin, several types of growth factors, inflammatory cytokines and peptides [112, 114], (iv) factors secreted by bone effect muscle, like osteocytes which function as a secretory endocrine organ and regulates phosphate thorough the fibroblast growth factor 23 (FGF23) which in turn maintains the normal bone mineral content. The Wnt1 and Wnt3 which are expressed by osteocytes due to mechanical loading and support myogenesis [112], (v) familiar associations of bone and muscle like the genetic influence[117], Vitamin D, sex hormones (estrogen and testosterone) [111], (vi) physical interaction of muscle and bones with structures like tendons, ligaments, cartilages and connective tissues [111], and (vii) nervous system plays a role in the bone and muscle cross talk.

The studies on the association of grip strength with muscle density have been inconclusive. The Health ABC study using CT scans of thigh observed that individuals in the lower quartiles of muscle attenuation / density had lower grip strength [234]. Another analysis in Health ABC study observed muscle density was not significantly correlated with grip strength among men and women [141]. In our analysis men had a significant independent association of muscle density and grip strength. In a cross sectional study in Australia among older adults, grip strength was significantly associated (β 0.507, [0.055, 0.96]) when adjusted for age, sex, BMI [246]. In another cross sectional study in Australia among adults (men and women), muscle density was not associated with grip strength (R^2 0.03, p – 0.198) unadjusted. In another cross sectional study in Australia among 50 years and over obese individuals, grip strength was not associated with muscle density[247]. One thing to note that these cross sectional studies had a small sample size (ranging from 50-85 participants). In contrast to these studies, our study observed significant independent association between grip strength and muscle density among both men and women.

To our knowledge one study among 85 community dwelling adults aged 50 years and more observed that SPPB score of ≤ 9 was associated with lower calf muscle density adjusting for age, sex, physical activity, fasting glucose, visceral adipose tissue and systemic inflammation (CRP) [247]. This was similar to our study where in SPPB score was independently positively associated with muscle density.

To our knowledge no studies have assessed muscle density association with 400-meter walk. In our study, it was observed that muscle density was associated with 400-meter walk completion in both unadjusted and minimally adjusted models (age, height and weight) but not in the fully adjusted models

Low muscle density is associated with systemic inflammation , hyperinsulinaemia and insulin resistance[250, 251]. High low grade chronic elevation of systemic markers of inflammation is associated with poor physical performance [252]. It is possible that the inflammatory markers may influence the associations as a residual confounder. Insulin resistance may also contribute to poor physical performance, however in this analysis we did adjust for diabetes status in all the fully adjusted models but had no specific measure of insulin resistance.

There are several limitations to our study; this is a cross sectional analysis and hence temporality cannot be established. Many confounders like inflammatory markers and insulin resistance have not been included in the study which may lead to residual confounding. The participants recruited for the study were required to be able to travel to the research clinic for the various examinations, this could have led to a selection bias. However, it was observed that the participants had lower physical performance measures including low SPPB score, low grip strength, lower completion of 400 meter walk test and lower BMI suggesting a frail population. It is to be noted that though the associations of physical performance measures and musculoskeletal

were independent and significant, the effect size was small, however the sample had enough power to identify small effect size. In this analysis we performed multiple comparisons, but as we were describing the Indian population and consider this to be a hypothesis generating study, we did not adjust for multiple comparisons.

However, there are several strengths of this study, this is the first study to our knowledge to explore these associations among older Indian population. The participants were randomly selected in the study which increases the external validity of the findings. To our knowledge we describe for the first time associations of pQCT bone measures and muscle density with 400-meter walk test.

In conclusion, among south Indian rural older population, grip strength was independently associated with muscle density among men and trabecular vBMD, cortical thickness, SSIp and muscle density among women. SPPB was an independent predictor of trabecular vBMD, cortical vBMD, cortical thickness, endosteal circumference and muscle density among men and; cortical density, SSIp and muscle density among women. The 400 meter walk completion was independently associated with cortical thickness among men and trabecular vBMD among women. These findings suggest that there is an association between lower physical performance measures and lower musculoskeletal measures among older adults. In the light of India being an aging nation and having higher physical disability rates, further epidemiological studies are required to understand the bone-muscle and physical performance relationship for designing interventions to decrease disability.

9.6 Tables and Figures

Table 9.1 Participants characteristics

Characteristics	Men (N=245)	Women (N=254)	p value
Age (years)	68.2 ± 6.62	67.2 ± 6.21	0.0749
Height (cms)	160.62 ± 5.6	147.01 ± 5.95	<0.0001*
Weight (Kg)	55.87 ± 11.51	49.96 ± 12.21	<0.0001*
BMI (kg/m^2)	21.58 ± 3.92	22.98 ± 4.8	0.0045*
Grip strength (Kg)	20 ± 6.04	12.45 ± 4.74	<0.0001*
SPPB score	8.78 ± 2.75	7.22 ± 2.82	<0.0001*
Health status (Good)	113 (46.1)	105 (41.3)	0.2814
ADL disability (difficulty of at least one activity)	197 (80.4)	199 (78.4)	0.5694
400 meter walk completed	179 (73)	147 (57.8)	0.0004*
Current smokers	107 (43.7)	1 (0.4)	<0.0001*
Consumes alcohol	175 (71.4)	146 (57.5)	0.0011*
Hypertension	149 (60.8)	157 (61.8)	0.8196
Diabetes	41 (16.7)	57 (22.4)	0.1087
Stroke	17 (6.9)	9 (3.5)	0.0880
Physical activity (>30 mins continuous walk per week)	65 (34.3)	18 (13)	<0.0001*

*Variables are significantly different between men and women at p<0.05

Table 9.2 pQCT parameters distribution among MILES men and women

pQCT parameter	Men (N=245)	Women (N=254)	P value
Trabecular VBMD (Radius 4%)	145.62 ± 43.71	84.81 ± 32.39	<0.0001*
Cortical vBMD (Radius 33%)	1184.22 ± 43.65	1132.81 ± 41.02	<0.0001*
Cortical Thickness (Radius 33%)	2.7 ± 0.51	1.73 ± 0.38	<0.0001*
Endosteal circumference (Radius 33 %)	21.12 ± 4.22	21.47 ± 3.06	0.1878
SSI p (Radius 33%)	266.73 ± 49.45	147.97 ± 29.96	<0.0001*
Trabecular VBMD (Tibia 4%)	170.71 ± 39.1	133.8 ± 37.23	<0.0001*
Cortical vBMD (Tibia 33%)	1168.42 ± 38.95	1127.46 ± 41.53	<0.0001*
Cortical Thickness (Tibia 33%)	4.48 ± 0.66	3.07 ± 0.66	<0.0001*
Endosteal circumference (Tibia 33 %)	40.4 ± 5.6	40.53 ± 4.82	0.6064
SSI p (Tibia 33%)	1496.41 ± 252.04	892.21 ± 168.1	<0.0001*
Muscle Density	67.6 ± 4.5	67.22 ± 4.16	0.1818

*pQCT measures significantly (p<0.05) different among men and women

Table 9.3 Association of pQCT measures with physical performance measures among men

pQCT measures with grip strength											
	Grip strength - model 1			Grip strength - model 2			Grip strength - model 3			
	β	95% CI limits		P value	β	95% CI limits		P value	β	95% CI limits		P value
Trabecular VBMD (Radius 4%)	0.03	0.01	0.05	0.0026*	0.01	-0.01	0.03	0.3153	0.01	-0.02	0.03	0.6072
Cortical vBMD (Radius 33%)	0.04	0.02	0.06	0.0001*	0.04	0.02	0.06	0.0011*	0.02	-0.01	0.05	0.1212
Cortical Thickness (Radius 33%)	0.05	0.03	0.07	<.0001*	0.03	0.01	0.05	0.0088*	0.02	0.00	0.05	0.0816
Endosteal circumference (Radius 33 %)	-0.03	-0.05	-0.01	0.0106*	-0.03	-0.05	0.00	0.018*	-0.02	-0.05	0.01	0.1621
SSI p (Radius 33%)	0.04	0.02	0.06	0.0005*	0.01	-0.01	0.03	0.3044	0.01	-0.01	0.04	0.3543
Trabecular VBMD (Tibia 4%)	0.05	0.03	0.07	<.0001*	0.02	0.00	0.04	0.0286*	0.02	-0.01	0.04	0.1306
Cortical vBMD (Tibia 33%)	0.02	0.00	0.04	0.076	0.02	0.00	0.05	0.0476*	0.00	-0.02	0.03	0.8242
Cortical Thickness (Tibia 33%)	0.05	0.03	0.07	<.0001*	0.04	0.02	0.06	0.0008*	0.02	0.00	0.05	0.0934
Endosteal circumference (Tibia 33 %)	-0.01	-0.04	0.01	0.1876	-0.03	-0.05	-0.01	0.0119*	-0.02	-0.04	0.01	0.2369
SSI p (Tibia 33%)	0.04	0.02	0.06	<.0001*	0.02	0.00	0.03	0.1155	0.01	-0.01	0.03	0.4771
Muscle Density [a]	0.03	0.01	0.05	0.0136*	0.05	0.03	0.07	<.0001*	0.03	0.002	0.05	0.0349*
pQCT measures with SPPB score											
	SPPB Score - model 1			SPPB Score - model 2			SPPB Score - model 3			
	β	95% CI limits		P value	β	95% CI limits		P value	β	95% CI limits		P value
Trabecular VBMD (Radius 4%)	0.03	-0.02	0.08	0.2008	0.02	-0.02	0.07	0.3601	0.05	-0.01	0.12	0.1102
Cortical vBMD (Radius 33%)	0.09	0.04	0.13	0.0003*	0.08	0.03	0.13	0.0016*	0.10	0.03	0.17	0.0079*
Cortical Thickness (Radius 33%)	0.07	0.02	0.11	0.0052*	0.05	0.00	0.10	0.035*	0.06	-0.01	0.13	0.1193
Endosteal circumference (Radius 33 %)	-0.04	-0.09	0.01	0.1152	-0.03	-0.07	0.02	0.2722	-0.03	-0.11	0.05	0.4389
SSI p (Radius 33%)	0.03	-0.01	0.08	0.1662	0.03	-0.02	0.07	0.2378	0.03	-0.04	0.10	0.4232
Trabecular VBMD (Tibia 4%)	0.06	0.01	0.10	0.018*	0.04	0.00	0.08	0.0669	0.07	0.01	0.14	0.0257*
Cortical vBMD (Tibia 33%)	0.08	0.04	0.13	0.0004*	0.08	0.03	0.13	0.0008*	0.06	-0.01	0.13	0.1195
Cortical Thickness (Tibia 33%)	0.09	0.05	0.14	<.0001*	0.08	0.04	0.13	0.0003*	0.10	0.03	0.17	0.0051*
Endosteal circumference (Tibia 33 %)	-0.07	-0.11	-0.02	0.0036*	-0.07	-0.11	-0.02	0.003*	-0.08	-0.15	-0.01	0.0326*
SSI p (Tibia 33%)	0.04	-0.01	0.09	0.0929	0.03	-0.01	0.07	0.1147	0.02	-0.04	0.09	0.4248
Muscle Density [a]	0.14	0.10	0.18	<.0001*	0.14	0.10	0.18	<.0001*	0.13	0.07	0.20	<.0001*

Table 9.3 Continued

pQCT measures with 400 meter walk completion status											
	400 meter - model 1				**400 meter - model 2**				**400 meter - model 3**		
	β	95% CI limits		P value	β	95% CI limits		P value	β	95% CI limits		P value
Trabecular VBMD (Radius 4%)	0.30	0.01	0.59	0.0419*	0.27	0.00	0.54	0.0494*	0.27	-0.18	0.72	0.235
Cortical vBMD (Radius 33%)	0.14	-0.15	0.43	0.3494	0.15	-0.14	0.44	0.3239	0.26	-0.22	0.74	0.2908
Cortical Thickness (Radius 33%)	0.16	-0.13	0.45	0.2678	0.16	-0.12	0.43	0.2643	0.22	-0.25	0.70	0.3559
Endosteal circumference (Radius 33 %)	-0.11	-0.40	0.18	0.4527	-0.08	-0.37	0.20	0.5741	-0.12	-0.63	0.38	0.6344
SSI p (Radius 33%)	0.02	-0.28	0.31	0.9135	0.05	-0.21	0.31	0.7295	0.24	-0.23	0.71	0.3178
Trabecular VBMD (Tibia 4%)	0.39	0.11	0.67	0.0072*	0.33	0.08	0.59	0.0099*	0.34	-0.11	0.78	0.1374
Cortical vBMD (Tibia 33%)	0.21	-0.07	0.50	0.1405	0.20	-0.08	0.49	0.1605	0.11	-0.38	0.59	0.662
Cortical Thickness (Tibia 33%)	0.44	0.16	0.72	0.0023*	0.42	0.15	0.69	0.0025*	0.65	0.19	1.11	0.0063*
Endosteal circumference (Tibia 33 %)	-0.24	-0.53	0.04	0.0934	-0.22	-0.49	0.05	0.1065	-0.38	-0.86	0.09	0.1113
SSI p (Tibia 33%)	0.16	-0.12	0.45	0.2639	0.20	-0.03	0.44	0.0914	0.34	-0.06	0.75	0.0935
Muscle Density [a]	0.61	0.33	0.88	<.0001*	0.62	0.37	0.88	<.0001*	0.42	0.00	0.85	0.0527

Model 1 – Unadjusted; Model 2- model 1 + age, height and weight; Model 3 = model 2+ opinion on health status, current smoking, alcohol consumption, ADL disability, hypertension, diabetes, stroke, physically activity.

* statistically significant represented by p <0.05

a – muscle density in model 3 has been additionally adjusted for muscle cross sectional area.

Table 9.4 Association of pQCT measures with physical performance measures among women

pQCT measures with grip strength										
	Grip strength - model 1			**Grip strength - model 2**			**Grip strength - model 3**			
	β	95% CI limits		P value	β	95% CI limits		P value	β	95% CI limits		P value
Trabecular VBMD (Radius 4%)	0.06	0.03	0.08	0.0001*	0.04	0.01	0.07	0.0113*	0.05	0.01	0.09	0.0095*
Cortical vBMD (Radius 33%)	0.06	0.03	0.08	<.0001*	0.03	0.00	0.06	0.0437*	0.03	0.00	0.07	0.0645
Cortical Thickness (Radius 33%)	0.08	0.05	0.10	<.0001*	0.04	0.01	0.06	0.0021*	0.04	0.01	0.07	0.0129*
Endosteal circumference (Radius 33 %)	-0.03	-0.06	0.00	0.062	0.00	-0.03	0.03	0.8201	-0.01	-0.05	0.03	0.7114
SSI p (Radius 33%)	0.07	0.04	0.09	<.0001*	0.04	0.01	0.07	0.0036*	0.04	0.002	0.08	0.0404*
Trabecular VBMD (Tibia 4%)	0.07	0.04	0.09	<.0001*	0.04	0.01	0.07	0.0034*	0.04	0.002	0.08	0.0417*
Cortical vBMD (Tibia 33%)	0.04	0.01	0.07	0.0063*	0.02	-0.01	0.05	0.1604	0.01	-0.03	0.04	0.5865
Cortical Thickness (Tibia 33%)	0.07	0.04	0.09	<.0001*	0.03	0.00	0.05	0.0227*	0.03	0.001	0.07	0.0409*
Endosteal circumference (Tibia 33 %)	-0.03	-0.05	0.00	0.0608	-0.02	-0.05	0.01	0.2429	-0.03	-0.07	0.01	0.1759
SSI p (Tibia 33%)	0.08	0.05	0.10	<.0001*	0.03	0.00	0.05	0.0162*	0.02	-0.01	0.05	0.1533
Muscle Density [a]	0.03	0.00	0.06	0.042*	0.05	0.02	0.07	0.0017*	0.05	0.01	0.09	0.0198*
pQCT measures with SPPB score											
	SPPB Score - model 1			**SPPB Score - model 2**			**SPPB Score - model 3**			
	β	95% CI limits		P value	β	95% CI limits		P value	β	95% CI limits		P value
Trabecular VBMD (Radius 4%)	0.03	-0.02	0.07	0.1984	0.01	-0.03	0.06	0.5378	0.03	-0.05	0.12	0.4457
Cortical vBMD (Radius 33%)	0.09	0.05	0.14	<.0001*	0.07	0.03	0.12	0.0007*	0.05	-0.03	0.13	0.2499
Cortical Thickness (Radius 33%)	0.11	0.07	0.15	<.0001*	0.07	0.04	0.11	<.0001*	0.02	-0.05	0.09	0.5379
Endosteal circumference (Radius 33 %)	-0.07	-0.11	-0.02	0.0028*	-0.05	-0.09	0.00	0.0398*	0.04	-0.06	0.13	0.4423
SSI p (Radius 33%)	0.05	0.01	0.10	0.0154*	0.03	-0.01	0.07	0.151	0.06	-0.03	0.15	0.1767
Trabecular VBMD (Tibia 4%)	0.07	0.03	0.11	0.0018*	0.05	0.01	0.09	0.0193*	0.00	-0.09	0.09	0.9834
Cortical vBMD (Tibia 33%)	0.09	0.05	0.14	<.0001*	0.09	0.04	0.13	0.0002*	0.08	0.01	0.15	0.0295*
Cortical Thickness (Tibia 33%)	0.13	0.09	0.18	<.0001*	0.11	0.07	0.14	<.0001*	0.06	-0.01	0.13	0.1078
Endosteal circumference (Tibia 33 %)	-0.09	-0.13	-0.05	<.0001*	-0.08	-0.13	-0.04	0.0003*	0.01	-0.08	0.10	0.8507
SSI p (Tibia 33%)	0.10	0.06	0.15	<.0001*	0.07	0.04	0.10	<.0001*	0.10	0.04	0.16	0.0019*
Muscle Density [a]	0.09	0.05	0.14	<.0001*	0.10	0.06	0.15	<.0001*	0.09	0.01	0.17	0.0338*

Table 9.4 Continued

pQCT measures with 400 meter walk completion status											
	400 meter - model 1				**400 meter - model 2**				**400 meter - model 3**		
	β	95% CI limits		P value	β	95% CI limits		P value	β	95% CI limits		P value
Trabecular VBMD (Radius 4%)	0.10	-0.16	0.36	0.4462	0.11	-0.14	0.36	0.3719	0.37	-0.06	0.80	0.0912
Cortical vBMD (Radius 33%)	0.38	0.12	0.63	0.0036*	0.33	0.09	0.58	0.0067*	0.13	-0.27	0.53	0.5215
Cortical Thickness (Radius 33%)	0.25	-0.01	0.50	0.056	0.19	-0.02	0.40	0.0819	-0.02	-0.36	0.33	0.9281
Endosteal circumference (Radius 33 %)	-0.18	-0.44	0.07	0.1617	-0.15	-0.40	0.10	0.2397	0.08	-0.37	0.54	0.7194
SSI p (Radius 33%)	0.01	-0.25	0.27	0.9412	-0.07	-0.30	0.17	0.5646	-0.02	-0.47	0.43	0.9302
Trabecular VBMD (Tibia 4%)	0.39	0.14	0.64	0.0022*	0.41	0.17	0.64	0.0007*	0.50	0.07	0.93	0.0244*
Cortical vBMD (Tibia 33%)	0.31	0.06	0.56	0.017*	0.33	0.08	0.58	0.0104*	0.15	-0.23	0.52	0.4407
Cortical Thickness (Tibia 33%)	0.42	0.17	0.67	0.0009*	0.41	0.20	0.62	0.0002*	0.11	-0.25	0.47	0.5526
Endosteal circumference (Tibia 33 %)	-0.40	-0.65	-0.15	0.0019*	-0.43	-0.68	-0.18	0.0009*	-0.21	-0.65	0.23	0.3414
SSI p (Tibia 33%)	0.18	-0.08	0.43	0.1719	0.11	-0.07	0.30	0.2344	-0.10	-0.41	0.22	0.5532
Muscle Density [a]	0.47	0.22	0.71	0.0003*	0.40	0.16	0.64	0.0014*	0.21	-0.20	0.63	0.31

Model 1 – Unadjusted; Model 2- model 1 + age, height and weight; Model 3 = model 2+ opinion on health status, current smoking, alcohol consumption, ADL disability, hypertension, diabetes, stroke, physically activity.

* statistically significant represented by p <0.05

a – muscle density in model 3 has been additionally adjusted for muscle cross sectional area.

10. Dissertation Summary and Public Health Importance

Globally there is an increase in the population of older adults with a steep increase in absolute numbers of older adults in the developing world. This places the developing nations in a vulnerable situation to address the various health concerns among these older adults.

India is the second most populous country (as per 2011 census) and has the second largest population of older adults ($\geq$ 60 years) [2]. More than two-thirds of the older adults live in rural areas with having low access to healthcare and also lower socioeconomic status, increasing their vulnerability to their health. Little is known about the musculoskeletal health of the older population. The focus of Indian healthcare priorities has been largely towards infectious diseases. With the transition to an aging society, the burden of musculoskeletal diseases like osteoporosis and disability will increase. Recently, the national initiative to start a national program for prevention and control of diabetes, cardiovascular diseases, and stroke (NPDCS) and establishment of Non Communicable Diseases (NCD) clinics at the district level is noteworthy. However musculoskeletal diseases, especially osteoporosis and disability are not yet recognized as national health priorities.

The population based cohort, Mobility and Independent Living in Elders Study (MILES) was established in Medchal region of Telangana state in south India, to define prevalence, incidence and risk factors for disability and age-related diseases. MILES has collected standardised information longitudinally on various objective measures of physical performance, pQCT, anthropometry including blood pressure, self-reported history of various medical conditions, access to healthcare, physical activity, ophthalmic testing, audiometry and socio-demographic data in a random sample of 562 men and women age 60 years and more. This information provides an opportunity for exploring several epidemiological insights for future directions among this

population. This dissertation focuses on the prior literature gaps of pQCT derived musculoskeletal measures and its associations with mortality and physical performance.

The cross cohort comparison of pQCT bone data suggests that rural south India older men had lower vBMD, geometry and strength measures. This is consistent with the earlier observation that aBMD was lower in the Indian population compared to US Caucasians [74]. Our results extend these findings that Indian older population apart from having lower BMD also have reduced bone strength and poor geometric measures. These observations suggest that Indian older men are vulnerable to osteoporotic fractures and increased future fracture burden in India. Future analyses will explore whether these results are similar in women.

We further evaluated the relationship of pQCT bone and muscle density measures with all-cause mortality. We observed that poor bone phenotypes (lower vBMD, geometry and strength) and lower muscle density (higher fat infiltration in muscle) were associated with increased mortality among older men and women. These findings were novel for pQCT bone measures, but were similar to the other global studies on bone and muscle density and mortality. These observations suggest that the lower bone phenotypes and lower muscle density may reflect biological mechanisms such as inflammation which leads to poor musculoskeletal health and may contribute to mortality. Efforts to understand these mechanisms could enhance the understanding of premature mortality among the older Indian population. We further studied cross sectional associations of physical performance measures as predictors for pQCT derived bone and muscle measures. We observed that the physical performance levels among Indian older population were lower compared to US Caucasians, thus making them more vulnerable to disability. We also observed that the grip strength, SPPB score and 400-meter walk were associated with lower bone phenotypes and lower muscle density.

This dissertation fills the gap in the prior literature among older Indian population and provides novel evidence of a potentially higher risk of disability associated with poor pQCT derived musculoskeletal measures which in turn are linked with early mortality, thus forming a vicious cycle.

India also has a unique systemic context where the vulnerability of the older adults increases due to reduced access to healthcare information and healthcare, sparse policies and enforcement of the existing policies protecting disabled, low socio-economic status, discrimination faced by older adults at the family, society and healthcare levels, and overall lack of information to the policy makers on the above issues thus impacting decisions or actions to be taken against these contextual issues.

There is a need for more research among older populations in India to further estimate the musculoskeletal diseases burden. Further studies are required to establish the association of poor musculoskeletal measures with mortality and explore the biological mechanisms influencing this relationship The physical performance measures which had an association cross sectionally with poor musculoskeletal measures have to be further explored longitudinally to establish the predictive ability of physical performance measures to identify the risk of future musculoskeletal weakness. India is a large country with several population level differences warranting further epidemiological studies to establish these relationships. India lacks a registry for osteoporotic fractures especially hip fractures which is critical to estimate the incident burden of fractures and also help in designing public health programs to address this growing public health burden. Physical performance measures like grip strength, SPPB and 400-meter walk test are easy to perform, for early identification of vulnerable population and could be included in the national program for elderly.

This dissertation provides important musculoskeletal epidemiological data of older Indian populations and can influence the incorporation of musculoskeletal screening or care for the older populations in the current national health programs or priorities and encourage further research in this domain.

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