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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 postmenopausal 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 osteoelasticogenesis 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 osteoelasticogenesis, 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 (Kcuusenti 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 osteoelasticogenesis (Lorenzo, 2000; Wei et al., 2005). Estrogen deficiency also induces T-cell activation and osteoelasticogenesis 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 (Ozogmen 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 (Khosa 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, hyperphosphatey, parathyroidectomy, Ar and Friedman, 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 (Lelowas et al., 2008). The similarities in pathophysiological 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 (Lelowas 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), Sirs 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 provided; (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; Lee et al., 2010; Waarsing et al., 2006; Park et al., 2009; 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; Lee 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; Sherman 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 (Th.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 (TbPf). 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 O VX 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 Kubler 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 new computational engineering methods 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. However, 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 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), TpPfs (Sampath et al., 2007; Kubler 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; Sang et al., 2007; Kubler 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, TpPfs, Conn. D and FEA were reduced whereas SMI was increased (rod-like structure) in the untreated OVX group.

According to Lab 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 3, 6, and 11 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 (SEb) 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; Kubler et al., 2008). In the study by Sampath et al. (2007), TbPfs 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 Kubler 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 (Kubler 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 Cibomet 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 (Lee 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 non-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).
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<thead>
<tr>
<th>Study</th>
<th>Authors (Year)</th>
<th>Type of study</th>
<th>Sample/population</th>
<th>Methodology</th>
<th>Results</th>
<th>Comments or outcomes</th>
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</table>
| Study 1 | Laib et al. (2001) | Animal study, bone microarchitecture, \textit{in vitro} \textmu CT | 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 limb tibiae were excised and scanned using \textmu C T20 (Scanco Medical) with 26 \mu m resolution  
- Region of interest (ROI) selected was at 1.04 mm below the growth plate \textmu CT 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) \textmu C T analysis capable of detecting early changes in OVX-induced bone loss and of monitoring the changes over time  
(b) \textmu C T 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 | Warsing et al. (2004) | Longitudinal animal study \textit{in vivo} \textmu CT | 10-month old female Wistar rats (n = 3) | Three rats were divided into:  
- Sham  
- OVX  
- Reproducibility control \textit{in vivo} \textmu CT protocols:  
  - SkyScan 1076  
  - 100 K V X-ray source  
  - 10 \mu m pixel size  
  - 0.4 Gy radiation dose  
  - Resolution: 20 \mu m  
  - ROI: Proximal epiphysis towards metaphyseal region  
- Rats were scanned at baseline, 4 and 14 weeks  
Only the tibiae were scanned to minimize bias and effects of radiation \textmu CT variables:  
  - BV/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 \textit{in vivo} \textmu C T 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 \textit{in vivo} \textmu C T 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, \textit{in vivo} \textmu CT | 8-months old Wistar Bone mineral density rats (n = 50) | Rats were randomly divided into 5 groups (10 rats each),  
- Sham+vehicle  
- Sham+1\textmu HPT 25 \mu g kg\(^{-1}\)  
- OVX+vehicle  
- OVX+zoledronic acid (ZA)  
- 5 \mu g kg\(^{-1}\) and  
  - OVX+17\textgamma-estradiol (eEE)  
- Rats were anesthetized and tibiae were scanned using \textit{in vivo} \textmu C T (X Vivo CT40) at 15 \mu m 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 \textmu CT variables:  
  - BV/TV, Tb.Th, Tb.N, SMI, CtTh  
Other parameters measured:  
- pQCT to monitor changes in cortical and bone mineral density  
- DXA  
- pQCT to monitor changes in cortical and cancellous bone | (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. eEE 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) \textmu C T allows an early detection in bone microarchitecture changes and the efficacy of therapeutic interventions compared to other diagnostic methods  
(c) \textmu C T able to save time that would otherwise be required for tissue processing in histomorphometry  
(d) Combination of \textmu C T, pQCT and DXA provides excellent information on changes in both cortical and cancellous bone |
<table>
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<tr>
<th>Study 4</th>
<th>Type of study/type of μCT Sample/population</th>
<th>Methodology</th>
<th>Results</th>
<th>Comments or outcomes</th>
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<tbody>
<tr>
<td>Yoon et al. (2012)</td>
<td>Animal study, BMD, bone microarchitecture, mechanical bone strength, <em>in vitro</em> micro-CT</td>
<td>11-weeks old female Sprague Dawley rats (n = 12)</td>
<td>- Rats were randomly divided into: • Sham • O VX&lt;br&gt;- Rats were euthanized at 8 weeks after surgery and the 4th lumbar vertebrae were removed for μCT analysis.&lt;br&gt;- <em>In vitro</em> μCT (Explore Locus SP)&lt;br&gt;- 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)</td>
<td>(a) O VX rats showed a 12% reduction in trabecular BMD and 6.2% reduction in cortical BMD&lt;br&gt;(b) O VX rats showed a significant decrease in BV/TV and Tb.Th and increase in Tb.Sp.&lt;br&gt;(c) There was no significant difference in BMC and Tb.N in both groups&lt;br&gt;(d) O VX group showed a 39% lower than sham&lt;br&gt;</td>
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| Study 5 | Longitudinal animal study, bone microarchitecture *in vivo* μCT | 8-months old female Wistar rats (n = 20) | - Rats were randomly divided into: • Sham • O VX<br>- *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.Sp, SMI, Conn.D. | (a) Structural parameters of O VX changed significantly with respect to time and were detected 1 month from the start of the study<br>(b) Most significant results were seen in the first 3 months<br>(c) Sham also showed a decline in structural parameters although less than O VX<br>(d) BV/TV for O VX decreased by 19 and 33% at 1 and 3 months respectively compared to Sham which lost only 3 and 7%, respectively<br>(e) SMI were altered 2 months after O VX 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 | Animal study *in vivo* μCT | 10-month-old female Wistar rats (n = 10) | - Rats were randomly divided into: • Sham • O VX<br>- The rats were scanned using *in vivo* micro CT system (Skyscan 1076) at week 0 (prior to operation), week 4, 34 and 54<br>Area of scanning was focused on proximal tibiae<br>- The effects of radiation on rat bone were investigated, by adding an additional group of sham-operated rats (non-irradiated control group)<br>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: - O VX group showed robust decrease in connectivity (80%) within the first 14 weeks. The decrease was slowed and remained constant between 34 to week 54<br>- Sham group showed almost linear decrease in connectivity with time<br>(b) Cortical bone: - In O VX group, resorption occurs on the periosteal tibial cortex between weeks 0-34. While, for week 35-54, no changes were observed<br>(c) Trabecular realignment: - In O VX 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

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<th>Study</th>
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<th>Methodology</th>
<th>Results</th>
<th>Comments or outcomes</th>
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| **Study 7** Saman 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 O VX)  
  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  
  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 Tb.Pf  
  - 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 Tb.Pf  
  - 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 | 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 |
| Study 9 | Kubert et al. (2008) | Animal study, BMD, microarchitecture, bone histomorphometry, bone strength, in vitro micro-CT | 6-months old female Sprague Dawley rats (n = 95) | - 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 |
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<td>a significant increase in BV/TV and Tb.Th. (a) Treated rats showed an increased in BMD</td>
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<td>(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</td>
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<td>(a) Sevelamer effects are more pronounced in O VX compared to sham due to</td>
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<td>(b) a higher bone turnover and a negative bone remodeling</td>
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<td>(c) Combination of μCT, Histomorphometry and biomechanical bone strength analysis provide a better insight and understanding of bone changes in osteoporotic condition</td>
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</table>
| 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 
- O VX 
- O VX+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 
| | | | | (a) At week 8, both meta-and epiphysis of O VX 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, O VX showed further deterioration except for Tb.Th which increased |
| | | | | (c) PTH treatment reversed the effects of O VX 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, O VX 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 O VX 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 |
| 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 • O VX • OVX+PTH (PTH-M) • OVX+PTH for 3 weeks then withdrawal (PTH-W) • OVX+PTH for 3 weeks followed by 17β-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 | (c) PTH group showed an increase in BMD over time (f) PTH caused an increase in load and energy compared to Sham group (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 • O VX • O VX+2.5 μg kg⁻¹ 17β-estradiol (E2) • O VX+5μg kg⁻¹ glucopyranosyl tetrahydroxylflavone (GTDF) • O VX+5.0 mg kg⁻¹ GTDF - Treatments were initiated immediately after surgery and given for 12 weeks μCT protocols: • Resolution: 64 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 mg 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 vitro* 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 O VX 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

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<th>Type of study</th>
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<tr>
<td>vibration (WBV) (n = 7)</td>
<td>decreased BV/TV, Conn D, Tb.N, Tb.Th and increased SMI and Tb.Sp. (a)</td>
<td>-WBV treatment was started 8 weeks postvarietomy 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.</td>
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<td>-In vivo μCT protocols: (Viva CT 40, Scanco medical AG Switzerland)</td>
<td>-After 8 weeks, the untreated OXV group showed further deterioration of bone structure except Tb.Th which improved. -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.</td>
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<td>(a)</td>
<td>&amp; (b)</td>
<td>μCT evaluation of the cortical bone was parallel with the 3-point bending result.</td>
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<td>(i) 6 mm μCT scan</td>
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<td>-At proximal tibia</td>
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<td>-70 kV, 14 μA, 1000 projections per 180°, 261 msec integration time</td>
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<td>-Isotropic resolution: 15 microns</td>
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<td>(ii) 3.15 mm μCT scan</td>
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<td>-At diaphysis</td>
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<tr>
<td>-70 kV, 114 μA, 250 projections per 180°, 350 mscn integration time</td>
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<td>-Isotropic resolution: 30 microns</td>
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<td>Follow up scans were made at 8, 10, 12, 14 weeks after OXV</td>
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<td>Ex-vivo μCT protocols: (used same apparatus and scan settings as for the in vivo scanning)</td>
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<td>Other parameters measured: 3 point-bending test</td>
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| Study 14 | 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 two groups:
- Sham
- OXV
- OXV + Fenofibrate 90 mg kg⁻¹ (FENO)
- OXV + Wylef 14643 90 mg kg⁻¹ (WY)
- OXV + Pioglitazone 35 mg kg⁻¹ (PIO)
- Treatment was initiated 1 week after surgery and given daily for 4 months μCT protocols:
- 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 7 days after surgery between the groups | (a) | DXA analysis was not able to detect early changes in BMD |
| (b) | After 4 months, of all groups except PIO showed an increase in whole body BMC and BMC | (b) | BMD changes were detected only after 4 months |
| (c) | After 4 months, sham and FENO had an increase femoral BMC. PIO showed a lower femoral BMD | (c) | The analysis of both μCT and DXA provide a stronger evidence of the efficacy of treatment used in this study |
| | Both cortical and trabecular of proximal femoral bone were analyzed μCT variables: |
| (d) | FENO maintained structural parameters at Sham levels | (d) | |
| (e) | WY showed an increase in connectivity density compared to FENO and OXV | (e) | |
Table 1: Continue

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| Study 15      | 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) 200 mg kg⁻¹ (n = 12) | (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  
Zhong 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 Acharaeantha bidentata root extract (ABRE) 100 mg kg⁻¹ (n = 10)  
  - OVx with Acharaeantha bidentata root extract (ABRE) 300 mg kg⁻¹ (n = 10)  
  - OVx with Acharaeantha 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

| Study 17 | Montero et al. (2012) | Animal study, bone microarchitecture, BMD, *in vitro* µCT | 6-month-old female Sprague Dawley rats (n = 36) | - 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  
  - 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  
  - µCT protocols:  
  - Scanning was performed on the distal region of the right femur  
  - 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  
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| - 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  
| (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 Tb.Sp, the OVX*K25 group attenuates the disconnection effect  
(c) For DEXA 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  
  - OVX+ZLN/CPC/CS₂O  
  - (0.025 mg zoledronate/calcium phosphate cement)  
  - OVX+ZLN/CPC/CS₂O  
  - (0.025 mg zoledronate/calcium)  
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| - The OVX control group showed significant decrease in BV/TV, Tb.N and Conn. Dm but significant increase in Tb.Sp and SMI compared to Sham group  
(b) Both the ZLN/CPC and ZLN/CPC/CS₂O showed significantly increased BV/TV, Tb.N and Conn. Dm 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 |
| Study 19 | 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 4 treatment groups 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)  
- 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 B/V of OVX rats as early as 2 weeks  
(b) 3 months after OVX, there were reductions in B/V, Tb.N and Tb.Th and increase in Conn.Dens and Tb.Sp.  
(c) Histology analysis showed a reduction in Tb.N 3 months after OVX. OA treatment for 3 months managed to increase Tb.Th.  
(d) OA treated rats showed a time-dependent improvement in bone mass  
(c) µCT is able to detect early changes in OVX-induced osteoporotic bone.  
(b) OA stimulated bone formation based on both 2D and 3D bone histomorphometric analysis |  

| Study 20 | 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+Saunusus chinesis (SC) 1 g kg⁻¹  
- Treatments were given for 10 weeks prior to euthanization. Femora were removed for BMD and BMC analysis using DEXA and trabecular microarchitecture using µCT.  
- The total BMD and BMC of the right femora were measured using DEXA (Norland Medical Systems, USA).  
- Proximal femora were scanned using in vitro µCT (NFR Polaris, Korea) with resolution of 18 µm  
- µCT variables: B/V, 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 B/V, 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 DEXA and µCT provide a clear information on the potential protective effects of SC on OVX-induced osteoporosis rats |
| Study 21 | 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 Xiu-gen-ling-gu-bao (XLGB), 0.5 g kg⁻¹ body weight) (n = 8)  
- OVX with 17β-estradiol (n = 8)  
- OVX with Echinodermata 30 mg kg⁻¹ (n = 8)  
- OVX with Echinodermata 50 mg kg⁻¹ (n = 8)  
- OVX with Echinodermata 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: B/TV, Tb.Th, Tb.N, Tb.Sp, SMI  
- Other parameters measured: Bone mineral density, bone biomechanical properties, bone histomorphology | (a) B/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 | 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 cm³  
- 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, B/TV, Tb.Th, Tb.N and Tb.Sp  
- Other parameters measured: Bone histology and biomechanical properties | (a) Deterioration of architecture in OVX groups was visualized after 12 weeks treatment  
(b) B/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 osteoclastoid marrow compared to OVX group (a) μCT analysis is able to show deterioration of architecture in ovariecetomized 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 humerus 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 use of tibiae and femora in osteoporotic studies. Tibiae or femora, 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; Nadis 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 Kubin 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 O VX 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 simulate 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 (Toccasio 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 microarchitectural 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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