MUSCLE-BONE UNIT PROPERTIES IN INDIVIDUALS WITH CHRONIC STROKE

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Muscle-bone Unit Properties in Individuals with Chronic Stroke

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Certificate of Originality

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Abstract of thesis entitled “Muscle-bone Unit Properties in Individuals with Chronic Stroke” submitted by YANG Zhenhui for the degree of Doctor of Philosophy at the Hong Kong Polytechnic University in May 2021.

ABSTRACT

The possible influence of stroke on bone properties has been well studied in a good number of studies. However, a comprehensive collation of the impact of stroke on bone properties has not been disseminated. In addition, whole-body vibration may be a useful method to improve the health status of the muscle-bone unit in stroke patients due to its reported effects on muscle-bone unit in other population, such as older adults, whereas no study to date has examined the effects of different WBV frequencies on the properties of the muscle-bone unit in stroke patients. Therefore, this thesis aimed to address the knowledge gap which was achieved through a series of integrated studies.

Study 1 (Chapter 2) is a systematic review aimed to synthesize the literature related to the impact of stroke on bone properties, and summarize the research evidence on the relationship between muscle function and bone properties in individuals with stroke. Based on extensive review of the literature, it was concluded that significant changes in bone mass and macrostructure occurred after stroke, and these changes were more compromised in the paretic sides and in first few months post-stroke. The paretic upper limb exhibited more pronounced bone properties compared with the paretic lower limb. Moreover, there was a strong relationship between muscle strength/power and bone quality, while the impact of muscle spasticity on bone quality remained unclear.

Exploring the relationship between bone quality and muscle strength for different types of contraction (i.e., dynamic Vs isometric) at different contraction velocities would be useful in guiding the design of physical activity or exercise programs for enhancing bone health in individuals with stroke. Study 2 (Chapter 3) aimed to investigate the association of bone strength index at the tibial diaphysis with strength measured during different types of muscle contraction

(i.e., isometric, concentric, eccentric) and at different contraction speeds. The results showed that concentric muscle contraction power was the most import determinant of bone strength index measured at the tibial diaphyseal site.

Research on the effects of different WBV frequencies on the properties of the muscle-bone unit in people with stroke is lacking. In the final study of this thesis (Chapter 4), a randomized controlled trial was conducted to investigate the effect of two different WBV frequencies on leg muscle strength and rate of bone resorption after stroke. The results showed that while both the 30 Hz frequency and 20 Hz frequency WBV protocols induced a significant increase in concentric and eccentric knee muscle strength and reduction in rate of bone resorption, the 30 Hz frequency protocol was more effective than the 20 Hz protocol in improving eccentric knee extension strength on the paretic side following 8 weeks of training.

Overall, the thesis indicates that stroke has considerable impact on bone health, and that leg muscle strength, particularly concentric muscle strength, is independently associated with tibial bone strength index among persons with chronic stroke. This thesis also showed that WBV is a safe training modality in people with chronic stroke, and a frequency of 30 Hz should be the more appropriate choice for enhancing leg muscle strength in individuals with chronic stroke.

PUBLICATIONS

A. Peer-reviewed journal articles

1. Yang, Z., Miller, T., Xiang, Z., & Pang, M.Y.C. (2021) Effects of different vibration frequencies on muscle strength, bone turnover and walking endurance in chronic stroke. *Scientific Reports*, *11*(1), 1-10.

2. Yang Z., Miller, T., & Pang, M.Y.C. (2020) Relationship between bone strength index of the hemiparetic tibial diaphysis and muscle strength in stroke patients: influence of muscle contraction type and speed. *Osteoporosis International*, 1-9.

3. Yang, F.Z., Jehu, D.A.M., Ouyang, H., Lam, F.M.H., & Pang, M.Y.C. (2020). The impact of stroke on bone properties and muscle-bone relationship: a systematic review and meta-analysis. *Osteoporosis International*, *31*(2), 211-224.

B. Conference Abstracts

1. Yang Z-H., & Pang M.Y.C. The impact of stroke on bone mass and marcostructure properties: a systematic review. *The 5th HKASMSS Student Conference on Sports Medicine, Rehabilitation and Exercise Science*, 26 November 2016, Hong Kong.

2. Yang Z-H., & Pang M.Y.C. Skeletal muscle area is associated with tibial bone strength index in people with chronic stroke: implications for rehabilitation. *The 11th International Society of Physical and Rehabilitation World Congress*, 30 April-4 May 2017, Argentina.

3. Yang Z-H., & Pang M.Y.C. Muscle strength is associated with tibial bone strength index in women with chronic stroke: implications for rehabilitation. *The 11th International Society of Physical and Rehabilitation World Congress*, 30 April-4 May 2017, Argentina. **(Won the best paper award)**

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AIC	akaike information criterion
BIC	bayesian information criterion
BMC	bone mineral content
BMD	bone mineral density
BMI	body mass index
BSI	bone strength index
CBSI	compressive bone strength index
CI	confidence interval
CMSA	Chedoke-McMaster Stroke Assessment
CONSORT	Consolidated Standards of Reporting Trials
CSA	cross-sectional area
CVA	cerebro-vascular accident
CXD	computed x-ray densitometer
DXA	dual energy x-ray absorptiometry
EMG	electromyography
FAC	Functional Ambulation Category
FMA	Fugl-Meyer motor assessment
HWBV	high frequency whole-body-vibration group
IQR	interquartile range
LWBV	low frequency whole-body-vibration group

MAS	Modified Ashworth Scale
MRI	magnetic resonance imaging
NIHSS	National Institute ff Health Stroke Scale
NS	non-significant
PASE	Physical Activity Scale For The Elderly
PQCT	peripheral quantitative computed tomography
PSSI	polar stress strain index
WBV	whole body vibration
WHO	World Health Organization
SD	standard deviation

1. Chapter 1: General Introduction

1.1 Epidemiology of stroke

Stroke, also known as a cerebro-vascular accident (CVA), has been defined by the World Health Organization's MONICA project as "rapidly developing clinical signs of focal or global disturbance of cerebral function lasting more than 24 hours with no apparent non-vascular cause", unless interrupted by surgery or death" [1]. According to the nature of the cerebral lesion, stroke can be classified into three main categories: ischemic stroke (80% of cases), intra-cerebral hemorrhage (16%) and subarachnoid hemorrhage (4%) [2].

Stroke ranks one of the most common life-threatening neurological conditions worldwide. In the United States, the prevalence of stroke was approximately 2.7% [3]. In the United Kingdom, about 130,000 people suffer a stroke each year, and approximately three-fourths of these cases involve population aged over 65 [4]. In developing countries such as Mainland China, the incidence of stroke is approximately 159.9 per 100,000 person-years [5]. More recently, the incidence of stroke in rural China has increased swiftly, particularly among middle-aged adults [6]. Locally in Hong Kong, there were 25,730 new cases of stroke in 2015, which translates to a stroke incidence of 368 per 100,000 person-years [7].

Stroke is one of the leading causes of long-term disability among the elderly, affecting not only the individual who sustains a stroke, but also the society at large. Stroke also imposes a substantial financial burden on the health care system. Currently, the mean cost of ischemic stroke per person, which includes inpatient care, rehabilitation, and follow-up care, is estimated at US$ 140,048 in the United States [8]. The American Heart

Association projects the direct and indirect costs of stroke to increase from US$105.2 billion in 2012 to US$240.7 billion by 2030 [9]. The high economic cost of stroke makes the alleviation of disability in people after stroke of major interest to healthcare providers and researchers.

1.2 Muscle weakness after stroke

Muscle weakness, defined as " the ability to voluntarily generate muscular force"[10], is one of the primary clinical characteristics among people with stroke [11] [12]. Multiple factors may account for the muscle weakness in stroke populations, such as the interruption of descending motor input to the spinal centers, which reduces the ability to voluntarily generate muscular force [13]. Apart from that, physiological changes in the paretic muscles may also contribute to the compromised ability to generate force among individuals with stroke. For example, McComas et al. found that the motor units in the extensor digitorum brevis muscles were decreased by 50% in patients between two and sixth months post-stroke [14]. Muscle atrophy [15], which is a common result of decreased physical activity levels and disuse [16, 17], may also account for the muscle weakness post-stroke. Previous research using dual energy X-ray absorptiometry (DXA) and computerized tomography also provide evidence of decreased lean tissue mass and increased intramuscular fat deposition in the paretic side in stroke survivors, which may partially explain the reduced ability to generate force [18].

Muscle weakness has been associated with reduced peak muscle torque, decrease force development velocity, fast onset of fatigue, and ineffective rate of force development within the context of a task [19, 20]. It has also been shown that muscle weakness has

been strongly correlated with compromised physical functioning and activity limitations among people with stroke [21].

Muscle force is a main source of mechanical strain applied to the bone. Muscle weakness post-stroke could substantially decrease the amount of mechanical loading applied to the bone, leading to bone loss. Therefore, it is not surprising that mounting evidence have shown a close link between muscle strength and bone health in the paretic side among stroke survivors [22-27]. For example, in the paretic upper extremity, a study using a sample of 56 patients with chronic stroke, Pang & Eng [15] found a moderate, positive relationship between total arm areal bone mineral density (aBMD) and the composite muscle strength score of upper extremity on the paretic side (r=0.60). A similar phenomenon was also reported in the paretic lower extremity. The proximal femur aBMD exhibited a significant relationship with isometric knee extension muscle strength (r=0.39) [28]. Many studies using peripheral quantitative computed tomography (pQCT) also disclosed such a muscle-bone link. For example, in the upper limb, the side-to-side difference in cortical thickness of the radius diaphysis has been found to be highly associated with a side-to-side difference in grip strength, accounting for 25.9% of the variance, after controlling for the effects of age, gender, body mass index (BMI) and post-stroke duration [27]. In the lower limb, at the tibial diaphysis and epiphysis, muscle strength has been found to be significantly associated with bone strength index among stroke populations [22, 24].

1.3 Changes in bone health after stroke

Secondary bone loss is another complication after stroke [29, 30]. Numerous studies used DXA to examine bone health among the stroke population. DXA is currently a gold

standard for diagnosing osteoporosis aBMD (aBMD in g/cm^2), is calculated by "dividing the total bone mineral content (BMC) in grams (g) by the projected area of the specified region (cm^2) [29]. The aBMD value of an individual is then compared with a young reference population and a T-score is generated. For example, a T-score of -1.0 denotes that the aBMD value of the individual is one standard deviation below the mean aBMD value of the young reference population. According to the world health organization (WHO) criteria, a T-score between -1.0 and 2.5 is defined as ostepenia, while a T-score at -2.5 or below is defined as osteoporosis [31].

A number of cross-sectional studies using DXA have consistently shown that aBMD was significantly lower on the paretic side than the non-paretic side at various skeletal sites [15, 28, 32-35]. For example, in a group of 63 patients with chronic stroke (mean post-stroke duration=4.1 years), Pang & Eng [15] showed that the total arm BMC and aBMD on the paretic side was significantly lower by 13.8% and 4.5% respectively than that on the non-paretic side. In addition, prospective studies have also reported that the paretic side has significantly more bone loss as stroke recovery progressed compared with the non-paretic side [36-40]. For example, Ramnemark et al. [39] followed a group of 24 patients with acute stroke for one year and showed that the aBMD reduced by 7.6%,17.4%, and 8.6% in the paretic total arm, humerus, and ultra-distal radius, respectively, during the follow-up period. In another DXA study, Jorgensen et al. [36] found that the aBMD value of the paretic proximal femur was reduced by 12% within the first year post-stroke, while the aBMD value of the corresponding skeletal site on the non-paretic side only declined by 5%, clearly demonstrating the pronounced effect of stroke on bone loss.

The main limitation of DXA is its planar nature, as the aBMD is only a two-dimensional bone density measure of a 3-dimensional bone structure [41, 42]. In the past few years, pQCT is a relatively new bone imaging technique which has been used to examine bone health post-stroke. Unlike DXA, pQCT provides a volumetric bone mineral density (vBMD) measure (mg/cm^3). In addition, it has the capacity to perform separate analyses of cortical and trabecular bone and yields valuable information on bone geometric properties. Bone geometry is a main determinant of bone strength and therefore is an important target of research investigations [43, 44]. In the distal end of long bones (i.e., epiphysis), increasing the total cross-sectional area would increase the resistance of the bone against compressive forces [45], and at the mid-shafts of long bone (i.e., disaphysis), if the cortical bone material is distributed further away from the center (i.e. over a greater total area), the bone strength against torsional and bending forces would be increased even if the bone mass and vBMD values remain constant, owing to an increase in the cross-sectional moment of inertia [46].

Numerous cross-sectional studies used pQCT to examine the vBMD and geometry of skeletal sites in patients with stroke [22, 23, 25, 40, 47-51]. For example, Ashe et al. [23] used pQCT in a small sample of 15 patients after chronic stroke, it was found that the cortical bone area, cortical BMC and cortical vBMD were all significantly lower on the paretic side than the non-paretic side by 6%, 8% and 3%, respectively at the radius diaphysis (i.e., 30% site, primarily a cortical bone site). At the distal radius epiphysis (i.e., 4% site, primarily trabecular bone), the total vBMD was significantly lower on the paretic side by 15%. On the other hand, Pang et al. [49] used a larger sample size of 55 patients after chronic stroke and found that the cortical area, cortical BMC, cortical

thickness and the bone strength index were all significantly lower on the paretic side than that on the non-paretic side at the tibial diaphysis (30% site).

While the cross-sectional studies provide some insight into the possible influence of stroke on bone, they could not give information on the real changes in bone outcomes on both sides as stroke recovery progressed, owing to the nature of cross-sectional studies. For example, the significant side-to-side difference in bone outcomes may be attributable to changes on both the paretic and non-paretic sides. Research studies that use a prospective longitudinal design would give a more accurate picture of stroke induced bone changes [24, 40, 52]. For example, Lazoura et al. [40] used pQCT to measure vBMD at the radius diaphysis (20% site) and distal radius epiphysis (4% site) during one-year follow-up period in patients with sub-acute stroke and found that the cortical vBMD was significantly reduced by 4% at the 20% site, and the trabecular vBMD at the 4% site was significantly declined by 14% and 9.3% among male and female subjects with stroke, respectively on the paretic side, whereas, it was also found that the non-paretic side also sustained a significant but less severe reduce in vBMD. In another study [24], Lam et al. found that the bone strength index was significantly reduced by 2.7% on the paretic side in one year, while the non-paretic side displayed no significant reduction in the same parameter.

Compromised bone health, especially reduced bone strength is an important risk factor of fractures, which are much more common in stroke patients than their age-and sex-matched counterparts [53]. Fragility fractures post-stroke can lead to devastating consequence. Feng et al. [54] found that among hip-fracture patients with a stroke history, the mortality rate was significantly higher by 24.8% in stroke patients compared

with those without a previous stroke (10.8%). The prolonged length of hospital stay has also been found to be associated with hip-fractured patients with stroke history [55]. Feng et al. [54] reported that the mean days of hospitalization after hip fracture was significantly increased in stroke patients (mean rank, 963.59) than that in patients without stroke (mean rank, 668.53). Last but not least, fractures can also have a negative impact at the societal level. For example, fractures post-stroke can impose a tremendous financial burden on the health care system [56].

Aside from bone mineral density (BMD) and bone geometric measurements by bone scan, the level of biochemical markers of bone turnover is also widely used to indicate bone health in stroke population [57]. Bone remodeling is a process which involves the removal of mineralized bone by osteoclasts and the formation of bone matrix through the osteoblasts that subsequently become mineralized [58]. Biochemical markers of bone turnover provide useful information on the dynamic process of bone turnover, whereas bone imaging only provides a static measurement of BMD and bone geometry [59]. Another advantage of using biochemical bone markers is that change in the levels of these markers can be observed in a relatively short period of time after intervention [60]. The relationship between bone turnover and BMD has been established in other populations, such as post-menoopausal women [61].

In a study by Paker et al. [57], 106 patients with stroke and 33 age-and sex-matched healthy subjects were evaluated for the serum level of osteocalcin (OC, a bone formation marker) and C telopetide of type 1 collagen (CTx, a bone resorption marker). Each participant also underwent DXA scanning of the proximal hip region on both sides. The results revealed that the proximal femur aBMD values on both sides were negatively

related with the serum OC and CTx levels. When compared with the healthy group, the bone resorption rate was higher in the stroke group whereas the bone formation rates were similar in both groups, indicating that the bone loss detected during the post-stroke period is mainly related to increased bone resorption. In another study, increased bone resorption and its relationship with the decline in proximal femur aBMD in patients with stroke survivors was also demonstrated by Levendoglu et al [62]. In this study, when compared with the control group, the proximal femur aBMD was significantly lower on the paretic side and the serum level of deoxypyridinoline (Dpd, a bone resorption marker) was significantly higher in the stroke group. Additionally, the deoxypyridinoline level, Barthel Index, Motricity Index Leg score, and 25-and 1,25-dihydroxyvitamin D were all significantly associated with the proximal femur BMD, which implied that impaired mobility, low vitamin D, and increased bone resorption rate were critical factors underlying bone loss on the paretic side among stroke survivors [62].

1.4 The muscle-bone unit and clinical relevance in stroke population

According to Wolff's Law, bone tissue adapts itself to the external loads under which it is placed [63]. Frost's mechanostat theory also highlights that bone strength adapts to meet mechanical needs [44]. However, the mechanostat theory makes no presumption about the nature of the mechanical forces resulting in bone strain. Later, Eckhard Schoenau put forward the "muscle-bone" unit theory, which was first applied in development of the skeletal system during childhood and adolescence [64]. In this theory, bone and muscle tissue are considered as one functional unit. The muscle-bone unit concept has now been utilized in bone research on other age groups [65, 66]. It is of particular interest in older people [66, 67] and patient populations who often have deficits

in muscle strength and bone quality [49, 50, 68]. Ashe et al. (2008) showed that leg extensor muscle power was shown to be the most important determinant of bone strength index of the tibial diaphysis in older adults [66]. Muscle cross-sectional area measured at the mid-femoral and mid-tibial sites were also strongly associated with tibial bone strength among middle-aged and older men [67]. An intimate muscle-bone link has also been demonstrated in people with stroke [49, 50, 68]. Using pQCT, Pang et al. found that muscle mass was independently associated with bone strength index of the distal tibial diaphysis among people with chronic stroke, after adjusting for the effects of potential confounders [49]. MacIntyre et al. [68] further showed that tibial bone strength index at the diaphyseal site was significantly associated with calf muscle density in sub-acute stroke patients.

Another important manifestation related to muscle function post-stroke is spasticity, however, the role of spasticity on bone loss in stroke patients is not as clear. For example, Pang et al. [25] found that more severe spasticity was correlated to a greater side-to-side difference in cortical thickness and cortical BMC of the radius epiphysis on the paretic limb (i.e., more compromised bone status on the paretic limb) in people with chronic stroke. In another pQCT study, the same group of researchers also identified spasticity as a significant determinant of bone strength index of the tibial epiphysis on the paretic side in patients with chronic stroke [50]. But not all studies found this, some studies did not find a significant correlation of aBMD with spasticity [15, 28]. The conflicting findings indicate that the relationship between spasticity and secondary bone loss in stroke patients may not be a straightforward one. While spasticity may impair limb function, the tonic muscle activity associated to spasticity may also provide a source of mechanical

loading to the bone and may have a protective effect on bone. The relationship between spasticity and bone properties unquestionably requires further investigations.

1.5 Dynamic mechanical stimulation and bone formation in animal models

It is common belief that mechanical strain is a powerful stimulant of osteogenesis [69]. According to Wolff's Law, bone growth and remodeling occur in response to forces placed upon bone [70, 71]. During physical activity, mechanical forces are applied to the bones through ground reaction forces and by the contractile activity of muscles [72].

Bone adapts more strongly to dynamic loads than static loads [73]. For example, in an animal study by Robling et al [74]. Rats were divided into 3 groups (group1: static loading at 8.5N, group 2: static loading at 17 N, and group3: dynamic loading at 17 N with a frequency of 2 Hz) and each group received the different loading protocol on the ulnae for 10 minutes a day for 2 weeks. It was found that the dynamic loading significantly increased the osteogenic responses on the periosteal and endocortical surfaces, whereas the two static loading protocols had no significant effect on the endocortical bone formation rates, and in fact, it has also been suppressed the periosteal bone formation [74]. Therefore, the evidence from animal studies indicates that dynamic loads enhance osteogensis rather than static loads.

Animal studies have also revealed that the frequency of the mechanical strain has important influence on ostegenesis [75, 76]. One important finding is that osteogenesis can be elicited by mechanical signals with relatively small amplitudes as long as a high strain rate is applied. The positive results obtained from these experiments have led to development of low magnitude, high-frequency whole body vibration (WBV) protocols to promote osteogenesis and changes in bone morphology in animal models, and the

results are quite promising [76-78]. In a mice model, Ozcivici et al. [77] showed that 6 weeks of WBV (90 Hz, 15min/day) led to better outcomes in greater osteogenic marrow stromal cell population, smaller osteoclast surface, greater osteoblast surface and greater trabecular bone volume fraction. These promising results obtained have prompted researchers to explore the use of WBV in humans to modify bone health in patients with compromised mobility or at risk of loss of bone mass/strength for other reasons, such as patients confined to bed, post-menopausal women and stroke patients. The intimate muscle–bone link also suggests the potential of modifying bone turnover by improving muscle function in stroke patients. An intervention such as WBV that provides mechanical loading to the bone tissue as well as augmenting muscle activation is hypothesized to achieve this.

1.6 Whole body vibration and its effect on muscle and bone outcomes in compromised populations including stroke

Whole-body vibration (WBV) has emerged and has gained increasing popularity in clinical practice over the past two decades. There are two major types of WBV platform: (1) vertical (synchronous), in which the whole platform oscillates up and down simultaneously; and (2) side-alternating platforms with reciprocating vertical displacements on the left and right sides of a fulcrum [79]. The vibration signals can be described in the following physical terms (Figure 1-1): (1) frequency (f) (i.e., the number of occurrences of a repeating oscillation per second [unit: Hz]); amplitude (A) (i.e., the maximum displacement from equilibrium [unit: mm]); peak displacement (D) (i.e., the displacement from the lowest to the highest point of the total vibration excursion [unit: mm]); period duration (T) (i.e., the duration of one oscillation cycle [unit:s]) [80].

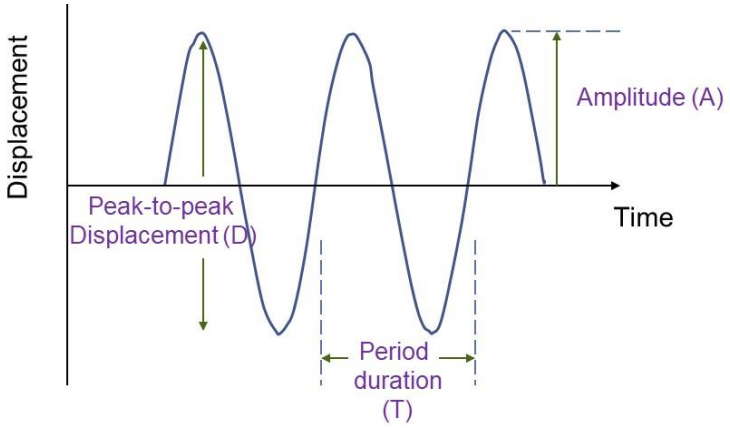


Figure 1-1 Illustration of sinusoidal WBV signal.

The peak acceleration (a_{peak}), which is often used to indicate the WBV intensity, is calculated by the formula:

$$a_{peak}=(2\pi f)^2 A$$

where the peak acceleration represented as peak acceleration (a_{peak}) in multiples of Earth's gravity (symbol: g; $1g=9.81 m/s^2$).

WBV therapy involves intermittent exposure to vibration stimulation while performing a few simple movements such as semi-squats, weight shifting and single-leg standing on a WBV platform [60, 81]. The mechanical signals of varying frequencies and amplitudes are delivered to the person's body from the oscillating platform via the feet, and the resulting mechanical loading may cause various physiological responses [82, 83].

Two studies investigated the effects of WBV on bone health in people subjected to prolonged bed rest. Belavy et al. [84] showed that WBV added to resistance training resulted in better retention of bone mass in the tibial diaphysis and proximal femur than resistance training alone during 60 days of bed rest, and the effects upon bone recovery

were still apparent up to 3 months after the bed rest period. In another study by Wang et al. [85], and it was found that resistive vibration exercise also led to significant reduction in hydroxyproline (a bone resorption marker) and increase in osteocalcin-N (a bone formation marker) following 60 days of bed rest, when compared with the control group.

Human muscles are also very sensitive to vibratory stimuli. It is well known that muscle spindle afferents, particularly the Ia afferents, are known to be highly sensitive to vibration stimuli. Therefore, WBV may elicit muscle contractions by activating the muscle afferents. Mounting evidence showed that muscle activity, as measured by electromyography (EMG), was augmented during WBV [86, 87]. For example, Eckhard et al. [87] showed that the EMG activity of the vastus lateralis was significantly increased when WBV was added during squatting exercise, indicating that WBV augmented the recruitment of muscle tissue during squatting exercise [87]. Roelants et al. [86] also studied muscle activity during different squat exercises with and without WBV. The results revealed that, various exercises (including high squat, low squat, and one-legged squat) with WBV induced significant increased muscle activity in the rectus femoris, vastus lateralis, vastus medialis, and gastrocnemius muscles, when compared with the control condition without WBV. Vibration exercise has been shown to be effective in preserving muscle structure and function in soleus muscle (e.g., increased type I and II myofiber cross-sectional area, increase in activity-dependent expression of nitric oxide synthase type 1 immunofluorescence) after prolonged bed rest [88].

A number of studies have investigated WBV training in older adults and mixed results have been obtained [89-96]. However, the effects of WBV intervention on muscle outcomes are generally positive. A meta-analysis by Lau et al. [97] showed that WBV

induced significant beneficial effect on increasing knee extension dynamic strength, leg extension isometric strength and jumping height and performance in sit-to-stand compared with no intervention among older adults, all with medium effect sizes. Another meta-analysis by Osawa et al. [89] concluded that WBV has significant additional beneficial effects on countermovement jump performance and knee extension muscle strength compared with control conditions without WBV. There is also some evidence that WBV can improve higher-level neuromotor function. A meta-analysis by Lam et al. [98] showed that WBV intervention induced beneficial effects on balance and mobility function, as revealed by a significant improvement in Tinetti Total Score, Tinetti Body Balance Score and Timed-Up-and-Go test. The treatment effects were more apparent for those who are frailer [98]. Overall, WBV may be effective in improving muscle strength, and relatively basic balance ability and mobility among older adults with more compromised neuromotor function. Nevertheless, more good-quality WBV trials are required to establish effective treatment protocols for improving different functional outcomes.

The research on the effects of WBV on bone outcomes has produced promising results in older adults. For example, Zheng et al. [90] showed that the levels of the bone formation marker (osteocalcin) and the bone resorption marker (tartrate-resistant acid phosphatase isoform 5b) significantly reduced after 6 months of WBV training, when compared with the control group, indicating that WBV therapy may induce a decrease in the overall bone turnover rate. Turner et al. [96] also found that the bone resorption marker (N-telopeptide X normalized to creatinine) significantly reduced after 8 weeks of low-frequency, low-magnitude vibration training in post-menopausal women when

compared with sham vibration exposure. A recent meta-analysis by Marin-Cascales et al. [99] showed that WBV was effective in improving lumbar spine BMD in postmenopausal and older women. In addition, WBV was also found to increase femoral neck BMD among postmenopausal women younger than 65 years. However, the results across individual studies demonstrated great heterogeneity. The discrepancies in results across the different studies may be partly explained by the great diversity of the training protocols (i.e. training frequency and duration, vibration frequency and amplitude etc.) adopted and the differences in sample characteristics (age, bone status at baseline, etc.). The optimal protocol for enhancing bone health remains uncertain.

WBV may be a potentially viable method to improve the health status of the muscle-bone unit in stroke patients, who often sustain more extensive muscle weakness and bone loss than the typical elderly population. It is known that leg muscle activity can be significantly enhanced during WBV exposure [100]. However, interventional researches on changes in muscle function and bone tissue after WBV treatment among stroke patients are scarce [60, 101-103]. A systematic review concluded that there is inadequate evidence to support or refute the use of WBV in people after stroke, mainly due to the limited number of studies and lack of high-quality trials [104]. The generalizability of the results is also in question, as most of the previous research had mainly studied patients with mild stroke only.

1.7 Knowledge gaps and rationale of the present study

The possible influence of stroke on bone outcomes has been well studied in a good number of either cross-sectional or longitudinal studies. It would be clinically important to consolidate the knowledge on the characteristics (i.e., magnitude, time course, site-

specific differences) of bone changes post-stroke, and since it would shed light on the proper therapeutic strategies to enhance bone health in individuals with stroke. Additional insights into patient treatment may be gained by reviewing the literature to identify the specific muscle outcomes that are highly associated with bone health post-stroke. Therefore, In Chapter 2 of the thesis, a systematic review was undertaken to examine the bone changes after stroke and their relationship to various muscle outcomes.

According to Schoenau's muscle-bone unit theory [64], muscle and bone are considered as a functional unit. Animal work have revealed that high-frequency dynamic mechanical loads are more potent than static loads in promoting osteogenesis [73, 105]. It is thus postulated that bone quality may be more correlated with the ability to generate muscle forces during dynamic (i.e., concentric, eccentric) contractions at higher velocities, compared with that during static (i.e., isometric) contractions. By studying the relationship between bone quality and muscle strength for different types of contraction (i.e., dynamic Vs isometric) at different contraction velocities would be useful in guiding the design of physical activity or exercise programs for enhancing bone health in individuals with stroke. Therefore, in Chapter 3 of the thesis, a cross-sectional study was conducted to investigate the association of bone strength index at the tibial diaphysis with strength measured during different types of muscle contraction (i.e., isometric, concentric, eccentric) and at different contraction speeds.

To date, research on the effects of different WBV frequencies on the properties of the muscle-bone unit in people with stroke is lacking. Research is also scarce for those with moderate disability after stroke, who are found to have the highest risk of fragility fractures compared with those with mild and severe disability [42]. Therefore, in Chapter

4 of the thesis, a randomized controlled study was undertaken to examine the effects of a high-frequency versus a low-frequency WBV protocol on muscle strength and rate of bone resorption in individuals with chronic stroke.

Overall, the series of the inter-related studies included in this thesis are intended to address the gaps of knowledge in the fields of muscle-bone unit and WBV application in stroke rehabilitation.

1.8 Research objectives and hypotheses

1.8.1 Research objectives

This thesis has the following objectives:

(1) To systematically review the literature concerning bone properties post-stroke and their relationship to muscle outcomes (Chapter 2);

(2) To examine relationship between various aspects of muscle function (e.g., strength measured during different muscle contraction types and speeds) and bone properties in people with chronic stroke (Chapter 3);

(3) To compare the efficacy of different vibration frequencies on muscle strength and bone turnover among people with chronic stroke in the form of a randomized controlled trial (RCT) (Chapter 4);

1.8.2 Research hypotheses

(1) Muscle strength measured during dynamic (concentric and eccentric) contractions at higher speeds would yield stronger correlations with bone strength index measured at the tibial diaphysis compared with those at lower speeds and isometric contractions (corresponding to objective 2);

(2) The higher-frequency WBV protocol would lead to significantly more improvement in leg muscle strength and reduction in rate of bone resorption than the low-frequency WBV protocol in individuals with chronic stroke (corresponding to objective 3).

2 Chapter 2: The impact of stroke on bone properties and muscle-bone relationship: a systematic review

2.1 Abstract

To systematically review available evidence related to the characteristics of bone changes post-stroke and the relationship between various aspects of muscle function (e.g., strength, spasticity) and bone properties after stroke onset. An extensive online database search was undertaken (last search in January 2019). Articles that examined the bone properties in stroke patients were included. The quality of the studies was evaluated with the National Institutes of Health (NIH) Study Quality Assessment Tools. Publication bias of meta-analyses was assessed using the Egger's regression asymmetry test. The selection and evaluation of the articles were conducted by two independent researchers. Fifty-nine studies were identified. In sub-acute and chronic stroke studies, the skeletal sites in the paretic limbs sustained a more pronounced decline in bone quality than their counterparts in the non-paretic limbs. The rate of changes showed a decelerating trend as post-stroke duration increased, but the timing of achieving the steady rate differed across skeletal sites. The magnitude of bone changes in the paretic upper limb was more pronounced than the paretic lower limb. There was a strong relationship between muscle strength/mass and bone density/strength index. Muscle spasticity seemed to have a negative impact on bone integrity in the paretic upper limb, but its influence on bone properties in the paretic lower limb was uncertain. Substantial bone changes in the paretic limbs occurred particular in the first few months after stroke onset. Early intervention, muscle strength training, and long-term management strategies may be important to enhance bone health post-stroke. This review has also revealed the knowledge gaps which should be addressed in future research.

2.2 Introduction

Stroke is one of the most prevalent chronic diseases among older adults [106]. One of the most common complications observed following stroke is secondary hemi-osteoporosis [36, 39, 107]. This area has been largely overlooked in research and clinical practice until the 2000s. Mounting evidence has demonstrated not only substantial reduction in bone mineral density (BMD), but also unfavorable changes in bone geometric properties on the hemi-paretic side after stroke [22-27, 49, 50, 52, 108]. Bone geometry is an important determinant of bone strength [43, 109]. Alterations in both bone mineral density and bone geometry have contributed to an exaggerated risk of fragility fractures in individuals with stroke [53, 110-112]. For example, the relative risk of fractures after hospitalization for stroke is more than seven times the rate of fracture in the age- and sex-matched populations [110]. Fragility fractures can lead to detrimental consequences, including prolonged hospital stay, as well as increased morbidity and mortality [113]. The medial cost related to the treatment of fracture also imposes an economic strain on the health care sector [114]. Thus, it is clinically important to search for proper therapeutic strategies to enhance bone health in individuals with stroke. In order to achieve this, there is a need to consolidate the knowledge on the characteristics (i.e., magnitude, time course, site-specific differences) of bone changes post-stroke.

Another important issue pertinent to post-stroke bone health is related to the muscle-bone link. According to Wolff's Law of transformation of bone, the skeletal system adapts itself to the external loads under which it is placed [63]. Muscle contractions provide a rich source of mechanical loading to bone, which may, in turn, induce bone adaptations. According to the muscle-bone unit theory proposed by Schoenau [64], muscle and bone are considered as a functional unit. Muscle function and integrity of bone tissue may thus be closely linked. After stroke, morphological and functional changes occur in skeletal muscles, including reduced

muscle mass and density [28, 68], intramuscular fat infiltration [115], muscle weakness [116], contracture [117]and spasticity [118]. These changes in muscle characteristics may have important influence on bone tissue.

To date, a comprehensive collation of the impact of stroke on bone properties has not been disseminated. Therefore, the primary objective of this systematic review was to synthesize the literature related to the impact of stroke on bone properties. The secondary objective was to summarize the research evidence on the relationship between muscle function and bone properties in individuals with stroke.

2.3 Methods

2.3.1 Study objectives

We systematically reviewed the literature to address the following questions: (1) What are the characteristics of bone changes in the paretic and non-paretic limbs after the onset of stroke (i.e., magnitude, time course, side-to-side differences, site-specific differences)? (2) Is there a relationship between different aspects of muscle function (e.g., muscle strength, spasticity) and bone properties (e.g., bone mineral density, bone geometry) in individuals with stroke? For the purpose of this review, acute, subacute and chronic stages of stroke were defined as occurring within one month after the onset of stroke, within six months after the onset of stroke, and more than six months after the onset of stroke, respectively. This systematic review was registered in PROSPERO (registration number: CRD42015026828).

2.3.2 Search strategy

The following databases were searched online through the university's library from inception to January 2019: Cochrane, Ovid (Medline, Embase), CINAHL, Scopus and Pubmed. Search terms were based on the participants of interest (e.g. stroke, cerebrovascular accident,

hemiplegia, hemiplegic, brain injury), and the construct of interest (e.g. bone density, bone mineral density, bone mineral content, bone geometry and bone loss). Search terms were truncated in accordance with each database and combined. The specific search strategy for the MEDLINE database is described in Appendix (section 7.4).

2.3.3 Selection criteria

Studies were included if they met the following criteria: (1) adult participants whose primary diagnosis was stroke, and (2) included measures of bone mass or geometry using single or dual photon absorptiometry, dual energy x-ray absorptiometry (DXA), peripheral quantitative computed tomography (PQCT), magnetic resonance imaging (MRI) or ultrasound. Exclusion criteria were: (1) case reports, (2) articles written in languages other than English, (3) grey literature.

2.3.4 Data extraction and quality assessment

Two reviewers independently evaluated the list of potential articles. The titles and abstracts were first reviewed to screen out irrelevant articles. The remaining articles were then read in full to identify the eligible articles. The reference lists of the eligible articles were examined to identify more relevant articles. In addition, a forward search was conducted using Web of Science to obtain the potential relevant articles that had referenced the eligible articles identified using the above search strategy (last searched in February 2019). Any disagreements on article selection were resolved by involving a third reviewer, and consensus was reached after discussion. Reporting quality of the selected articles was assessed using a standardized Study Quality Assessment Tool designed by the National Heart, Lung, and Blood Institute under the National Institutes of Health (NIH) [119]. Two reviewers independently appraised each study for risk of bias, and where disagreements occurred, a consensus was reached through discussion

with the principal investigator. Each study was rated either as good (most methodological criteria met, low risk of bias), fair (some criteria met, moderate risk of bias), or poor (few criteria met, high risk of bias).

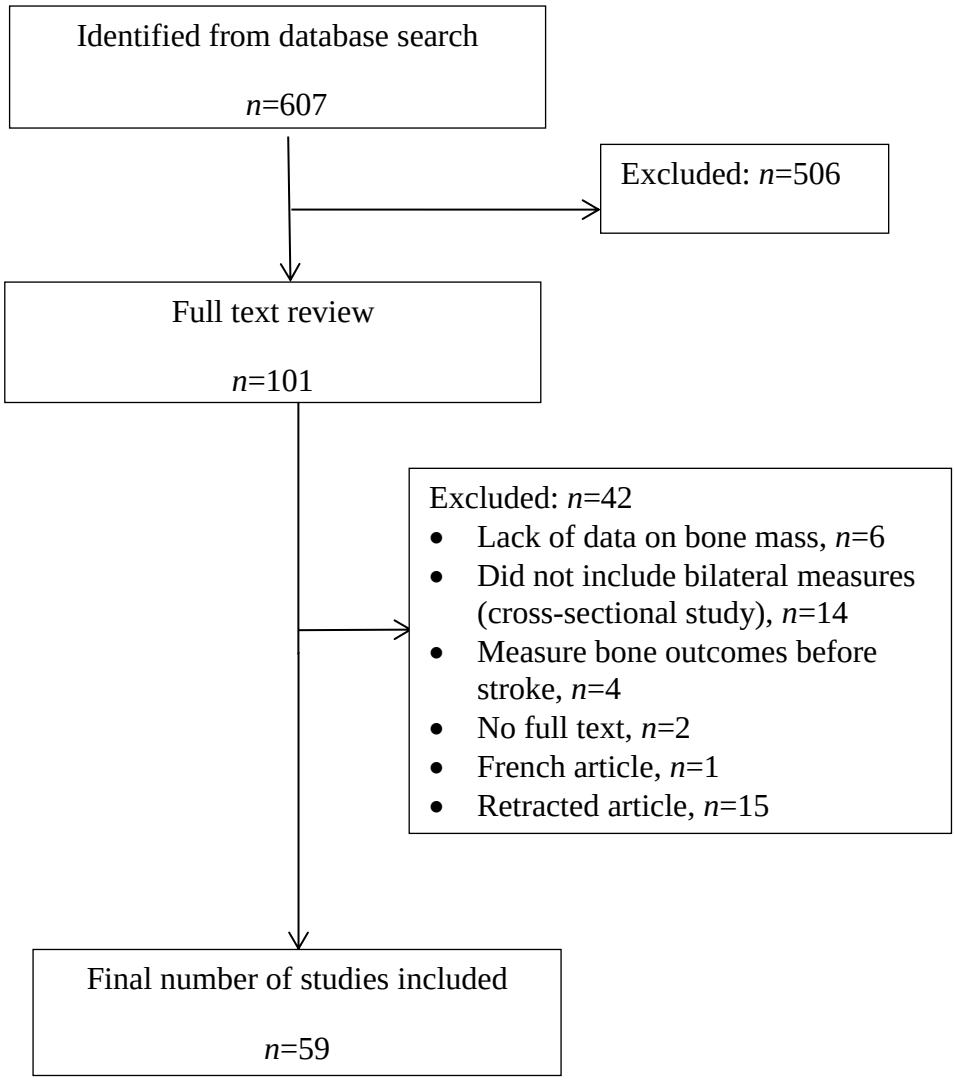
2.3.5 Quantitative analysis

For outcomes that were measured in 4 studies or more, meta-analyses were performed using the review software package *RevMan5 (The Nordic Cochrane Center, Copenhagen, Denmark)*. The generic inverse variance meta-analysis method was used as the data were paired (i.e., difference between the paretic and non-paretic side of the same individuals, or change over time within the same individuals) [120]. This required determining the mean inter-limb difference and the standard error of this difference. The standard error was calculated using the accepted formula [120] and involved imputing an assumed correlation of r=0.9 between the bone mass of the paretic and non-paretic limbs. It is reasonable to assume a high correlation between measures of bone mass taken bilaterally within subjects [121]. Statistical heterogeneity was assessed using the I^2 statistic. Where I^2 >50 %, a sensitivity analysis was performed to determine the source of heterogeneity. To assess publication bias, each meta-analysis was examined using Egger's regression asymmetry test (Comprehensive Meta-analysis version 3, Biostatc, Inc., Englewood, NJ, USA). A p-value of <0.1 (two-tailed test) was indicative of publication bias. Where meta-analyses were not appropriate, results were synthesized narratively.

2.4 Results

Figure 2-1 shows the flowchart of article selection. A total of 607 records were generated by the search strategy used, but only 59 articles fulfilled the criteria for review (intervention studies: n=4; observation studies: n=55). Among the observation studies, a total of 39 articles were cross-sectional, in which measures were taken from the paretic and non-paretic sides at one time point. In the remaining 16 observational studies, measures were taken from the same individuals at several time points relative to stroke onset. Of the measurement tools used, four articles used both DXA and PQCT, and one article used both Pixi densitometer and ultrasound. In the remaining 54 studies, only one measurement tool was used (DXA, *n*=35; pQCT, *n*=13; CXD, *n*=3; Ultrasound, *n*=1; Dual photon absorptiometry, *n*=2).

Figure 2-1. Study flowchart



2.4.1 Side-to-side differences in bone properties (cross-sectional analysis)

The differences in bone properties between the paretic and non-paretic sides can be assessed by examining the data of cross-sectional observational studies, and baseline data of longitudinal observational studies and interventional studies. The results are reported separately below according to the chronicity of stroke (acute, subacute, and chronic).

2.4.1.1 Acute stroke studies

Eleven articles studied bone properties in individuals with acute stroke (722 participants; Table2-1). The mean timing of bone measures relative to stroke ranged from 2 to 17 days [36, 38, 107, 122-125]. The level of stroke severity was reported in 6 studies: (moderate to severe stroke: n=2, moderate stroke: n=4). DXA was used to measure areal bone mineral density (aBMD) and bone mineral content (BMC) in all of these studies [36-38, 107, 122-128], and the skeletal sites measured included: proximal humerus (1 study) [38], total hip (3 studies) [125, 126, 128], femoral neck (5 studies) [36, 37, 124, 126, 127], and total body (3 studies) [107, 122, 123]. PQCT was used in one study to measure the volumetric BMD (vBMD) and bone geometry (7% of distal tibia) [122]. In terms of study quality,three studies were rated as fair [122, 123, 128], and the rest were rated as good [36-38, 107, 124-127]. In all 11 studies, there were no differences found in aBMD, BMC and vBMD between the paretic and non-paretic sides, regardless of the bone imaging techniques and skeletal sites measured.

2.4.1.2 Subacute stroke studies

There were 10 subacute stroke studies [39, 40, 129-136] totaling 523 participants (Table 2-2). The average timing of bone measurements ranged from 1 month to 4.2 months post-stroke onset. Five of those studies reported the level of stroke severity to be moderate to severe [40, 129-132], and five of them moderate [39, 133-136]. DXA was used to measure aBMD and BMC

in 10 studies [39, 40, 129-136], with measurement sites including humerus (2 studies) [39, 134], total arm (1 study) [39], forearm (1 study) [131], radius (4 studies) [39, 132-134], femoral neck (2 studies) [40, 130], femur (3 studies) [132, 134, 136], total femur (1 study) [39], proximal femur (1 study) [39], total hip (1 study) [133], calcaneus (1 study) [134], and total body (4 studies) [129, 133-135]. PQCT was used in one study [40] to measure the volumetric BMD (vBMD) and bone strength index (4 % and 20 % distal radius). Of the 10 studies, five were considered as having fair quality [130-132, 135, 136], and the other five were rated as good [39, 40, 129, 133, 134].

In the upper limbs, aBMD values measured at the proximal humerus (2 studies) [39, 134] and radius (3 studies) [132-134] on the paretic side were found to be significantly lower than the corresponding sites on the non-paretic side by 4.1-11.6% and 1.4-11.1% respectively. The trabecular vBMD and bone strength index of the 4 % distal radius (1 study) derived from pQCT were significantly lower than the corresponding sites on the paretic side by 8.8 % and 11.5 %, respectively [40]. At the 20 % distal radius (1 study), the side-to-side difference in cortical vBMD and bone strength index was much smaller (by 1.3 % and 1.4 % respectively) [40].

In the lower limbs, aBMD values measured at the femur (3 studies) [132, 134, 136] and calcaneus (1 study) [134] on the paretic side were found to be significantly lower than the corresponding sites on the non-paretic side by 2.1-4.1% and 1.8% respectively.

2.4.1.3 Chronic stroke studies

There were 37 chronic stroke studies [15, 22-28, 32-35, 47-52, 57, 62, 121, 137-152] totaling 1902 participants (Table2-3). The mean timing of bone measures ranged from 0.5 years [47] to 13.5 years [141] after stroke onset. Only 15 studies reported the level of stroke severity, which was generally moderate [15, 22-25, 34, 57, 62, 121, 138, 140, 146, 147, 149, 150]. The

bone measurement techniques used included DXA [15, 24, 28, 34, 35, 57, 62, 121, 137, 139-142, 144-146, 151, 152] (18 studies), CXD [147, 149, 150] (3 studies), PQCT [22-27, 47-52, 138, 141] (14 studies), dual photon absorptiometry (2 studies) [32, 33], Pixi densitometer and quantitative ultrasound (1 study) [143], and Lunar Achilles Plus ultrasound densitometer (1 study) [148]. Eight of the studies were considered to have good quality [23, 25, 48-50, 140, 144, 145], and 29 were rated as fair [15, 22, 24, 26-28, 32-35, 47, 51, 52, 57, 62, 121, 137-139, 141-143, 146-152].

In the upper limbs, the aBMD values measured at the total arm (2 studies) [32, 145] and second metacarpal [147] was significantly lower on the paretic side than that on the non-paretic side by 4.5-8 %, and 4.5% respectively.

At the 4 % distal radius (i.e., distal radius epiphysis), the total vBMD, BMC, and bone strength index were significantly lower on the paretic side than that on the non-paretic side by 9.7-18.8 %, 9.5-18.0 %, and 15.4-31.3 %, respectively, whereas the total area consistently showed no significant differences between sides (4 studies) [23, 26, 27, 51]. The meta-analysis showed that (4 studies, 131 individuals) [23, 26, 27, 51] the BMC and total vBMD on the paretic side was significantly lower than that on the non-paretic side by 12.58 mg/mm (Fig. 2-2A) and 40.83 mg/cm^3 (Fig. 2-2B), respectively, in individuals whose stroke onset was at least 12 months.

At the 30 % or 33 % distal radius (i.e., radius diaphysis), the meta-analysis showed that (4 studies, 147 individuals) [23, 25, 27, 52] the cortical vBMD and cortical area on the paretic side was significantly lower than that on the non-paretic side by 23.74 mg/cm^3 (Fig. 2-3A) and 5.7 mm^2 (Fig. 2-3B), respectively, in individuals whose stroke onset was at least 12 months. However, there was a significant publication bias in the cortical vBMD analysis (Egger's

regression asymmetry test, p=0.031). There was a trend for the bone strength index at radius diaphysis to be lower on the paretic side among individuals whose stroke onset was at least 12 months (Fig. 2-3C) but it did not reach statistical significance (*p*=0.09).

In the lower limbs, six studies [35, 57, 141, 142, 151, 152] demonstrated a significant side-to-side difference in femoral neck aBMD (by 2.2-16.1 %). The meta-analysis (6 studies, 400 individuals) [35, 57, 141, 142, 151, 152] revealed a significantly lower femoral neck aBMD by an average of 0.04 g/cm^2 in the paretic compared with the non-paretic limb in individuals who had sustained a stroke for at least 6 months prior (Fig. 2-4).

At the 4 % tibial epiphysis, the total vBMD, trabecular vBMD, and bone strength index on the paretic side were significantly lower than their counterparts on the non-paretic side (6 studies) by 3.2-19.0 %, 2.8-4.7 %, and 6.6-31.0 %, respectively [22, 24, 50, 51, 138, 141]. The meta-analysis revealed (4 studies) [22, 24, 50, 138] similar findings among individuals who had suffered the stroke for 12 months or more (Fig.2-5A-C).

At the 50 % or 66 % tibial diaphysis, differences between cortical vBMD and bone strength index in paretic and non-paretic limbs were not consistent across studies. In 3 studies, the cortical vBMD was significantly lower on the paretic side than that on the non-paretic side by 1.6-2.2 % [22, 24, 138] but not in other 4 studies [48, 49, 51, 141]. Significant side-to-side differences in the bone strength index (4.3-10.3 %) was found in 4 [22, 24, 138, 141] out of 6 [22, 24, 48, 51, 138, 141] studies.

At the tibial diaphysis (66% site), the meta-analysis showed that the bone strength index of the paretic side was significantly lower than the non-paretic side by 304.11 mm^3 among individuals whose stroke onset was at 12 months ago or longer [22, 24, 68, 138, 141] (Fig. 2-6),

but a publication bias was found (Egger's regression asymmetry test, p=0.086), and the heterogeneity of this analysis was high (I^2=59 %).

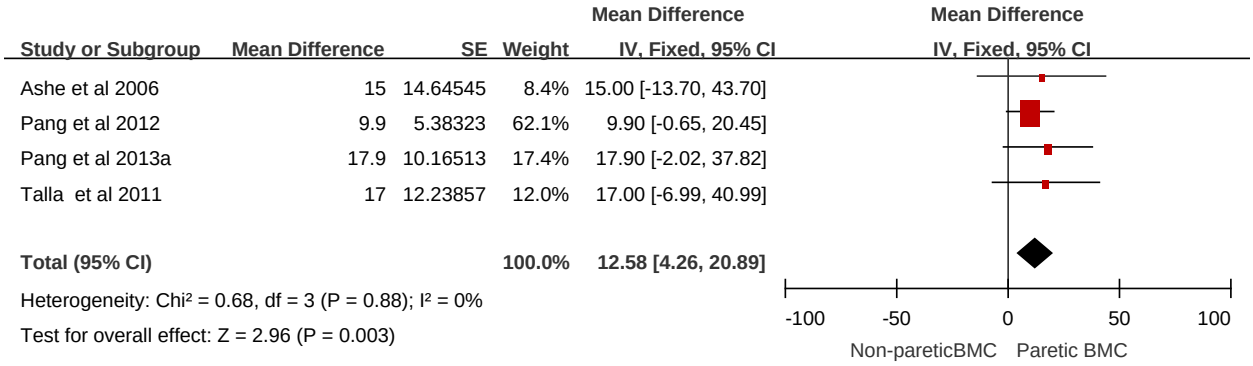
The amplitude-dependent speed of sound measured by quantitative ultrasound was significantly lower in the paretic os calcis than the non-paretic site by 2.5 % (1 study) [143], but there was no side-to-side difference in the index stiffness measured by a lunar Achilles Plus ultrasound densitometer (1 study) [148].

2.4.1.4 Mixed subacute and chronic stroke studies

One study used a mixed sample of subacute and chronic stroke patients [68] (22 participants; Table2-4). The average timing of bone measurements was 3.2 months for subacute participants and 60 months for chronic participants, respectively. This study did not report the level of stroke severity. PQCT was used to measure the volumetric BMD (vBMD) and bone strength index (66 % tibial). This study was rated as fair. Using pQCT, the side-to-side differences in bone variables measured at the tibial diaphysis were largely unremarkable, with only a small but significant side-to-side difference (1.5%) in bone strength index.

Figure 2-2. Meta-analysis: side-to-side differences in bone parameters at the 4% radius site

A. Bone mineral content (BMC in mg/mm)



B. Total volumetric bone mineral density (vBMD in mg/cm^3)

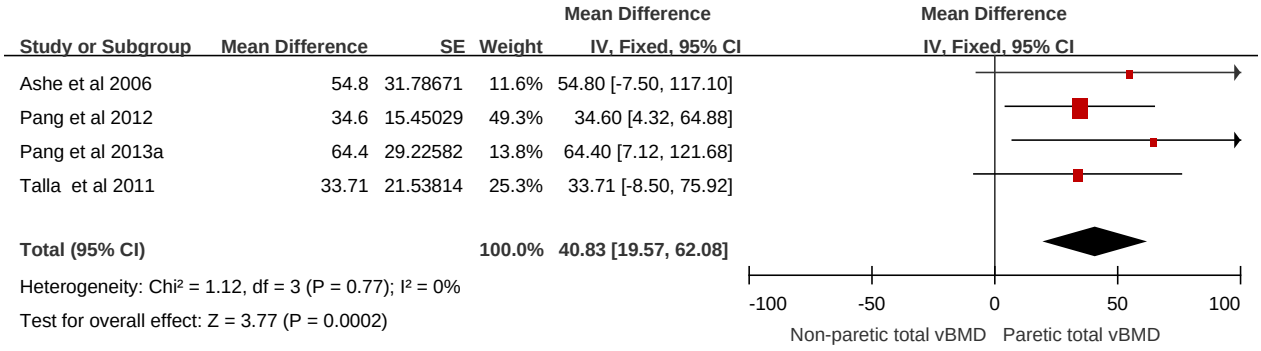
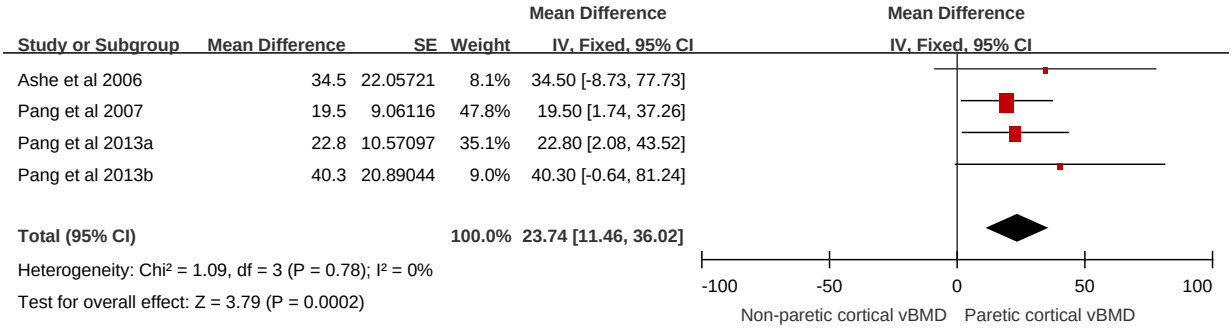
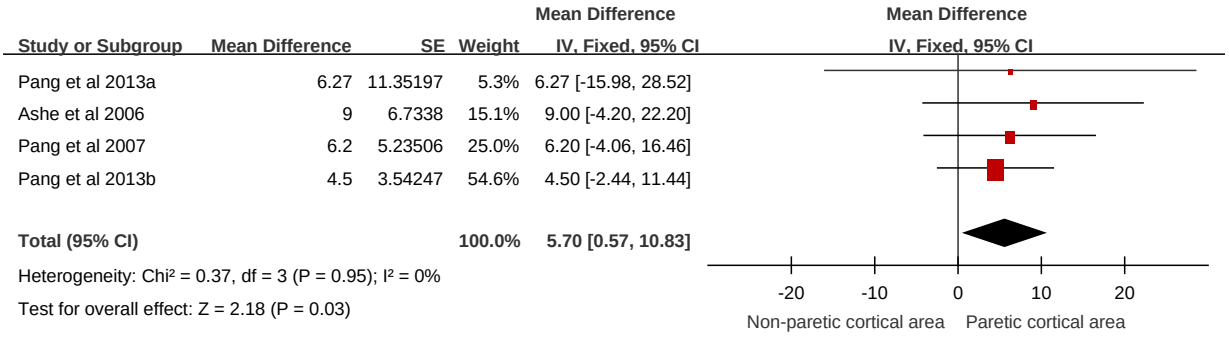


Figure 2-3. Meta-analysis: side-to-side difference in bone variables at the radius diaphysis

A. Cortical volumetric bone mineral density (vBMD in mg/cm^3)



B. Cortical area (mm^2)



C. Polar stress-strain index (p-SSI, mm^3)

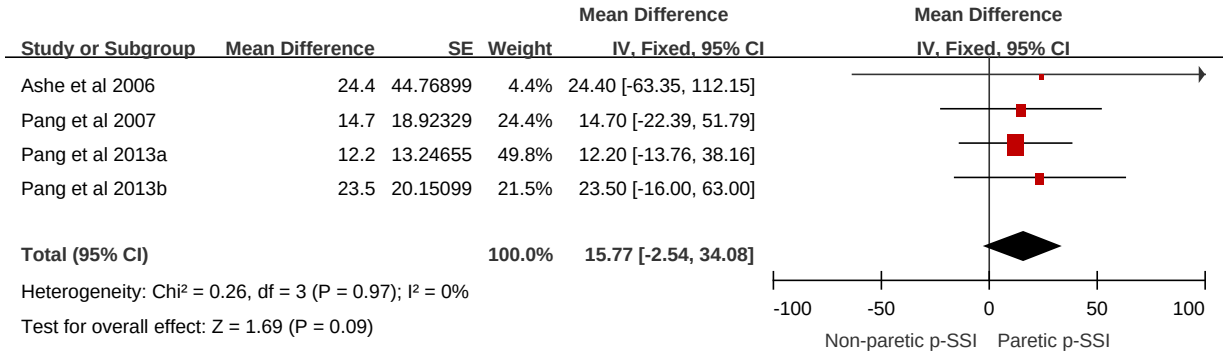


Figure 2-4. Meta-analysis: side-to-side difference in femoral neck areal bone mineral density (g/cm^2)

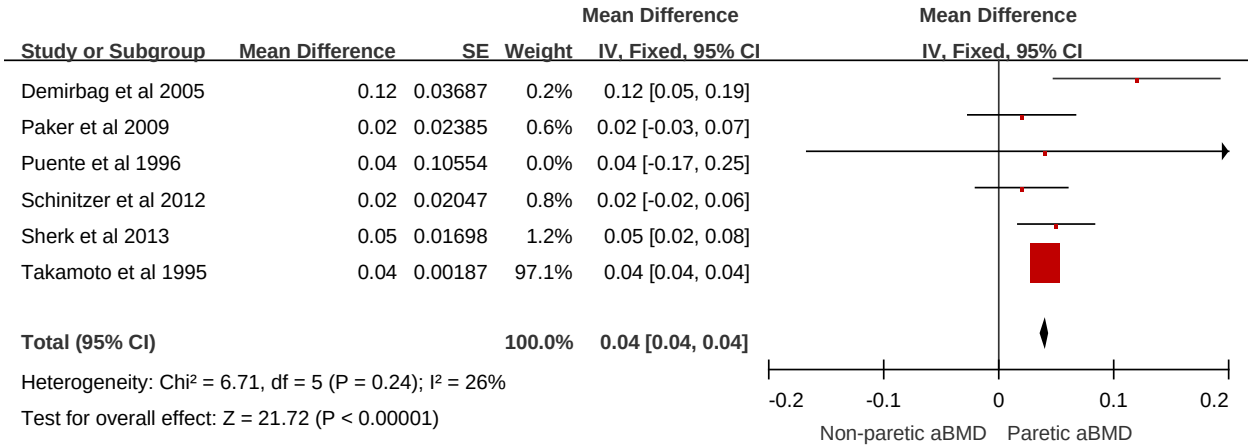
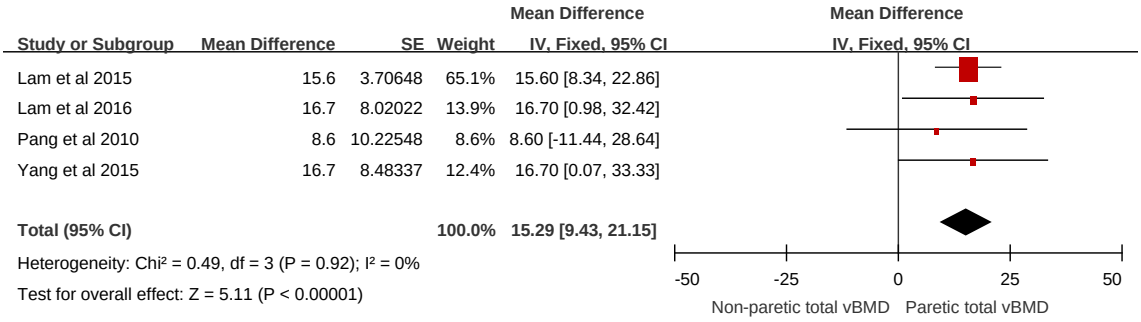
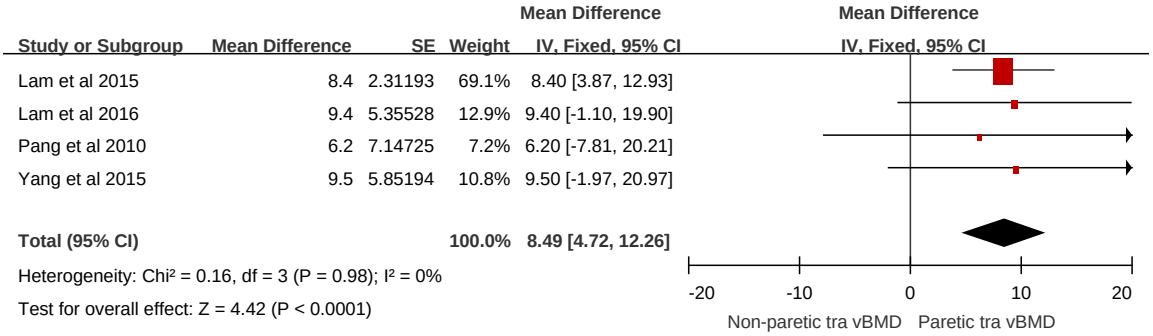


Figure 2-5. Meta-analysis: side-to-side difference in bone variables of the tibial epiphysis

A. Total volumetric bone mineral density (vBMD)(mg/cm^3)



B. Trabecular volumetric bone mineral density (vBMD)(mg/cm^3)



C. Bone strength index (BSI, g^2/cm^4)

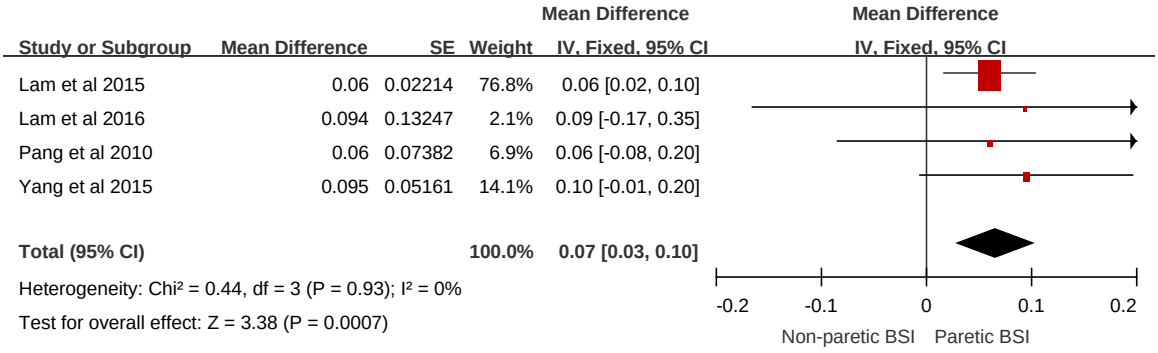
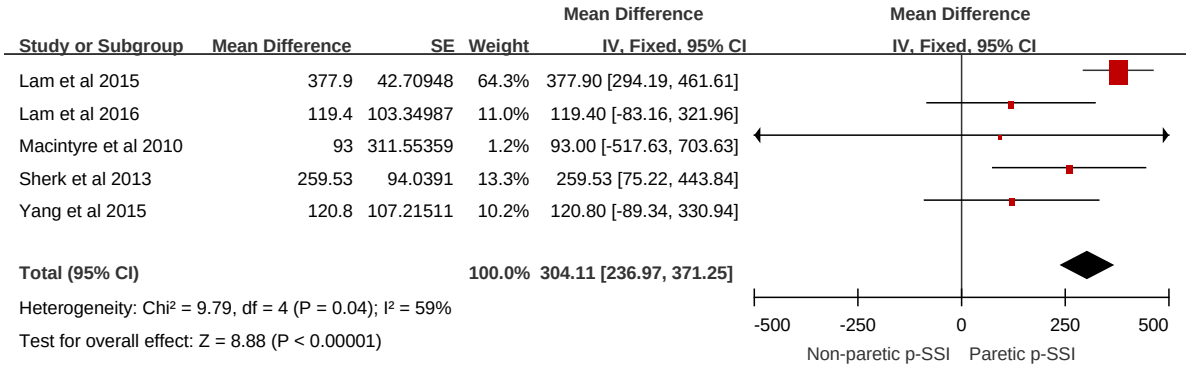


Figure 2-6. Side-to-side difference in polar stress-strain index (p-SSI, mm^3) at the tibial diaphysis



2.4.2 Actual bone changes over time (analysis of longitudinal data)

The actual changes in bone properties over time were assessed by comparing the baseline and follow-up data provided by the longitudinal observational studies.

Sixteen longitudinal studies [24, 36-40, 52, 107, 122, 123, 129, 130, 132, 134, 135, 137] (592 participants) examined bone changes during a follow-up period (Table 2-5). Only 8 studies reported the level of stroke severity, the overall level was moderate [24, 38, 39, 122, 130, 132, 134, 135]. The bone measurement techniques involved DXA (15 studies) [24, 36-40, 107, 122, 123, 129, 130, 132, 134, 135, 137] and PQCT (4 studies) [24, 40, 52, 122]. Seven of those studies were rated as fair [24, 122, 123, 130, 132, 135, 137], and 9 were rated as good [36-40, 52, 107, 129, 134].

In the upper limbs, there was no significant reduction in humerus aBMD on both sides during a period between the 1- and 4-month post-stroke onset. However, with a longer follow-up period (from 1 to 7 months or from 1 to 12 months post-stroke), there was a significant reduction in humerus aBMD only on the paretic side by 7.4% and 12%-17.4%, respectively [39, 134]. The non-paretic side showed no significant reduction in the same variable [39]. At the distal radius, the rate of reduction in aBMD on the paretic side was 1.8% (average duration of follow-up: 105.5 days; average time of first measurement post-stoke: 83 days) [134], and 12.4% (average duration of follow-up: 98 days; average time of first measurement post-stoke: 63 days) [132], whereas the non-paretic side showed only a significant reduction by 3.5% in the same variable [132].

At the 4% distal radius epiphysis, there was a significant reduction in the bone strength index on the paretic side by 25.6 % during a 1-year follow-up period in patients with subacute stroke (time of first measurement post-stoke: 3 months) [40] . However, the same variable showed less

reduction (by 6.7 %) in one year among patients with long-standing stroke (average time of first measurement post-stoke: 45 months) [52] . At the 20 % or 33 % distal radius (radius diaphysis), a similar phenomenon occurred. One study [40] reported a significant reduction in cortical vBMD (3.3 % on the paretic side, 1.5 % on the non-paretic side) and bone strength index (7.2 % on the paretic side, 5.6 % on the non-paretic side) during the 1-year follow-up period among subacute stroke patients, but the changes in these variables were not significant among chronic stroke cases (average time of first measurement post-stoke: 45 months) [52].

For the femoral neck and trochanter regions, the majority of studies showed a significant decline in aBMD within the first year post-stroke (1-year reduction in aBMD in femoral neck region: paretic side: 10-13%, non-paretic side: 5-10.9%; trochanter region: paretic side: 10-12.6%, non-paretic side: 5-10.9%), with most of the changes occurring during the first 6-7 months post-stroke (femoral neck region: 8-9% on paretic side, 2-8% on non-paretic side; trochanter region: 7-8% on the paretic side, 0-7.8% on non-paretic side) [37, 40, 130, 132].

Bone changes were less substantial in those whose stroke onset is more than 1 year. For example, for the total hip aBMD, Lam et al. [24] showed that chronic stroke patients exhibited a significant reduction by only 1.2 % on the paretic side in one year, while the non-paretic side displayed no significant reduction in the same parameter (average time of first measurement post-stoke: 48 months). However, the results may have differed across various bone sites. For example, at the 4 % tibial epiphysis, there was a significant reduction in the bone strength index by 2.7 % on the paretic side during the 1-year follow-up period among chronic stroke patients (average time of first measurement: 48 months post-stroke) but the 66 % tibial diaphysis revealed no significant reduction in bone strength index during the same period [24].

2.4.3 Muscle-bone relationship

The results regarding the muscle-bone relationship are illustrated in Table 2-6. The relationship between bone properties and muscle mass/strength was explored in 3 DXA studies [15, 28, 123] and 9 pQCT studies [22-27, 49, 50, 68]. In the upper limb, the total arm BMC and total arm aBMD were significantly correlated with the composite arm muscle strength score (r=0.60-0.62) [15, 123] and arm lean mass (r=0.86) [15]. At the 4 % radial epiphysis, grip strength had a significant relationship with bone strength index (r=0.69) [26]. The strong relationship between muscle strength and bone strength index at the 33% radial diaphysis was also quite consistent (r=0.71-0.85) [23, 27].

In the lower limb, the proximal femur BMC and aBMD presented a significant relationship with isometric knee extension muscle strength (r=0.41 and r=0.39, respectively) and leg lean mass (r=0.78 and r=0.61, respectively) [28]. At the 4 % tibial epiphysis, there was a significant relationship between muscle strength/mass and bone strength index/BMD in two out of three studies (r=0.45-0.73) [22, 24, 50]. At the 66 % tibial diaphysis, there was a significant relationship between bone strength index and eccentric knee extensor muscle strength (r=0.45) but not concentric knee extensor muscle strength [22].

The relationship between bone properties and muscle spasticity was assessed in 3 DXA studies [15, 28, 107] and 7 pQCT studies [22, 24-27, 49, 50]. In the upper limb, one study [15] revealed that total arm BMC and total arm aBMD had no significant relationship with spasticity as measured by the Modified Ashworth Scale (MAS) (r=0.197, and r=0.068 respectively). At the 4 % radial epiphysis, spasticity had a significant relationship with bone strength index (r=0.465) [26]. However, the relationship between spasticity and bone strength index at the radius diaphysis was not clear as some work showed a significant association (r=0.356) [27], while other work found no relationship [25]. In the lower limb, the results were inconsistent. Some

studies showed a significant correlation between MAS and proximal femur aBMD (r=-0.21, and r=-0.23 respectively) [28] and 4% tibial epiphysis (r=0.415) [50], other studies found no such relationship [22, 24, 49]. At the 66 % tibial diaphysis, one study found no significant relationship between bone strength index and spasticity [22].

Table 2-1. Side-to-side differences in bone parameters: cross-sectional studies and baseline data of longitudinal studies (acute stroke subjects)

Study	n	Age (years)	Severity of stroke	Timing of measures relative to stroke (days)	Measurement tool	Outcomes	Findings: Paretic vs Non-paretic limb	Study quality
Borschmann et al 2018[122]	37	69.7±11.6	NIHSS:12.6±4.7	⩽14	DXA	Total body BMC and Total body lean mass	Osteoporosis: *n*=1 Osteopenia: *n*=7 Healthy: *n*=21	Fair
						Total leg BMC	NS	
						Total leg lean mass	NS	
					PQCT	7% of distal tibia		
						Total vBMD	NS	
						Total bone mass	NS	
						Cort bone mass	NS	
						Trab bone mass	NS	
						Cort area	NS	
						Trab area	NS	
Kim et al 2016[128]	155	68.3±10.34	Not reported	⩽7	DXA	Total hip and lumbar spine BMD	Osteoporosis: *n*=31 Osteopenia: *n*=62	Fair

							Healthy: *n*=62	
Lee et al 2013[127]	191	69.8±11.1	NIHSS, median(interquartile range): 5.0(3-7):82 2.0(1-3):109	2.4±2.3	DXA	Femoral neck and lumbar spine BMD	Osteoporosis: *n*=86	Good
Kim et al 2008[126]	48	64.8±8.5(male,n=20) 68.9±8.7(female,n=28)	MBI: Male: 33.7±19.5 Female: 24.0±16.0	17±8	DXA	Total hip BMD	NS	Good
						Femoral neck BMD	NS	
Poole et al 2007[125]	31	70.8±10.6	Stroke severity scale,/48: 25.3±6.8	acute stroke, details not reported	DXA	Total hip BMD	NS	Good
Jørgensen et al 2001[107]	28	Not reported	Not reported	7±4	DXA	Total leg BMC	NS	Good
Jørgensen et al 2001[124]	63	75±8(male) 77±8(female)	Not reported	6±4	DXA	Femoral neck BMD	NS	Good
Jørgensen et al 2001[38]	28	Not reported	Scandinavian stroke scale arm score: 2 (range 0-5)	7±4	DXA	Proximal humerus BMD	NS	Good
Jørgensen et al 2000[37]	65	76±8	Not reported	6±4	DXA	Femoral neck	NS	Good
Jørgensen et al	65	76±8	FAC: FAC1:29	6±4	DXA	Femoral neck and	NS	Good

2000[36]			FAC2-6:36			trochanter BMD		
Hamdy et al 1995[123]	11	Not reported	Not reported	<7 days	DXA	Upper limb BMC Lower limb BMC	NS	Fair

BMC: bone mineral content; BMD: bone mineral density; Cort: cortical; DXA: dual-energy x-ray absorptiometry; FAC: functional ambulation category; MBI: modified bath index; NIHSS: national institute of health stroke scale; NS: non-significant; PQCT; peripheral quantitative computed tomography; Trab: trabecular; vBMD: volumetric bone mineral density.

Table2-2. Side-to-side differences in bone parameters: cross-sectional studies and baseline data of longitudinal studies (subacute stroke subjects)

Study	n	Age (years)	Severity of stroke	Timing of measures relative to stroke	Measurement tool	Outcomes	Report finds: Paretic vs Non-paretic limb	Study quality
Chang et al 2014[130]	30	65.9±9.2	Brunnstrom stage: Stage≤3:18 Stage≥4:12	2.3±2.2 months	DXA	Femoral neck BMD	NS	Fair
Lazoura et al 2010[129]	58	65.7(male) 62.3(female)	FAC: 1:52.7% in male,54.5% in female; 2-3:47.3% in male,45.5% in female	3 months	DXA	Total BMD of lower limb	NS	Good
Lazoura O et al 2008[40]	67	61.4±9.44(male) 64.75±9.31(female)	FAC: 1:53.5% in male,58.4% in female; 2-3:46.5% in male,41.6% in female	3 months	DXA	Femoral neck BMD	NS	Good
						Trochanter BMD	NS	
					PQCT	4% distal radius Trab vBMD	-8.8 %	
						4% distal radius p-SSI	-11.5 %	
						20% distal radius Cort vBMD	-1.3 %	
						20% distal radius p-SSI	-1.4 %	
Watanabe et al	83	65.7±1.5		43.1±27 (range: 14-113) days	DXA	Total hip BMD	Osteoporosis:32 Osteopenia: 40	Good

2004[133]			Mean Brunnstrom stage: Upper limb: 3.7±1.9 Hand: 3.6±2.1 Lower limb: 4.0±1.8				Normal: 11	
						Lumbar spine (L2-4)BMD	Osteoporosis: *n*=20 Osteopenia: *n*=31 Healthy: *n*=30	
						Distal radius BMD	-11.1 %	
Yavuzer et al 2002[132]	32	62.4±8.1	Brunnstrom stage: 2.1±1.2 (range 1-3)	63 days (median)	DXA	Femoral BMD	-2.1 %	Fair
						Distal radius BMD	-11.1 %	
Kumar et al 2001[131]	20	57.2 (range 45-75)	Canadian neurological score: Upper limb: 0.85(0.56)	4.15 months (range2-12)	DXA	Forearm BMD	NS	Fair
Ikai et al 2001[136]	81	67±8.9(control group) 66±8.1(treatment group)	Brunnstrom stage (lower limb): 3.5±1.4	2.6±1.3 months	DXA	Femoral BMD	-4.1%	Fair
Ramnemark et al 1999[39]	24	72.7±4.7	Motricity index: Paretic arm: 20±22/100 Paretic leg:34±35/100	1 month	DXA	Total arm BMD	-4.8 %*	Good
						Humerus BMD	-4.1 %*	
						Ultradistal radius BMD	NS	
						Total femur	NS	
						Proximal femur	NS	
Ramnemark et al	24	72.7±4.7	Motricity index:	1 month	DXA	Arm and leg BMC	NS	Fair

1999[135]			Paretic arm: 20±22/100 Paretic leg:34±35/100					
Liu et al 1999[134]	104	56.5±13.2	Stroke impairment assessment set: Upper extremity total: 2.9(2.6) Lower extremity total: 6.8(3.7)	116.2±56.8 (median 104) days	DXA	Raidus BMD	-1.4 %*	Good
						Humerus BMD	-11.6 %*	
						Upere limb BMD	-4.3 %*	
						Femur BMD	-2.3 %*	
						Calcaneus BMD	-1.8 %*	
						Lower limb BMD	NS	

BMC: bone mineral content; BMD: bone mineral density; Cort: cortical; DXA: dual-energy x-ray absorptiometry; FAC: functional ambulation category; NS: non-significant; PQCT; peripheral quantitative computed tomography; p-SSI: polar stress strain index; Trab: trabecular; vBMD: volumetric bone mineral density; -: decline on paretic side versus non-paretic side; *: statistically significant.

Table 2-3. Side-to-side differences in bone parameters: cross-sectional studies and baseline data of longitudinal studies (chronic stroke subjects)

Study	n	Age (years)	Severity of stroke	Timing of measures relative to stroke	Measurement tool	Outcomes	Findings: Paretic vs Non-paretic limb	Study quality
Tomasević-Todorović et al 2016[137]	40	66.5±9.8	Not reported	1.5±0.4 years	DXA	Femur total and Femoral neck BMD	NS	Fair
Lam et al 2016[138]	74	57.8±10.7	CMSA leg motor score:7.6(2.0)	5.1±4.0 years	PQCT	4%tibia:		Fair
						Total BMC	-6.5 %*	
						Total vBMD	-6.2 %*	
						Trab vBMD	-4.4 %*	
						Total area	NS	
						CBSI	-11.4 % *	
						66% tibia:		
						Cort BMC	-5.8 %*	
						Cort vBMD	-1.6 %*	
						Total area	NS	
						Cort bone area	-4.6 %*	
						Cort thickness	-3.3 %*	
						p-SSI	-4.9 %*	
Feng et al 2016[139]	61	64.3(male)	Not reported	⩾1 years	DXA	Upper limb BMC	-16.6%*	Fair

		65.8(female)				Lower limb BMC	-8.5%*	
Lam et al 2016[24]	20	63.2±1.8	CMSA leg motor (median(IQR)):4.5(4-6)	Mean: 49 months(range:12-166)	DXA	Total hip BMD	-3.6 %*	Fair
					PQCT	4%tibia:		
						Total BMC	NS	
						Total vBMD	-5.9 %*	
						Trab vBMD	-4.0 %*	
						Total CSA	2.1 %*	
						CBSI	-7.8 % *	
						66% tibia:		
						Cort BMC	-6.8 %*	
						Cort vBMD	-2.2 %*	
						Total CSA	NS	
						Cort CSA	-5.3 %*	
						Medullary CSA	NS	
						p-SSI	-6.1 %*	
Yang et al 2015[22]	66	58.5±9.9	CMSA leg motor score:7.6(2.0)	5.0±4.0 years	PQCT	4%tibia:		Fair
						Total vBMD	-6.3 %*	
						Trab vBMD	-4.7 %*	

						Total area	NS	
						CBSI	-11.6 % *	
						66% tibia:		
						Cort BMC	-5.5 %*	
						Cort vBMD	-1.6 %*	
						Total area	NS	
						Cort bone area	-4.4 %*	
						Marrow cavity area	NS	
						Cort thickness	-3.1 %*	
						p-SSI	-5.0 %*	
Marzolini et al 2014[140]	43	62.4±13.5	CMSA foot (range): 3.9±1.7 (1-7) CMSA leg (range): 4.9±0.9 (3-6)	17.9±32.8 months	DXA	Hip BMD	Osteoporosis or Osteopenia: *n*=15 Healthy: *n*=28	Good
Sherk et al 2013[141]	9	64.2±1.9	Not reported	13.5 years(4.4)	DXA	Leg BMC	-15.1 %*	Fair
						Total hip BMC	-10.1 %*	
						Femoral neck BMC	NS	
						Trochanter BMC	-14.3 %*	
						Total hip BMD	-9.3 %*	

						Femoral neck BMD	-6.0 %*	
						Trochanter BMD	-9.9 %*	
					PQCT	4%tibia:		
						Total BMC	-21 %*	
						Trab BMC	-22 %*	
						Total vBMD	-19 %*	
						Trab vBMD	-4.0 %*	
						Total area	NS	
						Trab area	NS	
						BSI	-31 % *	
						38% tibia:		
						Total BMC	-5.9 %*	
						Cort BMC	-7.5 %*	
						Total vBMD	-6.0 %*	
						Cort vBMD	-2.1 %*	
						Total area	NS	
						Cort area	NS	
						Endo_C	7.1 %*	
						Peri_C	NS	
						Cort thickness	-8.0 %*	
						SSI	NS	
						I_{max}	NS	
						I_{min}	NS	
						I_{max}/I_{min}	NS	

						SSI/BMC	NS	
						Total BMC4%/38%	-15.9 %*	
						66%tibia		
						Total BMC	-10.6 %*	
						Cort BMC	-10.4 %*	
						Total vBMD	-14.4 %	
						Cort vBMD	NS	
						Total area	NS	
						Cort area	-15.8 %*	
						Endo_C	6.4 %*	
						Peri_C	NS	
						Cort thickness	-16.0 %*	
						SSI	-10.3 %*	
						I_{max}	NS	
						I_{min}	-30.6 %*	
						I_{max}/I_{min}	38.8 %*	
						SSI/BMC	NS	
						Total BMC4%/66%	NS	
Pang et al 2013[27]	65	60.1±10.7	Not reported	47.8±46.0 months	PQCT	33% of the distal radius		Fair
						Cort BMC	-7.0 %*	
						Cort vBMD	-1.9 %*	
						Total area	NS	
						Cort bone area	-5.8 %*	

						Marrow cavity area	9.8 %*	
						Cort thickness	-6.7 %*	
						p-SSI	-5.1 %*	
Pang et al 2013[52]	28	62.6±9	Not reported	45.6±42.8months	PQCT	4% radius		Fair
						Total BMC	-18.0 %*	
						Total vBMD	-18.8 %*	
						Trab vBMD	NS	
						Total area	NS	
						CBSI	-31.3 %*	
						33% radius		
						Cort BMC	-16.0 %*	
						Cort vBMD	-3.4 %*	
						Total area	NS	
						Cort area	-13.5 %*	
						Cort thickness	-15 %*	
						p-SSI	-11.4 %*	
Pang et al 2012[26]	65	60.1±10.7	Not reported	47.8±46.0 months	PQCT	4% of the distal radius		Fair
						Total BMC	-9.5 %*	
						Total vBMD	-9.7 %*	
						Trab vBMD	-10.3 %*	
						Total area	NS	
						CBSI	-15.4 %*	

Schnitzer et al 2012[142]	87	58.2±11.8	Not reported	7.8±7.9 years	DXA	Total hip BMD	-4.3 %*	Fair
						Femoral neck BMD	-2.7 %*	
Pietraszkiewicz et al 2011[143]	71	64.5±8.3(male) 60.9±11.1(female)	Not reported	32.4±43.5 months (range 1-120 months)	Pixi densitometer	Forearm BMD	-13.1 %*	Fair
					Pixi densitometer	Calcaneus BMD	NS	
					Quantitative ultrasound	Amplitude dependent speed of sound (m/s)	-2.5 %*	
Talla et al 2011[51]	23	68±11	Not reported	5.4±3.2 years	PQCT	4% radius		Fair
						BMC	-13.5 %*	
						Total vBMD	-11.3%*	
						Trab vBMD	-11.3 %*	
						Total CSA	NS	
						66 radius		
						BMC	-10.8 %*	
						Total CSA	NS	
						Cort CSA	-12.2 %*	
						Cort wall thickness	-12.5 %*	
						Cort vBMD	-3.0 %*	
						p-SSI	NS	
						Muscle CSA	-6.4 %*	
						Fat CSA	NS	
						4% tibia		

						BMC	-6.4 %*	
						Total vBMD	-5.7 %*	
						Trab vBMD	-3.2 %*	
						Total CSA	NS	
						66% tibia		
						BMC	-3.1 %*	
						Total CSA	NS	
						Cort CSA	-4.7 %*	
						Cort wall thickness	-5.0 %*	
						Cort vBMD	NS	
						p-SSI	NS	
						Muscle CSA	NS	
						Fat CSA	NS	
Pang et al 2010[50]	45	64.6±8.1	Not reported	5.6±5.4 years	PQCT	4%tibia		Good
						Total BMC	NS	
						Total vBMD	-3.2 %*	
						Trab vBMD	-2.8 %*	
						Total area	NS	
						CBSI	-6.6 %*	
Paker et al 2009[57]	106	65.14±9.8	Brunnstrom stage: 3.7±1.4	16.9±9.1months	DXA	Femoral neck BMD	-2.2 %*	Fair
						Femur total BMD	-2.0 %*	
Pang et al 2008[144]	39	66.7±9.1	Not reported	6.5±5.7 years	DXA	Femoral neck BMD	Osteoporosis: *n*=8	Good

							Osteopenia: *n*=31	
Pang et al 2008[49]	55	65.7±9.2(male) 63.4±7.4(female)	Not reported	5.1±3.4 years in men 6.0±6.5 years in women	PQCT	30%tibia		Good
						Cort BMC	-3.1 %* in men, -5.6 %* in women	
						Cort vBMD	NS	
						Total bone area	NS in men, -2.8 %* in women	
						Cort bone area	-2.9 %* in men, -5.8 %* in women	
						Marrow cavity area	4.2 %* in men, NS in women	
						Cort thickness	-3.4 %* in men, -6.7 %* in women	
						BSI	-2.7 %* in men, -6.8 %* in women	
Celik et al 2008[121]	35	62.69±9.54	Brunnstrom motor stage: Stage1: 4 Stage2:9 Stage3:12 Stage5:6	1.31±1.85 years	DXA	Leg BMC	-5.0 %*	Fair

			Stage6:4					
Pang et al 2007[25]	47	64.5±8.6	Wolf Motor Function Test Score(0-5): 3.3(1.7)	5.3±8.6 years	PQCT	30% radius		Good
						Cort BMC	-17.6 %*	
						Cort vBMD	-1.6 %*	
						Total area	NS	
						Cort area	-6.2 %*	
						Cort thickness	-10.8 %*	
						p-SSI	-5.5 %*	
Pang et al 2006[48]	88	64.7±9.3	Not reported	⩾12 months	PQCT	4%tibia		Good
						Total area	NS	
						Trab BMC	NS	
						Trab area	NS	
						Trab BMD	NS	
						50%tibia		
						Total area	NS	
						Cort area	NS	
						Cort BMC	NS	
						Cort BMD	NS	
						p-SSI	NS	
						Cort thickness	NS	
Ashe et al 2006[23]	15	66.6±5.8	Fugl-Meyer Score (upper extremity): 64.5±16.1	46.8±22.6 months	PQCT	4% radius		Good
						Total density	-14.9 %*	
						Total content	-10.6 %*	
						Total area	NS	
						30%radius		
						Total area	NS	

						Total density	NS	
						Total content	-9.4 %*	
						Cort area	-5.9 %*	
						Cort vBMD	-3.1 %*	
						Cort content	-8.0 %*	
						Cort thickness	NS	
						p-SSI	-7.5 %*	
Worthen et al 2005[34]	33	65±8	Fugl-Meyer Lower-limb Score (max=100): 86±8	45.9±29.1months	DXA	Total femur BMD	-4.4 %*	Fair
Pang et al 2005[145]	63	65.8±9.1	Not reported	5.2 years	DXA	Femoral neck BMD	NS	Good
Pang et al 2005[15]	56	65.4±8.9	Fugl-Meyer upper limb score: 47.0±19.6	5.2±4.1 years	DXA	Total arm BMC	-13.8 %*	Fair
						Total arm BMD	-4.5 %*	
Pang et al 2005[28]	58	65.8±8.8	Not reported	5.6±5.1 years	DXA	Proximal femur BMC	NS	Fair
						Proximal femur BMD	-3.7 %* in men, -4.8 %* in women	
Demirbag et al 2005[35]	38	62.65±8.55	Not reported	8.28±5.81 months	DXA	Distal radius BMD	-12.5 %*	Fair

						Femoral neck BMD	-16.1 %*	
						Trochanter BMD	-3.5 %*	
Levendoglu et al 2004[62]	80	68.16±3.49	Motricity index leg score (range,1-100): 50.39±13.61	8.90±5.32months	DXA	Femoral neck and trochanter BMD(Z scores)	Paretic-side Z scores were significantly lower than the non-paretic side	Fair
Runge et al 2004[47]	26	Not reported	Not reported	⩾6 months	PQCT	4% tibia bone mass	-7.7 %	Fair
						38% tibia bone mass	-7.6%	
Sahin et al 2001[146]	30	57.6±10.8	Mean Brunnstrome stage: Upper limb: 3.20±1.4(range 1-6) Lower limb: 4.10±1.2(range 2-6)	361.5±270(range 180-1110) days	DXA	Proximal 1/3 portion radius BMD	NS	Fair
						Middle portion radius BMD	NS	
						Ultra-distal radius portion BMD	NS	
						Total radius BMD	NS	
						Femur neck BMD	NS	

						Femur trochanter BMD	NS	
						Femur inter-trochanter BMD	-3.1 %*	
						Femur ward's BMD	7.8 %*	
						Femur total BMD	NS	
Iwamoto et al 2001[147]	72	67 (range,48-83)	Mean Brunstrom stage: Hand: 2.8 (range 1-6) Arm: 3.0 (range 1-6) Leg: 3.3 (range 1-6)	19.4 months (range 3-98 months)	CXD	Second metacarpal BMD	-4.5 %*	Fair
Haddaway et al 1999[148]	28	73.9±21.2(male) 62.2±12.8(female)	Not reported	Not reported, time since stroke ranged from 1 month to 25 years	Achilles plus ultrasound densitometer	Os calcis index stiffness	NS	Fair
Kuno 1998[150]	88	70.9±9.1	Brunstrom's test: Finger: 3.7(1.7) Arm: 3.9(1.6) Leg: 4.3(1.3)	1709±1597 days	CXD	Second metacarpal BMD	The Z-scores: -1.648 on the paretic side, -0.453 on the intact side	Fair
Fujimatsu 1998[149]	54	68.2±8.4	Scandinavian Stroke Scale:	4.2±4.3years	CXD	Second metacarpal	The Z-scores: -	Fair

			Hand: 1.833(0.5757) Arm: 3.056(1.498) Leg: 3.148(1.352)			BMD	1.753 on the paretic side, 0.019 on the intact side	
Puente et al 1996[151]	48	59.0(male) 64.6(female)	Not reported	10.9months for men, 7.8 months for women	DXA	Femoral neck BMD	-4.3 %* (men), -5.9 %* (women)	Fair
Takamoto et al 1995[152]	112	68.3±10.6	Not reported	45.7±46.6 months	DXA	Femoral neck BMD	-6.6 %*	Fair
						Total femur BMD	-8.1 %*	
						Trochanter BMD	-9.7 %*	
						Ward's triangle BMD	-10.3 %*	
						Femoral neck BMC	-2.8 %*	
						Total femur BMC	-8.5 %*	
Hamdy et al1993[32]	30	61.7±9.39(recent past group) 67.5±10.89 (remote past group)	Not reported	11.3 weeks (range5.9-16.7weeks) in 15 subjects 483.6 weeks (range 210.5-756.6weeks) in 15 subjects	Dual photon absorptiometry	Arm BMC	-13.0 %*	Fair
						Arm BMD	-8.0 %*	
						Leg BMC	-4.0 %*	
						Leg BMD	-3.4 %*	

Iversen et al1989[33]	15	62.5 (range 39-78) years	Not reported	29.1 weeks (range 23-28weeks)	single photon absorptiometry	Proximal forearm BMC	-3.3 %*	Fair
						Distal forearm BMC	-5.8 %*	
					dual photon absorptiometry	Arm BMC	-10.3 %*	
						Leg BMC	-4.1 %*	

BMC: bone mineral content; BMD: bone mineral density; BSI: bone strength index; CBSI: compressive bone strength index; CMSA: Chedoke-McMaster stroke assessment; Cort: cortical; CSA: cross-sectional area; CXD: computed X-ray densitometer; DXA: dual-energy x-ray absorptiometry; Endo_C: endosteal circumference; Imax: maximum rotated moment of inertia; Imin:minimum rotated moment of inertia; NS: non-significant; Peri_C: periosteal circumference; PQCT; peripheral quantitative computed tomography; p-SSI: polar stress strain index; SSI: strength-strain index; Trab: trabecular; vBMD: volumetric bone mineral density; -: decline on paretic side versus non-paretic side; *: statistically significant.

Table 2-4. Side-to-side differences in bone parameters: cross-sectional studies (mixed subacute and chronic stroke subjects)

Study	n	Age (years)	Severity of stroke	Timing of measures relative to stroke	Measurement tool	Outcomes	Report finds: Paretic vs Non-paretic limb	Study quality
MacIntyre et al 2010[68]	11	69±9	Not reported	3.2 months (1.7)	PQCT	66% tibial Cort bone density	NS	Fair
						66% tibal Cort bone mass	NS	
						66% tibial p-SSI	-1.5 %*	
		72±12	Not reported	60 months (35.8)	PQCT	66% tibial Cort bone density	NS	
						66% tibal Cort bone mass	-3.0 %*	
						66% tibial p-SSI	-1.5 %*	

PQCT; peripheral quantitative computed tomography; Cort: cortical; NS: non-significant; p-SSI: polar stress strain index; -: decline on paretic side versus non-paretic side; *: statistically significant.

Table 2-5 Characteristics of included longitudinal studies

Study	n	Age (years)	Severity of stroke	Timing of measures relative to stroke (days)	Measurement tool	Outcomes	Findings: Change over time	Study quality
Borschmann et al 2018[122]	26	69.7±11.6	NIHSS:12.6±4.7	⩽2 weeks	DXA	Total leg BMC	6 months: Paretic side: -3.4 %* Non-paretic side: NS	Fair
						Total leg lean mass	6 months: Paretic side:NS Non-paretic side: NS	
	22	66.0±12.1	NIHSS:12.6±4.8	⩽2 weeks	PQCT	7% of distal tibia		
						Total vBMD	6 months: -2.4 %* on the paretic side, NS on the non-paretic side	
						Total bone mass	6 months: -2.5 %* on the paretic side, NS on the non-paretic side	
						Cort bone mass	6 months: -8.5 %* on the paretic side, NS on the non-paretic side	
						Trab bone mass	6 months: NS on the both sides	
						Cort area	6 months: -7.0 %* on the paretic side, NS on the non-paretic side	

						Trab area	6 months: 0.5 %* on the paretic side, NS on the non-paretic side	
Lam et al 2016[24]	20	63.2±1.8	CMSA leg motor (median(IQR)):4.5(4-6)	Mean: 49 months(range:12-166)	DXA	Total hip BMD	12 months: Paretic side: -1.2 %* Non-paretic side: NS	Fair
					PQCT	4 % tibia:	12-months:	
						Total BMC	NS on the both sides	
						Total vBMD	NS on the both sides	
						Trab vBMD	-1.8 %* on the paretic side, NS on the non-paretic side	
						Total CSA	NS on the both sides	
						CBSI	-2.7 % *on the paretic side, NS on the non-paretic side	
						66 % tibia:	12-months:	
						Cort BMC	-1.3 %* on the non-paretic side, NS on the paretic side	
						Cort vBMD	NS on the both sides	
						Total CSA	NS on the both sides	

						Cort CSA	NS on the both sides	
						Medullary CSA	NS on the both sides	
						p-SSI	NS on the both sides	
Tomasević-Todorović et al 2016[137]	40	66.5±9.8	Not reported	1.5±0.4 years	DXA	Femoral neck BMD	12 months: Paretic side: NS	Fair
Chang et al 2014[130]	30	65.9±9.2	Brunnstrom stage: Stage≤3:18 Stage≥4:12	2.3±2.2 months	DXA	Femoral neck BMD	Mean follow up time: 7.6 months: Paretic side: -8.2 %* Non-paretic side: -4.6 %*	Fair
Pang et al 2013[52]	20	63.2 ± 8.1	Not reported	45.6 ± 42.8 months	PQCT	4 % radius	12-months:	Good
						Total BMC	NS on the both sides	
						Total vBMD	-2.5 %* on the paretic side, NS on the non-paretic side	
						Trab vBMD	NS on the both sides	
						Total area	NS on the both sides	
						CBSI	-6.7 %* on the paretic side, NS on the non-paretic side	

						33 % radius	12-months:	
						Cort BMC	-2.1 % *on the paretic side, -1.0 % *on the non-paretic side	
						Cort vBMD	NS on the both sides	
						Total area	NS on the both sides	
						Cort area	-2.1 %* on the paretic side, NS on the non-paretic side	
						Cort thickness	-3.2 % *on the paretic side, NS on the non-paretic side	
						p-SSI	NS on the both sides	
Lazoura et al 2010[129]	58	65.7(male) 62.3(female)	Not reported	3 months	DXA	Leg BMD	6 months: Paretic side: -2.0 %* Non-paretic side: -1.0 %* Mean 12 months: Paretic side: -4.6 %* Non-paretic side: NS	Good
Lazoura	67	61.4±9.44(male)	Not reported	3 months	DXA	Femoral neck BMD	6 months: Paretic side: -7.3 %* in males,	Good

et al 2008[40]		64.75±9.31(female)					and -8.7 %*in females Non-paretic side: -5.6 %* in males, and -8.4 %*in females 12 months: Paretic side: -11.8 %* in males, and -13.0 %*in females Non-paretic side: -8.3 %* in males, and -10.9 %*in females	
						Trochanter BMD	6 months: Paretic side: -6.5 %* in males, and -8.0 %*in females Non-paretic side: -5.8 %* in males, and -7.8 %*in females 12 months: Paretic side: -10.4 %* in males, and -12.6 %*in females Non-paretic side: -8.4 %* in males, and -10.9 %*in females	

					PQCT	4 % radius:		
						Trab vBMD	6 months:-6.6 % *on the paretic side, -4.3 %* on the non-paretic side 12 months: -12.2 % *on the paretic side, -7.0 % *on the non-paretic side	
						p-SSI	6 months: -16.1 %* on the paretic side, -7.4 % *on the non-paretic side 12 months: -25.6 %* on the paretic side, -9.7 % *on the non-paretic side	
						20% radius:		
						Cort vBMD	6 months: -2.1 %* on the paretic side, -1.0 %* on the non-paretic side 12 months: -3.3 %* on the paretic side, -1.5 %* on the non-paretic side	

						p-SSI	6 months: -6.5 %* on the paretic side, -4.8 % *on the non-paretic side 12 months: -7.2 %* on the paretic side, -5.6 %* on the non-paretic side	
Yavuzer et al 2002[132]	32	62.4±8.1	Brunnstrom stage: 2.1±1.2 (range 1-3)	63 days (median)	DXA	Femoral neck BMD	median length of stay in days: 98 days Paretic side: -5.2 %* Non-paretic side: -2.1 %*	Fair
Jørgensen et al 2001[107]	28	Not reported	Not reported	7±4	DXA	Total leg BMC	12-months: Paretic side: -7.0 %* Non-paretic side: -2.0 %*	Good
Jørgensen et al 2001[38]	28	Not reported	Scandinavian stroke scale arm score: 2 (range 0-5)	7±4	DXA	Proximal humerus BMD	12-months: Paretic side: -17.0 %* Non-paretic side: NS	Good
Jørgensen et al 2000[36]	40	76±8	Not reported	6±4	DXA	Paretic femoral neck BMD	7 months: -8.0 %* (FAC1), -4.0 %* (FAC2-6) 12 months: -10.0 % * (FAC1), -3.0 % * (FAC2-6)	Good

						Non-paretic femoral neck BMD	7 months: -2.0 %* (FAC1), NS (FAC2-6) 12 months: -5.0 %* (FAC1), NS (FAC2-6)	
						Paretic trochanter BMD	7 months: -7.0 %* (FAC1), -4.0 % * (FAC2-6) 12 months: -10.0 %* (FAC1), NS (FAC2-6)	
						Non-paretic trochanter BMD	7 months: -4.0 %* (FAC1), 0 % (FAC2-6) 12 months: -5.0 %* (FAC1), 0 % (FAC2-6)	
Jørgensen et al 2000[37]	40	76±8	Not reported	6±4	DXA	Lower femoral neck BMD	12-months: Paretic side: -5.2 %* Non-paretic side: NS	Good
						upper femoral neck BMD	12-months: Paretic side: -4.4 %* Non-paretic side: NS	
Ramnemark et al1999[39]	24	72.7±4.7	Motricity index: Paretic arm: 20±22/100	1 month	DXA	Total arm BMD	4 months: -2.5 %* 7 months: -3.8 %* 12 months: -7.6 %*	Good

			Paretic leg:34±35/100				NS on the non-paretic side during the follow-up	
						Humerus BMD	4 months: NS 7 months: -7.4 %* 12 months: -17.4%* NS on the non-paretic side during the follow-up	
						Ultradistal radius BMD	4 months: -5.7 %* 7 months: -8.6 %* 12 months: -8.6 %* NS on the non-paretic side during the follow-up	
						Total femur	4 months: -0.8 %* 7 months: -4.0 %* 12 months: -7.2 %* NS on the non-paretic side during the follow-up	
						Proximal femur	4 months: -6.0 %* 7 months: -10.0 %* 12 months: -12.0 %* NS on the non-paretic side during the follow-up	

Ramnemark et al1999[135]	24	72.7±4.7	Motricity index: Paretic arm: 20±22/100 Paretic leg:34±35/100	1 month	DXA	Paretic arm BMC	4 months: -8.8 %* 7 months: -14.0 %* 12 months: -24.9 %	Fair
						Non-paretic arm BMC	4 months: NS 7 months: NS 12 months: -4.6 %*	
						Paretic leg BMC	4 months: -2.4 %* 7 months: -4.3 %* 12 months: -7.7 %*	
						Non-paretic leg BMC	4 months: NS 7 months: NS 12 months: NS	
Liu et al1999[134]	104	56.5±13.2	Stroke impairment assessment set: Upper extremity total: 2.9(2.6) Lower extremity total:6.8(3.7)	116.2±56.8 (median 104) days	DXA		Discharge BMD , median length of stay in days: 105.5 (range 20-262)	Good
						Proximal humerus BMD	Paretic side: -12.0 %* Non-paretic side: -11.6 %*	
						Distal radius BMD	Paretic side: -1.8 %* Non-paretic side: NS	
						Femoral neck BMD	Paretic side: -3.1 %* Non-paretic side: -2.3 %*	

						Calcaneus BMD	Paretic side: -2.9 %* Non-paretic side: -1.8 %*	
						Distal radius BMD	median length of stay in days: 98 days Paretic side: -12.4%* Non-paretic side: -3.5%*	
Hamdy et al 1995[123]	11	Not reported	Not reported	<7 days	DXA	Upper limb BMC	4-months: Paretic side: -9.3 %* Non-paretic side: NS	Fair
						Lower limb BMC	4-months: Paretic side: -3.7 %* Non-paretic side: NS	

BMC: bone mineral content; BMD: bone mineral density; CBSI: compressive bone strength index; CMSA: Chedoke-McMaster stroke assessment; Cort: cortical; CSA: cross-sectional area; DXA: dual-energy x-ray absorptiometry; FAC: functional ambulation category; IQR: interquartile range; NIHSS: national institute of health stroke scale; NS: non-significant; PQCT; peripheral quantitative computed tomography; p-SSI: polar stress strain index; Trab: trabecular; vBMD: volumetric bone mineral density; -: decline on paretic side versus non-paretic side; *: statistically significant.

Table 2-6 muscle-bone relationship of included studies measured by DXA and PQCT

study ID	Study design	Timing of measures relative to stroke	Severity of stroke	Measurement tool	Bone outcomes	Muscle outcomes	Results
Lam et al 2016[24]	longitudinal	49 months (range:12-166)	CMSA leg motor score (median(IQR)):4.5 (4-6)	PQCT	Trab vBMD at the 4 % tibia	Isometric knee extensor muscle strength	*r*=0.45*
						Ankle spasticity	NS
Yang et al 2015[22]	Cross-sectional	5 years (4.0)	CMSA leg motor score (SD): 7.6 (2.0)	PQCT	CBSI at the 4 % tibia	Concentric knee extensor muscle strength	NS
						Eccentric knee extensor muscle strength	NS
						ankle spasticity	NS
					p-SSI at the 66 % tibia	Concentric knee extensor muscle strength	NS
						Eccentric knee extensor muscle strength	*r*=0.45*
						ankle spasticity	NS
Pang et al 2013[27]	Cross-sectional	45.3 (43.7) months in men; 48.3 (41.8) months in women	Not reported	PQCT	p-SSI at the 33 % radius	Grip strength	*r*=0.71*
						Spasticity	*r*=-0.36*
Pang et al 2012[26]	Cross-sectional	45.3 (43.7) months in men; 48.3	Not reported	PQCT	CBSI at the 4% radius	Grip strength	*r*=0.69*
						Spasticity	*r*=-0.47*

		(41.8) months in women					
Pang et al 2010[50]	Cross-sectional	5.6 years (5.4)	Not reported	PQCT	total BMC	Leg muscle mass	*r*=0.79*
					total vBMD		*r*=0.50*
					Trab vBMD		*r*=0.45*
					Total area		*r*=0.68*
					CBSI		*r*=0.73*
					total BMC	Spasticity	*r*=-0.44*
					total vBMD		*r*=-0.33*
					Trab vBMD		*r*=-0.30*
					Total area		NS
					CBSI		*r*=-0.42*
MacIntyre et al 2010[68]	Cross-sectional	11 in subacute stroke: 3.2 months (1.7); 11 in chronic stroke: 60 months (35.8)	Not reported	PQCT	Cort bone density	Muscle density	*r*=0.78* in subacute stroke
					Cort bone mass		NS
					p-SSI		*r*=0.66* in chronic stroke
					Cort bone density	Muscle mass	NS at subacute and chronic stroke
					Cort bone mass		NS at subacute and chronic stroke

					p-SSI		NS at subacute and chronic stroke
Pang et al 2008[49]	Cross-sectional	5.1 years (3.4) in men, 6.0 years (6.5) in women	Timed-up-and go test(s): 14.6 (11.6) in men 13.6 (7.7) in women	PQCT	BSI	Leg lean mass	*r*=0.51 * in men, *r*=0.81* in women
						spasticity	NS
Pang et al 2007[25]	Cross-sectional	5.3 years (8.6)	Wolf Motor Function Test Score (0-5): 3.3 (1.7)	PQCT	Cort BMC	Percent side-to-side difference in muscle strength	*r*=0.47*
					Cort vBMD		NS
					Cort thickness		*r*=0.48*
					p-SSI		NS
					Cort BMC	Spasticity	*r*=0.46*
					Cort vBMD		NS
					Cort thickness		*r*=0.48*
					p-SSI		NS
Ashe et al 2006[23]	Cross-sectional	46.8 (22.6) months	Fugl-Meyer Score (upper extremity): 64.5 ± 16.1	PQCT	p-SSI	Composite muscle strength score	*r*=0.85*
Pang et al 2005[15]	Cross-sectional	5.2±4.1 years	Fugl-Meyer score: 47.0±19.6	DXA	Total arm BMC	Composite arm muscle strength score	*r*=0.60*
						Arm lean mass	*r*=0.86*

						Spasticity (Modified Ashworth scale)	*r*=-0.20
					Total arm BMD	Composite arm muscle strength score	*r*=0.43*
						Arm lean mass	*r*=0.74*
						Spasticity (Modified Ashworth scale)	*r*=-0.07
Pang et al 2005[28]	Cross-sectional	5.6±5.1 years	Not reported	DXA	Proximal femur BMC	Isometric knee extension muscle strength	*r*=0.41*
						Leg lean mass	*r*=0.78*
						Spasticity (Modified Ashworth scale)	*r*=-0.21
					Proximal femur BMD	Isometric knee extension muscle strength	*r*=0.39*
						Leg lean mass	*r*=0.61*
						Spasticity (Modified Ashworth scale)	*r*=-0.23
Jørgensen [107] et al 2001	Longitudinal	7±4 days	Not reported	DXA	The 1-year change in total leg BMC	Spasticity (Modified Ashworth scale)	r_s=-0.30

Hamdy et al 1995[123]	Longitudinal	⩽7 days	Not reported	DXA	Upper limb BMC	Arm muscle strength	*r*=0.62*

BMC: bone mineral content; BMD: bone mineral density; BSI: bone strength index; CBSI: compressive bone strength index; CMSA: Chedoke-McMaster stroke assessment; Cort: cortical; DXA: dual-energy x-ray absorptiometry; IQR: interquartile range; NIHSS: national institute of health stroke scale; PQCT; peripheral quantitative computed tomography; p-SSI: polar stress strain index; SD: standard deviation; Trab: trabecular; vBMD: volumetric bone mineral density; *: statistically significant

2.5 Discussion

The overarching goal of this systematic review was to provide insight into the impact of stroke on bone properties as well as examine the association between muscle function and bone properties post-stroke. From this review, four main findings were consolidated: 1) the rate of change in bone properties in the affected limbs was slower in chronic than subacute period after stroke; 2) the paretic upper limb exhibited more compromised bone properties compared with the paretic lower limb; 3) there was a strong relationship between muscle strength and bone quality in the upper and lower limbs; and 4) muscle spasticity seemed to have negative impact on bone integrity in the paretic upper limb, but its influence on the paretic lower limb was uncertain.

2.5.1 Rate of change in bone properties was slower in chronic than subacute stage

As revealed by the substantial side-to-side differences in bone properties found in cross-sectional studies, as well as the actual amount of bone changes in longitudinal studies, a consistent finding was that the skeletal sites in the paretic limbs sustained a more pronounced decline in bone quality than their counterparts in the non-paretic limbs. However, the rate of changes in bone properties showed a decelerating trend as post-stroke duration increased. Hamdy et al. [123] showed that most bone loss in the paretic upper and lower limbs occurred within the first 3-4 months post-stroke. After 1 year of stroke onset, the extent of bone change was minimal. For example, the decline in paretic total hip aBMD was 1.2% in 1 year for those whose stroke onset was more than one year ago [24]. This was much less than the 10% loss in femoral neck aBMD on the paretic side within the first year post-stroke [40]. This phenomenon was similar in the upper limbs. A good example is the radius diaphysis, in which the cortical vBMD showed a significant reduction in bone strength index by 7.2% during the period between 3 and 15 months

post-stroke, but the chronic stroke cases (average onset: 45 months post-stroke) no longer sustained significant changes.

The exact time point at which the bone changes reached a steady state was unclear and may have differed depending on the specific bone site measured. Lam et al. further showed that even within the same bone, the timing at which the bone reaches steady state may differ. The trabecular bone density of the paretic tibial epiphysis did not reach the steady state until 2 years post-stroke onset, but the cortical bone variables of the diaphysis region of the same bone showed no significant changes among those whose onset of stroke was earlier than 12-24 months ago [24]. The rate of change and timing of the plateau phase may also depend on the stroke severity as revealed by longitudinal studies [36]. For those who were wheelchair bound, the 1-year decline in hip BMD was much more severe (13%) than those who regained ambulatory function at 2-months post-stroke (8%) or had ambulatory function at stroke onset (3%) [36]. Nevertheless, the overall results suggested that the rate of change in bone properties between the paretic and non-paretic sides was slower in chronic than subacute stage. These results highlight the importance of therapeutic interventions mitigating the rapid decline in bone integrity within the first year following stroke.

2.5.2 Paretic upper limb sustains more pronounced changes in bone properties than paretic lower limb

Upon comparing the side-to-side differences in bone parameters of the upper limb and lower limb sites as well as the findings in longitudinal studies, it can be deduced that the magnitude of bone changes in the paretic upper limb was more pronounced than the paretic lower limb [40]. This observation could be partly explained by the difference in the course of recuperation between the hemiparetic upper and lower limbs post-stroke. Previous studies disclosed that only

44 % of stroke survivors with severe paralysis partially or completely recovered upper limb bone properties [24], while 75 % of stroke survivors recovered, in part or full, in the lower limbs [153]. It is also possible that because the affected lower limb was mechanically loaded during daily activities, such as standing and walking, a slower decline in function may have emerged relative to the upper site. Furthermore, the unaffected arm may have been used to compensate for the dysfunction of the paretic arm, thereby increasing the side-to-side differences in bone outcomes in the upper limbs. Altogether, a greater emphasis on recovering upper limb bone properties should be addressed in therapeutic interventions.

2.5.3 Association between muscle and bone properties post-stroke

Overall, there was a strong relationship between muscle strength/mass and bone density/strength index. This phenomenon was largely consistent in upper and lower limb skeletal sites. The results support the muscle-bone unit theory, which puts forward that muscle strength and bone properties form a functional biological unit [64]. Mechanical strains from muscle contractions provide a potent stimulus for osteogenesis. Following the initial paralysis after the onset of stroke, there may be decrease in physical activity [154], as well as learned non-use of the affected limbs [155], resulting in further muscle weakness and atrophy [156] and, ultimately, compromised integrity of bone tissue. The results also suggest that improving muscle strength may be a potentially effective method to enhance bone properties in this group. While no study has specifically examined the use of resistance/strength training on bone health post-stroke, Pang et al. [48, 145] did show in a chronic stroke exercise study that a mix of dynamic loading, resistance and aerobic exercises resulted in significant increase in paretic leg muscle strength and better bone outcomes in the hip and tibia on the affected side. Stroke is a chronic condition; thus, long-term care strategies in bone health management are essential. Health service providers,

especially physiotherapists, should have a major role in formulating and implementing long-term strategies to optimize bone health among chronic stroke survivors.

The relationship between muscle spasticity and bone outcomes, on the other hand, may not be straightforward, particularly in the affected lower limb. While some studies reported a negative relationship between spasticity and bone density/strength index [26, 27, 50], others did not show such relationship [22, 49, 157]. It is likely that the relationship between spasticity and bone quality is a non-linear one. For example, individuals with mild spasticity may have better bone outcomes than those who have complete flaccid paralysis, as the tonic muscle contraction involved in spasticity may exert a protective effect on bone tissue. However, as spasticity level continues to increase, a negative effect on bone may ensue, as the functional use of the affected limb becomes severely impaired [25]. Also, the MAS, a scale that was used in the reviewed studies to measure spasticity, cannot measure hypotonia (flaccid paralysis). It is only a 6-point ordinal scale and unable to provide a finer discrimination of different degrees of hypertonia. Overall, future studies with a larger sample that covers the full spectrum of muscle tone changes (hypotonia and hypertonia) and uses a better spasticity measure (e.g., electromyography) are required to decipher the relationship between spasticity and bone health.

2.5.4 Limitations of the studies reviewed

The numbers of longitudinal studies were low relative to cross-sectional studies. Longitudinal studies are better designed to assess actual bone changes in each skeletal site after stroke onset, whereas the side-to-side difference values obtained from cross-sectional studies represent a combination of changes in the paretic and non-paretic sides. The number of chronic stroke studies was also much greater than that in the acute and subacute phases. The impact of stroke on microstructural properties of bone is unknown (e.g., trabecular thickness and spacing).

2.5.5 Limitations of this systematic review

In order to obtain a comprehensive understanding of the impact of stroke on bone properties, as well as the association of bone and muscle properties post-stroke, we included a variety of study designs in our review, including interventional, observational, cross-sectional, and longitudinal studies. Inherent limitations exist with variability in the length of stroke duration across studies. Additionally, most of the studies investigating the association between bone and muscle properties post-stroke conducted different statistical analyses and investigated different outcomes; thereby limiting the studies to be included in the meta-analyses. While the influence of stroke severity is addressed in this review, the impact of functional capacity is understudied.

2.5.6 Future research directions

The limitations indentified above should provide opportunities for further research in various aspects of post-stroke bone health. More longitudinal studies with a long follow-up period after stroke onset are warranted. Future research should study changes in bone microproperties using bone measurement techniques such a high-resolution pQCT. The impact of functional capacity of stroke patients on bone health should be addressed in future research

2.6 Conclusion

In conclusion, significant changes in bone mass and macrostructure occurred after stroke, and these changes were more pronounced in the paretic limbs and in first few months post-stroke. The paretic upper limb sustained a substantial decline in bone quality relative to the paretic lower limb. There was a strong relationship between muscle strength/power and bone parameters, while the impact of muscle spasticity on bone quality remains unclear. The results of this review has important clinical implications, particularly issues related to early intervention, muscle strength training, and long-term management strategies to enhance bone health post-stroke. This

review has also revealed the knowledge gaps in the field which should be addressed in future research.

2.7 Citation

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3 Chapter 3: Relationship between bone strength index of the hemiparetic tibial diaphysis and muscle strength in stroke patients: influence of muscle contraction type and speed

3.1 Abstract

Summary This study was conducted to examine the association between the tibial bone strength index and leg muscle strength of different contraction types and speeds among people with chronic stroke. We found that concentric muscle power at moderate speed was more associated with tibial bone strength index than other types.

Introduction To compare the influence of muscle strength of different contraction types and speeds on the bone strength index of tibial diaphysis in chronic stroke patients.

Methods Eighty individuals with chronic stroke (age:62.6±8.0 years; men/women:46/34; post-stroke duration:9.0±5.4 years) underwent scanning of the tibia at the 66% site on both sides using peripheral quantitative computed tomography. Each participant was also evaluated for isometric and dynamic (at 60°/s and 120°/s) strength of knee flexors/extensors and ankle dorsiflexors/plantarflexors using an isokinetic dynamometer. For a given contraction type and speed, the strength values of the four muscle groups were summed to yield a composite score. Multiple regression analysis was used to identify the association of percent side-to-side difference (%SSD) in tibial polar-stress-strain index (p-SSI) with %SSD in muscle strength of different contraction types and speeds.

Results The p-SSI and all muscle strength parameters on the paretic side had lower values than the non-paretic side ($p \leqslant 0.001$). The %SSD in concentric muscle power at angular speed of 60°/s

(R^2=0.317, p=0.006) and 120°/s (R^2=0.298, p=0.020) remained independently associated with that in p-SSI, after controlling for age, sex, body mass index, post-stroke duration, motor impairment, spasticity and physical activity level. The effect of isometric strength and eccentric muscle power was not significant in multivariate analysis.

Conclusions Concentric muscle power was more predictive of %SSD in p-SSI at the tibial diaphysis than other contraction types and may be an important target for intervention to promote bone health in people with chronic stroke.

3.2 Introduction

Stroke is one of the most disabling conditions worldwide [158]. It is well known that people with stroke have an elevated risk of fragility fractures [53, 111], leading to complications such as increased morbidity [55], mortality [113], prolonged hospitalization [29] and decreased ability to regain independent mobility [55]. Exaggerated fracture rate after stroke can be partially attributable to compromised bone health status. Reduced bone strength is an important risk factor of fractures [29], which are much more common in individuals with stroke than their age-and sex-matched counterparts [53].

Several studies have shown a close link between muscle mass/strength and integrity of bone tissue among people with stroke [49, 50, 68]. For example, Pang et al. have shown muscle mass to be a significant determinant of the bone strength index measured at the distal tibial diaphysis among individuals with chronic stroke [49]. In another study, MacIntyre et al. further demonstrated that calf muscle density was significantly related to the bone strength index of the tibial diaphyseal site in individuals with sub-acute stroke [68]. However, the association between muscle contraction type (i.e., dynamic concentric/ eccentric versus isometric muscle contractions) and bone properties post-stroke is currently unknown. Moreover, the speed of

dynamic muscle contractions (e.g., fast versus slow) may also influence bone properties post-stroke. Animal studies have demonstrated that high-frequency dynamic loads are more effective than static loads in enhancing bone formation [73, 105]. Thus, it seems likely that bone tissue integrity may be more associated with the ability to generate force during dynamic muscle contractions at higher speeds rather than isometric contractions but this hypothesis is yet to be tested. This may have important implications for designing muscle strength training programs for enhancing bone health among people with stroke.

The objective of this study was to examine the association between the bone strength index measured at the tibial diaphysis with muscle strength measures consisting of different contraction types and speeds in people with chronic stroke. It was hypothesized that greater dynamic (concentric and eccentric) muscle strength measured at higher speeds would be more strongly associated with a higher bone strength index at the tibial diaphysis.

3.3 Methods

3.3.1 Sample size calculation

The sample size calculation was conducted using the Free Statistics Calculators version 4.0 (https://www.danielsoper.com/statcalc/calculator.aspx?id=16). A previous stroke study showed that leg mean mass was independently associated with tibial bone strength index, with R^2 value of 0.12 (equivalent to effect size f^2=0.13) [49]. Another study demonstrated that isometric knee muscle strength was significantly associated with 1-year change in trabecular volumetric bone mineral density (vBMD) at the distal tibia among people with chronic stroke (R^2=0.20,equivalent to f^2=0.25) [24]. A more conservative approach was taken by assuming the smaller effect size of f^2=0.13 attributable to the effect of muscle strength after adjusting for age, sex, post-stroke

duration, body mass index, physical activity level, severity of motor impairment, and spasticity. With an alpha of 0.05, power of 0.8, and attrition rate of 10%, a minimum of 76 individuals with stroke would be required.

3.3.2 Participants

Individuals with stroke were recruited between August 1, 2017, and April 30, 2018, from a stroke group organization in the community through convenience sampling. The screening of individuals and enrolment of participants were conducted by the research personnel through a telephone interview. The inclusion criteria were as follows: (1) a diagnosis of stroke with onset of 6 months or more, (2) medically stable, (3) aged ≥ 18 years, (4) able to walk >10 meters without physical assistance from other people (with or without walking aids) and (5) able to understand simple verbal commands. Exclusion criteria were: (1) other neurological conditions, (2) serious musculoskeletal conditions (e.g., amputations), (3) metal implants in the lower extremity, (4) fracture in the lower extremity within the past 1 year at the time of initial participant screening, (5) taking medications for the treatment of osteoporosis, and (6) other serious illnesses or contraindications that prevented the individual from participating in the study (e.g., neoplasms). Ethical approval was obtained from the Human Subjects Ethics Sub-committee of the University (Reference Number: HSEARS20140226001-03). The details of the study were explained to the participants before informed written consent was obtained. All of the experimental procedures were conducted in accordance with the Helsinki Declaration for human experiments.

3.3.3 Measurements

3.3.3.1 Demographics

Relevant demographic information (e.g., medications, stroke history) was collected through face-to-face interviews and a hospital discharge summary provided by the participants. The 10-item Abbreviated Mental Test (AMT) was used to assess cognitive function for each participant (possible score range: 0-10). Lower scores indicate greater cognitive impairment. The AMT has been previously validated among geriatric patients [159] and elderly in residential care homes [160] and has demonstrated excellent test-retest and inter-rater reliability (ICC=0.99) [160]. The 12-item Physical Activity Scale for the Elderly (PASE) questionnaire [161], which has been used in previous stroke research [22], was administered by the researcher to assess the participant's physical activity levels. Scores were calculated using weights and frequency values corresponding to the type of physical activity being assessed (e.g., leisure, occupational activity). Higher scores suggest greater daily physical activity. The Fugl-Meyer Motor Assessment (FMA) was administered to assess the degree of paresis of the leg and foot on the affected side [162]. It is a 17-item scale with each item rated on a 3-point ordinal scale (0-2). Hemiparesis was considered to be present if one was unable to attain the maximum FMA score of 34 [162].

3.3.3.2 Bone imaging

Each participant underwent scanning of the tibia on each side using peripheral quantitative computed tomography (pQCT) (XCT 3000, Stratec Medizintechnik GmbH; Pforzheim, Germany). The anatomical reference line was positioned at the cortical end plate of the distal medial edge of the tibia. Scans (2.3mm in thickness, scan speed at 25mm/s, voxel size of 500μm) were acquired at the tibial diaphysis (at 66% of the total bone length proximal to the reference

line, mainly a cortical bone site). Cortical bone analysis at the 66% site was performed using CORTBD (Model 1), with a threshold of 710 mg/cm^3. The variables of interest were total area, cortical bone area, cortical bone mineral content (BMC), cortical volumetric bone mineral density (vBMD), cortical thickness, marrow cavity area, and polar stress-strain index (p-SSI). The coefficients of variation for the aforementioned variables ranged from 0.47 to 1.73%. The marrow cavity area was calculated by subtracting the cortical area from the total area [49]. p-SSI reflects strength of long bone shaft against bending or torsional forces by considering densitometric and geometric properties of bone [49]. The equation for calculating the p-SSI of the tibial diaphysis has been described in a previous study [138] and is shown below:

Polar stress-strain index $= \sum_{d_{max}} [(A_z \times d_z^2)\ (\text{cortical bone mineral density/ND})]$

where A =area of each pixel, d_{max}=maximum distance to the center of gravity, d_z=distance between the pixel and the corresponding torsional (z) axis, and ND=normal physiological bone density (1200mg/cm^3).

3.3.3.3 Muscle strength

Participants underwent muscle strength testing of the bilateral knee extensors (i.e., quadriceps femoris) and flexors (i.e., biceps femoris) as well as ankle plantar flexors (i.e., gastrocnemius, soleus) and dorsiflexors (i.e., tibialis anterior) using an isokinetic dynamometer (HUMAC ®NORMTM Testing & Rehabilitation System, Computer Sports Medicine; USA), which allows good reliability of strength measurements (ICC=0.89-0.96) [163]. To test muscle strength during knee flexion-extension, participants were instructed to sit upright in a chair, the knee joint axis was aligned with the mechanical axis of the dynamometer, and straps were used to stabilize untested body part. Each participant was then instructed to perform maximal concentric/eccentric

knee flexion and extension throughout the range of 10°-70° knee flexion on each side at a constant angular speed of 60°/s and 120°/s. Each participant was also required to perform maximal isometric contraction of the same muscle groups at mid-range (45° flexion). To test muscle strength during ankle plantarflexion-dorsiflexion, participants were placed in a semi-reclined position with 30° knee flexion. Each participant then performed maximal concentric/eccentric ankle dorsiflexion and plantarflexion throughout the range of 0°-30° ankle plantarflexion on each side at a constant angular speed of 60°/s and 120°/s. Each participant also required performed a maximal isometric contraction of the same muscle groups at 10° ankle plantarflexion. The sequence of testing (i.e., paretic vs non-paretic side, type and speed of contractions) was randomized to minimize the order effect. Three trials were recorded for each test condition, and the data were averaged to obtain the mean peak torque for isometric testing (in newton-meters or Nm), and power for dynamic testing (in Watts or W) of each leg using customized software. As contraction type and speed were of primary interest, the strength values of knee flexion, knee extension, ankle dorsiflexion, and ankle plantarflexion were summed to yield a composite muscle strength score to reflect overall leg muscle strength for each of the five testing conditions (i.e., isometric, concentric at 60°/s and 120°/s, eccentric at 60°/s and 120°/s) [164].

3.3.3.4 H-reflex

The H-reflex was measured to assess the degree of excitability of the monosynaptic spinal reflex arc and was commonly used as an indicator of spasticity. Participants were instructed to lie in a comfortable supine position. The soleus H-reflex was induced and recorded using a Viking Quest device (Nicolet Biomedical, Madison, WI, USA). We used self-adhesive Ag–AgCl electrodes (1.0 cm diameter) to record surface electromyography signals. The recording

electrodes were fixed to the corresponding skin over the muscle bellies, and the reference electrode was secured to the skin over the muscle tendon. The ground electrode was secured between the recording and reference electrodes.

Before the application of the electrodes, the skin was properly prepared until a skin impedance of less than 20 kohm was obtained. The tibial nerve was stimulated with a rectangular electrical pulse of 1 ms duration and a stimulus frequency of 1 per 5s. The stimulation procedure described by Braddom and Johnson [165] was followed. Initially the optimal position for stimulating the tibial nerve in the popliteal fossa was determined by moving the stimulating electrode around until a visible contraction of the gastrocnemius muscle was seen. Then, the current was gradually increased until an H-reflex without an M response was recorded. The H reflex was identified as a triphasic wave with a small initial positive deflection followed by a larger negative one. The eleventh response with the largest amplitude was selected as the Hmax and other values were rejected. The stimulus intensity was then increased in small increments until the maximum M response was obtained. The maximum amplitudes of the H-reflex and the M wave were measured from the peak of the positive to the peak of the negative deflections. The Hmax to Mmax ratio was calculated by dividing the maximum amplitudes the H reflex by that of the M wave.

3.3.4 Statistical analyses

All statistical analyses were conducted using SPSS 23.0 software (IBM, Armonk, NY, USA). A significance level of 0.05 (2-tailed) was set for all statistical tests. Descriptive statistics were used to report all variables of interest. Paired t-tests were used to compare the pQCT and muscle strength parameters between the paretic and non-paretic sides. The percent side-to-side difference (%SSD) in pQCT and composite muscle strength parameters was obtained by

calculating the difference of values between the two sides (non-paretic minus paretic) divided by the value obtained from the non-paretic side and then multiplying it by a factor of 100. A positive value thus indicates a lower value on the paretic side when compared with the non-paretic side. Pearson's correlation coefficients were used to assess the association of %SSD in p-SSI with other variables. Multiple linear regression analyses were then performed to identify the association of %SSD in p-SSI with that in muscle strength outcomes. The %SSD in p-SSI was of interest here. It is derived from comparing the paretic and non-paretic side of the same individual. It can thus provide a more specific evaluation of the influence of stroke on bone strength index on the paretic side, while providing appropriate control for the different cofactors (i.e., genetic, age, nutrition, other environmental factors) which may affect bone metabolism across different people [28, 151]. Being a standardized score, it also facilitates the comparison of the degree of impact of stroke on bone properties between individuals. The %SSD has also been used in previous research assessing post-stroke bone status [25]. In each hierarchical regression model, age, sex, body mass index (BMI), years since stroke onset, physical activity level, and H-reflex were first forced into the model. Next, the muscle strength variable was entered.

Model comparisons were performed using the Bayesian Information Criterion (BIC) and Akaike Information Criterion (AIC) functions in R Studio (Version 1.2.5033, R Studio Inc., Boston, MA URL http://www.rstudio.com/). The AIC [166] and BIC [167] are commonly used criterion for determining model selection between two competing or comparable models. Given that the models arising from the analyses in the current study may be either true (asymptotically consistent) or a likely approximation of the data (asymptotically inconsistent), both BIC and AIC were interpreted [168].

The AIC value difference (ΔAIC) and the BIC value difference (ΔBIC) were compared between two models with equal numbers of predictor variables. The model with the lesser AIC or BIC value was considered optimal while the other was considered to be a secondary candidate model. ΔAIC was interpreted using the following criteria: < 2 = substantial evidence candidate model is likely to be as good as the optimal model; 4-7 = less evidence that the candidate model is as good as the optimal model; > 10 = no support for the candidate model over the optimal model [169]. ΔBIC was interpreted using the following criteria: <2 = negligible difference between models; 2-6 = evidence against the candidate model is positive; 6-10 = evidence against the candidate model is strong; >10 = evidence against the candidate model is very strong [170].

3.4 Results

Eighty individuals fulfilled all selection criteria. A summary of demographic and stroke-specific characteristics is provided in Table 3-1. Bilateral comparisons showed significant side-to-side differences in cortical area, thickness, BMC, vBMD and p-SSI, with higher values for the non-paretic side ($p \leq 0.001$) (Table 3-2). There was no difference observed for the total area between the two sides ($p = 0.773$).

All composite leg muscle strength variables showed significant side-to-side differences, indicating diminution of leg muscle strength on the paretic side during static and dynamic contractions at all measured speeds ($p \leq 0.001$) (Table 3-2).

A summary of bivariate correlations is provided in Table 3-3. The %SSD of p-SSI was significantly correlated with the %SSD of all composite muscle strength measures ($p \leq 0.026$). There were also significant correlations between the %SSD of p-SSI and body mass index (BMI) ($p \leq 0.027$) and motor impairment level (FMA) ($p \leq 0.038$).

To avoid multicollinearity among independent predictor variables, a series of separate regression models were used to predict the %SSD of p-SSI. After adjusting for the effects of sex, age, stroke duration, BMI, physical activity level, motor impairment level and spasticity (paretic leg H-reflex), each model also addressed the relative predictive contribution of the %SSD in composite muscle strength variables with regard to differences in contraction type and speed (Table 3-4). Overall, the models explained 25.6-31.7% of the variance in %SSD of p-SSI. Among the five models, model 2 and 3 accounted for the most variance of %SSD of p-SSI (29.8-37.1%). Also, only in these two models was the muscle power variable (concentric muscle contraction at 60°/s and 120°/s respectively) independently associated with %SSD of p-SSI ($p<0.05$), accounting for an additional 7.5% (model 2) and 5.6% (model 3) of the variance. The information criteria used to determine the optimal regression model (i.e., appropriate model complexity and explanatory power or fit of the model to the data) is summarized in Table 3-5. Model 2 had lower values (BIC = 550.84, AIC = 527.02) in comparison to model 3 (BIC = 553.07, AIC = 529.25). The difference in BIC and AIC also demonstrated positive evidence that the candidate model (model 3) was not comparable to the optimal model (model 2) (ΔBIC = 2.23, ΔAIC = 2.23). As there were an equal number of predictors between models with no unknown parameters, the use of the likelihood-ratio test was unwarranted. The distinguishing feature between these models was the speed of the muscle contraction variable used. The results of the model comparison suggest that the power output during concentric muscle contractions at a lower relative contraction velocity (60°/s, model 2) was more predictive of %SSD in p-SSI than at a higher contraction velocity (120°/s, model 3).

Table 3-1. Subject characteristics (n=80)

	Value[a]	Range
Demographics		
Age(years)	62.6 ± 8.0	38-81
Gender (male/female), n	46/34	--
Body mass index (BMI), kg/m^2	24.2 ± 3.2	17.7-32.2
Walking aid: none/cane/quad/frame, n	65/12/3/0	--
Stroke characteristics		
Hemiparesis side (right/left), n	44/36	--
Post-stroke duration, years	9.0 ± 5.4	1-24
Type of stroke (hemorrhagic/ ischemic), n	29/51	--
Fugl-Meyer lower limb score	23.7 ± 4.6	7-32
Paretic H-M ratio	0.39 ± 0.18	0-0.85
Non-paretic H-M ratio	0.25 ± 0.15	0-0.74
PASE score	114.4 ± 58.6	8.7-276.8
Comorbidities		
Hypertension, n	49	--
Diabetes mellitus, n	13	--
Hyperlipidemia, n	30	--
Total number of comorbidities, n	1.8 ± 0.9	--
Medications		
Antihypertensive, n	49	--
Antidiabetic, n	13	--
Anticonvulsants, n	32	--
Anticoagulants, n	31	--
Total number of medications, n	3.0 ± 1.7	--

[a]Means ± standard deviation presented unless indicated otherwise.

PASE: Physical Activity Scale for the Elderly

Table 3-2. Side-to-side comparisons of pQCT and muscle strength parameters

Parameter	Mean ± SD		%SSD
	Paretic	Non-Paretic	
pQCT variables			
Total Area (mm^2)	608.17 ± 115.24	608.97 ± 115.25	0.04 ± 3.94
Cortical BMD (mg/cm^3)	1095.20 ± 40.69	1104.19 ± 38.65**	0.81 ± 1.54
Cortical Area (mm^2)	246.30 ± 51.77	259.35 ± 49.82**	5.18 ± 6.40
Cortical BMC (mg/mm)	2.71 ± 0.61	2.87 ± 0.58**	5.94 ± 6.95
Marrow Cavity Area (mm^2)	361.87 ± 93.00**	349.63 ± 88.95	-3.89 ± 7.34
p-SSI (mm^3)	2164.44 ± 605.58	2277.68 ± 600.54**	5.19 ± 7.01
Leg Muscle Strength			
Isometric peak torque (Nm)	145.33 ± 65.66	221.63 ± 76.80**	34.04 ± 19.73
Concentric 60°/ second (Watt)	51.65 ± 27.02	87.68 ± 37.36**	39.12 ± 21.04
Concentric 120°/ second (Watt)	59.85 ± 30.22	112.49 ± 45.23**	44.26 ± 21.18
Eccentric 60°/ second (Watt)	135.11 ± 59.07	224.73 ± 72.14**	39.75 ± 17.11
Eccentric 120°/ second (Watt)	183.80 ± 75.65	286.80 ± 87.60**	34.86 ± 18.66

**$p \leqslant 0.001$ Statistically significant difference (paired t-test, 2-tailed)

%SSD = percent side-to-side difference, p-SSI = polar Stress-Strain Index, Cortical BMD = Cortical Bone Mineral Density, Cortical BMC = Cortical Bone Mineral Content

Table 3-3. Correlations between percent side-to-side difference in p-SSI and other variables

		r	p
Controlling variables	H-reflex (Paretic)	0.209	0.063
	H-reflex (Non-Paretic)	-0.024	0.631
	Body mass index	0.247*	0.027
	Height	-0.161	0.152
	Weight	0.093	0.412
	Fugl-Meyer Assessment	-0.232*	0.038
	Modified Ashworth Scale	-0.021	0.855
	Physical Activity Scale for the Elderly	-0.109	0.344
	Age	0.069	0.546
	Stroke Duration	-0.088	0.436
Composite Muscle Strength (%SSD)	Isometric peak torque	0.338**	0.002
	Concentric 60°/second	0.362**	0.001
	Concentric 120°/second	0.309**	0.005
	Eccentric 60°/second	0.282*	0.011
	Eccentric 120°/second	0.315**	0.004

* $p \leq 0.05$ Statistically significant correlation (Pearson's r, 2-tailed)

** $p \leq 0.01$ Statistically significant correlation (Pearson's r, 2-tailed)

Table 3-4. Regression models for predicting percent side-to-side difference in polar stress-strain index

Predictor	F	R^2	B	95%CI		Beta	p
				Lower	Upper		
Model 1							
Sex (Male=0, Female=1)			3.779	0.762	6.797	0.268	0.015*
Age			0.124	-0.055	0.303	0.143	0.171
BMI			0.587	0.133	1.042	0.270	0.012*
Stroke Duration	3.465	0.205	-0.165	-0.450	0.119	-0.126	0.251
FMA			-0.053	-0.427	0.322	-0.035	0.780
PASE			-0.009	-0.034	0.015	-0.078	0.456
H-reflex (Paretic)		0.242	6.232	-1.918	14.383	0.160	0.132
Isometric peak torque (%SSD)		**0.281**	**0.088**	**-0.002**	**0.178**	**0.249**	**0.054**
Model 2							
Sex (Male=0, Female=1)			4.597	1.624	7.570	0.326	0.003*
Age			0.160	-0.017	0.336	0.183	0.075
BMI			0.594	0.154	1.035	0.273	0.009*
Stroke Duration	4.129	0.205	-0.110	-0.385	0.166	-0.084	0.430
FMA			0.063	-0.316	0.442	0.042	0.741
PASE			-0.008	-0.032	0.016	-0.068	0.507
H-reflex (Paretic)		0.242	5.386	-2.592	13.364	0.138	0.183
Con60-Power (%SSD)		**0.317**	**0.121**	**0.035**	**0.208**	**0.364**	**0.006***
Model 3							
Sex (Male=0, Female=1)			4.372	1.373	7.370	0.310	0.005*
Age	3.772	0.205	0.124	-0.053	0.301	0.143	0.166
BMI			0.696	0.248	1.143	0.320	0.003*
Stroke Duration			-0.127	-0.406	0.151	-0.097	0.365

FMA			-0.020	-0.385	0.346	-0.013	0.914
PASE			-0.010	-0.034	0.015	-0.083	0.423
H-reflex (Paretic)		0.242	5.404	-2.739	13.547	0.139	0.190
Con120-Power (%SSD)		**0.298**	**0.098**	**0.016**	**0.180**	**0.296**	**0.020***
Model 4							
Sex (Male=0, Female=1)			3.933	0.868	6.998	0.279	0.013*
Age			0.120	-0.062	0.303	0.138	0.193
BMI			0.613	0.152	1.075	0.282	0.010*
Stroke Duration	3.052	0.205	-0.111	-0.399	0.178	-0.084	0.448
FMA			-0.160	-0.518	0.198	-0.106	0.375
PASE			-0.009	-0.034	0.017	-0.071	0.502
H-reflex (Paretic)		0.242	6.689	-1.641	15.019	0.172	0.114
Ecc60-Power (%SSD)		**0.256**	**0.058**	**-0.042**	**0.157**	**0.141**	**0.251**
Model 5							
Sex (Male=0, Female=1)			4.138	1.087	7.190	0.294	0.009*
Age			0.111	-0.070	0.293	0.128	0.226
BMI			0.556	0.087	1.025	0.256	0.021*
Stroke Duration	3.245	0.205	-0.078	-0.370	0.214	-0.060	0.595
FMA			-0.140	-0.487	0.206	-0.093	0.423
PASE			-0.008	-0.033	0.017	-0.070	0.509
H-reflex (Paretic)		0.242	6.915	-1.231	15.061	0.178	0.095
Ecc120-Power (%SSD)		**0.268**	**0.071**	**-0.018**	**0.161**	**0.189**	**0.118**

* $p < 0.05$ Statistically significant

B = Unstandardized regression coefficient, Beta = Standardized regression coefficient, 95%CI = 95% confidence interval, BMI = Body Mass Index, FMA = Fugl-Meyer Assessment, PASE = Physical Activity Scale for the Elderly, Con60 = concentric phase at 60°/second, Con120 = concentric phase at 120°/second, Ecc60 = eccentric phase at 60°/second, Ecc120 = eccentric phase at 120°/second

Table 3-5. Information criterion based model comparison

	AIC	BIC	ΔAIC	ΔBIC
Model 1	531.21	555.34	-	-
Model 2	527.02	550.84	2.23	2.23
Model 3	529.25	553.07		
Model 4	533.93	557.75	-	-
Model 5	532.65	556.47	-	-

ΔAIC = Akaike information criterion value difference, ΔBIC =

Bayesian information criterion value difference

3.5 Discussion

3.5.1 Side-to-side difference in bone strength index

Our findings suggest that the p-SSI of the tibial diaphysis on the paretic side was significantly lower than that on the non-paretic side. This was primarily due to the side-to-side differences in BMD, cortical bone mass and area, but not total area (Table 3-2). These findings are consistent with what were previously reported for the tibial diaphysis in people with chronic stroke [22, 49], and indicative of possible endosteal resorption.

3.5.2 Concentric strength at moderate speed is more strongly associated with bone strength index

An important finding of this study is that concentric muscle contraction power (models 2 and 3 in Table 3-4) was more associated with the %SSD of p-SSI than isometric muscle strength (model 1). Animal studies have revealed that dynamic loads are more effective than static loads in inducing bone formation [73, 105, 171]. The comparatively stronger association with concentric over isometric muscle contraction observed in our study confirms our initial hypothesis in this regard. A previous human study has also demonstrated a significant correlation between dynamic concentric muscle contraction power and bone strength index measured at the tibial mid-shaft among older adults [66].

Our results also showed that the concentric leg muscle power (model 2 and 3) yielded a stronger association with %SSD in p-SSI than eccentric muscle power (models 4 and 5). Eng et al. found that eccentric torque production in the paretic side was less affected by stroke than concentric torque production [172]. This is also largely in line with our findings, particularly at a higher speed of 120°/s, where a greater strength deficit was observed with concentric contraction

(44.2%) compared to eccentric contraction (34.8%)(Table3-2). The relative preservation of eccentric muscle strength may partly explain why it yielded a weaker association with %SSD in p-SSI compared to concentric muscle contraction.

Concentric muscle power at an angular speed of 60°/s (model 2) had a slightly stronger association with %SSD in p-SSI than that at 120°/s (model 3). Therefore, it did not support our hypothesis that a greater contraction speed was more strongly associated with %SSD in p-SSI. Theoretically, high speed muscle contractions produce more rapid fluid flow in bone canaliculi, thereby providing a stimulus that may magnify mechanotransduction [75, 173]. Animal work has also shown that mechanical stimulation at higher frequencies is more osteogenic than that at lower frequencies [105]. Nevertheless, the difference in contribution of the concentric muscle power at 60°/s versus 120°/s is modest (R^2 change: 7.5% vs 5.6%). The relationship between muscle power measured at different contraction speeds and bone strength index may not be linear. Future studies may benefit by testing more angular speeds in order to more accurately extrapolate the effect of contraction speed on the bone outcomes among people with stroke.

3.5.3 Clinical and research implications

Our findings may have important implications in designing muscle strength training programs for individuals with stroke. It is known that resistance training is effective in maintaining or enhancing bone health in different populations, including post-menopausal women [174, 175] and older adults [176-178]. A previous study [48] found beneficial effects on tibial bone architecture (using PQCT) and femoral neck BMD in people with chronic stroke as a result of a multi-dimensional exercise intervention which included a resistance training component. However, these studies did not specifically address the effectiveness of muscle strength training on bone outcomes. In addition, the issue concerning the type of strength training exercises

(isometric, concentric or eccentric contractions) was not considered. Our findings may help inform further research and clinical practice, particularly with regards to the approach and application of muscle strengthening in bone health management. Specifically, as concentric power at moderate speed shows the greatest deficit and demonstrates the strongest association with %SSD in p-SSI, more emphasis should be placed on concentric muscle training at similar speeds in the overall resistance exercise training protocol in order to enhance bone health post-stroke. This hypothesis will require further study.

3.5.4 Limitations

This study was a cross-sectional design and does not provide information on the changes in bone outcomes over time. While significant correlations between %SSD in p-SSI and muscle strength variables were found, cause-and-effect cannot be inferred. Our various regression models explained only 29.8-31.7% of the variance in %SSD of p-SSI, indicating that other potentially important factors underlying bone health post-stroke (e.g., nutrition) were under explored in the current study. Further studies should use a larger sample size and address the relationship between these factors and bone health in people with chronic stroke. There was a risk of recall bias with the use of the self-reported activity questionnaire used in the present study (i.e., PASE), although the reported activities undertaken by participants were limited to the previous 7 days. A more objective measurement of physical activity would strengthen future studies by providing information regarding the actual ambulatory and sedentary activities undertaken. Finally, it is possible that some of the participants may have undiagnosed osteoporosis. The purpose of excluding those who were taking osteoporosis medications was to minimize the influence of these medications on the results (e.g., bone properties). The possibility of having people with undiagnosed osteoporosis should not have major impact on the results,

especially when we were interested in %SSD, which was derived from comparing the two sides within the same individual. However, the results can only be generalized to individuals with stroke who have similar demographic and clinical characteristics to our sample (e.g., people not taking osteoporosis medications).

3.6 Conclusion

In summary, concentric muscle power was more predictive of %SSD in bone strength index at the tibial diaphyseal site than eccentric muscle power or isometric muscle strength in people with chronic stroke. Promoting concentric leg muscle power may be an important intervention strategy to improve or maintain lower extremity bone health post-stroke, and will need further investigation.

3.7 Citation

Material from: 'YANG Z, MILLER T, PANG MYC. RELATIONSHIP BETWEEN BONE STRENGTH INDEX OF THE HEMIPARETIC TIBIAL DIAPHYSIS AND MUSCLE STRENGTH IN PEOPLE WITH CHRONIC STROKE: INFLUENCE OF MUSCLE CONTRACTION TYPE AND SPEED, OSTEOPOROSIS INTERNATIONAL, published 2021, Springer'

4 Chapter 4: Effects of different vibration frequencies on muscle strength, bone turnover and walking endurance in chronic stroke

4.1 Abstract

This randomized controlled trial aimed to evaluate the effects of different whole body vibration (WBV) frequencies on concentric and eccentric leg muscle strength, bone turnover and walking endurance after stroke. The study involved eighty-four individuals with chronic stroke (mean age = 59.7 years, SD = 6.5) with mild to moderate motor impairment (Fugl-Meyer Assessment lower limb motor score: mean = 24.0, SD = 3.5) randomly assigned to either a 20 Hz or 30 Hz WBV intervention program. Both programs involved 3 training sessions per week for 8 weeks. Isokinetic knee concentric and eccentric extension strength, serum level of cross-linked N-telopeptides of type I collagen (NTx), and walking endurance (6-min walk test; 6MWT) were assessed at baseline and post-intervention. An intention-to-treat analysis revealed a significant time effect for all muscle strength outcomes and NTx, but not for 6MWT. The time-by-group interaction was only significant for paretic eccentric knee extensor work, with a medium effect size (0.44; 95% CI: 0.01, 0.87). Both WBV protocols were effective in improving leg muscle strength and reducing bone resorption. Comparatively greater improvement in paretic eccentric leg strength was observed for the 30 Hz protocol.

4.2 Introduction

Muscle weakness is a major impairment after stroke [179] and is associated with various aspects of physical functioning [180] and bone tissue integrity [50]. According to a recent systematic review [181], previous studies involving the use of bone imaging techniques such as

peripheral quantitative computed tomography (pQCT) [22, 24, 50] and dual-energy X-ray absorptiometry (DXA) [28] to investigate the impact of stroke on lower limb bone outcomes reported strong associations of muscle strength and mass with bone mineral density and indices of bone strength. Previous work has also demonstrated an increased rate of bone resorption in people with stroke, which was correlated with lower hip bone density [57, 182]. Therefore, effective interventions that target muscle strength and bone health are important for stroke rehabilitation.

Whole-body vibration (WBV) augments muscle activation during exercise [86, 100]. The mechanical vibration induces reflex muscle activation and increases motor cortex excitability [183, 184].WBV has also been shown to increase peak muscle torque in lower limb muscles [185], presumably through the recruitment of higher threshold motor units. Improved muscle contractility and force generating capacity have implications for bone health [75] as muscle contractions provide an important source of dynamic mechanical loading for maintaining bone tissue [75, 105]. There is evidence that WBV can reduce the rate of bone resorption in different populations (e.g., post-menopausal women, children with severe motor disabilities, and people with metabolic acidosis) [96, 186, 187].

WBV training has been identified as a potentially viable treatment modality in various patient groups with muscle weakness and consequent bone loss [84, 96, 97, 188], such as people after stroke [50, 103, 189, 190]. However, research on bone metabolism and muscle strength post-stroke after WBV intervention is scarce and the results are inconclusive [189-191]. Thus far, only one study has examined the effects of WBV on bone turnover in people with stroke, and found no significant change in both bone formation and resorption markers following an 8-week WBV intervention (9-15 min, 20-30 Hz) [60]. More research is needed before the use of WBV

for modifying bone turnover rate in people with stroke can be considered conclusive. A meta-analyses by Yang et al. demonstrated that the effects of WBV on maximal isometric knee extension strength (5 studies, SMD=0.23, 95%CI=-0.27 to 0.74, p=0.36), and maximal eccentric knee extension strength (2 studies, SMD=0.09, 95%CI=-0.38 to 0.56, p=0.71) yielded wide confidence intervals, indicating that the therapeutic value of WBV on improving knee muscle strength post-stroke requires further investigation [192].

Many factors may account for the discrepancies in results across previous studies in stroke (e.g., sample characteristics, WBV type, WBV frequency, treatment duration, etc.). As various studies differed on multiple factors, it was not feasible to delineate the effects of each factor by comparing the results of different studies. Nevertheless, among these factors, vibration frequency may be a particularly important parameter, as revealed by both animal and human studies. Animal studies have shown that higher frequency WBV can enhance osteogenesis more effectively than relatively lower frequency WBV [193]. In people with stroke, a greater level of leg muscle activation, as indicated by electromyography (EMG) findings, was found during exposure to higher WBV frequency (30 Hz) than lower frequency (20 Hz) [81, 100]. Therefore, repeated exposure to WBV of higher frequencies may lead to a greater strengthening effect of the muscles being stimulated. In a randomized controlled trial, Wei et al. showed that when controlling for the total number of vibrations, a 40 Hz frequency WBV protocol led to the best outcomes in terms of muscle size, strength and physical performance (i.e., 10-m walk test, timed-up-and –go, and sit-to-stand) in patients with sarcopenia [194, 195]. However, these findings are not necessarily generalizable to individuals with chronic stroke. Stroke-related impairments are heterogeneous in presentation, etiologically complex (compensatory movement patterns, learned disure, etc.) and are often inconsistent with typical muscle changes and performance deficits

associated with arthophy or aging alone [196, 197]. Only one study has compared the effects of two different WBV protocols in the same sample of people with stroke (20 Hz vs 30 Hz) and found difference in knee muscle strength after 10 weeks of intervention. However, the number of vibrations was not controlled and bone turnover was not measured [198].

To address these identified gaps in knowledge, we aimed to evaluate the effects of different WBV frequencies in stroke patients. In addition to leg muscle strength and bone turnover, the 6-min walk test (6 MWT), an indicator of walking endurance, was also used an outcome. Leg muscle strength has demonstrated a strong association with 6MWT distance in individuals with stroke. Therefore,any WBV-induced improvement in leg muscle strength was also thought to result in better walking endurance. We hypothesized that a higher WBV frequency (30 Hz) would induce larger improvements in muscle strength and walking endurance, and greater reduction in the level of bone resorption marker compared with a lower WBV frequency (20 Hz).

4.3 Methods

4.3.1 Study design

A single-blinded, randomized controlled trial was conducted.

4.3.2 Ethical approval

This study was registered on 06/11/2019 in clinicaltrials.gov (identifier: NCT03982251). Ethical approval for the study was granted by the Human Research Ethics Subcommittee of the Hong Kong Polytechnic University (reference number: HSEARS 20140226001-03), and all of the experiments were conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from each participant prior to data collection.

4.3.3 Participants

This study was conducted in a research laboratory at the University. Participants were recruited from a stroke patient organization in the community via convenience sampling. The screening and enrolment of potential participants were performed by an independent researcher.

The inclusion criteria were as follows: (1) a diagnosis of stroke with onset of 6 months or more, (2) patient age ≥ 50 years, (3) medically stable, (4) able to stand for at least 1 min with hand support, (5) able to walk > 10 meters without physical assistance from other people (with or without walking aids), and (6) able to understand simple verbal commands. Only individual aged 50 years or more were recruited. It was because stroke is more prevalent in older adults [199]. Setting an age limit would make the sample more homogeneous in terms of age thereby reducing the potential confounding effect of age on primary outcomes (i.e., muscle strength, bone turnover). The exclusion criteria were as follows: (1) additional neurological conditions, (2) musculoskeletal conditions affecting leg muscle performance (e.g., rheumatoid arthritis), (3) presence of metal implants in the lower extremity, (4) recent fracture in the lower extremity (within 1-year post-onset), (5) receiving medications to treat osteoporosis, (6) vestibular disorders, (7) peripheral vascular disease, and (8) other serious illnesses or contraindications to exercise.

4.3.4 Participant allocation

The participants were randomly allocated to one of two groups: a low frequency WBV group (frequency: 20 Hz; amplitude: 0.60 mm) or a relatively higher frequency WBV group (frequency: 30 Hz; amplitude: 0.60 mm). The allocation (with a 1:1 ratio) was completed by an off-site researcher who was not involved in other aspects of the trial, using an online

randomization program (http://rct.mui.ac.ir/q/). Prior to randomization, participants were stratified into three clinically meaningful groups according to walking speed (household amulators: <0.4m/s; limited community ambulators:0.4-0.8m/s; community ambulators: >0.8m/s) and sex [200]. These variables were used for stratification because they were shown to be associated with muscle strength and bone status [36, 49, 50, 201-203]. The stratified random allocation would ensure that the 20 Hz and 30 Hz groups were similar in terms of walking function and proportion of men/women. The reporting of results and procedures was done in accordance with the Consolidated Standards of Reporting Trials (CONSORT) guidelines. A diagram outlining the flow of participant screening, randomization and allocation is provided in Figure 4-1.

4.3.5 Intervention protocol

The two groups of participants completed training sessions 3 days a week for 8 weeks. A make-up session was provided for any missed appointments so that all participants eventually completed 24 training sessions. The duration of the intervention was based on a previously published study that reported a positive effect of a similar training dosage on bone turnover in post-menopausal women [96]. There is no established WBV protocol for enhancing muscle strength and bone health in people with stroke, and no stroke study has specifically examined the effect of WBV interventions involving different frequencies. Stroke patients also share similar bone health problems to those associated with post-menopausal women (i.e., increased rate of bone resorption and compromised bone density). Therefore, it is reasonable to take reference from this study on post-menopausal women when we developed our WBV protocol. WBV-induced changes in muscle strength and bone turnover marker levels were expected to be evident within this time frame [188, 204-207].

In each training session, the participants first performed warm-up exercises for ~10 min, which included general mobilization and upper limb stretching exercises performed in a sitting position. A Jet-Vibe System (Danil SMC Co. Ltd., Seoul, Korea) was then used to deliver the WBV. This device delivers synchronous vertical vibrations over a range of frequencies (20-55 Hz), which were adjusted by the researchers. Synchronous vertical WBV was used because it was thought to provide better stability during WBV exercise as it produces only vertical perturbations in comparison to both vertical and horizontal displacements associated with side-alternating or oscillating WBV [208, 209]. Frequencies >30 Hz were not used. The pilot data showed that high frequencies caused discomfort in this population. Frequencies <20 Hz were not used due to potential resonance [210] and sensorimotor coordination effects [211].

During the WBV treatment, the participants were instructed to remove their shoes and stand on the vibration platform with their feet placed a shoulder-width distance apart. Participants were instructed to flex the knee to 60° while standing on the vibration platform. This specified joint angle was chosen to reduce undesirable transmission of vibration to the head [212, 213]. Based on the results of a previous study, knee extensor muscle EMG activity during WBV exercise was shown to be greatest at 60° of knee joint flexion compared to 10° and 30° [100]. This angle was also determined to be safe and feasible during pilot testing. Participants were also asked to report any symptoms of pain or abnormal discomfort during WBV sessions. To facilitate a meaningful comparison and to delineate the effects of the WBV frequency, the number of loading cycles was matched between the 20 Hz and 30 Hz frequency groups. For both groups, exposure to vibration was provided in 1-min bouts, with a 1-min rest period between bouts. Twelve WBV bouts were delivered per training session to the 20 Hz frequency group, whereas 8 WBV bouts were delivered per training session to the 30 Hz frequency group (i.e., 14,400 loading cycles) so that

the total WBV dosage for each session was equivalent between groups. For standardization, all participants gently held onto the handrail of the WBV device only to maintain balance.

4.3.6 Outcome measures

Researchers who were blinded to the intervention groups conducted all of the outcome assessments. Relevant demographic information and clinical history were obtained from all participants through interviews conducted at baseline. During the baseline assessment session, the level of motor impairment of the leg and foot was evaluated using the Fugl-Meyer Motor Assessment (FMA) [162]. It is a 17-item scale with each item rated on a 3-point ordinal scale (0-2). Hemiparesis was considered to be present if one was unable to attain the maximum FMA score of 34 [162]. The Physical Activity Scale for the Elderly (PASE) [161] was used to measure participant physical activity level. Scores were calculated using weights and frequency values corresponding to the type of physical activity being assessed (e.g., leisure, occupational activity). Higher scores suggest greater daily physical activity. The spasticity of the paretic ankle joint was examined using the Modified Ashworth Scale (MAS) [214]. All of the following outcomes were assessed at baseline and also the end of the eight-week intervention period.

4.3.6.1 Isokinetic knee muscle strength

Participants underwent knee muscle strength testing on both sides using an isokinetic dynamometer (HUMAC ®NORMTM Testing & Rehabilitation System, Computer Sports Medicine Inc., U.S.A.), which provided good reliability of strength measurements (ICC = 0.89-0.96) [163]. In brief, participants maintained an upright sitting position while the knee joint was aligned with the mechanical axis of the dynamometer. Straps were used to stabilize the untested limb. Each participant was then instructed to perform maximal concentric/eccentric knee

extension throughout the range of 10°-70° knee flexion on each side at a constant angular speed of 120°/s. This range of motion was chosen based on the experience gained in our pilot testing. Some individuals with stroke patients had limited hamstrings flexibility, and were not able to reach the 0° knee flexion (i.e., full knee extension) in a sitting position. Some individuals experienced some discomfort if the knee (particularly on the paretic side) was flexed to more than 80°, potentially indicative of degenerative joint changes. Therefore, to ensure safety, we used a range of motion between 10° and 70° of knee flexion. This relatively high angular speed of 120°/s was chosen for several reasons. First, it was a speed commonly used in previous stroke studies [215, 216]. Second, individuals with stroke typically demonstrated severe muscle weakness at higher contraction speeds [11]. Also, knee movements at high speeds are involved in daily activities. Previous work showed that during walking over a wide range of speeds (0.4-1.39m/s), the angular velocity during knee flexion in the swing phase, and that of knee extension during terminal swing, exceeded 120°/s [217]. As walking speed approached 1.0 m/s, the angular velocity of knee flexion during the loading response also approximated 120°/s. During the sit-to-stand movement, the knee joint angular velocity has also been shown to be roughly 120°/s during the extension phase [218]. The sequence of testing (i.e., paretic side versus non-paretic side, or type of contraction) was randomized to minimize the order effect. Three trials were recorded for each test condition and the total work (in Joules; J) value was obtained using customized software. Three trials were conducted to obtain the mean value for statistical analysis. The total work represents the accumulated torque output produced as the joint moves through a specified range of motion [219]. Therefore, the measurement of total work takes into account the ability of the muscle to maintain contraction at a certain strength level through the range of motion. The measure was thus considered by some researchers to be more reflective of muscle function and

strength during movement than peak torque [220-222]. The percent standard error of measurement (%SEM) values established for the peak torque were 19.2% (eccentric) and 21.4% (concentric), which is an appropriate index for detecting change in a group of people [223].

4.3.6.2 Bone resorption analysis

Serum cross-linked N-telopeptides of type I collagen (NTx) was chosen as a surrogate marker of bone resorption to evaluate the dynamic process of bone turnover. In brief, a 5 ml fasting blood sample was collected from all participants in the morning (between 0900 and 1100 h) at defined investigational time points. All of the blood samples were then promptly centrifuged. The serum was separated and then immediately frozen at -80°C until further analysis. Serum levels of NTx were assessed using the Osteomark® NTx Serum assay (Alere Scarborough, Inc., Scarborough, U.S.A.) according to the protocol provided by the manufacturer. Essentially, appropriately diluted serum samples, together with NTx epitope-containing molecules that are conjugated with horseradish peroxidase, were added to the microplate wells that had been previously coated with antibodies against NTx. NTx in the patient sample thus competed with the conjugated NTx epitopes in the microplate well for antibody binding sites. Following a wash step, a chromogenic substrate solution was added for colour development. Absorbance was determined on a spectrophotometer and the NTx concentration was calculated against a standard calibration curve. The assay values were recorded in nanomoles Bone Collagen Equivalents per liter (nM BCE). The reference range was between 3.2 and 40.0 nM BCE. In each assay, three duplicate samples were used to determine the intra-assay coefficient of variation (% CV) (intra-assay %CV = 4.6%). The inter-assay %CV was established between two assays of a total of 18 samples (inter-assay %CV = 6.9%). An intra-assay and inter-assay %CV value of less than 10% is considered to be acceptable [224].

4.3.6.3 6 Minute walk test

This test was used to assess endurance [225]. Participants were asked to walk along a 15-m walkway and cover as much distance as possible in 6 min, using walking aids if necessary. The total distance walked (in meters) was recorded. The 6MWT has excellent reliability (ICC = 0.97-0.99) in individuals with stroke [225].

4.3.6.4 Compliance and adverse events

Participant attendance of the training sessions was recorded by the researcher who supervised the WBV training sessions. Any adverse events reported by the participants or observed by the researcher were also documented. The total time period taken to complete 24 training sessions (number of days) and the maximum time lapse between any two training sessions (number of days) for each participant were used for subsequent analysis.

4.3.7 Statistical analyses

The sample size was estimated using G*Power 3.1.9.2 software (Heinrich-Heine-Universität Düsseldorf, Germany). Tihanyi et al. found that WBV induced a significant increase in paretic knee muscle strength (Cohen's *d*) of 0.46-0.51 (i.e., medium) in stroke patients [102]. Another study by Wei et al. showed that medium-frequency WBV generated better knee muscle strength outcomes than low-frequency outcomes in patients with sarcopenia ($d = 0.24$) [195]. Another study by Turner et al. showed that a WBV protocol similar to that used in the HWBV group induced a significant change in bone resorption marker levels, with a large effect size of 0.96 [226]. Overall, a small to medium effect size was assumed ($f = 0.2$) for a 2 × 2 analysis of variance (ANOVA) with repeated measures. With an alpha of 0.05, a power of 90%, and

considering an attrition rate of 15%, the minimum sample size required to detect a significant group × time interaction effect was 80 participants (40 per group).

The Statistical Package for Social Sciences version 23.0 (SPSS; IB, Armonk, NY) was used for all analyses. The normality of the data was checked using the Kolmogorov-Smirnov test. Between-group differences in baseline characteristics were evaluated by an independent t test, a Mann-Whitney U test or a chi-square test, as appropriate. To compare the treatment effect between the two groups, a mixed-design, multivariate analysis of variance was used (within-subject factor: time; between-subject factor: group). An intention-to-treat analysis was conducted, in which the last observation carried forward method was used to substitute the missing data for participants who were lost to follow-up (i.e. dropout). This approach was considered to be more conservative but was less susceptible to bias arising from attrition [227]. Post-hoc analyses were conducted to examine the pre-test and post-test within-group scores (paired t-tests), and also between-group differences in change scores (independent t-test). The above analyses were repeated after eliminating drop-outs (on-protocol analysis). If both the intention-to-treat and on –protocol analysis approaches yielded similar findings, there would be strong confidence in the study results [227].

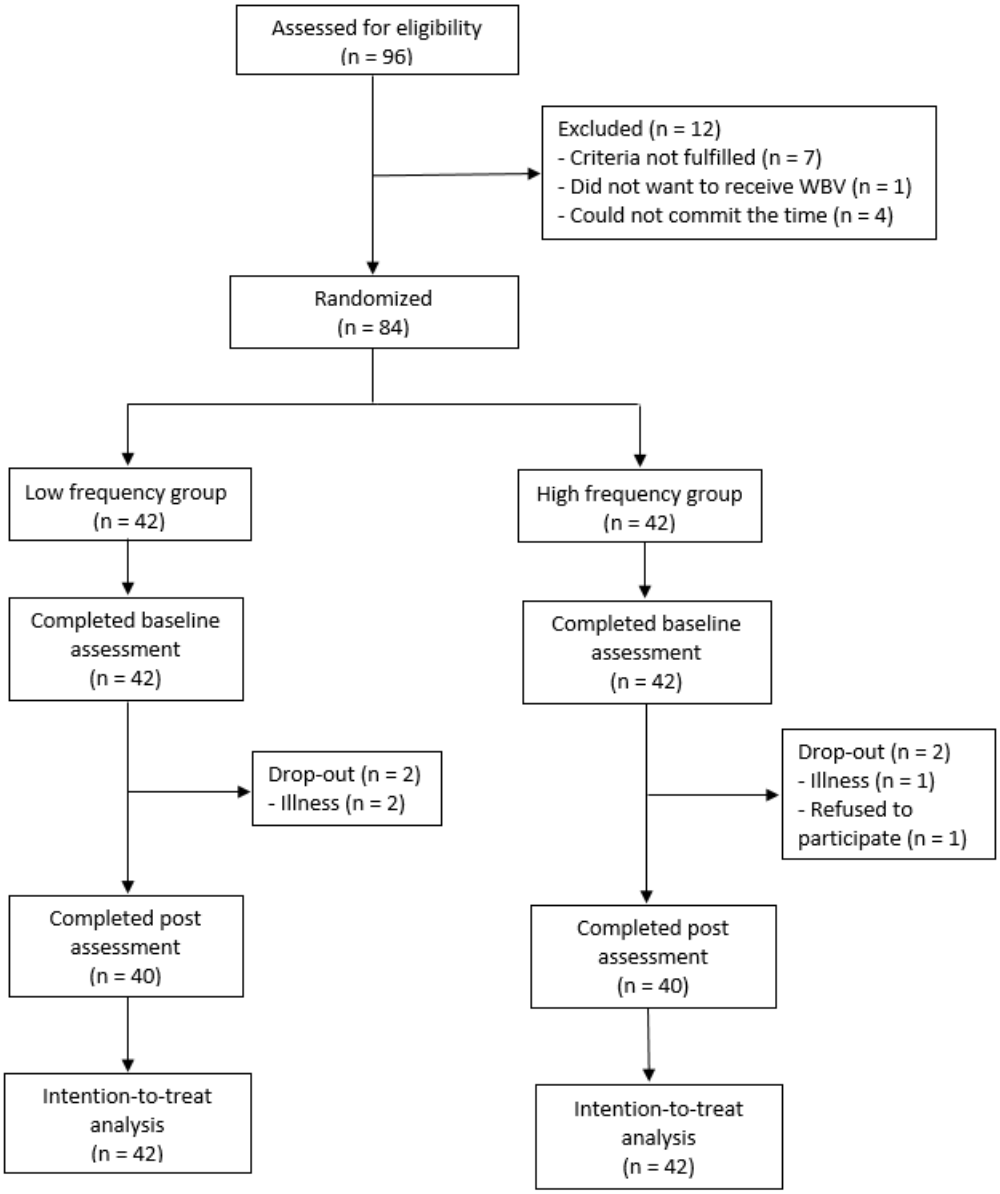
4.4 Results

4.4.1 Study group characteristics

Of the 96 individuals with stroke who were screened for eligibility, 84 fulfilled all of the selection criteria. This was greater than the estimated minimum number of participants required from our sample size calculation (n=80). As having a greater sample size could further increase statistical power, 84 individuals with stroke were ultimately enrolled in the study rather than 80.

They were randomly allocated to either the 20 Hz frequency (n = 42) or 30 Hz frequency (n = 42) groups. Four participants dropped out during the course of the study (2 in each of the treatment groups). By the end of the study, 80 participants had completed the training program and all outcome assessments (Figure 4-1). We found no significant between-group differences in terms of demographics variables or stroke characteristics at baseline (Table 4-1). Therefore, none of the variables shown in Table 4-1 were considered important confounding factors.

Figure 4-1. CONSORT flow diagram



4.4.2 Effect on outcome measures

The outcome measurements collected at baseline did not differ between the two groups (Table 4-2). We identified a significant main effect of time for all muscle strength and bone turnover outcomes ($p<0.001$), but not for the 6MWT outcome ($p=0.533$) (Table 4-2). We also identified a significant effect of time × group interaction for the paretic eccentric knee extensor work, with a medium effect size (0.44; 95% CI: 0.01, 0.87). The change of eccentric extensor work in the non-paretic leg also showed a similar trend, but the confidence intervals suggested a small chance that the 20 Hz frequency protocol might be superior (95% CI: -0.08, 0.78). The on-protocol analysis generated similar results (Table 4-2).

4.4.3 Compliance and adverse events

The time taken to complete 24 training sessions and the maximum time lapse between any two training sessions were similar between the two groups ($p>0.05$). No adverse events occurred during the intervention trial.

Table 4-1. Participant characteristics at baseline*

Characteristic	All (n = 84)	20Hz WBV (n = 42)	30Hz WBV (n = 42)	*p*†
Demographics				
Age (years)	59.7 ± 6.5	60.4 ± 5.9	59.0 ± 7.0	0.299
Sex (men/women)	54/30	29/13	25/17	0.362
Body mass index (kg/m^2)	23.5 ± 3.3	23.0 ± 3.1	24.0 ± 3.4	0.203
Walking aid: none/cane/quad/frame	68/13/3/0	35/6/1/0	33/7/2/0	0.791
PASE score	95.1 ± 50.3	97.2 ± 57.0	93.1 ± 43.2	0.715
Walking speed:<0.4m/s (n)	8	4	4	—
Walking speed:0.4-0.8m/s (n)	30	15	15	—
Walking speed:>0.8m/s (n)	46	23	23	—
Stroke characteristics				
Hemiparesis side, n (right/left)	37/47	17/25	20/22	0.510
Post-stroke duration (years)	4.6 ± 3.5	4.6 ± 3.7	4.5 ± 3.4	0.951
Type of stroke, n (hemorrhagic/ischemic)	40/44	17/25	23/19	0.190
Fugl-Meyer lower limb score	24.0 ± 3.5	24.6 ± 2.8	23.2 ± 4.1	0.060
Paretic ankle MAS score (0-4)	1.0 (0-4)‡	1.0 (0-4)‡	1.0 (0-4)‡	0.075
Comorbidities, n				
Hypertension	54	28	26	0.649
Diabetes mellitus	15	8	7	0.776
Hyperlipidemia	32	17	15	0.653
Number of comorbidities	1.7 ± 0.8	1.6 ± 0.9	1.8 ± 1.0	0.495
Medications, n				
Antihypertensives	54	28	26	0.649

Antidiabetic medications	15	8	7	0.776
Anticonvulsants	35	18	17	0.825
Anticoagulants	31	16	15	0.821
Number of medications	3.0 ± 1.8	3.1 ± 1.7	2.9 ± 1.8	0.462
Compliance				
Time taken to complete 24 training sessions (d)	65.4 ± 3.1	65.3 ± 3.2	65.5 ± 3.1	0.784
Maximum time lapse between training sessions (d)	8.0 ± 2.2	7.8 ± 2.3	8.1 ± 2.1	0.527

[*]Mean ± standard deviation presented unless indicated otherwise

[†]Between-group comparison

[‡]Median (interquartile range)

Abbreviations: 20Hz WBV: 20Hz whole-body-vibration group, 30Hz WBV: 30Hz whole-body-vibration group, PASE: Physical Activity Scale for the Elderly, MAS: Modified Ashworth Scale

Table 4-2. Outcome measurements

Variable	20Hz WBV (N = 42)			30Hz WBV (N = 42)			Between-group difference in change scores	Comparisons					
	Pre	Post	Change score	Pre	Post	Change score	Mean (95% CI)	p^a	p^b	p^c	p^d	p^e	p^f
Knee extensor work (J)													
Nonparetic concentric*‡§	28.1±11.1	35.8±17.4	7.8±12.4	23.5±11.1	36.3±23.4	12.8±17.6	5.0 (-1.6, 11.7)	0.061	0.000	0.135	0.000	0.000	0.135
Paretic concentric *‡§	17.4±8.0	22.4±9.0	5.0±5.1	15.3±8.2	21.0±10.0	5.7±4.8	0.7 (-1.4, 2.9)	0.242	0.000	0.496	0.000	0.000	0.496
Nonparetic eccentric *‡§	80.3±20.4	94.5±22.9	14.2±13.4	75.1±19.9	95.5±30.2	20.4±21.0	6.2 (-1.5, 13.8)	0.243	0.000	0.113	0.000	0.000	0.113

Paretic eccentric *†‡§¶	65.2±21.2	73.0±18.7	7.8±11.2	60.2±23.3	74.0±21.6	13.8±15.5	5.9 (0.05, 11.8)	0.311	0.000	0.048	0.000	0.000	0.048
Other Outcomes													
NTx (nM BCE)* ‡§	6.1±3.7	3.6±2.3	-2.2±3.4	6.3±4.2	3.6±1.8	-2.7±4.0	-0.5 (-2.1, 1.1)	0.792	0.000	0.540	0.000	0.000	0.540
6MWT distance (m)	248.7±89.8	250.6±90.3	1.9±11.9	257.2±111.4	256.8±112.5	-0.4±11.0	-2.3 (-7.3, 2.7)	0.701	0.533	0.365	0.302	0.835	0.365

*Significant time effect ($p<0.05$)

†Significant group × time interaction effect ($p<0.05$)

‡Significant within-group comparison (20Hz WBV group) ($p<0.05$)

§Significant within-group comparison (30Hz WBV group) ($p<0.05$)

¶Between-group comparison of change score ($p<0.05$)

[a]Baseline comparisons (independent t-test)

[b]Time effect (ANOVA)

[c]Group × time interaction effect (ANOVA)

[d]Within-group comparison (20Hz WBV group) (paired t-test)

[e]Within-group comparison (30Hz WBV group) (paired t-test)

[f]Between-group comparison of change score (independent t-test)

Abbreviations: 20Hz WBV: 20Hz whole-body-vibration group, 30Hz WBV: 30Hz whole-body-vibration group, CI: confidence interval, NTx: serum cross-linked N-telopeptides of type I collagen, 6MWT: 6-minute walk test

4.5 Discussion

The key finding is that both the 20 Hz and 30 Hz WBV protocols are effective in increasing knee muscle strength and reducing bone resorption, but the former is better at improving the paretic eccentric knee extensor strength than the latter.

Leg muscle activity measured by EMG can be augmented during WBV exposure [228-230]. Through regular WBV intervention, the stimulated muscles are repetitively "exercised". Over time, this may contribute to greater muscle strength [75]. Our study data confirm that leg muscle strength can be increased following regular WBV intervention over an 8-week period. Improved muscle coordination [102], and enhancement of intramuscular blood perfusion [231] are some of the proposed mechanisms underlying improved muscle strength following WBV reported in studies involving neurological populations. Other mechanisms associated with improved strength following WBV reported in healthy subjects include increased cortical excitability [183], reduced recruitment threshold, increased activation of fast-twitch muscle motor units [232], and motor unit reflex activation [209]. Overall, our findings are largely in line with previous studies showing increased muscle strength in elderly populations, both with and without sarcopenia, following WBV exercise [194, 233]. However, the positive improvement in muscle strength reported in this study cannot be attributed to the WBV stimulation alone. The participants assumed a static, semi-squatting posture (i.e., 60°of knee flexion) during WBV exposure which may have also contributed to the observed increase in muscle strength.

An interesting finding of our study is that the 30 Hz frequency protocol induced a greater gain in eccentric knee extension strength in the paretic leg. The mean increase in eccentric knee strength attained by the 30 Hz frequency group was 22.9%, which exceeded the %SEM value (i.e. 19.2%). Our results thus suggest that the 30 Hz frequency protocol produced a clinically

meaningful change in muscle strength. These results are accordant with a previous study that found a 30 Hz WBV frequency to be optimal for improving muscle strength among healthy adults [234]. Compared to relatively lower (20 Hz) and higher WBV frequencies (60 Hz), Wei et al. found that a 40 Hz frequency produced the largest improvement in isokinetic knee extension strength among older adults with sarcopenia [235]. Therefore, it seems that specific frequencies for producing optimal outcomes are different for various patient populations.

In our study, the eccentric strength results for the non-paretic leg also showed a similar trend to the paretic leg. However, the confidence intervals (-0.08, 0.78) indicate a slight probability that the 20 Hz frequency protocol might be superior to the 30 Hz frequency protocol. Baseline paretic muscle strength was also substantially lower than the non-paretic side (Table 4-2), suggesting there was more room for improvement in the former.

Only one randomized controlled study by Liao et al. has attempted to compare WBV protocols for stroke patients [198]. However, the addition of low-intensity (peak acceleration: 1.6 *g*) or high-intensity (3.6 *g*) WBV in the exercise protocol used in their trial did not improve concentric or eccentric muscle strength outcomes [198]. Differences in the WBV protocol [i.e., smaller (10°) knee flexion angle during WBV exercise, lower number of total bouts per session (2 for 20 Hz, 3 for 30 Hz), longer bout duration (1.5 min), and higher vibration amplitude (1 mm) used in the study by Liao et al.] may provide an explanation for this finding. The authors also compared WBV intensities but were unable to delineate the effect of frequency because of differences in the number of loading cycles involved in different treatment arms [198].

Both groups improved in concentric muscle strength on both sides after the intervention period but no significant between-group differences were found. Concentric muscle strength is

typically more compromised than eccentric muscle strength after stroke [179], and may require more intensive training to elicit a more pronounced difference in improvements between the two WBV protocols. This theory now requires further research.

Our results show that both the 20Hz and 30Hz frequency protocols promoted a significant reduction in the expression of NTx indicating that WBV exercise was beneficial in reducing the rate of bone resorption among chronic stroke patients. The amount of reduction in NTx was similar between the two groups (20Hz frequency: 36%, 30Hz frequency: 43%) and was not statistically significant. Perhaps a larger differential in WBV frequency and a larger sample size would be required to detect a significant between-group difference in reduction of NTx levels. Only one previous study has investigated the effects of WBV on bone turnover (indicated by C-telopeptide of type I collagen cross linking and bone-specific alkaline phosphatase levels) in people with stroke [189]. The level of these bone turnover markers showed no significant change after the 8-week intervention period (24 sessions) for both the WBV and control groups. The disparity in results may be related to the difference in WBV protocols [189]. In the previous study, the number of loading cycles was gradually increased as the training program progressed, and did not reach a level similar to the present study until week 5. The less intensive WBV stimulation in the initial period of the intervention program may partly explain why no significant change in bone resorption marker level was reported in their study [189]. As there was no sham WBV or no-intervention control group in this study, the difference in the change of NTx levels after WBV intervention versus a sham intervention is unknown. Previous work has shown that individuals with stroke have a higher level of bone resorption than their counterparts without a history of stroke [57]. Longitudinal studies are required to examine the temporal changes in bone resorption marker levels.

The two treatment protocols did not induce any significant change in the 6MWT distance. Apart from muscle strength, aerobic capacity has also been identified as a limiting factor related to 6MWT performance (i.e., walking endurance) among people with stroke [201, 236-238]. Previous results indicated that exposure to WBV produce only modest changes in cardiovascular parameters, such as heart rate and blood pressure [239]. This may explain, in part, why our intervention programs did not lead to any significant change in 6MWT distance.

This study has several limitations that should be noted. First, the findings cannot be generalized to those who are in the acute or sub-acute stages of stroke recovery, or to those who are wheelchair-bound or have a severe motor impairment. Second, while it is unlikely that age is an important confounding factor in this study due to the lack of significant between-group difference (Table 4-1), how younger stroke patients might respond to the two WBV frequencies remains to be investigated. Third, we did not measure the level of bone formation markers. Incorporating a bone formation maker in our study would have provided a more comprehensive evaluation of the effect of our WBV protocols on bone turnover. Fourth, only synchronous vertical vibrations were used in our study. It may not be meaningful to make a direct comparison between the results of this study with other using oscillating (side-alternating) vibrations as other factors also differed studies (i.e., participant demographics, body position, external load) [208]. Whether using side-alternating vibrations may result in greater muscle strength improvement in stroke patients will require further investigation. Finally, whether the beneficial effects can be sustained after the cessation of treatment also remains to be determined.

The results showed that while both the 20 Hz and 30 Hz WBV frequency protocols increased concentric and eccentric knee muscle strength and reduced bone resorption rate, the 30 Hz frequency protocol was more effective than the 20 Hz frequency protocol in improving

eccentric knee extension strength on the paretic side after treatment cessation. Therefore, a frequency of 30Hz may be more appropriate for enhancing leg muscle strength, with possible implications for maintaining bone health among individuals with chronic stroke.

4.6 Citation

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5 Chapter 5: Conclusions

5.1 Summary of findings

Secondary osteoporosis and fragility fractures are important health concerns among stroke survivors. The research work contained in this thesis aimed to investigate the relationship between muscle function and bone quality, and the therapeutic value of WBV on muscle strength and bone metabolism among individuals with stroke.

The systematic review reported in chapter 2 consolidated the knowledge on the characteristics (i.e., magnitude, time course, site-specific differences) of bone changes after stroke, and demonstrated that the rate of changes in bone mass and macrostructure on the paretic side was slower in the chronic than sub-acute period of stroke recovery, and that the paretic upper limb exhibited more compromised bone properties compared with the paretic lower limb. Additionally, it was also found that there was a strong relationship between muscle strength/power and bone quality in the upper and lower limbs, and muscle spasticity seemed to have negative impact on bone integrity in the paretic upper limb, but its influence on the paretic lower limb was uncertain. Based on the finding of the systematic review, there should be a greater emphasis on early intervention to prevent exaggerated bone loss post-stroke. Strategies to enhance muscle mass/strength should be explored as a potential intervention method to promote bone quality in individuals with stroke.

Chapter 3 reported on a cross-sectional study which evaluated the association of bone strength index at the tibial diaphysis with muscle strength measured during different types of muscle contraction (i.e., isometric, concentric, eccentric) and at different contraction speeds. The results

demonstrated that concentric muscle contraction power was the most import determinant of bone strength index measured at the tibial diaphyseal site.

Whole-body vibration (WBV), which has gained increasing popularity in rehabilitation and clinical practice and research, may have the potential effect on the properties of the muscle-bone unit in people with stroke, due to its reported effects on muscle-bone unit in other population, such as older adults. In the final study of this thesis (Chapter 4), a randomized controlled trial was conducted to investigate the effect of two different WBV frequencies on leg muscle strength and rate of bone resorption after stroke. The results showed that while both the high frequency WBV (HWBV; frequency=30 Hz) and low frequency WBV (LWBV; frequency=20 Hz) protocols induced a significant increase in concentric and eccentric knee muscle strength and reduction in rate of bone resorption, the HWBV protocol was more effective than an LWBV protocol in improving eccentric knee extension strength on the paretic side following 8 weeks of training. Overall, for clinical application, the thesis indicates that WBV is a safe training modality in people with chronic stroke, and a frequency of 30 Hz should be the more appropriate choice for enhancing leg muscle strength in individuals with chronic stroke.

5.2 Future research directions

The work arising from the thesis form the basis of future research in the field of post-stroke bone health. Firstly, the systematic review in Chapter 2 revealed that the impact of stroke on micro-structural properties of bone is unknown (e.g., trabecular thickness and spacing) and should require future studies using high-resolution pQCT. More research using a longitudinal design is also required to identify the trend of changes of different bone parameters over time and the related predictors. Secondly, based on the stronger relationship between concentric muscle power and tibial bone quality identified in Chapter 3, promoting concentric muscle power

may be an important rehabilitation strategy to enhance or maintain lower extremity bone health among chronic stroke survivors, but further investigation would be required to test this hypothesis. While the randomized controlled study in Chapter 4 showed that WBV of higher frequency (30Hz) was more effective in increasing eccentric muscle power and reducing rate of bone resorption, it is not known whether WBV training of a longer term (i.e., more than 6 months) is beneficial in maintaining or enhancing bone quality. Further work is required to examine the therapeutic effects of WBV on bone health in individuals with stroke.

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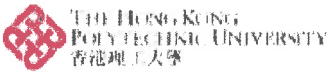
7 APPENDICES

7.1 *Ethical approval*



To	Pang Marco Yiu Chung (Department of Rehabilitation Sciences)
From	TSANG Wing Hong Hector, Chair, Departmental Research Committee
Email	rshtsang@ Date 07-Nov-2016

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-Apr-2014 to 30-Jun-2018:

Project Title:	The muscle-bone unit in people after chronic stroke: relationship to muscle contraction characteristics, spasticity and influence of vibration frequency.
Department:	Department of Rehabilitation Sciences
Principal Investigator:	Pang Marco Yiu Chung
Project Start Date:	01-Apr-2014
Reference Number:	HSEARS20140226001-03

Please note that it is the University's policy that all new research/teaching projects involving tests/trials on human subjects are required to take out appropriate insurance if deemed necessary by the Human Subjects Ethics Sub-committee. For such cases, investigators are not allowed to start any research/teaching projects involving tests/trials on human subjects if no appropriate insurance is or can be arranged.

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 the case of 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.

TSANG Wing Hong Hector

Chair

Departmental Research Committee

7.2 *Consent form*

The Hong Kong Polytechnic University
Department of Rehabilitation Sciences

Research Project Informed Consent Form

Project Title: The muscle-bone unit in people after chronic stroke: relationship to muscle contraction characteristics, spasticity and influence of vibration frequency.

Investigator(s):
Prof. Marco YC Pang (PhD), Professor, Department of Rehabilitation Sciences, The Hong Kong Polytechnic University.
Prof. Iris F.F. Benzie (PhD), Chair Professor, Department of Health Technology & Informatics, The Hong Kong Polytechnic University.
Dr. Raymond Chung (PhD), Scientific Officer, Department of Rehabilitation Sciences, The Hong Kong Polytechnic University.
Mr. Yang Zhenhui (MPhil), PhD student, Department of Rehabilitation Sciences, The Hong Kong Polytechnic University.

Project information: Increasing evidence has shown the close link between muscle mass/strength and integrity of bone tissue in people with stroke. Whole body vibration (WBV) has been found to improve bone health, postural control and muscle performance in the elderly and other patient populations. We would like to examine the relationship between various aspects of muscle function and bone properties in chronic stroke, and to compare how different WBV frequencies affect important aspects of the muscle-bone unit in chronic stroke.

You will have a series of clinical examinations on bone and muscle health. In addition, you may also be invited to participate in the WBV or physical exercise training.

Examination on Bone and Muscle Health
Except for bone imaging, you will undergo the following assessments at 3 different times at the Hong Kong Polytechnic University: (1) within one week before the training begins, (2) within one week after the 12-week intervention period ends, and (3) 12 weeks after termination of treatment. Each assessment will take a total of approximately 1.5~2 hours. Depending on your level of exercise tolerance, the evaluation may be done on two separate days. Intermittent rest periods will also be given in each assessment session if necessary to avoid fatigue. Bone imaging will be done only once within one week before the exercise training begins.

Bone imaging
You will undergo a bone scan called Peripheral Quantitative Computed Tomography (pQCT) to measure the bone density of your tibia bones on both sides. These procedures will take place at the Jockey Club Centre for Osteoporosis Care and Control. All the bone imaging procedures will be performed by the same technician who is well trained in performing the standardized scanning procedures. The bone imaging will take about 30 minutes.

Knee and Ankle Muscle Strength
We will measure how strong your muscles are. We will measure your bilateral knee and ankle muscle strength using an isokinetic dynamometer. When testing the knee muscles, you will be sitting comfortably in a chair. You will then be asked to maximally contract your knee muscles and hold the contraction for 5 seconds. When testing your ankle muscles, you will be lying on your back. You will then be asked to maximally contract your ankle muscles and hold the contraction for 5 seconds. Three trials will be performed on each side with a brief rest between trials.

H reflex
We will measure the H reflex with the Neuropack machine. When testing the H reflex, you will be lying in the prone position and your feet suspended over the end of the bed and your head resting on a pillow. Stimulating electrodes will be applied over the tibial nerve in the popliteal fossa, and active electrodes will be attached to the soleus or gastrocnemius muscles.

Balance control
We will evaluate your balance ability using a computerized system. You will stand on a platform. Unexpected external perturbations will be applied intermittently to test your balance reactions. To ensure safety, a suspended harness system will be used while standing on the platform.

Walking velocity
You will be required to walk along a walkway at your own self-selected and maximal walking velocities, using a walking aid as necessary.

Blood test
We will also collect a blood sample (about 6ml) from you to evaluate your bone metabolism. You should avoid consuming caffeine and alcohol on the day of testing. The blood sample will be sent to the Department of Health Technology and Informatics, the Hong Kong Polytechnic University for analysis.

Exercise Training
You will be randomly assigned to either the low or high intensity vibration groups, or the exercise group. If you are allocated to the low or high intensity vibration groups, you will receive WBV (up to 20 minutes of vibration per session, and 3 sessions per week) for 8 weeks. You will be instructed to perform a set of gentle leg exercises a few times. You can have a brief rest in between of each exercise. You will be informed of whether you have actually received the low-intensity or high-intensity vibration at the end of the study. If you are allocated to the exercise group, you will perform the same exercises on the platform, but no vibration will be given.

Benefits and risks of undertaking this study:
The major benefit from participating in this study is that you will have the opportunity to know the health status of your bone and muscle and you will receive exercise training. The results of this study will provide important information which will assist in the formulation of clinical guidelines for exercise prescription. Side-effects associated with WBV are extremely rare (e.g.

dizziness). The symptoms should subside following a brief rest period. If you experience some discomfort during exercise, you can request that the exercise be terminated. The radiation level associated with the leg bone scanning is extremely low (much lower than a chest X-ray) and should not cause any health hazards.

Confidentiality:

All information and data collected from this study will be treated in strict confidence. Your name and personal data will not be disclosed to anyone except the project investigators.

Consent:

I, _____, have been explained the details of this study. I voluntarily consent to participate in this study. I understand that I can withdraw from this study at any time without giving reasons, and my withdrawal will not lead to any punishment or prejudice against me. I am aware of any potential risk in joining this study. I also understand that my personal information will not be disclosed to people who are not related to this study and my name will not appear on any publications resulting from this study. I also understand that the video footage of me will be edited and used for educational purposes and for conference presentation.

I can contact the chief investigator, Prof. Marco Pang by telephone at 2766-7156 for any questions about this study. If I have complaints related to the investigator(s), I can contact Ms. Gloria Man, secretary of the Departmental Research Committee, at 2766-4394. I know I will be given a signed copy of this consent form.

Signature (subject): _____ **Date:** _____

Signature (witness): _____ **Date:** _____

香港理工大學康復治療科學系科研同意書

科研題目：關於慢性中風患者的肌肉骨骼單位研究：肌肉收縮特徵、痙攣和震動頻率 效果之間的關係

科研人員： 彭耀宗 教授 (香港理工大學康復治療科學系教授）
Iris F.F. Benzie 教授 (香港理工大學醫療科技及諮訊學系講座教授）
鐘志強 博士 (香港理工大學康復治療科學系科學主任)
楊振輝 先生 (香港理工大學康復治療科學系博士生)

科研內容：
過往研究顯示中風患者的肌肉質量/力量與骨骼組織完整性有密切關係。全身震盪訓練已被證實可有效改善長者和其他病患者的骨骼健康、姿勢控制和肌肉功能。這項研究旨在探討慢性中風患者的不同肌肉功能與骨組織之間的關係，並比較不同強度的全身震盪訓練對慢性中風病患的骨骼肌肉單位的作用。

你將會接受一些列的骨骼及肌肉方面的臨床檢查。此外，你亦可能將被邀請參加全身震或者體能訓練。

骨骼及肌肉檢查

你將在香港理工大學接受下列所有檢查(骨質掃描除外)。這些檢查共會進行三次，分別為：(1) 訓練開始前一周內； (2) 為期 12 周的訓練結束後的一周內； (3) 訓練中止後的第 12 周。完成每項檢查大約需要 1.5~2 小時。如果你感到疲憊，你可以在每項檢查期間進行休息。根據你的身體耐受情況，所有的檢查可被安排在兩天內完成。骨質掃描檢查只會進行一次，時間為運動訓練開始前約一周內。

骨質掃描
你的雙側下肢脛骨將會接受肢體定量計算機斷層掃描骨質密度儀 (pQCT) 的掃描，以量度骨質密度。這項檢查將會將會在香港中文大學賽馬會骨質疏鬆預防及治療中心進行。所有檢查將由一名經訓練的專業技術人員按規範操作程序進行，檢查需時約半小時。

膝部和足踝部肌肉力量
我們將會使用等速肌力測量器測試你的膝部和足踝部的肌肉力量。當測試膝部肌肉時，你將以舒適的姿勢坐在椅子上，然後以最大的力量收緊膝部大腿肌肉並維持 5 秒。當測試足踝肌肉時，你將以舒適的姿勢躺臥在床上，以最大的力量收緊足踝部肌肉並維持 5 秒。雙側下肢的每組肌肉力量測試需要重複量度 3 次，每次量度之間會 1 分鐘休息。

痙攣程度
我們將用與肌肉力量測試相同的等速肌力測量器和肌電圖儀評估你的患側膝部及足踝部肌肉的痙攣程度。等速肌力測量器的度量步驟與前述肌肉度量步驟相同。肌電圖儀的探測電極片將會被黏貼在你的患側大腿和小腿，以記錄膝關節及足踝關節活動時相應部位的肌肉活動。

平衡控制
我們會用專業的電腦系統評估閣下的平衡能力。測試平衡反應時，你會站在一個會間歇擾動的平臺上。為保證安全，當站在測試平臺上時，閣下需要需著上安全帶。

步行速度
你需要以平時舒適和最快的速度分別在走道上步行。如果需要，測可使用助行器。

血液測試
我們會為你收集大約 6 毫升血液樣本以評估骨格狀況。抽血當日，不可以食用含咖啡因或酒精的飲食。所收集的血液樣本將會在香港理工大學醫療科技及資訊學系部門進行生物化學分析。

運動訓練詳情
研究人員將會以隨機抽樣方式將閣下安排到低強度、高強度震盪訓練組或運動訓練組。如果你被安排到高或低強度震蕩訓練組，你將會接受為期 12 周，每週 3 次，每次約 7-16 分鐘的全身震蕩訓練。站在震蕩平臺上的同時，你也需要做一系列簡單的訓練動作。你可以在每個訓練動作之間做簡短休息。在研究結束時，我們將告知閣下是進行了低強度震盪訓練，或者是高強度震盪訓練。

如果你被安排到運動訓練組，你會站在震動平臺上做相同的體能訓練動作，但是該平臺不會有震動。

<u>對項目參的益處和潛在危險性</u>：
參與這項研究，閣下將有機會瞭解自己的骨骼和肌肉狀況，並接受運動訓練。研究的結果，將會提供重要的資料，有助於設計臨床上的運動處方。
大部分人進行震盪訓練均沒有出現如不適情況，但有很少部分人士可能會出現頭暈等不適現象。一般經休息後不適現象就會減退。若在訓練過程中感到不適，閣下可隨時要求訓練終止。骨質掃描所產生的輻射極低 (遠低於一次肺 X 光掃描所產生的輻射)，對健康應沒有影響。

<u>保密性：</u>
此項研究收集所得的個人資料及數據絕對保密；除相關研究人員之外，閣下的姓名或個人資料將不會被公開。

參加者同意書

本人_____已瞭解此次研究的具體情況。本人願意參加是項研究計劃，並有權在任何時候、不論任何原因放棄參與此項計劃，而此舉不會導致我受到任何懲罰或不公平對待。本人明白參與此項計劃的潛在危險性以及本人的資料將不會洩露給與此計劃無關的人員，我的名字不會出現在任何影帶或出版物上。本人亦明白製作人員可剪輯本人之訪問或錄音或錄影片段，而片段將製作成教學用具，作為教學用途或於學術會議中播放。

本人可以用電話 2766-7156 來聯絡此計劃負責人彭耀宗教授。若本人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文詠琴女士(電話:2766-4394)。本人亦明白，參與此計劃需要本人簽署一份同意書。

簽名（參與者） ：_____ 日期 ：_____

簽名（證人） ：_____ 日期 ：_____

7.3 *Sample of assessments*

7.3.1 Demographic information

Name: _____ Gender: Male/ Female Age_____

*Post menopausal year: _____

Body weight :_____(kg) Body Height:_____(cm)

First Onset of stroke:_____ Duration of stroke_____

Type of stroke: Ischemic / Hemorrhagic / Others (Please specify: ___) Paretic leg: L/ R

Orthosis: No/Yes (indoor_____/outdoor_____ during test_____)

Waling aids: No/Yes (indoor_____/outdoor_____/ during test_____)

(0,None / 1,cane,stick / 2,quadripod / 3,walking frame / 4,wheelchair)

Lesion area from MRI/CT _____ Dominant side: _____ Living status_____

Occupation (Pre/post)： ____/___ Smoking (Pre/post): ____ Drinking (Pre/post): _____

Exercise habit: Pre (Frequency/intensity/type): _____

Post: _____

Past 1 year fall history (time/numbers/ direction/cause/injury/follow medical care):

_____

_____

Present Medical Condition:

_____

_____

Medicine:

_____

_____

Surgical history:

_____

7.3.2 Intervention Record Form

WBV Intervention Record Sheet (Template) Week 1 – Round 1					26/03	27/03	28/03	29/03	30/03	31/03 (Make-up session)
Patient ID #	English Name	Chinese Name	* / #	Tally						
				Repetitions						
				Sessions (cumulative)						

Notes:

***** indicates group randomization to 8 repetitions with high frequency WBV

indicates group randomization to 12 repetitions with low frequency WBV

A tally of the number of repetitions and number of sessions completed by each participant is marked in the boxes under the corresponding date. If a participant is absent or unable to complete the entire training session, the corresponding box is marked with an **X**

7.3.3 Physical Activity Scale for the Elderly (PASE-Chinese Version)

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

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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

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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)

7.3.4 Fugl-Meyer Assessment (Lower Extremities)

E. LOWER EXTREMITY				
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)			
extended knee	more than 90° active flexion			2
Ankle dorsiflexion compare with unaffected side	no active motion limited dorsiflexion complete dorsiflexion	0	1	2
	Subtotal III (max 4)			

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)			

7.4 *Sample of Search Strategy for Chapter 2*

Search strategy (MEDLINE)

1. exp Cerebrovascular accident/
2. exp Stroke/
3. exp CVA/
4. exp cerebral vascular/
5. Exp Brain injuries/
6. Exp Hemiplegia/
7. Exp Hemiplegic/
8. Or/1-7
9. Exp bone/ or exp bone density/
10. Exp bone mineral density/
11. Exp bone geometry/
12. Exp bone strength/
13. Exp bone mass/
14. Exp bone volume /or exp bone area/
15. Exp bone turnover/
16. Exp bone densitometry/
17. Or/ 9-17
18. Exp Dual-energy X-ray absorptiometry/or exp DXA/or exp DEXA
19. Exp Ultrasound/
20. Exp absorptiometry/
21. Exp peripheral quantitative computed tomography/or exp PQCT/or exp QCT/
22. Or/18-22
23. 8 and 17 and 2