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An Evidence-Based Review of Micro-CT Assessments of the Postmenopausal Osteoporosis Rat Model

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ABSTRACT
Micro-CT (μCT) is a high resolution imaging tool that is generally used in animal studies. This review evaluates the effectiveness of μCT in assessing bone changes in the postmenopausal osteoporosis rat model. A systematic review of the literature was conducted to identify relevant studies on μCT and postmenopausal osteoporotic bone changes. A comprehensive search via the two databases; Medline via OVID Medline and Scopus was conducted for relevant studies published between 1994 and 2014. The results were limited to research articles published in English, that reported on the association between μCT findings and bone changes in the postmenopausal osteoporosis rat model. Studies were excluded if they were duplicated, did not use an ovariectomized-induced postmenopausal rat model and did not focus on μCT as the primary outcome. The literature search identified 182 potentially relevant articles that were later limited to 22 studies based on the inclusion and exclusion criteria. Fourteen *in vitro* μCT studies, 7 *in vivo* μCT studies and one report that combined both *in vitro* and *in vivo* μCT studies were included in this review. Of all these studies, 8 studies used μCT alone in assessing bone changes while the remaining studies used μCT analyses together with histomorphometry, DXA and pQCT which enabled a comparison of effectiveness. All the studies reported positive roles of μCT in evaluating bone quality. This evidence-based review highlights the ability of μCT to not only assess bone microarchitecture but also bone mineral density and bone strength.

Key words: Micro-CT, osteoporosis, postmenopausal, bone microarchitecture, bone quality

INTRODUCTION

Bone is a complex composite material made of a cellular and extracellular matrix. The extracellular matrix comprises of 40% organic components such as collagen type I, proteoglycan, proteins and cytokines, that contribute to bone structure and strength (Camozzi *et al*., 2010). The remaining 60% of the extracellular matrix is made from minerals including hydroxyapatite, calcium and phosphate, that help provide mechanical strength (Rusu *et al*., 2005). Both the cellular and extracellular matrices work together to maintain bone remodeling. Any disruption to these components will affect bone remodeling which will later lead to pathological bone conditions. The most common bone disease is osteoporosis. Osteoporosis is a progressive systemic skeletal disorder characterized by low bone mineral density (BMD), deterioration of the microarchitecture and susceptibility to fracture (WHO., 1994). It is a disease that causes bone loss and fractures which together lead to severe pain, deformity and in certain cases, secondary complications that result in death (Johnell and Kanis, 2006). As defined by the World Health Organization (WHO), osteoporosis occurs when BMD T-score is more than 2.5 standard deviation below the peak bone mass reference standard for young women (Nelson *et al*., 2002). The prevalence of osteoporosis is currently increasing globally due to the general increase in the population and in the proportion of aged individuals. This increase contributes to a higher economic burden due to the medical care expenditures.

Osteoporosis is classified clinically into primary and secondary osteoporosis. Primary osteoporosis refers to both bone loss due to sex hormone deficiency such as in post-menopausal women (type I) and bone loss due to the normal ageing process (type II) (McNamara, 2010). Secondary osteoporosis refers to bone loss that ensues as a secondary effect of other diseases or drug treatment. Post-menopausal osteoporosis is the most common form of the disease and is due to estrogen deficiency following menopause (Riggs *et al*., 2002). There are many factors that contribute to the reasons why women (75%) are affected by osteoporosis to a greater extent than men (25%). Firstly, women have a smaller skeletal size, lower bone mass (Nieves *et al*., 2005) and achieve a lower peak BMD compared with men (Avdagic *et al*., 2009). Secondly, women are prone to rapid bone loss due to estrogen reduction following menopause and thirdly, in almost every population, women have a longer life expectancy than men. As a consequence, there is a steadily increasing proportion of women at advanced ages (Barling, 2013).

Postmenopausal bone loss caused by both decreased ovarian production of sex steroids and an increase in Follicle Stimulation Hormone (FSH) secondary to estrogen deficiency (Sun *et al*., 2006). Estrogen is an important sex hormone that plays a fundamental role in modulating bone remodeling. Estrogen acts directly on bone via Estrogen Receptor (ER)-α and ER-β which are highly expressed on osteoblasts and osteoclasts (Komm and Bodine, 2001). Estrogen deficiency leads to accelerated bone resorption, primarily due to increased osteoclast differentiation and stimulation of osteoblast apoptosis. Consequently, high bone turnover stimulates osteoblastogenesis fuelled by an expansion of the pool of early mesenchymal progenitors and increased activities of pluripotent precursors toward the osteoblastic lineage (Jilka *et al*., 1998). Despite the stimulation of osteoblastogenesis, the net increase in bone formation is insufficient to compensate for the increase bone resorption due to the acceleration in osteoclast differentiation and osteoblast apoptosis (Kousteni *et al*., 2001). In addition to the direct effect of estrogen deficiency, an indirect effect due to an increase in FSH will lead to stimulation of tumor necrosis factor (TNF). The increased TNF production stimulates receptor activator of nuclear factor kappa-β ligand (RANKL) which further increases osteoclast formation. Simultaneously, a potent anti-osteoclastogenic factor, osteoprotegerin (OPG) will be inhibited (Cenci *et al*., 2000; Collin-Osdoby *et al*., 2001). The TNF also stimulates the production of inflammatory cytokines which further exacerbate bone loss by promoting osteoclastogenesis (Lorenzo, 2000; Wei *et al*., 2005). Estrogen deficiency also induces T-cell activation and osteoclastogenesis by downregulating the antioxidant defense pathway, leading to an upregulation of Reactive Oxygen Species (ROS). The ROS have been shown to be responsible in the development of bone loss in postmenopausal osteoporosis (Ozgocmen *et al*., 2007). Understanding the pathological mechanisms underlying postmenopausal osteoporosis will contribute to advances in the field of osteoporosis including its diagnostics and pharmacological interventions.

Bone loss affects both cortical and trabecular bone with trabecular bone loss more prominent in postmenopausal osteoporosis (Khosla *et al*., 2006). This is because women have thinner trabeculae and are more prone to trabecular thinning. Due to its large surface/volume ratio, trabecular bone shows a higher rate of turnover than cortical bone. Hence, trabecular bone is more responsive to risk factors causing deterioration and any interventions applied. Therefore, it has been studied more extensively to understand the mechanisms of osteoporosis, diagnostic methods and interventions with anti-osteoporotic agents. Experimentations on osteoporosis using both human and animal will lead to an improved understanding of this disease. It is important to not only understand its causes but also the mechanisms of bone deterioration, diagnostic methods and treatments, as well as preventative measures.

Experimentations using animal models not only discern bone loss mechanisms but also serve as a platform to investigate the efficacy of pharmacological interventions for osteoporosis. A wide variety of animals such as rodents, dogs and sheep have been used as animal models in osteoporosis studies. Laboratory rat is the most widely used in experimental protocols to induce bone loss using hormonal interventions (ovariectomy, orchidectomy, hyperphysectomy, parathyroidectomy) (An and Freidman, 1998; Iwamoto *et al*., 2004; Iwamoto *et al*., 2007) and dietary manipulations such as a low calcium diet (Koshihara *et al*., 2004). The ovariectomized (OVX) rat is commonly used as a postmenopausal osteoporosis model (Kalu, 1991; Turner, 2001). Following ovariectomy, the reduction in estrogen levels result in dramatic bone loss because bone resorption outweighs bone formation activity (Lelovas *et al*., 2008). The similarities in pathophysiologic responses between the human and rat skeleton, the fact that the rat is readily available and the financial advantages offered by the laboratory rat, have together made it a suitable model for osteoporosis research (Lelovas *et al*., 2008).

To date, the gold standard used to assess the risk of osteoporosis is by the Bone Mineral Density (BMD) measurement using dual X-ray absorptiometry (DXA) which was initially proposed by WHO (1994), Siris *et al*. (2004), Winzenberg and Jones (2011). It is versatile due to its high precision, short scan time and low radiation dose and it may be used to assess bone mineral density/bone mineral content of the entire skeleton as well as specific sites, such as the hip and vertebrae which are the most vulnerable to fracture. Although BMD is the cornerstone for the diagnosis of osteoporosis, the use of BMD alone is less than optimal for use as an intervention threshold for several reasons. For some cases, such as those with osteomalacia, a complication of poor nutrition in the elderly, DXA may underestimate total bone matrix due to decreased mineralization of the bone.

Osteoarthritis at the spine or hip is common in the elderly and contributes to density measurements, but not necessarily to skeletal strength (Kanis *et al*., 1997).

The operational definition of osteoporosis which involves BMD measurements using DXA, is discussed often because it focuses too much on bone density and bone mass rather than on structure. Although BMD is an important determinant of bone strength, it does not take into account the microarchitectural changes that occur in trabecular bone (Laib *et al*., 2001) hence, it is not an accurate predictor of the risk of osteoporotic fracture. It has been reported that the dominant features of the initial phase of rapid bone loss following the onset of estrogen deficiency is increased bone resorption, trabecular thinning and perforation and a loss in connections between remaining trabeculae. This phase is followed by a long-lasting period of slower bone loss where the dominant microarchitectural change is a loss of trabecular connections and trabecular thinning (Eriksen *et al*., 1990; Pacifici, 2008). This progression shows the importance of bone microarchitecture study in the field of osteoporosis. Regardless of the widespread use of DXA, its inability to analyze bone microarchitecture, has raised concerns over its reliability (Peter and Felix, 2008). Due to these limitations, ongoing studies endeavor to replace this conventional osteoporosis diagnostic tool with a more accurate bone assessment tool.

Assessment of the risk of osteoporosis should involve determining bone quality as a whole. To study bone quality which comprises bone mass, strength and microarchitecture, it is essential to develop an effective, sensitive and non-invasive tool that can analyze cortical and trabecular bone separately and detect early changes in bone. Previous studies have reported that bone microarchitectural structure is an important indicator of mass, strength and density which aids in diagnosing osteoporosis (Brandi, 2009; Neil, 2012). Traditionally, trabecular bone structure has been analyzed using histomorphometry which provides two-dimensional (2D) information on architectural parameters (Chappard *et al*., 2005). To overcome some of the limitations of 2D histological sections, several non-destructive three-dimensional (3D) techniques have been developed. In recent years, a highly-developed radiological tool, micro-computed tomography (μ-CT) has received attention in bone studies. This technique provides a new method of measuring bone microarchitecture and strength in 3D, replacing the tedious serial staining required for histomorphometric analyses. Measurement of 3D architecture provides improved insight into the underlying bone microarchitecture changes and on its biomechanical properties (Boyd *et al*., 2006). The development of μCT was first driven by the need for a highly precise and effective tool to reconstruct the complexity of bone architecture at high resolution. Later, it became a tool crucial for evaluating the pathophysiology of osteoporosis, to test the efficacy of pharmaceutical interventions and to estimate bone biomechanical properties.

High resolution μCT imaging is now becoming more applicable in the bone research field because it provides better and more accurate information on bone structural parameters. Compared to other radiological tools used in bone studies, μCT provides much greater accuracy when measuring bone mineralization. The μCT can measure changes in cortical thickness in the range of 10-20% which is undetectable when using other X-ray imaging tools. Previous studies have reported widely that μCT is capable of analyzing cortical and trabecular bone separately which cannot be done with other X-ray tools (Genant *et al*., 2008). This property is important for the investigation of osteoporosis. μCT may be used to analyze human bone and animal bone. Due to its high resolution, it is possible to analyze data from bone areas as small as the trabeculae of small rodents, such as mice and rats. It was originally developed for *in vitro* use which later led to an increase in the use of *in vivo* μCT.

The investigation of postmenopausal osteoporosis using μCT should be studied extensively in animals prior to embarking on clinical trials. The increasing number of bone studies using μCT which focus on the assessment of bone changes in the postmenopausal osteoporosis rat model, warrants a review. The aim of this evidence-based review is to explore original research articles to determine the efficacy and reliability of μCT in assessing bone changes in the postmenopausal osteoporosis rat model.

USE OF MICRO COMPUTED TOMOGRAPHY AND POSTMENOPAUSAL OSTEOPOROSIS

A systematic review of the literature was performed to identify relevant studies on the use of micro computed tomography and postmenopausal osteoporosis. To conduct a comprehensive search of health science journals, we used the Medline via Ovid Medline and Scopus databases (reports published between 1994 and 2014). The search strategy involved a combination of the following two sets of key words (1) Micro CT OR micro computed tomography and (2) Postmenopausal osteoporosis OR postmenopausal osteoporosis.

Selection of research articles: The results were limited to full research articles that were published in English language. Studies that complied with these following inclusion criteria were included (1) Reported μCT analysis of the postmenopausal osteoporosis rat model and (2) The postmenopausal osteoporosis-related bone changes should relate to lifestyle variables, aging or experimentally-induced conditions. Studies were excluded if they were (1) Duplicated studies (2) Reviews, news, letter, editorials or case studies (3) Did not use a control group (4) μCT was not the primary outcome (v) did not use the OVX-induced postmenopausal rat model (5) related to other diseases (e.g., chronic obstructive pulmonary disease, osteoarthritis) (6) Related to bone fracture healing.

Data extraction and management: Papers included in this review were selected based on three phases. Firstly, we excluded the papers that did not match the inclusion criteria based solely on their titles. Secondly, the abstracts of the remaining papers were screened and papers that did not match the inclusion criteria were excluded. Lastly, we scrutinized the remaining papers to exclude a second group of papers that did not match our inclusion criteria. The remaining papers were again screened by two reviewers prior to data extraction phase. Any discrepancy that arose was settled in discussions between the reviewers. We recorded (1) the type of study and micro-CT used and provides; (2) a brief description of the sample/population; (3) a brief description of the methods used; (4) a brief description of the results and (5) outcomes and comments on the study.

SEARCH RESULTS

The literature searches identified 234 potentially relevant articles. Two reviewers independently assessed all articles for inclusion or exclusion based on the title and abstract. A total of 182 articles were retrieved for further assessment and data extraction. Following these assessments, 160 of these articles were excluded because they did not involve postmenopausal osteoporosis and μCT was not the primary outcome. Frequent discussions between the two reviewers took place to resolve differences in opinion on the inclusion or exclusion of full articles. The remaining 22 articles that fulfilled all inclusion and did not fulfill exclusion criteria were included in this review. The process of paper selection from the beginning of the identification of relevant articles to articles selection based on the inclusion and exclusion criteria is summarized in a flow chart shown in Fig. 1.

Study characteristics: The characteristics of all studies are summarized in Table 1. All animal studies were conducted between the year 2000 and 2013, with majority conducted within the past five years. All studies used female rats, because this review focused on postmenopause-induced osteoporosis. Fifteen studies used Sprague-Dawley rats and the remaining 7 studies used Wistar rats. Rats were of varying age, with the majority being mature and aged 3 months. There were also some studies that used aged rats of 8-10 months. The number of rats for each study ranged from 3 up to 152 and in the majority of studies, rat numbers were kept to a minimal number due to animal ethics requirements.

Bone microarchitecture, a highly reliable indicator of osteoporosis was the primary outcome measured in reports used in this review. Bone microarchitecture parameters were measured using μCT. Out of 22, a total number of 14 studies used *in vitro* μCT, 7 used *in vivo* μCT and only one study used both *in vitro* and *in vivo* μCT. Six out of seven *in vivo* μCT studies examined in this review performed a longitudinal study to monitor time-dependent bone changes at different periods. Rats were scanned under anesthesia at different time periods. In the study by Wu *et al.* (2012) using *in vivo* μCT, femoral bone were scanned only after the completion of treatment, at 8 weeks (Wu *et al.*, 2012). Bone microarchitecture of trabecular bone is an important determinant of osteoporotic changes. However, cortical bone analysis also contributes to improved insight and understanding of the changes in bone structure following ovariectomized-induced osteoporosis. Of all the studies included in this review, a total of 15 studies focused only on trabecular bone as their primary parameter whereas the remaining seven studies measured both trabecular and cortical bone.

Eight studies used μCT alone to analyze bone loss in the postmenopausal osteoporotic rat model (Peyrin *et al.*, 1998; Waarsing *et al.*, 2004; Yoon *et al.*, 2012; Jee *et al.*, 2010; Waarsing *et al.*, 2006; Park *et al.*, 2008; Brouwers *et al.*, 2009; Rhee *et al.*, 2009). In the remaining studies, bone changes were studied using μCT, histomorphometry, DXA and biomechanical bone strength assessments. The outcomes of these methods were compared to determine the most efficient bone analysis method. Types of bone used for micro-CT analysis varied between the studies with the most widely used being the tibiae (Peyrin *et al.*, 1998; Waarsing *et al.*, 2004, 2006; Gasser *et al.*, 2005; Jee *et al.*, 2010; Brouwers *et al.*, 2009, 2010; Zhao *et al.*, 2011; Zhang *et al.*, 2012). Seven studies used the femora as their sample (Park *et al.*, 2008; Stunes *et al.*, 2011; Montero *et al.*, 2012; Wu *et al.*, 2012; Sung *et al.*, 2012; Li *et al.*, 2013; Zhao *et al.*, 2013), three used lumbar vertebrae (Yoon *et al.*, 2012; Rhee *et al.*, 2009; Bian *et al.*, 2012) and the remaining three studies (Sampath *et al.*, 2007; Kuber *et al.*, 2008; Sharan *et al.*, 2010) used both tibiae and femora. The Region of Interest (ROI) chosen in the majority of studies was below the growth plate extending proximally. However, in two of the studies, the ROI was not mentioned (Waarsing *et al.*, 2006; Park *et al.*, 2008). In studies by Kuber *et al.* (2008) and Li *et al.* (2013), the area scanned was mentioned, but not the specific ROI site.

The resolution used for μCT scanning in these studies ranged from 6-40 μm. However, in four studies, the resolution was not mentioned (Sampath *et al.*, 2007; Park *et al.*, 2008; Kuber *et al.*, 2008; Bian *et al.*, 2012). The majority studies in this review used protocols derived from Ruegsegger *et al.* (1996) with a resolution of 20 μm (Ruegsegger *et al.*, 1996). Micro-CT analysis is capable of measuring bone structural parameters similar to histomorphometry analysis, such as bone volume fraction (BV/TV), trabecular thickness (Tb.Th), trabecular number (Tb.N) and trabecular separation (Tb.Sp). In contrast to histomorphometry, μCT provides a more accurate 3D microarchitecture measure by providing non-metric parameters such as Structural Model Index (SMI), connectivity density (Conn.D), degree of anisotropy (DA) and trabecular bone pattern factors (TbPfs). In seven studies used for this review, μCT analysis measured only the directly assessed metric indices which are BV/TV, Tb.Th, Tb.N and Tb.Sp (Waarsing *et al.*, 2004; Gasser *et al.*, 2005; Yoon *et al.*, 2012; Park *et al.*, 2008; Montero *et al.*, 2012; Sung *et al.*, 2012; Zhao *et al.*, 2013). The remaining studies measured both metric and non-metric bone microarchitecture indices.

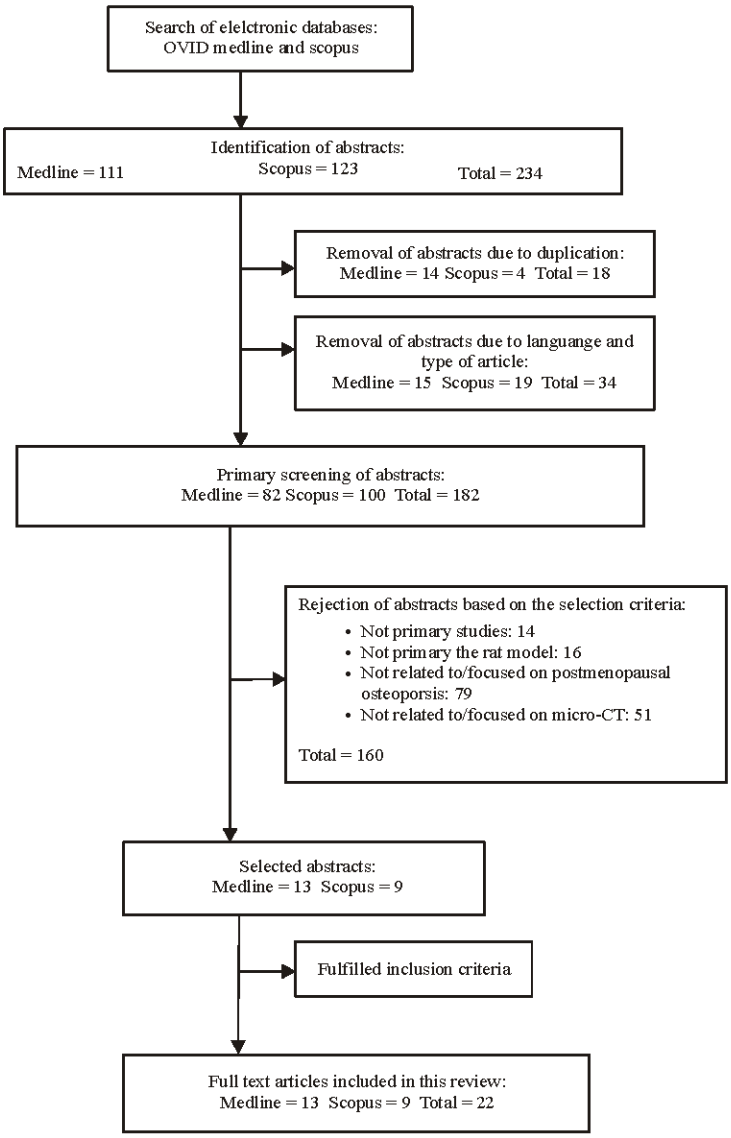

Fig. 1: Flow chart of the selection process of the articles used in this review

The directly assessed metric structural parameters provided by μCT are similar to histomorphometry. However, six studies used for this review performed the conventional bone histomorphometry analysis regardless of results obtained from μCT (Sampath *et al*., 2007; Kuber *et al*., 2008; Wu *et al*., 2012; Bian *et al*., 2012; Li *et al*., 2013; Zhao *et al*., 2013). In addition to structural parameters, bone histomorphometry analysis provides information on dynamic and static parameters. Dynamic parameters yield information on bone turnover such as Bone Formation Rate (BFR), mineral apposition rate (MAR), mineral formation rate, single labeled surfaces (sLs) and double labeled surfaces (dLs). In contrast, static parameters reflect bone cellular conditions, such as osteoblast and osteoclast volume. Out of these six studies, two measured only the structural bone parameters which paralleled the μCT analysis (Wu *et al*., 2012; Zhao *et al*., 2013). Two studies performed histomorphometry to measure structural and static parameters (Bian *et al*., 2012; Li *et al*., 2013). Sampath *et al*. (2007) measured both structural and dynamic parameters, whereas the remaining study by Kuber *et al*. (2008) measured only the dynamic bone parameters.

Apart from μCT scanning and histomorphometry, some studies performed dual X-ray absorptiometry (DXA) scanning to measure BMD. This analysis provides better insight into bone changes in the postmenopausal osteoporosis rat model. Ten studies used DXA as one of their parameters. In three other studies however, BMD was measured using the μCT itself (Yoon *et al*., 2012; Brouwers *et al*., 2009; Zhao *et al*., 2013). Yoon *et al*. (2012) measured Bone Mineral Content (BMC) and BMD of both trabecular and cortical bone and OVX rats showed a significant reduction in BMD whereas, BMC was not affected (Yoon *et al*., 2012). In another study by

Brouwers *et al.* (2009), μCT was used to measure BMD of both cortical and trabecular bone (Brouwers *et al.*, 2009). Furthermore, Zhao *et al.* (2013) reported that BMD analysis using μCT revealed a significant increase in the treated group compared to OVX control group (Zhao *et al.*, 2011). Based on the ability of μCT to measure BMD, these data may lead to the rise of μCT as the gold standard in accessing bone status.

Out of 22 studies used for this review, three studies demonstrated the use of pQCT as an outcome measurement. Gasser *et al.* (2005) employed pQCT and DXA to measure BMD of both cortical and cancellous bone of OVX rats (Gasser *et al.*, 2005). pQCT showed a decrease in BMD. In an additional two studies by Sampath *et al.* (2007) and Kuber *et al.* (2008), pQCT was used to measure the volumetric content and density of both cortical and cancellous bone. However, these results were not reported. In recent years, the rise of a new computational engineering method has led to the development of Finite Element Analysis (FEA) by μCT. The study by Rhee *et al.* (2009), Sharan *et al.* (2010) reported the use of FEA to convert 3D reconstructed μCT images into micro-finite elements. Indirectly, this technique enabled the measurement of bone mechanical properties, an indicator of bone strength. In addition to FEA, some studies performed conventional bone mechanical tests. Apart from μCT as their primary outcome, a total number of 11 studies used for this review performed the conventional 3-point bending biomechanical bone test.

ASSESSMENT OF BONE MICROARCHITECTURAL CHANGES USING MICRO-CT VARIABLES IN *IN VITRO* μCT

A total of 14 studies used this review used *in vitro* μCT. In general, bone structural parameters, such as BV/TV, Tb.Th, Tb.Sp and Tb.N were analyzed. In the majority of studies, BV/TV,Tb.Th,Tb.N were decreased whereas Tb.Sp increased in the untreated ovariectomized group, indicating deterioration in bone microarchitecture. For example, in the studies by Sung *et al.* (2012) and Li *et al.* (2013), the untreated OVX group showed significantly decreased BV/TV, Tb.Th, Tb.N and increased Tb.Sp (Sung *et al.*, 2012; Li *et al.*, 2013). In addition to measuring these typical variables, some studies used for this review measured additional variables, such as BMD and BMC (Yoon *et al.*, 2012; Brouwers *et al.*, 2009; Zhao *et al.*, 2013), TbPfs (Sampath *et al.*, 2007; Kuber *et al.*, 2008; Stunes *et al.*, 2011; Montero *et al.*, 2012), Conn.D (Peyrin *et al.*, 1998; Zhao *et al.*, 2011; Zhang *et al.*, 2012; Wu *et al.*, 2012), DA (Peyrin *et al.*, 1998), SMI (Peyrin *et al.*, 1998; Sampath *et al.*, 2007; Kuber *et al.*, 2008; Sharan *et al.*, 2010; Brouwers *et al.*, 2010; Wu *et al.*, 2012; Li *et al.*, 2013) and FEA (Rhee *et al.*, 2009). In the majority of studies, BMC, BMD, TbPfs, Conn. D. and FEA were reduced whereas SMI was increased (rod-like structure) in the untreated OVX group.

According to Laib *et al.* (2001) and Peyrin *et al.* (1998), *in vitro* μCT was capable of detecting bone microarchitectural changes in OVX rats as early as 12 days after surgery. Bone microarchitectural changes were measured over time because scans were performed at 35, 60 and 110 days post-ovariectomy. Within the first 12 days post-ovariectomy, all trabecular structural parameters in the OVX group showed a dramatic decrease which was then followed by a slower decline. In the study by Bian *et al.* (2012), bone microarchitectural changes were reported at different times; at 2 weeks and 3-months, after treatment of OVX-induced postmenopausal osteoporosis rats with Oleanolic Acid (OA). In this study, μCT analysis was capable of showing a reduction in metric structural indices (i.e., BV/TV,Tb.N and Tb.Th) and an increase in non-metric index (Conn. D.) as early as 2 weeks after surgery.

In studies by Yoon *et al.* (2012) and Zhao *et al.* (2013), BMD and BMC were determined using *in vitro* μCT. According to Yoon *et al.* (2012), BMD and BMC of the OVX group were significantly reduced when compared with the Sham group (Yoon *et al.*, 2012). According to Zhao *et al.* (2013), the OVX rats treated with bone-seeking estrogen compound (SE_2) showed similar BMD to the estrogen-treated and Sham-operated groups and this outcome was significantly increased compared with the OVX group (Zhao *et al.*, 2013).

Only two studies used for this review measured TbPfs and SMI simultaneously (Sampath *et al.*, 2007; Kuber *et al.*, 2008). In the study by Sampath *et al.* (2007), TbPf and SMI were measured in rats given various doses of Thyroid Stimulating Hormone (TSH) for the prevention and restoration modes of bone loss. The TbPfs and SMI values at all the TSH doses were decreased in both the prevention and restoration modes (Sampath *et al.*, 2007). In the study by Kuber *et al.* (2008), TbPfs and SMI were measured in rats given Sevalamer and TbPfs was significantly decreased compared with the OVX group, whereas the SMI value tended to decrease but was not significantly different from the OVX group (Kuber *et al.*, 2008). The TbPfs value also decreased in OVX rats treated with Kalsis, an antioxidant dietary supplement. The decrease in TbPfs attenuated the bone disconnection effect (Montero *et al.*, 2012). SMI was also measured in the study by Sharan *et al.* (2010), where SMI was shown to increase (more rod-like) in the OVX group and was lower in the 5.0 mg kg^{-1} GTDF-treated rats (Sharan *et al.*, 2010). According to Stunes *et al.* (2011), the ovariectomized group exhibited a significantly higher SMI value compared with the Sham group. The OVX group that was treated with Wyeth at 90 mg kg^{-1} showed a significantly higher Conn.D. value compared with the OVX group (Stunes *et al.*, 2011).

Conn. D. Which indicates connections between trabecular structures was measured in a study by Zhao *et al* (2011) where OVX rats treated with Cibotium Barometz Extract (CBE) showed a significant increase in Conn.D compared with the OVX control group. Other bone structural parameters, such as BV/TV, Tb.N and Tb.Th were also significantly increased when compared with the OVX which denotes an improvement in bone microarchitectural (Zhao *et al.*, 2011). In addition, a study by Zhang *et al.* (2012) reported a significant decreased

in Conn.D. in the OVX group compared with the Sham group. Other bone structural parameters, such as BV/TV, Tb.N and Tb.Th were also significantly decreased in OVX rats compared with the Sham rats. These data indicate trabecular deterioration in OVX rats (Zhang *et al*., 2012).

Laib *et al.* (2001) and Rhee *et al.* (2009) were the only two studies used for this review that used *in vitro* μCT to measure the Degree of Anisotropy (DA). According to Laib *et al.* (2001), DA was calculated by projecting the triangles of bone surfaces onto an ellipse, based on the methods described by Laib *et al.* (2000) and Harrigan and Mann (1984). DA in the OVX group at 12, 35, 60 and 110 days was significantly different from the Sham group (Peyrin *et al*., 1998). In the study by Rhee *et al.* (2009), DA was not significantly different between the control and treatment groups. These authors also performed a Finite Element Analysis (FEA) to measure mechanical parameters, such as stiffness and elastic modulus. The FEA of the OVX group was not significantly different from the Sham group for both mechanical parameters ($p>0.05$). PTH-treated rats showed higher mechanical properties than Sham rats (Rhee *et al*., 2009).

ASSESSMENT OF BONE MICROARCHITECTURAL CHANGES USING *IN VIVO* μCT

A total of 7 studies used for this review *in vivo* μCT. Notably, μCT variables measured using *in vivo* μCT were similar to those measured using *in vitro* μCT. By using *in vivo* μCT, longitudinal studies may be performed to determine bone microarchitectural changes overtime. In the study by Waarsing *et al.* (2004), a dramatic and progressive loss in epiphyseal and metaphyseal of the trabeculae bone in OVX group was reported during week 4 to week 14 after surgery. In the Sham group, no changes in bone volume were noted, however, after 14 weeks, thinning of the trabeculae in metaphyseal region may be observed (Waarsing *et al*., 2004). Gasser *et al.* (2005) performed a longitudinal study using *in vivo* μCT and reported significant microarchitectural changes at as early as 2 weeks after surgery and gradual changes of BV/TV and Tb.Th were observed for up to 12 weeks in the OVX group (Gasser *et al*., 2005). A longitudinal study by Boyd *et al.* (2006) used *in vivo* μCT over a longer period, by scanning only sham-operated and OVX groups for 6 months at 1-month intervals. The OVX group showed significant changes in all structural parameters, including BV/TV, BS/BV, Tb.Th, Tb.Sp and Tb.N. However, the Sham group also showed reduction in these structural parameters that were less prominent than in the OVX group (Jee *et al*., 2010).

Waarsing *et al.* (2006) performed a longitudinal study for over 54 weeks, where scanning was performed at week 0 (prior to OVX) and at weeks 4, 34 and 54 post-OVX. Changes in bone microarchitectural were obvious during the first 14 weeks with the trabecular bone of the OVX group showing a dramatic reduction in Conn.D. In addition, this study also reported on the microarchitectural changes in cortical bone, with cortical thickness of the OVX group being significantly reduced compared with the previous time point. Because *in vivo* μCT exposes live rats to radiation, the radiation may affect the results of the study and these authors also performed a comparison between irradiated and non-irradiated rats. In this study, small differences were observed in SMI and trabecular thickness. The non-irradiated controls showed slightly more rod-like trabeculae than age-matched irradiated animals and trabecular thickness in noon-irradiated controls was not significantly different from baseline at week 0 (Waarsing *et al*., 2006).

Park *et al.* (2008) used *in vivo* μCT to measure only three variables; BV/TV,Tb.Th and BMC to determine the effects of apigenin (API) and estrogen (E2) on OVX-induced osteoporotic rats. The rats were scanned at 7, 12 and 23 weeks. At week 7, the BV/TV and Tb.Th values of the OVX group were significantly decreased compared with the Sham, indicating deterioration in bone microarchitecture and proving that the induction of osteoporosis was successful. The BMC of the API group was increased compared to OVX group (Park *et al*., 2008). *In vivo* μCT can also be used to measure BMD, as studied by Brouwers *et al.* (2009). In this paper, the authors measured BMD of both cortical and trabecular bone. They reported that the PTH-treated group showed an increase in trabecular BMD whereas the cortical BMD remained unaffected (Brouwers *et al*., 2009).

Brouwers *et al.* (2010) used both types of μCT in their study. For the *in vivo* μCT, scanning was performed at weeks 8, 10, 12 and 14 post-OVX on the proximal tibia of rats. At week 8, both tibial meta-and epiphysis of the OVX group showed a deterioration in bone microarchitecture which were demonstrated by a decrease in BV/TV,Conn. D, Tb.N, Tb.Th and an increase SMI and Tb.Sp. Beyond 8 weeks, all variables worsen further, except in the case of Tb.Th which improved. For the *in vitro* μCT, epiphysis region of the femora was scanned to determine microarchitectural changes. All structural parameters including BV/TV, Tb.N, Tb.Th and Tb.Sp were significantly different in the OVX group when compared with the Sham group. However, the Conn. D. Value of the epiphysis region in OVX group was not significantly different from the Sham group (Brouwers *et al*., 2010). These data indicate that the epiphysis of the femora shows much slower changes compared with the epiphysis region of the tibiae.

In a study by Wu *et al.* (2012), *in vivo* μCT was not used for longitudinal study. Instead, the OVX rats treated with zoledronate-impregnated calcium phosphate (ZLN/CPC) were euthanized and femora were scanned after the completion of the treatment to measure bone structural parameters including BV/TV, Tb.N, Tb. Sp, SMI and Conn. D. The ZLN/CPC-treated group showed a significant increase in BV/TV, Tb.N and Conn. D and a decrease in Tb.Sp and SMI compared with the OVX group (Wu *et al*., 2012) (Fig. 1, Table 1).

Table 1: Review of literature for the type of study and type of µCt

	Type of study/type of µCT	Sample/population	Methodology	Results	Comments or outcomes
Study 1					
Laib *et al*. (2001)	Animal study, bone microarchitecture, *in vitro* µCT	6-month old female Sprague Dawley rats (n = 45)	-Rats were randomly divided into: • Sham • OVX euthanized at 12, 35, 60 and 110 days -Right proximal tibiae were excised and scanned using µCT20 (Scanco Medical) with 26 µm resolution -Region of interest (ROI) selected was at 1.04 mm below the growth plate µCT variables: BV/TV, Tb.N, Tb.Th Tb.Sp, SMI, DA, Conn. D	(a) There was a dramatic drop in all trabecular structural parameters within the first 12 days then the slope of reduction declined (b) Tb.Sp increased at the beginning prior to plateau, before rising again at 60 and 110 days (c) The trabeculae were more rod-like after 12 days and with further trabecular bone loss, the structure remained rod-like	(a) µCT analysis capable of detecting early changes in OVX-induced bone loss and of monitoring the changes over time (b) µCT not only provided 3D images and structural parameters but also provides non metric indices such as SMI, DA and connectivity density (c) These data provide a valuable insight into monitoring bone changes in postmenopausal osteoporosis and the efficacy of any interventions
Study 2					
Waarsing *et al*. (2004)	Longitudinal animal study *in vivo* µCT	10-month old old female Wistar rats (n = 3)	-Three rats were divided into: • Sham • OVX • Reproducibility control -*in vivo* µCT protocols: (Skyscan 1076) • 100 kV X-ray source • 10 µm pixel size • 0.4 Gy radiation dose • Resolution: 20 µm` • ROI: Proximal epiphysis towards metaphyseal region -Rats were scanned under anesthesia at baseline, 4 and 14 weeks Only the tibiae were scanned to minimize bias and effects of radiation µCT variables: TV, Tb.Th	(a) There was no change in bone volume for sham. However, after week 14, thinning of the metaphyseal trabeculae was observed (b) There was a gradual loss of both epiphyseal and metaphyseal trabeculae bone of the OVX rat from week 4 to week 14	(a) These new developments of *in vivo* µCT scanning enable possible to perform longitudinal studies in small animals, reducing the numbers of animals required, while accurately measuring local architectural changes in bone over time (b) This longitudinal *in vivo* µCT analysis may be used to monitor the efficacy of postmenopausal osteoporosis pharmacological interventions
Study 3					
Gasser *et al*. (2005)	Longitudinal animal study, Bone mineral density (BMD), bone microarchitecture, pQCT, DXA, *in vivo* µCT	8-months old Wistar rats (n = 50)	-Rats were randomly divided into 5 groups (10 rats each); • Sham+vehicle • Sham+hPTH 25 µg kg^{-1} • OVX+vehicle • OVX+zoledronic acid (ZA) • 5 µg kg^{-1} and OVX+17-α-ethinylestradiol (aEE) -Rats were anesthetized and tibiae were scanned using *in vivo* µCT (vivaCT40) at 15 µm resolution at baseline, 1, 2, 4, 8 and 12 weeks after surgery to assess cancellous bone structure. The ROI chosen was 1 mm below the growth plate µCT variables: BV/TV, Tb.Th, Tb.N, SMI, CtTh Other parameters measured: -pQCT to monitor changes in cortical and bone mineral density -DXA	(a) In OVX rats, significant differences began to appear at 2 weeks, gradually up to 12 weeks with a decrease in BV/TV and Tb.Th and increase in Tb.Sp (b) hPTH showed a gradual significant increase in BV/TV and Tb.Th at 2 weeks until 12 weeks (c) ZA caused a gradual significant increase in BV/TV, Tb.N and Tb.Th. aEE showed similar structure to sham after 12 weeks (d) pQCT analysis of OVX rats showed a decrease in BMD and cortical thickness after 2 weeks (e) DXA analysis showed a decrease in BMD	(a) Combination of BMD and bone microarchitecture provides a better insight of bone quality (b) µCT allows an early detection in bone microarchitecture changes and the efficacy of therapeutic interventions compared to other diagnostic methods (c) µCT able to save time that would otherwise be required for tissue processing in histomorphometry (d) Combination of µCT, pQCT and DXA provides excellent information on changes in both cortical and cancellous bone

Table 1: Continue

	Type of study/type of µCT	Sample/population	Methodology	Results	Comments or outcomes
Study 4					
Yoon *et al*. (2012)	Animal study, BMD, bone microarchitecture, mechanical bone strength, *in vitro* micro-CT	11-weeks old female Sprague Dawley rats (n = 12)	-Rats were randomly divided into: • Sham • OVX -Rats were euthanized at 8 weeks after surgery and the 4th lumbar vertebrae were removed for µCT analysis -*in vitro* µCT (Explore Locus SP) X-ray energy settings of 80 kV, 80 µA and a resolution of 15 µm µCT variables: BMC, BMD, Tb.Th, Tb.N, Tb.Sp) Additional parameter measured: bone mechanical test (3-point bending)	(a) OVX rats showed a 12% reduction in trabecular BMD and 6.2% reduction in cortical BMD (b) OVX rats showed a significant decrease in BV/TV and Tb.Th and increase in Tb.Sp. (c) There was no significant difference in BMC and Tb.N in both groups (d) OVX group showed a 39% lower load than sham	(a) µCT analyses can distinguish between cancellous and cortical bone Hence, it may be used to produce realistic depictions of BMDs and structural parameters which in turn reflect the degrees of bone mineralization (b) µCT is able to measure BMD which is typically measured using DXA (c) Both µCT and bone mechanical analyses provide a better insight into bone changes
Study 5					
Boyd *et al*. (2006)	Longitudinal animal study, bone microarchitecture *in vivo* µCT	8-months old female Wistar rats (n = 20)	-Rats were randomly divided into: • Sham • OVX -*In vivo* µCT (vivaCT40 Scanco Medical) was used to scan each rat (under anesthesia) at 1-month interval for 6 months -µCT protocols 55 kV, 145 µA, 0.5 Gy radiation dose and 15 µm resolution ROI: proximal tibial metaphysis µCT variables: BV/TV, Tb.Th, Tb.Th, Tb.Sp, SMI, Conn.D.	(a) Structural parameters of OVX changed significantly with respect to time and were detected 1 month from the start of the study (b) Most significant results were seen in the first 3 months (c) Sham also showed a decline in structural parameters although less than OVX (d) BV/TV for OVX decreased by 19 and 33% at 1 and 3 months respectively compared to Sham which lost only 3 and 7%, respectively (e) SMI were altered 2 months after OVX whereas Conn.Dens were altered at the end of the study	(a) The trabecular bone loss in Sham may due to normal aging as the rats used were 8 months old (b) *In vivo* µCT was able to detect a longitudinal changes and monitor any small changes in bone microarchitecture over time (c) µCT analysis began showing significant changes in the first 3 months. Intervening in the early stage prior to significant architectural changes would be advantageous for the treatment and prevention of postmenopausal osteoporosis
Study 6					
Waarsing *et al*. (2006)	Animal study *in vivo* µCT	10-month-old female Wistar rats (n = 10)	-Rats were randomly divided into: • Sham • OVX -The rats were scanned using *in vivo* micro CT system (Skyscan 1076) at week 0 (prior to operation), week 4, 34 and 54 Area of scanning was focused on proximal tibiae -The effects of radiation on rat bone were investigated, by adding an additional group of 4 sham-operated rats (non-irradiated control group) These rats were scanned at week 54 µCT variables: BV/TV, Tb.Th, Ct.Th, SMI, Conn.Dens µCT protocols: (skyscan 1076 *in vivo* system)	(a) Trabecular bone: -OVX group showed robust decrease in connectivity (80%) within the first 14 weeks. The decrease was slowed and remained constant between 34 to week 54 -Sham group showed almost linear decrease in connectivity with time (b) Cortical bone: -In OVX group, resorption occurs on the periosteal tibial cortex between week 0-34.While, for week 35-54, no changes were observed (c) Trabecular realignment: -In OVX group, the proximal	(a) µCT evaluation is able to show the bone loss occur differently at certain region of the long bones (b) µCT scanning caused a slight different trabecular structure when comparisons were made between irradiated and non irradiated rats (c) This longitudinal *in vivo* µCT study showed bone adaptation dynamics in age related bone loss and bone loss due to estrogen depletion leads to vertical alignment of trabeculae

Table 1: Continue

	Type of study/type of µCT	Sample/population	Methodology	Results	Comments or outcomes
			-20 min per scan -0.4 Gray radiation per scan -voxel size: 20 µm Micro CT variables: BV/TV, Tb.Th, SMI, Conn D, C.Th	metaphyseal trabeculae were preserved, while the distal metaphyseal trabeculae were resorbed -In OVX group, the proximal metaphyseal trabeculae were preserved, while the distal metaphyseal trabeculae were resorbed (d) The radiated sham rats showed no changes in SMI and showed an increase in Tb.Th. While, for the nonradiated sham controls had slightly more rod-like trabeculae (SMI = 3) than the age-matched radiated rats and showed insignificant difference in Tb.Th from the baseline control of week 0	
Study 7					
Sampath *et al.* (2007)	Animal study, BMD, histomorphometry, *in vitro* micro CT	6 months-old female Sprague Dawley rats (n = 152)	-Rats were divided into 2 modes: (a) Prevention mode (8 weeks) (Treated immediately after OVX) Sham, OVX, 0.1, 0.3, 1.0, 3.0 and 10 µg TSH (Sham group n = 20, OVX groups n = 12) (b) Restoration mode (16 weeks) (Left untreated for 7 months to develop osteopenia) Sham, OVX, 0.01, 0.1, 0.3 µg of TSH (Sham group n = 20, OVX groups n = 10) - µCT protocols: (µCT 40, SCANCO MEDICAL) • Site of scan: Distal femur • Thickness: 13 µm thick in the dorsoventral direction, 250 slices • 3D reconstruction of bone was performed using a triangulation algorithm µCT variables: BV, Tb.N, Tb.Th, Tb.Sp, TbPfs, SMI Other parameters measured: BMD using DXA, pQCT, histomorphometric (BV, Tb.N, Tb.Th, mineral apposition rate, bone formation rate) and biomechanical analyses	(a) Prevention mode - µCT measurement showed that all doses of TSH increased BV/TV, Tb.N, Tb.Th and decreased Tb.Sp and TbPf. -Histomorphometric analysis showed that TSH were dose-dependently preserved the bone volume and maintained the Tb.N and Tb.Th. (b) Restoration mode - µCT measurement showed that all doses of TSH increased BV/TV, Tb.N, Tb.Th and decreased SMI and TbPf -Histomorphometric analysis showed that BV, Tb.N, Tb.Th were increased in TSH treated rats. Moreover, the dynamic bone parameters were 2- to 3- fold higher mineral apposition rates in TSH treated rats.	(a) µCT assessments enable the 3D visualization of trabecular microarchitecture of bone (b) Comparison between both parameters of µCT and histomorphometry showed consistency in their results
Study 8					
Park *et al.* (2008)	Longitudinal animal study, bone mineral content, bone microarchitecture, *in vivo* µCT	3-months old female Sprague Dawley rats (n = 24)	-Rats were randomly divided into: • Sham • OVX • OVX+estrogen (E2) 0.5 mg kg^{-1} • OVX+apigenin (API) 10 mg kg^{-1}	(a) At week 7, a successful OVX-induced osteoporosis was seen through µCT scan (b) At week 12 (5 weeks after treatment), API and E2 had	(a) µCT successfully monitored a gradual bone changes in postmenopausal osteoporosis rat model (b) µCT is an effective tool for monitoring the efficacy of

Table 1: Continue

	Type of study/type of μCT	Sample/population	Methodology	Results	Comments or outcomes
			-Treatments started 7 weeks after surgery and given for 15 weeks -Rats were scanned for trabecular structure of left femur at 7, 12 and 23 weeks using *in vivo* μCT μCT variables: BV/TV, Tb.Th	a significant increase in BV/TV and Tb.Th. (c) At week 23, API and E2 had a significant increase in BV/TV and Tb.Th	therapeutic interventions API is effective in preventing and reversing bone loss
Study 9					
Kuber *et al*. (2008)	Animal study, BMD, microarchitecture, bone histomorphometry, bone strength, *in vitro* micro-CT	6-months old female Sprague Dawley rats (n = 95)	• Part I: Rats were randomly divided into groups of Sham (n = 15), OVX (n = 15) and OVX+Sevelamer 3% (n = 15). 12 weeks of treatment started 4 weeks after surgery • Part II: Sham (n = 10), OVX (n = 10) and OVX+Sevelamer 3% (n = 10). Four weeks of treatment started at 4 weeks after surgery • Part III: Sham (n = 10) and Sham+Sevelamer 3% (n = 10) -Treatment given for 25 weeks -Femur, tibia and lumbar were scanned using DXA at 4 weeks interval prior to euthanization at the end of each experiment -Femur and lumbar were scanned using pQCT and μCT -Structural parameters: BV/TV, Tb.Th, Tb.N, Tb.Sp, SMI, Ct.Th, TbPfs. -Femora were tested for histology (parameters: MAR, BFR, dL length) and 3-point bending test	(a) Treated rats showed an increased in BMD (b) μCT of treated group showed increase in structural parameters after 12 weeks of treatment. Sham group only showed a significant results after 24 weeks of treatment Histology analysis confirmed the results of μCT	(a) Sevelamer effects are more pronounced in OVX compared to sham due to (b) a higher bone turnover and a negative bone remodeling (c) Combination of μCT, Histomorphometry and biomechanical bone strength analysis provide a better insight and understanding of bone changes in osteoporotic condition
Study 10					
Brouwers *et al*. (2009)	Longitudinal animal study, BMD, bone microarchitecture, bone strength *in vivo* μCT	6-months old female Wistar rats (n = 25)	-Rats were randomly divided into; • Sham • OVX • OVX+PTH 60 μg kg^{-1}. -Treatment was initiated at 8 weeks after surgery and given daily for 6 weeks. Rats were scanned under anesthesia at 0, 8, 10, 12 and 14 weeks - μCT protocols: (vivaCT 40, Scanco Medical). • 70 kV, 114 μA and 15 μm resolution • Site of scan: proximal tibia. • The metaphyseal and epiphyseal area of both cortical and trabecular bone were analyzed for BMD and structural parameters μCT variables: BV/TV, Tb.Th, Tb.N, Tb.Sp, SMI, Conn.Dens Other parameter: biomechanical bone strength	(a) At week 8, both meta-and epiphysis of OVX group displayed a tremendous loss in BV/TV, Conn. Den, Tb.N and Tb.Th values and an increase in SMI and Tb.Sp values (b) After 8 weeks, OVX showed further deterioration except for Tb.Th which increased (c) PTH treatment reversed the effects of OVX and prevented further deterioration. These effects differed slightly between the meta- and epiphyseal trabecular bone which Tb.N increased only in the latter (d) PTH treatment increased cortical thickness over time compared to sham, OVX showed significantly lower increase in BMD of cortical bone over the first 8 weeks, but did not affect the trabecular bone significantly	(a) *In vivo* μCT enables longitudinal monitoring of both cortical and trabecular bone and the efficacy of PTH treatment (b) μCT is able to distinguish the effects of OVX and PTH treatment on both meta- and epiphyseal bone structure (c) μCT not only provides information on structural parameters but also able to measure bone mineral density

Table 1: Continue

	Type of study/type of μCT	Sample/population	Methodology	Results	Comments or outcomes
				(e) PTH group showed an increase in BMD over time (f) PTH caused an increase in load and energy compared to Sham group	
Study 11					
Rhee *et al*. (2009)	Animal study, bone microstructure, bone strength, *in vitro* micro-CT	5-months old female Sprague Dawley rats (n = 30)	Rats were randomly divided into: • Sham • OVX • OVX+PTH (PTH-M) • OVX+PTH for 5 weeks then withdrawal (PTH-W) • OVX+PTH for 5 weeks followed by 17 β-estradiol (PTH-E) • OVX+PTH for 5 weeks followed by zoledronate (PTH-Z) -Treatment initiated at 5 weeks after surgery -Rats were euthanized after 10 weeks of treatment and vertebrae were scanned using μCT at 21 μm resolution (Skyscan 1072) μCT variables: BV/TV, Tb.Sp, Tb.Th, Tb.N, SMI Other parameters measured: Finite element analysis (FEA), by converting 3D reconstructed images to micro-finite elements	(a) PTH-M and PTH-Z showed the highest significant increase in structural parameters (BV/TV, Tb.N and Tb.Th) and reduction in in Tb.Sp and SMI (b) All rats treated with PTH showed higher mechanical properties (stiffness and elastic modulus) than sham and OVX rats.	(a) μCT and FEA based on micro-images provide a useful tool that reflects the changes in micro-structural and mechanical properties of OVX-induced bone loss and the efficacy of its interventions (b) μCT has the potential of replacing the conventional biomechanical bone strength analyses
Study 12					
Sharan *et al*. (2010)	Animal study, BMD, bone microarchitecture, bone strength, *in vitro* micro-CT	3-4 months old female Sprague Dawley rats (n = 50)	-Rats were randomly divided into: • Sham • OVX • OVX+2.5 μg kg^{-1} 17 β-estradiol (E2) • OVX+ kg^{-1} glucopyranosyl tetrahydroxyflavanone (GTDF) • OVX+5.0 mg kg^{-1} GTDF. -Treatments were initiated immediately after surgery and given for 12 weeks μCT protocols: • Resolution: 6.4 μm • Site of scan: proximal tibia and distal femur μCT variables: BV/TV, Tb.Th, Tb.Sp, Tb.N, SMI -Other parameters measured: BMD assessment using DXA and 3-point bending analysis	(a) BMD and biomechanical strength of OVX rats were significantly lower than sham (b) All treatment groups showed significantly higher BMD than OVX (c) Both 1.0 and 5.0 amg kg^{-1} GTDF presented significant increase in biomechanical strength. (d) μCT analysis showed a microarchitectural deterioration in OVX control group (lower BV/TV, Tb.Th and higher Tb.Sp) (e) 5.0 mg kg^{-1} (but not 1.0 mg kg^{-1}) GTDF significantly reverse all the OVX-induced changes (f) μCT analysis also showed an increase SMI in OVX and lower SMI in 5.0 mg kg^{-1} GTDF	(a) Although 1.0 mg kg^{-1} GTDF increased both BMD and biomechanical strength, deterioration of microarchitecture is prevented at the dose of 5.0 mg kg^{-1} (b) μCT provides a better insight in determining dose-dependent effect of an intervention (c) Combination of BMD, biomechanical strength and μCT assessment provide a more reliable result of an intervention on osteoporotic bone
Study 13					
Brouwers *et al*. (2010)	Animal study, bone microarchitecture, bone strength, *In vivo* μCT	6 month-old female Wistar rats (n = 23)	-Rats were randomly divided into 3 groups: • Sham-operated (n = 8) • Ovariectomized control (n = 8) • Ovariectomized and whole body	(a) Trabecular bone OVX group: -At week 8, both meta-and epiphysis of ovariectomized groups showed	(a) μCT evaluation is able to detect changes of the trabecular bone structure in proximal metaphysis and epiphysis

Table 1: Continue

	Type of study/type of μCT	Sample/population	Methodology	Results	Comments or outcomes
			vibration (WBV) (n = 7) -WBV treatment was started 8 weeks postovariectomy where the rats were placed on a vibrating platform for 2 times 20 min per day at 0.3 g and 90 Hz, once in the morning and once in the afternoon. -The treatment was done 5 days a week for 6 weeks period. All rats were sacrificed at 14 weeks by cervical dislocation -*In vivo* μCT protocols: (Viva CT 40, Scanco medical AG Switzerland) i) 6 mm μCT scan -At proximal tibia -70 kV, 114 μA, 1000 projections per 180°, 261 msec integration time -isotropic resolution :15 microns ii) 3.15 mm μCT scan -At diaphysis -70 kV, 114 μA, 250 projections per 180°, 350 msec integration time. -isotropic resolution: 30 microns Follow up scans were made at 8, 10, 12, 14 weeks after OVX -*Ex-vivo* μCT protocols: (used same apparatus and scan settings as for the *in vivo* scanning) -scanned femoral epiphysis for each rat after sacrifice μCT variables: BV/TV, Conn. D., SMI, Tb.N, Tb.Sp. Other parameters measured: 3 point-bending test	decreased BV/TV, Conn D, Tb.N, Tb.Th and increased SMI and Tb.Sp. -After 8 weeks, the untreated OVX group showed further deterioration of bone structure except Tb.Th which improved. WBV-treated group: -At week 8, WBV treatment showed further deterioration of bone structure except for Tb.Th which significantly and gradually increased overtime for metaphysis and epiphysis respectively -During 8th weeks to 14th weeks, this group showed no significant difference compared to OVX group but had significant difference from the SHAM (b) Cortical bone -For both meta- and diaphysis region, no significant response was observed for WBV treatment and was also reflected by unaltered bending properties. (c) *In vivo* μCT -There was no significant difference between WBV and OVX groups for all structural parameters in 8-14 week (d) *In vitro* μCT -Both the SMI and Tb.Th variables showed small differences between WBV and OVX groups while other structural parameters were not significantly different	(b) μCT evaluation of the cortical bone was parallel with the 3-point bending result
Study 14					
Stunes *et al.* (2011)	Animal study, BMD, BMC, bone microarchitecture, bone strength, *in vitro* μCT	12-weeks old female Sprague Dawley rats (n = 55)	-Rats were randomly divided into • Sham • OVX • OVX+Fenofibrate 90 mg kg^{-1} (FENO) • OVX+Wyeth 14643 90 mg kg^{-1} (WY) • OVX+Pioglitazone 35 mg kg^{-1} (PIO) -Treatment were initiated 1 week after surgery and given daily for 4 months - μCT protocols: (*in vitro* μCT) • Resolution of 11.89 μm • Both cortical and trabecular of proximal femoral bone were analyzed μCT variables:	(a) There were no differences in BMD at 7days after surgery between the groups (b) After 4 months, of all groups except PIO showed an increase in whole body BMD and BMC (c) After 4 months, sham and FENO had an increase femoral BMD. PIO showed a lower femoral BMD (d) FENO maintained structural parameters at Sham levels (e) WY showed an increase in connectivity density compared to FENO and OVX	(a) DXA analysis was not able to detect early changes in BMD (b) BMD changes were detected only after 4 months (c) The analysis of both μCT and DXA provide a stronger evidence of the efficacy of treatment used in this study

Table 1: Continue

	Type of study/type of µCT	Sample/population	Methodology	Results	Comments or outcomes
			Ct.Th, BV/TV, Tb.Th, SMI, Conn.Dens Other parameters measured: -3-point bending method, -DXA scans were performed at baseline, after 2 and 4 months		
Study 15					
Zhao *et al*. (2011)	Animal study, bone microarchitecture, bone strength, BMD, *in vitro* µCT	3-month-old female Sprague Dawley rats (n = 72)	-Rats were randomly assigned to: Sham (n = 12) • OVx with vehicle (n = 12) • OVx with 17β-estradiol (n = 12) • OVx with Cibotum Barometz extract (CBE) 100 mg kg^{-1} (n = 12) • OVx with Cibotum Barometz extract (CBE) 100 mg kg^{-1} (n = 12) • OVx with Cibotum Barometz extract (CBE) 100 mg kg^{-1} (n = 12) -All treatments were orally given started on week 4 after OVx. The duration was 16 weeks - µCT protocols: (eXplore Locus SP Pre-clinical Specimen) -Scanning was performed from the proximal growth plate in the distal direction (16 µm $slice^{-1}$) -Volume of interest : 25 slices away from growth plate 125 slices away from proximal end of tibia. (includes 350 images) -isotropic voxel resolution: 22 $µm^3$ µCT variables: BV/TV, Tb.Th, Tb.N, Tb.Sp, Conn. D., SMI. Other parameters measured: dual-energy X-ray absorptiometry (DEXA), Biochemical markers and three-point bending test	(a) BV/TV, Conn. D., Tb.N and Tb.Th of OVX group were decreased compared to SHAM group (b) BV/TV, Conn. D., Tb.N and Tb.Th in 500 mg kg^{-1} CBE treated group was significantly increased compared to OVX group (c) The structure model index (SMI) was decreased in CBE and E2 treated group (d) For bone mineral density evaluation, OVX group showed significant decreased of femoral BMD compared with the SHAM group ($p<0.01$)	(a) µCT evaluation is capable to show deterioration in trabecular bone microarchitecture of OVX rats where the variables showed reduced values (b) The result of µCT evaluation consistent with DXA, where both of the parameters showed that ovariectomized group had significant bone loss
Study 16					
Zhang *et al*. (2012)	-Animal study, bone microarchitecture, BMD, bone strength, *in vitro* µCT	3-month-old female Sprague Dawley rats (n = 80)	-Rats were randomly assigned to: • Sham (n = 10) • OVx with vehicle (n = 20) • OVx with 17β-estradiol (n = 20) • OVx with Achyranthes bidentata root extract (ABRE) 100 mg kg^{-1} (n = 10) • OVx with Achyranthes bidentata root extract (ABRE) 300 mg kg^{-1} (n = 10) • OVx with Achyranthes bidentata root extract (ABRE) 500 mg kg^{-1} (n = 10) -All treatments were orally given started on week 4 after OVx. The duration was 16 weeks	(a) 3D images reconstructed by micro CT showed differences among the various groups in trabecular microarchitecture of femoral metaphysis (b) BV/TV, Conn. D., Tb.N and Tb.Th in ovariectomy (OVX) group was significantly decreased (c) SMI and Tb.Sp in OVX group were significantly increased (d) For DEXA measurement, total bone mineral density (t-BMD) of OVX group was significantly lower	(a) µCT evaluation is able to visualize the microarchitecture of trabecular structure. (b) µCT evaluation can show consistent result with the DXA evaluation and biomechanical evaluation

Table 1: Continue

	Type of study/type of µCT	Sample/population	Methodology	Results	Comments or outcomes
			- µCT protocols: (eXplore Locus SP Pre-clinical Specimen) • Scanning was performed from the proximal growth plate in the distal direction (16 µm/slice) • Volume of interest: 25 slices away from growth plate 125 slices away from proximal end of tibia • isotropic voxel resolution: 22 μm^3 µCT variables: BV/TV, Tb.Th, Tb.N, Tb.Sp, Conn.D., SMI Other parameters measured: dual-energy X-ray absorptiometry (DEXA), Biochemical markers and three-point bending test	compared to SHAM group (e) For biomechanical result, the OVX group showed significantly decreased stress and Young's modulus compared to SHAM	
Study 17					
Montero *et al.* (2012)	Animal study, bone microarchitecture, BMD, *in vitro* µCT	6-month-old female Sprague Dawley rats (n = 36)	-Rats were randomly assigned to: • Sham (n = 12) • OVx with vehicle (n = 12) • OVx with Kalsis 25 mg kg^{-1} day^{-1} (n = 12) -All treatments were daily given orally started a day after surgery for the duration of 12 weeks -µCT protocols: • Scanning was performed on the distal region of the right femora • 100 kV, 100 µA, isotropic voxel size of 11 µm Region of interest: -Trabecular region -Starting position 1.00 mm from growth plate extending to 2.50 mm longitudinally in proximal direction -226 images analyzed, cortical bone excluded µCT variables: TV, BV, BV/TV, Tb.Th, Tb.N, Tb.Sp, TbPf. Other parameters measured: DXA, biochemical parameters	(a) For 3D microarchitecture the Tb.SP and BV/TV of OVX group showed significant increase and decrease value, respectively when compared to SHAM group (b) For TbPf, the OVX+K25 group attenuates the disconnection effect (c) For DXA measurement, the OVX group presented a significant decrease both in femoral BMD and lumbar BMD	(a) Differences in bone mass between OVX-treated and intact rats can only be detected by micro CT and not by conventional measurement of BMD by DEXA (b) In comparison with DXA, detection of differences in µCT was more sensitive than BMD, where µCT is able to demonstrate on the condition of trabecular area and determine other structural parameters such as BV/TV
Study 18					
Wu *et al.* (2012)	Animal study, bone microarchitecture, histomorphometry, *in vivo* micro CT	10-11 weeks-old female wistar rats (n = 40)	-Rats were divided into four groups consisted of 10 rats per group: • Sham • OVX • OVX+ZLN/CPC • (0.025 mg zoledronate/calcium phosphate cement) • OVX+ZLN/CPC/$CaSo_4$ (0.025 mg zoledronate/calcium	(a) The OVX control group showed significant decrease in BV/TV, Tb.N and Conn. Dn but significant increase in Tb.Sp and SMI compared to Sham group (b) Both the ZLN/CPC and ZLN/CPC/$CaSo_4$ showed significantly increased BV/TV, Tb.N and Conn. Dn but significantly	(a) µCT evaluation is able to show that the cancellous bone architectures were restored, with decreased bone porosity which is parallel with histologic examinations for zoledronate impregnated CPC group

Table 1: Continue

	Type of study/type of µCT	Sample/population	Methodology	Results	Comments or outcomes
			phosphate cement/calcium sulfate) -The blocks were implanted at greater omentum under general anaesthesia after 4 weeks of ovariectomy procedure for 8 weeks -µCT protocols: (SkyScan 1176 on-vivo µCT, Kontich, Belgium) • Scanning site: Femoral metaphysis • Analytical condition: • 65 kV with 385 µA, 9 mm • Isotropic resolution of 15 µm • VOI: 3.0 mm below growth plate. µCT variables: BV/TV, Tb.Th, Tb.N, Tb.Sp, SMI, Conn. Dn Other parameters measured: Histology, serum bone turnover markers	lower in Tb.Sp and SMI compared to OVX group. (c) Histologically, the ZLN/CPC/CaSO4 group showed restoration of bony architecture and healthy cancellous bone	
Study 19					
Bian *et al*. (2012)	Animal study, bone microarchitecture, histomorphometry, *in vitro* µCT	2-months old female Sprague Dawley rats (n = 48)	-Rats were randomly divided into: • Sham • OVX • OVX+oleanolic acid (OA) 20 mg kg^{-1} -Each of these groups were further divided into duration of 2 weeks and 3 months. Rats were sacrificed after the end of treatment period. L4 vertebrae were scanned using *in vitro* µCT (µCT80, Scanco Medical) µCT variables: BV/TV, Tb.Th, Tb.N, Tb.Sp, Conn.Dens Histomorphometry analysis was done on L4 and L5 vertebrae for static parameters (osteoblast number, trabecular bone area, bone perimeter)	(a) µCT analysis showed a significant decrease in BV/TV of OVX rats as early as 2 weeks (b) 3 months after OVX, there were reduction in BV/TV, Tb.N and Tb.Th and increase in Conn.Dens and Tb.Sp. Treatment with OA for 3 months managed to increase Tb.Th. (c) Histology analysis showed a reduction in Tb.N 3 months after OVX OA treatment for 3 months managed to increase osteoblasts number	(a) OA treated rats showed a time-dependent improvement in bone mass (b) µCT is able to detect early changes in OVX-induced osteoporotic bone, compared to histology which showed a significant result after 3 months. (c) OA stimulated bone formation based on both 2D and 3D bone histomorphometric analysis
Study 20					
Sung *et al*. (2012)	Animal study, bone microarchitecture, BMD, *in vitro* µCT	6-7 months old female Sprague Dawley rats (n = 40)	-Rats were randomly divided into: • Sham • OVX • OVX+alendronate (AD) 0.028 mg kg^{-1} OVX+ Saururus chinensis (SC) 1 g kg^{-1} -Treatments were given for 10 weeks prior to euthanization. Femora were removed for BMD and BMC analysis using DXA and trabecular microarchitecture using µCT -The total BMD and BMC of the right femora were measured using DXA (Norland Medical Systems, USA). Proximal femora were scanned using *in vitro* µCT (NFR Polaris, Korea) with resolution of 18 µm µCT variables: BV/TV, Tb.Th, Tb.N, Tb.Sp	(a) BMD and BMC of the OVX group were significantly lower than sham (b) BMD and BMC of AD and SC group were significantly higher than OVX (c) OVX group showed significantly lower BV/TV, Tb.Th and Tb.N and higher Tb.Sp. (d) AD and SC prevented the changes in those parameters induced by OVX	(a) Low bone mass is a major risk factor for fractures. Hence, preservation of trabecular microarchitecture significantly contributes to bone strength and may reduce fracture risk beyond BMD and BMC. (b) Both DXA and µCT provide a clear information on the potential protective effects of SC on OVX-induced osteoporosis rats

Table 1: Continue

	Type of study/type of μCT	Sample/population	Methodology	Results	Comments or outcomes
Study 21					
Li *et al*. (2013)	Animal study, bone microarchitecture, BMD, bone strength, *in vitro* μCT	6-month-old female Sprague Dawley rats (n = 56)	Rats were randomly assigned to: • Sham (n = 8) • OVx with vehicle (n = 8) • OVx with Xian-ling-gu-bao (XLGB, 0.5 g kg^{-1} body weight) (n = 8) • OVx with 17β-estradiol (n = 8) • OVx with Echinoside 30 mg kg^{-1} (n = 8) • OVx with Echinoside 90 mg kg^{-1} (n = 8) • OVx with Echinoside 270 mg kg^{-1} (n = 8) -All treatments were orally given started on week 4 after OVx. The duration was 12 weeks -μCT protocols: Scanning was performed on the right distal femora 60 kV, 40 W, isotropic voxel size of 22 μm μCT variables: BV/TV, Tb.Th, Tb.N, Tb.Sp, SMI. Other parameters measured: Bone mineral density, bone biomechanical properties, bone histomorphology	(a) BV/TV, Tb.N, Tb.Th, were significantly decreased while Tb.Sp and SMI were significantly increased in OVX group. (b) All ECH-treated groups showed enhanced bone quality compared to OVx group after 12 weeks treatment (c) The biomechanical parameters such as ultimate load, stiffness and energy absorption were significantly decreased in OVX group compared to SHAM group. (d) OVX groups had decreased BMD by 7.83% after 12 weeks postovariectomy	(a) μCT is a reliable method to measure bone microarchitecture of trabecular bone where it shows consistency with other parameters measured
Study 22					
Zhao *et al*. (2013)	Animal study, bone microarchitecture, histomorphometry, bone strength, *in vitro* μCT	3-month-old female Sprague Dawley rats (n = 96)	-Rats were randomly assigned to: • Sham (n = 24) • OVx with vehicle (n = 24) • OVx with 17β-estradiol (n = 24) • OVx with bone-seeking estrogen compound (SE2) (n = 24) -All treatments were daily injected subcutaneously in a volume of 0.2 cm^3 The duration of treatment was 12 weeks -μCT protocols: Scanning was performed on the distal metaphysis femora 55 kV, 145 μA, isotropic voxel size of 15-40 μm Region of interest: -2.5 mm thick regions of femur proximal to the growth plate of knee joint μCT variables: BMD, BV/TV, Tb.Th, Tb.N and Tb.Sp Other parameters measured: Bone histology and biomechanical	(a) Deterioration of architecture in OVX groups was visualized after 12 weeks treatment (b) BV/TV, Tb.Th, Tb.N were significantly increased while Tb.Sp was significantly decreased in SE2 group compared to OVX group (c) SE2 group showed similar BMD as in the E2- and sham-treated groups group and was significantly increased compared with the OVX group (d) The intertrabecular space of SE2 group was filled with significantly lower fat cells and increased trabecular bone and erythropoietic marrow compared to OVX group	(a) μCT analysis is able to show deterioration of architecture in ovariectomized group effectively (b) μCT result is in parallel with the histomorphometric analysis result

USE OF μCT IN BONE ASSESSMENTS

Our primary aim is to review the use of μCT in bone assessments of ovariectomized-induced osteoporosis rat models. Based on the chosen studies, μCT has shown beneficial advantages as a bone-assessment tool. The initial type of μCT made commercially available for bone studies was *in vitro* μCT. Osteoporotic studies using *in vitro* μCT are typically performed using the proximal tibia and distal femur. In this review, the majority of studies performed μCT analysis using tibiae although seven studies used femora, three studies used lumbar and the remaining studies analyzed a combination of these three types of bones. The most common complication of postmenopausal osteoporosis is hip fracture (Cummings and Melton, 2002). The annual incidence of hip fractures has been increased worldwide during the last five decades and that the current total number of women who sustain a hip fracture is estimated to be one million annually (Gullberg *et al*., 1997). In comparison with other types of fractures, such as vertebral and wrist fractures, hip fracture is associated with serious disability and excess mortality. Women who have sustained a hip fracture have a 10-20% higher mortality for their age (Cummings and Melton, 2002). This fact underlies the wide used of tibiae and femora in osteoporotic studies. Tibiae or femora area, in particular, the area approximately 1.5 mm below the epiphyseal growth plate that extends towards the proximal direction is the region of interest selected most often for osteoporotic studies (Martin *et al*., 2003). This ROI is the trabecular region rich in blood supply and with high bone turnover activity, thus, it is sensitive to changes caused by any stimulation (Judith, 2009).

This review demonstrated that *in vitro* μCT bone assessment requires rats to be euthanized at the end of the study, followed by excision of the bone for scanning. This protocol demands indirectly that a large number of animals must be used to overcome variability to obtain statistically significant results. The results of *in vitro* μCT are often consistent with structural parameters assessed using traditional histomorphometric method. *In vitro* μCT is commonly used to assess the effects of therapeutic interventions such as in the studies by Sampath *et al*. (2007) and Rhee *et al*. (2009) which investigate the effects of Thyroid Stimulating Hormone (TSH) and zoledronic acid respectively. In contrast, *in vivo* μCT is always used to perform longitudinal studies such as in the study by Waarsing *et al*. (2004). The OVX rats were scanned at baseline and at, 4 and 14 weeks later to observe changes in trabecular structure. This study reported a progressive dramatic loss of both epiphyseal and metaphyseal trabeculae over weeks 4-14. In another study by Gasser *et al*. (2005), OVX rats were scanned at baseline and at, 1, 2, 4, 8 and 12 weeks. Significant gradual changes were detected at 2 weeks and up to 12 weeks (Gasser *et al*., 2005).

Based on this review, it can be postulated that *in vivo* μCT is designed to enable monitoring of bone changes over time without euthanizing the animal. The advantage of longitudinal studies is that each rat acts as its own control, hence, control groups are not required (Woo *et al*., 2005). This design indirectly reduces the number of animals required for study. Brouwers *et al*. (2010) employed the use of both *in vitro* and *in vivo* μCT to monitor the bone changes following OVX and the effect of Whole Body Vibration (WBV) treatment (Brouwers *et al*., 2010). *In vivo* μCT analysis revealed a significant difference between the OVX and the treated group at only week 8. Over the period of week 8-14, there was no significant difference found between the two groups. This finding is in contrast to the *in vitro* μCT analysis which revealed a significant difference between the groups at 14 weeks after OVX. This finding may be due to several reasons. Compared with *in vitro* scanning, it is difficult to obtain a consistent orientation during *in vivo* μCT because the rat leg cannot be positioned exactly the same during each scan (Waarsing *et al*., 2005). Another limitation of *in vivo* μCT is that the presence of soft tissues around the bone during scanning may reduce image quality and the actual volume of the bone may be difficult to ascertain (Cendre *et al*., 2002; Nadia *et al*., 2013). Both *in vitro* and *in vivo* μCT have their own distinct advantages and disadvantages that make them effective tools in monitoring bone changes in postmenopausal osteoporosis.

In osteoporosis studies, it is important to determine bone quality as a whole which comprises all characteristics, such as bone mass, microarchitecture and strength. Although BMD has long been known as an important determinant of bone status and is a gold standard for assessing the risk of osteoporosis, bone microarchitecture also plays an important factor in bone studies. DXA limitations that focused too much on bone mass have led to the rise of other radiological imaging tools. Prior to the development μCT, the radiological tools used for bone assessment included peripheral quantitative CT (pQCT), high resolution CT (hrCT) and Magnetic Resonance Imaging (MRI). Many previous studies have reported the use of pQCT in longitudinal osteoporotic studies due to its advantages over DXA (Brunader and Shelton, 2002).

In this review, three studies used pQCT as one of their imaging protocols. Gasser *et al*. (2005) reported on the use of DXA, pQCT and μCT in assessing osteoporotic rats. DXA measurements showed a decrease in BMD of cortical tibia whereas pQCT measurements revealed a decrease in cancellous BMD and cortical thickness in OVX rats. In contrast, μCT analysis revealed a significant osteoporotic changes in microarchitectural parameters which were reversed by parathyroid hormone (PTH) and Zoledronic Acid (ZA). The strong anabolic effect of PTH also caused a reduction in SMI values, indicating that the bone is no longer plate-like but more like a solid block of bone with pores (Gasser *et al*., 2005). Other studies by Sampath *et al*. (2007) and Kuber *et al*. (2008) also reported on the use of DXA and pQCT together with μCT and showed decreased BMD in the proximal tibia, distal femur and the spine of OVX rats. These changes were reversed following treatment. The high resolution provided by pQCT is sufficient to distinguish cortical and trabecular bone but remain too low to resolve trabecular microarchitecture which

consequently may result in underestimations of trabecular thickness (Hangartner, 2007). Due to the high resolution provided by μCT (1-100 μm), it is feasible to analyze areas of bone as small as the trabeculae of small rodents. μCT analysis in these studies showed restoration of structural parameters which also included SMI and trabecular bone pattern factors (TbPf), following treatment. In a latter study, μCT analysis also revealed an increase in cortical thickness (Ct.Th).

In the majority of studies, μCT analysis also revealed non-metric indices. SMI indicates the relative prevalence of rods and plates in a 3D trabecular structure and involves measurement of the surface convexity (Hildebrand and Ruegsegger, 1997). SMI values of a pure plate-shaped bone and pure rod-shaped bone are 0 and 3, respectively. A negative SMI value indicates a solid and very dense trabecular structure. TbPf is a quantitative ratio of inter-trabecular connectivity (Hahn *et al*., 1992), where positive values denote concave structures and negative values denote convex structures (Odgaard, 1997). In addition to SMI and TbPf, another non-metric index that may be derived from μCT analysis is the Degree of Anisotropy (DA). DA is a measure of how substructures are oriented within a volume and it is calculated using Mean Intercept Length (MIL) method (Van der Linden and Weihans, 2007). Bone is known to be anisotropic, meaning that it is stronger when loaded in one direction. Higher DA suggests greater anisotropy resulting from loss of directional trabeculae. This is in accordance with previous studies which reported that increase of bone resorption in osteoporosis leads to thinner trabeculae, resulting in an increase of anisotropy (Newitt *et al*., 2002; Odgaard *et al*., 1990). In this review, the two studies by Laib *et al*. (2001) and Rhee *et al*. (2009) demonstrated DA in OVX rats using μCT (Peyrin *et al*., 1998; Rhee *et al*., 2009).

In this review, some studies also demonstrated the use of histomorphometry and biomechanical bone analyses. Bone architecture has long been performed using conventional histomorphometric methods. In contrast to μCT, histomorphometry provides only two-dimensional (2D) information on trabecular bone parameters (Iwaniec *et al*., 2008). Histomorphometry is also time-consuming because it requires tedious processing of thin bone sections and serial staining (Vidal *et al*., 2012). In comparison between 2D histomorphometry and 3D μCT, many previous studies have demonstrated the advantages of μCT in detecting earlier changes in bone architecture (Jiang *et al*., 2005). These data are consistent with data presented by Laib *et al*. (2001) which reported a dramatic decrease in trabecular structural parameters of OVX rats as early as 12 days post-OVX. The trabeculae also began to change into rod-like structure after 12 days post-OVX (Peyrin *et al*., 1998). Another study by Gasser *et al*. (2005) reported significant trabecular changes at 2 weeks post-OVX that progressed gradually up to 12 weeks (Gasser *et al*., 2005). Bian *et al*. (2012) who used both histomorphometric and μCT analyses, reported significant changes in trabecular parameters using μCT as early as 2 weeks post-OVX. In contrast, histomorphometric analysis only showed a reduction in Tb.N at 3 months after OVX (Bian *et al*., 2012). Another report by Sampath *et al*. (2007) also revealed that histomorphometric analysis showed that the Tb.N and Tb.Th were maintained where, μCT showed an increase in trabecular structural parameters (Sampath *et al*., 2007). These results support the fact that μCT is capable of detecting early changes in bone structure compared with conventional histomorphometric analyses.

Previous studies have reported that BMD and structural parameters measured by DXA, histomorphometry and μCT correlate significantly (Barou *et al*., 2002; Yeom *et al*., 2008). Although μCT is well known for its primary use in generating information on bone structure, in recent years, it has been proposed for use for non-destructive, 3D measurements of bone mineralization using a linear calibration of mineral density and x-ray attenuation. This is to measure BMD which is typically measured using DXA. In contrast to DXA which produces two different energy peaks to distinguish between soft tissue and bone absorption, μCT uses mixtures of polymers with hydroxyapatite (HA) crystals for calibration of x-ray attenuation to density (Kazakia *et al*., 2008; Burghardt *et al*., 2008). Phantom models comprising HA are desirable because HA exhibits a similar composition and x-ray attenuation to bone mineral. The measured x-ray attenuation versus HA density then undergo in linear regression which will then be calibrated to estimate bone mineral density (Nazarian *et al*., 2008). In this review, three studies measured BMD using μCT rather than DXA. The study by Yoon *et al*. (2012) showed a significant BMD reduction in both the cortical and trabecular bone of OVX animals and studies by Brouwers *et al*. (2009) and Zhao *et al*. (2013) demonstrated a significant increase in BMD only in trabecular bone of the treated group. Based on these results, it may be postulated that μCT is capable of replacing DXA as the gold standard for assessing bone mineral density.

BMD and bone microarchitecture are not the sole determinant of osteoporosis and fracture risks. A combination of bone mass, microarchitecture and intrinsic material determine a bone's ability to withstand loading (Dempster, 2003). In some studies, bone strength was reported to play an important role in predicting early fracture risk (Sambrook *et al*., 2007). Hence, it is important to assess bone strength to have a better insight into bone quality. DXA, the gold standard for assessing osteoporotic risk does not measure bone strength accurately. Bone strength has long been measured using biomechanical tests. The gold standard for bone-strength assessment is performed by exerting a load on the bone until it fractures (Voide *et al*., 2006). In this review, a total of 11 studies performed biomechanical bone tests using the 3-point bending method. As expected, in the majority of papers, the OVX group showed weaker mechanical bone properties. In animal models, biomechanical bone testing, where the bone can be dissected out and tested biomechanically until it fractures, presents no problem. Another limitation of this conventional test is that it produces large errors and significant uncertainty due to the sensitivity of

the mechanical measurements to friction between the sample and the load. Therefore, this test may not detect small or even large changes in mechanical properties of bone. In human studies, this method is not a viable option. Bone strength may only be assessed indirectly using computer software such as Finite Element Analysis (FEA) via μCT (9 Joshua and Steven, 2008; Grant and Tony, 2009). These limitations have led to an increase in effort to developing this feasible and advanced technique.

In this review, we noted that one study, by Rhee *et al.* (2009), performed a finite element analysis using *in vitro* μCT Skyscan 1072. All treated rats showed higher mechanical properties (stiffness and elastic modulus) compared with the sham and OVX rats (Rhee *et al.*, 2009). For this technique, micro-images of the bone obtained from μCT scanning are converted into Finite Element (FE) models, presenting the bone tissue as equally shaped 8-node brick elements using the hexahedron meshing technique to stimulate real mechanical tests (Keyak and Rossi, 2000). These FE elements are described as elastic properties. Simulated compression tests of FE models are performed following a compressive displacement of 0.5% strain application (Torcasio *et al.*, 2012). This advanced technique has enabled the calculations of bone mechanical properties, such as load, stress, strain and Young modulus which may replace conventional biomechanical bone testing. FEA has not been feasible for bone *in vivo* due to insufficient resolution, however, recently, *in vivo* imaging has been introduced with FEA (Ulrich *et al.*, 19993). A combination of bone microarchitecture and bone biomechanical properties may reflect bone turnover and bone microdamage more effectively.

STRENGTHS AND LIMITATIONS OF THIS REVIEW

The μCT assessments of the postmenopausal osteoporosis rat model have revealed the capability of μCT to measure bone microarchitecture changes in several variables. To seek alternative treatments for postmenopausal osteoporosis, numerous studies have been conducted using rat osteoporosis model that used μCT for evaluation. Thus, a critical review is highly relevant to identify relevant articles published so far. Our search identified 22 research articles that were included in this systematic review. To the best of our knowledge, this is the first evidence-based review that focuses on the bone microarchitectural changes in the OVX-induced postmenopausal osteoporosis rat model. We have included both types of μCT *in vivo* and *in vitro* μCTs to provide a better overview of the most recent and reliable evidence presented on this subject. In one of the studies examined, investigations using the *in vivo* μCT were followed with *in vitro* μCT to compare the differences in μCT analysis methods (Brouwers *et al.*, 2010).

A number of limitations were identified in this review. Many studies used different resolutions for their μCT protocols. Due to the differences in resolution used, the outcomes of μCT images are not uniform and may influence the interpretation of the results. In addition, the number of the animals per group was quite limited. For example, only one rat per group was used in Waarsing *et al.* (2004) and this limited number of rats may not be sufficient to examine bone microarchitectural changes. We have restricted our study solely to animal studies due to the limited use of μCT in humans to date. Thus, we cannot study μCT assessment of bone changes in humans systematically.

RECOMMENDATIONS

Based on the μCT protocols examined in this review, in the future, it will be important to use a standardized protocol for each specific μCT model to determine bone microarchitectural changes in rodents more uniformly. Furthermore, the use of the efficient, high resolution and sensitive μCT technique should not be restricted to animal experiments. It should be used widely on humans to provide better understanding and a true picture of microarchitectural changes in human bone.

CONCLUSION

This evidence-based review has shown the potential of μCT to be effective tool in monitoring bone changes in the postmenopausal osteoporosis rat model. It is highlighted that μCT is not only capable of assessing bone microarchitecture but is also effective in measuring bone mineral density and bone strength. Therefore, μCT is a practical tool for osteoporosis studies, where it may be used as a diagnostic tool and for monitoring the efficacy of therapeutic interventions. Further studies particularly in the clinical field are warranted to verify the reliability and effectiveness of μCT.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interests. The authors are responsible for the writing and content of this paper.

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