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Differential Age-Related Bone Architecture Changes between Female and Male STR/Ort Mice

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Abstract: The incidence of spontaneous osteoarthritis (OA) in female STR/Ort mice is much lower than that observed in male STR/Ort mice; however, the reason for the differential incidence of OA between sexes has not been elucidated. Here, we investigated and compared age- and sex-related bone mineral density and architectural changes in male and female STR/Ort mice. Bone architecture and bone mineral density (BMD) of femurs were examined in 5-, 10-, 15-, 20-, and 35-week-old male and female STR/Ort mice by microscopic computed tomography (μ CT). Angular degrees of internal tibial torsion (ADITT) were also measured in mice at 5, 15, and 35 weeks of age. Earlier decreases of cancellous volume and BMD were found in male STR/Ort mice. Using μ CT, an age-related decline of bone marrow space in femoral diaphysis was observed in both males and females but was more dramatic in females. In addition, an earlier increase of ADITT was observed in male STR/Ort mice, suggesting that internal rotation of the tibia may contribute to OA. Age- and sex-related bone architectural changes clearly differ between male and female STR/Ort mice. These differences in bone structure, particularly ADITT, may explain the differential incidence of OA in STR/Ort mice.

Key words: bone architecture, osteoarthritis, STR/Ort

Introduction

Male STR/Ort mice, which were initially derived from the parent strain STR/1N following a period of noninbreeding [23], are used as a spontaneous osteoarthritis (OA) model [20, 22, 28]. Interestingly, the incidence of

OA in female STR/Ort mice is markedly lower than that in males [15, 28], which is opposite to the situation in humans. As the cause of the differential incidence of OA between males and females has not been elucidated, examining OA progression in STR/Ort mice may help reveal the mechanisms responsible for these sex-related

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differences.

Age- and sex-related bone changes are often associated with severe musculoskeletal diseases, including OA and osteoporosis. Studies examining the relationships between age-related bone changes and OA development have revealed that elderly patients with OA of knees or hips were more likely to have a higher bone mass and bone mineral density (BMD) compared with healthy individuals of similar age [9, 13, 21]. Higher BMD has also been detected in animal models of OA compared with healthy age-matched controls [5, 14, 22]. Beuf *et al.* [2] reported that femoral trabecular bone loss was observed in knee OA patients, suggesting that altered bone metabolism and/or architecture is important not only for the development of OA, but also for its progression.

Varus, valgus, and other axial deformities are frequently observed in human OA of the knee joint. In clinical practice, adduction of the foot in a patient standing with the patella faced directly anterior is a result of torsional deformity and is referred to as internal tibial torsion (ITT). Torsional abnormalities are considered by numerous researchers to be either a possible cause or effect of OA [3, 10, 11, 16, 25, 32]. Recently, we found that osteoarthritic changes in the patellofemoral joint in STR/Ort mice may be the result of increasing ITT with aging [20]. Therefore, examining age- and sex-related bone changes in STR/Ort mice may provide useful information for understanding the relationship between OA and bone metabolism.

Here, we investigated and compared bone mineral density and architectural changes in male and female STR/Ort mice from 5 to 35 weeks of age.

Materials and Methods

Animals

All procedures involving the handling of animals were in accordance with the guidelines of the animal ethics committee of Kitasato University. An SPF colony of male and female STR/Ort mice was maintained at Charles River Laboratories Japan (Kanagawa, Japan). The mice were housed in a semibarrier system with a controlled environment ($23 \pm 2^\circ\text{C}$, $55 \pm 10\%$ humidity, and a 12-h light/dark cycle) throughout the study. All mice were

fed a diet of standard rodent chow (CRF-1, Oriental Yeast Co., Ltd., Tokyo, Japan). At 5, 10, 15, 20, and 35 weeks of age, mice were sacrificed by deep anesthesia with diethyl ether. At the time of death, mice were weighed, and radiographic OA was evaluated using soft X-rays. Femurs were then dissected and cleaned of soft tissue. Right femurs were stored in 70% ethanol for peripheral quantitative computed tomography (pQCT), and left femurs were stored in 4% paraformaldehyde until microscopic computed tomography (μCT) analysis.

Radiographic analysis

Radiographs of knee joints (lateral projections) were taken at 15 and 35 weeks using a Softex-CMB4 X-ray system (Softex Co., Ltd., Kanagawa, Japan) at 25 kV and 10 mA and IX Industrial X-Ray Film (Fujifilm Corporation, Tokyo, Japan) with a 10-s exposure time. OA changes on soft X-ray radiographs were evaluated using standard criteria consisting of six abnormal parameters, as previously described [20].

Peripheral quantitative computed tomography (pQCT)

The distal metaphysis (1.4 mm proximal to the growth plate) and mid-diaphysis of excised femurs (5 mm proximal to the midpoint) were scanned using an XCT Research SA + pQCT instrument (Stratec Medizintechnik GmbH, Pforzheim, Germany) with a tube voltage and current of 50 kV and 550 μA , respectively, and a voxel size of $80 \times 80 \times 46 \mu\text{m}$. Cortical bone was defined as areas of $\text{BMD} > 690 \text{ mg/mm}^3$, while a threshold value of 395 mg/mm^3 in contour mode 1 was set to define trabecular bone in the bone marrow. Cancellous and cortical BMD (mg/cm^3) were then calculated as previously described [19].

Microscopic computed tomography (μCT)

μCT (inspeXio SMX-90CT, Shimadzu Corporation, Tokyo, Japan) was used to assess trabecular bone morphology in the distal femoral metaphysis and femoral diaphysis using a $12\text{-}\mu\text{m}$ isotropic voxel size, as previously described [12]. We assessed the trabecular bone volume per total tissue volume ($\text{BV/TV, \%}$), trabecular thickness ($\text{Tb.Th, }\mu\text{m}$), and trabecular number (Tb.N, mm^{-1}) of cancellous bone. In addition, we determined

the cortical bone volume per total tissue volume (cBV/TV, %), external line length (ELL, μm), and medullary volume (MV, mm^3) of cortical bone. As age- and sex-specific patterns of bone architectural changes were nearly identical between the tibia and femur of STR/Ort mice, we selected to report data for only the femur.

Ovariectomy

Female STR/Ort mice at 10 or 16 weeks of age were divided into two groups ($n=8$), which received bilateral ovariectomies or sham operations, respectively. Six weeks after surgery, femurs were harvested and stored in 4% paraformaldehyde or 70% ethanol until they were subjected to μCT or pQCT analysis.

Measurement of the angular degree of ITT (ADITT)

ADITT was determined by superimposing the proximal tibial posterior condylar axis onto the distal bimalleolar axis using μCT images [20]. The tibial posterior condylar axis was defined as the line connecting the two most posterior points of the medial and lateral condyle of the tibia at the joint surface level. The bimalleolar axis was defined as the line connecting the medial malleolus and lateral malleolus of the ankle.

Statistical analysis

All statistical analyses were performed using SPSS software (Version 11.0; SPSS, Inc., Chicago, IL, USA). One-way ANOVA with Tukey's multiple comparison test was used to examine differences between sex-matched mice. An unpaired t -test was used to examine differences between age-matched male and female mice. A P value of <0.05 was considered statistically significant.

Results

Incidence of radiographic OA

Radiographic OA changes at 15 weeks were not detected for either male or female STR/Ort mice (Fig. 1A and 1C). Joint space narrowing, subchondral bone sclerosis, and osteophyte formation were noted in males at 35 weeks (Fig. 1B). At 35 weeks, osteoarthritic changes were observed in 80% of male STR/Ort mice (Table 1). However, no osteoarthritic changes were found in females at 35 weeks (Fig. 1D).

Fig. 1. Typical radiographs of the lateral projections of the knees of STR/Ort mice at 15 and 35 weeks of age ($n=20$). A. Male STR/Ort mouse at 15 weeks. B. Male STR/Ort mouse at 35 weeks. C. Female STR/Ort mouse at 15 weeks. D. Female STR/Ort mouse at 35 weeks.

Table 1. Incidence of radiographic osteoarthritis in STR/Ort mice ($n=10$)

	Age (weeks)	
	15	35
Male	0/10	8/10
Female	0/10	0/10

Age-related changes in cancellous and cortical BMD of femurs

We first examined changes in the density of cancellous and cortical bone in the femurs of STR/Ort mice between 5 and 35 weeks of age (Fig. 2). Femoral cancellous BMD increased between 5 and 10 weeks of age and declined thereafter in male mice but increased until 20 weeks of age in females and then exhibited a relatively rapid decline (Fig. 2A). ANOVA revealed a significant age-by-sex interaction, indicating that patterns of age-related changes in cancellous BMD differed in male and female STR/Ort mice. Between 15 and 20 weeks of age, cancel-

Fig. 2. Age-related changes in cancellous and cortical bone marrow density (BMD) in the femurs of male (open triangles) and female (closed triangles) STR/Ort mice (n=10). A. Cancellous BMD in the femoral metaphysis. B. Cortical BMD in the femoral diaphysis. Values are presented as means $\pm$ SE. The asterisks (*) indicate significant differences at $P<0.05$ compared with age-matched female mice.

lous BMD in females was markedly higher than that in males. In contrast, cortical BMD in the femoral diaphysis displayed nearly identical increases between 5 and 10 weeks of age in both males and females and remained stable until 35 weeks of age (Fig. 2B). No significant age-related changes were detected between males and females.

Age-related changes in trabecular bone architecture by μ CT

In female STR/Ort mice, no significant changes in femoral BV/TV were observed until 15 weeks of age, at which point BV/TV drastically increased until 35 weeks of age (Fig. 3A). In males, BV/TV decreased at 10 weeks of age and was then relatively stable until 35 weeks of age. ANOVA revealed a significant age-by-sex interaction among adults, indicating that patterns of age-related change in trabecular bone architecture differed in males and females. Specifically, trabecular BV/TV in females at 10, 20, and 35 weeks of age were significantly higher than that in males.

Tb.N in females showed a pattern similar to that of BV/TV, increasing between 15 and 20 weeks of age, with higher values detected at 10, 20, and 35 weeks of age than those in males (Fig. 3B). Tb.Th was also significantly higher in females than males at 20 and 35 weeks of age (Fig. 3C). In addition, age-related changes in trabecular architecture at the proximal tibia followed patterns similar to those of the distal femur (data not

Fig. 3. Age-related changes in cancellous bone architecture in the femurs of male (open triangles) and female (closed triangles) STR/Ort mice (n=10). A. BV/TV. B. Tb.th. C. Tb.N. Values are presented as means $\pm$ SE. The asterisks (*) indicate significant differences at $P<0.05$ compared with age-matched female mice.

shown). To estimate the effect of bone structural differences (tibial torsional angle), data for the distal femur was evaluated.

Fig. 4. Age-related changes in cortical bone architecture in the femurs of male (open triangles) and female (closed triangles) STR/Ort mice (n=10). A. Marrow volume. B. External line length. C. Cortical BV/TV (cBV/TV). Values are presented as means $\pm$ SE. The asterisks (*) indicate significant differences at $P<0.05$ compared with age-matched female mice.

Age-related cortical bone changes in the femoral diaphysis by μ CT

To evaluate potential concomitant changes in cortical architecture, we next assessed the cortical bone morphology in the femoral diaphysis of STR/Ort mice between 5 and 35 weeks of age (Fig. 4). At the femoral diaphysis, MV in females decreased dramatically from 10 to 15 weeks of age and was significantly lower than that of males at 15, 20, and 35 weeks of age (Fig. 4A). ELL increased with aging (Fig. 4B). Although ELL in 15-week-old males was higher than that in age-matched females, no differences were observed in mice of any other ages examined. However, cBV/TV in females increased dramatically from 10 to 15 weeks of age and was significantly higher than that of males at 15, 20, and 35 weeks of age (Fig. 4C).

Table 2. Age- and sex-related changes in the angular degree of internal tibial torsion (ADITT) in STR/Ort mice

	Age (weeks)		
	5	15	35
Male	-2.0 ± 1.1	$7.1 \pm 1.1^{a, b)}$	$7.1 \pm 1.0^{a, b)}$
Female	-3.2 ± 1.0	-3.0 ± 0.6	$1.9 \pm 0.9^a)$

ADITT data are presented as means $\pm$ SE (n=20). ^{a)} $P<0.05$ compared with sex-matched 5-week-old mice. ^{b)} $P<0.05$ compared with age-matched female mice.

Age-related changes in ADITT

Changes of ADITT with aging were examined in 5-, 15-, and 35-week-old STR/Ort mice (Table 2). ADITT of female STR/Ort mice at 35 weeks of age was higher than that in 5- and 15-week-old females. In male STR/Ort mice, ADITT increased with age and was significantly higher in 15- and 35-week-old males than that in 5-week-old males. Sex-based differences were also detected, as the ADITTs of male STR/Ort mice at 15 and 35 weeks of age were significantly higher than those of age-matched female mice.

Effect of ovariectomy on bone architecture

To investigate the involvement of osteoclasts in bone marrow narrowing in female mice, ovariectomies were performed in 10-week-old female STR/Ort mice with confirmed narrow bone marrow space. The MVs of these ovariectomized (OVX) mice significantly increased, whereas cBV/TV and BMD significantly decreased (Fig. 5A, 5C, and 5D, respectively). No differences in external line length were found between the sham-operated and OVX groups (Fig. 5B). Ovariectomies were also performed in 15-week-old female STR/Ort mice, in which the bone marrow space had disappeared, and significant increases in MV and decreased BMD were also observed (Fig. 5A, 5C, and 5D, respectively).

Discussion

In this study, we examined differences in BMD and bone architectural changes with aging between male and female STR/Ort mice. Earlier decreases of cancellous BMD were observed in male STR/Ort mice, while no differences in cortical BMD were detected between males and females. An age-related decline in the bone

Fig. 5. Effect of ovariectomy on cortical bone architecture of female STR/Ort mice (n=8). A. Cortical bone marrow volume. B. External line length. C. Cortical BV/TV (cBV/TV). D. Bone marrow density (BMD). Values are presented as means $\pm$ SE. The asterisks (*) indicate significant differences at $P < 0.05$ compared with sham-operated mice.

marrow space of the femoral diaphysis occurred in both males and females but was more dramatic in female mice. In addition, an earlier increase in ADITT was observed in male STR/Ort mice. Differences in age- and sex-related bone architecture changes between female and male STR/Ort mice may explain the differential incidence of OA observed in STR/Ort mice.

In humans, subchondral bone changes are important in the progression of OA, while in small animals, femoral shaft BMD also influences OA progressions [5, 14, 22]. For example, a comparative analysis of bone and cartilage metabolism in Hartley guinea pigs with naturally occurring OA showed that femoral shaft BMD was higher in guinea pigs with OA than in controls [14]. Calvo *et al.* [5] demonstrated that lower BMD increased the severity of cartilage damage in an experimental model of knee OA in rabbits, while Sarukawa *et al.* [22] reported that an increase in femoral BMD prior to the establishment of radiographic OA in male STR/Ort mice may be related to the development of OA. In the present study, no differences in the BMD of the femoral diaphysis were detected between male and female STR/Ort mice. Therefore, the BMD of the femoral diaphysis does not appear related to the differential incidence of OA

between males and females.

In addition to subchondral and femoral shaft BMD changes, modifications to cancellous bone have also been reported in OA patients and model animals [2, 8, 9, 17]. In a guinea pig OA model, Layton *et al.* [17] observed with μCT that an initial bone loss of trabecular volume fraction led to initiation of OA. In a canine OA model induced by anterior cruciate ligament (ACL) transection, Dedrick *et al.* [7] demonstrated that an increase in subchondral bone thickness was accompanied by a decrease in trabecular thickness, while Beuf *et al.* [2] reported that a reduction in BV/TV was observed in human knee OA patients. Here, we found that decreasing cancellous volume and BMD occurred earlier in male STR/Ort mice than in females, a result which may explain the earlier initiation and progression of OA in male STR/Ort mice.

Several studies have reported that increased bone resorption occurs in OA patients [1, 18]. In our present study, in addition to differences of cancellous volume and BMD, we found that the bone marrow space in female STR/Ort mice decreased with age and had nearly completely disappeared by 15 weeks of age. Therefore, we speculated that lower bone resorption is a characteristic phenotype of female STR/Ort mice. To investigate the effects of bone resorption in differential bone structure changes, we accelerated bone resorption using ovariectomy. Acceleration of osteoclastogenesis by ovariectomy significantly increased the bone marrow space, even when ovariectomy was performed at 15 weeks of age, at which time the bone marrow space had nearly entirely disappeared. Recently, peroxisome proliferator-activated receptor- γ (PPAR- γ) signaling, which plays an important role for pro-osteoclastogenic differentiation [29], was shown to be downregulated in male STR/Ort mice [30]. Tie2Cre-mediated hematopoietic cell-specific PPAR- γ deletion leads to osteopetrosis, which is characterized by markedly increased bone volume and a reduced medullary cavity space in Tb.th and Tb.N. Our observed changes in female STR/Ort mice were remarkably similar to those found in Tie2Cre-mediated PPAR- γ knockout mice [29]. Osteopetrosis occurs when the rate of bone formation exceeds the rate of bone resorption. Our findings suggest that the lower rate of bone resorption in female STR/Ort may have participated in osteo-

petrotic changes.

In contrast, bone marrow narrowing in male STR/Ort mice was relatively minimal. Notably, patella dislocation increased with age in male, but not female, STR/Ort mice [28]. Our preliminary data suggest that surgical patella dislocation in normal rats induces cancellous and cortical bone loss and an increase of endosteal circumference; therefore, differential bone architectural changes may be associated with patella dislocation in male STR/Ort mice. Taken together with the findings of Masell *et al.* [18], who reported that hip OA cancellous bone has markedly higher bone metabolism activity compared with normal tissues, and Bettica *et al.* [1], who observed increased bone resorption in patients with progressive knee OA, our results suggest that sex-related differences in bone resorption activity contribute to the different incidence of OA in STR/Ort mice.

Prior to our present study, we speculated that male STR/Ort mice may have higher bone resorption than females. However, although acceleration of bone resorption by ovariectomy changed the bone structure in female mice, the incidence of OA was not affected, a finding that is consistent with previous reports [6]. Therefore, we propose that other factors may contribute to OA progression in male STR/Ort mice. We also previously reported that osteoarthritic changes in male STR/Ort mice may be caused by ITT [20]. Turner [24] identified that the knees of OA patients with patellofemoral instability had greater external tibial torsion than normal subjects, whereas patients with panarticular OA of the knee exhibited reduced external torsion or true internal torsion. In addition, Yagi *et al.* [31, 32] reported the occurrence of tibial torsional deformities in association with medial-type osteoarthritic knees. Here, the internal rotation of the tibia markedly increased with age in male STR/Ort mice. Taken together, these results suggest that increased ITT contributes to OA in male STR/Ort mice.

A few limitations of the study warrant mention. A primary shortcoming of our study is that we inferred age-related changes by cross-sectional analysis. In future studies, the use of *in vivo* μ CT systems will facilitate longitudinal assessment of changes in bone architecture with aging [4, 26, 27]. In addition, we only examined age-related bone changes, and relationships between OA

initiation and/or progression were not investigated.

In conclusion, age- and sex-related bone architectural changes differ between male and female STR/Ort mice. These differences in bone structure may explain the differential incidence of OA observed in STR/Ort mice.

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