USING HIGH-RESOLUTION PERIPHERAL QUANTITATIVE COMPUTED TOMOGRAPHY TO STUDY THE LONG BONE STRUCTURAL CHANGES AFTER STROKE: A LONGITUDINAL STUDY

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Using High-Resolution Peripheral Quantitative Computed Tomography to Study the Long Bone Structural Changes after Stroke: A Longitudinal Study

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A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy

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CERTIFICATE OF ORIGINALITY

I hereby declare that this thesis is my own work and that, to the best of my knowledge and belief, it reproduces no material previously published or written, nor material that has been accepted for the award of any other degree or diploma, except where due acknowledgement has been made in the text.

_____ (Signed)

HUIXI OUYANG (Name of student)

Dedication

This thesis is dedicated to the study participants.

Abstract of thesis entitled “Using High-Resolution Peripheral Quantitative Computed Tomography to Study the Long Bone Structural Changes after Stroke: A Longitudinal Study” submitted by OUYANG Huixi for the degree of Doctor Philosophy at the Hong Kong Polytechnic University in September 2023.

ABSTRACT

Background (Chapter 1) and Study 1 (Chapter 2)*:*

Secondary stroke-induced osteoporosis is often overlooked. Study 1 (systematic review and meta-analysis) revealed moderate evidence supporting that exercise was beneficial for hip bone health in post-stroke individuals. However, individualized effective interventions to alleviate post-stroke bone loss have not been well established, especially for the radius and tibia. Therefore, knowing how the long bone health changes measured by the High Resolution – peripheral Quantitative Computed Tomography (HR- pQCT) and their associated factors during different stages of stroke recovery were crucial. Thus, the overall aims of this thesis project were (1) to study the changes in long bone properties during different stages of stroke recovery using HR-pQCT; (2) to identify the contributing factors to compromise bone strength during different stages of stroke recovery.

Study 2-3 (Chapter 3-4):

***Methods*:** Forty-six people with chronic stroke and 45 age and gender-matched healthy controls were recruited. Bone properties and failure load of the bilateral distal radius

and tibia were measured twice with a 24-month interval period using HR-pQCT.

***Results*:** On the paretic side, the decline in trabecular bone density (1.18%-2.29%), cortical vBMD (0.53-1.28%), cortical thickness (1.95%-3.61%) exceeded precision error in both bones after two years of follow up. The decrease in the latter two bone variables contributed most to the decrease in failure load of the corresponding bone. The blood flow volume significantly improved the regression model to predict the change in failure load in both paretic limbs. In addition, hand sensory ($\Delta F = 4.26$, $p = 0.046$) at baseline significantly improved the regression model to predict the change in failure load of the radius. As to lower limb, the change in walking speed (F change = 5.10, $p = 0.029$) and baseline factors (gait speed, physical activity level, and muscle strength) (F change = 6.47, $p = 0.015$) significantly improved the regression model.

***Conclusions**:* Changes in cortical bone properties accounted most for the deterioration of the failure load of both bones in people with chronic stroke. More compromised vascular health and hand sensation at baseline were associated with a greater decline in failure load of the hemi-paretic distal radius. Individuals with lower levels of mobility and less physical activity tended to show a greater decline in the estimated tibial bone strength on the paretic side.

Study 4 (Chapter 5):

Methods: Bone properties and failure load of the bilateral distal radius and tibia were measured twice: within 2 weeks post-stroke onset with 6-month follow-up using HR-pQCT. ***Results and conclusion***: Due to the Covid pandemic, this pilot study only included six individuals with acute stroke in analysis. In the paretic distal radii and tibiae, the reduction in the trabecular vBMD (1.18%-1.25%), cortical vBMD (0.4%-

0.73%) and estimated failure (2.18%-3.96%) load was similar to the decline reported in Chapter 3 and 4. However, an increase was observed in the trabecular thickness (0.80%) of the distal radius and in the trabecular number (1.53%) of the distal tibia on the paretic side. During the follow-up period, the reduction in the failure load was related to wrist range of motion and hand grip strength in the paretic upper limb, whereas it was related to vascular health at baseline in the paretic lower limb.

Conclusion*:* In summary, the evidence obtained from the studies performed in this thesis reveals the key aspects of bone changes at different sites during different stages of stroke recovery (i.e., acute to subacute, chronic stage). They also reveal the modifiable clinical factors that contribute to compromised bone strength at different stages of stroke. These findings will guide the design of treatment protocols for future intervention trials.

Publication List

1. **Publications arising from this thesis**

Journal Publications

1. **Ouyang HX,** Lee TC, Chan FYF, Li X, Lai KY, Lam WY, Yung TY, Pang MYC. Non-pharmacological and pharmacological Treatment on bone health post stroke: systematic review and meta-analysis. 2023, *Annals of Physical and Rehabilitation Medicine*. Under review.

2. **Ouyang HX,** Miller T, Qin L, Ying MTC, Hung VWY, Leung TWH, Pang MYC. Using high-resolution peripheral quantitative computed tomography to study tibial bone changes during chronic stage of stroke recovery: a 2-year prospective cohort study. 2023, *Brazilian Journal of Physical Therapy*, Under review.

3. **Ouyang HX,** Miller T, Qin L, Ying MTC, Hung VWY, Leung TWH, Pang MYC. Contributing factors to decreased failure load of radius in the hemi-paretic limb after chronic stroke: a prospective cohort study with two-year follow-up. 2023, *Annals of Physical and Rehabilitation Medicine*, Under review.

4. **Ouyang HX,** Qin L, Ying MTC, Hung VWY, Leung TWH, Pang MYC. Longitudinal changes in the bone properties of the distal radius and tibia within the first 6 months post-stroke: a pilot study. 2023, In preparation.

Conference Abstracts

1. **Ouyang HX,** Miller T, Pang MYC. Interventions for Bone Loss in Post-Stroke Individuals: A Systematic Review and Meta-Analysis. *The Hong Kong Physiotherapy Association Conference 2023*, June 23-24, Hong Kong (PPT Presentation)

2. **Ouyang HX,** Miller T, Pang MYC. Using high-resolution peripheral quantitative computed tomography to study tibial bone changes during

chronic stage of stroke recovery: a 2-year prospective cohort study. *World Physiotherapy Congress 2023*, June 2-4, Dubai (PPT Presentation)

3. **Ouyang HX,** Miller T, Pang MYC. Contributing factors to decreased failure load of radius in the hemi-paretic limb after chronic stroke: a prospective cohort study with two-year follow-up. *World Physiotherapy Congress 2023*, June 2-4, Dubai (Poster)

4. **Ouyang HX,** Miller T, Pang MYC. Using High-Resolution Peripheral Quantitative Computed Tomography to Study the Tibia Structural Changes in Acute and Chronic Stroke individuals: A Longitudinal Study. *World Physiotherapy Asia Western Pacific Reginal Congress with HKPA Conference 2022*. June18-19, HK (Virtual Platform Presentation)

5. **Ouyang HX,** Miller T, Pang MYC. Using High-Resolution Peripheral Quantitative Computed Tomography to Study the Long Bone Structural Changes in Acute and Chronic Stroke individuals: A Longitudinal Study. *The Hong Kong Physiotherapy Association Conference 2021*. December 12-13, Hong Kong (Poster)

2. Additional co-authored manuscripts and abstracts published while affiliated with Department of Rehabilitation Sciences of The Hong Kong Polytechnic University

Journal Publication

1. **Ouyang HX**, Tsang CSL, Li X, Pang MYC. Dual-task effect of stride length, gait variability and trunk stability can differentiate the dual task walking capability between individuals after stroke and healthy elderly. *Clinical Rehabilitation.* 2023; In preparation.

2. **Ouyang HX**, Tsang CSL, Zhuang JW, Lin S, Pang MYC. Test-Retest Reliability and Concurrent Validity of Dual Task Effect on Gait Variability and Trunk Stability with Four Cognitive Tasks in People after Chronic Stroke. *Gait and Posture.* 2023; In preparation.

3. Shi JH, Wang W, Wang LY, Bo XK, Chan L, **Ouyang HX**, Pang M, Li WJ, Daoud W. A hybrid sensor system for upper extremity stroke rehabilitation assessment. *Science Advances*.2023; under review.

4. Leung A, **Ouyang HX**, Pang MYC. Mechanical stimulation on mastectomy scars improves upper limb function and quality of life: monocentric single-blinded randomized controlled trial. *Annals of Physical and Rehabilitation Medicine.* 2023; 66(5):101724.

5. Luk KY, **Ouyang HX**, Pang MYC. Low-Frequency rTMS over Contralesional M1 Increases Ipsilesional Cortical Excitability and Motor Function with Decreased Interhemispheric Asymmetry in Subacute Stroke: A Randomized Controlled Study. *Neural Plasticity*. 2022:3815357.

6. Miller T, Qin L, Hung VWY, Ying MTC, Tsang CSL, **Ouyang HX**, Chung RCK, & Pang MYC. Gait speed and spasticity are independently associated with estimated failure load in the distal tibia after stroke: an HR-pQCT study. *Osteoporosis International*. 2022 ;33(3):713-724.

7. Miller T, Ying MTC, Hung VWY, Tsang CSL, **Ouyang HX**, Chung RCK, Qin L, & Pang MYC. (2021) Determinants of estimated failure load in the distal radius after stroke: an HR- pQCT study. *Bone*. 2021; 144:115831.

8. Chiu CY, Ng MY, Lam SC, Hui KY, Keung CH, **Ouyang HX**, Li X, Pang MY. Effect of physical exercise on fear of falling in patients with stroke: A systematic review and meta-analysis. *Clinical rehabilitation*. 2023; 37(3):294-311.

9. Yuen M, **Ouyang HX**, Miller T, Pang MYC. Baduanjin Qigong Improves Balance, Leg Strength, and Mobility in Individuals with Chronic Stroke: A Randomized Controlled Study. *Neurorehabilitation and Neural Repair*. 2021; 35(5):444-456.

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9. List of abbreviation

aBMD	areal BMD
ADL	activities of daily living
ALP	alkaline phosphatase
ANOVA	analysis of variance
AOU	amount of use
BAP	bone alkaline phosphatase
BESTest	Balance Evaluation Systems Test
BMD	bone mineral density
CBSI	compressive bone strength index
CI 95%	95% Confidence Interval
CSS	Composite Spasticity Scale
CTx	crosslinked C-telopeptide of type I collagen
CV	coefficient of variation
CV%RMS	root-mean-square of the coefficient of variance
DALYs	disability-adjusted life years
DXA	dual-energy X-ray absorptiometry

FAC	Functional Ambulation Categories
FAME	fitness and mobility exercise
FIM	Functional Independence Measure
FMA	Fugl–Meyer Motor Assessment
FRAX	fracture risk assessment tool
GRADE	Grading of Recommendation, Assessment, Development, and Evaluation
GRF	ground reaction force
HR	hazard ratio
HR-pQCT	High-resolution peripheral quantitative computed tomography
ICC	intraclass correlation coefficient
IL	interleukin
LSC	least significant change
MAL	Motor Activity Log
MG	medial gastrocnemius
μFE	micro finite element
MRI	magnetic resonance imaging
MRS	Modified Rankin Scale
NFT	neuromuscular facilitation training
NTx	crosslinked N-telopeptide of type I collagen
OPG	osteoprotegerin
PASE	Physical Activity Scale for the Elderly
P1NP	procollagen type 1 C-terminal propeptide
pQCT	Peripheral quantitative computed tomography
p-SSI	polar stress–strain index
PTH	parathyroid hormone
PWV	pulse wave velocity
QOM	quality of movement
RANKL	receptor activator of nuclear factor κ-B ligand
RCTs	randomized controlled trials
ROM	range of motion
RST	medial reticulospinal tract
SMD	standardized mean difference
%SSD	side-to-side difference
SSRIs	selective serotonin reuptake inhibitors

SWMT	Semmes–Weinstein monofilament test
TNF	tumor necrosis factor
vBMD	volumetric BMD
VIF	variance inflation factor
VOI	volume of interest
WHO	World Health Organization
WBV	whole-body vibration

1. Chapter 1

General introduction

Ouyang Huixi

1.1 Bone health in aging population

The global population is aging. According to the World Health Organization (WHO), globally, the percentage of people aged 60 years or above will increase from 12% in 2015 to 22% in 2050, corresponding to a total size of 2.1 billion. Over the past two decades, due to the overall advancement of healthcare coverage, healthy life expectancy (average number of years that a person can expect to live in "full health") has increased from 58.3 to 63.7 years globally. Meanwhile, the global life expectancy has experienced a more rapid increase (from 66.8 to 73.3 years between 2000 and 2019), resulting in a larger number of years lived with disability [1].

1.1.1 Epidemiology of fracture in the aging population

There is a progressive increase in fracture risk with increasing age, particularly for those aged 65 years or above [2]. According to a study of 550,000 participants (27,169 fractures, age range 16–105 years), 44% of distal radius fractures (n = 4,445) and 92.3% of proximal femur fractures (n = 3,993) occur in people older than 65 years [3]. It has been predicted that the global incidence of hip fracture will increase by 275% from 1990 to 2050 [4].

In Europe, fragility fracture is the fourth leading cause of chronic disease morbidity, after ischemic heart disease, dementia, and lung cancer [5]. Osteoporosis is one of the major factors contributing to fragility factures in older adults. Even modest bone loss is associated with a pronounced increase in fracture risk. For example, a 10% loss in bone mass was found to be associated with a 2.5

times greater hip fracture risk [6]. Annually, over 1.5 million fractures are caused by osteoporosis in the US. Approximately 40 million American adults older than 50 are at risk of osteoporotic fracture in 2019, according to the US National Osteoporosis Foundation.[7] And by 2030, this number will reach 61 million. Moreover, it has been estimated that one third of women and one fifth of men aged 50 years or above will experience osteoporotic fractures in their lifetime [8, 9]. The incidence rate of distal radius fracture in males and females in Europe is 1.7 and 7.3 per 1,000 person-years, respectively [10].

1.1.2 Epidemiology of osteoporosis in elderly individuals

Osteoporosis, one of the most common type of metabolic bone disease, is a disorder characterized by low bone mass (≤2.5 standard deviations below the peak bone mass). In the adult skeleton, bone remodeling is the core process to maintain bone strength. Bone remodeling involves mineralized bone resorption by osteoclasts followed by bone matrix formation by osteoblasts and mineralization of the bone matrix. In elderly individuals, estrogen deficiency or other metabolic disorders can lead to more bone resorption than bone formation. Because of an increase in osteoclast number and/or activity and a decrease in osteoblast number and/or activity, the bone resorption rate exceeds the bone formation rate [11], resulting in a decrease in bone mass, compromised bone microarchitecture, and ultimately, osteoporosis [12].

The prevalence of osteoporosis is increasing, and this is mainly attributed to the aging population and changing lifestyles. Globally, one in three women and

one in eight men will have osteoporosis, making the total number of people with osteoporosis at 200 million in 2019.[13] Furthermore, osteoporosis affected 32 million (5.6%) individuals over the age of 50 years in Europe in 2019 [14]. In the US, more than 50% of older people had a high risk of osteoporosis in 2020 [15]. Meanwhile, in China, it was estimated that more than 60.2 million people had osteoporosis in 2018 [16].

The high incidence of osteoporosis and related fractures have imposed a great burden on the healthcare system. By 2050, about $130 billion will be spent on hip fractures each year globally because of bone loss [17]. In the US, the accumulated amount spend on osteoporotic fractures will reach $228 billion from 2016 to 2025 [18].

1.1.3 The physiological foundation of bone health

Seventy percent of bone consists of bound minerals, while the rest is a flexible matrix, of which 90%–95% consists of elastic collagen fibers [19]. While the elasticity of collagen makes it resistant to fractures, mineralization gives it rigidity [20]. Binding with calcium phosphate, an inorganic mineral salt, hardens the matrix. Ongoing bone remodeling, also known as bone turnover, is a process of resorption followed by the formation of new bone. Annually, approximately 10% of the skeletal mass of adults is remodeled [21]. Two types of bone cells, osteoblasts and osteoclasts, mediate bone reconstruction and remodeling throughout life.

To begin bone formation, osteoblasts secrete both collagen and ground substance. The collagen fibers are further polymerized to collagen strands.

Mineralization then progresses with the precipitation of calcium and phosphate around the collagen strands within days to weeks [19]. Concurrently, osteoblasts produce alkaline phosphatase, which facilitates calcium and phosphate deposition by cleaving phosphate groups [22]. During the process of bone absorption, hydrogen protons and chloride ions are generated by carbonic anhydrase in osteoclasts to acidify the resorption lacuna of bone. Osteoclasts further secrete enzymes to degrade the demineralized bone matrix [23].

This bone turnover process helps regulate calcium homeostasis and repair the microdamage of bones from daily stress [24]. The activities of osteoblasts and osteoclasts are modulated by systemic regulation and growth factors that either promote or inhibit their activity, leading to increased, maintained, or decreased bone mass [25].

Osteoid formation is stimulated by a growth hormone, namely thyroid hormone, and two sex hormones, namely estrogens and androgens, from the circulatory system [25, 26]. Osteoblasts also secrete osteoprotegerin (OPG), which is also stimulated by the abovementioned hormones, to inhibit osteoclast activity by binding receptor activator of nuclear factor κ-B ligand (RANKL) [25, 26]. Conversely, by secreting numerous cytokines (e.g., RANKL and interleukin [IL]-6) that stimulate osteoclast activity and differentiation, osteoblasts may also be induced to promote bone reabsorption. Such cytokine secretion is induced by vitamin D, parathyroid hormone, and stimulation by osteocytes. The absorption rate of osteoclasts is also inhibited by calcitonin (produced by the thyroid gland)

through binding to receptors on osteoclasts [26].

1.1.4 The biomechanical foundation of bone strength

When an external force is added to a solid object, micro-deformation (also known as “strain”) occurs. Meanwhile, internal forces (also known as “stress”) will occur within the object [27]. A stress–strain curve describes the change in strain with increasing stress, as shown in Figure 1.1. When bone is compressed over its elastic range and exceeds the yield point, plastic (permanent) deformation occurs. If the stress continues to increase beyond the ultimate strength, a fracture will occur [28]. The level of stress required to cause a fracture is much lower in osteoporotic bone than healthy bone [29].

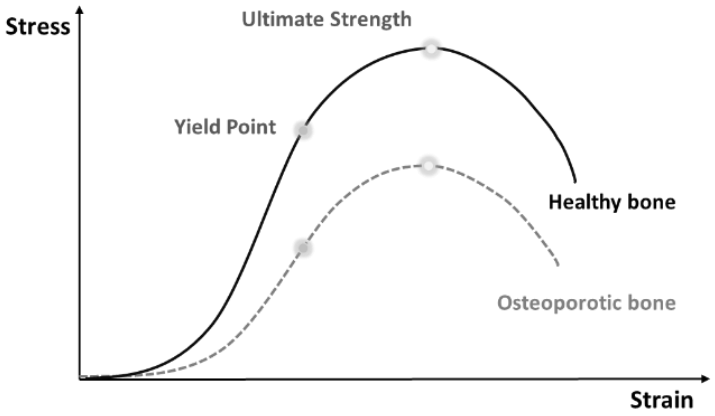


Figure 1.1: Stress–strain curve (adapted from Boughton et al., 2018 [29])

The factors that influence the material strength of bone tissue include the distribution of the cross-sectional area, the type of loading, the loading direction, and bone mineral density (BMD). Given the same bone cross-sectional area, a hollow cylinder with a larger diameter has greater bending strength than a solid

cylinder. This is because a hollow cylinder has a greater moment of inertia, as the distance between the bone materials and the center is greater for a hollow cylinder than a solid cylinder [27]. Therefore, the polar stress–strain index (p-SSI, mm^3) is estimated as an index of bone strength against torsional forces, and is calculated using the following formula [30, 31]:

$$\frac{\sum[(A_z \times d_z^2)(\text{cortical bone mineral density/ND})]}{d_{\max}},$$

where A is the area of the voxel, d is the distance between the voxel and the corresponding torsion axis, ND is the normal physiological bone density (1200 mg/cm^3), and $d_{\max}$ is the maximum distance to the center of gravity. This formula considers both the densitometric and geometric properties of bone. It has been validated by mechanical testing in a cadaver study, and shown to account for 89% of actual fracture load in response to bending forces [32].

The stress–strain curve is different for trabecular and cortical bone [27], as shown in Figure 1.2. Trabecular bone fractures occur when yielding occurs at the same time as fracture. A long plateau after the yielding point is seen with progressing fracture in the trabeculae. In addition to BMD and cross-sectional area, the orientation, spacing, thickness, and number of trabeculae affect the elastic modulus and failure stress of cancellous bone [33]. For example, a 20% decrease in bone mass by adding resorption cavities (increased trabecular separation) through bone resorption may lead to a 50% decrease in bone strength [34]. Moreover, a 10% reduction in BMD through a decrease in trabecular thickness or trabecular number results in a 20% or 65% reduction in bone strength, respectively

[35]. In a previous study, the compressive bone strength index (g^2/cm^4) was used as an indicator of bone strength against compressive force using the formula *total area* × *(total volumetric BMD)* [36]. This indicator explained 85% of the variance in the failure load in a human cadaver study [37].

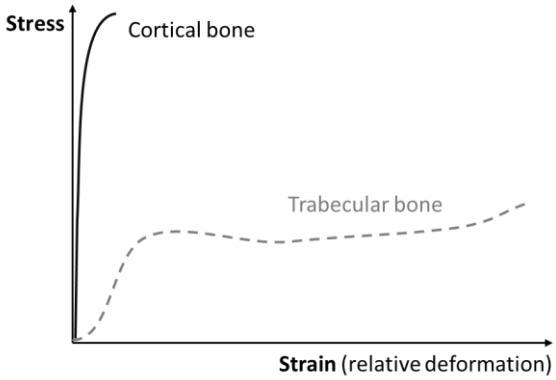


Figure 1.2: Stress–strain curves for cortical and trabecular bone (adapted from Hayes et al., 1985 [27])

With aging, there is endosteal resorption with decreased BMD and cortical thickness, and increased trabecular separation [38]. And these changes contribute to decreased bone strength. On the other hand, subperiosteal apposition also occurs, with an increase in the cortical perimeter and total cross-sectional area [38], which essentially increases the moment of inertia, and thus, bone strength. Therefore, such bone remodeling is considered to be a compensatory mechanism to partially counteract the deleterious effect of bone loss during the aging process. Overall, changes in bone remodeling in elderly individuals lead to crucial changes in both the elastic modulus and geometric features of bone tissue, which affect the structural strength and rigidity of the whole bone [39].

1.1.5 Physiological mechanisms whereby exercise affects bone remodeling

According to Wolff's law, devised in 1892, external stresses imposed on bone can influence both its internal architecture and external shape [40]. Trabecular alignment first involves adaptive changes along the direction of force, after which secondary changes occur to the shape of the cortical bone. Eventually, the bone structure becomes thicker and denser to resist the deformation caused by these forces. It has been shown that weight-bearing exercise causes repeated stress, resulting in bone thickening. During physical exercise, ground reaction forces (GRFs) from impact activities and strain forces induced by muscle contractions (e.g., interval [41], treadmill [42], and resistance training [43]) are also sources of mechanical stress to the bone tissue.

The beneficial effect of exercise on bone health arises from two aspects: an increase in bone formation and a decrease in bone resorption. These effects may be achieved by (1) facilitating osteoblast formation, (2) inhibiting osteoclast differentiation and activity, and (3) activating and protecting osteocytes. The mechanisms involved are (1) direct mechanical stress to the bone, (2) increased systemic hormone levels, and (3) altered local inflammatory cytokine levels.

First, mechanical stress applied to the bone is an important factor in promoting bone health. Animal studies have shown that bone marrow mesenchymal stem cells are induced to differentiate into osteoblasts, instead of adipocytes, by endurance exercise in rats [44]. Moderate exercise (e.g., treadmill and vibration stimulation training) also inhibits osteoclast differentiation and activity by increasing the

production of OPG and inhibiting the expression of RANKL in osteoblasts in rats [45, 46]. Mechanical loading down-regulates the expression of osteoclast-specific genes (tartrate-resistant acid phosphatase, matrix metalloproteinase-9, cathepsin-K, and calcitonin receptor), thereby decreasing the production of osteoclasts in mice [47]. Such benefits have been verified in human studies. Upregulated serum osteogenic marker expression and decreased bone resorption biomarker levels have been found after 2 months of physical training (resistance or combined aerobic and resistance exercise) in women [48] and after a jumping intervention in men [49].

Mechanical stress from physical exercise promotes mesenchymal stem cells to differentiate into osteoblasts and chondrocytes [50-53]. For example, low-magnitude, high-frequency vibrations can improve osseointegration by promoting osteoblast differentiation, matrix synthesis, and mineralization [54]. Mechanical stress can also improve bone strength by altering collagen alignment [55]. In addition, mechanical stimulation from exercise can activate osteocytes and protect them from apoptosis, partly because osteocytes can accommodate mechanical stimuli by reorganizing their cytoskeletons [56].

Second, increased levels of certain hormones (parathyroid hormone [PTH], estrogen, and prostaglandin E2) also contribute to osteogenesis. There is evidence that exercise can influence bone formation and resorption rates by modulating hormone levels [50]. Animal studies have shown that enhanced osteoblast differentiation and inhibited adipocyte differentiation of bone marrow mesenchymal stem cells are related to increased expression levels of PTH type-1

receptor after climbing exercise. Moreover, the inhibition of PTH signaling during exercise has been reported to reduce the changes in the geometrical and mechanical properties of bone [57].

Finally, exercise may also regulate bone metabolism by altering cytokine levels. It has been suggested that pro-inflammatory cytokines, such as IL-1, IL-6, IL-7, and tumor necrosis factor (TNF)-α, may promote osteoclast proliferation and bone resorption activity. In contrast, anti-inflammatory cytokines (e.g., IL-2, IL-4, IL-10, IL-12, and interferon-γ) promote bone formation [50, 58, 59]. It has been shown that long-term (6 weeks to 6 months) moderate exercise may modulate bone resorption by increasing the levels of anti-inflammatory cytokines (IL-2, IL-10) and decreasing the levels of pro-inflammatory cytokines (IL-6, TNF-α) [60, 61]. Furthermore, a study involving a single session of exercise found that increased anti-inflammatory cytokine (IL-10) levels were positively correlated with increased levels of bone alkaline phosphatase (BAP; bone formation turnover marker) at 5 minutes post-exercise. The immediate increase in pro-inflammatory cytokine (IL-1α) levels after exercise was negatively correlated with the increase in BAP levels 24 h after exercise [62].

1.1.6 Treatment for osteoporosis in the aging population

The use of drugs to treat osteoporosis can be traced back to the 1940s, when estrogen was used to reverse the negative calcium balance for post-menopausal women. Since then, driven by discoveries in animal studies, advances in the fundamental knowledge of bone biology, clinical observations, clues from rare

bone diseases, and the opportunistic repurposing of existing compounds, many osteoporosis drugs have been discovered. These drugs can basically be classified into three types: 1) anti-resorptive (i.e., romosozumab, selective estrogen receptor modulators, calcitonin, bisphosphonates, and anti-RANKL antibodies); 2) anabolic (PTH or PTH-related peptide analogs); and 3) mixed (anti-sclerostin antibody) drugs [63]. Antiresorptive medications inhibit osteoclast function to prevent bone resorption, whereas anabolic medications facilitate bone formation. For example, taking abaloparatide, a PTH-related peptide analog, at 80 μg per day for 24 weeks has been found to increase BMD by 6.7% in the lumbar spine and by 3.1% in the femoral neck [64]. Furthermore, in a phase 3 trial, vertebral and non-vertebral fractures were reduced by 86% and 43%, respectively, in postmenopausal women taking 80 μg of abaloparatide per day for 18 months [65].

Despite advances in the development of effective drugs to treat osteoporosis, many people who may benefit from pharmacological treatment are either not provided these drugs or are not compliant with their prescriptions [66]. These phenomena occur in those with frailty fracture, for whom the benefits of treatment far outweigh the risks [67, 68]. There are two major factors contributing to these low levels of prescription and compliance. The first factor is concern about the potential side-effects of anti-resorptive drugs, especially bisphosphonates (i.e., atypical femur fractures and jaw osteonecrosis). The second factor is a lack of clear evidence supporting the long-term efficacy of these drugs [63]. Therefore, it is important to explore other adjunct intervention methods, such as exercise training.

A recent systematic review showed the beneficial effect of resistance exercise, aerobic exercise, and mind–body exercise on lumbar and hip BMD in older adults with osteoporosis [69]. Moreover, positive evidence from 20 interventional studies showed that long-term exercise (≥3 months) reduces the incidence rate of major osteoporotic fractures (of the hips, spines, forearms, and humerus) in middle-aged and older adults [70]. In addition, fall prevention and risk factor modification (reducing smoking and excessive alcohol intake), together with regular weight-bearing exercise, have been shown to be crucial to mitigate bone loss [11].

1.2 Stroke: a high-risk disease in the aging population

1.2.1 Epidemiology of stroke

According to the World Stroke Organization Global Stroke Fact Sheet (2022), stroke remains the third-leading cause of death and disability globally [71]. During the past three decades, the absolute number of stroke cases increased by 70%, resulting in 101 million prevalent cases and 142 million DALYs lost in 2019. Each year, 12.2 million new strokes occur, which translates to one new stroke diagnosed every 3 seconds. Furthermore, from 1990 to 2019, although the overall age-standardized rates of stroke incidence and prevalence have decreased by 17% and 6%, respectively, the corresponding prevalence and incidence rates have increased by 22% and 15%, respectively, in people younger than 70 years [72]. This means that more younger people are suffering from this disease. Specifically, in 2019, 37% of strokes occurred in people younger than 70 [71]. Meanwhile, in the US, 38% of

stroke survivors were younger than 65 in 2014 [73]. The lifetime risk of stroke also increased by 50% from 1990 to 2016. It has been estimated that one in four people will have a stroke in their lifetime [74]. In China, it was estimated that there were 17.8 million stroke cases in 2020, among which 3.4 million was first-ever stroke.[75] In Hong Kong, stroke prevalence rates remain at 4.9% and 30.9% in community-dwelling and institutionalized older adults, respectively. [76] Of note, stroke-induced disability results in approximately 106,000 lost DALYs in the elderly every year. Annually, the direct medical cost from stroke is also notable, at greater than HK$1,300 million.[76]

People with hemiplegia usually suffer from voluntary movement dysfunction, mobility deficits, and sensory disturbances. Stroke has been shown to impair mobility in more than 50% of survivors aged 65 and above [77].

1.2.2 Epidemiology of post-stroke fracture

Stroke is accompanied by an increased fracture risk. The relative fracture risk for stroke survivors after they are discharged from hospital is more than seven times the rate in healthy control individuals [78]. Within the first year after a stroke, the fracture risk is approximately 3%–10%, with hip fracture being the most common type of fracture. Thus, the risk of hip fracture is approximately 1.4–4 times higher for stroke survivors than for healthy control individuals [79-83]. Moreover, a large cohort study of 23,751 stroke cases found that the 2-year rate of fracture was 5.7% in stroke survivors, compared with 4.1% in age- and sex-matched control subjects (adjusted hazard ratio for those with stroke vs. control subjects, 1.47; 95%

confidence interval, 1.35–1.60) [84]. Based on a study involving a retrospective cohort of 40,000 participants, approximately 1 in 12 people experience a hip fracture in the 5 years following an acute stroke [85].

Post-stroke fractures most commonly occur in the wrist and hip [86]. They may lead to a poorer prognosis, and require longer rehabilitation times compared with fractures in individuals without stroke [87]. Fractures can also hinder recovery after a stroke, as they pose further restrictions on performing activities of daily living [80]. After a hip fracture, 69% of healthy control subjects, but only 38% of stroke survivors, regain independent mobility [87]. The mortality rate 3 months after fracture surgery is twice as high in the stroke population as in healthy older adults [88]. Thus, preventing fractures in individuals with stroke is an important public health concern.

Identifying the factors contributing to post-stroke fractures deserves attention. A recent cohort study found that the increased fracture risk after a stroke is mainly attributable to moderate stroke severity, a fall history, and osteoporosis, after controlling for age, sex, and hyperparathyroidism [84]. In fact, these demographic characteristics (age, sex, and whether taking glucocorticoid) have been included in the commonly used fracture risk prediction tool, FRAX [89, 90]. It is interesting that those with a moderate stroke, rather than those with a severe stroke, have the highest fracture risk. While individuals with a moderate stroke should have less bone loss and better balance function than those with a severe stroke, they may have more falls and subsequent fractures due to more frequent mobility activities

and transfer practices in their daily living [84].

1.2.3 Epidemiology and characteristics of post-stroke falls

It is widely known that falling is one of the most common issues after a stroke. The fall incidence is approximately 30% within the first 6 months post-stroke, especially during the setting transition stage immediately after discharge from the hospital to the community [91-93]. Moreover, the fall incidence increases to 40%–50% from the sixth to the twelfth month [94, 95]. One year after a stroke, the reported fall incidence continues to increase to 50%–73% [96, 97]. A recent systematic review found that the fall prevalence was seven times higher in individuals with stroke than in healthy control subjects [98].

Post-stroke falls usually occur at times of transfer or during mobilization in ambulatory individuals with stroke [99]. These falls tend to occur in the paretic side [100, 101], where bone strength is compromised in both the upper and lower limbs, resulting in fractures or other injuries [102]. In fact, a large cohort study found that more than one quarter of falls resulted in fractures during the 2 years after a stroke [84].

Post-stroke falls have multiple risk factors. They may occur as a result of an interaction between physical impairments (e.g., muscle weakness, sensorimotor deficits, attention decline, and balance impairment), cognitive dysfunction (e.g., misjudgment, reaction delay, and reduced attention), and participation level (e.g., dependence level) [103] after a stroke [104]. In addition, compromised cognitive–motor dual-task ability has been found to be associated with post-stroke falls [105].

1.2.4 Mobility and stability deficits as fall risk factors in individuals after a stroke

The adaptation of and alterations in the central nervous system, together with secondary peripheral skeletal muscle changes, contribute to mobility and stability deficits after a stroke [106]. Stroke may cause damage to various brain regions (e.g., the motor cortex, internal capsular-basal ganglia, frontal and parietal cortex, and descending motor pathways), resulting in a variety of motor deficits, including muscle weakness, muscle spasticity [107, 108], balance deficits [98], and improper GRF during walking. All of these impairments have been associated with an increased fall risk and they alter the mechanical loading on the bone tissue [104].

1.2.4.1 Muscle weakness and spasticity

Muscle weakness is the foundation of most motor deficits after a stroke (e.g., impaired muscle control, muscle coordination, and balance and walking deficits). It results from an interruption of the corticospinal tract and damage to the motor cortex, where sensorimotor information from the peripheral neuromuscular system is transported and analyzed in the brain after a stroke [109]. Post-stroke muscle weakness can also be attributed to decreased muscle mass, fiber cross-sectional area, fiber length, pennation angle, and a more elongated tendon. Alterations in these four aspects change the length–tension curve, torque–angle curve, and force–velocity curve, a combination of which eventually contributes to weaker forces generated by muscles [110]. Other causes of post-stroke muscle loss include immobilization and dysfunctional atrophy, inflammation, sympathetic overactivation, and denervation [111]. Impaired torque-generation capacity of

voluntary muscles may also arise from the reduced number of motor units, a lower firing rate, and prolonged electromechanical delay [106]. Previous studies have shown that a loss of muscle strength [112] and motor control deficits [113] are predictive of falls in community-dwelling, ambulatory people with stroke.

Spasticity (increased resistance to joint movement) is estimated to exist in approximately 20%–40% of individuals with stroke [114]. Within the first month after a stroke, spasticity occurs in approximately 4%–27% of individuals, whereas the incidence rate of spasticity increases to 17%–43% 3 months after a stroke [115]. When the brainstem descending pathways and the intraspinal motor network, which are responsible for automatic motor processes, are disinhibited by a loss of supraspinal inhibition and become hyperexcitable, muscle spasticity and diffuse spastic synergistic activation occur [116]. The hyperexcitability of medial reticulospinal tract (RST) in the brainstem has been suggested to be the most likely contributor to post-stroke spasticity [117]. An adaptive exaggerated stretch reflex, velocity-dependent resistance to stretch, muscle overactivity, or the spontaneous firing of motor units are caused by elevated excitability of spinal motor neurons resulting from unopposed excitatory descending inputs from a hyperexcited RST [116]. The increased excitability of the alpha motor neurons results from an imbalance between the excitatory and inhibitory effects from the vestibulospinal and reticulospinal projections [118]. The muscle groups most commonly affected by spasticity are elbow flexors, wrist flexors, and ankle plantar flexors.

The influence of post-stroke spasticity can have two different aspects [118].

First, it may bring potential benefits to them. For example, spasticity of the extensor muscles can help with body weight support during walking by keeping the lower limb rigid, despite severe leg muscle weakness. Moreover, spasticity has been reported to be beneficial, to some extent, as it maintains muscle bulk and bone strength and may decrease the risk of deep-vein thrombosis in the lower leg. However, spasticity may contribute to muscle tissue shortening, further deteriorating post-stroke muscle weakness. Moreover, spasticity in the lower limb impairs the flexibility to modify the location of the center of pressure beneath the paretic leg in a time-sensitive manner to maintain whole-body stability [119]. This may partly explain why more severe spasticity in the lower limb [113, 120], in addition to gait asymmetry [120] and gait instability [113], has been found to be predictive of an increased number of falls in ambulatory people with stroke.

1.2.4.2 Inter-limb asymmetry and balance control

Temporal and spatial asymmetry between the two lower limbs during gait is common in stroke survivors, with a prevalence ranging from 44% to 82% [121-124]. The imbalance in mechanical power in the skeletal muscle between limbs may contribute to such asymmetry. This leads to a more deviated center of mass during walking. A larger displacement of the center of mass brings challenges in maintaining balance. A previous study investigating the difference in the kinetic modulation asymmetry index (center of pressure velocity from both legs) found that the paretic leg accounted for only one third of the total kinetic modulation activity [125]. Likewise, the paretic leg was found to help with 11%–45% of

balance control after analyzing the ankle joint torque of both sides [126].

Inter-leg asymmetry can contribute to the balance deficits seen after hemiplegic stroke. A significant association has been found between gait asymmetry and upright stability after a stroke [121, 127, 128]. Specifically, there is a negative association between the Berg Balance Score and step length and swing duration asymmetry in people with stroke [121].

Balance impairment is also common in individuals with stroke [129-132]. Balance function has six domains: (1) biomechanical constraints, (2) stability limits, (3) reactive postural adjustment, (4) anticipatory postural corrections, (5) sensory orientation, and (6) gait stability [133]. There is evidence that all six domains are impaired after a stroke. First, in terms of biomechanical constraints, previous studies have found that the distance of the excursion of the center of pressure is 1.5 to 5 times greater in people with stroke than in healthy control subjects, particularly in the frontal plane [125, 134-139]. This suggests that people with stroke require a larger base of support to maintain stability. Second, deficits in stability limits have also been observed in individuals with stroke, who demonstrate decreased excursion of lateral weight transfer (stroke: 65%–85%; control: 95%) [129, 131] and prolonged lateral weight transfer time (stroke: 3.5–4.3 s; control: 2.6 s). Third, impairment in reactive postural adjustment manifests as delayed muscle onset latency in reaction to external perturbations [140-145]. Fourth, anticipatory postural corrections are also impaired, with abnormal muscle co-activation, when attempting to maintain equilibrium while performing self-initiated limb

movements [121-124]. Fifth, post-stroke balance impairment may also arise from compromised sensory orientation (sensory information integration, perceptual deficits, and compromised spatial cognition) [146]. Finally, impaired gait stability is also common after a stroke. According to a recent systematic review of fall risk factors in people with stroke, gait stability (e.g., as determined by the Timed Up and Go Test) was one of the common risk factors in addition to other factors (e.g., age, gender, motor control, and cognitive impairment) [98].

1.2.4.3 Ground reaction force

Mechanical loading to the lower limb can also be indicated by the GRF during walking, which can be measured using a force platform. Three main directions are included in the GRF: upward, forward, and backward. After a stroke, the upward GRF, particularly to the paretic limb, is significantly decreased. The forward and backward GRF generate anterior–posterior propulsion for walking [147-149].

The compromised propulsive impulse during walking mainly arises from the altered activity of three muscles, the gastrocnemius, tibial anterior, and rectus femoris [150]. An impaired and disorganized muscle propulsion system contributes to decreased push-off during the pre-swing stage and increased braking during the loading response phase of the paretic leg during a gait cycle [151]. Furthermore, the foot contact pattern (forefoot-, flat-foot-, and heel-initial contact) also influences the GRF pattern [152]. Approximately 83% of individuals with stroke have either a flat foot or forefoot contact during gait, resulting in a reduction in the vertical potential energy and GRF [152-155].

Finally, the relative locations of the foot and the center of mass (also the angle between the foot and the ground when contacting it) can affect acceleration and deceleration [156-159]. After a stroke, the declined posterior position of the affected foot in the pre-swing phase and a prolonged loading response phase duration lead to reduced propulsion and increased braking power, respectively [147, 157, 160]. Taken together, these data indicate that muscle weakness and spasticity in the propulsion system decrease both the gait velocity and the interaction angle between the foot and the floor after a stroke, resulting in a weakened GRF in the three directions. As a result, an apparatus enabling perturbations (eight directions in the transversal plane) and assessing postural responses through the GRF and center of pressure has been recommended for directional fall risk indication [161].

1.3 Epidemiology of post-stroke bone health

One of the health issues experienced by stroke survivors that is often overlooked in both research and clinical practice is secondary hemi-osteoporosis. Compromised bone health may contribute to fragility fractures [79]. It has been documented that individuals with stroke have approximately a two-fold increased risk of fracture compared with an age-matched reference population [79]. Fractures in people post stroke are strongly associated with a reduced ability to regain independent walking function, a longer rehabilitation hospital stay, and increased mortality [162, 163]. Therefore, fragility fractures are serious events that have a major impact on persons living with a stroke, their caregivers, and society [164].

Thus, research on post-stroke bone health is of great importance. The problems that we are currently facing with the aging population also make studying this topic particularly timely and relevant [165].

1.3.1 Studies using dual-energy X-ray absorptiometry

Post-stroke bone health issues did not garner much attention from researchers until the late 1990s and early 2000s, when dual-energy X-ray absorptiometry (DXA) was used to assess BMD in individuals with stroke [162, 166-169]. These studies consistently found significant bone loss in hemi-paretic limbs after stroke onset. For example, within the first year after a stroke, the proximal femur in the hemi-paretic leg was found to sustain an average of a 12%–13% reduction in BMD, compared with only a 4%–8% reduction in the non-paretic leg [166, 167].

Such a reduction in BMD is much greater than the typical <1.5% annual bone loss for a healthy person older than 50 years [170]. The rate of bone loss in the hemi-paretic upper limb is often more severe and may reach as high as 25% within the first year after a stroke [166, 169]. Progressive bone loss often leads to osteopenia and even osteoporosis, which are common among stroke survivors [164]. A review using meta-regression to analyze 38 placebo-controlled trials indicated that for a 2% or 6% decrease in total hip BMD, a 16% or 40% increase in hip fracture risk is expected, respectively [171].

In the chronic stroke stage, one longitudinal study using DXA (mean post-stroke duration: 33.4 months) did not find a significant difference in femoral bone loss between the two legs [172]. In another study, Lam et al. found a significant

change in total hip areal BMD (aBMD) on the paretic side (1.2%) after 1 year of follow up. However, the change was within the least significant change (LSC) value of the DXA device, indicating that the change observed was within the margin of measurement error [30]. In summary, previous studies using DXA have provided solid evidence demonstrating that secondary bone loss and hemi-osteoporosis is serious issue for individuals with stroke. There is some evidence suggesting that the rate of bone loss is faster in the acute to subacute stages than the chronic stage.

The use of DXA to evaluate bone density in individuals with stroke has provided important insights into the effect of stroke on skeletal health. However, one of the major limitations of the DXA technique is its planar nature. Bone is a three-dimensional structure, but DXA can only generate a BMD value based on the area scanned (i.e., aBMD in g/cm^2). Moreover, DXA does not provide any information on bone geometry. Studying bone geometric properties is clinically important, because, in addition to BMD, bone geometry is also a key determinant of bone strength [173]. Although DXA-derived aBMD is a significant predictor of fracture risk, its predictive value is limited because aBMD in most older individuals who experience a fracture is outside the osteoporotic range (T-score < -2.5) [174, 175]. This may be because the aBMD values produced from the two-dimensional images of DXA lack sufficient sensitivity to identify the full spectrum of fracture risk, as they do not include cortical porosity or cortical thickness [38, 39]. Another concern about this technique is that the measured cross-sectional plane may change

with time. Thus, the reliability of the measurements may be affected, especially in longitudinal studies [27].

1.3.2 Cross-sectional studies using first-generation peripheral quantitative computed tomography devices

Peripheral quantitative computed tomography (pQCT) is a bone imaging technique that measures volumetric BMD (mg/cm^3) and bone geometry. Bone strength indices can also be derived from pQCT, based on densitometric and geometric measures. Bone geometry measured by pQCT has been shown to be useful at predicting fracture risk [176]. Thus, pQCT may be a useful tool to evaluate bone volumetric density, geometry, and mechanical properties after a stroke.

Stroke trials that involve the use of pQCT began to emerge in the mid-2000s [30, 31, 177-184]. The first- generation pQCT device, with a pixel size of 0.2–1.0 mm, was used in these studies. These studies, which were mostly cross-sectional, showed that the trabecular and cortical volumetric BMD (vBMD) values were significantly lower in the hemi-paretic radius and tibia than the non-paretic radius and tibia. In terms of bone geometry, the cortical bone area and cortical thickness of the tibia and radius diaphysis on the hemi-paretic side were also significantly smaller than the corresponding sites on the non-paretic side. Distinct side-to-side differences in the marrow cavity area were observed in the radius diaphysis [180, 184]. However, the side-to-side difference in the marrow cavity area in the tibia diaphysis was found to be more prominent in men than in women [177]. The total cross-sectional area in both the radius and tibia diaphysis demonstrated no

significant side-to-side difference. Taken together, these results indicate that endosteal resorption of the radius and tibia diaphysis (i.e., bone loss on the endosteal surface) may have taken place after stroke onset [30, 31, 177-183].

1.3.3 Longitudinal studies using first- generation pQCT devices

As noted previously, most previous pQCT studies of stroke have been cross-sectional, and thus, have not assessed temporal changes in bone properties after stroke onset. The side-to-side difference in bone variables observed may be attributable to changes in the hemi-paretic and/or non-paretic limb. Longitudinal studies are required to determine the time course of changes in different bone variables on each side after stroke onset. Three longitudinal studies have used first-generation pQCT devices to investigate bone changes after a stroke [30, 167, 181], and two of these studies measured individuals who were well into the chronic stage of stroke recovery (mean post-stroke duration: 48.5 months; measurement gap: 1 year) [30, 181]. The third study is the only longitudinal pQCT study that assessed bone at an earlier stage of stroke recovery using a first-generation PQCT device [167].

From a preventive point of view, it is necessary to study trends in bone changes starting from the very early stages after stroke onset. During a 9-month follow-up period (from 3 to 12 months after stroke onset), Lazoura et al. found that the trabecular BMD of the distal epiphysis of the radius on the hemi-paretic side was reduced by 14% and 9% in women and men, respectively [167]. However, information on geometric properties was not reported. The lower limbs were also

not measured in their study.

In a study of chronic stroke, Pang et al. found a significant decrease in cortical thickness (3.2%) in the radial diaphysis on the paretic side after 1 year of follow-up in a group of 20 participants (mean post-stroke onset: 4 years), but the changes in all pQCT variables were unremarkable in the epiphyseal site of the radius during the same follow-up period [181]. For the tibia on the affected side, a significant reduction in trabecular vBMD (1.8%) was found in the epiphyseal site at 1 year (20 participants, mean post-stroke onset: 4 years), whereas none of the bone variables showed significant changes in the diaphyseal site [30]. Taken together, these findings indicate that differential bone changes in the epiphyseal and diaphyseal sites may persist in the chronic stage, although with a less substantial degree of change compared with the subacute stage of stroke recovery. A longer follow-up period (i.e., 2 years) may be needed to determine whether a steady state is reached for the different bone variables.

1.3.4 High-resolution pQCT studies

While the abovementioned pQCT studies have shed some light on how stroke influences long-bone density and structure, the earlier generation of the pQCT device does not have adequate resolution to accurately measure the cortical thickness at sites where the cortex is relatively thin. However, these sites are often where fractures are more prevalent (e.g., the distal radius). The low resolution also renders it impossible to study bone microstructure (e.g., cortical porosity and trabecular number and spacing), which is important for determining bone fragility

[185].

High-resolution peripheral quantitative computed tomography (HR-pQCT) (pixel size: 41–246 μm) was developed in the mid-2000s. It provides reliable and valid measurements of bone microstructure *in vivo* at peripheral bone sites (i.e., tibia and radius), which allows the estimation of bone strength via the reconstruction of three-dimensional bone images [186, 187]. In human cadaver studies, microstructural measurements of the radius and tibia assessed by HR-pQCT have been shown to correlate with bone stiffness and yield strength, as determined by mechanical testing [188]. HR-pQCT is considered to be superior to DXA in fracture discrimination [186]. The microarchitecture of the tibia and radius can discriminate fragility fracture status in postmenopausal women, independent of the DXA-derived aBMD [189]. Cortical porosity, which can be assessed by HR-pQCT *in vivo*, predominantly contributes to fragility fracture in postmenopausal women with type-2 diabetes [190]. In addition, the Bone Microarchitecture International Consortium (BoMIC), which includes data from eight cohorts (n = 7,254), reported that total vBMD or trabecular vBMD, tibial cortical vBMD, and cortical thickness improve fracture prediction in elderly individuals compared with femoral neck aBMD or FRAX scores alone [191]. HR-pQCT is now commonly used in large population-based studies of people across different age groups [192] and for evaluating treatment efficacy [193].

Only two recent cross-sectional studies [194, 195] and one longitudinal study [196] have used HR-pQCT to evaluate post-stroke bone health. The two cross-

sectional studies used second-generation HR-pQCT (XtremeCT II: isotropic voxel size of 61 μm), and the longitudinal study used first-generation HR-pQCT (XtremeCT I: isotropic voxel size of 82 μm). In addition to trabecular bone and cortical bone vBMD and bone geometry, trabecular microstructure (e.g., trabecular separation and trabecular thickness) and cortical microstructure (e.g., cortical thickness) are also analyzed by HR-pQCT.

Miller et al. found that the side-to-side difference (%SSD) in trabecular vBMD and cortical vBMD was 23.1% and 4.5%, respectively, in the distal radius of people with chronic stroke. In contrast, the corresponding values were only 0.7% and 0.2% in age-matched healthy control subjects [194]. Much larger trabecular separation (%SSD: 24.3%) and smaller cortical thickness (%SSD: 14.4%) were found in the distal radius of the paretic side compared with the non-paretic side. No obvious between-limb differences in HR-pQCT variables were demonstrated in the healthy control group. As a result, the between-limb difference in estimated failure load of the radius was eight times higher in the chronic stroke group (23.8%) than in the control group (2.9%) [194].

The same group of researchers also used HR-pQCT to study bone properties at the distal tibial site. The side-to-side differences in trabecular vBMD (4.1%) and cortical vBMD (4.7%) in the stroke group were significant, whereas those in the control group were unremarkable (0.1%–0.3%) [195]. In addition, significant side-to-side differences were observed in the cortical area (11.6%) and cortical thickness (11.9%), with the non-paretic side showing greater values than the paretic side. The

inter-limb difference in the estimated failure load of the distal tibia was 10.3% in the stroke group, compared with only 0.1% in the control group [195]. These findings from recent cross-sectional HR-pQCT studies indicate that stroke may have a substantial impact on the macro- and microstructural properties of bone. A longitudinal study of stroke individuals using HR-pQCT is warranted.

Borschmann et al. investigated changes in the distal tibia from 2 weeks to 6 months after a stroke. Greater reductions in the total vBMD and cortical area were found in the paretic side than the non-paretic side during the follow-up period [196]. However, changes in the distal radius (another common fracture site) starting from the very early phase of stroke (acute stage) were not evaluated. Moreover, the estimated failure load (a bone strength index generated by pQCT, based on finite element analysis) was not provided. Human cadaver studies have shown that the estimated failure load is more related to the actual failure load than are other bone variables measured by pQCT (e.g., vBMD or cortical thickness) [188]. The estimated failure load's predictive ability for fractures in older adults is also superior to those of other bone variables [191].

In summary, the effect of stroke on bone structure during different stages is still understudied. It is essential to study the patterns of change in various bone properties and their related factors in the acute, subacute, and chronic stages of stroke. Such information would help to identify key interventional strategies for improving bone health during different stages of stroke recovery.

1.3.5 Factors related to compromised bone health after a stroke

Another key issue is related to the identification of predictors of bone outcomes in individuals with stroke. This area is poorly understood due to the limited number of longitudinal studies. Ambulatory status immediately after a stroke [162], the ability to regain walking function at 2 months post-stroke [162], and the degree of asymmetry in weight-bearing during standing measured at 7 months post-stroke [168] have been found to be associated with the extent of change in hip BMD (measured by DXA) during the first year after stroke onset. The initial functional level of the upper limb is also associated with the change in proximal humerus BMD within the first year post-stroke [169]. These results suggest that the decline in mobility after a stroke plays an important role in post-stroke bone loss. However, these results are from DXA studies, which do not provide any information on predictors of other important bone variables.

In the only longitudinal study that involved the use of first-generation pQCT in earlier stages of stroke recovery (3–12 months post-stroke), Lazoura et al. found that spasticity was not significantly related to vBMD loss on the paretic side [167]. It is unknown whether other stroke impairments and their recovery (e.g., muscle strength and sensory function) are predictive of bone structural changes over time, as these factors were not measured in this study. The only longitudinal study that involved the use of first-generation HR-pQCT for acute stroke (within 2 weeks of onset) found that better motor control recovery was significantly correlated with less decline in the total vBMD of the distal tibia during the first 6 months after a

stroke [196].

Meanwhile, the three longitudinal studies on chronic stroke (onset > 1 year) found that the factors associated with bone loss differ between different sites [30, 172, 181]. In contrast to the findings within the first year post-stroke reported by de Brito et al., spasticity was found to be positively related to femoral bone loss after 1 year post-stroke [172]. Leg muscle strength is negatively related to BMD decline in paretic tibial distal epiphysis during the chronic stage [30]. In the radius, Pang et al. found that disuse of the paretic arm, vascular elasticity, and the level of physical activity were associated with decreased BMD at this site [181].

More recently, studies using second-generation HR-pQCT devices (XtremeCT II: isotropic voxel size of 61 μm) found that the inter-limb difference in the estimated failure load of the distal tibia was independently associated with gait speed and ankle spasticity [195] and that the same outcome for the distal radius was associated with motor control impairment, upper limb disuse, and spasticity [194]. However, these studies used a cross-sectional design. Apart from the above factors, the drugs used for the management of comorbidities in people with stroke (e.g., selective serotonin reuptake inhibitors and antidepressants) may independently mediate bone loss [197].

In summary, the influence of these important stroke characteristics on bone changes have been underexamined in longitudinal studies using HR-pQCT. We have very little knowledge of the predictors of bone structural changes as the clients go through the acute, subacute, and chronic phases of stroke recovery. Determining

the key predictors of bone outcomes will be instrumental for the early identification of those individuals with a greater risk of secondary hemi-osteoporosis following a stroke event. In addition, identifying the key modifiable factors has important implications for the formulation of intervention strategies to prevent or reverse deleterious bone changes.

1.3.6 Treatment for secondary osteoporosis in individuals after a stroke

According to a recent systematic review, only one experimental trial has investigated the efficacy of exercise and two studies have applied pharmacological treatment to manage secondary osteoporosis after a stroke [198]. Pang et al. showed that 19-week training (a mix of dynamic loading, resistance, and aerobic exercises) resulted in significant increases in paretic leg muscle strength and better bone outcomes in the hip (femoral neck aBMD) and tibia (trabecular bone content of 4% at the tibial site and cortical bone thickness of 50% at the tibial site) on the affected side in people with chronic stroke [199, 200].

Ikai T et al. (2001) found that 2 weeks of etidronate (anti-bone absorption agent) treatment before 3 months of rehabilitation mitigated bone loss by 4.6%, compared with the same rehabilitation alone, in females with subacute stroke. This beneficial effect was only seen in the low activities of daily living group with a baseline Functional Independence Measure score less than 71. The smaller decrease in the aBMD in the femoral neck was accompanied by reduced levels of bone resorption markers (i.e., urinary pyridinoline and deoxypyridinoline) [201]. Another study examined a single infusion of zoledronate, another anti-resorptive agent, in people

with subacute stroke. A protective effect was found for the total hip aBMD of the paretic side in the treatment group [202].

In summary, considering that stroke is a chronic condition with long-lasting disability, long-term care interventions for bone health management are crucial. More investigation is required to help establish individualized long-term strategies to optimize bone health in people with chronic stroke.

1.4 Knowledge gaps and study rationale

Research on post-stroke bone health is scarce but is of great importance. However, only a small number of people are screened or treated for osteoporosis after a stroke [203]. One important reason may be the lack of a comprehensive understanding of the phenomenon of bone loss and its modifiable factors during different stages after a stroke. Longitudinal studies using HR-pQCT would be useful to examine the changes in different bone outcomes at various stages post-stroke. This may help to identify the bone properties (e.g., BMD, geometry, microstructure, estimated failure load, etc.) that sustain the most severe deficits at different stages post-stroke. The findings of such a study will further help develop a comprehensive model to predict bone changes during different stroke stages. Such information would be useful in informing the design of clinical interventions to improve post-stroke bone health.

This PhD thesis describes a series of studies that were designed to address these knowledge gaps. First, a systematic review was conducted to consolidate the evidence for pharmacological and non-pharmacological interventions for

managing secondary osteoporosis post-stroke (Chapter 2). Next, two longitudinal cohort studies involving the use of HR-pQCT were undertaken to investigate bone changes in the distal radius (Chapter 3) and tibia (Chapter 4) and the factors that influence these changes during chronic stroke stages. Finally, a pilot study examining the changes in HR-pQCT variables of the distal tibia and radius from the acute to the subacute stage post-stroke is described in Chapter 5.

1.5 Research objectives

1. To consolidate the research evidence for pharmacological and non-pharmacological treatments aimed at improving post-stroke bone health (Chapter 2)
2. To assess the changes in bone density, macrostructure, and microstructure of the radius and tibia during the chronic post-stroke stage (Chapters 3 and 4)
3. To identify the predictors of bone structural changes during the chronic post-stroke stage (Chapters 3 and 4)
4. To assess the changes in bone density, macrostructure, and microstructure of the radius and tibia within the first 6 months post-stroke (Chapters 5)
5. To assess the factors that are associated with bone changes in the radius and tibia during a 6-month follow-up period (Chapters 5)

1.6 Research hypotheses

Hypothesis #1: For both the radius and tibia, the extent of change in bone variables is significantly greater on the hemi-paretic side than the non-paretic side during the chronic post-stroke stage (addressing Objective 2).

Hypothesis #2: For both the radius and tibia, the extent of change in bone variables is significantly greater on the hemi-paretic side than the non-paretic side during the first 6 months post-stroke (addressing Objective 4).

Hypothesis #3: Muscle strength has also been consistently shown to be a strong clinical correlate of the bone strength index value in people post-stroke in previous cross-sectional studies [17–21]. It is thus hypothesized that, of the various impairment variables, muscle strength measured at baseline and the recovery of muscle strength during the follow-up period are the strongest predictors of bone structural changes during the same follow-up period (addressing Objectives 2 and 5).

2. Chapter 2

Treatment for bone loss in post-stroke individuals: a systematic review and Meta-analysis

Ouyang Huixi

2.1 Abstract

Objective: Examine the effects of pharmacological and non-pharmacological interventions on different bone health outcomes in post-stroke individuals and its influential factors.

Design: A systematic review and meta-analysis of randomized controlled trials and quasi-experimental trials.

Participants: People diagnosed with stroke at any stage.

Intervention: Any form of physical exercise, supplements, or medications.

Outcome measures: Bone health-related outcome measures.

Results: Thirteen trials involving 6,335 participants (362 in non-pharmacological and 5,973 in pharmacological studies) were included in this review. Meta-analysis revealed moderate-quality evidence for the beneficial effect of exercise on the bone mineral density (BMD) of the paretic hip (standardized mean difference [SMD]: 0.50, 95% Confidence Interval [CI]: 0.16–0.85). Very low-quality evidence showed that exercise may improve the BMD of the paretic side radius (SMD: 0.41, 95% CI: -0.10–0.91, I^2 = 0%). Exercise (two trials, 73 participants) had either no or a small effect on the cortical BMD of the paretic side tibia (SMD: 0.04, 95% CI: -0.42–0.50, I^2 = 0%; low-quality evidence). The effect of exercise on the cortical thickness of the paretic side tibia was inconclusive due to the wide CI (SMD: 0.30, 95% CI: -0.32–0.92, I^2 = 0%; very low-quality evidence). The effects of medications on the BMD of the paretic hip were also uncertain (SMD: 0.31, 95% CI: -0.20–0.83; low-quality evidence). High-quality evidence (three trials, 5,973 participants) showed that the administration of the

antidepressant fluoxetine increased the risk of fracture (risk ratio: 2.28, 95% CI: 1.58–3.30) in post-stroke individuals.

Conclusion: Exercise under supervision was beneficial for hip bone health in post-stroke individuals. However, the skeletal benefits of exercise and medication for the radius and tibia remain uncertain. The adverse effects of antidepressants on the risk of fracture among post-stroke individuals warrant further attention. Further high-quality studies are required to better understand this issue.

2.2 Introduction

Stroke is a leading cause of long-term disability in adults [204]. Hemi-osteoporosis is a common secondary complication among stroke survivors [166]. Bone mineral density (BMD) of the paretic leg and arm are known to decrease by more than 10% and 20%, respectively, within the first year post-stroke [205]. Decreased BMD, combined with an increased risk of falling, contributes to a highly exaggerated risk of post-stroke fractures (i.e., up to 4-fold more than healthy controls) [79, 206-208]. Hence, the implementation of effective interventions to mitigate post-stroke bone loss is crucial.

The literature on post-stroke osteoporosis treatment spans more than 20 years. A narrative review conducted by Sundeep and Hoffbauer in 2017 provided a summary of the beneficial effects of medications on osteoporosis management in older adults and menopausal women [209]. However, no recent systematic reviews have investigated the effects of pharmacotherapy on bone health in the post-stroke population. A commentary-based article containing evidence from two randomized controlled trials

(RCTs) and retrospective studies highlighted potential benefits of zoledronate, calcium, and vitamin D for osteoporosis management in the post-stroke population [210].

Post-stroke bone loss has been associated with disuse of the paretic limb, muscle weakness, and alterations of the mechanical structure and material properties of bone; therefore, exercise interventions may be a promising approach for maintaining or improving post-stroke bone health by modifying these associated factors [177, 194, 198-200, 211-213]. A systematic review in 2011 [207] investigating the skeletal effects of post-stroke exercise showed that physical activities significantly improved the tibial cortical thickness and femoral neck BMD of the paretic lower limb. However, the review only demonstrated a small treatment effect of exercise on these bone parameters; in addition, the number of studies included in the review was limited and the quality of the evidence was not evaluated systematically. Recently, in 2022, Sallehuddin et al. [214] conducted another systematic review of non-pharmacological interventions for post-stroke bone health and found that physical and vibration therapies reduced bone loss in post-stroke individuals.

However, neither of the above-mentioned reviews of pharmacological or non-pharmacological interventions for post-stroke bone health provided any information on the effect size estimate and its precision, making the interpretation of results difficult. In the past decade, many studies have explored the use of exercise interventions to modify bone health outcomes. Therefore, an updated systematic review is required to interpret the evidence generated in this important area of research.

The objectives of this systematic review were to investigate the effects of

pharmacological and non-pharmacological interventions on the bone health outcomes of post-stroke individuals and determine the most effective exercise protocols and medications that could improve these outcomes.

2.3 Methods

This study was registered on PROSPERO on September 20, 2022 (registration number: CRD42022359186). The review protocol can be accessed via the following link: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42022359186.

Identification and selection of trials

The PubMed, PEDro, CINAHL Complete, Cochrane Library, Embase, and SCOPUS databases were searched using keywords related to stroke, bone health, physical exercise, pharmacotherapy and supplementation. (Appendix 1). The inclusion and exclusion criteria for article selection are provided in Appendix 2. Two researchers individually screened each article and determined eligibility for inclusion. In the case of a disagreement, the opinion of a third researcher was sought. However, if the disagreement persisted, the senior researcher was consulted for a final decision. Relevant reviews and the reference list of each eligible study were screened to find other potentially relevant studies. Forward reference searching of all of the relevant studies identified in the above search was conducted using the Science Citation Index (last search was conducted in September 2022).

Assessment of study characteristics and methodological quality

All types of physiotherapy and medication were included in this review. Characteristics of the study populations, outcome measures, and intervention protocols (i.e., dosage of medications and type, frequency, intensity, and duration of exercise) were extracted from each included study. Bone health parameters were classified based on the skeletal site and type of bone structure (e.g., cortical and trabecular), as appropriate. The methodological quality of each non-pharmalogical study was assessed using the PEDro score, which was directly obtained on the PEDro website (www.pedro.org.au), except one included both human and animal participants. For the one with animals and all of the pharmacological studies, the PEDro score was independently rated by two experienced researchers. Any discrepancies were discussed until a consensus was reached. PEDro scores of 0–3, 4–5, 6–8, and 9–10 indicated poor, fair, good, and excellent methodological quality, respectively [215].

Data analysis and assessment of strength of evidence

Two researchers independently extracted relevant data from the included studies. One of them used Tabula (v1.2.1) [216] to export tables from PDF to Excel. No discrepancies were found after data extraction and conversion. The standardized mean difference (SMD) was calculated as a summary measure of the treatment effects for interval data. Risk ratio was used to synthesize the risk of fracture. As suggested by the Cochrane Handbook for Systematic Reviews of Interventions (v6.3), the missing change in standard deviation (SDs) was calculated using the following formula:

$$SD_{E,\ change}=\sqrt{SD_{E,\ baseline}+SD^2_{E,\ final}-(2\times Corr\times SD_{E,\ baseline}\times SD_{E,\ final})}$$

where *Corr* represents the correlation coefficient between baseline characteristics and post-intervention outcomes, and was set as 0.5.

Meta-analysis was conducted using Review Manager 5.4 software (The Cochrane Collaboration, London, UK) when at least two similar trials tested the same measured construct. Meta-analysis was performed separately for pharmacological and non-pharmacological intervention studies. Considering the variation across study designs, a random-effects model was used for all of the meta-analyses. Egger's regression asymmetry test was performed in R software (v4.2.1, R Core Team) to assess publication bias. $p < 0.1$ (two-tailed test) indicated the existence of publication bias. Forest plots generated by Review Manager were used to display the synthesized results.

The Grading of Recommendation, Assessment, Development, and Evaluation (GRADE) approach was used to evaluate the quality of evidence for each outcome measure. No observational studies were included in the review. Hence, the initial evidence rating for each outcome was set at "high quality." For each condition specified in Appendix 3, the quality of evidence was downgraded by one level. When a substantial effect size or dose–response association was reported, the quality of evidence was increased by one level.

2.4 Results

Flow of studies through the review

The electronic searches generated 3,976 records. After screening and removing duplicates, 3,019 potentially relevant articles were extracted. Sixteen eligible papers were identified after full-text screening. These papers were derived from 13 trials because three of the studies generated two papers each (Pang 2005 and Pang 2006 [199, 200]; Pool 2007 and Pool 2009 [202, 217]; Hankey 2020 and Hankey 2021[218, 219]). Forward reference searching identified two additional retrospective cohort studies [220, 221], which examined the risk of fracture after statin administration post-stroke. They were eventually excluded due to the potential risk of bias arising from their retrospective nature. Finally, 13 trials (seven non-pharmacological and six pharmacological trials) involving a total of 6,335 participants (362 in non-pharmacological and 5,973 in pharmacological studies) fulfilled both our inclusion and exclusion criteria (Figure 1).

Characteristics of the included trials

Detailed study characteristics have been summarized in Table 2-1. The mean age of the participants in individual trials ranged from 57 to 73 years. The disability level of participants varied across studies that investigated non-pharmacological interventions (able to walk with or without aids: three trials; able to stand with or without aids: one trial; not reported: two trials). Among studies that investigated exercise interventions, four involved people with chronic stroke only (≥ 6 months since

onset); one involved a mix of individuals with subacute and chronic stroke (≥ 3 months since onset); and one involved only people with acute stroke (≤ 3 days since onset). In contrast, all of the pharmacological studies involved participants in the acute or subacute stroke stage with a mild-to-severe level of disability.

A wide range of exercise types were adopted in the studies, such as the fitness and mobility exercise (FAME) program (a mix of aerobic, balance, and strength training exercises; one trial) [199, 200], daily upright bed training (one trial) [222], exercise on a whole-body vibration (WBV) platform (two trials) [211, 223], progressive body weight-supported treadmill exercise (one trial) [224], neuromuscular facilitation training (NFT; one trial), [225] and isotonic finger flexor exercise (one trial) [226].

Seven studies investigated the treatment effect of various drugs and supplements. Of these, one study investigated the effect of 2 years of calcitonin supplementation, and another examined the effect of 2 weeks of etidronate administration. Three recent multi-center studies investigated the effect of 6-month fluoxetine treatment on the risk of fracture, whereas another tested the effect of one dose of zoledronate in combination with daily calcium and vitamin D supplementation on post-stroke bone health (Table 2-1 provides the specific protocols).

The methodological quality of the trials is shown in Tables 2-2 and 2-3. Of the eight non-pharmacological studies included in this review, one had excellent and three had good methodological quality, as indicated by their PEDro scores. Of the seven included pharmacological trials, the methodological quality of six was good-to-excellent and of one was fair.

Effects of exercise on bone health outcomes

Many of the included studies used either dual-energy X-ray absorptiometry (DXA) or peripheral quantitative computed tomography (pQCT) to assess the effects of exercise on femur, lumbar spine, tibia, and radius parameters, whereas others used serum bone biomarkers to assess the effects of exercise on the bone turnover rate.

Hip BMD measured using DXA (g/cm^2)

Three exercise trials used hip BMD as the outcome measure. The exercise programs were a 5-month FAME program (one trial) [200], a 6-month progressive treadmill training program (one trial) [224], and conventional training with NFT (one trial) [225]. As illustrated in Figure 2A, meta-analysis (three trials, 136 participants) revealed that 5–6 months of exercise significantly mitigated the decline in hip BMD after stroke (SMD: 0.50, 95% confidence interval [CI]: 0.16–0.85).

A study by Han et al. [222] was not included in the meta-analysis because it compared the efficacies of different exercise durations, and not the exercise intervention with the control condition. Significant effects were only seen in male participants. The study demonstrated that improvement in the paretic femoral neck BMD following daily bedside weight training for 30 min was significantly smaller than that following the same training for 90 min (Hedge's g: 1.16, 95% CI: 0.56–1.76) among men, with a medium-to-large effect size [222].

Lumbar BMD measured using DXA (g/cm^2)

Only one study examined the effect of exercise (daily bedside weight training) on lumbar areal BMD (aBMD) [222]. The results showed that longer training durations per session were more effective in improving the BMD of L1–L4 lumbar vertebra in men (60 min vs. 30 min: Hedge's g: 0.62, 95% CI: 0.05–1.18; 90 min vs. 30 min: Hedge's g: 1.08, 95% CI: 0.49–1.68). Among female participants, a stronger treatment effect on the same outcome was observed with the 90-min protocol than with the 30-min protocol (Hedge's g: 0.63, 95% CI: -0.04–1.30), whereas no difference in outcome was observed between the 30-min and 60-min protocols.

Tibia cortical BMD (g/cm^3), cortical thickness (mm), and total cross-sectional area (mm^2) measured using pQCT

Two trials assessed the effects of exercise on cortical BMD and cortical thickness in post-stroke individuals [199, 224]. One study was an RCT involving the FAME program, whereas another was a non-RCT involving 6 months of treadmill walking training. Meta-analysis of these two trials (73 participants) showed that exercise had no or a small effect on the cortical BMD of the paretic mid-shaft tibia (SMD: 0.04; 95% CI: -0.42–0.50; low quality of evidence) (Figure 2B). The result on the cortical thickness of the mid-shaft paretic tibia was inconclusive because of the wide CI (SMD: 0.43; 95% CI: -0.43–1.30; very low quality of evidence) (Figure 2C). The meta-analysis (two trials, 84 participants) also revealed a small or no effect of exercise on the total area of mid-shaft tibia on the paretic side (SMD: 0.018, 95% CI: -0.441–

0.477; low quality of evidence) (Figure 2D).

Forearm/radius BMD (DXA, g/cm^2)

Only two exercise studies measured the total BMD of the forearm/radius. Shimizu et al. [226] studied the effect of paretic upper limb resistance training, in addition to physical therapy, among individuals with chronic stroke; Si et al. [225] examined the effect of 6 months of NFT in individuals with acute stroke. Shimizu et al. [226] did not find a treatment effect on the forearm BMD (Hedge's g: -0.17, 95% CI: -1.41–1.08) despite the relatively long training duration (average: 1.8 years). In contrast, Si et al. [225] showed a potential beneficial effect on the radius BMD (Hedge's g: 0.52, 95% CI: -0.03–1.08). However, both studies had poor methodological quality (PEDro score = 3).

Serum bone biomarkers

Three trials assessed serum bone biomarkers to study the effect of exercise training on post-stroke individuals [211, 223, 225]. Pang et al. [223] assessed the serum concentrations of crosslinked C-telopeptide of type I collagen (CTx; a bone resorption marker) and bone-specific alkaline phosphatase (BAP; a bone formation marker) after 8 weeks of WBV exercise training in individuals with chronic stroke. In contrast, Si et al. [225] examined the effect of 6-month NFT, in addition to conventional training, on the total alkaline phosphatase (a bone formation marker) and serum concentrations of interleukin (IL-6; a bone resorption marker) in individuals with acute stroke.

Meta-analysis of these two studies (134 participants) revealed that the effect of exercise on bone formation (SMD: 0.34, 95% CI: -0.41–1.10) and resorption biomarkers (SMD: -0.91, 95% CI: -2.87–1.05) was inconclusive, as indicated by the wide CIs (Figure 2E and 2F). The heterogeneity between the two studies for serum bone biomarkers was also very large ($I^2 > 70\%$).

A study by Yang et al. [211] was not included in the meta-analysis as it compared the effects of similar WBV protocols with different frequencies (20 Hz vs. 30 Hz). The study investigated the influence of 8 weeks of WBV training on the serum concentration of crosslinked N-telopeptide of type I collagen (NTx; a bone resorption marker) [211] and found a significant reduction in NTx concentration following training in both groups ($p < 0.001$), with no between-group difference in the pre- to post-training changes (Hedge's g: 0.13, 95% CI: -0.29–0.56).

Effects of pharmacotherapy on bone health outcomes

Six studies investigated the effects of medication on bone health in post-stroke individuals. All of these studies recruited participants in the acute or subacute stroke stage.

Hip BMD (DXA, g/cm^2)

Two studies examined the effect of medications on hip BMD in individuals with subacute stroke [201, 202]. Ikai et al. [201] examined the effect of 2-week etidronate administration, whereas Pool et al. [202] administered a single dosage of zoledronate,

in addition to daily supplements of calcium and vitamin D. The effects of these two anti-resorptive drugs (zoledronate and etidronate, 99 participants) on paretic hip BMD were uncertain, as indicated by the wide CIs (SMD: 0.34; 95% CI: -0.41–1.10; Figure 3A). Subgroup analysis by Ikai et al. showed that an additional 2-week etidronate intake had a positive effect on paretic femoral neck BMD in individuals with a poor activities of daily living (ADL) ability (Functional Independence Measure [FIM] ≤ 70) (Hedge's g: 0.83, 95% CI: 0.15–1.52), but not in those with relatively better ADL ability [201].

Fracture risk

High-quality evidence from our meta-analysis (three multi-center trials, 5,907 participants) indicated that administration of the antidepressant fluoxetine for 6 months, starting from the acute stroke stage, increased the risk of fracture (risk ratio: 2.28, 95% CI: 1.58–3.30).

Adverse effects

Among the exercise trials, four [211, 222, 224, 226] reported no adverse events and three [199, 200, 223] reported the following adverse events: two falls (two studies) [199, 200] and soreness in the limbs in a small number of participants (two studies) [199, 223]. The reported falls did not result in any injury [199, 200], and the soreness was alleviated in a few weeks [199, 223]. None of these three trials specified whether the adverse events were related to their respective exercise interventions [199, 200, 223].

Among the pharmacological studies, Uebelhart et al. [227] reported no adverse

effects. Two participants (including one who subsequently dropped out) reported stomach discomfort in the study by Ikai et al. [201]. A single dose of zoledronate infusion led to more cases of acute malaise and pyrexia in the experimental group (three cases) than in the control group (one case). The consumption of fluoxetine resulted in more cases of hyponatremia (< 130 mmol/L) (11 cases vs. one case) [228] and epileptic seizures (10 cases vs. two cases) [219] in the treatment group than in the control group.

Compliance

All of exercise studies, except for that conducted by Han et al. [222], reported the attrition and training session attendance rates. Generally, the attrition rate was low (< 15%) and training session attendance rate was high (> 80%). Of the 60 participants in the study by Pang et al. [199], 24 and eight were excluded from analysis of the tibial epiphysis and diaphysis bone parameters, respectively, because of poor pQCT image quality arising from movement artifacts.

In the medication studies, adherence to medication was generally high (≥ 84%) and almost identical in both experimental and control group, except in the studies by Si et al. [225] and Dennis et al. [229]. The compliance rate was unspecified in the former study, and only 67% of participants completed at least 150 days of treatment in the latter. However, no significant between-group differences in compliance rate were identified in either study.

2.5 Discussion

Although interventions for secondary osteoporosis post-stroke are an underexplored area of research, moderate-quality evidence suggests that exercise protects from hip bone loss after stroke. The effects of exercise training on bone health outcomes in other body parts (tibia and radius) and on serum bone turnover biomarkers remain uncertain. The consumption of antidepressants can increase the risk of fracture among post-stroke individuals. In addition, evidence shows that, although the treatment efficacy of exercise may be influenced by training duration and sex, the beneficial effect of anti-absorptive medication on paretic hip BMD may be influenced by the ADL level.

Effect of exercise on hip BMD

Our meta-analysis showed that hip BMD was preserved after approximately 5–6 months of exercise training compared with the control condition. Pang et al. [200] reported a significant improvement in the paretic femoral neck BMD of individuals with chronic stroke after 5 months of the FAME program (Hedges' g: 0.56; a mix of aerobic, standing, and dynamic balance, and muscle strengthening exercises. This is consistent with previous findings showing the positive effect of exercise on femoral neck BMD in older men and osteoporotic people [230, 231]. The combination of aerobic, muscle strengthening, and impact activities can effectively improve bone health as cardiovascular health [181], muscle weakness [30], and lack of weight-bearing [232] are known to be associated with poor bone health outcomes in people with stroke. In individuals with acute stroke, Si et al. [225] showed that daily NFT, in addition to

conventional weight-bearing training, for 6 months improved femoral neck BMD (Hedge's g: 0.63). The benefits of NFT for hip bone health are attributable to active muscle contractions facilitated by the sensorimotor stimulation, paretic limb weight-bearing, and reflex inhibition. These approaches may be useful in inducing muscle activity during the acute and subacute stroke stages, when active and voluntary muscle activities are most likely to be impaired.

Of the three exercise studies included in the meta-analysis, the study by Pang and Lau [224] showed no significant improvement in total hip BMD after a 6-month treadmill exercise program. The lack of significant findings may be due to low statistical power and certain participant characteristics. The sample size was small (n = 10 in each group), rendering the study underpowered to detect significant changes in bone health outcomes after training.

Effects of exercise on tibia bone health outcomes

Our meta-analysis showed that the effect of exercise on the cortical thickness of the mid-shaft tibia was inconclusive. The evidence was of very low quality because of the small sample size and risk of bias in the two included studies. Pang and Lau [199] demonstrated a large effect size (Hedge's g: 0.96) of a 6-month treadmill training program on the cortical thickness of the paretic side tibia. This was in line with other studies suggesting that enhanced endocortical surface thickness and/or BMD after mechanical loading could underlie exercise-induced skeletal benefits [231, 233]. Progressive treadmill walking exercises may improve bone health by providing

mechanical stimulation and enhancing cardiopulmonary health [234, 235]. In contrast, a study involving a 5-month integrated exercise program reported no effects on tibia bone health [199]. This negative finding may have arisen due to the large variation (SD) in the results. Thus, a future study targeting a population with prominent bone loss is warranted.

Small or no effects of exercise were found on cortical volumetric BMD (vBMD). This is inconsistent with the findings of a meta-analysis with six RCTs involving postmenopausal women, which showed a moderately beneficial effect on tibia cortical vBMD, especially among women who exercised for long durations (12 months) and had a relatively short menopausal period [236]. The discrepancy is partly attributable to two factors: the inclusion of small samples and short durations of the exercise interventions (e.g., 6 months).

Effects of exercise on forearm/radius bone health outcomes

Si et al. [225] found that NFT during the first half-year after stroke decreased bone loss (aBMD) in the radius with a medium effect size (Hedge's g: 0.52). A meta-analysis of two studies showed a beneficial effect of upper extremity exercise on the cortical area of the radial shaft in post-menopausal women [236]. In contrast, a study by Shimizu et al. [226] involving 10 post-stroke individuals found no protective effects of progressive isotonic finger flexor exercises on forearm aBMD in individuals with chronic stroke. This discordance is attributable to differences in sample size and participants' stroke recovery. In particular, the study by Shimizu et al. [226] lacked precision and power because of the small sample size of 10. Moreover, potential for the

functional recovery required to promote bone health is less in individuals with chronic stroke [226] than in those with acute stroke [225]. Thus, whether exercise has beneficial effects on radius bone loss in individuals with chronic stroke remains uncertain and should be investigated in a study with a sufficiently large sample size. Whether the affected limb was the dominant side or non-dominant side may be a confounding factor but was unknown in both included studies. Handedness should be controlled in future research. The degree of functional impairment at baseline may also be an important factor that may influence the response to treatment. Further studies with clear documentation of motor impairment level could help identify those who would benefit most from exercise intervention in future studies.

Effects of exercise on serum bone biomarkers

Our meta-analysis found that the effects of exercise on bone formation and resorption biomarkers were inconclusive. Si et al. [225] found a significantly more decline in a bone resorption biomarker (interleukin-6 [IL-6]; Hedge's g: 1.93) and more increase in a bone formation biomarker after an additional 6 months NFT exercise, compared with after conventional training alone. In contrast, Pang et al. [223] found no significant changes in bone formation and resorption markers following an 8-week WBV program. The discrepancies between the findings of these two studies are attributable to differences in treatment durations and participants' stroke durations. A narrative review of the application of serum biomarkers to osteoporosis management reported that biomarker concentrations reflected the effectiveness of treatments within

3–6 months of starting exercise [237]. This indicates that the treatment duration used in the study by Pang et al. (8 weeks vs. 6 months) may be insufficient, especially for participants with chronic stroke. The treatment frequency and duration per session in the study by Pang et al. (3 times/week, 15 min/time) were also much less than those in the study by Si et al. (almost daily, 1–2 times/day, 60–90 min/time). In addition, the two studies evaluated different serum bone biomarkers (Pang et al.: BAP and CTx; Si et al.: alkaline phosphatase [ALP] and IL-6). A previous study found that the sensitivity of two bone formation markers differed across populations (e.g., BAP: 83% and ALP: 74% in individuals with renal osteodystrophy) [238]. Moreover, a review suggested that BAP was a superior biomarker to ALP as the latter was not specific to bone turnover, despite having been widely used for this purpose [239]. Another recent review [240]compared different bone biomarkers based on applicability in four aspects—diagnosis, progression, monitoring, and predictive value —and found that the two most specific and sensitive biomarkers for osteoporosis pharmacotherapy were CTx-1 (for bone resorption) and procollagen type 1 C-terminal propeptide (P1NP; for bone formation) [239-241]. Further research is required to evaluate the effects of different exercise protocols on bone biomarkers in post-stroke individuals.

Effects of pharmacological interventions on hip BMD and fracture risk

The beneficial effects of pharmacotherapy for osteoporosis have long been established in non-post-stroke populations such as post-menopausal women [11]. However, only two studies have investigated the efficacy of anti-resorption agents

(etidronate and zoledronate) on proximal femur BMD in individuals with acute stroke, and only one study has examined the effects of a single infusion of zoledronate [202]. Our meta-analysis revealed that such a treatment effect on hip BMD was uncertain and that the results should be interpreted with caution considering the small sample sizes of the included studies. Notably, in the study by Ikai et al. [201], in which participants were classified into two groups based on ADL independency, a significant treatment effect was only seen in individuals with low ADL independency (Hedge's g: 0.83, 95% CI: 0.15–1.52). This finding indicates the importance of osteoporosis screening to identify individuals who may benefit more from pharmacotherapy.

Furthermore, we found that the consumption of antidepressants increased the risk of fracture by two to three times. Wadhwa et al. [242] suggested that reduction of osteoblast proliferation by gut-derived selective serotonin reuptake inhibitors (SSRIs) may a mechanism underlying bone loss. Mixed results were found regarding the relationship between the consumption of antidepressants and BMD change in post-menopausal women [243-245]. Studies by Rauma et al. and Diem et al. [244, 245]demonstrated that SSRIs increased bone loss in post-menopausal women, but another study by Diem [243] showed no association between the use of SSRIs and an increased rate of bone loss . This discrepancy is mostly attributable to differences in the age and post-menopausal duration of the participants. Diem [243] investigated middle-aged women (mean age: 49 years, 24.1% post-menopausal), whereas the mean ages of the women in the other two trials were 63 [245] and 79 years (menopause age: 49 years) [244], respectively. In summary, SSRIs may be more detrimental to bone health in

women of advanced age and with a relatively long post-menopausal period.

Influential factors and exercise recommendations

A close examination of the results of the reviewed studies suggested that several factors likely influence treatment success, such characteristics of the exercise program (exercise type and training duration), characteristics of the participants (stage of stroke recovery and sex), and the bone health outcomes evaluated.

The three exercise programs that showed positive effects were 6-month NFT with conventional training (Hedge's g: 0.52–1.93) [225], 5-month mixed fitness and mobility exercise (Hedge's g: 0.56, 95% CI: 0.05–1.06) [200], and 6-month progressive treadmill exercise (Hedge's g: 0.96, 95% CI:0.05–1.87) [224]. All of these programs included muscle strengthening or weight-bearing exercises. In contrast, the 2-month WBV training program had no beneficial effects on bone turnover (as indicated by serum bone biomarkers) in individuals with chronic stroke [223]. This negative finding is partly attributable to the type of exercise implemented; as the WBV program mainly involved static posture maintenance with vibratory stimulation, the exercise intensity was low and engendered only a moderate cardiovascular training effect and inadequate active muscle work. Another factor may be the shorter exercise training duration of the WBV program (15 min per session, 2 months) than of the three beneficial exercise programs reported above (60 min per session, 5–6 months). A review of the application of serum biomarkers to osteoporosis management stated that at least 3 months of exercise training was required to detect a treatment effect, as measured by serum bone

biomarkers [237].

Further, a study by Han et al. [222] suggested a dose–response relationship. They showed that a longer duration of weight-bearing exercises (60–90 min per day) led to higher gains in the femoral neck and lumbar spine BMD than 30 min of training per day. Taken together, these results suggest that a long exercise training session (60 min or more per day) for a minimum of 5–6 months is essential for improving bone health in post-stroke individuals.

The chronicity of stroke may also be a determinant of treatment success. Our results revealed a larger treatment effect on the hip BMD, radius BMD, and serum biomarkers in individuals with acute stroke [225] than in those with chronic stroke [200, 223, 224, 226]. For the hip and radius BMD, the effect size was moderate (Hedge's g: 0.5–0.6) in acute stroke individuals [225]. In individuals with chronic stroke, we found no to a moderate treatment effect (Hedge's g: 0.1–0.6) [200, 224, 226]. For the serum bone resorption biomarker, the effect size was large in acute stroke (Hedge's g: 1.9) [225] but small in chronic stroke (Hedge's g: 0.1) [223]. These results suggest that it can be more difficult to modify bone outcomes in the chronic stroke stage than in the acute stroke stage, highlighting the importance of early intervention in the acute and subacute stroke stages to minimize bone loss in post-stroke individuals.

Han et al. [222] also demonstrated that women required longer training durations than men to show improvements in bone outcomes. For men, increasing the training duration from 30 min to 60 or 90 min enhanced improvements in the femoral

neck BMD (Hedge's g: 0.5–1.2). In contrast, in females, the effect sizes were much smaller (Hedge's g: 0.1–0.5) for the same increases in training duration. A similar phenomenon was observed for the lumbar vertebra BMD. In contrast, Pang et al. [199] found no sex differences in the percentage change in tibia BMD after exercise. This result should be interpretated with caution as the number of females in their study was small.

Lastly, the review showed that the benefits of exercise varied for different bone health indicators. For example, the 6-month progressive treadmill training improved cortical thickness of the tibia diaphysis but not hip BMD in individuals with chronic stroke [224]. This finding indicates that the response to a particular type of exercise may be site-specific, highlighting the importance of evaluating a bone health indicator that is responsive to exercise training.

Limitations of the included studies

The methodological quality of half of the included non-pharmacological studies was either poor or fair due to a lack of assessor blinding, allocation concealment, and intention-to-treat analysis. Among the pharmacological trials, substantial differences in the baseline characteristics of participants were identified as concerns in three studies that examined the treatment effects of anti-resorptive agents [201, 202, 227]. The sample sizes used in these trials were also small (range: 27–36 participants). Advanced bone imaging techniques, such as high-resolution pQCT or magnetic resonance imaging (MRI), or highly sensitive and specific serum bone biomarkers, such as P1NP

and CTx-1, were not commonly used in the reviewed studies. Furthermore, none of the studies investigated the retention effects of exercise. Although previous studies have suggested that the degree of loss severity [184, 194] and time course [183] can differ between cortical and trabecular bone, only one study reported trabecular bone.[199] Thus, little is understood about the site-specific effect of exercise on trabecular bone.

Limitations of the review

This review has several limitations. The overall number of participants in the meta-analysis was relatively small, which may have reduced the precision of analysis. The effect size estimate lacked precision, as indicated by the wide 95% CIs. The heterogeneity in the meta-analysis was also substantial, with the I^2 value being ≥ 50%. These factors explain why the quality of evidence was generally either low or very low. The relatively small number of studies also made it unfeasible to perform sensitivity analyses to examine the differential effects of exercise based on exercise type and participant characteristics (e.g., acute vs. chronic stroke, walking independently vs dependently). Specifically, only one included study examined the effect of exercise from the acute to subacute stroke stage. Only one study specifically included post-stroke individuals with the ability to independently ambulate, representing a higher than normal level of functioning commonly seen in chronic stroke survivors. In addition, Among the included pharmacotherapy trials, only one study investigated whether medication had any effect on the bone turnover rate. Therefore, further research is warranted in this area. As only English publications were included, potentially relevant

articles published in other languages might have been omitted. Future reviews should not use the publication language as an exclusion criterion.

Future research directions

Further research is needed to examine the site-specific effects of exercise on different bone tissues [246]. Studies with long-term follow-ups should be conducted to better illustrate the dynamic process of exercise-induced osteogenesis. As a recent study highlighted that DXA-measured BMD alone may have limitations as a research tool [231], future research should consider employing a combination of measurements such as serum bone markers, pQCT, DXA-based hip structural analysis, and MRI. All but one reviewed study assessed people in the chronic stroke stage. Thus, more research is needed in people in the acute and subacute stroke stages, wherein bone loss is more substantial [166]. Another important research direction is the identification of the determinants of treatment success (e.g., exercise parameters and participant characteristics) and the dose–response relationship. In addition, more pharmacological trials are required to identify effective drugs that reduce or prevent bone loss after stroke.

2.6 Conclusion

In conclusion, supervised exercise intended to improve bone health is safe for post-stroke individuals. The evidence for the effect of exercise on the paretic hip BMD after stroke is of moderate quality. Whereas, with limited number of included studies/participants, the evidence for the effect of integrated exercise post-stroke to maintain or improve bone health of radius or tibia is of low or very low quality. The

consumption of SSRIs or antidepressants can have adverse effects on the bone health of post-stroke individuals.

2.7 List of Tables

Table 2-1: Table summarizing study characteristics

Study (year) Quality	Key participant characteristics mean (SD)	Intervention protocol	Outcome measure
Non-pharmacological intervention			
Yang et al., 2021 PEDro score =9 (excellent)	**n=**84 (34 females) **Age**: 59.7(6.5) **Years since stroke onset**: 4.6(3.5) **Impairment/disability level:** **Fugl-Meyer Assessment lower limb motor score:** 24.0 (3.5) Able to stand for at least 1 min with hand support	**Low frequency (20Hz) group:** Whole-body vibration training on Jet-Vibe System. Synchronous vertical vibrations of 20Hz: 1-min bouts, with 1-min rest between bouts, 12 bouts/session **High frequency (30Hz) group:** Whole-body vibration training on Jet-Vibe System Synchronous vertical vibrations of 30Hz: 1-min bouts, with 1-min rest between bouts, 8 bouts/session **Frequency** in both groups**:** 3 days/week for 8 weeks	Serum cross-linked N-telopeptides of type I collagen (NTx) **No adverse events occurred.**
Shimizu, Ishizaki & Nakamura, 2002 PEDro score	**n=**10 (2 females) **Age**: EXP: 62.6 (9.2) CON: 63.0 (8.1) **Years since stroke onset**:	**Exercise group:** Type of exercise: Ball squeezing exercise in sitting, elbow flexed, arm resting on the armrest. **Frequency:** 10 times x 2 sets/day; 3 times/week or more	Bilateral upper extremity: total bone area, BMC, and BMD (DXA) Adverse events not reported.

=3 (poor)	EXP: 4.6 (3.8) CON: 4.4 (5.4) **Impairment/disability leve**l: Unspecified	In addition to standard Physical therapy. **Control group:** Standard Physical therapy only.	
Han et al., 2017 PEDro score =4 (fair)	**n=**129 (54 females) **Age**: 66.6 (/) **Months since stroke onset**: 5.1 **Impairment/disability leve**l: Able to stand with dynamic standing bed	Type of exercise: Daily upright bed weight training for different durations **M30, F30 groups:** 30 minutes **M60, F60 groups:** 60 minutes (with an interval of 6h) **M90, F90 groups:** 90 minutes (with an interval of 3h) In addition to basic treatment: i) lifestyle adjustment ii) basic dietary supplements for bone health **Frequency:** 5 days/week, 3 months	BMD at the anteroposterior lumbar spine (L1-L4) and ipsilateral femoral neck Adverse events not reported.
Pang et al., 2006 PEDro score =6 (good)	**n=**63 (26 females) **Age:** 65.48 (/) **Years since stroke onset**: 5.2 (5.0) **Impairment/ disability level**: American Heart Association Functional classification (I/II/III/IV/V) 4% site: EXP (n=18) 2/12/4/0/0 CON (n=18) 1/12/5/0/0 50% site EXP (n=26) 3/16/7/0/0 CON (n=26) 1/15/8/2/0	**Exercise group:** Fitness and mobility exercise (FAME) program designed to improve cardiorespiratory fitness, mobility, leg muscle strength, balance, and hip BMD **Control group:** Seated upper extremity exercise program include upper extremity muscle strengthening exercises, passive of self-assisted range of motion exercises, and functional training **Frequency** in both groups**:** 1 hour per session, 3 sessions/week for 19 weeks	Total bone area; Trabecular bone area; Trabecular BMD; Trabecular BMC; Cortical bone area; Cortical BMC; Cortical BMD; Cortical thickness; Polar stress-strain index (p-SSI) **Five low-impact falls happened in EXP.**

Pang & Lau, 2010 PEDro scode =5 (fair)	**n=**21 (7 females) **Age**: EXP: 64.6(7.2); CON: 64.5(6.2) **Years since stroke onset**: EXP: 7.3 (4.2); CON: 9.3 (3.2) **Impairment/disability leve**l: CMSA Leg Score (1-7) EXP: 4.3 (0.9); CON: 4.3 (1.3) CMSA Foot Score EXP: 3.2 (1.5); CON: 2.7 (1.3)	**Exercise group:** 6-month progressive treadmill exercise intervention program with bodyweight supported (BWS). o Initial training speed: individualized comfortable speed. o With intermittent 2-minute rest periods, warm-up and cool-down exercises **Frequency:** 1-hour x 2 sessions/week, 52 sessions, 6 months **Control group:** usual activities (eg, leisure walking, light household tasks)	Hip BMD (using DXA) Tibial BMD and geometry **No adverse events were reported.**
Pang et al., 2005 PEDro score =8 (good)	n=63 (26 females) 32/31 Age: 65.2(8.75) Years since stroke onset: 5.15(4.25) **Impairment/disability level**: American Heart Association Stroke Functional Classification: I/II/III/IV/V EXP: 5/17/9/1/0 CON: 2/19/8/2/0	**Exercise group:** Fitness and mobility exercise (FAME) program designed to improve cardiorespiratory fitness, mobility, leg muscle strength, balance, and hip BMD (Appendix 1) **Control group:** Seated upper extremity program **Frequency** in both groups: 1 hour per session, 3 sessions/week for 19 weeks	femoral neck BMD (using DXA) **Five low-impact falls happened in EXP.**
Pang, Lau & Yip, 2013 PEDro score =8 (good)	**n=**82 (24 females) **Age**: 57.35 **Years since stroke onset**: 4.95 **Impairment/disability leve**l: Abbreviated Mental Test score >= 6; able	**Intervention group:** o Exercise training with vertical WBV stimulation, with progression on WBV intensity and duration o Exercise protocol on the WBV platform:	Bone turnover markers(ng/ml): Serum cross-linked C-telopeptides of type I collagen (CTx); Serum level of bone-specific alkaline phosphatase (BAP)

	to stand 1.5 minutes with or without aids **Functional ambulatory category out of 5** EXP: 5;5-5; CON: 5;5-5 **CMSA leg/foot/arm/hand score out of 7** EXP:4(0)/3(1)/3(2)/3(3) CON:4(0)/3(2)/3(2)/3(3)	Side to side weight shift/Semi squat/Forward and backward weight shift/Forward lunge/Standing on one leg/Deep squat **Control group:** The same exercises as the experimental group on the WBV platform without WBV **Frequency** in both groups**:** maximum of 15 minutes, 3 days per week, 8 weeks	**No major adverse events were reported** by the participants, although three felt **mild dizziness** during first few sessions of the WBV, and four (two from the WBV group) experienced lower limb **soreness** and **fatigue** which lessened within a few weeks.
Si 2016 PEDro score =3 (Poor)	N=52 (female=unspecified); Age:45–75 Time since stroke onset: ≤3 days Impairment/disability level: **EXP**: FMA-UL=8.84 ± 4.36, FMA-LL=13.52 ± 6.39 **CON**: FMA-UL=8.97 ± 4.57, FMA-LL=13.46 ± 6.53	**Intervention group:** conventional treatment + neuromuscular facilitation technique (NFT). NFT included the Bobath, Brunnstrom and Rood techniques. **Control group:** conventional treatment **Frequency** in both groups: 60–90 minutes every time, 1–2 times a day, for 5–6 days a week,6 months	DXA: radius & femur neck BMD, serum Osteocalcin, IL-6 and leptin, serum alkaline phosphatase (ALP) Adverse events not reported.
Pharmacological intervention			
Uebelhart 1999 PEDro score =6 (Fair)	**N**=34 (13 female) **EXP**: n=18; Age: 62 ± 10; Days since stroke onset: 23 ± 1 **CON**: n=16; Age: 52 ± 15; Days since stroke onset: 22 ± 8	**Intervention group:** 1 0 0 IU of salmon calcitonin as a nasal spray +1 g daily oral supplementation of calcium + Bobath rehabilitation. **Control group:**	**Serum biochemical markers**: total alkaline phosphatase (TAP), osteocalcin (OC), carboxy-terminal extension peptide of type I procollagen (PICP), cross-linked carboxy-terminal telopeptide of type I collagen (ICTP),

	Impairment/disability level: unspecified	placebo nasal spray+1 g daily oral supplementation of calcium+ Bobath rehabilitation. **Frequency**: twice a day for two years. Two stages: Hospital (4-6 months), Discharged (2 years)	amino-terminal extension peptide of type III procollagen (PIIINP) **Urinary biomarkers**: calcium/creatinine (Ca/cr) ratio, hydroxyproline/creatinine (HOP/cr) ratio. **No abnormal biological profile was noticed** and none of the participants presented with any fracture.
Ikai 2001 PEDro score =4 (Fair)	**All female** **EXP**: n=40; Age: 66.0 ±8.1; Months since stroke onset: 2.7 ± 1.4 Impairment/Disability level: Brunnstrom stage (lower limb):3.6 ± 1.3 **CON**: n=41; Age: 67.0 ± 8.9; Months since stroke onset: 2.6 ± 1.2 Impairment/disability level: Brunnstrom stage (lower limb):3.4 ± 1.4	**Intervention group:** a dose of 200 or 400 mg/ day of etidronate for a 2-wk period +3 months rehabilitation exercise (PT+OT) + dietary calcium. **Control group:** Rehabilitation exercise (PT+OT) + dietary calcium **Frequency**: rehabilitation exercise 5 days a week, in 40min sessions, 3 months	**BMD**: lumbar spine and both sides of the femoral neck (DXA), FIM (ADL) **Bone formation markers**: Serum levels of alkaline phosphatase (ALP) and osteocalcin were measured as markers of bone formation, **Bone absorption markers**: urinary pyridinoline, urinary deoxypyridinoline, and serum crosslinked carboxy-terminal telopeptide of type 1 collagen (ICTP). Low ADL: FIM ⩽70 points **2 reported stomach discomfort** (1 dropout)
Poole 2007 & Pool 2009 PEDro score =8 (Good)	**N=6 females** **EXP**: n=14; Age: 66.9 (11.7); Days since stroke onset: within 35 days of stroke; Impairment level: SSS=27.4 (6.6) **CON**: n=13; Age:72.9 (10.4); Days since stroke onset: within 35 days of stroke;	**Intervention group:** single dose of zoledronate 4mg with daily calcium (1g) and vitamin D (800 IU) **Control group:** placebo infusion in 50-mL coded sachets with daily calcium	Hip BMD (DXA), Barthel index, Scandinavian Stroke Scale, FAC In 4 subjects, “acute phase” symptoms **of post infusion malaise and pyrexia** were observed (3 EXP, 1 CON)

	Impairment level: SSS= 24.7 (7.5)	(1g) and vitamin D (800 IU)	
Dennis 2019 PEDro score =10 (excellent)	**EXP**: n=1564; Age: 71.2 (12.4); Days since stroke onset:6.9 (3.6) Impairment/disability level: NIHSS(Median)=6 (3–11) **CON**: n=1563; Age=71.5 (12.1): Days since stroke onset:7 (3.6) Impairment/disability level: NIHSS(Median)= 6 (3–11)	**Intervention group:** oral fluoxetine 20 mg **Control group:** placebo **Frequency** in both groups: once daily for 6 months	mRS, SIS, SF36, EQ5D-5L **Adverse events reported.**
Hankey 2020 & Hankey 2021 PEDro score =10 (excellent)	**EXP**: n=642 (female=36%), Age= 63.5 (12.5), Days since stroke onset= 6.1 (3), Impairment/disability level: NIHSS Median (IQR)= 6.0 (3.0–9.0) **CON**: n=638 (female=38%), Age = 64.6 (12.2), Days since stroke onset= 6.3 (3), Impairment/disability level: NIHSS Median (IQR)=6.0 (3.0–9.0)	**Intervention group:** oral fluoxetine 20 mg **Control group:** placebo **Frequency** in both groups: once daily for 6 months	Measured at 6-month post treatment: Fractures, mRS, mood (Patient Health Questionnaire 9 score), and health-related quality of life using the Euro QoL EQ-5D-5 L **Adverse events reported.**
Lundström 2020 PEDro score =10 (excellent)	**EXP**: n=750 (female=38%), Age=70.6 (11.3), Days since stroke onset (Median)=5.0 (4.0–8.0), Impairment/disability level: NIHSS Median (IQR)= 3.0 (2.0–6.0) **CON**: n=750 (female=38%), Age =71.0 (10.5), Days since stroke onset (Median)=5.0 (3.0–8.0), Impairment/disability level: NIHSS	**Intervention group:** oral fluoxetine 20 mg **Control group:** placebo **Frequency** in both groups: once daily for 6 months	MRS, SIS, Safety outcomes (fractures) at 6 months Followed-up at 12 months by postal questionnaire or telephone by one research nurse **Adverse events reported.**

	Median (IQR)=3.0 (2.0–6.0)

Note: EXP: Experimental group; CON: Control group; BMC: Bone mineral content; BMD: Bone mineral density; DXA: Dual-energy X-ray Absorptiometry; CMSA: Chedoke McMaster Stroke Assessment; SSS: Scandinavian Stroke Scale; WBV: Whole body vibration; UL: upper limb; LL: lower limb.

Table 2-2: Methodological quality of reviewed non-pharmacological articles (PEDro)

	Pang et al., 2005	Pang et al., 2013	Pang, & Lau 2010	Pang et al, 2006	Si 2016	Han et al., 2017	Shimizu et al., 2002	Yang et al., 2021
Eligibility	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Subject random allocation	Yes	Yes	No	Yes	No	Yes	No	Yes
Allocation concealment	Yes	Yes	No	Yes	No	No	No	Yes
Baseline similarities	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Subject Blinding	No	No	No	Yes	No	No	No	Yes
Therapist blinding	No	No	No	No	No	No	No	No
Assessor blinding	Yes	Yes	Yes	No	No	No	No	Yes
>85% of outcomes obtained	Yes	Yes	Yes	Yes	No	No	Yes	Yes
Intention to treat	Yes	Yes	No	No	No	No	No	Yes
Between-group comparison	Yes	Yes	Yes	No	Yes	Yes	No	Yes
Point measures of variability	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Pedro score	**8**	**8**	**5**	**6**	**3**	**4**	**3**	**9**
Quality	good	good	fair	good	poor	fair	poor	excellent

Table 2-3: Methodological quality of reviewed pharmacological articles (PEDro)

	Uebelhart 1999	Ikai 2001	Poole 2007	Hankey 2020	Lundström 2020	Dennis 2019	Hankey 2021
Eligibility	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Subject random allocation	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Allocation concealment	NR	NR	Yes	Yes	Yes	Yes	Yes
Baseline similarities	No	No	No	Yes	Yes	Yes	Yes
Subject Blinding	Yes	No	Yes	Yes	Yes	Yes	Yes
Therapist blinding	Yes	NR	Yes	Yes	Yes	Yes	Yes
Assessor blinding	Yes	NR	Yes	Yes	Yes	Yes	Yes
>85% of outcomes obtained	No	Yes	Yes	Yes	Yes	Yes	Yes
Intention to treat	No	No	No	Yes	Yes	Yes	Yes
Between-group comparison	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Point measures of variability	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Pedro score	**6**	**4**	**8**	**10**	**10**	**10**	**10**
Quality	good	fair	good	excellent	excellent	excellent	excellent

Table 2-4: Change score, Standardized effect size and confidence intervals of bone outcomes

			Experimental Group			Control Group			Hedge's	95% CI	
Study	**Site**	**Outcome**	**Mean**	**SD**	**N**	**Mean**	**SD**	**N**	**G**	**Lower**	**Upper**
Non-pharmacological intervention studies											
Pang 2006	Tibia	P- area total (mm^2) 4% tibial-pQCT	21.20	23.00	18	-10.90	126.92	18	0.34	-0.31	1.00
	Tibia	P- Trab BMC (mg) 4% tibial-pQCT	11.00	54.67	18	0.50	47.01	18	0.20	-0.45	0.86
	Tibia	P- Trab area (mm^2) 4% tibial-pQCT	37.20	21.00	18	-3.30	152.35	18	0.36	-0.29	1.02
	Tibia	P- Trab BMD (mg/cm^3) 4% tibial-pQCT	2.70	34.60	18	0.10	36.46	18	0.07	-0.58	0.73
	Tibia	NP- area total (mm^2) 4% tibial-pQCT	27.30	20.00	18	-11.50	129.99	18	0.41	-0.25	1.07
	Tibia	NP- Trab BMC (mg) 4% tibial-pQCT	10.30	47.70	18	-0.60	45.87	18	0.23	-0.43	0.88
	Tibia	NP- Trab Area (mm^2) 4% tibial-pQCT	33.70	18.00	18	-8.10	175.12	18	0.33	-0.33	0.99
	Tibia	NP- Trab BMD (mg/cm^3) 4% tibial-pQCT	2.50	30.61	18	0.60	41.32	18	0.05	-0.60	0.70
	Tibia	P-Total area (mm^2) 50% tibial-pQCT	-1.10	90.10	26	0.80	90.00	26	-0.02	-0.56	0.52
	Tibia	P- Area(cortical) (mm^2) 50% tibial-pQCT	1.70	74.71	26	-1.00	55.30	26	0.04	-0.50	0.58
	Tibia	P- Cort BMC (mg) 50% tibial-pQCT	1.30	87.01	26	-1.50	65.65	26	0.04	-0.51	0.58
	Tibia	P-Cortical BMD (mg/cm^3) -P 50% tibial-pQCT	-2.20	37.90	26	-1.40	30.89	26	-0.02	-0.57	0.52
	Tibia	P-Cortical thickness (mm) -P 50% tibial-pQCT	0.03	1.08	26	-0.04	0.95	26	0.07	-0.48	0.61
	Tibia	P- p-SSI (mm^3): women 50% tibial-pQCT	3.41	339.28	26	-0.20	197.03	26	0.01	-0.53	0.56
	Tibia	P- p-SSI (mm^3): men 50% tibial-pQCT	24.40	313.56	26	6.00	441.09	26	0.05	-0.50	0.59
	Tibia	NP- area total (mm^2) 50% tibial-pQCT	-5.20	86.85	26	-4.80	91.48	26	0.00	-0.55	0.54
	Tibia	NP- Area(cortical) (mm^2) 50% tibial-pQCT	-1.30	73.86	26	-0.30	56.30	26	-0.01	-0.56	0.53
	Tibia	NP- cortical BMC (mg) 50% tibial-pQCT	-0.60	86.85	26	-0.80	65.10	26	0.00	-0.54	0.55
	Tibia	NP- Cort BMD (mg/cm^3) 50% tibial-pQCT	1.10	47.64	26	-1.80	36.12	26	0.07	-0.48	0.61
	Tibia	NP- Cortical thickness (mm) 50% tibial-pQCT	-0.02	1.07	26	0.02	0.93	26	-0.04	-0.58	0.50
	Tibia	NP- p-SSI (mm^3): women 50% tibial-pQCT	-37.20	429.54	26	-14.90	236.45	26	-0.06	-0.61	0.48
	Tibia	NP- p-SSI (mm^3): men 50% tibial-pQCT	6.70	296.47	26	9.20	407.09	26	-0.01	-0.55	0.54

			Experimental Group			Control Group			Hedge's	95% CI	
Study	**Site**	**Outcome**	**Mean**	**SD**	**N**	**Mean**	**SD**	**N**	**G**	**Lower**	**Upper**
Pang 2005	Femoral neck	P-BMD (gm/cm^2) of femoral neck-DXA	-0.00	0.04	32	-0.02	0.03	31	**0.56***	**0.06**	**1.06**
	Femoral neck	NP-BMD (gm/cm^2) of femoral neck-DXA	0.01	0.03	32	0.00	0.03	31	0.36	-0.14	0.86
Pang 2010	Tibia	P-cortical thickness (mm) of 66% tibia-pQCT	0.10	0.10	10	0.00	0.10	11	**0.96***	**0.06**	**1.86**
Pang 2010	Tibia	P-cortical BMD (mg/cm^3) of 66% tibia -pQCT	5.70	59.10	10	-7.50	76.10	11	0.18	-0.67	1.04
	Tibia	P-Total area (mm^2) of 66% site of tibia -pQCT	-1.90	14.30	10	-1.60	6.10	11	-0.03	-0.88	0.83
	Femur	P-BMD (g/cm^2) -total hip-P -DXA	0.00	0.01	10	-0.01	0.02	11	0.06	-0.79	0.92
Shimizu 2002	Upper limb	P-Upper extremity bone area-DXA (cm^2)	-2.00	40.06	5	10.80	24.90	5	-0.35	-1.60	0.90
	Upper limb	P-BMC (g) of upper limb-DXA	-4.60	34.87	5	2.80	29.54	5	-0.21	-1.45	1.04
	Upper limb	P-BMD (gm/cm^2) -UL-DXA	-0.02	0.10	5	0.00	0.12	5	-0.17	-1.41	1.07
	Upper limb	NP-Upper extremity bone area-DXA (cm^2)	-4.00	32.16	5	4.00	31.49	5	-0.23	-1.47	1.02
	Upper limb	NP-BMC (g) of upper limb-DXA	-5.80	33.02	5	-7.40	38.80	5	0.04	-1.20	1.28
	Upper limb	NP-BMD (gm/cm^2) -UL-DXA	-0.01	0.07	5	-0.02	0.17	5	0.09	-1.15	1.33
Yang 2021	serum	Cross-linked N-telopeptides of type I collagen (NTx)	-2.2	3.40	42	-2.7	4.00	42	0.13	-0.29	0.56
Pang 2013	serum	Cross-linked C-telopeptides of type I collagen (CTx)	0.27	0.27	41	0.25	0.25	41	0.08	-0.36	0.51
	serum	BALP-alkaline phosphatase (ng/ml)	-0.09	8.06	41	0.09	8.65	41	-0.02	-0.45	0.41
Han 2017	Femoral neck	P-BMD (gm/cm^2) of femoral neck of M30 group-DXA[a]	0.00	0.11	25	0.07	0.12	25	-0.54	-1.11	0.02
	Femoral neck	P-BMD (gm/cm^2) of femoral neck of M60 group-DXA[b]	0.07	0.12	25	0.14	0.12	25	-0.58	-1.15	-0.02
	Femoral neck	P-BMD (gm/cm^2) of femoral neck of M90 group-DXA[c]	0.14	0.12	25	0.00	0.11	25	1.16	0.56	1.76
	Femoral neck	P-BMD (gm/cm^2) of femoral neck of F30 group-DXA[a]	0.00	0.12	18	0.01	0.12	18	-0.07	-0.72	0.59
	Femoral neck	P-BMD (gm/cm^2) of femoral neck of F60 group-DXA[b]	0.01	0.12	18	0.06	0.13	18	-0.41	-1.07	0.25
	Femoral neck	P-BMD (gm/cm^2) of femoral neck of F90 group-DXA[c]	0.06	0.13	18	0.00	0.12	18	0.47	-0.19	1.13
	lumbar	BMD (gm/cm^2) of lumbar vertebra of M30 group-DXA[a]	0.00	0.14	25	0.09	0.14	25	-0.62	-1.18	-0.05
	lumbar	BMD (gm/cm^2) of lumbar vertebra of M60 group-DXA[b]	0.09	0.14	25	0.15	0.14	25	-0.48	-1.04	0.08
	lumbar	BMD (gm/cm^2) of lumbar vertebra of M90 group-DXA[c]	0.15	0.14	25	0.00	0.14	25	**1.08***	**0.49**	**1.68**

			Experimental Group			Control Group			Hedge's	95% CI	
Study	**Site**	**Outcome**	**Mean**	**SD**	**N**	**Mean**	**SD**	**N**	**G**	**Lower**	**Upper**
	lumbar	BMD (gm/cm^2) of lumbar vertebra of F30 group-DXA[a]	0.00	0.11	18	0.01	0.13	18	-0.04	-0.69	0.61
	lumbar	BMD (gm/cm^2) of lumbar vertebra of F60 group-DXA[b]	0.01	0.13	18	0.08	0.12	18	-0.55	-1.21	0.12
	lumbar	BMD (gm/cm^2) of lumbar vertebra of F90 group-DXA[c]	0.08	0.12	18	0.00	0.11	18	0.63	-0.04	1.30
Si 2016	Radius	P-BMD (g/cm^2) of Radius 33%-DXA	-0.05	0.12	26	-0.12	0.14	26	0.52	-0.03	1.08
	Femur neck	P-BMD (g/cm^2) of femural neck-DXA	-0.09	0.12	26	-0.17	0.12	26	**0.63***	**0.07**	**1.18**
	Radius	NP-BMD (g/cm^2) of Radius 33%-DXA	0.01	0.11	26	-0.03	0.12	26	0.33	-0.22	0.87
	Femur neck	NP-BMD (g/cm^2) of femoral neck-DXA	-0.02	0.13	26	-0.04	0.15	26	0.14	-0.40	0.69
Si 2016	Serum	Leptin	-1.84	2.64	26	-3.78	3.13	26	**0.66***	**0.10**	**1.22**
	Serum	ALP-alkaline phosphatase	34.31	20.79	26	18.14	21.62	26	**0.75***	**0.19**	**1.31**
	Serum	Osteocalcin	6.18	2.42	26	1.93	2.52	26	**1.69***	**1.06**	**2.33**
	Serum	IL-6-Interleukin-6	-19.39	30.44	26	43.61	33.88	26	**-1.93***	**-2.58**	**-1.27**
Pharmacological intervention studies											
Uebelhart 1999	Serum	TAP-total alkaline phosphatase (U/litre)	7.00	24.02	15	-25.00	61.02	15	0.67	-0.06	1.41
	Serum	OC-osteocalcin (ng/ml)	-4.00	6.41	15	2.80	6.29	16	**-1.04***	**-1.79**	**-0.29**
	Serum	PICP-carboxy-terminal extension peptide of type I procollagen (µg/litre)	4.00	37.47	15	35.00	26.85	16	**-0.93***	**-1.67**	**-0.19**
	Serum	ICTP-cross-linked carboxy-terminal telopeptide of type I collagen (µg/litre)	-0.90	4.00	15	-3.10	9.20	16	0.30	-0.41	1.01
	Serum	Ca/cr-calcium/creatinine ratio	-0.07	0.49	15	-0.09	0.33	15	0.05	-0.67	0.76
	Serum	OHP/cr-hydroxyproline/creatinine ratio	1.10	18.40	15	-9.40	23.68	15	0.48	-0.24	1.21
	Serum	PIIINP-amino-terminal extension peptide of type III procollagen (U/ml)	-0.06	0.54	15	0.18	0.54	16	-0.43	-1.15	0.28
Ikai 2001	lumbar	Low ADL-BMD in lumbar spine-DXA (g/cm^2)	-0.40	3.70	18	-0.30	3.40	18	-0.03	-0.68	0.63
	Femur neck	Low ADL-BMD of the femoral neck-P-DXA (g/cm^2)	-4.00	7.20	18	-9.60	5.90	18	**0.83***	**0.15**	**1.51**

			Experimental Group			Control Group			Hedge's	95% CI	
Study	**Site**	**Outcome**	**Mean**	**SD**	**N**	**Mean**	**SD**	**N**	**G**	**Lower**	**Upper**
	Femur neck	Low ADL-BMD of the femoral neck-NP-DXA (g/cm^2)	-0.80	5.40	18	-3.70	5.20	18	0.53	-0.13	1.20
	lumbar	High ADL-BMD of the lumbar spine-DXA (g/cm^2)	-0.40	4.70	17	-0.10	3.90	19	-0.07	-0.72	0.59
	Femur neck	High ADL-BMD of the femoral neck-P-DXA (g/cm^2)	-4.10	6.40	17	-4.60	6.50	19	0.08	-0.58	0.73
	Femur neck	High ADL-BMD of the femoral neck-NP-DXA (g/cm^2)	-0.50	5.40	17	-2.60	4.40	19	0.42	-0.24	1.08
Poole2007	Hip	**Total hip BMD-P-DXA (g/cm^2)**	0.00	2.25	14	-0.06	4.47	13	0.02	-0.74	0.77
	Hip	Total hip BMD-NP (g/cm^2)-DXA	0.01	2.25	14	-0.03	3.46	13	0.01	-0.74	0.77
Hankey 2020	NA	New bone fractures (No. events, Relative risk)	19		642	6		638	**3.15***	**1.27**	**7.83**
Lundström 2020	NA	New bone fractures (No. events, Relative risk)	28		750	11		750	**2.55***	**1.28**	**5.08**
Dennis2019	NA	New bone fractures (No. events, Relative risk)	45		1564	23		1563	**1.96***	**1.19**	**3.22**

Note: P: paretic side; NP: non-paretic side; BMC: Bone mineral content; BMD: Bone mineral density; pQCT: peripheral Quantitative Computed Tomography; DXA: Dual-energy X-ray Absorptiometry; Trab: Trabecular, Cort: Cortical; Hedge' G in bold: 95% CI not spanning zero. ADL: Activities of Daily Living.

[a]: 30min VS 60min; [b]: 60min VS 90min; [c]: 30min VS 90min. [*]: Significant results.

Table 2-5: Quality of evidence: Grades of Recommendation, Assessment, Development, and Evaluation (GRADE)

Sub-group (Scanning machine used)	Outcome	Number of studies	Risk of bias	Inconsistency	Indirectness	Imprecision	Publication bias	ES	Plausible residual confounding	Dose response gradient	Grade rating
Outcomes included in the meta-analysis: non-pharmacological interventions											
Others (pQCT)	Total cross-sectional area (mm^2) of tibia	2	-1 a	0	0	-1 b c	0	0	0	0	Low
Cortical (pQCT)	Cortical thickness (mm) of tibia	2	-1 a	-1 e	0	-1 b c	0	0	0	0	Very Low
	Cortical BMD (mg/cm^3) of tibia	2	-1 a	0	0	-1 b c	0	0	0	0	Low
Total (DXA)	Total BMD (mg/cm^3) of hip	3	-1a	0	0	-1 b	0	0	0	1	Moderate
	Upper Extremity BMD (gm/cm^2)	2	-2a	0	0	-1 b c	0	0	0	0	Very low
Serum bone biomarkers	Serum level of bone-specific alkaline phosphatase (BAP)	2	0	-1 e	0	-1 b c	0	0	0	0	Low
	Serum resorption biomarkers	2	0	-1 e	0	-1 b c	0	0	0	0	Low
Outcomes included in the meta-analysis: pharmacological interventions											
Events	Fracture risk	3	0	0	-1g	0	0	+1	0	0	High
Total (DXA)	Total BMD (mg/cm^3) of hip	2	0	0	-1g	-1	0	0	0	0	Low

Sub-group (Scanning machine used)	Outcome	Number of studies	Risk of bias	Inconsistency	Indirectness	Imprecision	Publication bias	ES	Plausible residual confounding	Dose response gradient	Grade rating
Outcomes not included in the meta-analysis: non-pharmacological interventions											
Cortical (pQCT)	Cortical BMC of 50% Tibia (mg)	1	0	0	0	-2 d	0	0	0	0	Low
	Polar stress-strain index (mm^3) of 50% tibia	1	0	0	0	-2 d	0	0	0	0	Low
Trabecular (pQCT)	trabecular BMC (mg) of 4% tibia	1	0	0	0	-2 d	0	0	0	0	Low
	trabecular area (mm^2) 4% tibia	1	0	0	0	-2 d	0	0	0	0	Low
	trabecular BMD (mg/cm^3) 4% tibia	1	0	0	0	-2 d	0	0	0	0	Low
Other bone measures (DXA)	Upper extremity bone area (cm^2)	1	-1 a	0	0	-2 d	0	0	0	0	Very Low
	Upper extremity BMC (g)	1	-1 a	0	0	-2 d	0	0	0	0	Very Low
	femoral neck BMD (gm/cm^2)	2	0	0	0	-2 d	0	0	0	0	Low
	BMD (gm/cm^2) of lumbar vertebra	1	-1 a	0	0	-2 d	0	0	0	0	Very Low
Serum bone markers (/)	Serum level of cross-linked N-telopeptides of type I collagen	1	0	0	0	-2 d	0	0	0	0	Low
	Serum level of C-telopeptide of type I collagen cross-links (CTx)	1	0	0	0	-2 d	0	0	0	0	Low

Note: ES: effect size; BMC: Bone mineral content; BMD: Bone mineral density; pQCT: peripheral Quantitative Computed Tomography; DXA: Dual-energy X-ray Absorptiometry.(a) For outcomes if less than half of the participants were from trials with PEDro scores larger or equal to 6; (b) Insufficient studies for meta-analysis and the number of subjects included in the meta-analysis was below 400; (c)The 95% CI spanned 0; (d) Insufficient studies for meta-analysis (Imprecision); (e) For outcomes included in the meta-analysis, I^2 value is larger or equal to 50% in the meta-analysis; (g) The participants, intervention, comparator intervention, outcome measure, or study design did not match between the included studies and the eligibility criteria for this revie

2.8 List of Figures

Figure 2-1. Flow of studies through the review process

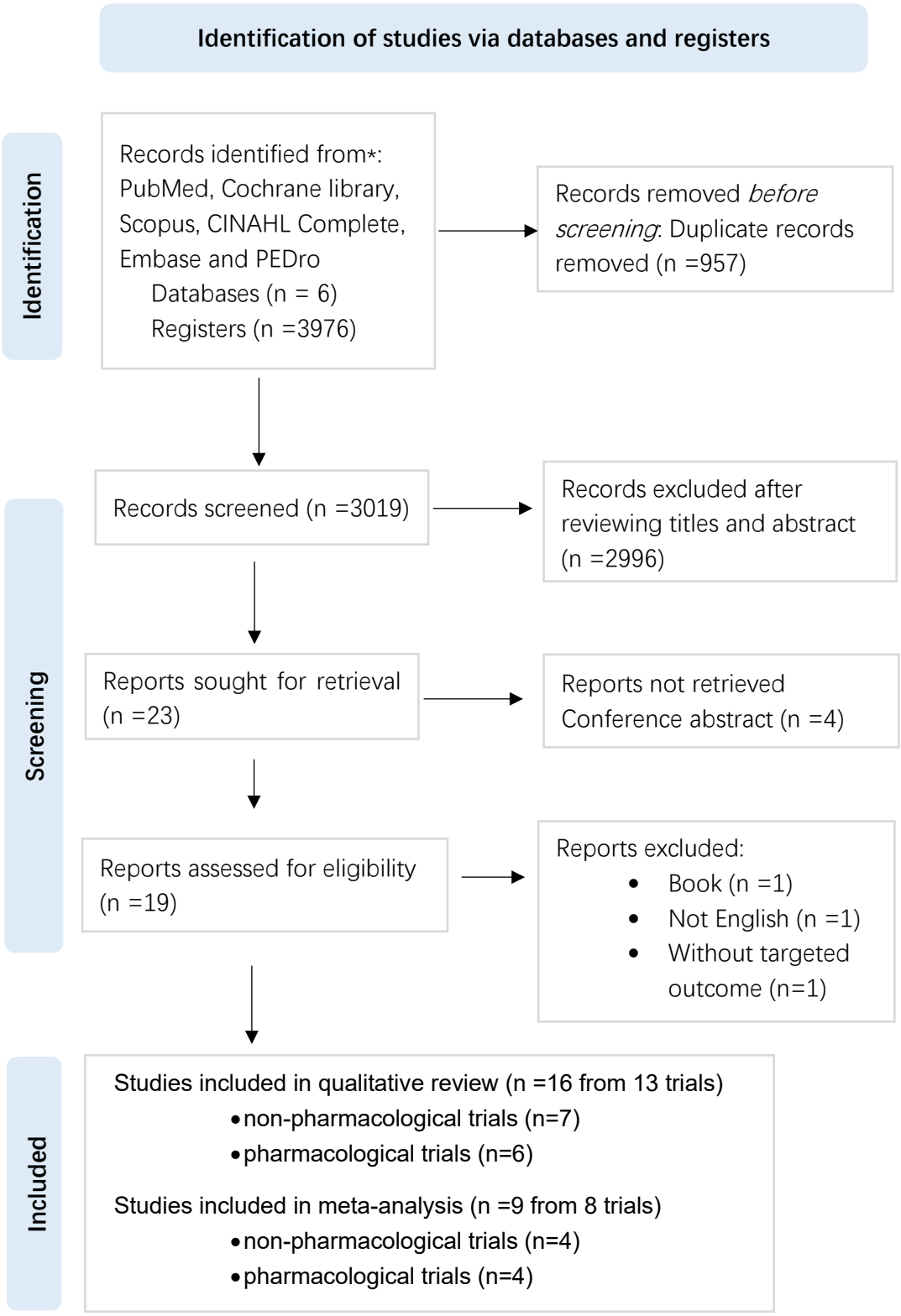
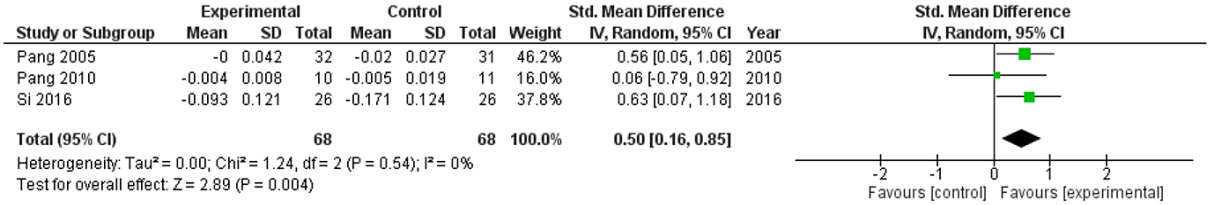
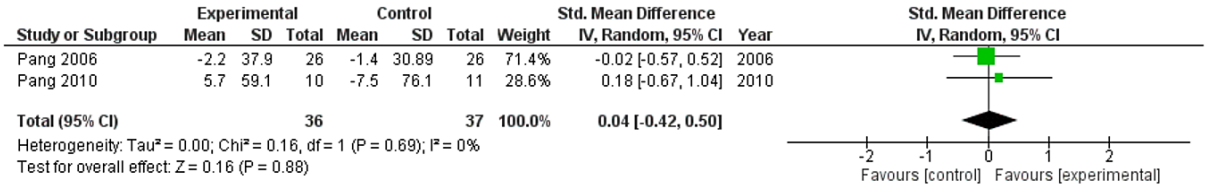


Figure 2-2. Meta-analysis: Effect of exercise on bone parameters measured at mid-shaft tibia on the paretic side

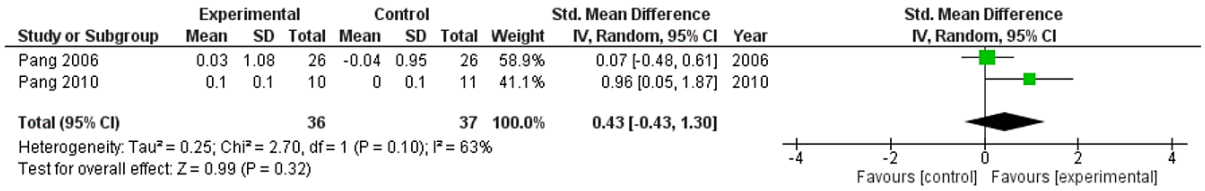
A. Hip: Total BMD (g/cm^2, DXA)



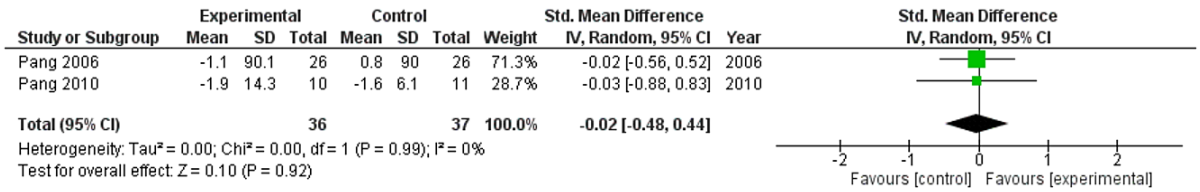
B. Tibia (mid-shaft): Cortical BMD (g/cm^3, pQCT)



C. Tibia (mid-shaft): Cortical thickness (mm, pQCT)



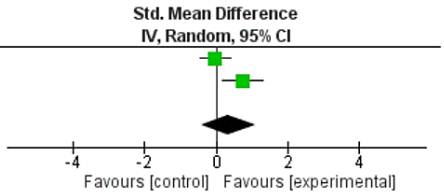
D. Tibia (mid-shaft): Total cross-sectional area (mm^2, pQCT)



E. Bone formation biomarker (serum total alkaline phosphatase & BAP)

Study or Subgroup	Experimental Mean	SD	Total	Control Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI
Pang 2013	-0.09	8.059	41	0.09	8.646	41	52.9%	-0.02 [-0.45, 0.41]
Si 2016	34.31	20.79	26	18.14	21.62	26	47.1%	0.75 [0.19, 1.31]
Total (95% CI)			**67**			**67**	**100.0%**	**0.34 [-0.41, 1.10]**

Heterogeneity: Tau² = 0.23; Chi² = 4.53, df = 1 (P = 0.03); I² = 78%
Test for overall effect: Z = 0.89 (P = 0.37)



F. Bone absorption biomarker (serum)

Study or Subgroup	Experimental Mean	SD	Total	Control Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI	Year
Pang 2013	0.27	0.27	41	0.25	0.25	41	50.8%	0.08 [-0.36, 0.51]	2013
Si 2016	-19.39	30.44	26	43.61	33.88	26	49.2%	-1.93 [-2.59, -1.26]	2016
Total (95% CI)			**67**			**67**	**100.0%**	**-0.91 [-2.87, 1.05]**	

Heterogeneity: Tau² = 1.92; Chi² = 24.41, df = 1 (P < 0.00001); I² = 96%
Test for overall effect: Z = 0.91 (P = 0.36)

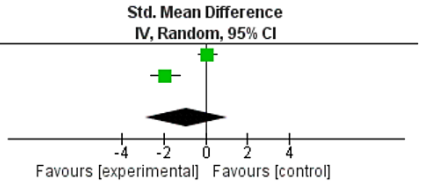
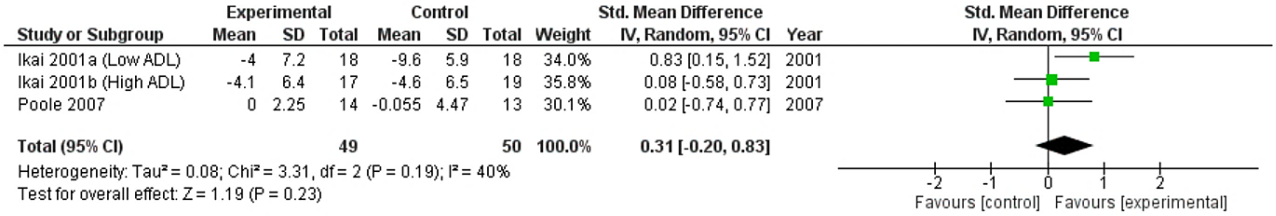
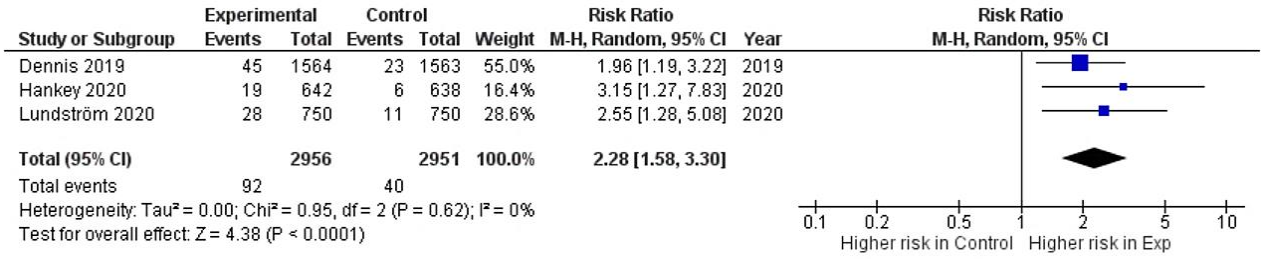


Figure 2-3. Meta-analysis: Effect of medication on bone parameters

A. Total BMD of paretic hip (DXA, g/cm2)



B. Fracture risk



2.9 Appendix

Appendix 2-1. keyword strategy

1# "Stroke" or "Cerebrovascular accident" or "Hemiparesis" or "hemiplegia" or "hemiplegic"

2# "Bone" or "Osteoporosis"

3# "Exercise*" or "Physical Activit*" or "Fitness" or "Rehabilitation" or "Muscle Strengthening" or "intervention" or "treatment" or "therapy" or "physical therapy" or "physiotherapy" or "training"

4# "pharmacological" or "Pharmacotherapy" or "medication" or "drug" or "supplement" or "supplements" or "supplementation"

5# LA- Language English

6# PL-clinical trial OR Randomized controlled Trial

7# Limit to: Human

8# 1# AND 2# AND (3# OR 4#) AND 5# AND 6# AND 7#

Appendix 2-2. Eligibility criteria

Inclusion criteria	
Design	• Randomized controlled trials • Experimental based studies • Quasi-experimental trials
Participants	Any age, diagnosed with any form of stroke for any duration
Intervention	Physical Exercise / pharmacotherapy/supplements
Outcome measures	Standardized or objective scale test
Comparisons	• Experimental treatment versus no intervention/placebo/active control • Experimental treatment plus other intervention versus the same other intervention only

Exclusion criteria
• Books • Systematic reviews • Meta-analyses • Conference proceedings • Non-English publications

Appendix 2-3. GRADE criteria

Key: Explanations for downgrading criteria	
Risk of bias	**(a)** For outcomes if less than half of the participants were from trials with PEDro scores larger or equal to 6.
Imprecision	**(b)** Insufficient studies for meta-analysis and the number of subjects included in the meta-analysis was below 400 **(c)** The 95% CI spanned 0 **(d)** Insufficient studies for meta-analysis (Imprecision)
Inconsistency	**(e)** For outcomes included in the meta-analysis, I^2 value is larger or equal to 50% in the meta-analysis. **(f)** For outcomes not included in the meta-analysis, mixed results were reported.
Indirectness	**(g)** The participants, intervention, comparator intervention, outcome measure, or study design did not match between the included studies and the eligibility criteria for this review
Publication bias	**(h)** $p < 0.1$ on the two-tailed Egger's regression asymmetry test

Appendix 3. GRADE criteria

Key: Explanations for downgrading criteria	
Risk of bias	**(i)** For outcomes if less than half of the participants were from trials with PEDro scores larger or equal to 6.
Imprecision	**(j)** Insufficient studies for meta-analysis and the number of subjects included in the meta-analysis was below 400 for continuous outcome and below 2000 for dichotomous outcome. **(k)** The wide 95% CI spanned 0 **(l)** Insufficient studies for meta-analysis (Imprecision)
Inconsistency	**(m)** For outcomes included in the meta-analysis, I^2 value is larger or equal to 50% in the meta-analysis. **(n)** For outcomes not included in the meta-analysis, mixed results were reported.
Indirectness	**(o)** The **participants**, intervention, comparator intervention, outcome measure, or study design did not match between the included studies and the eligibility criteria for this review (e.g., only includes part of full spectrum of targeted participants)
Publication bias	**(p)** $p < 0.1$ on the two-tailed Egger's regression asymmetry test

3. Chapter 3

Longitudinal bone changes in the radius of individuals with chronic stroke

Ouyang Huixi

3.1 Abstract

Background Secondary osteoporosis is common after stroke, particularly in the hemi-paretic limbs. Previous study found that bone failure load was a significant risk factor to facture post stroke.

Purpose This prospective cohort study aimed to explore how bone density, macrostructure, and microstructure of the distal radius changed during chronic stage of stroke recovery (≥ 6 months duration) and to identify the factors associated with change in failure load of the distal radius.

Methods A total of 46 people with chronic stroke (mean age: 60 Years; mean post-stroke onset: 6 years, 21 females). Bone properties (e.g., bone mineral density, geometry, microstructures) and failure load of the bilateral distal radius were measured twice using high-resolution peripheral quantitative computed tomography (HR-pQCT), first at the time of study enrolment and again at 2-year follow-up. Blood flow volume of brachial artery was measured by Doppler Ultrasound. Multiple hierarchical regression analysis was applied to identify the potential predictors (e.g., physical activity, peripheral vascular health, hand sensation, etc.) of failure load.

Results All participants included in the analysis (n=90) were right-handed. Nearly half of the participants in stroke group had right paresis (n=25). There was a slight but significant increase in arm usage at 2-year follow up (p<0.01). On the paretic side, the change in trabecular bone density (2.29%), cortical vBMD (0.53%), cortical thickness

(3.61%), cortical area (3.45%), and the trabecular area (1.06%) exceeded precision error after two years of follow up. Among these, the change of cortical bone density (standardized Beta =0.39, p =0.004) and cortical thickness (standardized Beta =0.34, p =0.012) contributed most to the decrease in failure load of the hemi-paretic radius. In addition, both blood flow volume of the brachial artery (ΔF =5.31, p =0.027) and hand sensory (ΔF =4.26, p =0.046) at baseline significantly improved the regression model to predict the decline in failure load of the radius over the 2-year follow-up period.

Conclusions: Changes in cortical bone properties accounted most for the deterioration of failure load of the radius in people with chronic stroke. More compromised vascular health and hand sensation at baseline were associated with greater decline in failure load of the hemi-paretic distal radius.

Implications: Cortical bone properties continued to show deterioration during the chronic stage of stroke. Improving vascular health and hand sensation may be a potential target to mitigate secondary osteoporosis in individuals with chronic stroke.

Key words: bone, stroke, Peripheral Quantitative Computed Tomography

3.2 Introduction

The wrist is the second most common site of post-stroke fragility fracture.[86] Fractures in individuals with stroke often have poorer prognosis and require prolonged rehabilitative treatment compared with those in individuals without pre-existing disability.[87] The two major reasons for the increased rate of post-stroke fractures are

compromised bone strength and increased fall incidence.[84] Studies have shown that the rate of bone loss in the hemi-paretic upper limb can reach as high as 25% within the first year post-stroke.[166, 169] In one study, the difference in bone strength of the distal radius between the paretic and non-paretic sides 6 years after stroke onset reached as high as 23%, while the corresponding difference in healthy controls without stroke history was just 3%.[194]

Research has demonstrated a strong relationship between the bone strength index and muscle strength and has also found that the functional level of the hemi-paretic arm is associated with the decrease in proximal humerus bone mineral density (BMD) during the first year post-stroke.[169] Pang et al. found that in addition to physical activity, arm disuse and vascular elasticity were associated with BMD decline in this site.[181] More recently, Miller et al. found that apart from motor control impairment and arm disuse, upper limb spasticity was related to the relative side-to-side difference in estimated failure load in the distal radius during the chronic stage of stroke recovery.[194] However, one common limitation of these studies is their cross-sectional design. Moreover, BMD is not the only determinant of bone strength. Other bone characteristics such as bone geometry and microstructure are also important. Thus, research using a longitudinal design with measurement of different bone characteristics and clinical factors is warranted. A more comprehensive understanding of post-stroke changes in various bone properties and related modifiable factors is crucial for informing the design of exercise intervention trials for future research.

Estimated failure load, a surrogate measure of bone strength, is computed using a

multitude of bone variables (e.g., BMD, microstructure, and geometry) derived from high-resolution peripheral quantitative computed tomography (HR-pQCT).[247] In cadaver studies, estimated failure load has been found to be more strongly correlated with the actual failure load than with other bone variables measured using pQCT (e.g., vBMD or cortical thickness).[188] The ability of estimated failure load to predict fractures in older adults is also superior to that of other bone variables.[191] However, the extent to which the changes in different bone variables contribute to the changes in estimated failure load remains unknown. Accordingly, the associations of different post-stroke impairments with the changes in estimated failure load post-stroke are also uncertain.

To address the knowledge gaps identified above, a prospective cohort study was conducted to achieve the following objectives: 1) to assess the changes in bone density, geometry, and microstructure of the radius during the chronic stage post-stroke; 2) to assess how the changes in different bone variables contribute to the changes in estimated failure load; and 3) to assess the associations of changes in estimated failure load of the radius during the chronic stage post-stroke with post-stroke impairment variables.

3.3 Methods

Study design and ethics

This was a 2-year longitudinal study involving individuals with chronic stroke. Each participant provided written informed consent, and ethical approval was obtained

from the Joint Chinese University of Hong Kong – New Territories East Cluster Clinical Research Ethics Committee. All study procedures were conducted in accordance with the Declaration of Helsinki.

Recruitment of participants

Individuals with chronic stroke were recruited from community self-help groups, existing participant databases, and the general public. The inclusion criteria were diagnosis of a unilateral stroke confirmed by the individual's physician, age ≥ 18 years, stroke onset more than 6 months ago, medically stable, Modified Rankin Scale score of 2–5, ability to obey motor commands, and competency in providing informed consent. The exclusion criteria were other neurological conditions (e.g., Parkinson's disease), receptive aphasia, recurrent stroke, pregnancy, other conditions that had had a substantial impact on bone health (e.g., rheumatoid arthritis), taking medications for osteoporosis (e.g., bisphosphonates) currently and/or prior to stroke, known diagnosis of osteoporosis prior to stroke, fragility fractures prior to stroke, metal implants in the limb to be scanned, or another serious illness (e.g., cancer). Age- and sex-matched individuals with no history of stroke were also recruited, to serve as the control group.

Sample size estimation

The sample size estimation was based on an alpha of 0.05 (two-tailed) and power of 0.8. Based on a previous study,[30] the difference in percentage change in various HR-pQCT variables between the hemi-paretic and non-paretic sides during the late chronic stage post-stroke yielded Cohen's d values ranging from 0.25 to 1.67 (i.e.,

equivalent to f = 0.13–0.84). Using the smallest yielded effect size (f = 0.15) with two within-subject factors [side (non-paretic vs. hemi-paretic) and time (two assessments)], a sample size of 90 individuals (45 per group) would be needed to detect a significant side × time interaction effect.

Another line of analysis would involve multivariate regression to examine the associations of impairment variables with the estimated bone failure load. Our previous cross-sectional studies had generated R^2 values from 0.17 to 0.81 (equivalent to effect sizes f^2 = 0.20–4.26).[31, 177-180, 182] Using the smallest yielded effect size (f^2 = 0.20), an alpha of 0.05, and power of 0.80, a sample size of 42 would be needed to detect a significant association of estimated failure load with other factors.

In summary, assuming a 15% attrition rate, we aimed to recruit 54 individuals with chronic stroke and 54 healthy control participants for this study.

Demographic information

Basic demographic data (e.g., sex, age and supplementation) was collected from participants. Brain scan findings (e.g., computed tomography and magnetic resonance imaging that provided information on stroke type and location) were obtained from the medical records of the participants. In addition to the bone measurements, a list of clinical variables were measured. These outcomes were selected because they were implicated in post-stroke bone health in previous cross-sectional studies.[31, 177-180, 182] The bone scan was performed by a technician at a local bone imaging center with extensive experience in osteoporosis research. Two other qualified researchers with more than 5 years of research experience, and one research assistant performed the other

clinical measurements. All measurements, except the stroke-specific assessments, were administered to both groups.

Bone scan protocol

The bilateral distal radii of each study participant were assessed twice: at baseline and 2 years later. This time frame was chosen for two reasons. First, two previous longitudinal studies showed that while the bone masses of the hemi-paretic and non-paretic sides were substantially different in people who were well into the chronic stage (onset > 1 year ago), the temporal changes in bone variables within a 1-year period in these people were somewhat modest.[30, 181] Thus, the present study aimed to evaluate the changes in bone status during the chronic stage with a 2-year assessment interval. Given that the effective radiation dose for each HR-pQCT scan is only 3–4 μSv, the total effective radiation dose during the 2-year follow-up period would be approximately 12–16 μSv (4 scans: 2 bone sites × 2 time points), which is similar to that received during 1–1.3-h flight. The protocol here was thus very safe and did not pose any health hazards.

Bone imaging and image registration

Imaging and image registration: Volumetric BMD (vBMD), cross-sectional geometry, and microstructural properties of the bilateral distal radii were measured using HR-pQCT (XtremeCT II, Scanco Medical AG, Brüttisellen, Switzerland). The distal radius scan region was fixed at 9.5 mm proximal from the mid-joint line.[248] The length of the scan region spanned 9.02 mm proximally, which was equivalent to a

stack of 110 slices.[249] The analyses of bone images of corresponding sites were subjected to the same volume of interest (VOI) which matched between the baseline and 24-month measurements. A software program provided by the HR-pQCT manufacturer was used to identify the same region by matching the corresponding images with their total cross-sectional area. Image analyses were conducted only for the bone volume common to the measurements at the two time points.[249]

Image analysis: Analyses of 3D scan data were conducted using the Image Processing Language software (IPL v5.08b, Scanco Medical AG). First, bone images were analyzed using a standard (default) protocol.[187] Next, extended cortical analyses were conducted using a cortical compartment segmentation technique adapted from the study by Buie et al.[250] (µCT Evaluation v6.0, Scanco Medical AG).[192] This was done to achieve improved accuracy in the analyses of very porous or thin cortical shells.

Micro-finite element (mFE) analyses: All mFE analyses were performed using the FE-solver included in the built-in Image Processing Language software of HR-pQCT (IPL-FE v1.15, Scanco Medical).[247] A special peeling algorithm specifying a minimum cortical thickness of six voxels was used to identify cortical and trabecular bone tissue. mFE analyses were performed by converting the binary image data into a mesh of isotropic brick elements. For all elements, a Poisson's ratio of 0.3 was specified. Elements representing cortical and trabecular bone were both assigned a Young's modulus of 10 GPa.[247] A uniaxial compression test with a 1000 N load was performed with an applied strain of 1%. Whole-bone stiffness (kN/mm) was calculated.

Estimated failure load (N) was calculated based on the assumption that bone failure occurred if >2% of the elements were strained beyond 0.7% strain.[175]

The intra-rater reliability of these parameters was good (linear weighted kappa = 0.855).[38] Our HR-pQCT scanner had excellent reproducibility. The coefficient of variation (CV) was calculated as the standard deviation (SD) of the two repeated measurements of each participant divided by their mean. The short-term precision error was calculated as the root-mean-square average of the CV ($CV\%_{RMS}$) for each participant. According to our unpublished data from 32 individuals, the short-term reproducibility of the vBMD parameter, expressed as the $CV\%_{RMS}$, ranged from 0.46% to 0.89%, and the short-term reproducibility of the microarchitectural parameters (excluding cortical porosity) ranged from 0.88% to 1.99%. The 95% confidence least significant change (LSC) values (calculated as 2.77 × the precision error) are also provided in Table 3-1.

$$LSC = 1.96 \cdot \sqrt{CV\%_{RMS}^2 + CV\%_{RMS}^2} = 2.77 \cdot CV\%_{RMS}$$

Other clinical assessments

Muscle strength: Bilateral handgrip strength was measured using a hand-held Jamar dynamometer (Sammons Preston, Mississauga, Ontario, Canada).[251] During testing, the participants were instructed to squeeze the handgrip with the maximal effort. Verbal encouragement from the assessor was provided to elicit maximal effort from the participants. The test was conducted with the participants sitting with their shoulders in neutral position and elbows in 90° flexion. This technique has demonstrated good to excellent intra-rater (intraclass correlation coefficient [ICC] = 0.88–0.96) and test–

retest reliability (ICC = 0.71–0.94) in individuals with stroke and those with closed brain damage.[252]

Another dynamometer system (Humac Norm Systems, Stoughton, Massachusetts, USA) was used to measure the isometric peak torque (N/m) of the elbow flexors.[253] The elbow flexor was tested at 60° flexion because it has been shown that the greatest elbow flexion torque on the paretic side of individuals with stroke occurs at 60° flexion.[254] The test–retest reliability of the isometric elbow flexor test has previously been shown to be excellent (ICC = 0.97).[255] The tests were repeated three times with 1-minute rest intervals. The grip and elbow flexion strength values obtained from the three trials were averaged and used for subsequent analyses.

Muscle stiffness: A hand-held MyotonPRO device (Myoton AS, Tallinn, Estonia) was used to measure the dynamic stiffness of the flexor carpi ulnaris (in N/m), an important muscle involved in the gripping function of the hand. Measurements were performed by placing the probe applicator perpendicularly to the center point of the muscle belly. Then, a mechanical impulse with quick release to the muscle was applied percutaneously. The durometer measured the stiffness of muscle tissue by applying mechanical deformation to the surface. The device has previously demonstrated excellent test–retest reliability for the medial gastrocnemius in individuals with spinal cord injury (ICC = 0.88–0.97)[256] and for hand grip muscle (the flexor carpi ulnaris) in people with stroke (ICC = 0.87–0.90)[257].

Sensory function: The touch pressure threshold was tested using the Semmes–Weinstein monofilament test (SWMT).[258, 259] In the upper limb, the dorsum of the

hand (at the metacarpophalangeal joint of the middle finger and mid-length of the third metacarpal bone) was tested, because these are the sites where the greatest deficit in cutaneous sensation tends to occur in people with stroke.[258] In individuals with chronic stroke, the intra-rater agreements on the SWMT grade score were substantial to perfect (Cohen's kappa: thumb = 0.89, index finger = 0.80) and the inter-rater agreements were substantial (Cohen's kappa: thumb = 0.75, index finger = 0.79).[259]

Physical activity level: The Physical Activity Scale for the Elderly (PASE)[260] was used to evaluate the physical activity level (score range: 0–400). Twelve questions were asked regarding the frequency and duration of leisure activities (e.g., jogging, swimming, and strength and endurance exercise), household activity, and work-related activity during the last 7 days. Higher scores indicated higher activity levels. The Chinese version of the PASE has demonstrated good test–retest reliability in older adults (ICC = 0.81).

Vascular health: The Aixplorer ultrasound scanner (Aixplorer, SuperSonic Imagine, Aix en-Provence, France) was employed with a linear array probe (4-15 MHz, SuperLinear, 15-4, Vermon, France). The blood flow velocity (cm/s) and blood flow volume (cc/min) of the brachial artery on each side were measured using a Doppler ultrasound.[261] Arterial diameter (cm) was measured by placing the calipers at each end of the superior and inferior borders of the endothelial wall. The settings of the Doppler ultrasound were standardized for high sensitivity, medium wall filter, a pulsed repetition frequency of 7229 Hz. To ensure that the blood flow was measured with the participants in a resting state, all participants were rested in a supine position for at least

10 minutes before the ultrasound examination. The room temperature was set at 23℃. The scanning of the two sides was conducted in a randomized order.

The participants were in a supine position throughout the ultrasound examination. The scan location of the brachial artery was standardized at the distal third point (i.e., 33%) between the coracoid process and the crease of cubital fossa of the medial side.[194, 262] To exclude the influence of stiffness of elongated biceps and considering that the elbow joint range of motion was limited because of spasticity in some individuals with stroke, the elbow joint was kept at 60° flexion using a custom arm immobilization device. To expose enough skin area for scanning with the ultrasound probe, the shoulder was maintained at 45° abduction. The following steps were applied to obtain the optimal image of the brachial artery:

Step 1: B-mode was applied in the beginning to find the artery with best image resolution. After finding the distal one-third points of the upper arm, the probe was initially placed perpendicularly to the humerus to find the cross-sectional plane of the artery.

Step 2: Color Doppler was used for visual confirmation of the artery. Thereafter, the probe was slightly moved medially or laterally to obtain the cross-sectional area in the middle of the visualization window, such that it was beneath the middle of the probe.

Step 3: The probe was rotated in the sagittal plane and tilted to be parallel with the artery. Fine adjustments were made to obtain an image with the longest continuity and the highest contrast between the blood and artery wall.

Step 4: An electronic measurement point on the visualization window was located

at the center of the artery with the smallest insonation angle, which is the acute angle between the ultrasound beam and the flow direction of the artery.

Step 5: Pulse wave mode was selected. The angle-to-flow was optimized with an insonation ≤ 60° by modulating with Doppler steering and fine angle correction. The sample volume was set at 0.5 mm. To detect the full range of positive and negative flow, the auto-trace function was applied.

Three consistent spectral waveform cycle readings were used in one measurement. Each measurement was performed three times to calculate the average outcomes for both sides. Based on our pilot trial involving 15 individuals with chronic stroke, good to excellent inter-rater (ICC = 0.87–0.92) and intra-rater reliability (ICC = 0.92–0.98) were demonstrated for the peak systolic velocity and diameter of the brachial artery. As illustrated in Table 3-2, moderate inter-rater reliability (ICC = 0.59–0.60) and good to excellent intra-rater reliability (ICC = 0.82–0.93) were shown for the peak volume flow of the brachial artery.

Stroke-specific assessments

Motor Activity Log (MAL): The MAL was used to measure the usage frequency and movement quality of the affected arm and hand during 30 daily activities.[263] Two scores were rated for each activity: the amount of use (AOU) and the quality of movement (QOM) of the paretic arm. The AOU was assessed based on the question "How much was the paretic upper arm used in this activity?" Scores ranged from 0 (never used the affected arm for this activity) to 5 (always used the affected arm for this activity). The QOM was scored based on the question "How well did the paretic arm

participate in the activity?" Each item/activity was scored from 0 (the affected arm could not be used for this activity) to 5 (the affected arm could be used as well as before stroke for this activity). We calculated the mean MAL score by averaging the scores for the 30 items. The MAL has demonstrated high internal consistency in participants with stroke (AOU: Cronbach's alpha = 0.88; QOM: Cronbach's alpha = 0.91).[263] The test–retest correlation coefficient (Pearson's r) was 0.91 for the QOM and 0.50 for the AOU. This scale has good convergent validity. The correlation between the QOM scale and wrist accelerometer recordings (3 days) was 0.7 ($p < 0.01$).[264] The correlation between the MAL (QOM and AOU) and Action Research Arm Test was moderate (Spearman's rho = 0.63).[263]

Motor recovery: The Fugl-Meyer Motor Assessment was used to assess the degree of motor recovery of the hemi-paretic upper limb in terms of movement quality, movement coordination, and reflex action of the shoulder, elbow, forearm, wrist, and hand. Items were scored using a 3-point ordinal scale (0 = could not perform, 1 = performed partially, and 2 = performed fully). The total score ranged from 0 to 66, with lower scores indicating more severe motor impairment. This tool has previously shown excellent inter-rater reliability (ICC = 0.96).[265]

Spasticity: Spasticity of the wrist/finger flexors was measured using the Composite Spasticity Scale (total score range: 1–16).[266] Higher scores indicate higher spasticity. The scale includes three items: 1) bicep tendon jerk (score range: 0–4); 2) resistance to full range of motion for elbow flexion (score range: 0–8); 3) the duration and amount of wrist clonus elicited (score range: 1–4). This scale has

previously shown excellent test–retest reliability (ICC = 0.97).[266]

Statistical analysis

First, the data in both the groups were summarized separately using descriptive statistics as appropriate. An independent t-test or Mann–Whitney U test or chi-square test was used to assess the between-group differences in demographic variables, depending on whether the criteria for parametric statistics were fulfilled.

Second, for each bone outcome and functional outcomes with two sides (muscle strength, muscle stiffness, sensory, and vascular ultrasound measurement), generalized estimating equation analysis [within-subject factors: side (2 levels: stroke: hemi-paretic vs. non-paretic; healthy: dominant vs. non-dominant); time (2 levels: baseline, 2-year follow-up)] were used to detect whether there was an interaction effect between the two factors in these two groups. A separate paired t-test and/or Wilcoxon signed-rank test was performed for each side if an interaction effect was found. The independent t-test and/or Mann–Whitney U test was also applied to compare the 2-year bone changes in each side between the two groups.

Third, we were interested in determining which aspects of bone (e.g., densitometric, microstructure, and cortical vs. trabecular) contributed more to the relative change in estimated failure load. To that end, we first used Pearson's correlation analysis to assess the correlation between the change in failure load and the changes in other bone variables in the stroke group. Next, those bone variables that showed correlations with the relative change in failure load ($p \leq 0.1$) were entered into a multivariate regression model to identify their associations with the change in estimated

failure load. To avoid multicollinearity, independent variables with a variance inflation factor (VIF) above 10 or strong intercorrelations ($r > 0.7$)[267] were eliminated from the prediction model.

Fourth, a second hierarchical multiple regression analysis was used to identify which clinical variables at baseline were predictive of relative changes in estimated failure load. Likewise, bivariate correlations between the change in estimated failure load on the paretic side in the stroke group (i.e., dependent variables) at the 2-year follow-up and potential influencing clinical variables (e.g., muscle strength, spasticity, and upper arm disuse) measured at baseline were examined. The variables that showed significant correlations with the dependent variable ($p < 0.1$) were selected as the independent variables and entered into the subsequent hierarchical multivariate regression analysis, while adjusting for potentially confounding factors (i.e., demographics). Because many demographic factors (e.g., age, sex, and stroke duration) were measured, these were entered into the principal component analysis (i.e., data reduction). Factors extracted from the principal component analysis were first entered into the regression model, followed by the clinical impairment variables identified above.[58]

Lastly, a third multivariate regression analysis was used to examine the associations of the *changes* in clinical variables with the change in estimated failure load during the 2-year follow-up period, after accounting for the demographic factors mentioned above. The relative change values of all clinical outcome measures except hand sensory function were calculated using the formula $[(T1 - T2)/T1]$. As the sensory threshold

does not have a zero point, it was not appropriate to use the relative change value for hand sensory function, and therefore, the absolute change value (T1 − T2) was used instead. The clinical variables were selected following the same principles as those for selecting the independent variables as described above.

In addition, the percentage change in height (to adjust for the potential variation in the location of the scan region) was added to the three prediction models to check whether the results remained stable.

3.4 Results

Participants' characteristics

The current study recruited 64 individuals with chronic stroke, 18 of whom dropped out, and 64 healthy older adults as controls, 19 of whom dropped out for various reasons. In addition, the bone data of one participant from the stroke group were deemed to be invalid because of motion artifacts. Thus, 45 stroke individuals and 45 healthy older adults were included in the final analysis. The details of study flow are illustrated in Figure 3-1. All participants included in the analysis (n=90) were right-handed. Nearly half of the subjects in stroke group had right-sided paresis (n=25). Calcium or vitamin D supplementation was seldom taken by our participants. More details about participant characteristics are provided in Table 3-3.

Changes in bone outcomes

Significant time effects were observed in total vBMD, trabecular area, cortical area, cortical vBMD, cortical perimeter, and cortical thickness in both the stroke and control

groups ($p < 0.05$). Significant time effects in trabecular vBMD (Wald Chi-square = 4.44, $p = 0.035$), bone stiffness (Wald Chi-square = 30.23, $p < 0.001$), and estimated failure load (Wald Chi-square = 23.27, $p < 0.001$) were only apparent in the stroke group, whereas significant time effects in trabecular number (Wald Chi-square = 11.32, $p = 0.001$), trabecular separation (Wald Chi-square = 6.28, $p = 0.012$), and cortical porosity (Wald Chi-square = 13.65, $p < 0.001$) were only found in the control group. A significant time × side interaction effect in trabecular vBMD was only seen in the stroke group (Wald Chi-square = 7.52, $p = 0.006$). Detailed results are provided in Table 3-4 and Table 3-5.

Post-hoc analysis revealed that in both the stroke and control groups, total vBMD, cortical area, cortical thickness, and trabecular area decreased significantly on both sides ($p < 0.001$). A significant decline in failure load in both arms was found in the stroke group only ($p < 0.001$). Notably, trabecular vBMD decreased on the paretic sides of stroke survivors only ($p < 0.001$). Further details are provided in Table 3-6.

The relative changes in bone variables in both the groups are illustrated in Table 3-7. The relative change in total vBMD exceeded the LSC on both sides in both the groups. The relative changes in cortical area and cortical thickness above the LSC were on the paretic side in the stroke group only. A comparison of the relative changes on the paretic side of the stroke group with those on the non-dominant side in the control group revealed a greater decline in cortical thickness (stroke: 3.61%, controls: 1.72%, $p = 0.009$), bone stiffness (stroke: 3.81%, controls: 0.30%, $p = 0.006$), and estimated failure load (stroke: 3.32%, controls: 0.39%, $p = 0.017$) in the stroke group.

Changes in clinical variables

In the stroke group, significant time effects were found in bicep stiffness (Wald Chi-square = 24.13, $p < 0.001$), brachial artery diameter (Wald Chi-square = 30.11, $p < 0.001$), and hand sensation (Wald Chi-square = 11.04, $p < 0.001$). The time × side interaction effects of the peak volume flow (Wald Chi-square = 3.18, $p = 0.075$) and diameter (Wald Chi-square = 3.37, $p = 0.066$) of the brachial artery did not quite reach statistical significance (Tables 3-8 and 3-9)

In the stroke group, as illustrated in Table 3-10, post-hoc analysis revealed that the peak volume flow of the brachial artery tended to increase on the paretic side ($p = 0.055$), but not the non-paretic side, during the 2-year follow-up period ($p = 0.938$). After 2 years, there were slight increases in the AOU and QOM of MAL scores ($p < 0.001$), together with slightly improved motor control in the paretic arm ($p = 0.005$), but muscle stiffness had increased significantly on both sides ($p < 0.001$) without a significant change in bicep muscle strength. In contrast, light touch sensation became less sensitive on both the paretic ($p = 0.032$) and non-paretic sides ($p = 0.001$). There was a decreasing trend in the physical activity level from baseline to the 2-year follow-up ($p = 0.061$).

In the control group, a significant main effect of time was observed in bicep strength (Wald Chi-square = 94.39, $p < 0.001$), bicep stiffness (Wald Chi-square = 7.26, $p < 0.001$), peak volume flow (Wald Chi-square = 28.96, $p = 0.075$), and diameter (Wald Chi-square = 91.01, $p = 0.066$) of the brachial artery and hand sensation (Wald Chi-square = 16.39, $p < 0.001$). A significant time × side interaction effect was also found

in the peak volume flow (Wald Chi-square = 3.18, p = 0.075) of the brachial artery. Additionally, significant increases were observed in bicep strength and peak volume flow of the brachial artery on both the dominant and non-dominant sides ($p < 0.001$) and in bicep stiffness on the dominant side ($p = 0.006$). Sensitivity for light touch sensation was also decreased in both hands at the 2-year follow-up compared with baseline ($p < 0.001$). Additional details are provided in Table 3-11.

Prediction of the change in estimated failure load by other bone variables

The relative change in failure load was found to be significantly correlated with the relative changes in total vBMD ($r = 0.68$, $p < 0.001$), cortical area ($r = 0.61$, $p < 0.001$), trabecular area ($r = -0.60$, $p < 0.001$), cortical vBMD ($r = 0.59$, $p < 0.001$), and cortical thickness ($r = 0.53$, $p < 0.001$). The association between the changes in trabecular vBMD and failure load was marginally significant ($r = 0.25$, $p = 0.100$) (Table 3-12).

The changes in both cortical bone density (standardized Beta = 0.39, p = 0.004) and cortical thickness (standardized Beta = 0.34, p = 0.012) were significant contributors to the decrease in failure load of the hemi-paretic radius. The changes in cortical vBMD and cortical thickness significantly increased the accuracy of the regression model ($R^2 = 0.46$) in predicting the change in failure load, accounting for 35% and 9% of the variance, respectively (Table 3-13). The changes in cortical area (VIF > 10) and trabecular area ($r = -0.76$) were not included in the prediction model because of concern over multicollinearity due to their strong correlations with the change in cortical thickness.

Prediction of the change in estimated failure load by clinical factors measured at baseline

According to Table 3-14, only the baseline blood flow volume ($r = -0.31$, $p = 0.036$) and hand sensation ($r = 0.32$, $p = 0.032$) were significantly correlated with the change in estimated failure load. After adjusting for the influence of relevant demographic factors (factors 1–4: e.g., age, gender, and supplementation), both vascular health and hand sensation at baseline significantly improved the prediction of the change in estimated failure load, accounting for 10% and 11% of the total variance, respectively. Smaller blood flow volume of the brachial artery on the paretic side at baseline (standardized Beta = −0.35, $p = 0.027$) emerged as a significant predictor of a greater decline in the estimated failure load of the radius over the 2-year follow-up (Table 3-15).

Next, the relationships between the *changes* in clinical factors and the change in estimated failure load was explored. A greater decline in physical activity ($r = 0.27$, $p = 0.076$) was found to be associated with a greater decrease in the failure load of the radius on the paretic side. The subsequent multivariate hierarchical regression analysis showed that after adjusting for the effects of demographic factors (factors 1–4), the effect of the change in physical activity level on the change in estimated failure load was diminished (Table 3-16).

3.5 Discussion

In summary, a significantly greater decline was observed in the estimated failure load of the distal radius on the paretic side of stroke participants than in their healthy

counterparts over the 2-year follow-up period. Among the various bone parameters, declines in cortical vBMD and cortical thickness were the most important contributing factors to the overall reduction in the estimated failure load. A higher blood flow volume and poorer hand sensation measured at baseline were predictive of less decline in estimated failure load during the 2-year follow-up period.

Differences in bone changes between the stroke and control groups

There was a significantly greater decline in cortical area, cortical thickness, and estimated failure load on the paretic side in the stroke group than in the control group (*cortical area*: stroke participants 3.45%, healthy controls 1.79%; *cortical thickness*: stroke participants 3.61%, healthy controls 1.72%; *failure load*: stroke participants 3.32%, healthy controls 0.39%). In summary, a greater loss in cortical bone, rather than trabecular bone, was observed in the stroke group than in the control group. As a non-weight-bearing bone, the compressive stimuli to both cortical bone and trabecular bone may not be as substantial a factor in the radius as in weight-bearing bones. Thus, the major mechanical force resources originate from muscle contraction. As the superficial cortical bone is directly connected with muscle tendons, which transmit the mechanical stimulation from muscle contraction, unlike the profundal trabecular bone, the decreased tensile forces from compromised muscle contractions after stroke can have more adverse effects on cortical bone than on trabecular bone.

A previous study by Pang et al. also found a significant decline in total vBMD (2.5%) and compressive bone strength index (CBSI; 6.7%) in the epiphysis radius on the paretic side at 1-year follow up.[268] However, the current study showed that the

decline in total vBMD at the distal radius was similar (3.3%) despite the follow-up period being double of that in the study by Pang et al. As the post-stroke duration in the current study was longer (mean: 6 years) than that in the study by Pang et al. (mean: 4 years), the overall results may imply that the rate of change slows down as chronicity increases.

Pang et al. reported a much greater change in the bone strength index (CBSI) than the change observed in estimated failure load (3.3%) in the current study. This can be explained by two factors. First, although both the CBSI (r = 0.92) and estimated bone stiffness (r = 0.94) have been shown to be strongly related to the exact failure load during mechanical testing in human cadaver studies,[37, 188] the precision error (4.5%) of the CBSI[268] generated from the first generation of pQCT with lower resolution used in the study by Pang et al. was larger than the estimated failure load (precision error: 2%–3.3%) obtained in our study using HR-pQCT with higher resolution (pixel size: 0.2–1 mm vs. 82 μm, respectively).[269] Second, the difference in the computation methods may also have contributed to the discordance in the results. While only the total area and total vBMD were considered in the computation of the CBSI,[270] the estimated failure load was calculated based on factors including density, geometry, and microstructure (e.g., trabeculae separation and thickness).[247]

Percentage bone change, failure load, and fracture risk

The current study found that the changes in three bone properties (cortical vBMD, trabecular vBMD, and cortical thickness) accounted for 46% of the variance in

estimated failure load among participants with chronic stroke. A review conducted by the Bone Microarchitecture International Consortium in 2019, which included eight prospective cohort studies (n = 7,254), found an improved fracture prediction in older adults through the addition of trabecular vBMD, cortical vBMD, and cortical thickness rather than only using femoral neck areal BMD or fracture risk assessment tool (FRAX) scores.[191] Additionally, known group validity was established for total vBMD, cortical vBMD, and cortical area for the prediction of forearm fracture risk in children (approximately 200 children per group).[176] In that study, the total vBMD values in 4% of the radius (3.4%; p = 0.02) and cortical vBMD (0.9%, p = 0.01) and cortical area (2.8%, p = 0.03) in 20% of the radius were lower in children with fracture than in healthy controls.

Considering the strong correlation between the estimated failure load and actual failure load and the superior ability of estimated failure load to predict fracture risk compared with other bone parameters,[191] the relationship between the percentage decline in estimated failure load and actual fracture risk has also been investigated in previous studies. A recent review with four prospective HR-pQCT studies found that the radial failure load in people after fracture was lower than that in people without fracture by 9.1% (6.7%–11.5%).[269] Moreover, forearm fracture risk was most strongly associated with radial failure load. The 5-year hazard ratio (HR) was 2.13 (1.77–2.56) per SD decrease in failure load (811 N) and 1.58 (1.45–1.72) per SD decrease in radius vBMD.[191] This suggested that for every 811 N decrease in failure load, the fracture risk would increase twice from baseline in older adults. Considering

that the estimated failure load was already 24.6% (990 N) lower on the paretic side than on the non-paretic side at baseline and that it further decreased by 3.3% (97 N) during the 2-year follow-up period, it is presumed that the forearm fracture risk among individuals with chronic stroke would be substantially increased.

Factors predictive of bone loss: vascular health at baseline

In this study, the decreased failure load during the chronic stage of stroke recovery was independently associated with baseline blood flow volume. Only two longitudinal studies have investigated the changes in bone health and its related factors.[172, 268] Similar to the current findings, Pang et al. found that lower vascular elasticity ($\rho = 0.620$, $p = 0.004$) at baseline was significantly associated with a greater decline in cortical thickness on the paretic side of the radius diaphysis at 1-year follow-up.[268] In addition, a recent large cohort study on older people ($n \approx 160{,}000$, mean age = 58 years) also supported the association between bone quality (i.e., ultrasound travelling speed through bone) and vascular health (arterial stiffness).[271] As bone is a highly vascularized structure, it is not entirely surprising that compromised vascular function may have detrimental effects on bone health. The mechanism underlying this association is still unclear, although studies have suggested that cytokines and oxidized lipids may play a role.[268, 272] Further research is warranted to elucidate the physiological mechanisms underlying the link between vascular health and bone health.

Factors predictive of bone loss: hand sensation at baseline

Poorer hand sensation on the paretic side at baseline was correlated with a greater

decline in estimated failure load of the distal radius on that side. A recent review on the physiological interactions between the nervous and skeletal systems reported evidence of a bone–nerve crosstalk.[273] Specifically, the peripheral sensory nerve in the skeleton may play an essential role in promoting bone regeneration by providing trophic factors (e.g., neuropeptides: calcitonin gene-related peptide, vasoactive intestinal peptide, and substance P).[274] Among these molecules, calcitonin gene-related peptide was indicated to enhance osteoblast activity,[274] whereas vasoactive intestinal peptide was proved to enable bone resorption.

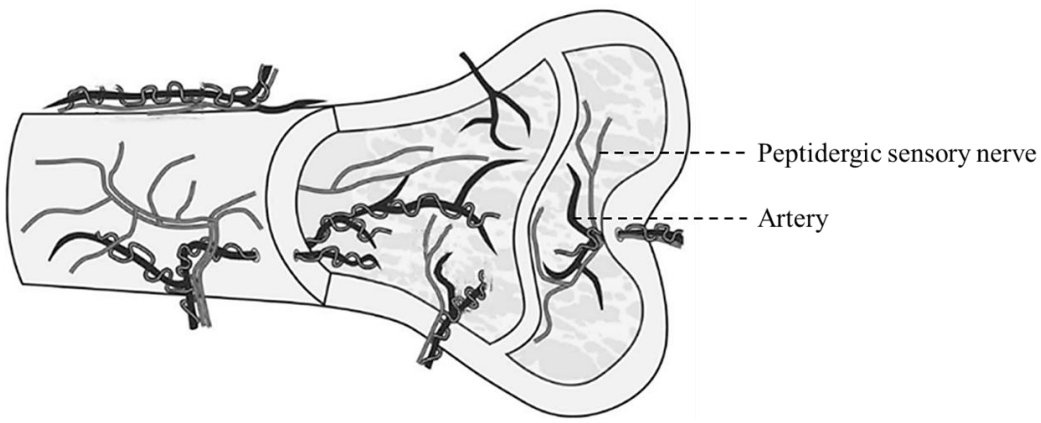


Figure 5-1 Distribution of sensory nerves in bone. (Adapted from Brazill et al, 2019) [275]

Similar to the skin sensory nerves, the skeletal sensory nerves originate from the dorsal root ganglion next to the spinal cord and can sense pain, pressure, and other stimuli.[275] Furthermore, the nerve supply to the long bones continues from the sensory nerve supply to the muscles and skin.[274] It has also been shown that stroke, as a central nervous system disorder, may impair skeletal sensory nerves in addition to cutaneous sensory function. Such a stroke-induced loss in skeletal sensation may impair its protective role in bone formation. This indicates that the skeletal sensory nerve could

be a potential target for interventions to improve bone health.

Clinical implications

Vascular health and sensory function may be the potential targets of future interventional studies that aim to improve upper limb bone health in individuals with chronic stroke. Thus, it is recommended that evaluations of peripheral vascular health and sensation be added to the clinical practice routine. Vascular health and hand sensation may be potential targets for interventions aimed at improving post-stroke bone health. For example, 6 months of aerobic treadmill exercise training has been shown to improve both resting and reactive hyperemic blood flow in the paretic limb.[276] A perceptual learning-based sensory discrimination program has been shown to be effective in improving sensory capacity.[277] Future research can investigate the efficacy of these programs in improving bone outcomes in individuals with stroke.

Limitations

The study period overlapped with the COVID-19 pandemic period. The level of physical activity reported may not truly reflect the typical physical activity level on a typical day before the onset of the pandemic. The study had a relatively high attrition rate (28% in both stroke participants and controls), probably because of the long follow-up period, which may have reduced the statistical power. Because of the large number of variables involved, we used factor analysis as a data reduction method for our regression analysis. Future studies with larger samples are warranted to examine the

independent effects of individual variables on the decline in failure load.

3.6 Conclusion

In conclusion, this study found that the estimated bone strength of the distal radius on the paretic side continues to decline in people with chronic stroke, mostly because of the decrease in both the BMD and thickness of cortical bone. Better vascular health, as indicated by a higher blood flow volume, and better hand sensation at baseline were predictive of less decline in failure load over the 2-year follow-up period. These findings should be useful in guiding the design of treatment strategies to address post-stroke bone loss in future intervention research trials.

3.7 List of Tables

Table 3-1 Short-term reproducibility and least significant change of bone outcomes measured by HR-pQCT.

Unpublished data for radius	**Bone variables**	**Precision error ($CV\%_{RMS}$)**	**Least significant change**
Bone Density	Total vBMD	0.59%	1.63%
	Cortical vBMD	0.46%	1.27%
	Trabecular vBMD	0.89%	2.47%
Bone Morphometry	Cortical Area	0.94%	2.60%
	Cortical Perimeter	0.31%	0.86%
	Cortical Thickness	0.88%	2.44%
	Trabecular Area	0.47%	1.30%
Trabecular and cortical Microarchitecture	Trabecular Number	1.99%	5.51%
	Trabecular Thickness	0.58%	1.61%
	Trabecular Separation	1.77%	4.90%
	Cortical Porosity	12.46%	34.51%

Table 3-2 Intra-rater and inter-rater reliability of Doppler Ultrasound measurement for brachial artery (n=15).

		Peak systolic velocity (cm/s)		Diameter (mm)		Peak volume flow (cc/min)	
		P	NP	P	NP	P	NP
Inter-rater (ICC 2,3)		0.87	0.88	0.96	0.92	0.59	0.60
Intra-rater (ICC 3,3)	Rater 1	0.97	0.92	0.99	0.97	0.93	0.82
	Rater 2	0.95	0.96	0.97	0.98	0.86	0.93

Note: P: paretic side; NP: non-paretic side.

Table 3-3 Demographic information.

	Stroke (n=45)	**Control (n=45)**	***p***
Basic demographics			
Age (year)	60.7± 7.2	57.7± 6.3	0.041*
Body Mass Index (kg/m^2)	24.2±3.1	23.4±2.8	0.052
Stroke duration (year)	6.4±4.2	NA	NA
Gender (Female/male, n)	20/25	17/28	0.669
Dominant hand (L/R, n)	0/45	0/45	1.000
Paretic hand (L/R, n)	25/20	NA	NA
Postmenopausal women (Yes/no, n)	18/27	15/30	0.662
Postmenopausal (year, women only)	15.2±13.5	10.3±9.0	0.299
Alcohol history (non-drinker/drinking history, n)	36/9	28/17	0.103
Smoking history (non-smoker/ smoking history, n)	35/10	33/12	0.807
Modified Rankin Scale (1/2/3, n)	2/31/12	NA	NA
Abbreviated Mental Test (Max:10)	9.33±1.02	9.93±0.25	<0.001*
Co-morbid conditions			
Hypertension, n	25	16	0.090
Diabetes, n	10	6	0.409
High cholesterol, n	15	4	0.009*
Total number of comorbidities	1.4±1.4	0.7±1.0	0.007*
Medications/supplements			
Antihypertensive agents, n	26	12	0.005*
Anticoagulants, n	16	0	<0.001*
Anticonvulsive agents, n	4	0	0.117
Hypolipidemic agents, n	26	7	<0.001*
Hypoglycemic agents, n	7	4	0.522
Antidepressants, n	5	0	0.056
Proton Pump Inhibitors, n	19	0	<0.001*
Calcium, n	1	3	0.616
Vitamin D, n	2	0	0.494
Total number of medications	4.0±2.6	0.8±1.3	<0.001*

Note: Mean ± SD. *: $p<0.05$.

Table 3-4 Generalized estimating equation: effect of time and side on bone variables in the stroke group (n=45)

	Main effect				Interaction	
	Time		**Side**		**Side*time**	
	Wald Chi-square	p	Wald Chi-square	p	Wald Chi-square	p
Total vBMD (mg HA/cm3)	29.30	<0.001*	139.53	<0.001*	0.90	0.343
Trabecular area (mm2)	33.60	<0.001*	8.34	0.004*	0.00	0.966
Trabecular vBMD (mg HA/cm3)	4.44	0.035*	72.60	<0.001*	7.52	0.006*
Trabecular number (1/mm)	2.01	0.157	35.38	<0.001*	0.81	0.369
Trabecular thickness (mm)	0.49	0.486	0.65	0.422	1.19	0.276
Trabecular separation (mm)	0.01	0.911	23.77	<0.001*	0.00	0.959
Cortical Area (mm2)	47.70	<0.001*	81.56	<0.001*	0.42	0.515
Cortical vBMD (mg HA/cm3)	5.52	0.019*	65.12	<0.001*	0.34	0.558
Cortical Perimeter ((mm)	7.46	0.006*	0.07	0.793	0.57	0.449
Cortical Porosity (%)	0.87	0.352	0.84	0.361	0.14	0.709
Cortical Thickness (mm)	47.40	<0.001*	75.42	<0.001*	0.60	0.437
Stiffness (kN/mm)	30.23	<0.001*	101.17	<0.001*	0.79	0.373
Failure load (N)	23.27	<0.001*	107.11	<0.001*	0.00	0.952

Note: *: $p < 0.05$.

Table 3-5 Generalized estimating equation: effect of time and side on bone variables in controls. (n=45)

	Main effect				Interaction	
	Time		**Side**		**Side*time**	
	Wald Chi-square	p	Wald Chi-square	p	Wald Chi-square	p
Total vBMD (mg HA/cm3)	33.43	<0.001*	2.54	0.111	0.05	0.820
Trabecular area (mm2)	27.46	<0.001*	7.28	0.007*	0.38	0.540
Trabecular vBMD (mg HA/cm3)	3.33	0.068	0.25	0.617	0.02	0.901
Trabecular number (1/mm)	11.32	0.001*	3.08	0.079	0.83	0.362
Trabecular thickness (mm)	1.12	0.291	6.18	0.013*	1.14	0.287
Trabecular separation (mm)	6.28	0.012	1.77	0.183	3.22	0.073
Cortical Area (mm2)	27.42	<0.001*	3.33	0.068	0.03	0.873
Cortical vBMD (mg HA/cm3)	46.33	<0.001*	0.84	0.359	0.05	0.831
Cortical Perimeter ((mm)	10.01	0.002*	15.27	<0.001*	4.33	0.038*
Cortical Porosity (%)	13.65	<0.001*	0.15	0.703	0.06	0.811
Cortical Thickness (mm)	31.87	<0.001*	0.09	0.766	0.05	0.832
Stiffness (kN/mm)	2.71	0.100	6.67	0.010*	2.38	0.123
Failure load (N)	3.06	0.080	5.87	0.015*	2.22	0.136

Note: *: $p < 0.05$.

Table 3-6 Radius HR-pQCT variables for the stroke and control groups

	Stroke (n=45)						Control (n=45)					
	Baseline		2-Year Follow up		*p*		Baseline		2-Year Follow up		*p*	
	P	NP	P	NP	P	NP	ND	D	ND	D	ND	D
Total vBMD (mg HA/cm^3)	294.67±87.53	359.37±67.77	287.71±85.46	351.88±67.89	<0.001**	<0.001**	339.86±63.86	334.80±65.14	332.87±63.51	328.04±65.53	<0.001**	<0.001**
Trabecular area (mm^2)	186.91±50.52	179.48±51.19	188.72±51.94	181.15±51.19	<0.001**	<0.001**	195.16±59.70	201.54±54.75	196.45±59.61	202.68±54.61	<0.001**	<0.001**
Trabecular vBMD (mg HA/cm^3)	117.61±51.51	149.42±36.98	117.00±51.06	149.44±37.75	0.015*	0.964	135.12±37.36	135.80±39.57	134.15±37.96	134.90±40.12	NA	NA
Trabecular number (1/mm)	1.10±0.28	1.27±0.17	1.11±0.30	1.28±0.19	NA	NA	1.20±0.20	1.23±0.20	1.19±0.20	1.21±0.19	0.144	0.012*
Trabecular thickness (mm)	0.23±0.02	0.23±0.02	0.23±0.02	0.23±0.02	NA	NA	0.23±0.02	0.23±0.02	0.23±0.02	0.23±0.02	NA	NA
Trabecular separation (mm)	0.95±0.36	0.74±0.12	0.94±0.34	0.74±0.13	NA	NA	0.81±0.19	0.78±0.15	0.81±0.18	0.79±0.16	0.125	0.008**
Cortical Area (mm^2)	55.49±14.25	65.57±13.32	53.95±14.31	64.02±13.58	<0.001**	<0.001**	65.86±12.19	67.01±13.83	64.70±12.15	65.89±13.87	<0.001**	<0.001**
Cortical vBMD (mg HA/cm^3)	870.70±76.42	914.17±53.77	865.68±68.01	906.82±50.90	0.164	0.001**	916.61±57.54	913.89±55.11	907.18±56.96	904.14±53.53	<0.001**	<0.001**
Cortical Perimeter ((mm)	64.99±8.20	65.06±7.89	65.12±8.33	65.22±7.96	0.012*	0.001*	66.97±8.81	68.19±8.16	67.00±8.73	68.41±8.19	0.424	0.007**
Cortical Porosity (%)	0.01±0.01	0.01±0.01	0.01±0.01	0.01±0.01	NA	NA	0.01±0.01	0.01±0.01	0.01±0.01	0.01±0.00	0.013	0.005**
Cortical Thickness (mm)	1.03±0.26	1.21±0.22	1.01±0.27	1.18±0.22	<0.001**	<0.001**	1.18±0.18	1.18±0.21	1.16±0.18	1.16±0.21	<0.001**	<0.001**
Stiffness (kN/mm)	53787.96±17976.30	69471.39±17754.57	52172.11±17589.57	67801.21±17783.85	<0.001**	<0.001**	66857.11±15487.10	69159.36±17170.48	66765.00±16152.75	68150.52±17517.99	NA	NA
Failure load (N)	2863.13±960.62	3782.85±963.03	2793.82±945.27	3689.89±964.24	<0.001**	<0.001**	3625.03±865.37	3744.53±932.78	3615.93±897.15	3684.97±953.10	NA	NA

Note: P: paretic side; NP: non-paretic side; ND: non-dominant side; D: dominant side. *: $p < 0.05$, **: $p<0.01$.

Table 3-7 The relative change of radius HR-pQCT variables over the 2-year follow-up period

	Stroke (n=45)			**Control (n=45)**			**Between-group**	
	Paretic	**Non-paretic**	**Between-side *p***	**Non-dominant**	**Dominant**	**Between-side *p***	***p*- Paretic**	***p*- non-paretic**
Total vBMD (mg HA/cm^3)	3.32%±4.40%*#†	2.07%±2.86%*#†	0.027‡	2.06%±2.74%*#†	2.06%±2.52%*#†	0.988	0.534	0.980
Trabecular area (mm^2)	-1.06%±1.26%*#	-1.01%±1.19%*#	0.763	-0.70%±0.91%*#	-0.60%±0.99%*#	0.427	0.238	0.084
Trabecular vBMD (mg HA/cm^3)	2.29%±5.72%*#	0.08%±2.97%	0.039‡	0.84%±3.29%	0.80%±2.97%	NA	0.625	0.234
Trabecular number (1/mm)	-0.30%±5.72%	-1.05%±3.93%	NA	0.67%±2.96%	1.24%±3.29%	0.664	0.313	0.007
Trabecular thickness (mm)	-0.09%±2.59%	-0.46%±1.71%	NA	0.26%±1.31%	0.01%±0.91%	NA	0.414	0.106
Trabecular separation (mm)	-0.57%±5.54%	0.25%±2.78%	NA	-0.56%±2.15%	-1.33%±3.16%	0.407	0.990	0.009
Cortical Area (mm^2)	3.45%±3.98%*#†	2.46%±2.85%*#	0.116	1.79%±2.40%*#	1.72%±2.53%*#	0.836	0.047§	0.261
Cortical vBMD (mg HA/cm^3)	0.53%±3.65%#	0.77%±1.46%*#	0.897	1.02%±1.30%*#	1.05%±1.08%*#	0.822	0.401	0.299
Cortical Perimeter ((mm)	-0.23%±0.59%*	-0.25%±0.46%*	0.768	-0.08%±0.38%	-0.34%±0.74%*#	0.054	0.151	0.650
Cortical Porosity (%)	-14.00%±63.75%#	-20.69%±91.58%#	NA	-16.46%±44.68%*#	-20.89%±55.70%*#	0.804	0.195	0.250
Cortical Thickness (mm)	3.61%±3.89%*#†	2.38%±2.77%*#	0.046‡	1.72%±2.36%*#	1.82%±2.37%*#	0.744	0.009§	0.296
Stiffness (kN/mm)	3.81%±5.68%*	2.44%±3.93%*	0.090	0.30%±5.52%	1.57%±3.70%	0.138	0.006§	0.279
Failure load (N)	3.32%±6.10%*	2.48%±4.25%*	0.316	0.39%±5.96%	1.70%±4.00%	NA	0.017§	0.370

Note: relative change=(T1-T2)/T1, $p<0.05$; Positive values suggest decline from baseline to follow-up.

*: significant change from T1 to T2.

#: relative change above precision error.

†: relative change above Least significant change (LSC).

‡: Significant difference between two sides, $p<0.05$.

§: Significant difference between stroke and control group, $p<0.05$.

Table 3-8 Generalized estimating equation: effect of time and side for clinical variables in stroke group.

	Main effect				Interaction	
	Time		**Side**		**Side*time**	
	Wald Chi-square	p	Wald Chi-square	p	Wald Chi-square	p
Biceps muscle strength (Nm)	1.15	0.283	127.44	<0.001*	1.54	0.214
Biceps stiffness (N/m)	24.13	<0.001*	40.94	<0.001*	0.32	0.569
PSV-Brachial artery (cm/s)	0.94	0.333	1.11	0.293	0.07	0.789
PVF-Brachial artery (cc/min)	1.77	0.184	0.69	0.406	3.18	0.075
Diameter-Brachial artery (cm)	30.11	<0.001*	80.60	<0.001*	3.37	0.066
Hand sensation	11.04	0.001*	27.54	<0.001*	0.16	0.686

Note: *: $p < 0.05$.

Table 3-9 Generalized estimating equation: effect of time and side for clinical variables in controls

	Main effect				Interaction	
	Time		**Side**		**Side*time**	
	Wald Chi-square	p	Wald Chi-square	p	Wald Chi-square	p
Biceps muscle strength (Nm)	94.39	<0.001*	0.90	0.342	0.85	0.357
Biceps stiffness (N/m)	7.26	0.007*	0.23	0.633	2.18	0.140
PSV-Brachial artery (cm/s)	1.96	0.161	9.29	0.002*	2.24	0.134
PVF-Brachial artery (cc/min)	28.96	<0.001*	3.28	0.070	7.19	0.007*
Diameter-Brachial artery (cm)	91.01	<0.001*	13.84	<0.001*	2.24	0.134
Hand sensation	16.39	<0.001*	2.65	0.104	0.01	0.931

Note: *: $p < 0.05$.

Table 3-10 Changes of clinical status over the follow-up period in stroke group.

	Baseline	**2-year follow up**	*p*
Biceps muscle strength (Paretic Side, Nm)	17.42±8.86	17.38±9.39	0.969
Biceps muscle strength (Non-Paretic Side, Nm)	29.14±10.82	30.73±11.06	0.118
Composite Spasticity Scale - Upper Limb Total (Max:16)	8.67±2.41	8.20±2.16	0.102
MAL- Amount of Use (Frequency, Max:5)	1.28±1.40	1.60±1.43	<0.001*
MAL- Quality of Movement (Quality, Max:5)	1.40±1.43	1.66±1.43	<0.001*
Fugl-Meyer Assessment - Upper Limb (Max:66)	34.46±18.87	40.09±16.03	0.005*
Bicep stiffness (Paretic Side, N/m)	229.22±30.40	255.31±40.50	<0.001*
Bicep stiffness (Non-Paretic Side, N/m)	206.89±33.21	229.29±31.42	<0.001*
Peak systolic velocity (Paretic Side, cm/s)	76.67±15.00	78.96±18.58	NA
Blood flow volume (Paretic Side, cc/min)	42.82±24.99	52.85±35.39	**0.055**
Arterial diameter (Paretic Side, cm)	0.33±0.06	0.36±0.07	<0.001*
Peak systolic velocity (Non-Paretic Arm, cm/s)	75.50±16.36	77.08±17.73	0.506
Blood flow volume (Non-Paretic Arm, cc/min)	50.19±23.72	50.54±26.11	0.938
Arterial diameter (Non-Paretic Arm, cm)	0.38±0.06	0.42±0.07	<0.001*
Hand light touch sensation (Paretic Side, Max:6.65)	3.81±1.49	4.27±1.45	0.032*
Hand light touch sensation (Non-Paretic Side, Max:6.65)	2.78±0.61	3.18±0.41	0.001*
Physical Activity Scale for the Elderly (Max:400)	120.91±79.16	101.55±76.97	**0.061**

Note: Mean±SD. * $p < 0.05$ statistically significant change.

Table 3-11 Changes of clinical variables over the follow-up period in controls.

	Baseline	**2-Y Follow up**	*p*
Biceps muscle strength (non-Dominant, Nm)	26.99±9.75	34.22±10.56	<0.001*
Biceps muscle strength (Dominant, Nm)	27.85±10.17	34.24±11.42	<0.001*
Bicep stiffness (non-Dominant, N/m)	205.11±23.99	212.20±22.67	0.080
Bicep stiffness (Dominant, N/m)	201.02±25.18	213.82±28.31	0.006*
Peak systolic velocity (non-Dominant, cm/s)	72.38±17.19	76.66±16.48	0.062
Blood flow volume (non-Dominant, cc/min)	30.59±21.97	64.44±39.30	<0.001*
Arterial diameter (non-Dominant, cm)	0.34±0.06	0.38±0.07	<0.001*
Peak systolic velocity (Dominant Arm, cm/s)	78.51±15.07	78.83±15.73	NA
Blood flow volume (Dominant Arm, cc/min)	41.41±23.35	62.86±37.03	<0.001*
Arterial diameter (Dominant Arm, cm)	0.35±0.06	0.39±0.07	<0.001*
Hand light touch sensation (non-Dominant, Max:6.65)	2.76±0.25	2.93±0.35	<0.001*
Hand light touch sensation (Dominant, Max:6.65)	2.80±0.30	2.97±0.38	0.001*
Physical Activity Scale for the Elderly (Max:400)	148.26±82.16	147.43±72.06	0.928

Note: Mean±SD. * $p < 0.05$ statistically significant change.

Table 3-12 Correlation between the change of failure load and the change of other bone variables in stroke (n=45).

	Estimated failure load: %change Pearson's r	***p***
Total vBMD-%change	0.68**	<0.001
Cortical vBMD-%change	0.59**	<0.001
Trabecular vBMD-%change	0.25	0.100
Cortical area-%change	0.61**	<0.001
Trabecular area-%change	-0.60**	<0.001
Cortical thickness -%change	0.53**	<0.001
Cortical perimeter -%change	0.02	0.876

Note: P: paretic side. **: $p < 0.01$.

Table 3-13 Regression analysis: Relative contributions of different bone parameters to %change in estimated failure load of the paretic distal radius for the stroke group.

	Model Summary				Standardized Regression Coefficients	
Independent variables	**R2**	**ΔR2**	**ΔF**	***p* (ΔF)**	**Beta**	***p***
Cortical vBMD %change	0.35	0.35	23.26	<0.001*	0.45	<0.001†
Trabecular vBMD %change	0.37	0.02	1.32	0.258	0.04	0.735
Cortical thickness %change	0.46	0.09	6.94	0.012*	0.34	0.012†

Note: R2 = total variance, ΔR [2] = additional predictor variance, ΔF = F-value change, Beta = standardized regression coefficient, %change = percent change; vBMD = volumetric bone mineral density. * $p \leq 0.05$ Statistically significant F-value change; † $p \leq 0.05$ Statistically significant predictor; Add %change of height to the regression model didn't change the result.

Table 3-14 Correlation between the %change of estimated failure load on the paretic side and baseline demographic and clinical variables in the stroke group.

	Failure load (% change)	
	r	***p***
Demographic information	-0.18	0.243
Age (years)	-0.18	0.251
Stroke Duration (years)	0.15	0.323
Total number of medications	0.11	0.493
Antihypertensive agents	0.02	0.891
Anticoagulants	-0.24	0.115
Hypolipidemic agents	-0.15	0.332
Proton Pump Inhibitors	-0.12	0.441
Total number of comorbidities	0.01	0.977
Hypertension	0.29	**0.050**
Hyperlipidemia	0.06	0.695
Diabetes Mellitus	-0.11	0.488
BMI	0.03	0.863
Sex	-0.02	0.919
Calcium Supplementation	0.15	0.323
Vitamin D Supplementation	0.16	0.304
Smoking history	-0.07	0.670
Alcohol history	-0.18	0.243
Paretic side	-0.21	0.189
Baseline clinical impairment variables		
Physical Activity Scale for Elderly	-0.038	0.805
Biceps strength	-0.17	0.274
Composite Spasticity Scale- upper limb	0.013	0.932
Fugl-Meyer Assessment-upper limb	0.03	0.835
Hand sensation	0.32*	**0.032**
Blood flow volume	-0.31*	**0.036**
Artery diameter	-0.13	0.394
Bicep stiffness	-0.20	0.184
Motor Activity Log- frequency	-0.02	0.882
Motor Activity Log- quality	-0.07	0.643
The relative change of clinical impairment variables		
MAL frequency-% change	<0.01	0.979
MAL quality-% change	0.08	0.595
Physical activity for elderly-% change	0.27	**0.076**
Fugl-Meyer Assessment-upper limb-% change	-0.03	0.823
Hand sensation- absolute change [a]	0.22*	0.152
Blood flow volume-% change	-0.23	0.124
Artery diameter-% change	-0.08	0.588
Bicep stiffness-% change	0.16	0.288

Note: %Change=(T1-T2)/T1; [a]: absolute change=T1-T2; *: $p<0.05$; p value in bold: $p \leqslant 0.1$.

Table 3-15 Regression analysis: Associations between clinical impairment variables at baseline and % change in estimated failure load of radius on paretic side.

	Model Summary				Standardized Regression Coefficients	
Predictor variables	**R2**	**ΔR2**	**ΔF**	***p* (ΔF)**	**Beta**	***p***
Factor 1	0.01	0.01	0.30	0.584	0.00	0.993
Factor 2	0.01	0.00	0.07	0.795	0.13	0.404
Factor 3	0.01	0.01	0.24	0.626	0.14	0.35
Factor 4	0.02	0.00	0.00	0.957	-0.01	0.959
Baseline hand sensory	0.11	0.10	4.26	0.046*	0.30	0.059
Baseline Blood flow volume	0.22	0.11	5.31	0.027*	-0.35	0.027†

Note: Factor 1 = Age, gender, smoking history, alcohol history.

Factor 2 = Calcium supplementation status; Vitamin D supplementation status.

Factor 3 =Total number of medications, total number of comorbidities, BMI.

Factor 4 =stroke duration.

R2 = total variance, ΔR [2] = additional predictor variance, ΔF = F-value change, Beta = standardized regression coefficient, CI = confidence interval, %change = percent change.

* $p \leq 0.05$ Statistically significant F-value change.

† $p \leq 0.05$ Statistically significant predictor.

Add %change of height to the regression model didn't change the result.

Table 3-16 Regression analysis: Associations between the %change of clinical impairment variables and %change in estimated failure load of radius on paretic side

	Model Summary				Standardized Coefficients	Regression
Independent variables	**R2**	**ΔR2**	**ΔF**	***p* (ΔF)**	**Beta**	***p***
Factor 1	0.01	0.01	0.30	0.584	0.05	0.752
Factor 2	0.01	0.00	0.07	0.795	0.03	0.845
Factor 3	0.01	0.01	0.24	0.626	0.05	0.746
Factor 4	0.02	0.00	<0.01	0.957	-0.04	0.798
PASE- %change	0.08	0.06	2.73	0.107	0.26	0.107

Note:

Factor 1 = Age, gender, smoking history.

Factor 2 = Calcium supplementation status; Vitamin D supplementation status.

Factor 3 =Total number of medications, total number of comorbidities.

Factor 4 =stroke duration, alcohol history.

R2 = total variance, ΔR [2] = additional predictor variance, ΔF = F-value change, Beta = standardized regression coefficient, CI = confidence interval, %change = percent change; PASE: Physical Activity Scale for the Elderly.

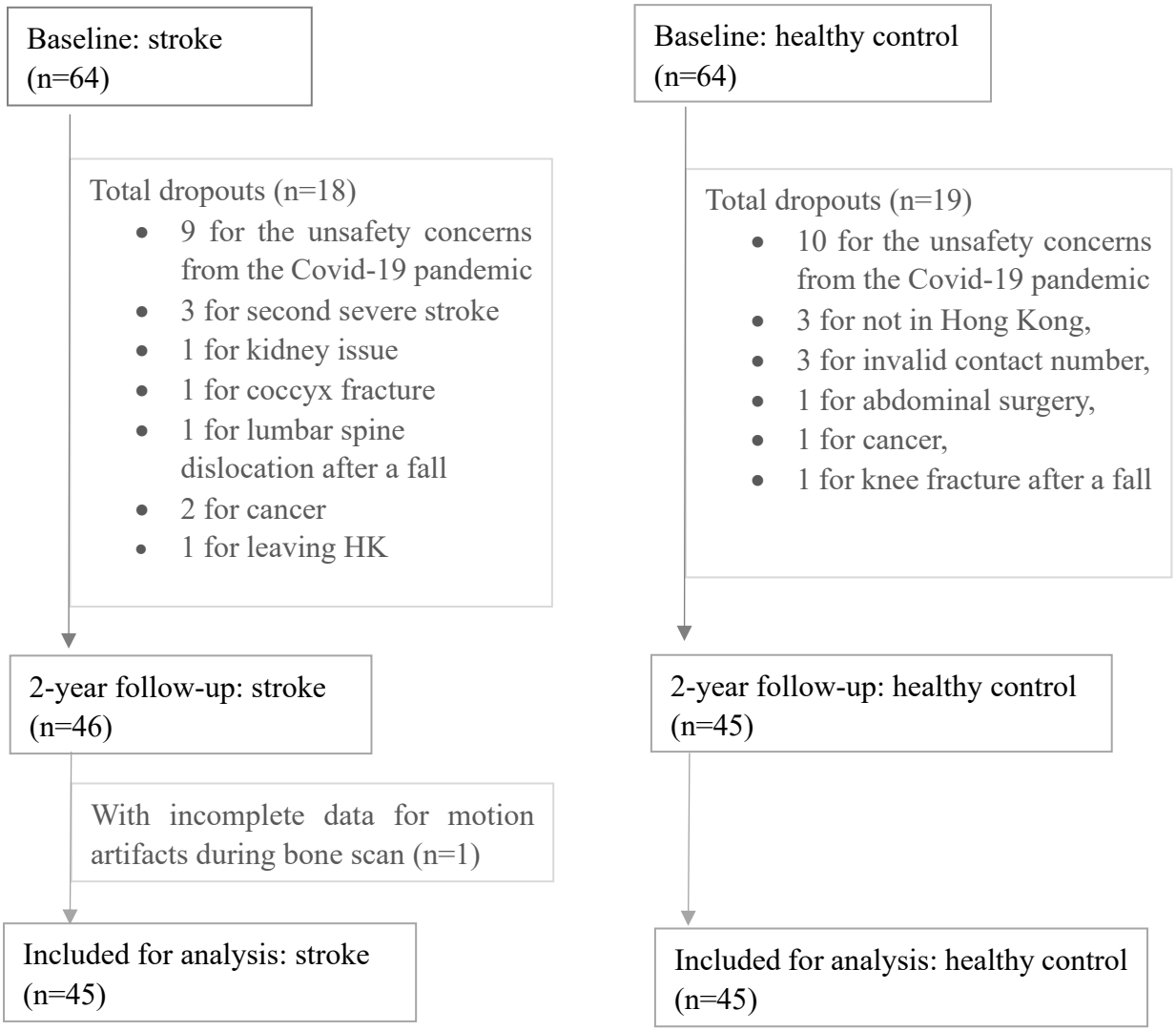
* $p \leq 0.05$ Statistically significant F-value change.

† $p \leq 0.05$ Statistically significant predictor.

Add %change in height to the regression model didn't change the result.

3.8 List of Figures

Figure 3-1 Flowchart of measurements.



4. Chapter 4

Longitudinal changes in the tibia and its contributing factors in individuals with chronic stroke

Ouyang Huixi

4.1 Abstract

Background: Secondary osteoporosis is common after a stroke, particularly in the hemi-paretic limbs. A previous stud found that failure load was a significant risk factor for fracture after a stroke.

Purpose: To examine how the bone density, macrostructure, and microstructure of the distal tibia change in people with chronic stroke (≥ 6 months duration) and to identify the factors associated with the change in the estimated failure load of the hemi-paretic distal tibia.

Methods: Forty-six individuals with chronic stroke (21 women, mean age = 60 years; mean post-stroke onset = 6 years) participated in the study. The distal tibia was measured twice bilaterally using high-resolution peripheral computed tomography (HR-pQCT). The first measurement was made at the time of study registration and the second measurement was made 2 years later to assess the change in bone density, geometry, and microstructure and the estimated failure load (an indicator of bone strength). The blood flow volume of the popliteal artery was measured by Doppler ultrasound. The 10-meter walking test was used to assess walking speed. Multiple hierarchical regression analysis was used to identify the predictors of the change in the estimated failure load of the hemi-paretic distal tibia.

Results: In the paretic leg, there was a significant decline in cortical bone density (1.28%), trabecular bone density (1.18%), cortical thickness (1.95%), cortical area

(1.83%), and trabecular thickness (0.58%) after 2 years of follow-up. The changes in cortical bone density, trabecular bone density, and cortical thickness were significantly associated with a reduction in the failure load during the 2-year follow-up period. After accounting for relevant factors, the change in walking speed (F change = 5.10, p = 0.029) and baseline blood flow volume (F change = 4.49, p = 0.041) and baseline factors, including gait speed, physical activity level, and muscle strength (F change = 6.47, p = 0.015), improved the regression model's prediction of the 2-year change in estimated failure load of the hemi-paretic distal tibia.

Conclusions: The reduction in the failure load of the hemi-paretic distal tibia during the 2-year follow-up period, as evaluated by HR-pQCT, was mostly explained by decreases in cortical and trabecular bone density and cortical thickness. Individuals with lower levels of mobility, less physical activity, and poorer vascular health tended to show a greater decline in the estimated tibial bone strength on the paretic side.

4.2 Introduction

Due to the aging trend of the global population, stroke continues to be a public health concern. There have been 101 million prevalent cases (an increase of 70% from 1990 to 2019) and 142 million disability-adjusted life-years lost as a result of stroke over the past three decades. [71] Even more alarming is the fact that approximately 1 in 12 people would experience a hip fracture in the 5 years following a stroke. [85] A

fracture following a stroke may have a negative effect on the recovery of function and may necessitate a longer period of rehabilitation. [87] Thus, the prevention of post-stroke fracture is an important area that merits more attention.

Secondary osteoporosis is one of the two major factors, along with fall history, contributing to the increased risk of fracture after a stroke. [84] Previous studies using dual-energy x-ray absorptiometry (DXA) as the bone measurement tool have found a 10-12% reduction in areal bone mineral density (aBMD) of the proximal femur on the paretic side and a 4-8% reduction on the non-paretic side during the first year after a stroke. [166, 167] Such an annual bone loss, especially on the affected side, was approximately 10 times greater than the amount of bone loss in a healthy person aged greater than 50 years. [170] However, mixed results have been reported for changes in bone properties during the chronic stage of stroke recovery. Lam et al. used both DXA and peripheral computed tomography (pQCT) to investigate changes in bone properties in individuals with chronic stroke (mean stroke duration: 4 years; time interval between measurements: 1 year). [30] Of the various outcome variables measured at the distal tibia by pQCT, changes were only found in the trabecular volumetric bone mineral density (vBMD) and bone strength index. In addition, no significant changes in the aBMD of the proximal femur (measured by DXA) were found in the same study. Similarly, De Brito et al. did not find further changes in femoral aBMD (measured by DXA) after 16 months of follow-up in individuals with chronic stroke (stroke duration: approximately 3 years). [172] Taken together, these

findings suggest that further bone loss can still occur during the chronic stage, but in a site-specific manner.

One of the limitations of previous DXA and pQCT studies is that they were unable to assess the bone microstructure due to insufficient resolution. The development of HR-pQCT has allowed for the accurate measurement of cortical bone properties, even at distal sites of the long bones in compromised populations (e.g., stroke) where the cortical bone shell is often very thin. HR-PQCT, which has a voxel size of 82 μm, is capable of assessing cortical and trabecular bone microstructure, which are also important determinants of overall bone strength. [30] However, no longitudinal studies have applied HR-pQCT to investigate changes in bone properties in people with chronic stroke.

Another important issue is related to the identification of factors that predict the deterioration of bone properties after a stroke. The early identification of these factors may help guide the design of targeted intervention programs to mitigate the decline in bone health in individuals with stroke. In a longitudinal study, Lam et al. found that leg muscle strength and motor recovery were negatively related to the decline in paretic tibial distal epiphysis during the chronic stage of stroke. [30] A positive relationship was found between spasticity and femoral bone loss 1 year after stroke in another longitudinal study. [172] However, the relative contribution of these factors to the change in bone health is unknown. As multiple factors may influence progressive bone loss after a stroke, a comprehensive prediction model including demographic factors

(e.g., age, sex, and calcium or Vitamin D supplementation) is necessary, but is still lacking.

The objective of the current study was to 1) assess the changes in bone density, geometry, and microstructure of the tibia during the chronic stage after a stroke and 2) identify the factors associated with changes in bone density, geometry, and microstructure of the tibia during the chronic stage of stroke recovery.

4.3 Methods

Study design and ethics

This was a 2-year prospective cohort study involving individuals with chronic stroke and age-matched control participants. Each participant provided written informed consent, and ethics approval was obtained from the Joint Chinese University of Hong Kong – New Territories East Cluster Clinical Research Ethics Committee and the Hong Kong Polytechnic University-Human Subjects Ethics Sub-Committee. All of the study procedures were conducted in accordance with the Declaration of Helsinki.

Subject recruitment

Individuals with chronic stroke were recruited from community self-help groups, existing participant databases, and the general public. The inclusion criteria were: 1) a diagnosis of a unilateral stroke confirmed by the individual's physician, 2) an age ≥ 18 years, 3) a stroke onset more than 6 months previously, 3) a medically stable condition, 4) a Modified Rankin Scale score of 2-5, 5) the ability to obey motor commands, and 6) competency in providing informed consent. The exclusion criteria were: 1) other

neurological conditions (e.g., Parkinson's disease), 2) receptive aphasia, 3) a recurrent stroke that led to a significant impairment in daily activities (Modified Rankin Scale score ≥ 2), 4) pregnancy, 5) other conditions with a substantial effect on bone health (e.g., rheumatoid arthritis), 6) taking medication to treat osteoporosis (e.g., bisphosphonates) currently and/or prior to the stroke, 7) a known diagnosis of osteoporosis prior to the stroke, 8) fragility fractures prior to the stroke, 9) metal implants in the scanned limb, or 10) other serious illnesses (e.g., cancer). An age- and sex-matched control group with no history of stroke was recruited from the community using the same inclusion and exclusion criteria, but without a stroke history. A technician at a local bone imaging center with extensive experience in osteoporosis research performed the scan. Clinical measurements were performed by two other researchers with over five years of experience and one research assistant.

Sample size estimation

All sample size estimations were based on an alpha of 0.05 (two-tailed) and a power of 0.8. To address objective 1 (i.e., comparing HR-pQCT data between the stroke and control groups), the sample size estimation was based on a previous study [30] examining the difference in the percentage change in various HR-pQCT variables between the two sides during the late chronic stage after a stroke, which yielded Cohen's d values ranging from 0.25 to 1.67 (i.e., equivalent to f = 0.13 to 0.84). Using the smallest effect size yielded (f = 0.15), with two within-subject factors (side [non-paretic vs. hemi-paretic] and time [two assessments]), an alpha value of 0.05, and a power of 0.80, a sample size of 90 individuals (45 per group) was needed to detect a

significant side × time interaction effect.

To address objective 2, generalized estimating equations were used to identify the determinants of bone variables. Previous cross-sectional studies generated R^2 values ranging from 0.17 to 0.81 (equivalent to effect sizes of $f^2 = 0.20$ to 4.26). [31, 177-180, 182] Using the smallest effect size yielded ($f^2 = 0.20$) and an alpha value of 0.05 and power of 0.80, a sample size of 42 individuals with stroke was needed to detect a significant association between the bone variables and other factors.

Taken together, assuming a 15% attrition rate, we aimed to recruit 54 individuals with chronic stroke and 54 control participants for this study.

Demographic information

Basic demographic data (e.g., sex and age), and brain scan results (e.g., computed tomography, magnetic resonance imaging) were obtained from the participants' medical records. The participants were also asked whether they were living independently prior to their stroke. The measurements of bone properties and other clinically relevant variables were performed at the initial assessment (T1) and again at the 2-year follow-up (T2). [31, 177-180, 182] This time frame was chosen for two reasons. First, two previous longitudinal studies showed that, while the bone mass of the hemi-paretic and non-paretic sides were substantially different in person who were already well into the chronic stage (onset > 1 year), the temporal changes in bone variables within a 1-year period in this population were quite modest. [30, 181] Thus, this study aimed to evaluate the change in bone status during the chronic stage, using a 2-year assessment interval. Two qualified researchers with at least 5 years of research experience, together with

one research assistant, performed these measurements. The bone scan was performed by an experienced technician at a local bone imaging center. Previous studies showed that the temporal changes in some bone variables within a 1-year period were quite modest. [30, 181] Thus, this study used a 2-year assessment interval to observe detectable and clinically significant change.[278] The bone scans were performed by a technician at a local bone imaging center with extensive experience in osteoporosis research. Two other qualified researchers with ≥5 years of research experience, and one research assistant performed the other clinical measurements. All measurements, except the stroke-specific assessments, were administered to both groups.

Bone scan protocol

The effective radiation dose for each HR-pQCT scan is only 3-4 μSv. Thus, the total effective radiation dose during the 2-year follow-period would be approximately 12-16 μSv (four scans: two legs × two time points), which is similar to the radiation dose received during a 1-1.3-hour flight. The participants underwent scanning of the bilateral distal radius for the study described in Chapter 3, and therefore, the total effective radiation dose for each participant was 24-32 μS, which was still safe.

Although the hip, rather than the tibia, is the most common site of post-stroke lower-limb fracture, [79] the hip region cannot be scanned using HR-pQCT due to its central location. The tibia, however, is a long bone that can be easily accessed by HR-pQCT. In a recent study, Lam et al. further verified the clinical relevance of tibial measurements with pQCT, by demonstrating moderate to strong correlations between pQCT-derived tibial bone parameters and DXA-derived hip BMD measurements in

individuals with stroke (r = 0.51-0.83). [183] Similarly, a high correlation (r = 0.70) has been found between hip aBMD (determined by DXA) and distal tibia vBMD (determined by HR-pQCT) in post-menopausal women. [279] Therefore, the distal tibia was chosen as the site of HR-pQCT measurements.

The vBMD, cross-sectional geometry, and microstructural properties of the bilateral distal tibiae were measured using HR-pQCT (Scanco Medical AG, Brüttisellen, Switzerland). The scan region was fixed at 22.5 mm (distal tibia) proximal from the mid-joint line. [248] The length of the scan region spanned 9.02 mm proximally, which was equivalent to a stack of 110 slices. [249] The analyses of bone images acquired during the follow-up period from each individual were subjected to the same volume of interest (VOI) matching as the baseline and 24-month measurements. A software program provided by the manufacturer was used to identify the same region by matching the corresponding images based on their total cross-sectional area. Image analyses were only conducted for the bone volumes common to the measurements at the two time points. [249]

Analyses of the three-dimensional scan data were conducted using Image Processing Language (IPL v5.08b; Scanco Medical AG, Brüttisellen, Switzerland). First, bone images were analyzed using a standard (default) protocol. [187] Extended cortical analysis was then conducted, [192] using a cortical compartment segmentation technique adapted from Buie et al. [250] (μCT Evaluation v6.0, Scanco Medical AG). This was done to achieve better accuracy in the analysis of very porous or thin cortical shells. The details of the HR-pQCT variables are shown in Table 4-1.

All micro finite element (μFE) analyses were performed using the FE-solver included in the built-in Image Processing Language software of the HR-pQCT scanner (IPL-FE v1.15, Scanco Medical AG). [247] A special peeling algorithm that specified a minimum cortical thickness of 6 voxels was used to identify the cortical and trabecular bone tissue. μFE analyses were performed by converting the binary image data to a mesh of isotropic brick elements. For all elements, a Poisson's ratio of 0.3 was specified. Elements representing cortical and trabecular bone were assigned a Young's modulus value of 10 GPa. [247] A uniaxial compression test, with a 1,000 N load applied, was performed with an applied strain of 1%. Whole-bone stiffness (kN/mm) was calculated. The estimated failure load (N) was calculated based on the assumption that bone failure occurred if >2% of the elements were strained beyond 0.7% strain. [175]

Our HR-pQCT scanner has excellent reproducibility, based on unpublished data from 30 cases re-scanned within 1 month. Short-term reproducibility can be expressed as the root-mean-square of the coefficient of variance ($CV\%_{RMS}$). The $CV\%_{RMS}$ values of the geometric measures and vBMD parameters were in the range of 0.22-0.89% and 0.41-0.65%, respectively, and the corresponding values for microarchitectural parameters ranged from 0.60 to 1.44%. The intra-rater reliability of the parameters of these three aspects (geometry, vBMD, and microarchitecture) is good (linear weighted kappa = 0.855). [38] The least significant change (LSC) is a metric used to determine whether a measurement is reliable, based on its signal-noise ratio. [280] The 95% confidence LSC value (calculated from 2.77 × the precision error) is calculated based on (1) and provided in Table 4-1.

$$LSC = 1.96 \cdot \sqrt{CV\%_{RMS}^2 + CV\%_{RMS}^2} = 2.77 \cdot CV\%_{RMS} \quad (1)$$

Other assessments

Muscle strength: A dynamometer system (Humac Norm Systems, Stoughton, MA, USA) was used to measure the isometric peak torque (N/m) of the ankle plantar flexor muscle. [253] The ankle plantar flexors were tested at 0° (neutral position). The optimal ankle joint positions for isometric force production were determined using anatomical models, while accounting for force-length properties.[281] During testing, the participants were instructed to perform isometric contraction with maximal effort. Verbal encouragement from the assessor was provided to elicit maximal effort from the participants. The tests were performed three times with a 1-minute rest interval. The average strength values of the three trials were calculated.

Muscle stiffness: A hand-held MyotonPRO device (Myoton AS, Tallinn, Estonia) was used to measure the stiffness (N/m) of the medial gastrocnemius (MG) muscle. Measurements were performed by placing the probe applicator perpendicularly to the center point of the muscle belly. A mechanical impulse with a quick release to the muscle was then added percutaneously. A durometer in this device was used to measure the stiffness of the muscle tissue by applying mechanical deformation to the surface. This device have demonstrated excellent test–retest reliability for the MG muscle in people with spinal cord injury (intraclass correlation coefficient [ICC] = 0.88-0.97) [256] and for the hand-grip muscle (flexor carpi ulnaris) in individuals with chronic stroke (ICC = 0.87-0.90). [257]

Balance: Functional balance was measured using the 6-item Brief Balance Evaluation Systems Test (BESTest). The following six categories were included in the test: biomechanical constraints, stability limit, anticipatory postural responses, postural responses, sensory orientation, and gait stability. Each item was rated on a 3-point ordinal scale, with the total score ranging from 0 to 24. A higher score indicated better balance ability. The Brief BESTest has demonstrated excellent intra-rater and inter-rater reliability in individuals with stroke (ICC > 0.90). [282]

Walking status: The Functional Ambulation Categories (FAC) test (range: 0-5) was used to assess ambulatory status. High test–retest reliability (Cohen's kappa = 0.950) and inter-rater reliability (kappa = 0.905) have been found in people with stroke. [283] The 10-meter walk test at a safe maximal speed was also implemented. The test–retest reliability for this test is excellent (ICC = 0.97). [284]

Sensory function: The touch pressure threshold was tested using Semmes–Weinstein monofilaments (SWMT). [258, 259] The plantar surface of the foot was tested because sensory deficit in this area has an important contribution to balance impairments. In people with chronic stroke, intra-rater agreements of the SWMT grade score have been shown to be substantial to perfect (Cohen's kappa values: thumb = 0.89, index finger = 0.80). Inter-rater agreements were also substantial (Cohen's kappa values: thumb = 0.75, index finger = 0.79). [259]

Physical activity level: The Physical Activity Scale for the Elderly (PASE) [260] was used to evaluate the physical activity level (score range: 0 to 400). Twelve questions were asked regarding the frequency and duration of leisure activity (e.g., jogging,

swimming, and strength and endurance exercises), household activity, and work-related activity during the last 7 days. Higher scores indicated higher activity levels. Good test–retest reliability of the Chinese version of the PASE has been shown in healthy elderly individuals (ICC = 0.81).

Vascular health: The Aixplorer ultrasound scanner (Aixplorer, SuperSonic Imagine, Aix en-Provence, France) was employed with a linear array probe (4-15 MHz, SuperLinear, 15-4, Vermon, France). The blood flow velocity (cm/s) and blood flow volume (cc/min) of the popliteal artery were measured on each side using Doppler ultrasound. [261] Within the same image, the arterial diameter (cm) was measured by placing the calipers at each end of the superior and inferior borders of the endothelial wall. The settings of the Doppler ultrasound were standardized and set for high sensitivity, a medium wall filter, a pulsed repetition frequency of 4162Hz. Measurements for the lower limb assessments were performed while the participants were lying in the prone position. The ankle joint was maintained in a neutral plantar dorsiflexion position (0^0) fixed by an anchored device without eversion or inversion. To ensure that blood flow was measured in a resting state, the participants rested in the supine position for at least 15 minutes before the ultrasound examination commenced.

To measure the popliteal artery, the probe was placed in a sagittal orientation on the popliteal fossa at the approximate level of the popliteal crease. After visual confirmation of the artery was confirmed with color Doppler ultrasound in the transverse image plane, the probe was then rotated sagittally and tilted to visualize the artery longitudinally. An electronic caliper was then positioned in the center of the artery.

The sample volume was standardized at 1.0 mm for the popliteal artery. Doppler steering and fine angle correction were adjusted to optimize angle-to-flow at an insonation ≤ 60°. The auto-trace function was applied to estimate the full range of positive and negative flow. Three consistent spectral waveform cycle readings were used to calculate the average measures. The specific steps we applied to find the optimal image of the popliteal artery were similar to the steps used for brachial artery, as described above.

Based on our pilot trial involving 15 individuals with chronic stroke (Table 4-2), good to excellent inter-rater (ICC = 0.85-0.93) and intra-rater reliability (ICC = 0.90-0.98) were demonstrated for the peak systolic velocity, peak volume flow, and diameter of the popliteal artery.

Stroke-specific assessments

Motor recovery: The Fugl–Meyer Motor Assessment (FMA) was used to assess the degree of motor recovery of the hemi-paretic lower limbs (range: 0-34). Lower scores indicated more severe motor impairment. The FMA assessed movement quality, movement coordination, and reflex action of the hip, knee, and ankle. Items were scored using a 3-point ordinal scale where 0 indicated could not perform, 1 indicated performed partially, and 2 indicated performed fully. The interrater reliability of the FMA was excellent (ICC = 0.96). [265]

Spasticity: Spasticity of the ankle plantar flexors was measured using the Composite Spasticity Scale (CSS; score range: 1-16). [266] Higher scores were indicative of higher spasticity. The CSS included three items: 1) Achilles tendon jerk

(score range: 0-4), 2) resistance to full range of motion for ankle dorsiflexion (score range: 0-8), and 3) the duration and amount of ankle clonus elicited (score range of 1-4). The test–retest reliability of the CSS was excellent (ICC = 0.97). [266]

Statistical analysis

First, descriptive statistics were used to summarize and tabulate the data. An independent samples Student's t test, Mann–Whitney U test, or Chi-square test was used to compare the measured variables between the stroke and control groups, depending on whether the data fulfilled the criteria for parametric statistics.

Second, for each bone outcome and sided functional outcome (muscle strength, muscle stiffness, sensory, and vascular ultrasound measurement), generalized estimating equations (within-subject factors: side [two levels: stroke: hemi-paretic vs. non-paretic; healthy: dominant vs. non-dominant]; time [two levels: baseline, 2-year follow-up]) were used to determine if there was an interaction effect between the two factors. Separate paired Student's t tests or Wilcoxon signed-rank tests were performed to compare baseline and follow-up data if necessary. An independent samples Student's t test or Mann–Whitney U test were also used to compare the between-group differences in relative change scores for each variable, where relative change = (T1-T2)/T1. Positive values indicated a decrease in the value from baseline to follow-up.

Third, we were interested in determining which bone properties (e.g., densitometric, microstructure, and cortical vs. trabecular) contributed more to the relative change in estimated failure load on the paretic side in individuals with stroke. First, associations of the bone variables that showed a significant change with the

change in estimated failure load during the follow-up period were explored using Pearson's correlation analysis. Next, the variables that showed significant bivariate correlation were entered into a hierarchical regression model to identify their associations with the relative change in estimated failure load.

Fourth, we were interested in identifying the baseline demographic and clinical variables that were predictive of the change in estimated failure load of the distal tibia on the paretic side. Bivariate Pearson's correlation analysis was first used to analyze the correlation between the change in estimated failure load (from the initial assessment to the 24-month follow-up assessment) and impairment variables (e.g., muscle strength and spasticity) measured at baseline. Variables that yielded a correlation with $p < 0.1$ were chosen to be the predictor variables in the subsequent regression analysis.

The first hierarchical multiple regression analysis was used to identify the *baseline* clinical variables that were predictive of the change in estimated failure load on the paretic side (dependent variable) for the stroke group, while adjusting for potentially confounding factors (e.g., demographics). First, as there are many demographic variables that may influence bone health, principal component analysis was used to extract the demographic factors to use as independent variables in the regression analysis. To avoid overfitting in the multivariate regression analysis, if four or more clinical variables showed significant bivariate correlations with the dependent variable, a separate principal component analysis (dimension reduction) was performed to determine the clinical factors to use as independent variables in the subsequent regression model. The demographic and clinical factors extracted from the principal

component analysis were entered into the model using a hierarchical regression series procedure. [285] In addition, the percentage change in height (to adjust for the potential variation in the location of the scan region) was added to the prediction model to determine if it changed the prediction model.

We were also interested in exploring whether the *2-year change* in any of the clinical variables was associated with the change in the estimated failure load of the distal tibia on the paretic side. A separate hierarchical regression analysis was performed to identify the associations between the percentage change in estimated failure load and the percentage *change in clinical variables* during the 2-year follow-up period. The selection of independent variables followed the principles described above.

4.4 Results

Participant characteristics

One hundred and twenty-eight individuals (64 people with stroke and 64 controls) were recruited. There were 18 dropouts in the stroke group and 19 dropouts in the control group. Half of the dropouts in each group dropped out due to safety concerns related to the COVID-19 pandemic. More details about those who dropped out can be found in Figure 4-1. Thus, 46 stroke individuals and 45 controls completed the two measurements and were included in the final analysis. The age, sex ratio, and body mass index (BMI) were similar between the two groups ($p > 0.05$). Most of the participants

with stroke (93.5%) were able to at least walk independently on level surfaces according to the FAC test results. More details about the participants' characteristics can be found in Table 4-3.

Changes in bone outcomes

There was a significant time effect for total vBMD, cortical vBMD, cortical porosity, bone stiffness, and estimated failure load in both groups (Wald Chi-square = 6.90-44.81, $p < 0.01$; Tables 4-4 and 4-5). Significant time effects for trabecular area (Wald Chi-square = 6.6, $p = 0.01$), cortical area (Wald Chi-square = 6.08, $p = 0.01$), and cortical thickness (Wald Chi-square = 9.46, $p < 0.01$) were only seen in the stroke group, while a significant time effect for trabecular thickness (Wald Chi-square = 8.68, $p < 0.01$) was only seen in the control group. There were significant interaction effects between side and time for total vBMD (Wald Chi-square = 5.04, $p = 0.025$), trabecular vBMD (Wald Chi-square = 11.05, $p < 0.01$), trabecular thickness (Wald Chi-square = 10.59, $p < 0.01$), and estimated failure load (Wald Chi-square = 4.48, $p = 0.03$) in the stroke group only.

As illustrated in Table 4-6, post-hoc analysis showed that a decline in cortical vBMD, cortical porosity, and estimated failure load were observed for both sides of the two groups. However, significant declines in trabecular vBMD, trabecular thickness, and cortical area occurred on the paretic side in the stroke group only.

The relative changes in bone variables in the two groups are also shown in Table 4-7. It was found that the decline in cortical vBMD exceeded the LSC (1.14%) in both the stroke and control groups after 2 years, whereas the changes in total vBMD and

cortical thickness exceeding the LSC (1.25% and 1.77%) were found in the paretic tibia only in those with stroke. Moreover, the changes in trabecular area, trabecular vBMD, and cortical area in the stroke group, albeit statistically significant, were greater than the precision error, but less than the corresponding LSC values.

Changes in other clinical outcomes

As shown in Table 4-8 and Table 4-9, the main effect of time was significant for gastrocnemius muscle strength, hallux sensation, and vascular health (Wald Chi-square = 7.65-24.43, $p < 0.01$) in both the stroke and control groups. Time × side interaction effects were only significant for muscle strength (Wald Chi-square = 4.42, $p = 0.036$) and blood flow volume (Wald Chi-square = 4.26, $p = 0.039$) in the control group.

Post-hoc paired comparisons showed that there was slightly better vascular health ($p < 0.01$), gastrocnemius muscle strength ($p < 0.05$), and walking speed ($p < 0.05$) in both the stroke and control groups at the 2-year follow-up compared to the baseline. However, more severe spasticity and worse motor control and sensation were demonstrated in the paretic leg than baseline measures in participants with stroke ($p < 0.01$). In addition, there was a trend of a decline in physical activity level (based on PASE scores) in the stroke group ($p = 0.061$). More information about these comparisons is shown in Table 4-10 and Table 4-11.

Bone properties that contributed most to changes in failure load during follow-up

Table 4-12 illustrates the associations between the relative change in various bone properties and that in the estimated failure load on the paretic side. The correlation

coefficient was largest between the relative change in failure load and total vBMD ($r = 0.82$, $p < 0.01$), followed by cortical thickness ($r = 0.68$, $p < 0.01$), trabecular area ($r = -0.64$, $p < 0.01$), and cortical area ($r = 0.64$, $p < 0.01$).

As demonstrated in Table 4-13, hierarchical regression found that prediction model 1, which included the change in cortical vBMD, trabecular vBMD, and cortical thickness as predictor variables, accounted for 72% of the change in failure load of the paretic tibia. Among the five related bone variables, cortical vBMD (standardized Beta = 0.44, $p < 0.01$), trabecular vBMD (standardized Beta = 0.40, $p < 0.01$), and cortical thickness (standardized Beta = 0.38, $p < 0.01$) were significant predictors of the failure load and explained 28%, 27%, and 17% of the change in estimated failure load, respectively. The relative changes in cortical area (variance inflation factor > 10) and trabecular area (strongly correlated with the change in cortical thickness, $r = -0.82$) were not included in the prediction model to avoid multicollinearity. [267]

Clinical variables at baseline that were predictive of the change in estimated failure load during follow-up

Among the clinical variables, a higher walking speed ($r = -0.30$, $p = 0.043$) and greater blood flow volume ($r = -0.36$, $p = 0.013$) *at baseline* were associated with less of a decline in failure load. In addition, a greater level of physical activity ($r = -0.27$, $p = 0.071$) and greater muscle strength ($r = -0.28$, $p = 0.059$) also showed a marginally significant association with less of a change in the estimated failure load (Table 4-14). As there were four clinical variables that showed significant bivariate correlations with the dependent variable, a separate principal component analysis (dimension reduction)

was performed to determine the clinical factors that would be used as independent variables for the subsequent regression model to avoid overfitting in the multivariate regression (factor 5 and 6, Table 4-15). After accounting for relevant demographic factors, both the baseline blood flow volume (standardized Beta = -0.33, p = 0.041) and factor 5 (including baseline walking speed, muscle strength, and blood flow volume; standardized Beta = -0.37, p = 0.011) were significant predictors of failure load and explained 8% and 13% of the change in estimated failure load, respectively.

Changes in clinical variables that were predictive of the change in estimated failure load during follow-up

The next line of correlation analysis was performed to examine the relationship between the percentage *change* in the clinical variables and the estimated failure load in the paretic tibia during the 2-year follow-up period (Table 4-16). The results showed that a greater increase in the popliteal artery diameter (r = 0.30, p = 0.041) during the 2-year follow-up period was associated with less of a reduction in the estimated failure load on the paretic side. There was also a tendency for those with a greater improvement in walking speed to have less of a decline in the estimated failure load (r = 0.26, p = 0.077). As only two clinical variables (i.e., the change in popliteal artery diameter and walking speed) showed bivariate correlations with the percentage change in the estimated failure load, no principal component analysis of the clinical variables was required. Regression analysis revealed that including the change in walking speed significantly improved the prediction model (F change: 5.10, p = 0.029), after adjusting for relevant factors. More details can be found in Table 4-17.

4.5 Discussion

Overall, a decline was observed in both cortical and trabecular bone variables in the paretic distal tibia of people with chronic stroke. Specifically, the estimated failure load demonstrated a greater decline in the stroke group (paretic side) than the control group (non-dominant side), which was mainly attributed to the decline in the vBMD of the cortical and trabecular bone and decreased cortical thickness. Baseline vascular health and walking velocity were predictive of the change in the estimated failure load during the 2-year follow-up period. In addition, an improvement in walking speed during follow-up was independently associated with the change in the estimated failure load.

Differences in bone property changes between the stroke and control group

The relative change in failure load was significantly larger in the stroke group (3.4%) than the control group (1.9%) in this study. This was similar to the normal change over 2 years in healthy women (1.8%) of a similar age. [38] In line with our findings, Lam et al. also found a clinically significant decline in the bone strength index in the paretic distal tibia in a previous pQCT study (XCT 3000, voxel size: 500 μm). [30]

This study found that cortical thickness and cortical vBMD on the paretic side experienced the most pronounced reduction (above LSC), but with a maintained cortical perimeter. This suggested that bone loss continued during the chronic stage of

stroke, but mainly occurred in the cortical bone. These results may suggest that cortical bone deteriorated over a longer time span than trabecular bone in the distal tibia. Such degradation in cortical bone may be due to endocortical trabecularization, during which intracortical and endocortical remodeling erode the cortical bone. [23] This is different from the aging process, in which decreased cortical thickness occurs together with an enlarged cross-sectional area through periosteal remodeling, as part of a compensatory mechanism to partially counteract the detrimental effect of bone loss on overall bone strength. [38]

Lam et al. found a decrease in trabecular vBMD, greater than the LSC, after 1 year of follow-up (1.8%). [30] In contrast, our results showed a reduction in trabecular vBMD (1.2%) that was smaller than the LSC. One important reason for this discrepancy may be the shorter stroke duration in the study of Lam et al. (4 years) compared with our study (6 years). As our participants were more chronic, the extent of their bone loss may have been less. Indeed, the baseline trabecular vBMD was much higher in the study of Lam et al. (203.6 mg/cm^3) [30] than in the current study (147.1 mg/cm^3). Another reason may be the difference in the bone site that was measured. The site of skeletal measurement used by Lam et al. was more distal (and thus, more dominated by trabecular, rather than cortical, bone tissue) than the site used in our study.

Percentage bone change, failure load, and fracture risk

We found that changes in three HR-pQCT variables (trabecular vBMD, cortical vBMD, cortical thickness) accounted for 72% of the reduction in the estimated failure load over the 2-year follow-up period in individuals with chronic stroke. Of these five

variables, the decline in cortical vBMD and trabecular vBMD contributed the most to the decline in the estimated failure load. A review of eight prospective cohort studies (n = 7,254) published by the Bone Microarchitecture International Consortium in 2019 supports our findings to some extent. They reported that, in addition to using femoral neck aBMD or fracture risk assessment tool (FRAX) scores, adding cortical vBMD, cortical area, trabecular number, and trabecular thickness improved fracture prediction in elderly individuals. [191]

Available research evidence suggests that a small reduction in bone mass leads to a substantial increase in fracture risk. According to a meta-regression analysis of 38 placebo-controlled trials, a 2% or 6% improvement in total hip BMD may lead to a 16% or 40% reduction in hip fracture risk. [171] In this study, there was an average of a 2.1% decrease in total vBMD and a 3.4% decrease in the estimated failure load of the distal tibia after the 2-year follow-up period in the stroke group. The failure load estimated by HR-pQCT is an important variable, because its ability to predict fracture has been established in other populations. Zhu et al. found that, after adjusting for age and BMI, the tibial estimated failure load (odds ratio = 3.85, 95% confidence interval [CI] 1.47 to 10.0, per SD change) was strongly associated with hip fracture in postmenopausal women. [286] Furthermore, another longitudinal study with 5 years of follow-up found that, after adjustment for demographic variables (age, sex, cohort, height, and weight), the estimated failure load of the tibia was the bone measure most strongly associated with the risk of fracture in older adults (hazard ratio = 2.46, 95% CI: 1.81, 3.34, per SD decrease in failure load). [191] This indicates that every SD decrease in tibial failure

load from baseline in these populations results in a prominent increase in femoral fracture risk. Taken together, it is reasonable to presume that fracture risk is substantially increased as a result in individuals with chronic stroke.

Factors predictive of bone loss: vascular health

We found that baseline blood flow volume was predictive of the reduction in the estimated failure load in the hemi-paretic distal tibia. This concurs with the findings in the distal radius reported in Chapter 3. A previous 1-year longitudinal study of individuals with chronic stroke (post-stroke duration: 4 years) also found a negative relationship between vascular elasticity index and the decline in cortical thickness in the radius diaphysis. [268] The relevance of vascular function in bone health has also been demonstrated in older adults. A significant association was revealed between vascular health, as indicated by arterial stiffness, and bone quality (travelling speed of ultrasound through bone) in a large cohort study. [271] As bone is a highly vascularized structure, better vascular function may have a protective effect on the integrity of bone tissue. According to a large cross-sectional study of menopausal women (n = 386), adipokines (e.g., adiponectin and vaspin) may mediate the association between vascular health and osteoporosis. [287] Overall, the mechanisms underlying this relationship are still uncertain and require more research.

Factors predictive of bone loss: walking speed

In the present study, a higher walking speed at baseline and a greater increase in

walking speed were found to be associated with less of a decline in the estimated failure load. In a cross-sectional study, Miller et al. found that gait speed was independently associated with the between-limb difference in the estimated failure load of the distal tibia in individuals with chronic stroke, after adjusting for other relevant factors. [212] A faster walking speed is indicative of better mobility function and is highly related to higher ground reaction forces generated during walking [288] and more frequent ambulatory activity. [212, 289] These factors may contribute to greater loading to the bone tissue, leading to more of a protective effect against deleterious changes in bone properties.

Similar results were found during the acute to early chronic stroke stages. The recovery of independent walking function has been found to be associated with reduced bone loss in the distal tibia (vBMD; from 2 weeks to 6 months post-stroke) [196] and hip (aBMD; from 1 week to 12 months post-stroke). [162] The reason why the level of independent walking function (FAC score) was not correlated with the change in bone loss in our study may be that most of our participants in the stroke group (85%) had already gained independent walking at the initial assessment (refer to Table 4-3). Moreover, the FAC is only a 6-level ordinal scale, which may not be sensitive enough to differentiate the walking performance levels among community-dwelling participants or capture changes in walking performance over time. Our study demonstrated that walking speed is a valuable assessment that is predictive of bone strength.

Factors predictive of bone loss: muscle strength and physical activity

Muscle strength and physical activity, measured at baseline, were found to be associated with the change in the estimated failure load in our regression model. In a previous study of people with chronic stroke, Lam et al. also attempted to identify the clinical factors associated with the 1-year change in tibia epiphysis. They found that a more severe decline in trabecular vBMD of the distal tibia was associated with poorer baseline quadricep strength ($\rho = 0.45$, $p = 0.048$). [30] In addition, a relationship between physical activity and changes in bone turnover markers was also found during the first 6 months post-stroke. [196] Considering its multi-influential-factor nature, the multivariate regression used in this study can be superior to univariate correlation analysis for bone strength prediction.

The protective effect of greater muscle strength and higher levels of physical activity on bone strength and BMD may be attributable to the mechanical loading from muscle contraction. Muscle contractions during exercise can activate osteocytes and protect them from apoptosis [41-43], partially by reorganizing their cytoskeletons. [56] However, only one randomized interventional study has assessed the effect of exercise on bone health after stroke. Using a first-generation pQCT device with a lower resolution, a positive effect of training on the cortical thickness of the midshaft tibia was found. [199] More interventional studies using HR-pQCT are warranted.

Potential factors predictive of bone loss: muscle spasticity

Muscle spasticity was not found to be related to changes in any of the bone variables in this study. Mixed results have been found in previous studies. A recent

cross-sectional study found that ankle clonus (mean: 1.3 ± 0.6; 1: clonus not elicited, 2: 1 to 3 beats of clonus elicited) was a significant determinant of the between-limb difference in failure load in individuals with chronic stroke. [212] However, Lam et al. and Lazoura et al. found no relationship between spasticity of the ankle plantar flexors (MAS) and bone loss in the tibia (chronic stroke) or hip (subacute stroke), respectively. [30, 167] The participants in the studies of Lam et al. (75%) and Lazoura et al. (100%) were quite homogeneous in that most had mild spasticity. This lack of variance in spasticity partly explains the lack of a significant correlation with changes in bone properties. De Brit et al. found that the level of spasticity in people with chronic stroke was higher in those with greater bone loss (greater than the LSC) than those with less bone loss (less than the LSC). [172] However, their results should be interpreted with caution. The sample size was much smaller for the group with greater bone loss than the group with less bone loss (12 vs. 41). [30] It is possible that the relationship between spasticity and bone loss may not be linear. It may be detrimental or beneficial to bone health, depending on certain thresholds. [30] Nevertheless, future studies should include more participants with severe spasticity to explore its relationship with changes in bone properties.

Clinical implications

Walking speed, peripheral vascular health, and motor factors (muscle strength and physical activity) in the lower limb were shown to be important factors predicting the decline in estimated tibial bone strength in people with chronic stroke. One of the clinical implications is that assessments of walking speed, vascular health, muscle

strength, and physical activity should be incorporated in overall stroke assessments. Another clinical implication is that interventions that target these associated clinical factors (e.g., treadmill aerobic walking [290], exoskeleton-assisted gait training [291], overground walking training [292-294], proprioceptive training [295], ankle-foot orthoses [296], or mixed exercise training [297]) may be beneficial in improving bone health in individuals with chronic stroke, but this requires further investigation.

Limitations

The results presented herein can be only generalized to individuals with chronic stroke who share similar characteristics as the participants of this study. The social distancing and other restrictive measures implemented due to the COVID-19 pandemic limited the opportunities to engage in outdoor and community activities. Therefore, the levels of physical activity reported here may have been substantially lower than the measurements performed before the onset of the pandemic. The dropout rate was 28%, which may have reduced the statistical power. A future study with a larger sample size is recommended.

4.6 Conclusions

In conclusion, there was a significant deterioration in the estimated bone strength, even in people with long-standing stroke. The bone variables that showed the greatest changes in individuals with stroke were total vBMD, cortical vBMD, and cortical thickness in the paretic distal tibia. The decline in trabecular thickness, cortical area,

and estimated failure load was much larger in the stroke group than the control group. The decrease in the estimated failure load was mainly explained by reductions in cortical vBMD, trabecular vBMD, and cortical thickness. Better lower-limb vascular health, a higher walking velocity, better muscle strength, and a higher level of physical activity at the initial assessment, and a greater improvement in walking speed during the 2-year follow-up period, were associated with less of a decline in the estimated failure load. Intervention programs that target these factors may be viable methods to improve bone health in individuals with chronic stroke, but further research is required to prove this hypothesis.

4.7 List of Tables

Table 4-1 Short-term reproducibility and least significant change in bone outcomes measured by HR-pQCT.

Unpublished data	Bone variables	Tibia precision error (%CV $_{RMS}$)	LSC of tibia	Norms: male	Norms: female
Bone density	Total vBMD	0.45%	1.25%	0.36%	2.30%
	Cortical vBMD	0.41%	1.14%	0.46%	2.03%
	Trabecular vBMD	0.65%	1.80%	0.94%	1.32%
Bone morphometry	Cortical area	0.89%	2.47%	-0.10%	2.17%
	Cortical perimeter	0.22%	0.61%	-0.04%	-0.14%
	Cortical thickness	0.64%	1.77%	-0.55%	2.25%
	Trabecular area	0.27%	0.75%	-0.01%	-0.43%
Trabecular and cortical Microarchitecture	Trabecular number	1.44%	3.99%	0.75%	0.64%
	Trabecular thickness	0.60%	1.66%	0.17%	-0.13%
	Trabecular separation	1.36%	3.77%	-0.97%	-0.65%
	Cortical porosity	26.61%	73.71%	-3.68%	-12.65%
	Stiffness			0.68%	1.88%
	Estimated failure load			0.68%	1.84%

Note: Norms were established based on the change over 2 years in healthy elderly individuals aged 55-65 years. HR-pQCT: high-resolution peripheral computed tomography; LSC: least significant change; vBMD: volumetric bone mineral density.

Table 4-2 Intra-rater and inter-rater reliability of Doppler ultrasound measurements of the popliteal artery (n = 15).

		Peak systolic velocity (cm/s)		Diameter (mm)		Peak volume flow (cc/min)	
		P	NP	P	NP	P	NP
Inter-rater (ICC 2,3)		0.86	0.93	0.85	0.91	0.87	0.86
Intra-rater (ICC 3,3)	Rater 1	0.94	0.96	0.95	0.96	0.90	0.96
	Rater 2	0.98	0.96	0.97	0.95	0.95	0.95

Note: P: paretic side; NP: non-paretic side; ICC: intraclass correlation coefficient.

Table 4-3 Demographic information

Variable	**Stroke (n=46)**	**Control (n=45)**	***p***
Basic demographics			
Age (year)	60.4± 7.8	57.7± 6.3	0.074
Stroke duration (years)	6.3±4.2	NA	NA
Body Mass Index (kg/m^2)	24.2±3.1	23.4±2.8	0.056
Body weight distribution asymmetry	13.6%±16.4%	1.5%±6.7%	<0.001*
Dominant leg (Equivalent /L/R, n)	1/44/1	0/44/1	1.000
Paretic hand (L/R, n)	26/20	NA	NA
Gender (Female/male, n)	21/25	17/28	0.446
Walking aids (No/Outdoor Cane or Stick/Outdoor Quadripod/Both Indoor & Outdoor Cane or Stick/Both Indoor & Outdoor Quadripod /Both Indoor & Outdoor Wheelchair, n)	11/27/1/1/4/2	45/0/0/0/0/0	<0.001*
Oxfordshire Classification (Anterior Circulation Syndrome/Partial Anterior Circulation Syndrome/Lacunar Syndrome/Hemorrhage, n)	7/16/7/16	NA	NA
Alcohol history (non-drinker/drinking history, n)	37/9	28/17	0.055
Smoking history (non-smoker/ smoking history, n)	36/10	33/12	0.583
Functional ambulation Category (Dependent/Dependent with Supervision/Independent on Level Surfaces Only/Independent, n)	1/2/4/39	NA	NA
Modified Rankin Scale (1/2/3, n)	2/31/13	NA	NA
Medications/supplements			
Calcium (no supplementation/yes, n)	1	3	0.361
Vitamin D (no supplementation/yes, n)	2	0	0.495
Antihypertensive agents, n	27	12	0.002*
Anticoagulants, n	16	0	<0.001*
Anticonvulsive agents, n	4	0	0.117
Hypolipidemic agents, n	27	7	<0.001*
Hypoglycemic agents, n	7	4	0.354
Antidepressants, n	5	0	0.056
Proton Pump Inhibitors, n	20	0	<0.001*
Total number of medications	4.4±3.4	0.8±1.3	<0.001*
Co-morbid conditions			
Hypertension, n	25	16	0.072
Diabetes, n	10	6	0.292
High cholesterol, n	15	4	0.005*
Total number of comorbidities	1.3±1.4	0.7±1.0	0.016*

Note: Mean ± SD. *: $p < 0.05$. NA: not applicable.

Table 4-4 Generalized estimating equations for the effect of time and side to tibia in stroke group.

	Main effect				**Interaction**	
	Time		**Side**		**Side*time**	
Bone variables	Wald Chi-square	p	Wald Chi-square	p	Wald Chi-square	p
Total vBMD (mg HA/cm3)	20.45	<0.001*	53.22	<0.001	5.04	0.025*
Trabecular area (mm2)	6.60	0.010*	3.55	0.059	2.16	0.142
Trabecular vBMD (mg HA/cm3)	1.51	0.220	6.99	0.008	11.05	0.001*
Trabecular number (1/mm)	0.03	0.874	0.22	0.636	0.59	0.443
Trabecular thickness (mm)	1.12	0.290	3.47	0.062	10.59	0.001*
Trabecular separation (mm)	1.81	0.178	0.93	0.336	0.10	0.751
Cortical Area (mm2)	6.08	0.014*	83.57	<0.001	2.62	0.105
Cortical vBMD (mg HA/cm3)	20.47	<0.001*	60.53	<0.001	0.36	0.550
Cortical Perimeter ((mm)	3.22	0.073	5.43	0.020	3.55	0.060
Cortical Porosity (%)	8.13	0.004*	4.48	0.034	0.43	0.512
Cortical Thickness (mm)	9.46	0.002*	63.94	<0.001	0.27	0.603
Stiffness (kN/mm)	44.81	<0.001*	57.81	<0.001	3.52	0.061
Failure load (N)	47.82	<0.001*	57.57	<0.001	4.48	0.034*

Note: *: $p < 0.05$.

Table 4-5 Generalized estimating equations for the effect of time and side to tibia in controls.

	Main effect				**Interaction**	
	Time		**Side**		**Side*time**	
Bone variables	Wald Chi-square	p	Wald Chi-square	p	Wald Chi-square	p
Total vBMD (mg HA/cm3)	6.90	0.009*	0.06	0.802	1.44	0.230
Trabecular area (mm2)	0.16	0.685	0.31	0.576	2.23	0.136
Trabecular vBMD (mg HA/cm3)	0.01	0.939	0.50	0.482	1.02	0.313
Trabecular number (1/mm)	1.06	0.303	4.67	0.031	0.34	0.562
Trabecular thickness (mm)	8.68	0.003*	0.03	0.865	1.28	0.257
Trabecular separation (mm)	3.32	0.069	3.43	0.064	1.31	0.253
Cortical Area (mm2)	0.09	0.769	0.52	0.469	2.24	0.135
Cortical vBMD (mg HA/cm3)	23.67	<0.001*	0.00	0.951	2.24	0.135
Cortical Perimeter ((mm)	1.85	0.174	0.82	0.364	1.04	0.307
Cortical Porosity (%)	16.96	<0.001*	3.67	0.055	0.15	0.695
Cortical Thickness (mm)	2.19	0.139	1.46	0.228	0.59	0.444
Stiffness (kN/mm)	38.63	<0.001*	0.18	0.673	0.58	0.447
Failure load (N)	37.03	<0.001*	0.39	0.532	1.30	0.254

Note: *: $p < 0.05$.

Table 4-6 HR-pQCT tibia variables for the stroke and control groups

	Stroke group (n= 46)						Control group (n=45)					
	Baseline		2-Year Follow up		*p*		Baseline		2-Year Follow up		*p*	
	P	NP	P	NP	P	NP	ND	D	ND	D	ND	D
Total vBMD (mg HA/cm^3)	263.22±70.01	286.02±58.33	258.34±71.65	283.20±59.05	<0.001**	0.002**	278.95±54.16	279.85±55.73	276.32±54.69	276.48±55.31	0.013*	0.017
Trabecular area (mm^2)	545.75±126.51	539.14±122.34	547.41±126.18	540.07±121.80	0.007**	0.085	583.66±135.57	585.54±135.46	583.55±134.95	586.03±134.24	NA	NA
Trabecular vBMD (mg HA/cm^3)	147.05±41.16	152.61±35.68	145.80±42.09	152.87±35.87	0.024*	0.502	143.01±34.96	143.80±32.67	142.80±35.74	143.96±33.04	NA	NA
Trabecular number (1/mm)	1.15±0.17	1.15±0.15	1.14±0.19	1.15±0.18	NA	NA	1.08±0.17	1.10±0.17	1.08±0.19	1.10±0.17	NA	NA
Trabecular thickness (mm)	0.25±0.02	0.25±0.02	0.25±0.02	0.26±0.02	0.012*	0.261	0.25±0.02	0.25±0.02	0.25±0.02	0.25±0.02	0.113	0.010**
Trabecular separation (mm)	0.86±0.15	0.85±0.14	0.87±0.19	0.86±0.16	NA	NA	0.91±0.18	0.89±0.15	0.93±0.24	0.90±0.16	NA	NA
Cortical Area (mm^2)	110.56±32.82	124.18±29.75	108.92±33.75	123.35±30.49	0.007**	0.137	130.36±24.12	129.99±24.23	130.52±25.22	129.55±24.91	NA	NA
Cortical vBMD (mg HA/cm^3)	813.32±87.54	847.81±69.42	802.29±82.98	837.88±66.13	<0.001**	<0.001**	870.48±59.82	871.50±59.55	858.41±56.95	857.69±57.13	<0.001**	<0.001**
Cortical Perimeter ((mm)	100.01±9.37	100.55±9.36	100.06±9.46	100.73±9.38	0.489	0.011*	103.82±10.42	103.83±10.19	104.04±10.55	105.33±13.43	0.001**	0.001**
Cortical Porosity (%)	0.04±0.02	0.03±0.01	0.04±0.02	0.04±0.02	0.017*	0.046*	0.03±0.02	0.03±0.02	0.04±0.02	0.03±0.02	<0.001**	0.004**
Cortical Thickness (mm)	1.33±0.40	1.47±0.35	1.31±0.41	1.46±0.36	0.011*	0.009**	1.48±0.28	1.47±0.31	1.48±0.29	1.46±0.30	NA	NA
Stiffness (kN/mm)	152678.67±45479.37	171850.84±42025.99	148128.19±46358.33	168563.58±41820.75	<0.001**	<0.001**	180350.09±39369.91	181118.19±38505.39	177352.45±40689.99	177732.52±39896.72	<0.001**	<0.001**
Failure load (N)	8285.13±2390.70	9278.31±2182.68	8037.26±2434.01	9102.36±2165.22	<0.001**	<0.001**	9754.45±2059.24	9813.21±2015.50	9596.26±2140.51	9624.58±2102.79	<0.001**	<0.001**

Note: P: paretic side; NP: non-paretic side; ND: non-dominant side; D: dominant side. *: $p < 0.05$, **: $p<0.01$. NA: not applicable for insignificant time effect.

Table 4-7 The relative change of Tibia over the 2-year follow-up period

	Stroke group (n=46)			Control group (n=45)			Between-group	
	Paretic	Non-paretic	*P (between-side)*	Non-dominant	Dominant	*P (between-side)*	*P* (Paretic)	*P* (Non-paretic)
Total vBMD (mg HA/cm^3)	2.14%±3.24%*#†	1.02%±2.20%*#	0.008‡	0.99%±2.43%*#	1.17%±2.97%*#	0.666	0.038§	0.659
Trabecular area (mm^2)	-0.32%±0.74%*#	-0.20%±0.76%	0.232	0.00%±0.68%	-0.14%±0.81%	0.133	0.036§	0.207
Trabecular vBMD (mg HA/cm^3)	1.18%±3.65%*#	-0.15%±2.06%	0.001‡	0.36%±2.21%	-0.05%±2.44%	0.190	0.294	0.831
Trabecular number (1/mm)	0.33%±4.29%	-0.18%±5.78%	NA	0.69%±3.86%	0.23%±2.10%	0.937	NA	0.370
Trabecular thickness (mm)	0.58%±1.58%*	-0.29%±1.40%	0.002‡	-0.28%±1.24%	-0.60%±1.50%*	0.233	0.005§	0.299
Trabecular separation (mm)	-0.83%±4.51%	-0.68%±5.52%	NA	-1.24%±4.34%	-0.69%±1.82%	0.913	NA	0.319
Cortical Area (mm^2)	1.83%±4.92%*#	0.77%±3.58%	0.069	-0.01%±2.74%	0.41%±2.83%	0.137	0.013§	0.306
Cortical vBMD (mg HA/cm^3)	1.28%±2.36%*#†	1.13%±1.65%*#	0.517	1.36%±1.86%*#†	1.55%±2.19%*#†	0.143	0.617	0.943
Cortical Perimeter ((mm)	-0.04%±0.54%	-0.18%±0.42%*	NA	-0.20%±0.32%	-1.43%±8.19%	0.643	NA	0.566
Cortical Porosity (%)	-12.04%±24.12%*	-7.89%±19.71%*	0.238	-9.79%±15.79%*	-13.59%±27.62%*	0.316	0.601	0.880
Cortical Thickness (mm)	1.95%±5.29%*#†	1.22%±3.37%*#	0.714	0.46%±2.50%	0.57%±2.76%	0.576	0.228	0.322
Stiffness (kN/mm)	3.40%±3.61%*	1.97%±2.60%*	0.004‡	1.92%±2.42%*	2.11%±2.79%*	0.352	0.024§	0.578
Failure load (N)	3.39%±3.46%*	1.93%±2.48%*	0.002‡	1.89%±2.44%*	2.18%±2.84%*	0.312	0.019§	0.703

Note: relative change=(T1-T2)/T1; Positive values suggest decline from baseline to follow-up. NA: not applicable for insignificant time effect.

*: significant change from T1 to T2, $p<0.05$. Positive values suggest decline from baseline to follow-up.

#: relative change above precision error.

†: relative change above Least significant change (LSC).

‡: Significant difference between two sides, $p<0.05$.

§: Significant difference between stroke and control group, $p<0.05$.

Table 4-8 Generalized estimating equation (GEE): the effect of time and side for clinical variables in stroke group (n=46)

GEE	**Main effect**				**Interaction**	
	Time		**Side**		**Side*time**	
	Wald Chi-square	p	Wald Chi-square	p	Wald Chi-square	p
Gastrocnemius muscle strength (Nm)	8.41	0.004*	57.44	<0.001*	1.12	0.290
Gastrocnemius stiffness (N/m)	0.78	0.379	1.72	0.189	0.00	0.947
Blood flow velocity (cm/s)	7.65	0.006*	1.22	0.270	1.01	0.314
Blood flow volume (cc/min)	24.43	<0.001*	0.08	0.776	0.10	0.751
Diameter (cm)	17.99	<0.001*	40.95	<0.001*	0.01	0.923
Hallux sensation	18.89	<0.001*	24.23	<0.001*	0.10	0.753

Note: *: $p < 0.05$.

Table 4-9 Generalized estimating equation (GEE): the effect of time and side for clinical variables in controls (n=45)

GEE	Main effect				Interaction	
	Time		**Side**		**Side*time**	
	Wald Chi-square	p	Wald Chi-square	p	Wald Chi-square	p
Gastrocnemius muscle strength (Nm)	59.02	<0.001*	4.39	0.036	4.42	0.036*
Gastrocnemius stiffness (N/m)	0.63	0.428	0.54	0.465	0.49	0.486
Blood flow velocity (cm/s)	14.07	<0.001*	2.06	0.152	0.14	0.711
Blood flow volume (cc/min)	91.51	<0.001*	0.23	0.629	4.26	0.039*
Diameter (cm)	137.05	<0.001*	1.61	0.205	1.38	0.240
Hallux sensation	44.72	<0.001*	2.33	0.127	2.33	0.127

Note: *: $p < 0.05$.

Table 4-10 Changes of clinical variables over the follow-up period in stroke group (n=46)

	Baseline	**2-Y follow up**	**p**
Gastrocnemius strength (Paretic Side, Nm)	41.02±15.89	44.31±18.24	0.027 *
Gastrocnemius strength (Non-Paretic Side, Nm)	65.53±23.23	72.41±28.58	0.019*
Gastrocnemius stiffness (Paretic Side, N/m)	310.52±51.50	315.72±50.73	NA
Gastrocnemius stiffness Non-Paretic Side, N/m)	303.09±41.60	308.85±37.09	NA
Peak systolic velocity (Paretic Side, cm/s)	47.62±14.64	50.70±12.97	0.102
Blood flow volume (Paretic Side, cc/min)	37.22±22.08	60.58±41.70	<0.001*
Arterial diameter (Paretic Side, cm)	0.53±0.12	0.57±0.10	0.005*
Peak systolic velocity (Non-Paretic Arm, cm/s)	45.36±13.07	50.32±14.34	0.004*
Blood flow volume (Non-Paretic Arm, cc/min)	35.45±25.28	60.58±37.88	<0.001*
Arterial diameter (Non-Paretic Arm, cm)	0.59±0.12	0.62±0.11	<0.001*
Hallux light touch sensation (P, Max:6.65)	3.90±1.14	4.62±1.43	0.002*
Hallux light touch sensation (NP, Max:6.65)	3.35±0.93	4.01±0.89	<0.001*
10-meter walk Test (m/s)	0.80±0.39	0.86±0.48	0.045*
BMI (kg/m^2)	24.23±3.06	26.62±16.34	0.009*
Physical Activity Scale of the Elderly (Max:400)	120.91±79.16	101.55±76.97	0.061
Brief-BEST (Max:24)	10.76±5.23	7.13±3.26	<0.001*
Composite Spasticity Scale - lower Limb Total (Max:16)	7.33±2.43	8.78±2.56	<0.001*
Fugl-Meyer Assessment - lower Limb (Max:34)	26.65±4.71	25.13±4.68	0.001*
Total number of comorbidities	1.33±0.20	1.35±0.20	0.685
Total number of medications	4.37±0.50	4.02±0.48	0.154

Note: Mean±SD. * $p < 0.05$ statistically significant change. NA: not applicable for insignificant time effect.

Table 4-11 Changes of clinical variables over the follow-up period in controls (n=45)

	Baseline	2-Y follow up	p
Gastrocnemius strength (non-Dominant, Nm)	75.61±36.65	98.22±42.60	<0.001*
Gastrocnemius strength (Dominant, Nm)	75.27±34.15	104.83±40.98	<0.001*
Gastrocnemius stiffness (non-Dominant, N/m)	307.11±40.27	301.07±31.04	NA
Gastrocnemius stiffness (Dominant, N/m)	307.24±38.74	305.32±34.04	NA
Peak systolic velocity (non-Dominant, cm/s)	44.19±7.61	48.40±10.00	0.003*
Blood flow volume (non-Dominant, cc/min)	28.64±12.67	78.74±39.88	<0.001*
Arterial diameter (non-Dominant,cm)	0.52±0.10	0.62±0.08	<0.001*
Peak systolic velocity (Dominant, cm/s)	44.94±9.04	49.70±9.19	0.003*
Blood flow volume (Dominant, cc/min)	32.23±15.96	72.81±42.98	<0.001*
Arterial diameter (Dominant, cm)	0.53±0.09	0.62±0.07	<0.001*
Hallux light touch sensation (non-Dominant, Max:6.65)	3.26±0.63	3.89±0.72	<0.001*
Hallux light touch sensation (Dominant, Max:6.65)	3.19±0.64	4.05±0.84	<0.001*
10-meter walk Test (m/s)	1.93±0.27	2.38±0.54	<0.001*
BMI (kg/m^2)	23.04±2.76	23.05±3.16	0.980
Physical Activity Scale of the Elderly (Max:400)	148.26±82.16	147.43±72.06	0.928
Brief-BEST (Max:24)	15.04±2.24	15.84±2.27	0.013*
Total number of comorbidities	0.69±0.15	0.73±0.14	0.740
Total number of medications	0.82±0.19	0.98±0.20	0.251

Note: Mean±SD. * $p < 0.05$ statistically significant change. NA: not applicable for insignificant time effect.

Table 4-12 Correlation between the % change of estimated failure load and the %change of other bone variables of tibia.

	Estimated failure load (%change)	
	r	p
Total vBMD-%change	0.82**	<0.001
Trabecular area-%change	-0.64**	<0.001
Trabecular vBMD-%change	0.54**	<0.001
Trabecular thickness-%change	0.21	0.155
Cortical area-%change	0.64**	<0.001
Cortical vBMD-%change	0.56**	<0.001
Cortical thickness -%change	0.68**	<0.001
Cortical porosity -%change	-0.22	0.139

Note: *: $p < 0.05$, **: $p < 0.01$.

Table 4-13 Regression analysis: Relative contributions of different bone parameters to %change in estimated failure load for the stroke group.

	Model Summary				**Standardized regression coefficients**	
Independent variables	**R2**	**ΔR2**	**ΔF**	**p (ΔF)**	**Beta**	**p**
Cortical vBMD %change	0.31	0.28	16.69	<0.001*	0.42	<0.001†
Trabecular vBMD %change	0.61	0.27	25.83	<0.001*	0.43	<0.001†
Cortical thickness- %change	0.72	0.17	26.19	<0.001*	0.44	<0.001†

Note: R2 = total variance, ΔR [2] = additional predictor variance, ΔF = F-value change, Beta = standardized regression coefficient, %change = percent change; vBMD = volumetric bone mineral density.

* $p \leq 0.05$ Statistically significant F-value change

† $p \leq 0.05$ Statistically significant predictor

Add %change of height to the model didn't change the finding.

Table 4-14 Correlation between the %change of estimated failure load and demographic information and clinical variables in the stroke group.

	Failure load (% change)	
	r	***p***
Demographic information		
Age	-0.01	0.969
Stroke Duration	-0.36	**0.013**
Total number of medications	-0.05	0.735
Antihypertensive agents	-0.17	0.274
Anticoagulants	-0.26	**0.080**
Hypolipidemic agents	-0.18	0.223
Proton Pump Inhibitors	-0.16	0.302
Total number of comorbidities	0.02	0.881
Hypertension	-0.02	0.905
Hyperlipidemia	0.12	0.431
Diabetes Mellitus	-0.13	0.390
BMI	-0.24	0.117
Sex	0.32	**0.030**
Calcium Supplementation	-0.28	**0.057**
Vitamin D Supplementation	0.14	0.351
Smoking History	-0.14	0.352
Alcohol History	-0.02	0.898
Paretic side	0.13	0.396
Baseline clinical variables		
Physical Activity Scale for Elderly	-0.27	**0.071**
Gait Velocity	-0.30	**0.043**
Gastrocnemius strength	-0.28	**0.059**
Composite Spasticity Scale	0.08	0.620
Fugl-Meyer Assessment-Lower limb	-0.22	0.134
Blood flow volume	-0.36	**0.013**
Artery diameter	-0.06	0.705
Hallux sensory	-0.01	0.936
Gastrocnemius stiffness	0.05	0.724
The relative change of clinical variables over the follow-up period		
Physical Activity Scale for Elderly	0.03	0.856
Gait Velocity	0.26	**0.077**
Gastrocnemius strength	0.04	0.809
Composite Spasticity Scale	0.11	0.465
Fugl-Meyer Assessment-Lower limb	0.03	0.863
Blood flow volume	-0.18	0.228
Artery diameter	0.30*	**0.041**
Hallux sensory	-0.05	0.724

Note: p value in bold: p<0.1. Relative change=(T1-T2)/T1.

Table 4-15 Regression analysis: Associations between clinical variables at baseline and % change in estimated failure load of paretic tibia.

	Model Summary				Standardized regression coefficients	
Predictors	**R2**	**ΔR2**	**ΔF**	***p* (ΔF)**	**Beta**	***p***
Factor 1	0.03	0.03	1.28	0.264	-0.10	0.486
Factor 2	0.03	0.00	0.13	0.721	0.05	0.719
Factor 3	0.06	0.03	1.15	0.290	-0.04	0.768
Factor 4	0.07	0.01	0.53	0.472	-0.16	0.258
Factor 5	**0.20**	**0.13**	**6.47**	**0.015***	**-0.37**	**0.011†**
Factor 6	**0.28**	**0.08**	**4.49**	**0.041***	**-0.33**	**0.041†**

Note:

Factor 1 = Age, gender, smoking history, alcohol history.

Factor 2 = Calcium supplementation status; Vitamin D supplementation status.

Factor 3 = BMI, total number of medications, total number of comorbidities.

Factor 4 = stroke duration.

Factor 5 = Gait speed, muscle strength, physical activity scale of elderly

Factor 6 = Blood flow volume

R2 = total variance, ΔR [2] = additional predictor variance, ΔF = F-value change, Beta = standardized regression coefficient, CI = confidence interval, %change = percent change; vBMD = volumetric bone mineral density.

* $p \leq 0.05$ Statistically significant F-value change

† $p \leq 0.05$ Statistically significant predictor

Add %change of height to the model didn't change the findings.

Table 4-16 Correlation between the %change of estimated failure load and relative change in clinical variables in the stroke group.

	Estimated failure load (% change)	
	r	***p***
The relative change of clinical variables over the follow-up period		
Physical Activity Scale for Elderly	0.03	0.856
Gait Velocity	**0.26**	**0.077**
Gastrocnemius strength	0.04	0.809
Composite Spasticity Scale	0.11	0.465
Fugl-Meyer Assessment-Lower limb	0.03	0.863
Blood flow volume	-0.18	0.228
Artery diameter	**0.30***	**0.041**
Hallux sensory	-0.05	0.724

Note: p value in bold: p<0.1. Relative change=(T1-T2)/T1.

Table 4-17 Regression analysis: Associations between the %change of clinical variables and %change in estimated failure load of paretic tibia.

	Model Summary				**Standardized regression Coefficients**	
Independent variables	**R2**	**ΔR2**	**ΔF**	***p* (ΔF)**	**Beta**	***p***
Factor 1	0.03	0.03	1.18	0.284	0.22	0.146
Factor 2	0.04	0.01	0.65	0.426	-0.10	0.509
Factor 3	0.04	0.00	0.16	0.695	-0.14	0.356
Factor 4	0.06	0.02	0.82	0.370	-0.11	0.441
Gait speed-%change	**0.17**	**0.11**	**5.10**	**0.029***	**0.26**	**0.124**
Arterial diameter -%change	0.20	0.03	1.38	0.247	0.19	0.247

Note:

Factor 1 = Age, gender, smoking history, alcohol history.

Factor 2 = Calcium supplementation status; Vitamin D supplementation status.

Factor 3 = BMI, total number of medications, total number of comorbidities.

Factor 4 = stroke duration.

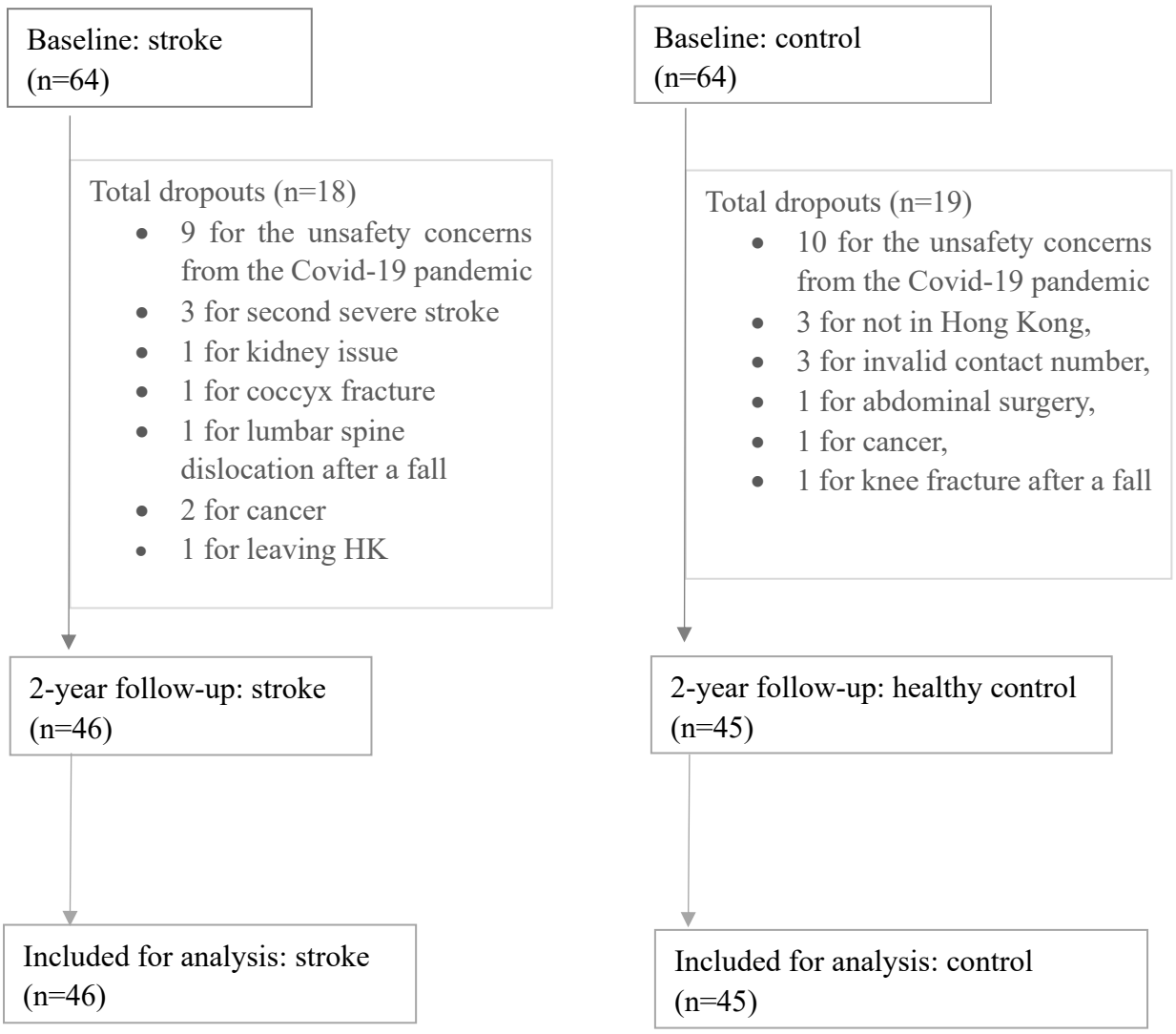
R2 = total variance, ΔR [2] = additional predictor variance, ΔF = F-value change, Beta = standardized regression coefficient, CI = confidence interval, %change = percent change; vBMD = volumetric bone mineral density.

* $p \leq 0.05$ Statistically significant F-value change

Add %change of height to the model didn't change the findings

4.8 List of Figures

Figure 4-1 Study flowchart.



5. Chapter 5

Longitudinal changes in the bone properties of the distal radius and tibia within the first 6 months post-stroke

Ouyang Huixi

5.1 Abstract

Background: Secondary bone loss appears in early period after stroke. However, few study has applied the high-resolution peripheral computed tomography (HR-pQCT) to investigate the related factors of such bone loss from acute stage to early chronic stage of stroke recovery.

Purpose: To examine how the bone density, macrostructure, and microstructure of the distal tibia and radius change in people with acute stroke and to identify the factors associated with the change in the estimated failure load of these two sites.

Methods: Thirteen individuals with acute stroke participated in the study. The distal tibia and radius were measured twice bilaterally using HR-pQCT. The baseline measurement was made within 2 weeks post stroke onset and the second measurement was made 6 months later. The vascular health of two peripheral arteries was measured by Doppler ultrasound. Hand grip strength and quadricep strength was measured by portable dynamometer. Active range of motion of ankle and wrist joint were examined as well.

Results: Six subjects completed the follow-up and were included in the analysis. In the paretic side, reduction in the trabecular vBMD (1.18%-1.25%), cortical vBMD (0.4%-0.73%) and estimated failure (2.18%-3.96%) load was observed in both the distal radii and tibiae. However, an increase was observed in the trabecular thickness (0.80%) of the distal radius and in the trabecular number (1.53%) of the distal tibia on the paretic

side. More decline in cortical vBMD in both sites were associated with more reduction in estimated failure load (r=0.76-0.89). Better hand-grip strength (r=-0.59) and greater active ROM (r=-0.57) on the paretic side were associated with less of a decline in the estimated failure load of the distal radius. Higher peak systolic velocity (r=0.66) at baseline was associated with a greater decline of estimated failure load in the distal tibia during the follow-up period.

Conclusions: From acute to early chronic stage post stroke, the reduction in the failure load were mostly explained by decreases in cortical bone density. Individuals with less wrist range of motion and worse hand grip strength tended to show a greater decline in the estimated tibial bone strength on the paretic radius. Whereas people with poorer vascular health at baseline was associated with a greater decline in the estimated failure load of the paretic tibia.

5.2 Introduction

Stroke is a debilitating condition that often results in long-lasting comorbidities. Among these comorbidities, secondary stroke-induced hemi-osteoporosis has become an increasing concern, because it poses an exaggerated risk of fragility fractures.[79] From a preventive point of view, determining the changes in different bone variables on the paretic side and their associated modifiable factors early after stroke onset is essential. A number of earlier longitudinal studies applied dual-energy X-ray absorptiometry (DXA) to study the trend in bone changes starting from the very early

stage after stroke onset.[166-168] The magnitude of the decline in the areal bone mineral density (aBMD) of the proximal femur and distal radius was found to be higher during the first 6 months than during the second 6 months post-stroke, suggesting that the rate of deterioration of bone health is higher in the former period.[166] Further, stroke severity and the level of walking independence recovery were found to be related to the degree of bone loss in the humerus and proximal femur.[167, 168]

Using DXA to evaluate bone properties has several limitations. First, it only provides a two-dimensional measure of aBMD, whereas bone is a three-dimensional (3D) structure. Second, it cannot measure cortical and trabecular bone properties separately. Later, peripheral quantitative computed tomography (pQCT) was developed, which allows for the measurement of 3D bone features (e.g., volumetric BMD [vBMD]).[31, 177] pQCT has been shown to have a better ability to predict fracture than DXA.[176] According to the only pQCT study that investigated bone changes early after stroke, the decline in the polar stress–strain index (a bone strength indicator) of the distal radius was greater during the first 6 months than during the second 6 months.[167] Considering that most motor recovery occurs within the first 6 months post-stroke, this may also be the optimal time window for investigating bone changes.[169]

While the abovementioned pQCT studies have shed some light on how stroke influences bone density and structure, the pQCT devices used in these studies did not have adequate resolution (pixel size: 0.2–1.0 mm) to accurately measure the cortical thickness at sites where the cortex is relatively thin. However, these sites are often

where fractures are more prevalent (e.g., the distal radius) than at sites where the cortex is relatively thick. The low resolution also renders it impossible to study the bone microstructure (e.g., cortical porosity and trabecular number and spacing), which is important for determining bone fragility.[185] Evidence from human cadaver studies supports the notion that microstructural measurements of the radius and tibia assessed by high-resolution pQCT (HR-pQCT) are highly correlated with actual bone stiffness and yield strength, as determined by mechanical testing.[188] Therefore, it is worthwhile to evaluate bone microstructure in people with acute stroke.

To date, only one study has used HR-pQCT (voxel size: 82 μm) to investigate the change in vBMD within the first 6 months post-stroke and it found an association between the change in the distal tibia vBMD and the impairment in motor control.[196] Moreover, Borschmann et al. did not study another skeletal site that may also be susceptible to fragility fracture, such as the distal radius. However, other bone microstructure parameters, such as cortical thickness and estimated failure load, which have been suggested to be important predictors of fracture risk,[269] were not examined in this study.

Determining the key predictors of bone outcomes is instrumental in the early identification of individuals who are at a high risk of secondary hemi-osteoporosis following stroke. These predictors may also have important implications for the development of interventions to prevent or reverse deleterious bone changes. The objectives of the work described in this chapter were: 1) to assess the changes in bone density, geometry, and microstructure of the distal radius and tibia during the first 6

months post-stroke and 2) to investigate the associations of the changes in bone density, geometry, and microstructure post-stroke with potential clinical factors.

5.3 Methods

Study design and ethics

This was a 6-month longitudinal study involving participants with acute stroke. Each participant provided written informed consent prior to data collection. Ethics approval was obtained from the Joint Chinese University of Hong Kong – New Territories East Cluster Clinical Research Ethics Committee and the Institutional Review Board of the Hong Kong Polytechnic University. All study procedures were conducted in accordance with the Declaration of Helsinki.

Participant recruitment

Participants with acute stroke were recruited from the Prince of Wales Hospital. The inclusion criteria were as follows: diagnosis of a unilateral stroke confirmed by the individual's physician, age ≥ 18 years, stroke duration < 2 weeks, medically stable, Modified Rankin Scale (MRS) score: 2–5, ability to obey motor commands, and ability to provide informed consent (including consent of family members). The exclusion criteria were as follows: other neurological conditions (e.g., Parkinson's disease), receptive aphasia, recurrent stroke that had led to significant impairment in daily activities (MRS score ≥ 2), pregnancy, other conditions that had a substantial effect on bone health (e.g., rheumatoid arthritis), taking medications for osteoporosis treatment (e.g., bisphosphonates) currently and/or prior to stroke, a known diagnosis of

osteoporosis prior to stroke, fragility fractures prior to stroke, metal implants in the limb to be scanned, or other serious illnesses (e.g., cancer).

Demographic information

The basic demographic data (e.g., sex and age) and brain scan findings (e.g., computed tomography and magnetic resonance imaging findings), which provided information on stroke type and location, were obtained from the participants' medical records. The pre-stroke physical activity level was estimated using a six-level scale for the classification of physical activity level.[298] Two qualified physiotherapists with more than 10 years of clinical experience, and an research student operated the other clinical measurements. The bone scans were performed by a technician at a local bone imaging center with extensive experience in osteoporosis research.

Bone scan protocol

The bilateral distal radii and distal tibiae of each participant were scanned twice: within 2 weeks after stroke onset and again at 6 months after stroke onset. Thus, this study evaluated the change in bone status during the first 6 months since stroke onset. The effective radiation dose in each HR-pQCT scan was 3–4 μSv. The total effective radiation dose during the 6-month follow-up period was thus approximately 24–32 μSv (8 scans: 4 bone sites × 2 time points), which is similar to the radiation dose received during a 4–5-hour flight. The protocol of this study was thus very safe and did not pose any health hazards.

Bone imaging and image registration

Imaging and image registration: The vBMD, cross-sectional geometry, and microstructural properties of the bilateral distal radii and distal tibiae were measured using HR-pQCT (Scanco Medical AG, Brüttisellen, Switzerland). The scan region was fixed at 9.5 mm (distal radius) or 22.5 mm (distal tibia) proximal from the mid-joint line.[248] The length of the scan region spanned 9.02 mm proximally, which was equivalent to a stack of 110 slices.[249] For analyses, the bone images acquired during the follow-up period from each individual were subjected to same–volume-of-interest (VOI) matching between the baseline and 6-month measurements. A software program provided by the manufacturer was used to identify the same region by matching the corresponding images by their total cross-sectional areas. Image analyses were conducted only for the bone volume common to the measurements at the two time points.[249]

Image analysis: Analysis of 3D scan data was conducted using the Image Processing Language (IPL v5.08b; Scanco Medical AG, Wangen-Brüttisellen, Switzerland). First, bone images were analyzed using a standard (default) protocol.[187] Next, extended cortical analysis was conducted using a cortical compartment segmentation technique adapted from the study by Buie et al.[250] (μCT Evaluation v6.0, Scanco Medical AG).[192] This was done to improve the accuracy of the analysis of very porous or thin cortical shells. The details of the HR-pQCT variables are shown in Appendix 1.

Micro finite element (mFE) analyses: All mFE analyses were performed using the

FE-solver included in the built-in Image Processing Language software of the HR-pQCT system (IPL-FE v1.15, Scanco Medical AG).[247] A special peeling algorithm specifying a minimum cortical thickness of 6 voxels was used to identify cortical and trabecular bone tissue. mFE analyses were performed by converting the binary image data into a mesh of isotropic brick elements. For all elements, a Poisson's ratio of 0.3 was specified. Elements representing cortical and trabecular bone were both assigned a Young's modulus of 10 GPa.[247] A uniaxial compression test with a 1,000-N load and an applied strain of 1% was performed. Whole-bone stiffness (kN/mm) was calculated. The estimate failure load (N) was calculated based on the assumption that bone failure occurred if >2% of the elements were strained beyond 0.7% strain.[175]

Our HR-pQCT scanner has excellent reproducibility. The coefficient of variation (CV%) was calculated as the standard deviation (SD) of the two repeated measurements divided by the subject mean. Short-term precision error was calculated as the root-mean-square average of the CV ($CV\%_{RMS}$) for each subject. According to our unpublished pilot data from 32 healthy older adults (mean age = 58.1 years, 81% women), the precision error ($CV\%_{RMS}$) was 0.22%–0.94% for geometric measures (Table 3-1 and Table 4-1). The corresponding values for density and microstructural variables (not including cortical porosity) were 0.41%–0.89% and 0.58%–1.99%, respectively. The least significant change (LSC) value at 95% confidence (calculated from 2.77 × precision error) was also calculated as given below. The intra-rater reliability of these parameters was good (linear weighted kappa = 0.855).[38]

$$LSC = 1.96 \cdot \sqrt{CV\%_{RMS}^2 + CV\%_{RMS}^2} = 2.77 \cdot CV\%_{RMS}$$

Table 3-1 & 4-1 Short-term reproducibility and least significant change in bone outcomes measured by HR-pQCT.

Unpublished data	Bone variables	Radius precision error (%CV)	LSC of radius	Tibia precision error (%CV)	LSC of tibia
Bone density	Total vBMD	0.59%	1.63%	0.45%	1.25%
	Cortical vBMD	0.46%	1.27%	0.41%	1.14%
	Trabecular vBMD	0.89%	2.47%	0.65%	1.80%
Bone morphometry	Cortical area	0.94%	2.60%	0.89%	2.47%
	Cortical perimeter	0.31%	0.86%	0.22%	0.61%
	Cortical thickness	0.88%	2.44%	0.64%	1.77%
	Trabecular area	0.47%	1.30%	0.27%	0.75%
Trabecular and cortical microarchitecture	Trabecular number	1.99%	5.51%	1.44%	3.99%
	Trabecular thickness	0.58%	1.61%	0.60%	1.66%
	Trabecular separation	1.77%	4.90%	1.36%	3.77%
	Cortical porosity	12.46%	34.51%	26.61%	73.71%

Note: HR-pQCT: high-resolution peripheral computed tomography; LSC: least significant change; vBMD: volumetric bone mineral density. CV%: coefficient of variation.

Other clinical assessments

In addition to the bone measurements, the following variables were also measured. These outcomes were selected because they were implicated to be associated with bone health post-stroke in our previous cross-sectional studies.[31, 177-180, 182]

Muscle strength: Bilateral handgrip (lbs) was measured using a hand-held Jamar dynamometer (Sammons Preston, Mississauga, Canada).[251] The test was performed with the participants in a sitting position with their shoulder in a neutral position and their elbow flexed at 90°. This technique has previously shown good to excellent intra-rater reliability (intraclass correlation coefficient [ICC] = 0.88–0.96) and test–retest

reliability (ICC = 0.71–0.94) in people after stroke and closed brain damage.[252] Another portable dynamometer (DS2-500N; ZHIQU, Dongguan, China) was used to measure isometric peak torque (lbf) of the quadriceps. The measurement error of this device is ±1%. The knee extensor was tested at 90° flexion. During the isometric test for the quadriceps, the dynamometer was fixed to the leg of the chair in which the participant was sitting to eliminate the measurement error caused by unstable dynamometer attachment.

During the test, the participants were instructed to perform contraction with maximal effort. Verbal encouragement from the assessor was provided to elicit maximal effort from the participants. The test was repeated three times with 1-minute rest intervals. The strength values from the three trials were averaged.

Walking status: The Functional Ambulation Category test (range: 0–5) was used to assess ambulatory status. This test has previously demonstrated high test–retest reliability (Cohen's kappa = 0.950) and inter-rater reliability (kappa = 0.905) in individuals with stroke.[283] The 10-meter walk test at a safe maximal speed was also performed. This test has previously shown excellent test–retest reliability in stroke population (ICC = 0.97).[284]

Sensory function: The touch pressure threshold [evaluator size = Log_{10} of (10 × force in milligrams)] was tested using the Semmes–Weinstein monofilament test (SWMT).[258, 259] The score ranges from 1.65 to 6.65. In the upper limb, the dorsum of the hand was tested at the mid-length of proximal phalange of the middle finger and at the mid-length of the third metacarpal bone, because these are the sites where the

greatest deficits in cutaneous sensation tend to occur in stroke survivors.[258] The plantar surface of the foot was tested because sensory deficit in this area has an important contribution to balance impairments. The SWMT score has previously shown substantial to perfect intra-rater agreement (Cohen's kappa: thumb = 0.89, index finger = 0.80) and substantial inter-rater agreement in people with chronic stroke (Cohen's kappa: thumb = 0.75, index finger = 0.79).[259]

Physical activity level: The Physical Activity Scale for the Elderly [260] was used to evaluate the participants' physical activity level (possible score range: 0–400). Twelve questions were asked regarding leisure activity frequency and duration (e.g., jogging, swimming, and strength and endurance exercise), household activity, and work-related activity during the last 7 days. Higher scores indicated higher activity levels. The Chinese version of this test has previously shown good test–retest reliability in healthy older adults (ICC = 0.81).

Vascular health: As a surrogate measure for arterial stiffness,[299] the pulse wave velocity (PWV) of the bilateral brachial and popliteal arteries was measured using Doppler ultrasound. The PWV is influenced by several factors, as shown in the formula below:

$$PWV^2 = 2E \cdot h / r \cdot p, \text{ [300]}$$

where E is the elastic modulus, h is the arterial wall thickness, r is the artery diameter, and p is the blood density. Thus, a higher PWV value is indicative of greater arterial stiffness (i.e., poorer vascular health).

Settings of the Doppler ultrasound were standardized and set for high sensitivity,

medium wall filter, a pulsed repetition frequency of 6.1 kHz for brachial artery and 3.5 kHz for popliteal artery, and medium persistence. To ensure that blood flow was measured with the participants in a resting state, all of the participants were rested in the supine position for at least 10 minutes before the ultrasound examination. The room temperature was maintained at 23℃. The sequence of the scan of the two sides was randomized.

The participants were in a supine position during the ultrasound examination of the brachial artery. The scan location for the brachial artery was standardized at the distal third point (i.e., 33%) between the coracoid process and the crease of the cubital fossa of the medial side.[194, 262] To exclude the influence of stiffness of the elongated biceps and considering that some individuals have limited elbow joint range of motion (ROM) because of spasticity, the elbow joint was maintained at 60° flexion using a custom arm immobilization device. To expose enough skin area for the scan by the ultrasound probe, the shoulder was maintained at 45° abduction. For ultrasound examination of the popliteal artery, the probe was placed in a sagittal orientation on the popliteal fossa at the approximate level of the popliteal crease.

After visual confirmation of the artery with color Doppler in the transverse image plane, the probe was sagittally rotated and tilted to visualize the artery longitudinally. An electronic caliper was then positioned in the center of the artery. The sample volume was standardized at 1.0 mm for the popliteal artery and 0.5 mm for the brachial artery. Doppler steering and fine angle correction were adjusted to optimize angle-to-flow at an insonation angle ≤ 60°. The auto-trace function was applied to estimate the full range

of positive and negative flow. Three consistent spectral waveform cycle readings were used to calculate the average peak systolic velocity (cm/s). The specific procedures performed to obtain the optimal images of the arteries were the same as those described in Chapters 3 and 4.

Based on our preliminary analysis in 15 individuals with chronic stroke (as in Chapter 3 and 4), good to excellent inter-rater reliability (ICC = 0.87–0.93) and intra-rater reliability (ICC = 0.92–0.98) were demonstrated for peak systolic velocity in both arteries.

Table 3-2 & Table 4-2 Intra-rater and inter-rater reliability of Doppler ultrasound measurement of the popliteal artery (n = 15).

		Peak systolic velocity (cm/s)	
		P	NP
Popliteal artery			
Inter-rater (ICC 2,3)		0.86	0.93
Intra-rater (ICC 3,3)	Rater 1	0.94	0.96
	Rater 2	0.98	0.96
Brachial artery			
Inter-rater (ICC 2,3)		0.87	0.88
Intra-rater (ICC 3,3)	Rater 1	0.97	0.92
	Rater 2	0.95	0.96

Note: P: paretic side; NP: non-paretic side; ICC: intraclass correlation coefficient.

Stroke-specific assessments

Motor recovery: The Fugl-Meyer Motor Assessment (FMA) was used to assess the degree of motor recovery in the hemi-paretic lower limbs (range: 0–34) and upper limbs (range: 0–66). Lower scores indicate more severe motor impairment. The FMA assesses the movement quality, movement coordination, and reflex action of the

shoulder, elbow, wrist, hand, hip, knee, and ankle. Items were scored on a 3-point ordinal scale, where 0 = cannot perform; 1 = can perform partially; and 2 = can perform fully. This test has previously shown excellent inter-rater reliability (ICC = 0.96).[265]

Spasticity: Spasticity of the lower leg and arm was measured using the Composite Spasticity Scale (score range: 1–16).[266] Higher scores indicate higher spasticity. The scale includes three items: 1) Achilles/wrist flexion tendon jerk (score range: 0–4); 2) resistance to full ROM for ankle dorsiflexion/elbow flexion (score range: 0–8); and 3) the duration and amount of ankle/wrist clonus elicited (score range: 1–4). This scale has previously shown excellent test–retest reliability (ICC = 0.97).[266]

Motor Activity Log: The Motor Activity Log (MAL) was used to measure the usage frequency and movement quality of the affected arm and hand during 30 daily activities.[263] Two scores were rated for each activity: the amount of use (AOU) and the quality of movement (QOM) of the paretic arm. The AOU was determined by the answer to the question "How much was the paretic upper arm used in this activity?" The score ranges from 0 (never used the affected arm for this activity) to 5 (always used the affected arm for this activity). The QOM was determined by the answer to the question "How well did the paretic arm participate in the activity?" The score of each item/activity ranges from 0 (cannot use the affected arm for this activity) to 5 (can use the affected arm for this activity as well as before stroke). We calculated the mean MAL score by averaging the scores for the 30 items. This scale has previously demonstrated high internal consistency in hemiplegic participants (AOU: Cronbach's alpha = 0.88; QOM: Cronbach's alpha = 0.91).[263] The test–retest correlation coefficients

(Pearson's r) for the QOM and AOU were 0.91 and 0.50, respectively. This scale has good convergent validity. The correlation coefficient between the QOM scale and wrist accelerometer recordings (3 days) is 0.7 ($p < 0.01$),[264] while the MAL (QOM and AOU) and Arm Reach Arm Test scores are moderately correlated (Spearman's rho = 0.63).[263]

Statistical analysis

First, the demographic information was described and assessed. An independent Student's t test, Mann–Whitney U test, or Chi-square test (depending on whether the criteria for parametric statistics were fulfilled) was used to determine whether there were any differences in the demographic variables between those who completed follow-up assessments and those who dropped out.

Second, for each bone outcome, a two-way repeated-measures analysis of variance (ANOVA; within-subject factors: side [two levels: stroke: hemi-paretic vs. non-paretic]; time [two levels: baseline vs. 6-month follow-up]) was used to determine whether the main effect or interaction effect between the two factors existed. Post-hoc paired Student's t tests were performed if necessary to examine the changes over time. The relative changes in bone outcomes between the two sides were also compared using a paired Student's t test. Relative change was calculated as (T1 − T2)/T1, where T1 and T2 represent the values measured at baseline and at the 6-month follow-up, respectively. A paired Student's t test was also used to explore the time effect on functional outcomes (e.g., muscle strength, muscle stiffness, sensory, and vascular ultrasound measurement).

In addition, the effect size (Cohen's d) was calculated (0.2: small; 0.5 medium; 0.8: large) for these comparisons.

Third, bivariate analysis (Pearson's r) was used to analyze the correlation between the relative changes in key bone outcomes (with Cohen's d > 0.5 or change > the precision error) and potential clinical factors at baseline (e.g., muscle strength, spasticity, motor control impairment, and sensation). To determine which bone variables contributed the most to the change in estimated failure load, the correlations between the change in estimated failure load and those in other bone variables were also examined. Associations with Pearson's correlation coefficients (r) greater than 0.5 were reported with further discussion. The following criteria depicted the strength of the association: little or no: $r < 0.25$; fair: $r = 0.25–0.5$; moderate to good: $r = 0.5–0.75$; good to excellent: $r > 0.75$.[301]

5.4 Results

Participant characteristics at baseline

Thirteen participants (three women) with acute stroke were recruited. Because of the pandemic, only six participants completed the bone scans and only four of them completed the physical assessment at the 6-month follow-up. Figure 5-1 shows the participants' characteristics. The average stroke duration was 5 days (SD = 2.7 days). Two participants had a slight disability (mRS score = 2), three had a moderate disability (mRS score = 3), seven had a moderately severe disability (mRS score = 4), and one had a severe disability (mRS score = 5). Seven participants did not need walking aids,

whereas four required a wheelchair for indoor mobility at baseline. During the acute stage, the hand grip strength of the paretic side was 63% of the hand-grip strength of the non-paretic side, while the knee extension strength of the paretic side was 44% of the corresponding strength of the non-paretic side. The seven participants who dropped out had significantly weaker knee extension strength in both the paretic and non-paretic legs than those who completed the follow-up measurements ($p < 0.05$). More details are provided in Table 5-1.

Change in the distal radius

The two-way repeated-measures ANOVA revealed a significant effect of side on trabecular number and trabecular separation ($p < 0.05$; Table 5-2), with the paretic side showing greater trabecular separation and less trabecular number than the non-paretic side at baseline ($p < 0.05$).

The main effect of time was significant for total vBMD ($p = 0.009$) and marginally significant for cortical vBMD ($p = 0.063$). Post-hoc comparisons showed a significant decline only in the cortical vBMD of the paretic radius in the first 6 months post-stroke (−0.73%). During the same period, the total vBMD of the paretic radius declined by 1.33% ($p = 0.062$).

Additionally, the interaction effect of time × paretic side was marginally significant for estimated failure load ($p = 0.066$) and stiffness ($p = 0.076$). During the first 6 months post-stroke, the estimated failure load decreased by 3.96% ($p = 0.091$) on the paretic side only. Overall, three bone outcomes (i.e., three vBMD variables) on the paretic side demonstrated a reduction that exceeded the precision error. Among

these, the total vBMD (d = 0.58) and cortical vBMD (d = 0.75) showed a substantially greater percentage decline on the paretic side than on the non-paretic side. In contrast, trabecular thickness on the paretic side showed a significant increase that exceeded the precision error. More information is provided in Table 5-3.

Change in the distal tibia

A significant time × side interaction effect was found in the cortical vBMD (p = 0.020), bone overall stiffness (p = 0.007), and estimated failure load (p = 0.007). A marginally significant interaction effect was also shown for total vBMD (p = 0.099), trabecular vBMD (p = 0.067), and trabecular number (p = 0.071) (Table 5-4).

No significant difference was found between the paretic and non-paretic tibiae at baseline. A post-hoc comparison test showed a significant deterioration in cortical vBMD −0.40%, p = 0.03), stiffness (−2.49%, p = 0.046), and estimated failure load (−2.19%, p = 0.028) in the first 6 months post-stroke on the paretic side (Table 5-5). Two bone outcomes (total vBMD, trabecular vBMD) showed a reduction that exceeded their corresponding precision errors, whereas the trabecular number demonstrated an increase exceeding the precision error. Together with estimated failure load and bone stiffness, five variables demonstrated a substantially greater change (d > 0.5) on the paretic side than on the non-paretic side. Specifically, a greater percentage increase was found in the trabecular number on the paretic side than on the non-affected side (d = −0.89). In contrast, the other four variables showed a greater decline on the paretic side than on the non-paretic side (d > 0.5). More details are provided in Table 5-5.

Changes in the other clinical outcomes

The paretic upper limbs (Table 5-6) showed general improvements in hand light touch sensation (d = 0.68), active ROM of wrist extension (d = −0.68), hand-grip strength (d = −0.75), and motor control (FMA: d = −0.83) in the first 6 months post-stroke. However, there was a decline in the peak systolic velocity of the bilateral brachial arteries (Cohen's d: paretic side = 1.03, non-paretic side = 1.37). Muscle spasticity of the paretic arm did not show a significant change during this period.

The paretic lower limbs showed improvements in hallux sensation (d = 2.11), active ankle ROM (d = −1.16), and quadricep strength (d = −0.49) at the 6-month follow-up. Additionally, there was a reduction in the peak systolic velocity of the popliteal artery on the paretic side (d = 0.95) but not on the non-paretic side. Neither motor control nor muscle spasticity showed a significant change in the paretic lower limbs.

Correlation between the change in estimated failure load and changes in other bone variables

In both the distal radius (r = 0.89) and tibia (r = 0.76), the greater decrease in cortical vBMD was related to a greater decrease in the estimated failure load (Table 5-7). Interestingly, the greater increase in trabecular thickness was associated with a greater reduction in the estimated failure load in the distal radius on the paretic side (r = −0.73).

In the distal tibia of the paretic side, a greater decline in the estimated failure load was associated with a greater increase in trabecular number (r = −0.41).

Correlation between the change in estimated failure load and baseline clinical factors

Among the various clinical factors measured at baseline, better wrist active ROM ($r = -0.57$) and greater hand-grip strength ($r = -0.59$) on the paretic side showed the strongest correlations with less of a decline in the estimated failure load of the paretic radius (Table 5-8).

In the distal tibia on the paretic side, a higher peak systolic velocity of the popliteal artery at baseline was associated with a greater decline in trabecular vBMD ($r = 0.66$; Table 5-9).

5.5 Discussion

Overall, although the physical function recovered in the first 6 months post-stroke, a decline in bone health was observed in both the paretic radii and tibiae of our acute stroke participants. Specifically, a reduction in the total vBMD, trabecular vBMD, cortical vBMD, bone stiffness, and estimated failure load was observed in both the distal radii and tibiae. However, an increase was observed in the trabecular thickness of the distal radius and in the trabecular number of the distal tibia on the paretic side. Better hand-grip strength and greater active ROM on the paretic side were associated with less of a decline in the estimated failure load of the distal radius. Higher peak systolic velocity at baseline was associated with a greater decline of estimated failure load in the distal tibia during the follow-up period.

Change in the bone properties of the distal radius

The relative change in the radius in the first 6 months post-stroke observed in

our pilot study was smaller in magnitude than the change reported by Lazoura et al.[167] In their study, the trabecular vBMD of the distal radius showed a change of 7.67% during a 3-month follow-up period (from 3 months to 6 months after stroke onset), while we only found a reduction of 1.25% in that variable during the first 6 months post-stroke. There are several possible reasons for the lower magnitude of change observed in the current study. First, the sample size was smaller in our study than in the study by Lazoura et al. (67 participants). A larger sample size would enable us to obtain a better estimate of the changes in bone properties within the first 6 months post-stroke. Second, functional status may influence bone loss in the upper arm.[169] Indeed, the upper-extremity motor control of our participants was only mildly impaired overall, as revealed by the relatively high FMA score (mean score was 54 out of 66). Unfortunately, Lazoura et al. did not measure the FMA score of the upper limb, and thus, no direct comparison can be made. Nevertheless, participants in the current study had higher functional status than those in the study by Lazoura et al. For example, the Functional Ambulation Category scores of all of their participants were ≤ 3, indicating that none of them could walk independently, with or without supervision. In contrast, half of our participants were able to ambulate independently, with or without supervision, at baseline. These findings suggest that our participants had better overall motor function than their participants.

We observed a decline in the estimated failure load (3.96%) and cortical vBMD (0.75%) on the paretic side within the first 6 months post-stroke. In the chronic stroke study discussed in Chapter 3, the same variables showed a similar degree of decline

(3.81% and 0.53%, respectively) over a much longer follow-up period (24 months). Hence, we have indirect evidence that the rate of bone changes is greater in the first 6 months post-stroke than in the late chronic stage.

Changes in the bone properties of the distal tibia

Borschmann et al. used pQCT to compare the changes in bone properties of the tibia in the early stage of stroke recovery and found a 2.4% decrease in total vBMD between 2 weeks and 6 months after stroke.[196] We found a smaller reduction in total vBMD (0.87%), albeit a reduction that exceeded the precision error (0.45%). This discordance in results may be partially explained by the fact that our participants had less muscle weakness at baseline. In the study by Borschmann et al., the percentage side-to-side difference in quadricep strength at baseline was 66%, whereas the corresponding value was just 26% in our participants.

The change in the estimated failure load in the paretic tibiae was similar in this study (2.19%) and during the late stage of stroke recovery (3.39%) described in Chapter 4. This is consistent with the findings in the radii, for which there is evidence of a greater rate of decline in the estimated bone strength within the first 6 months post-stroke.

In both the paretic radius and tibia, the reduction in the estimated failure load during the follow-up period was strongly associated with the reduction in cortical vBMD. In addition, the reduction in the estimated failure load was associated with an *increase* in the trabecular thickness in the radius and an *increase* in the trabecular number in the tibia. There may be two reasons for these negative associations. The increased trabecular number during the early period after stroke may be caused by endocortical

trabecularization of the bone. Overall, the results seem to suggest degradation of cortical bone arising from intracortical and endocortical remodeling.[23] This process of decorticalization should typically result in an enlargement of the trabecular area with a decrease in cortical thickness. This was observed in our individuals with chronic stroke, as described in Chapters 3 and 4. However, in the current pilot study, the increase in trabecular area (0.03%) and the decrease in cortical thickness (0.12%) were minimal. A larger sample is needed to confirm our hypothesis that endocortical trabecularization occurs in the early period post-stroke.

Clinical factors predictive of bone loss—baseline muscle strength and wrist ROM

A new finding of this study is that, among the various clinical variables assessed, greater active ROM and muscle strength of the paretic upper limb at baseline showed the strongest correlations with less of a reduction in the estimated failure load. No previous pQCT study has examined the relationship between motor recovery and bone changes in the distal radius after stroke. The association of less bone loss with better muscle strength and active ROM immediately after stroke may be due to a direct increase in mechanical loading[168] from muscle contraction. Previous studies have indicated that mechanical stress can improve osteoblast differentiation[50-52] and decrease osteoclast production by downregulating the expression of related genes.[47]

The results of this study may highlight the importance of the severity of initial impairment/recovery of motor function in the early stage post-stroke in determining the degree of subsequent decline in bone strength, suggesting that muscle strength and motor control are potential treatment targets for improving upper-limb bone health in

individuals with acute stroke. There is a lack of research on non-pharmacological interventions to improve upper-limb bone outcomes in the early stage of stroke recovery, as indicated in our systematic review in Chapter 2. The findings of this pilot study provide some useful information to guide the design of rehabilitative interventions in future investigations.

Clinical factors predictive of bone loss—baseline blood flow velocity

Higher baseline peak systolic velocity in the popliteal artery detected using pulsed wave Doppler was significantly associated with a greater decline in the estimated tibia strength. As PWV is a widely used indicator of arterial stiffness,[299] this finding suggests that the increased arterial stiffness exerts adverse effects on bone health post-stroke. The greater decline in bone strength may be caused by decreased blood flow accompanied by increased impairment in the transportation of supporting factors (e.g., oxygen, nutrients, and systemic hormones) and immune and precursor cells necessary for normal bone function.[302] Likewise, previous evidence suggests that a reduction in bone mass is related to impaired vasodilator function and flow volume of blood vessels in bone in rats and humans.[303, 304] Impaired vasodilator function would lead to a narrowed artery diameter, resulting in elevated blood flow velocity.

In this study, peak systolic velocity decreased in both arms and in the paretic leg during the first 6 months post-stroke. This pathological elevation of the peak systolic velocity in the acute stage after stroke may be caused by decreased arterial wall compliance, which may be attributable to changes in the autonomic nervous control of vascular smooth muscle post-stroke. In fact, autonomic dysfunction with elevated

arterial blood pressure has been reported during acute stroke.[305] In people with chronic stroke, a similar phenomenon may occur, as we found a smaller brachial and popliteal artery diameter on the paretic side than on the non-paretic side, as shown in Chapters 3 and 4. Overall, our findings highlight the potential importance of improving vascular health in people with acute or subacute stroke to prevent lower-limb bone loss. The use of aerobic exercises to improve bone health in the early stage after stroke remains an unexplored area of research.

Limitations

We were only able to collect data from a small sample because all in-ward research activities were suspended during the COVID-19 pandemic. Thus, caution should be exercised when interpreting our data. A study with a larger sample size is needed to obtain a better estimate of the actual bone changes over the first 6 months post-stroke and the related factors. These findings can only be generalized to participants with similar characteristics to those in this study.

5.6 Conclusions

This pilot study found that the estimated failure load declined significantly in both the distal radius and tibia on the paretic side during the first 6 months post-stroke and that this decline was most strongly correlated with a decrease in cortical vBMD. Baseline grip strength and active wrist ROM on the paretic side at baseline were predictive of a subsequent decline in the estimated failure load of the distal radius,

whereas poorer vascular health at baseline, as indicated by a greater blood flow velocity, was associated with a greater decline in the estimated failure load of the paretic tibia. A longitudinal study with a larger sample size is warranted to verify these findings.

5.7 List of Tables

Table 5-1 Demographic information.

	All (n=13)	With Follow Up (n=6)	Drop-outs (n=7)
Age	63.23±9.80	62.67 ±10.15	63.71 ±10.28
BMI	23.00±3.19	22.98 ±3.57	23.02 ±3.11
Comorbidity Total	2.00±1.08	2.33 ±0.82	1.71 ±1.25
Medication Total	3.85±1.82	4.50 ±2.07	3.29±1.50
Years post menopause	14.67±4.62	NA	14.67 ±4.62
Stroke Duration (days)	5.15±2.67	4.00 ±1.27	6.14 ±3.24
Abbreviated Mental Test (Max:10) [a]	8.00±2.61	7.00 ±3.58	8.86±1.07
Physical Activity Scale for Elderly [a]	136.2±113.8	120.0 ±71.73	150.1 ±145.4
Sex (female/male, n)	3/10	0/6	4/3
Education Level (No/Primary/Secondary/tertiary, n)	1/3/7/2	0/0/4/2	1/3/3/0
Alcohol Class (non-drinker/ previous social drinker/ previous habitual drinker, n)	5/5/3	2/3/1	3/2/2
Smoking Class (non-smoker/previous social smoker/previous habitual smoker, n)	7/1/5	2/1/3	5/0/2
Receiving PT &OT (yes/no, n)	7/6	3/3	4/3
Walking Aid indoor (No/ Cane or Stick/ Walking Frame/ Wheelchair, n)	7/1/1/4	4/1/0/1	3/1/0/3
Paretic Side (L/R, n)	8/5	3/3	5/2
Dominance-hand (L/R, n)	1/12	0/6	1/6
Dominance-leg (L/R/non-specific, n)	1/9/3	0/6/0	1/3/3
Stroke Type (Ischemic/Hemorrhagic/Other, n)	10/2/1	6/0/0	4/2/1
Stroke location (Cortical/Subcortical/Mixed, n)	4/8/1	2/3/1	2/5/0
FAC (Nonfunctional/Dependent /Dependent with Supervision)/ Conditional Independent, n)	4/5/2/2	2/1/1/2	4/4/1/0
Modified Rankin Scale (2/3/4/5, n)	2/3/7/1	1/1/3/1	1/2/4/0
SGPALS (1/2/3, n)	4/8/1	2/3/1	2/5/0

[a]: Man-Whitney U test for baseline comparison between dropouts and those with follow-up; FAC: Functional Ambulatory Categories; SGPALS: Saltin-Grimby Physical Activity Level Scale.

Table 5-2 Two-way ANOVA for the effect of time and side to radius. (n=6)

	Time effect	Side effect	Time × side
	p	*p*	*p*
Total vBMD (mgHA/cm^3)	0.009**	0.299	0.227
Trabecular area (mm^2)	0.272	0.416	0.346
Trabecular vBMD (mgHA/cm^3)	0.125	0.128	0.630
Trabecular number (1/mm)	0.746	0.016**	0.125
Trabecular thickness (mm)	0.172	0.261	0.702
Trabecular separation (mm)	0.683	0.019**	0.251
Trabecular inhomogeneity (mm)	0.422	0.319	0.822
Cortical area (mm^2)	0.381	0.535	0.196
Cortical vBMD (mgHA/cm^3)	0.063*	0.363	0.146
Cortical perimeter (cm)	0.316	0.620	0.074*
Intra-cortical porosity (%)	0.410	0.856	0.862
Cortical thickness (mm)	0.456	0.741	0.691
Stiffness (kN/mm)	0.365	0.462	0.076*
Estimated failure load (N)	0.355	0.494	0.066*

vBMD: volumetric bone mineral density

*: $p<0.1$. **: $p<0.05$.

Table 5-3 HR-pQCT radius variables. (n=6)

	NP-Baseline	P-Baseline	NP-6 Months	P-6 Months	ES-NP[a]	ES-P[a]	ES [b]	ES [c]	*% Change-Non-paretic*	*% Change-Paretic*	ES [d]
Total vBMD (mgHA/cm^3)	311.05 ±26.19	299.70 ±27.20	310.23 ±27.17	295.68 ±27.05	0.03	-0.15	0.44	-0.54#	-0.30%	1.33%‡	0.58 ǂ
Tb. area (mm^2)	250.42 ±30.17	253.57 ±33.88	250.67 ±29.86	254.22 ±34.21	-0.01	0.02	-0.34	0.11	0.12%	-0.23%	-0.31
Tb. vBMD (mgHA/cm^3) [e]	145.52 ±31.28	132.20 ±17.42	145.05 ±32.35	130.65 ±18.24	0.02	-0.09	0.78#	-0.55#	-0.43%	1.25%‡	0.23
Tb. number (1/mm)	1.28 ±0.20	1.19 ±0.15*	1.25 ±0.17	1.20 ±0.12	0.15	0.10	1.76#	-0.34	-1.80%	-1.55%	-0.72 ǂ
Tb. thickness (mm)	0.234 ±0.01	0.229 ±0.01	0.231 ±0.01	0.235 ±0.01	-0.18	0.21	0.56#	-0.53#	0.45%	-0.80%‡	-0.32
Tb. separation (mm)	0.75 ±0.12	0.80 ±0.09*	0.76 ±0.12	0.79 ±0.08*	-0.08	-0.04	-1.61#	0.39	1.45%	0.23%	0.65 ǂ
Ct. area (mm^2)	72.13 ±8.02	70.98 ±8.76	72.03 ±7.75	70.35 ±9.08	0.01	-0.07	0.24	-0.20	-0.10%	0.88%	0.68 ǂ
Ct. vBMD (mgHA/cm^3)	880.58 ±47.48	889.95 ±46.16	880.23 ±47.17	883.50 ±44.55†	0.01	-0.14	-0.66#	0.07	-0.03%	0.73%‡	0.75 ǂ
Ct. perimeter (cm)	76.47 ±3.43	76.20 ±3.41	76.83 ±3.65	76.13 ±3.46	-0.10	-0.02	0.12	-0.20	0.47%‡	0.12%	0.92 ǂ
Intra-Ct. Porosity (%)	0.01 ±0.01	0.01 ±0.01	0.01 ±0.00	0.01 ±0.01	0.17	-0.11	0.10	-0.07	4.38%	4.83%	0.35
Ct. thickness (mm)[e]	1.15 ±0.14	1.14 ±0.18	1.15 ±0.14	1.13 ±0.19	0.03	-0.04	0.13	-0.09	-0.28%	0.75%	0.30
Stiffness (kN/mm)	74.85 ±7.46	73.56 ±5.03	75.82 ±8.25	70.78 ±5.94	-0.12	-0.51§	0.15	-0.70#	-1.26%	3.96%	0.42
Estimated failure load (N)	4042.41 ±410.61	4010.98 ±293.11	4101.66 ±434.29	3849.93 ±320.93	-0.14	-0.52§	0.07	-0.66#	-1.48%	3.96%	0.39

Ct: cortical; ES: effect size; NP: non-paretic side; P: paretic side; Tb: trabecular; vBMD: volumetric bone mineral density

[a]: Effect size for comparison between baseline and follow-up; [b]: Effect size for comparison between two sides at baseline. [c]: Effect size for comparison between two sides at follow-up. [d]: Effect size for comparison between the relative change of two sides; [e]: Wilcoxon test for comparison between baseline and follow-up

*: $p<0.05$, comparison between two sides.

†: $p<0.05$, comparison between baseline and follow-up.

#: Cohen's $|d|>0.5$, comparison between two sides.

§: Cohen's $|d|>0.5$, comparison between baseline and follow-up.

‡: Relative change above precision error.

ǂ: Cohen's $|d|>0.5$, comparison between two sides for relative change.

% Change= (Baseline-6 month)/Baseline, positive value suggests decline.

Table 5-4 Two-way ANOVA for the effect of time and side: distal tibia.

	Time effect	Side effect	Time × side
	p	*p*	*p*
Total vBMD (mgHA/cm^3)	0.295	0.417	0.099*
Tb. area (mm^2)	0.659	0.294	0.936
Tb. vBMD (mgHA/cm^3)	0.386	0.454	0.067*
Tb. number (1/mm)	0.680	0.456	0.071*
Tb. thickness (mm)	0.696	0.874	0.352
Tb. separation (mm)	0.687	0.391	0.111
Ct. area (mm^2)	0.650	0.469	0.913
Ct. vBMD (mgHA/cm^3)	0.910	0.091	0.020**
Ct. perimeter (cm)	0.708	0.746	1.000
Intra Ct. Porosity (%)	0.291	0.290	0.788
Ct. thickness (mm)	0.418	0.346	0.848
Stiffness (kN/mm)	0.133	0.558	0.007**
Estimated failure load (N)	0.107	0.527	0.007**

Ct: cortical; Tb: trabecular; vBMD: volumetric bone mineral density

*: $p<0.1$. **: $p<0.05$.

Table 5-5 HR-pQCT tibia variables (n=6).

	NP-Baseline	P-Baseline	NP-6 Months	P-6 Months	ES -NP[a]	ES -P[a]	ES [b]	ES [c]	% Change-non-paretic	% Change-Paretic	ES [d]
Total vBMD (mgHA/cm^3)	264.47 ±24.34	269.92 ±24.02	264.58 ±25.97	267.48 ±22.40	-0.05	0.77§	-0.46	-0.25	0.00%	0.87%‡	0.79 ǂ
Tb. area (mm^2)	666.42 ±104.22	651.75 ±86.73	666.58 ±103.80	651.98 ±86.40	-0.09	-0.38	0.47	0.48	0.03%	-0.03%	0.00
Tb. vBMD (mgHA/cm^3) [e]	147.12 ±23.18	150.67 ±27.90	146.60 ±22.07	148.75 ±26.78	0.17	0.56§	-0.42	-0.25	-0.27%	1.18%‡	0.76 ǂ
Tb. number (1/mm)	1.10 ±0.11	1.10 ±0.11	1.09 ±0.12	1.12 ±0.13	0.37	-0.72§	0.16	-0.87#	-0.97%	-1.53%‡	-0.89 ǂ
Tb. thickness (mm)	0.25 ±0.01	0.25 ±0.02	0.25 ±0.01	0.25 ±0.01	0.00	0.26	-0.14	0.03	-0.03%	0.47%	0.43
Tb. separation (mm)	0.87 ±0.10	0.87 ±0.11	0.88 ±0.10	0.86 ±0.11	-0.58§	0.38	0.11	0.75#	0.75%	0.50%	0.81 ǂ
Ct. area (mm^2)	127.50 ±16.14	129.33 ±14.79	127.33 ±16.95	129.08 ±14.90	0.09	0.42	-0.37	-0.28	-0.22%	0.20%	-0.01
Ct. vBMD (mgHA/cm^3)	860.75 ±60.57	857.35 ±67.54	864.43 ±62.05	853.98 ±67.40*†	-0.72§	1.19§	0.36	1.41#	0.42%‡	0.40%	1.38ǂ
Ct. perimeter (cm)	109.63 ±6.83	109.27 ±5.81	109.72 ±6.91	109.35 ±5.60	-0.16	-0.16	0.14	0.14	0.05%	-0.08%	-0.15
Intra-Ct. Porosity (%)	0.04 ±0.02	0.04 ±0.02	0.03 ±0.01	0.04 ±0.02	0.45	0.37	-0.46	-0.47	-0.90%	0.27%	-0.05
Ct. thickness (mm)[e]	1.35 ±0.20	1.37 ±0.19	1.34 ±0.21	1.37 ±0.19	0.24	0.25	-0.46	-0.40	-0.28%	0.12%	-0.14
Stiffness (kN/mm)	191.26 ±27.89	195.32 ±27.51	190.95 ±26.11	190.25 ±24.72†	0.08	1.29§	-0.68#	0.10	-0.06%	2.49%	1.68 ǂ
Est. failure load (N)	10353.94 ±1449.10	10545.21 ±1441.93	10330.31 ±1367.88	10302.81 ±1295.65†	0.14	1.29§	-0.75#	0.08	-0.15%	2.19%	1.78 ǂ

Ct: cortical; ES: effect size; NP: non-paretic side; P: paretic side; Tb: trabecular; vBMD: volumetric bone mineral density

[a]: Effect size for comparison between baseline and follow-up; [b]: Effect size for comparison between two sides at baseline. [c]: Effect size for comparison between two sides at follow-up. [d]: Effect size for comparison between the relative change of two sides; [e]: Wilcoxon test for comparison between baseline and follow-up

*: $p<0.05$, comparison between two sides.

†: $p<0.05$, comparison between baseline and follow-up.

#: Cohen's $|d|>0.5$, comparison between two sides.

§: Cohen's $|d|>0.5$, comparison between baseline and follow-up.

‡: Relative change above precision error.

ǂ: Cohen's $|d|>0.5$, comparison between two sides for relative change.

% Change = (Baseline-6 month)/Baseline, positive value suggests decline.

Table 5-6 Clinical outcomes

	Baseline-All (n=13)	Baseline-with follow Up (n=6)	Baseline-Dropouts (n=7)	6-month Follow up (n=4)	*P*[c]	*ES* [d]
Upper Limb						
Touch Pressure Threshold - P Hand (g)	3.70±1.05	3.51±1.02	3.85±1.14	2.78 ±0.52	0.588	**0.68**
Touch Pressure Threshold - NP Hand(g)	3.08±0.60	2.86±0.74	3.26±0.42	2.83 ±0.28	0.245	-0.28
Active ROM wrist - Extension P (°) [a b]	40.46 ±29.69	45.83 ±32.00	35.86 ±29.25	56.00 ±19.44	0.445	**-0.68**
Active ROM wrist - Extension NP (°) [b]	61.85 ±14.00	60.33 ±15.32	63.14 ±13.86	65.50 ±17.06	0.735	-0.49
Peak systolic velocity - P Arm (cm/s)	88.65 ±15.74	90.21 ±14.55	87.32 ±17.73	82.43 ±5.95	0.758	**1.03**
Peak systolic velocity - NP Arm (cm/s)	88.85 ±13.85	94.00 ±13.54	84.43 ±13.48	85.36 ±7.85	0.229	**1.37**
Strength hand grip - P [a b]	17.37 ±20.46	22.64 ±24.78	12.86 ±16.55	49.17 ±27.53	0.366	**-0.75**
Strength hand grip - P	52.90 ±26.38	57.78 ±26.86	48.72 ±27.33	72.58 ±12.79	0.560	-0.07
Composite Spasticity Scale - UL (Max:16) [a b]	4.38 ±1.19	4.50 ±0.84	4.29 ±1.50	4.75 ±0.50	0.731	0.00
FMA - upper extremity (Max:66) [a b]	47.85 ±21.28	53.83 ±14.35	42.71 ±25.84	60.25 ±6.90	0.445	**-0.83**
Lower Limb						
Touch pressure threshold - P hallux(g)	4.40±1.13	4.59±1.14	4.23±1.18	3.61 ±0.70	0.594	**2.11**
Touch pressure threshold - NP hallux(g) [a]	3.77±0.78	3.56±0.91	3.96±0.66	2.73 ±0.74	0.295	**0.96**
Active ROM Ankle - DF P (°) [a]	7.92 ±9.03	9.33 ±6.68	6.71 ±11.06	33.00 ±26.50	0.295	**-1.16**
Active ROM Ankle - DF NP (°)	16.85 ±8.96	16.33 ±8.64	17.29 ±9.89	36.00 ±23.04	0.858	**-1.09**
Peak systolic velocity - P knee (cm/s)	63.27 ±12.17	66.72 ±10.77	60.32 ±13.33	58.80 ±3.85	0.367	**0.95**
Peak systolic velocity - NP knee(cm/s)	62.28 ±13.11	66.86 ±10.80	58.36 ±14.41	65.05 ±1.83	0.261	-0.21
Strength Knee - P	25.64 ±20.68	40.28 ±18.57	13.09 ±13.03	55.33 ±8.09	0.010*	-0.49
Strength knee - NP	43.11 ±24.78	58.45 ±28.14	29.97 ±11.45	72.00 ±23.01	0.031*	-0.18
Composite Spasticity Scale - LL (Max:16) [a b]	6.00 ±1.68	6.67 ±1.51	5.43 ±1.72	5.50 ±2.38	0.181	0.36
FMA-lower extremity (Max:34)	25.00 ±8.61	27.17 ±5.91	23.14 ±10.51	28.75 ±6.18	0.425	-0.23
Walking speed	NA	NA	NA	6.72 ±1.04	NA	NA
Physical Activity Scale for Elderly [b]	136.2±113.8	120.0 ±71.73	150.1 ±145.4	57.10±28.55	0.731	-0.23

Note: P: paretic side; NP: non-paretic side; ROM: range of motion; DF: dorsi-flexion; FMA: Fugl-Meyer Assessment. UL: upper limb; LL: lower limb.

[a]: Man-whitney U test for baseline comparison between dropouts and those with follow-up.

[b]: Wilcoxon test for comparison between baseline and follow-up.

[c]: between-group baseline comparison: dropouts and those completed the follow-up.

[d]: within-group comparison for those completed the follow-up.

*: $p<0.05$. Numbers in bold: Cohen's $|d|>0.5$.

Table 5-7 Association between the relative change of estimated failure load and other bone variables.

	Estimated failure load- %change (**radius**)		Estimated failure load- %change (**tibia**)	
	r	p	r	p
Total vBMD (mgHA/cm^3) - %change	**0.53**	0.279	**0.63**	0.179
Tb. vBMD (mgHA/cm^3) - %change	-0.09	0.87	**0.54**	0.266
Tb. thickness (mm) - %change	**-0.73**	0.102	NA	NA
Tb. number (1/mm) - %change	NA	NA	-0.41	0.424
Ct. vBMD (mgHA/cm^3) - %change	**0.89***	0.017	**0.76**	0.081

Note: Ct: cortical; Tb: trabecular; vBMD: volumetric bone mineral density

Values in bold, r>0.5. % Change = (Baseline-6 month)/Baseline.

Table 5-8 Association between the relative change of estimated failure load of paretic distal radius and baseline clinical factors (n=6).

	Estimated failure load -%change	
	r	p
Hand sensory	0.36	0.483
Composite Spasticity Scale- Total	0.43	0.397
Peak systolic velocity- Brachial artery	0.30	0.564
Physical activity scale for elderly	-0.46	0.364
Active ROM-wrist extension	**-0.57**	0.237
Hand grip strength	**-0.59**	0.217
FMA- upper extremity	-0.39	0.45

ROM: range of motion; FMA: Fugal-Meyer Assessment.

% Change = (Baseline-6 month)/Baseline.

Values in bold: r >0.5.

Table 5-9 Association between the relative change of estimated failure load of paretic distal tibia and baseline clinical factors (n=6).

	Estimated failure load -%change	
	r	p
Peak systolic velocity- Popliteal artery	**0.66**	0.155
Hallux sensory	0.42	0.413
Physical Activity Scale for Elderly	-0.23	0.664
Active ROM - ankle dorsiflexion	-0.34	0.513
Quadricep strength	-0.06	0.915
Composite Spasticity Scale-lower limb	0.17	0.747
Functional ambulation category	-0.37	0.476
FMA- lower extremity	-0.24	0.644

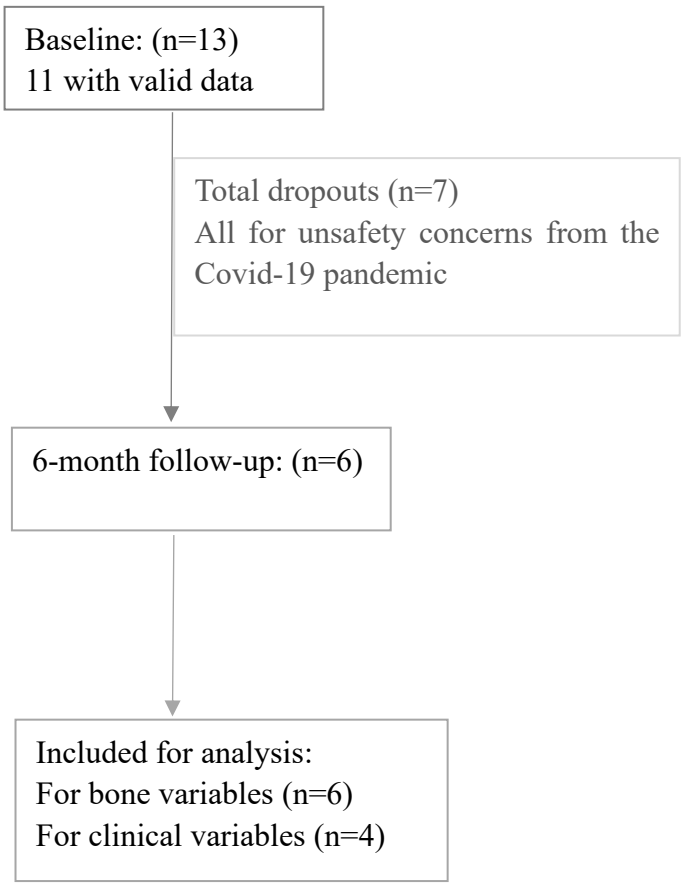
ROM: range of motion; FMA: Fugal-Meyer Assessment.

% Change= (Baseline-6 month)/Baseline.

Values in bold: r>0.5.

5.8 List of Figures

Figure 5-1 Study flowchart.



5.9 Appendix

Appendix 1. Bone variables generated by HR-pQCT

HR-pQCT variables	Description
Default analyses	
Cortical area (Ct. Ar), mm^2	Cross-sectional area for cortical compartment
Trabecular area (Tb. Ar), mm^2	Cross-sectional area for trabecular compartment
Total volumetric BMD (Tot. vBMD), mg HA/cm^3	Average bone density of the whole volume of interest (VOI)
Trabecular volumetric BMD (Tb. vBMD), mg HA/cm^3	Average bone density of the VOI of trabecular compartment
Trabecular bone volume fraction (BV/TV)	Trabecular bone volume fraction derived from Tb. vBMD assuming fully mineralized bone to have a mineral density of 1.2 g HA/cm^3
Trabecular number (Tb. N), 1/mm	Measure of the average number of trabeculae per unit length
Trabecular thickness (Tb. Th), mm	Mean thickness of trabeculae
Trabecular separation (Tb. Sp), mm	Mean distance between trabeculae
Extended cortical analyses	
Cortical volumetric BMD (Ct. vBMD), mg HA/cm^3	Average bone density of the VOI of cortical compartment
Cortical thickness (Ct. Th), mm	Average cortical thickness, disregarding intracortical pore surfaces
Cortical porosity (Ct. Po), %	The mineral content of the pure cortical bone within a 1mm slice.
Finite element analysis	
Bone stiffness (kN/mm)	Whole-bone stiffness
Estimated ailure load (N)	Estimated maximum load

6. Chapter 6

Conclusions

Ouyang Huixi

6.1 Project scope

Secondary hemi-osteoporosis is a significant health problem experienced by stroke survivors but is often overlooked in both research and clinical practice. The risk of fragility fractures increases when bone health is compromised [79], especially in a population with compromised balance function and thus an increased rate of falls. Considering the adverse impact of post-stroke fractures on stroke survivors, their caregivers and society [164], there is a substantial need for research on post-stroke bone health. Moreover, to enable the establishment of effective interventions to alleviate post-stroke bone loss, the changes in different bone parameters and their associated factors must be understood. Thus, the aim of the current project was to understand how various bone characteristics and related clinical factors change and their interaction during different stages of stroke recovery.

6.2 Summary of study findings and future research directions

The systematic review and meta-analysis described in Chapter 2 reveals that exercise is helpful for maintaining post-stroke hip bone health. Although exercises improve the BMD of the radius on the paretic side, the quality of the evidence for this finding is very low. The impact of exercise on the cortical thickness of the tibia on the paretic side is also uncertain. In contrast, there is high-quality evidence that the use of the antidepressant fluoxetine increases the incidence of fracture in stroke survivors. Thus, the effects of exercise on post-stroke bone health warrant further investigation. To guide the design of future intervention strategies, longitudinal cohort studies should be performed to investigate micro- and macrostructural changes during different stages

of stroke recovery and these changes' influential modifiable factors.

The 2-year longitudinal cohort study described in Chapter 3 shows that the bone strength of the paretic distal radius continues to deteriorate in people with chronic stroke, primarily as a result of decreases in cortical bone thickness and cortical vBMD. Better baseline hand sensation and better vascular health which was indicated by a larger blood flow volume was associated with less decline of estimated failure load. Therefore, vascular health and sensory function may be potential targets for future intervention studies aimed at improving upper-extremity bone health in people with chronic stroke.

Similar to the findings in the distal radius, the data presented in Chapter 4 show that even in individuals who regain independent walking during the chronic stroke stage, the estimated failure load of the distal tibia on the paretic side is significant reduced. This was mainly attributed to decreases in cortical vBMD, trabecular vBMD and cortical thickness. Lower-limb vascular health, walking velocity, muscle strength and physical activity level are predictive of the decline in estimated failure load during follow-up periods. Future intervention studies are recommended to target these outcome measures to improve lower-limb bone health for people with chronic stroke.

The pilot study of people with acute stroke presented in Chapter 5 reveals that there is a general decline in the vBMD of both the cortical and trabecular bone and the estimated failure load, at rates greater than those observed in the chronic stroke stage. Worse baseline hand muscle strength and less active wrist ROM of the affected upper limb were found to be associated with a greater decline in the estimated failure load of the distal radius. A higher peak systolic velocity of the popliteal artery (i.e., greater

arterial stiffness) on the paretic side was correlated with a greater decline in the estimated failure load of the distal tibia. Future studies with larger sample sizes are warranted to attain a better understanding of the changes in different HR-pQCT variables during the first 6 months post-stroke.

6.3 Concluding remarks

This was the first longitudinal study which applied HR-pQCT to find influential clinical factors that are associated with the decline of bone strength during different stages of stroke recovery. In summary, the evidence obtained from the studies performed in this thesis reveals the key aspects of bone changes at different sites during different stages of stroke recovery. They also reveal the modifiable clinical factors that contribute to compromised bone strength at different stages of stroke. These findings will guide the design of treatment protocols for future intervention trials.

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APPENDICES

Appendix 1. The Ethical approval

THE HONG KONG POLYTECHNIC UNIVERSITY
香港理工大學

To	Pang Marco Yiu Chung (Department of Rehabilitation Sciences)		
From	Yee Kay Yan Benjamin, Delegate, Departmental Research Committee		
Email	benjamin.yee@	Date	22-Aug-2020

Application for Ethical Review for Teaching/Research Involving Human Subjects

I write to inform you that approval has been given to your application for human subjects ethics review of the following project for a period from 01-Jan-2018 to 30-Jun-2022:

Project Title:	Using high-resolution peripheral quantitative computed tomography to study the long bone structural changes after stroke: a longitudinal study.
Department:	Department of Rehabilitation Sciences
Principal Investigator:	Pang Marco Yiu Chung
Project Start Date:	01-Jan-2018
Reference Number:	HSEARS20161229002-02

You will be held responsible for the ethical approval granted for the project and the ethical conduct of the personnel involved in the project. In case the Co-PI, if any, has also obtained ethical approval for the project, the Co-PI will also assume the responsibility in respect of the ethical approval (in relation to the areas of expertise of respective Co-PI in accordance with the stipulations given by the approving authority).

You are responsible for informing the Human Subjects Ethics Sub-committee in advance of any changes in the proposal or procedures which may affect the validity of this ethical approval.

Yee Kay Yan Benjamin

Delegate

Departmental Research Committee (on behalf of Human Subjects Ethics Sub-Committee)

香港中文大學醫學院
Faculty Of Medicine
The Chinese University Of Hong Kong

醫院管理局
新界東醫院聯網
Hospital Authority
New Territories East Cluster

Joint Chinese University of Hong Kong-New Territories East Cluster Clinical Research Ethics Committee
香港中文大學-新界東醫院聯網 臨床研究倫理 聯席委員會

8/F, Lui Che Woo Clinical Sciences Building, Prince of Wales Hospital, Shatin, HK
Tel : (852) 3505 3935 / 2144 5926 Fax : (852) 2646 6653 Website : http://www.crec.cuhk.edu.hk

The Joint CUHK-NTEC CREC is an independent committee established by CUHK/NTEC and authorized to perform ethics and scientific review and oversight of clinical studies within the jurisdiction of CUHK/NTEC in accordance with its standard operating procedure and the principles of the Declaration of Helsinki and ICH Good Clinical Practice.

27 NOV '20

CREC Ref. No.: 2017.023

To: Prof. Ling QIN
Dept. of Orthopaedics & Traumatology
Prince of Wales Hospital

This notice is issued by the Joint CUHK-NTEC CREC with respect to the application/submission by you, being the principal investigator of the following study at your study site:

- **Study Protocol Title:** Using high-resolution peripheral quantitative computed tomography to study the long bone structural changes after stroke: a longitudinal study
- **Investigator(s):** Ling QIN, Thomas LEUNG, Adrian WONG, Marco Yiu Chung PANG, Raymond Chi Keung CHUNG, Michael YING and Huixi OUYANG

In accordance with our standard operating procedure, we have duly performed ethics and scientific review of your application/submission as detailed below:

- **Nature of Your Application/Submission:** ☐ Initial application ☒ Amendments/changes ☐ Others: ☐ Renewal
- **Mode of Review:** ☒ Full review ☐ Expedited review
- **Date of Initial/Renewal Approval:** 28 February 2020
- **Date of Amendment Approval:** 06 November 2020
- **Document(s) Reviewed:** See Schedule 1
- **Reviewer(s):** See Schedule 2

After due review by our reviewer(s), we hereby write to inform you of our decision on your application/submission as follows:

- **Decision:**
  - ☒ Application/Submission approved
  - ☐ Application/Submission approved with condition(s) (see condition(s) below)
  - ☐ Application/Submission approved with remark(s) (see remark(s) below)
  - ☐ Application/Submission approved with condition(s) and remark(s) (see condition(s) and remark(s) below)
- **Regular Progress Report(s) Required:** Every 12 months from the date of initial/renewal approval and during the period of the study if required

香港中文大學醫學院
Faculty Of Medicine
The Chinese University Of Hong Kong

醫院管理局
新界東醫院聯網
Hospital Authority
New Territories East Cluster

Joint Chinese University of Hong Kong-New Territories East Cluster Clinical Research Ethics Committee
香港中文大學-新界東醫院聯網 臨床研究倫理 聯席委員會

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You, being the principal investigator of the study at your study site, are reminded to comply with our requirements and to maintain communication with us during the period of the study by undertaking the principal investigator's responsibilities including (but not limited to):

- if the study is an industry-sponsored clinical study, submitting to us a copy of the fully executed indemnity agreement satisfying the Hospital Authority's requirement prior to commencement of the study (if it has not been submitted yet);
- observing and complying with all applicable requirements under our standard operating procedure ("IRB/REC SOP"), the Declaration of Helsinki and the ICH GCP (if applicable);
- submitting regular progress report(s) at the required intervals (as specified above) in accordance with the requirements in the IRB/REC SOP;
- not implementing any amendment/change to any approved study document/material without our written approval, except where necessary to eliminate any immediate hazard to the subjects or if an amendment/change is only of an administrative or logistical nature;
- notifying us of any new information that may adversely affect the rights, safety or well-being of the subjects or the proper conduct of the study;
- reporting any deviation from the study protocol or compliance incident that has occurred during the study and may adversely affect the rights, safety or well-being of any subject in accordance with the requirements in the IRB/REC SOP;
- submitting safety reports on all SAEs observed at your study site or SUSARs reported from outside your study site in accordance with the requirements in the IRB/REC SOP; and
- submitting a final report in accordance with the requirements in the IRB/REC SOP upon completion or termination of the study at your study site.

In addition to the above, you are also reminded to observe and comply with other applicable regulatory and management requirements including (but not limited to):

- if required by Hong Kong laws or regulations, obtaining a certificate for clinical trial through the Hong Kong Department of Health and complying with the associated requirements;
- obtaining the necessary consent from the management of your institution/department in accordance with the requirements of your institution/department;
- if required by local laws or regulations at conducting site out of IRB/REC's jurisdiction, obtaining an approval and complying with associated requirements;
- not representing to any third party or in any way likely to mislead any third party forming the view that the approval from the IRB/REC has any extraterritorial effect; and
- with due diligence ensuring your teams, staff, agents or whosoever connected with you to comply with the preceding requirements.

Yours sincerely,

Envy Lee (Secretary)
for and on behalf of
The Joint CUHK-NTEC CREC

EL/ci

Appendix 2. Consent form

香港理工大學，復康治療科學系

香港中文大學，矯形外科及創傷學系,骨質量檢測和健康評定中心

Bone Quality and Health Assessment Centre
Department of Orthopaedics & Traumatology, CUHK

透過使用高像數外周定量電腦斷層掃瞄，研究中風後長骨結構的轉變：縱向研究。

Using high-resolution peripheral quantitative computed tomography to study the long bone structural changes after stroke and associated factors: a longitudinal study.

科研人員:

秦嶺教授　(香港中文大學矯形外科及創傷學系教授)
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Prof. Ling Qin, Department of Orthopaedics and Traumatology, The Chinese University of Hong Kong.
Prof. Marco Yiu Chung Pang, Department of Rehabilitation Sciences, The Hong Kong Polytechnic University.
Dr. Michael Ying, Department of Health Technology and Informatics, The Hong Kong Polytechnic University.
Prof. Thomas Leung, Acute Stroke Unit, Prince of Wales Hospital.
Prof. Adrian Wong, Research Assistant Professor, Department of Medicine and Therapeutics, The Chinese University of Hong Kong.
Dr. Chi Keung Raymond Chung, Senior Scientific Officer, Department of Rehabilitation Sciences, The Hong Kong Polytechnic University.
Ms. Huixi Ouyang, PhD student, Department of Rehabilitation Sciences, The Hong Kong Polytechnic University.

參與者須知

Patient Information Sheet

前言

Introduction

繼發性骨質疏鬆為常見中風後的情況，特別在患側的上肢及下肢。中風後骨組織完整性的轉變是令中風人士出現骨折機會上升至為重要的因素。據資料顯示，在早期的研究中大多使用傳統的雙能 X 線骨質密度測量儀 (Dual-energy X-ray

Absorptiometry，DXA)評估骨質密度。然而骨強度的決定因素，除了骨密度和骨大小以外，還包括骨內部三維微結構，例如外層骨皮質的厚度和疏鬆程度，以及內部骨小梁的立體網狀結構。傳統的雙能 X 線骨質密度測量儀雖然廣泛用於臨床上診斷骨質疏鬆，但由於受到影像為二維的限制，由雙能 X 線骨質密度測量儀計算得的面積性骨質密度，不能夠反映全部的骨強度和完全準確地預測骨折的風險。香港中文大學矯形外科及創傷學系骨質量檢測和健康評定中心是亞洲第一所研究中心設有高解像度肢體定量計算斷層掃瞄器(High-resolution peripheral quantitative computed tomography，HR-pQCT)。高解像度外周骨定量斷層掃描能以非入侵性方法量度人類外周骨的容積性骨質密度和骨微結構。而這些資料將會幫助我們更深入瞭解骨中風後骨結構的變化，從而更全面地評估骨的強度和骨折的風險。本研究的目的，就是(1)要使用高解像度肢體定量計算斷層掃瞄器，評估中風後一年內及中風慢性期兩年期間手腕骨及腳腕骨的骨質密度、幾何學及微觀結構上的轉變，以及(2)評估人口統計數據、中風的類型及位置，以及各種身體功能的損傷程度等因素與中風後一年內及中風慢性期手腕骨及腳腕骨的骨質密度、幾何學及微觀結構上轉變之間的關係。

該研究是由香港中文大學矯形外科及創傷學系骨質量檢測和健康評定中心的秦嶺教授主理，已經獲得香港中文大學-新界東醫院聯網臨床研究倫理聯席委員會批核，該委員會旨在保障於新界東醫院聯網醫院參與研究的患者的權益。

Secondary osteoporosis is a common phenomenon after stroke, particularly in the hemi-paretic limbs. The compromised integrity of bone tissue is an important contributing factor to the increased incidence of fragility fractures after stroke. Earlier studies have used dual-energy X-ray absorptiometry (DXA) to assess bone mineral density (BMD). However, bone strength depends not only on bone mass and bone size, but also the microarchitecture of the bone, including the thickness and porosity of the cortices as well as the integrity of the trabecular network. The traditional DXA is now widely used in clinical setting for the diagnosis of osteoporosis. Limited by the two-dimensional imaging, the areal bone mineral density (BMD) provided by DXA scan cannot fully reflect the strength of the bone or accurately predict the fracture risk. The Bone Quality and Health Assessment Centre at the Department of Orthopaedics & Traumatology of the Chinese University of Hong Kong is the first research centre in Asia that is equipped with the high-resolution peripheral quantitative computed tomography (HR-pQCT). HR-pQCT is a non-invasive technology which measures volumetric bone mineral density and microarchitecture of the peripheral bone. These measures have the potential to further our understanding of the structural changes of the bone post-stroke and help us predict fracture more accurately. The objectives of this research study are to assess the changes in bone density, geometry and microstructure of the radius and tibia within the first year post-stroke and during chronic stage, and to assess the association of changes in bone density, geometry and microstructure of the radius and tibia during these two stages with demographic factors, type and location of stroke, and severity of impairment in various bodily functions.

The principal investigator of this research is Prof. Ling Qin, Bone Quality and Health Assessment Centre, Department of Orthopaedics & Traumatology, The Chinese university of Hong Kong. This research has been review and approved by the Joint Chinese University of Hong Kong (CUHK) – New Territories East Cluster (NTEC) Clinical Research Ethics Committee (CREC). The mission of the Joint CUHK-

NTEC CREC is protecting the rights, safety and well-being of human subjects with respect to their participation in clinical studies in the hospitals of NTEC.

研究目的
Objective

1. 評估中風後一年內及慢性期(>1 年)手腕骨及 腳 腕骨的骨質密度、幾何學及微觀結構上的轉變。
2. 評估中風的位置以及肌無力嚴重程度等 因素與中風後一年內及慢性期(>1 年)手腕骨及 腳 腕骨的骨質密度、幾何學及微觀結構上轉變之間的關係。
3. 建立一個能全面預測中風後一年內及慢性期(>1 年)骨結構變化的模型。

1. To assess the changes in bone density, geometry and microstructure of the radius and tibia within the first year post-stroke and during late chronic stroke stage (>1 year).
2. To assess the association of changes in bone density, geometry and microstructure of the radius and tibia during the follow-up period in these two stages with location of stroke, and severity of muscle weakness.
3. To develop a comprehensive and empirically-based model to predict bone structural changes during both subacute and chronic phases.

參與者的權利和責任
Your rights and responsibilities

您的參與完全為自願性質。您參與這項研究與否將不影響您所應得的醫療服務及法律權利。即使在您參與這項研究後，您仍可在任何時候退出，而無需任何理由。如果你決定參與此研究，你需要在此同意書上簽署。研究人員會幫你安排時間進行研究所涉及的各項檢查，請你在檢查的當日準時到達檢查地點。如果您不能應約，請盡早聯絡研究人員，以更改時間。

Your participation in this study is entirely voluntary. Your decision whether or not to participate will not prejudice you or your medical care. If you decide to participate, you are free to withdraw your consent and to discontinue participation at any time. If you decide to participate, you need to sign on this consent form. Our research staff will arrange the time and date for you to conduct the procedures related to this research. Please attend your arranged visit on time. If you cannot visit on time, please contact our research staff as soon as possible for changing the appointment.

研究對象
Participants

急性期中風人士: 納入資格:被醫生診斷為單側中風人士而病發不多於兩星期、年滿 18 歲或以上、醫療情況穩定、改良 Rankin 量表在 2 至 5 分、能執行運動指令，有能力簽署研究同意書（包括家屬）。排除資格: 患有其他神經系統疾病(例如：柏金遜患者) 、接收性失語症、懷孕, 以前的中風中風導致日常生活功能受損：改良 Rankin 量表 2 分或以上），其他身體情況導致對骨骼健康有實質性影響(例如：類風濕性關節炎) 、中風前後服用過治療骨質疏鬆的藥物（例如雙膦酸鹽）、中風前有診斷為骨質疏鬆或脆性骨折、或有任何金屬植入物於受掃瞄的肢體中、其他嚴重疾病 (例如：癌症) 。
慢性期中風人士：除病發多於一年外，納入及排除資格與急性期一致。**控制組**: 除無中風史外，納入及排除資格與急性期一致。

Individuals with acute stroke: *Inclusion criteria*: diagnosis of a unilateral stroke confirmed by your physician, aged ≥18 years, stroke onset within 2 weeks, medically stable, Modified Rankin Scale score: 2-5, can obey motor command , ability to provide informed consent by yourself or your family member. *Exclusion criteria*: other neurological conditions (e.g., Parkinson's disease), receptive aphasia, pregnancy, previous stroke(s) that led to significant impairment in daily living: Modified Rankin Scale ≥2), other conditions that have substantial impact on bone health (e.g., rheumatoid arthritis), taking medications for treatment of osteoporosis currently or/and prior to stroke (e.g., bisphosphonates), known diagnosis of osteoporosis prior to stroke, fragility fractures prior to stroke, or metal implants in the limb to be scanned, or other serious illnesses (e.g., cancer).

Individuals with chronic stroke：inclusion and exclusion criterion are the same to acute stroke subjects except with a stroke duration of more than one year. Control group: except with no history of stroke, same inclusion and exclusion criteria.

研究小組將會進行以下檢查
Research group will carry out the following measurements

參與此研究你需要親身前往香港中文大學矯形外科及創傷學系,骨質量檢測和健康評定中心進行幾項檢查，包括高解像度肢體定量計算和斷層掃瞄以及體格測驗。急性期：這些檢查將會在四次不同時段進行，分別在中風後兩星期內，三個月，六個月以及十二個月時。慢性期：這些檢查將會進行兩次，分別為首次檢查及兩年後進行。

You are required to go to Bone Quality and Health Assessment Centre, Department of Orthopaedics & Traumatology, The Chinese University of Hong Kong, in person, for the assessment related to this research. The assessment will include high-resolution quantitative computed tomography and a physical examination. The assessment will be performed at 4 different times, at 2 weeks, 3 months, 6 months and 12 months after stroke onset or during chronic stage with a two-year gap between two measurements.

骨骼掃瞄:
高解像度肢體定量計算和斷層掃瞄器是在身體兩邊的手腕骨及腳腕骨進行非入侵性骨結構測量(合共 4 處骨骼的掃瞄)。參加者會坐在椅子，手和腳會分別放在特製的架上。工作人員會為參加者調校椅子到舒適的位置進行檢查。每處骨骼的掃瞄需時約 3 分鐘（4 處骨骼共 12 分鐘)。為確保影像清晰，掃瞄時請避免任何身體移動。

Bone imaging:
The assessment of high-resolution peripheral quantitative computed tomography will be performed at the distal tibia and distal radius on both sides (total of 4 scans in each appointment) for non-invasive bone microarchitectural measurement. You will be asked to sit on a chair. Arm or leg will be positioned in a cast and the position of the chair will be adjusted to make it comfortable for you. Each scan will take approximately 3 minutes (12 minutes in total for 4 scans). To ensure good quality of the bone images, you should avoid any movement when the scanning is taking place.

體格檢查:
此項檢查大約需要 2 小時。
研究人員會詳細詢問你的健康狀況，你的身高體重、吸煙喝酒的習慣等。我們也會問你關於中風之前體能活動水平和獨立生活能力的問題。
體能活動水平：我們將會以問卷調查方式評估您過去 7 天的體能活動水平。
肌肉強度:手握力測試是一項測試你前臂肌肉力量的測試，你需要在無其他手部動作下用盡全力握緊測試儀的手柄。進行下肢股四頭肌力量的時候，你將會端坐在

座椅上，膝關節呈屈曲狀態，請以最大力度對抗放
置於您小腿上的測力機。肌肉力量測試將會左右手和腳各做三次。

關節活動範圍：我們將會測量你手腕，腳腕主動及被動的活動範圍。

血管健康：你將會被要躺在床上和放鬆 ，我們將以手持式多普勒超聲探頭測量您位於兩側手臂及腿部的血流情況。
活動能力的回復：我們將對你中風後受影響上肢和下肢的活動能力進行評估。
痙攣程度：我們將移動您患側的腳踝，手腕和手指，從而量度您腳踝和手腕/手指的痙攣程度。 我們亦會使用反射錘
測試患側手臂及腳踝肌肉的反射動作。在測試過程中，請盡量放鬆身體。
步行狀態： 我們將評估您的獨立步行的水平，及是否需要助行器。(例如: 手扙，助行架)。另外，我們亦會在步行 10 米距離中量度您最快安全步行速度。
感覺功能：我們將以不同粗幼的單絲輕觸您手背和腳底的皮膚。過程中您會被要求閉上雙眼。當你感覺到皮膚被觸碰時，您需要告知研究員。
此外，我們還會檢查中風慢性期人士的平衡能力及上肢在日常生活中的使用情況。

Physical examination:
This part of the assessment will take approximately 2 hours.
Our staff will conduct interview regarding your past medical history and obtain demographics such as your body weight and height, and your smoking and drinking habit. We will also ask you questions regarding your physical activity level and independence of living prior to stroke.
Physical activity level: You will also have to fill in a questionnaire concerning your physical activity levels.
Muscle strength: You will also undergo upper and lower limb strength test. The handgrip test is a test to test your forearm muscle strength. You will be instructed to squeeze a dynamometer as hard as you can. The knee extension test is a test to test your quadriceps strength. You will be instructed to seat up straight with hip and knees at 90°. A dynamometer will be placed on your shin, and you will be instructed to forcefully push against the dynamometer as strongly as possible. These tests will be repeated for three times for each forearm/foot.
Joint range of motion: We will measure the active and passive range of motion of two joints: wrist and ankle joint.
Vascular health: You will be asked to lie on your back and relax. We will use a hand-held Doppler ultrasound probe to measure your measure your blood flow in your arm and leg on each side.
Motor recovery: We will assess how well you move your upper and lower limbs on the affected side.
Spasticity: We will measure the spasticity level of the ankle and wrist/finger muscles by passively moving your ankle, wrist and fingers on the affected side. We will also test the reflex response of the muscles in your affected elbow and ankle by tapping the tendon using a reflex hammer. You will be asked to relax as much as possible during testing.
Walking status: We will assess your independence level in walking and whether you require any walking aid (e.g., cane, walking frame). We will also measure your safe maximal speed in walking a 10-meter path.
Sensory function: We will test your light touch sensation in the back of your hand, and also the sole of your foot. You will be asked to keep your eyes closed during this test. We will use monofilaments of different sizes to touch your skin in the above regions. You need to indicate whether you feel the monofilament touching your skin to the researcher.
Additionally, we will measure balance and the usage frequency of the affected upper limb during daily activities for individuals during chronic stage.

益處，風險和副作用
Benefits, risk and side effect

我們希望你在參與該項研究中進行的檢查能夠讓你更瞭解你的個人健康，但我們不能保證或承諾您會從這項研究當中得益。該研究所涉及的影像學檢查均為無創性檢查，不需要注射顯影劑。**影像學檢**查涉及少量輻射。手腕骨或者腳腕骨高解像度肢體定量計算和斷層掃瞄器檢

查的輻射量為 3-4μSv，比日常背景輻射 (8μSv)低。因此，一年跟進期中的總幅射劑量約為 50-60 μSv (4 處骨骼 × 4 次 = 16 次掃瞄) ，此劑量相約於 8-10 小時的飛行旅程的幅射水平。由於 X 射線對孕婦和胎兒是有害的，因此您應該向研究人員表明您是否懷孕或者懷疑懷孕。為確保您的安全，研究醫生和技術人員會評估您是否適合做以上檢查。

We hope that the assessments you get from this research will help you understand more your personal health. However, we cannot guarantee benefits of taking part in any of these studies. Both the dual-energy X-ray absorptiometry and the high-resolution peripheral quantitative computed tomography assessments are non-invasive and they do not involve injection of contrast into your body. The radiation for the standard measurement of a single distal radius/tibia is 3-4μSv, which is lower than the background dose (8μSv per day). The total radiation dose during the 1-year follow-period will thus be around 50-60 μSv (16 scans: 4 bone sites × 4 time points), which is similar to that received during an 8-10 hour flight. However, X-rays are known to post risk on unborn babies. Therefore, it is very important to tell your doctor or our research staff if you are pregnant or you think you may be pregnant before you have these assessments.

付款，費用和研究相關的傷害
Fees, payment and harm related to research

您不需要為該研究中的檢查支付費用。對於在一年跟進期內會有四次評估的人士，其中第二、第三和 第四次評估均在出院後進行，所以您將收取每次港元 100 元正作為 交通費補貼。故此在完成所有評估後，您將總共收取港元 300 元正的交通費補貼。對於在兩年內進行兩次檢查的人士，在完成每次評估後，您將收取港元 100 元正的交通費補貼（共 200 元)。因參與這項研究而受到傷害將不會獲得特定賠償。但若您的損傷是由於他人疏忽而造成，您可以以醫療人員失誤引起身體傷害為由自費循法律途徑索取賠償。如您欲就您接受的治療提出投訴，您可循醫院管理局現行的投訴機制向有關部門申訴。

You are not required to pay for the bone imaging or physical examinations. As the second, third and fourth assessment will take place after you are discharged from the hospital for acute stroke individuals, you will receive a travel subsidy of HK$100 after the completion of each of these assessments. Therefore, you will receive a maximum of $300 at the end of the study if you complete all the required assessments. Likewise, for the chronic stroke patients, we will provide a travel subsidy of HK$100 for each of the two assessment during the two-year follow-up period (total of HK$200). If you are harmed by taking part in this study, there are no special compensation arrangements. If you are harmed due to someone's negligence, then you may have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to complain about any aspect of the way you have been approached or treated during the course of this study, the normal health service complaints mechanisms may be available to you.

私隱權及記錄的保密
Privacy and confidentiality

您享有保障個人私隱的權利。所有研究期間收集的資料將按法例保密。除了法例要求外，您將不會因為您的名字、住址、電碼號碼或其他識別號碼，包括身份證號碼而被辨認。在研究期間獲得任何有關您的資訊，包括對您進行的所有測試結果將會被保存在電腦和人工歸檔系統中，但是這些系統都不會標識您的姓名。您將會被編配一組獨特號碼。此號碼的資訊將會被儲存在安全的地方，只限研究人員存取。您參與此研究內的這些醫療記錄僅根據需要來決定保存時間，在保存期間將保密，並且在適用法律和法規允許範圍內，將不會對外公開。研究數據將由研究者進行分析。某些統計學測試將會在本研究中使用。本研究結果有可能在學術期刊或會議上發表。如果公佈研究結果，您的身份仍會保密。在任何與這項研究相關的陳述或者報告中都不會標識您的身份。

您的醫療紀錄將不會被研究人員公開。機構審查委員會/倫理委員會和監管機構在適用法律及規例許可的範圍內，有權直接查看您原本的醫療紀錄來驗證臨床測試或其中包含的資料。在這情況下，您的身份可能會被披露，但仍然保持絕對機密。

You have a right to privacy, and all information that is collected because of this study is confidential to the limit that is possible by law. Except as required by law, you will not be identified by name, address, telephone number, or any other direct personal identifier such as your Identity Card number. The information obtained from this study, including the all the results of the tests carried out during the study will be held in both computerized and manual filing system, although these will not identify you by name. You will be identified by a unique code number and information about the code will be kept in a secure location and access limited to research study personnel. These records will only be kept for as long as necessary and during that time will be kept confidential and, to the extent permitted by applicable laws and regulations will not be made publicly available. Data collection upon you for this study will be analyzed by study investigators. Certain statistical tests will be carried out on your data, along with that collected from the others patients who entered the study. The results of this study may be published on journals or be presented at conferences. If the results are published, your identity will remain confidential. Any identifying information (name, address, etc.) will be removed from all records. You will not be identified personally in any presentation or report dealing with this study.

Your medical records will not be voluntarily disclosed by the study investigators. However, at any time during or after the study, direct access to your medical records will be given to regulatory authority, such as institution review board or ethics committee so that they can verify that the information collected for the study is accurate. This will be done without violating your confidentiality to the extent permitted by the applicable laws and regulations.

聯絡資料

我們鼓勵您在研究期間隨時提出問題。如果您對有關這項研究、它的步驟、風險和益處有疑問，或者你需要更改已經提供的資料，請於辦公時間內連絡計劃負責人，秦嶺教授，香港中文大學矯形外科及創傷學系，電話: 3505 3071。或者彭耀宗教授, 香港理工大學康復治療科學系，电话 2766-7156。若您對此計劃之研究人員有任何投訴，可以聯絡部門科研委員會秘書鍾靜妍女士(電話: 2766 4329)。

如果您對作為參與者的權利有疑問，您可以聯絡沒有利益衝突的第三者：香港中文大學 - 新界東醫院聯網臨床研究倫理聯席委員會，電話: 3505 3935。

Contact information

You are encouraged to ask questions at any time during the study. In case you have any question regarding this research, the procedure, risk and side effect, or you need to update your personal information provided in this research, you are welcome to contact the principal investigator of this research, Prof. Ling Qin, Department of Orthopaedics and Traumatology, The Chinese University of Hong Kong, Tel: 3505 3071, or Prof. Marco Yiu Chung Pang, Department of Rehabilitation Sciences, The Hong Kong Polytechnic University. Tel: 2766 7156, during our office hour. If I have complaints related to the investigator(s), I can contact Ms. Vangie Chung, secretary of Departmental Research Committee, at 2766-4329.

If you have any questions about your rights as a study subject, you can contact a third party without conflict of interest: The Joint Chinese University of Hong Kong – New Territories East Cluster Clinical Research Ethics Committee. (Tel: 2632 3935)

透過使用高像數外周定量電腦斷層掃瞄，研究中風後長骨結構的轉變以及其相關因素：縱向研究。

Using high-resolution peripheral quantitative computed tomography to study the long bone structural changes after stroke and associated factors: a longitudinal study.

參加者同意書
Consent Form

我在此聲明：
I declare that:

1. 本人已閱讀及明白這份參與研究同意書，並且已經獲得提問的權利。我已經被知會我可能遇到的風險和得益。我所提出的問題已得到滿意的解答。
   I confirm that I have read and understood the information sheet for the above study and have had the opportunity to ask questions. I have been told about the risks and benefits and I have had my questions answered to my satisfaction.
2. 我明白我的參與完全出於自願並且可以在任何時候退出，而無需任何理由。我的決定不會影響我所受到的醫療服務和法律權利。如果我決定退出這項研究，我同意之前收集到的資料可繼續被使用。
   I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. If I decide to withdraw from this study, I agree that the information collected about me up to the point when I withdraw may continue to be processed.
3. 我明白機構審查委員會、倫理委員會或監察機構的成員可能會查閱我的醫療紀錄，我同意授權有關人員查閱我的記錄。
   I understand that sections of any of my medical notes may be looked at by responsible individuals from regulatory authorities where it is relevant to my taking part in research. I give permission for these individuals to have access to my records.
4. 我同意參與這項研究。
   I agree to take part in the above study.
5. 簽署這同意書並不表示我放棄任何權利。
   I do not waive any liability rights by signing this form.
6. 我的簽署表示我已得到這檔的副本。我會保留這副本直至我參與完結為止。
   My signature indicates that a copy of this form has been given to me and I will keep it until the end of my participation in the study.

_____	_____	_____
參與者的簽署 (Signature)	參與者姓名 (Name of participant)	簽署日期 (Date)
_____	_____	_____
法定代表或監護人簽署, 如適用 (Signature of legal representative, if applicable)	法定代表或監護人姓名 (Name of legal representative, applicable)	簽署日期 (Date)
_____	_____	_____
研究者/代表簽署 (Signature of investigator or delegate)	研究者/代表姓名 (Name of investigator or delegate)	簽署日期 (Date)

Appendix 3. Sample of assessments

Appendix 3.1 Demographic information

Name: _____ **Subject code:** _____

Date of Assessment: _____ **(dd/mm/yy)**

Assessors: _____

1. Demographics & Stroke Characteristics

Household Income: $0-$15,000 / $15,001-$30,000 / $30,001-$45,000 / >$45,000

Age:_____(Date of Birth:___/___/___) Education Level: _____

Gender: Male/ Female/ Non-binary sex designation at birth

Body weight:____(kg) Body Height:___(cm) Body fat composition:_____(%)

Menstruation period (Female only):_____(months/yearspost-menopausal)

Medication currently taking:

_____(Last taken at:_____)

Surgical history:_____(Date of surgery:_____)

Medical conditions (e.g.HT/DM/SCI):_____

Orthosis: No / Yes (Indoor:_____/Outdoor:_____)

Walking aids: No / Yes (Indoor:_____/Outdoor:_____)

if yes, Stick / Tripod / Quadripod / Frame / wheelchair

Smoking: _____ (past 2 yr) **Drinking:** _____

Calcium: _____ **Vitamin D:** _____

Body weight distribution: Paretic: _____kg Non-paretic:_____kg

Dominant foot: Left / Right Dominant hand: Left / Right

First onset of stroke: Duration of stroke:

Location of stroke: anterior circulation syndrome / partial anterior circulation syndrome / lacunar syndrome / posterior circulation syndrome

Type of stroke: ischemic / hemorrhagic Hemiparetic Side: Right / Left

Receiving PT or OT past half years: _____Living status:_____

Appendix *3*.2 Functional Ambulatory Categories

1. Level surface: Tile, rugs, pavement
2. Non-level surface: Grass, gravel, dirt, snow, ice
3. Stairs: Up and down at least seven steps with rail
4. Incline: Up and down 5-ft (1.52-m) incline of 30 degrees or greater

FAC Level	**Ambulation Description**	**Definition**
1	Nonfunctional	Unable to ambulate Ambulates only in parallel bars Requires supervision or physical assistance from > 1 person
2	Dependent, level II	Requires manual contact of one person during ambulation on level surfaces Manual contact is continuous and necessary to support body weight and/or to maintain balance or assist coordination
3	Dependent, level I	Requires manual contact of one person during ambulation on level surfaces Manual contact is continuous or intermittent light touch to assist balance or coordination
4	Dependent, supervision	Ambulation occurs on level surfaces without manual contact of another person Requires stand-by guarding of one person because of poor judgment, questionable cardiac status, or the need for verbal cuing to complete the task
5	Independent, level surfaces Only	Ambulate is independent on level surfaces Requires supervision/physical assistance to negotiate stairs, inclines, or unlevel surfaces.
6	Independent, Level and Non- level surfaces	Ambulation is independent on unlevel and level surfaces, stairs, and inclines

*Ask patients subjectively the FAC level first, if patients come independently, assume level 6; if patients come with companions, ask subjectively, and confirm by asking the patients to demonstrate if suspected.

Appendix 3.3 Motor Activity Log

家居活動紀錄表

姓名 _____ **日期**_____

	使用頻率(甲)	**動作質素(乙)**	**(丙)**
1. 開燈掣	_____	_____	如沒有進行此活動，請說明(填代號) 備註 _____
2. 開櫃桶	_____	_____	如沒有進行此活動，請說明(填代號) 備註 _____
3. 從櫃桶取衣物	_____	_____	如沒有進行此活動，請說明(填代號)) 備註 _____
4. 接聽電話	_____	_____	如沒有進行此活動，請說明(填代號) 備註
5. 抹檯面	_____	_____	如沒有進行此活動，請說明(填代號)) 備註 _____
6. 落車 *(只包括當車門打開後，**從坐到站**之動作)*	_____	_____	如沒有進行此活動，請說明(填代號) 備註 _____
7. 開雪櫃	_____	_____	如沒有進行此活動，請說明(填代號) 備註
8. 通過扭把手開門	_____	_____	如沒有進行此活動，請說明(填代號) 備註 _____
9. 使用電視搖控器	_____	_____	如沒有進行此活動，請說明(填代號) 備註 _____

	使用頻率(甲)	**動作質素(乙)**	**(丙)**
10. 洗手 *(包括使用皂液及沖洗，但**不包括**開關水龍頭)*	_____	_____	如沒有進行此活動，請說明(填代號)備註 備註 _____
11. 開關水龍頭	_____	_____	如沒有進行此活動，請說明(填代號) 備註 _____
12. 抹幹手	_____	_____	如沒有進行此活動，請說明(填代號) 備註 _____
13. 穿襪子	_____	_____	如沒有進行此活動，請說明(填代號) 備註 _____
14. 脫下襪子	_____	_____	如沒有進行此活動，請說明(填代號) 備註 _____
15. 穿鞋子 *(包括綁鞋帶)*	_____	_____	如沒有進行此活動，請說明(填代號) 備註 _____
16. 脫下鞋子 *(包括解開鞋帶)*	_____	_____	如沒有進行此活動，請說明(填代號) 備註 _____
17. 從有扶手的椅子起身	_____	_____	如沒有進行此活動，請說明(填代號) 備註 _____
18. 坐下前將椅子拉開	_____	_____	如沒有進行此活動，請說明(填代號) 備註 _____
19. 坐下後把椅子拉近桌子	_____	_____	如沒有進行此活動，請說明(填代號) 備註 _____
20. 拿起杯子或罐頭 *(**不包括**飲用)*	_____	_____	如沒有進行此活動，請說明(填代號) 備註 _____

	使用頻率(甲)	**動作質素(乙)**	**(丙)**
21. 刷牙 (***不包括****預備牙刷或洗刷假牙*)	_____	_____	如沒有進行此活動，請說明(填代號)) 備註 _____
22. 搽潤膚露/ 美容用品/ 剃鬚膏	_____	_____	如沒有進行此活動，請說明(填代號) 備註 _____
23. 用鎖匙開門	_____	_____	如沒有進行此活動，請說明(填代號) 備註 _____
24. 在紙上寫字 (*如中風前即****使用健側手寫字****，****則可忽略此題*)	_____	_____	如沒有進行此活動，請說明(填代號) 備註
25. 手拿物件 （***不包括****用手臂夾物品*）	_____	_____	如沒有進行此活動，請說明(填代號) 備註 _____
26. 使用叉子、筷子或湯匙進食	_____	_____	如沒有進行此活動，請說明(填代號) 備註 _____
27. 梳頭	_____	_____	如沒有進行此活動，請說明(填代號) 備註 _____
28. 通過杯柄拿起杯	_____	_____	如沒有進行此活動，請說明(填代號) 備註 _____
29. 扣鈕	_____	_____	如沒有進行此活動，請說明(填代號) 備註 _____
30. 食三文治或小食	_____	_____	如沒有進行此活動，請說明(填代號) 備註 _____

(甲) 患側上肢的使用頻率

0 沒有使用

1 極少使用

2 偶爾使用：大部份時間使用好手

3 間中使用：程度約為中風前之**一半**

4 經常使用：程度約為中風前之 7~8 **成**

5 常常使用：程度與中風**之前一樣**

(乙) 患側上肢的動作質素

0 **沒有使用**患側上肢進行活動。

1 在進行活動時，患肢可輕微移動，但對該活動**沒有幫助**。

2 在進行活動時，患肢在**健側協助**之下才可完成此活動。

3 用患肢可**慢慢**地或**困難**地**完成**活動。

4 用患肢可將活動完成，但比正常**慢一點**或不是很準確。

5 用患肢可將活動完成，並與中風前**表現一樣**。

(丙) 說明沒有進行此活動之代號

1 完全使用好手進行此活動（標 0 分）

2 別人替我完成此活動（標 0 分）

3 從來不做此活動，因為沒有需要﹝例如：光頭並不需要梳頭﹞（標 NA）

4 有時會從事此活動，但自上次填寫此問卷後並沒有機會再做

5 非慣用手偏癱（僅適用於第 24 項：在紙上寫字）（標 NA）

Appendix *3*.4 Physical Activity level (Physical Activity Scale for Elderly)

體能活動

以下幾條問題喺問你喺過去７日內嘅體能活動。如果喺過去７日因為唔舒服或者天氣唔好而影響你往常嘅活動，請你根據兩三個星期前嘅活動嚟做估計。

1. 喺過去７日，你用咗幾多時間嚟做一啲坐喺度嘅活動，例如閱讀、睇電視、做手工藝、打麻雀、捉棋、玩啤牌、玩電腦等？

○ 冇 ○ 好少(1 至 2 日) ○ 有時(3 至 4 日) ○ 經常(5 至 7 日)

直去第 2 題 係啲乜嘢活動呢？ _____

你每日平均會用幾多個鐘頭嚟做呢啲坐喺度嘅活動？

○ 少過 1 個鐘 ○ 1 至 2 個鐘 ○ 2 至 4 個鐘 ○ 多過 4 個鐘

2. 喺過去７日，你通常用幾多時間喺屋外行路(唔理為咗乜嘢原因)？例如：去玩或者做運動、行路返工、帶狗散步、去買餸、掉垃圾、去飲茶、去行街等？

○ 冇 ○ 好少(1 至 2 日) ○ 有時(3 至 4 日) ○ 經常(5 至 7 日)

直去第 3 題 係啲乜嘢活動呢？ _____

你每日平均會用幾多個鐘頭嚟行路？

○ 少過 1 個鐘 ○ 1 至 2 個鐘 ○ 2 至 4 個鐘 ○ 多過 4 個鐘

3. 喺過去７日，你用咗幾多時間嚟做一啲輕量嘅運動或者消遣嘅活動？例如：打保齡球、打高爾夫球(乘車)、在碼頭或坐船釣魚、耍太極、氣功、打乒乓球或者其他類似嘅活動。

○ 冇 ○ 好少(1 至 2 日) ○ 有時(3 至 4 日) ○ 經常(5 至 7 日)

直去第 4 題 係啲乜嘢活動呢？ _____

你每日平均會用幾多個鐘頭嚟做一啲輕量嘅運動或者消遣嘅活動？

○ 少過 1 個鐘 ○ 1 至 2 個鐘 ○ 2 至 4 個鐘 ○ 多過 4 個鐘

4. 喺過去７日，你用咗幾多時間嚟做一啲溫和嘅運動同消閒活動，例如：網球雙打、羽毛球、社交舞、高爾夫球(冇乘車) 、拎重嘢行平路(少過 5 公斤)或者做其他類似嘅活動？

○ 冇 ○ 好少(1 至 2 日) ○ 有時(3 至 4 日) ○ 經常(5 至 7 日)

直去第 5 題 係啲乜嘢活動呢？ _____

你每日平均會用幾多個鐘頭嚟做呢啲溫和嘅運動同消閒活動？

○ 少過 1 個鐘 ○ 1 至 2 個鐘 ○ 2 至 4 個鐘 ○ 多過 4 個鐘

體能活動

5. 喺過去7日，你用咗幾多時間嚟做一啲劇烈嘅運動同消閒活動，例如：跑步、游泳、踩單車、網球單打、跳健康舞、咩背囊行山、壁球、籃球、健身單車、划船、拎重嘢上樓梯(例如一包5公斤嘅米)或者做其他類似嘅活動？

○ 冇 ○ 好少(1至2日) ○ 有時(3至4日) ○ 經常(5至7日)

直去第6題 係啲乜嘢活動呢？ _____

你每日平均會用幾多個鐘頭嚟做呢啲劇烈嘅運動同消閒活動？

○ 少過1個鐘 ○ 1至2個鐘 ○ 2至4個鐘 ○ 多過4個鐘

6. 喺過去7日，你用咗幾多時間嚟特登做一啲增強肌肉力量同持久力嘅運動？例如：舉重、掌上壓或者其他類似嘅活動。

○ 冇 ○ 好少(1至2日) ○ 有時(3至4日) ○ 經常(5至7日)

直去第7題 係啲乜嘢活動呢？ _____

你每日平均會用幾多個鐘頭嚟做一啲增強肌肉力量同持久力嘅運動？

○ 少過1個鐘 ○ 1至2個鐘 ○ 2至4個鐘 ○ 多過4個鐘

7. 喺過去7日，你有冇做過一啲輕巧嘅家務，例如：打掃或者洗碗、手洗、熨、晾衫、煮飯、買餸？ ○ 有 ○ 冇

8. 喺過去7日，你有冇做過一啲粗重嘅家務或者雜務，例如：吸塵、擦地板、拖地、洗窗、洗車、搬傢俬或者石油氣？ ○ 有 ○ 冇

9. 喺過去7日，你有冇做以下任何嘅活動？(請答有或者冇)

A. 家居維修，例如：油漆油、貼牆紙、整電器等等	○ 有	○ 冇
B. 草地或者庭院工作，例如：剪草、掃樹葉、斬木等	○ 有	○ 冇
C. 戶外園藝	○ 有	○ 冇
D. 照顧其他人，例如：小孩、配偶、或者其他成人	○ 有	○ 冇

10. 喺過去7日，你有冇做工(包括有支薪水或係義工)？

○ 有 ○ 冇

過去一個星期，你做咗幾多個鐘頭有支薪水嘅工作或者係義工 □□ 小時

以下邊一個類別最好用嚟形容你做工時(受薪或係義工)，所需要嘅體能活動？

○ 坐喺度為主，只有少量嘅手部活動
(例如：文員、手錶匠、生產線工人、巴士司機等。)

○ 坐或者企，有時需要行
(例如：收銀員、文員、技工、信差)

○ 需要行，有時要搬唔超過50磅重嘅嘢
(例如：郵差、侍應、建築工人、重機械工人)

○ 需要行同經常要搬超過50磅重嘅嘢
(例如：建築工人、搬運工人、農夫、漁夫、礦工、修路工人)

Appendix *3*.5 Composite Spasticity Scale

Composite Spasticity Scale		
	Upper limb	Lower limb
Tendon jerk	Tendon jerk (**biceps**) 0 No response 1 Normal response 2 Mildly hyperactive response 3 Moderately hyperactive response	Tendon jerk (**achilles**) 0 No response 1 Normal response 2 Mildly hyperactive response 3 Moderately hyperactive response
Resistance to full range displacement	Resistance to full range passive joint displacement (**elbow extension**) 0 No resistance (hypotonic) 2 Normal resistance 4 Mildly increased resistance	Resistance to full range passive joint displacement (**ankle dorsiflexion**) 0 No resistance (hypotonic) 2 Normal resistance 4 Mildly increased resistance
Clonus	Clonus (**wrist**) 1 Clonus not elicited 2 1–3 beats of clonus elicited 3 4–10 beats of clonus elicited 4 Sustained clonus	Clonus (**ankle**) 1 Clonus not elicited 2 1–3 beats of clonus elicited 3 4–10 beats of clonus elicited 4 Sustained clonus
Composite Spasticity Score:___/ 16		

Appendix *3*.6 Touch Pressure Threshold (sitting and supine)

Non-paretic		Paretic	
Site	Threshold (gram)	Site	Threshold (gram)
1		1	
2		2	
3		3	
4		4	
5		5	
6		6	
7		7	

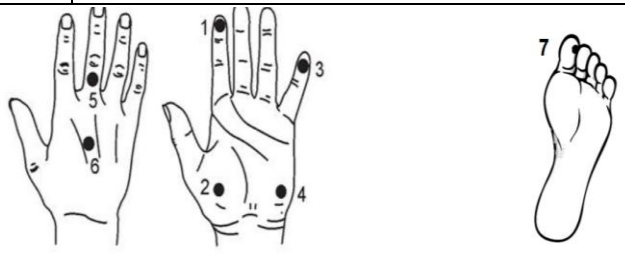

Figure 1. Cutaneous sensation testing sites. Sites 1–4 reflect glabrous skin and sites 5–6 hairy skin. Sites 1–2 have median nerve innervation, 3–4 ulnar nerve, and 5–6 radial nerve.

Appendix 3.7 Fugl-Meyer Assessment (Upper Extremities)

Repeat each movement 3x on the affected side and score best performance. If full score is attained on trials 1 or 2, do not have to repeat 3 times. Only test Coordination/speed, one time (allow one practice trial)

A. UPPER EXTREMITY, sitting position				
I. Reflex activity		**none**	**can be elicited**	
Flexors: biceps and finger flexors (at least one) **Extensors**: triceps		0 0	2 2	
Subtotal I (max 4)				
II. Volitional movement within synergies, without gravitational help		**none**	**partial**	**full**
Flexor synergy: Hand from contralateral knee to ipsilateral ear. From extensor synergy (shoulder adduction/ internal rotation, elbow extension, forearm pronation) to flexor synergy (shoulder abduction/ external rotation, elbow flexion, forearm supination). **Extensor synergy:** Hand from ipsilateral ear to the contralateral knee	Shoulder retraction	0	1	2
	Shoulder elevation	0	1	2
	Shoulder abduction (90°)	0	1	2
	Shoulder external rotation	0	1	2
	Elbow flexion supination	0	1	2
	Shoulder adduction/internal rotation	0	1	2
	Elbow extension	0	1	2
	Forearm pronation	0	1	2
Subtotal II (max 18)				
III. Volitional movement mixing synergies, without compensation		**none**	**partial**	**full**
Hand to lumbar spine hand on lap	cannot perform or hand in front of ant-sup iliac spine hand behind ant-sup iliac spine (without compensation) hand to lumbar spine (without compensation)	0	1	2
Shoulder flexion 0°- 90° elbow at 0° pronation-supination 0°	immediate abduction or elbow flexion abduction or elbow flexion during movement flexion 90°, no shoulder abduction or elbow flexion	0	1	2
Pronation-supination elbow at 90° shoulder at 0°	no pronation/supination, starting position impossible limited pronation/supination, maintains starting position full pronation/supination, maintains starting position	0	1	2
Subtotal III (max 6)				
IV. Volitional movement with little or no synergy		**none**	**partial**	**full**
Shoulder abduction 0 - 90° elbow at 0° forearm pronated	immediate supination or elbow flexion supination or elbow flexion during movement abduction 90°, maintains extension and pronation	0	1	2
Shoulder flexion 90° - 180° elbow at 0° pronation-supination 0°	immediate abduction or elbow flexion abduction or elbow flexion during movement flexion 180°, no shoulder abduction or elbow flexion	0	1	2
Pronation/supination elbow at 0° shoulder at 30°- 90° flexion	no pronation/supination, starting position impossible limited pronation/supination, maintains start position full pronation/supination, maintains starting position	0	1	2
Subtotal IV (max 6)				
V. Normal reflex activity assessed only if full score of 6 points is achieved in part IV; compare with the unaffected side		**0 (IV), hyper**	**lively**	**normal**
biceps, triceps, finger flexors	2 of 3 reflexes markedly hyperactive or 0 points in part IV 1 reflex markedly hyperactive or at least 2 reflexes lively	0	1	

B. WRIST support may be provided at the elbow to take or hold the starting position, no support at wrist, check the passive range of motion prior testing		**none**	**partial**	**full**
Stability at 15° dorsiflexion elbow at 90°, forearm pronated	less than 15° active dorsiflexion dorsiflexion 15°, no resistance tolerated maintains dorsiflexion against resistance	0	1	2
Repeated dorsifexion / volar flexion elbow at 90°, forearm pronated shoulder at	cannot perform volitionally limited active range of motion full active range of motion, smoothly	0	1	2
Stability at 15° dorsiflexion elbow at 0°, forearm pronated slight	less than 15° active dorsiflexion dorsiflexion 15°, no resistance tolerated maintains dorsiflexion against resistance	0	1	2
Repeated dorsifexion / volar flexion elbow at 0°, forearm pronated slight shoulder flexion/abduction	cannot perform volitionally limited active range of motion full active range of motion, smoothly	0	1	2
Circumduction elbow at 90°, forearm pronated shoulder at 0°	cannot perform volitionally jerky movement or incomplete complete and smooth circumduction	0	1	2

C. HAND support may be provided at the elbow to keep 90° flexion, no support at the wrist, compare with unaffected hand, the objects are interposed, active grasp		**none**	**partial**	**full**
Mass flexion	from full active or passive extension	0	1	2
Mass extension	from full active or passive flexion	0	1	2
GRASP				
a. Hook grasp flexion in PIP and DIP (digits II-V), extension in MCP II-V	cannot be performed can hold position but weak maintains position against resistance	0	1	2
b. Thumb adduction 1-st CMC, MCP, IP at 0°, scrap of paper between thumb and 2-nd MCP joint	cannot be performed can hold paper but not against tug can hold paper against a tug	0	1	2
c. Pincer grasp, opposition pulp of the thumb against the pulp of 2-nd finger, pencil, tug upward	cannot be performed can hold pencil but not against tug can hold pencil against a tug	0	1	2
d. Cylinder grasp cylinder shaped object (small can) tug upward, opposition of thumb and fingers	cannot be performed can hold cylinder but not against tug can hold cylinder against a tug	0	1	2
e. Spherical grasp fingers in abduction/flexion, thumb opposed, tennis ball, tug away	cannot be performed can hold ball but not against tug can hold ball against a tug	0	1	2

D. COORDINATION/SPEED, sitting, after one trial with both arms, eyes closed, tip of the index finger from knee to nose, 5 times as fast		**marked**	**slight**	**none**
Tremor	at least 1 completed movement	0	1	2
Dysmetria at least 1 completed movement	pronounced or unsystematic slight and systematic no dysmetria	0	1	2
		≥ 6s	**2 - 5s**	**< 2s**
Time start and end with the hand on the knee	at least 6 seconds slower than unaffected side 2-5 seconds slower than unaffected side less than 2 seconds difference	0	1	2

Appendix 3.8 Fugl-Meyer Assessment (Lower Extremities)

Repeat each movement 3x on the affected side and score best performance. If full score is attained on trials 1 or 2, do not have to repeat 3 times. Only test Coordination/speed, one time (allow one practice trial)

E. LOWER EXTREMITY				
I. Reflex activity sitting position		**none**	**can be elicited**	
Flexors: knee flexors (Medial hamstrings)		0	2	
Extensors (at least one): Patellar		0	2	
Achilles		0	2	
Subtotal I (max 4)				
II. Volitional movement within synergies supine position		**none**	**partial**	**full**
Flexor synergy: Maximal hip flexion (abduction/external rotation), maximal flexion in knee and ankle joint (palpate distal tendons to ensure active knee flexion).	Hip flexion	0	1	2
	Knee flexion	0	1	2
	Ankle dorsiflexi	0	1	2
Extensor synergy: From flexor synergy to the hip extension/adduction, knee extension and ankle plantar flexion. Resistance (against hip extension & ankle plantarflexion) is applied to ensure active movement, evaluate both movement and strength (compare with the unaffected side)	Hip extension	0	1	2
	adduction	0	1	2
	Knee extension	0	1	2
	Ankle plantar	0	1	2
Subtotal II (max 14)				
III. Volitional movement mixing synergies sitting position, knee 10cm from the edge of the chair/bed		**none**	**partial**	**full**
Knee flexion from actively or passively extended knee	no active motion	0		
	less than 90° active flexion, palpate tendons of hamstrings		1	
	more than 90° active flexion			2
Ankle dorsiflexion compare with	no active motion	0		
	limited dorsiflexion		1	
Subtotal III (max 4)				
IV. Volitional movement with little or no synergy standing position, hip at 0°		**none**	**partial**	**full**
Knee flexion to 90° hip at 0°, balance support is allowed	no active motion or immediate, simultaneous hip flexion less than 90° knee flexion and/or hip flexion during movement at least 90° knee flexion without simultaneous hip flexion	0	1	2
Ankle dorsiflexion compare with unaffected side	no active motion limited dorsiflexion complete dorsiflexion	0	1	2
Subtotal IV (max 4)				

V. Normal reflex activity supine position, assessed only if full score of 4 points is achieved in part IV, compare with the unaffected side		**0 (IV), hyper**	**lively**	**normal**
Reflex activity knee flexors, Patellar, Achilles,	0 points on part IV or 2 of 3 reflexes markedly hyperactive 1 reflex markedly hyperactive or at least 2 reflexes lively maximum of 1 reflex lively, none hyperactive	0	1	2
	Subtotal V (max 2)			
	Total E (max 28)			

F. COORDINATION/SPEED supine, after one trial with both legs, eyes closed, heel to knee cap of the opposite leg, 5 times as fast as possible		**marked**	**slight**	**none**
Tremor	at least 1 completed movement	0	1	2
Dysmetria at least 1 completed movement	pronounced or unsystematic slight and systematic no dysmetria	0	1	2
		≥ 6s	**2 - 5s**	**< 2s**
Time start and end with the hand on the knee	at least 6 seconds slower than unaffected side 2-5 seconds slower than unaffected side less than 2 seconds difference	0	1	2
	Total F (max 6)			

Appendix 3.9 The Brief-BESTest: A Suggested Brief Version of the BESTest

Flat heel or shoes and socks off.

Note: “instability” is defined as using more than an ankle strategy to maintain balance (eg, a hip strategy is used).

Section 1. Biomechanical Constraints		
Item 1: Hip/Trunk Lateral Strength “Rest fingertips in my hands while you lift your leg to the side and hold, keep trunk vertical. You will hold for 10 s.” Count 10 s, watch for straight knee; if they use moderate force on your hands, score as “without keeping trunk vertical.”	(3) Normal (10 s with trunk vertical) bilateral (2) Mild (10 s without trunk vertical) bilateral (1) Moderate (10 s with 1 hip abducts with trunk vertical) (0) Severe (neither hip, 10 s and vertical or not vertical) -- cannot abduct either hip 10 s, with or without	trunk vertical
Section II. Stability Limits		
Item 2: Functional Reach Forward “Stand normally; lift both arms straight in front of you; reach as far forward as you can with arms parallel to the ruler without lifting your heels.” 2 attempts Observe that patient does not lift heels, rotate trunk, or protract scapula. Watch for vertical initial alignment. Record best reach.	(3) >32 cm (12.5 in) (2) 16.5–32 cm (6.5–12.5 in) (1) <16.5 cm (6.5 in) (0) No measurable lean (or must be caught)	Trial 1 (cm) Trial 2 (cm)
Section III. Transitions–Anticipatory Postural Adjustment		
Items 3 and 4: Stand on One Leg–Left and Right “Look ahead; hands must stay on hips; bend one leg behind you; stand on 1 leg as long as you can for up to 30 s. Do not let your lifted leg touch the other leg.” Allow 2 attempts, record best attempt; record time up to 30 s (stop time if hands off hips or leg on floor or leg touches supporting leg).	(3) Normal (stable >20 s) (2) Trunk motion OR 10–20 s (1) Stand 2–10 s (0) Unable	L Sec R Sec
Section IV. Reactive Postural Response		
Items 5 and 6: Compensatory Stepping–Lateral, Left and Right “Stand with feet nearly together; lean into my hands; I will remove my hands; do whatever necessary to keep balance, trying to take 1 step.” Note: Stand next to and behind participant. Place hand on greater trochanter and brace yourself to hold the person’s weight shifted to supported leg.	(3) Recovers with 1 side/crossover step (2) Several steps to recover independently (1) Steps but needs assist to prevent fall (0) No step OR falls	
Section V. Sensory Orientation		
Item 7: Stance With Eyes Closed, on Foam Surface “Stand on foam with your eyes closed, your hands on your hips, and your feet close but not touching. Start by looking straight ahead, and I will start timing when you close your eyes. Stay as stable as possible and try to keep your eyes closed for the entire time. The goal is 30 s.” Two trials, if necessary. Patient must step off foam between trials.	(3) 30 s stable (2) 30 s unstable (1) <30 s (0) Unable	Trial 1 (s) Trial 2 (s)
Section VI. Stability in Gait		
Item 8: Timed “Up & Go” Test “When I say ‘go,’ stand up and walk quickly but safely to the tape, turn, and walk back and sit in chair.” Start with back against chair, stop timing when buttocks hit the chair; chair should have arms to push from, if necessary. Imbalance might include trips or lateral/backward stumbles or crossovers.	(3) Fast, <11 s, good balance (2) Slow, >11 s, good balance (1) Fast, <11 s, imbalance (0) Slow, >11 s, imbalance	Time (s)

Appendix *3*.10 Modified Rankin Scale (MRS)

0 No symptoms at all

1 No significant disability despite symptoms; able to carry out all usual duties and activities

2 Slight disability; unable to carry out all previous activities, but able to look after own affairs without assistance

3 Moderate disability; requiring some help, but able to walk without assistance

4 Moderately severe disability; unable to walk without assistance and unable to attend to own bodily needs without assistance

5 Severe disability; bedridden, incontinent and requiring constant nursing care and attention

6 Dead