ORIGINAL

Effects of unilateral sciatic neurectomy on growing rat femur as assessed by peripheral quantitative computed tomography, Fourier transform infrared spectroscopy and bending test

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Abstract : We studied the effects of unilateral sciatic neurectomy (USN) on the development of the femoral shaft in the 30 growing Wistar-derived rats aged 5 weeks. Rats were allocated to three groups. One of these was immediately used for measurements, and the remaining 2 groups underwent USN of internal control. Specimens obtained from each group were divided into 2 subgroups : left femurs of each group served as the control subgroup (CONT) and right femurs from each group as the USN-operated subgroup (USN-OP). The bone mineral density (BMD), bone mineral content (BMC), bone area, periosteal circumference and endosteal circumference were measured by peripheral quantitative computed tomography (pQCT) and the mineral / matrix ratio was evaluated by Fourier transform infrared spectroscopy (FTIR). A three-point bending test was performed to analyze the biomechanical effects of sciatic neurectomy. USN-OP showed a significant decrease in cortical BMC, bone area, and periosteal circumference compared with CONT. The mineral / matrix ratio of cortical bone did not differ significantly between USN-OP and CONT. Strength and stiffness were significantly decreased in USN-OP compared with CONT. The results showed that USN inhibited periosteal bone formation, but has no significant effects on the mineral / matrix ratio of cortical bone in femurs. J. Med. Invest. 51 : 96-102, February, 2004

Keywords : *sciatic neurectomy, bone mineral density, pQCT, FTIR, bone strength*

INTRODUCTION

Unilateral sciatic neurectomy (USN) is often used in experiments as a model of immobilization, which causes osteopenic changes localized to the affected limb. Several investigators have demonstrated changes in the bone mineral density (BMD) and bone strength with osteopenic animal models (1-5). Our previous study (6) showed that cortical bone mineral content (BMC), bone area and periosteal circumference at the diaphysis of the femur 8 weeks after USN were significantly reduced, whereas cortical bone mineral density (BMD), mineral / matrix ratio by Fourier transform infrared spectroscopy (FTIR), endosteal circumference at the diaphysis were not decreased. These results suggest that USN for 8 weeks does not affect bone quality shown as mineral / matrix ratio, and BMD at the diaphysis of the femur, and that USN causes reduction of outer diameter at the diaphysis of the femur.

The purpose of this study was to investigate and com-

Received for publication November 28, 2003 ; accepted January 13, 2003.

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pare the effects of USN on the development of femoral shaft between 4 weeks and 8 weeks after the UNS, in growing male rats aged 5 weeks. The mineral phase and organic matrix in cortical bone was analyzed by FTIR. As for the biomechanical parameters, the strength and stiffness of the femoral shaft were measured by the three point bending test. BMC, BMD, periosteal and endosteal circumferences of femoral shaft were measured by peripheral quantitative computed tomography (pQCT).

Recently, pQCT has been introduced to both clinical and laboratory investigations, and it is the only non-invasive assessment of bone mineral of the long bones to measure a three-dimensional density and allow separate determination of the cortical and cancellous bone areas (7-11). The most frequently used technique for measure to BMD is dual energy X-ray absorptiometry (DXA)(12, 13). DXA provides measurements of the so-called areal or surface BMD. The unit of BMD with DXA is grams of ashed bone per unit of bone area scanned (g/cm^2). Therefore, the values generated by DXA directly depend on both the size of bone and the integrated BMD of the scanned skeletal tissue.

FTIR is useful for examining the molecular structure and conformation of biological macromolecules because it measures absorption in vibrational or rotational energy of atoms or groups of atoms within the molecule (14-16).

MATERIALS AND METHODS

Animal care and Experimental Design

Thirty, male Wistar-derived albino rats aged 5 weeks (135-150g) were divided into three groups (each group : n=10). And one of the these groups was immediately used for pQCT, FTIR and bending test. In the remaining 2 groups, the animal underwent unilateral sciatic neurectomy of their right hindlimbs. The rats were anesthetized by an intraperitoneal injection of pentobarbital sodium. An incision was made on the posterior aspect of the proximal femur. The sciatic nerve was identified and a 5 mm segment was resected. The wound was closed immediately and the contralateral leg was left intact as the control. The rats were fed a standard lab chow (MF ; Oriental Yeast Co., Ltd., Tokyo, Japan) and allowed to have a free access to tap water. They were maintained with normal cage activity during the experimental period. At 4 and 8 weeks after the surgery, the animals in each group were sacrificed, and the bilateral femurs were removed for measurement of pQCT, FTIR and bending test. Specimens obtained from each group were divided into 2 subgroups. The left femurs from each group were used as the control subgroup (CONT) and the right femurs from each group as the USN-operated subgroup (USN-OP).

Peripheral Quantitative Computed Tomography (pQCT)

Five rats of each group were prepared for pQCT. The bone mineral density (BMD), bone mineral content (BMC), bone area, periosteal circumference and endosteal circumference were measured by pQCT (XCT-960 A, Norland / Stratec, Fort Atkinson /Pforzheim, USA / Germany). The total bone, cancellous bone and cortical bone were examined using a 1 mm-thick slice, at the midshaft of the femur. Hence, the unit of BMC with pQCT is milligrams of ashed bone per 1 mm-thick slice scanned (mg/mm).

Fourier Transform Infrared Spectroscopy (FTIR)

The remaining 5 rats of each group were evaluated by using the FTIR. Femurs were removed and soft tissue, periosteum, and bone marrow were immediately cleaned off. The midshaft of the diaphysis was frozen in liquid nitrogen and lyophilized for 48 hours to remove all water and then ground in liquid nitrogen. The infrared spectra were recorded in the FTIR spectrometer

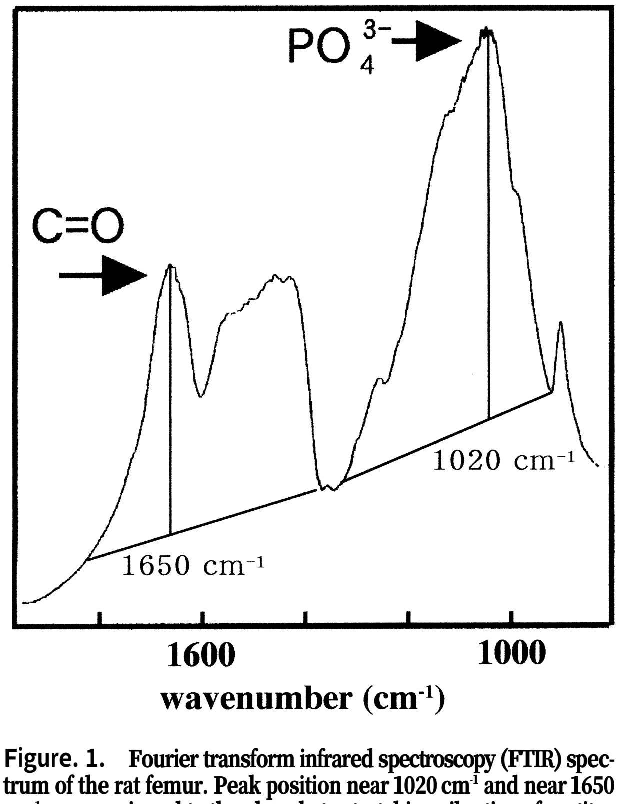

Figure. 1. Fourier transform infrared spectroscopy (FTIR) spectrum of the rat femur. Peak position near 1020 cm^{-1} and near 1650 cm^{-1} were assigned to the phosphate stretching vibration of apatites and the amide I stretching vibration of bone organic matrix, respectively. The mineral / matrix ratio was calculated from the ratio of absorbance of the phosphate band at 1020cm^{-1} to the amide I band at 1650 cm^{-1}.

(FTIR-1720, Perkin-Elmer, Norwalk, USA) equipped with a triglycine sulfate detector provided with the KBr windows. The samples were examined by the KBr technique (17). Figure 1 shows typical FTIR spectrum of the rat femur. Peak position near 1020 cm^{-1} and near 1650 cm^{-1} were assigned to the phosphate stretching vibration of apatites and the amide I stretching vibration of bone organic matrix, respectively (6). The spectral resolution was 4 cm^{-1}. The abscissa range was 900-1800 cm^{-1}, covering the phosphate band and the amide 1 band. Data were Fourier transformed and averaged after 50 scans. The mineral/matrix ratio was calculated from the ratio of absorbance of the phosphate band at 1020 cm^{-1} to the amide I band at 1650 cm^{-1}.

Measurements of bone biomechanical parameters (bending test)

After pQCT, the femurs were subjected to the three-point bending test. Measurements were made with a bone strength tester (Model MZ-500S, Marto, Tokyo, Japan) according to the method of Molster *et al.*(18). Each femur was positioned with the flexor surface side up on the top of two metal supports located at a distance of 13 mm in the tester, and the bending force was applied midway at the rate of 10 mm/min until fracture occurred. Bone biomechanical parameters were determined from a load-deflection curve. Stiffness (N/mm) was calculated from the slope of the load deflection curve between 20% and 70% of the maximum load value. Energy absorbed (N•m) was determined from the area under the maximum load-deflection curve. These data were calculated automatically with the apparatus.

Statistical analysis

Results were expressed as the mean ± standard deviation (SD). A one-way analysis of variance (ANOVA) was used to show a significant difference, and this was further evaluated using Fisher's protected least significant difference (PLSD)(19). A probability (p) of less than 0.05 was considered statistically significant.

RESULTS

Femoral length

Femoral length increased with time in both unilateral sciatic neurectomy-operated (USN-OP) and control (CONT) groups. At 4 and 8 weeks after the surgery, femoral length did not differ significantly in both groups (Figure 2).

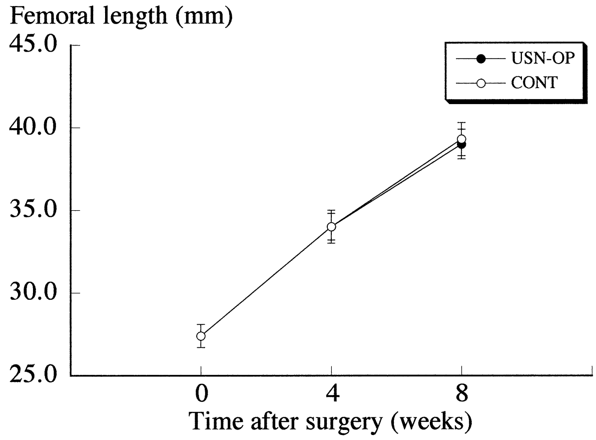

Figure. 2. Time course of changes in femoral length. Femoral length increased with time in both unilateral sciatic neurectomy-operated (USN-OP) and control (CONT) groups, which did not produce significant difference between the two.

pQCT

The cortical bone mineral content (BMC) and bone area increased with time in both USN-OP and CONT. Such time-dependent increase of these parameters was significantly slower in USN-OP compared with CONT (cortical BMC: $p<0.01$ USN-OP vs CONT at the 4th week, $p<0.01$ USN-OP vs CONT at the 8th week, cortical bone area : $p<0.05$ USN-OP vs CONT at the 4th week, $p<0.05$USN-OP vs. CONT at the 8th week). BMD also increased as the rats grew older, but there were no significant differences between USN-OP and CONT (Figure 3).

The periosteal circumference of USN-OP was significantly decreased compared with CONT, whereas the endosteal circumference did not vary significantly between USN-OP and CONT (periosteal circumference : $p<0.001$ USN-OP vs. CONT at the 8th week) (Figure 4).

The cancellous BMC, BMD and bone area at the diaphysis did not differ significantly between USN-OP and CONT.

FTIR

The mineral / matrix ratio increased with time in both USN-OP and CONT. And the difference between the ratio of the USN-OP and that of CONT was not statistically significant at the 4th week and the 8 the week (Figure 5).

Measurement of bone biomechanical parameters (bending test)

Table 2 shows the results of the bending test. The strength of the femur increased as the rat grew older. And there were significant changes between USN-

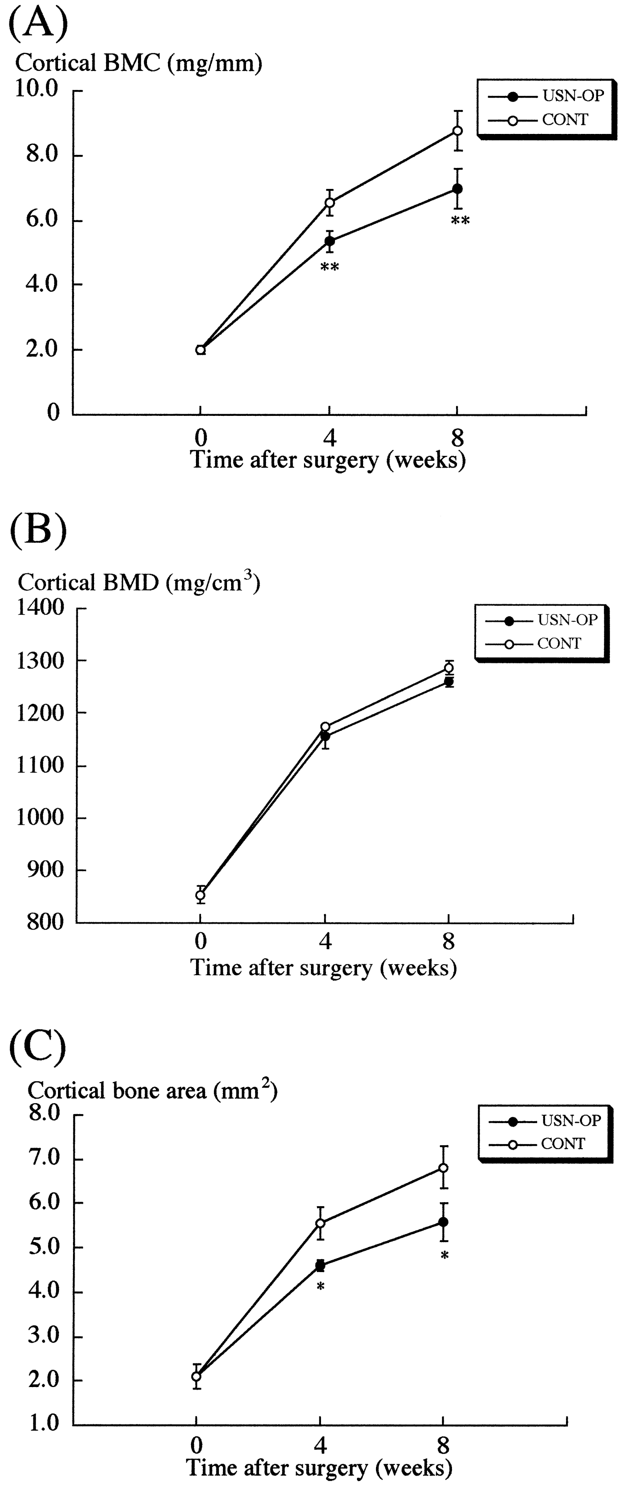

Figure. 3. (A, B, C) Time course of changes in cortical bone mineral content (BMC) (A), cortical bone mineral density (BMD) (B), and cortical bone area (C) of the femur in USN-OP (closed circles) and CONT (open circle). Cortical BMC showed significant difference between these two groups at 4 and 8 weeks after the surgery, whereas there was no significant difference of cortical BMD. Each point is the mean±SD.
***p<0.05,**p<0.01 vs same-age control subgroup.**

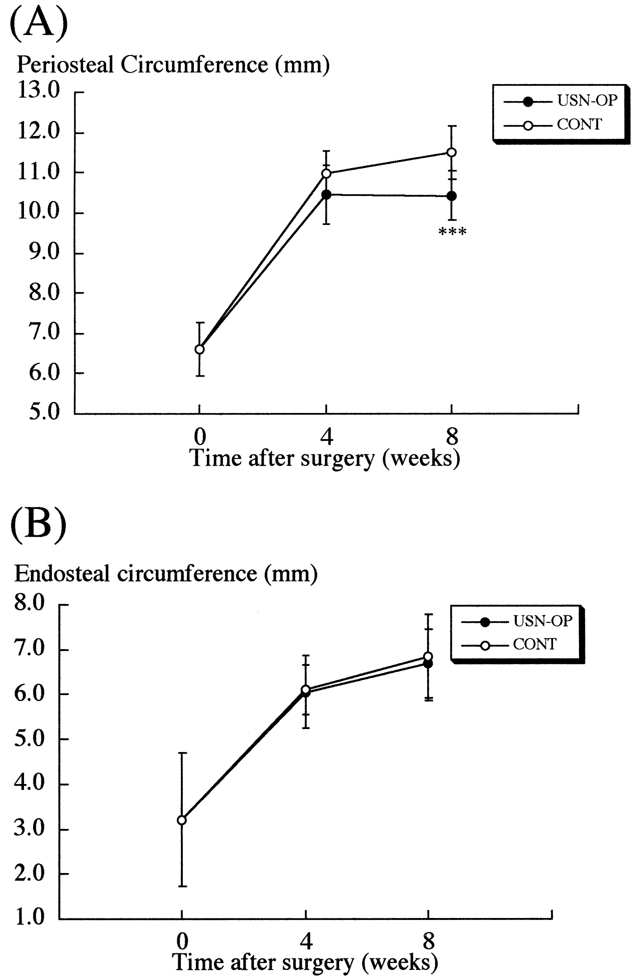

Figure. 4. Time course of changes in periosteal circumference (A), and endosteal circumference (B) of the femur in USN-OP (closed circles) and CONT (open circles). Periosteal circumference of the femur showed significant difference between these two groups at 8 weeks after the surgery. In contrast, endosteal circumference of the femur showed no significant difference. Each point is the mean±SD.
*****p<0.001 vs same-age control subgroup.**

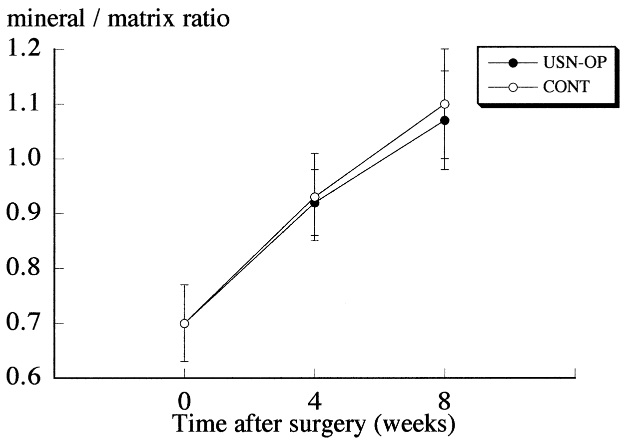

Figure. 5. Time course of changes in mineral / matrix ratio of the femur in USN-OP (closed circles) and CONT (open circles). There were no significant difference of mineral/matrix ratio between these two groups at 4 and 8 weeks after the surgery. Each point is the mean±SD.

OP and CONT (p<0.05USN-OP vs. CONT at the 4th week, p<0.01 USN-OP vs. CONT at the 8th week). The stiffness also increased as the rats grew older. The stiffness of USN-OP was significantly decreased compared with CONT only at the 8th week (p<0.05 USN-OP vs. CONT at the 8th week). However, the energy absorbed in the bone to make the fracture did not differ significantly between USN-OP and CONT (Figure 6).

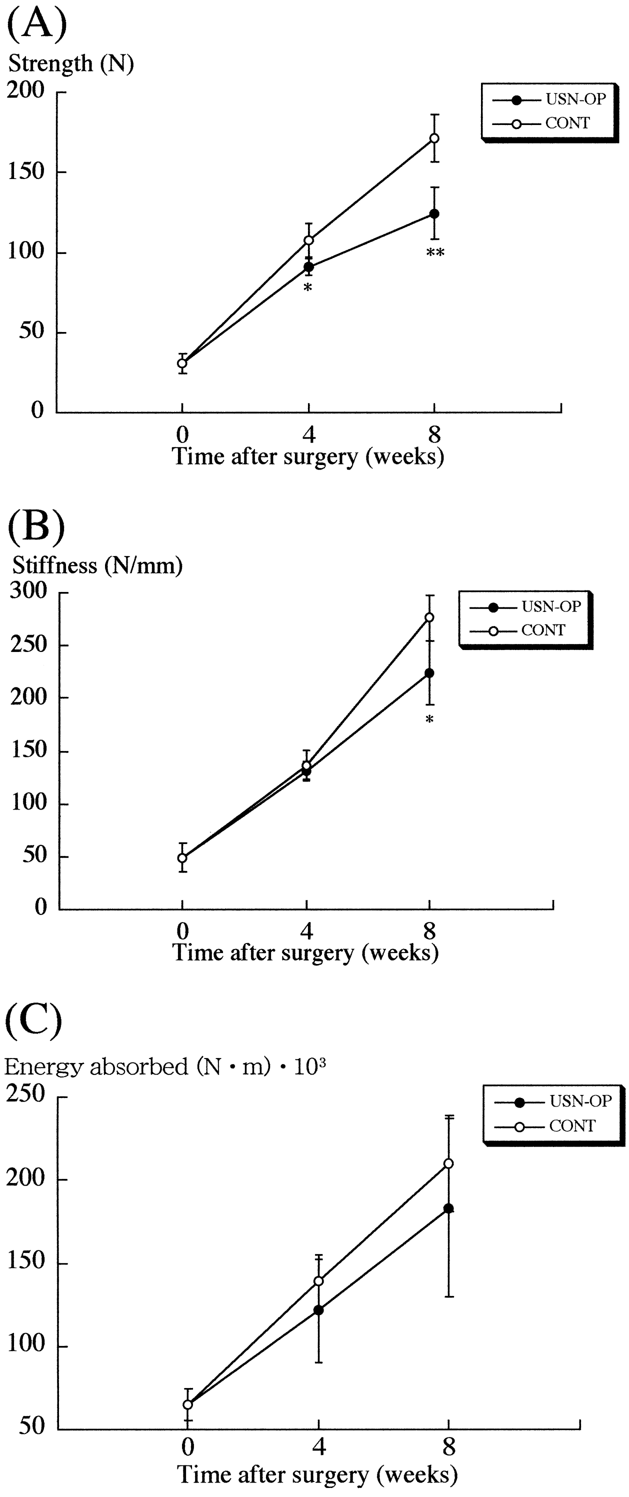

Figure. 6. Time course of changes in strength (A), stiffness (B), and energy absorbed (C) of the femur in USN-OP (closed circles) and CONT (open circles). Strength of rat femur showed significant difference between these two groups at 4 and 8 weeks of the surgery, whereas stiffness of the femur showed significant difference at 8 weeks after the surgery. Each point is the mean±SD. *p<0.05, **p<0.01 vs same-age control subgroup.

DISCUSSION

The major findings of this study were that USN reduced cortical BMC and bone area association with the decrease of the bone strength, and that USN did not cause a significant change in the mineral / matrix ratio of cortical bone of the developing femur.

This study showed an age-related increase in cortical bone and a decrease in the cortical BMC, cortical bone area, and periosteal circumference of the diaphysis after USN. The reduction of cortical BMC was reflected in a decrease in the cortical bone area, and was followed by a decrease of the periosteal circumference. The bone balance is in favor of the bone formation on the periosteal surface, where osteoclasts do not appear in any appreciable numbers (20). Osteoblasts are sensitive to the mechanical load and their function is impaired after the start of immobilization (21). Thus, USN inhibits the periosteal bone formation. The endosteal bone resorption, however, is constant after USN, and is nearly matched by the endosteal bone formation. The decrease in cortical BMC, bone area and periosteal circumference, therefore, may be due to inhibition of the periosteal bone formation.

The strength of the femoral shaft depends on the cortical bone, because the diaphyses of long bones are formed mostly by the cortical tissue (22, 23). In addition, a decrease in the cortical bone mass appears to play an important role in reduction in the strength of long bones (12). Although several roentogengraphic techniques have been employed to measure changes in the bone metabolism, it has been impossible to resolve the heterogeneous response of the cancellous and cortical bone. And these techniques measure the areal bone density, which is influenced by the bone size. A recently developed technique for measuring a bone mass is pQCT. It can selectively resolve total bone into the cancellous bone and cortical bone, and it is the only non-invasive assessment of bone mineral of the long bones to measure a three-dimensional density.

We used FTIR to examine whether USN affects the quality of cortical bone. FTIR allows us to characterize both the mineral and organic parts of bones. According to a previous study (15), the information obtained from FTIR analysis of bone includes a measure of the relative amount of mineral and matrix content and the arrangement of apatite and organic matrix. Therefore, FTIR is a promising method to evaluate the bone quality. The mineral / matrix ratio increased with time in both UNS-OP and CONT. However, the difference between the ratio of USN-OP and that of CONT was not statistically significant. Therefore, it can be said that USN did not cause a significant change in the quality of cortical bone of the developing femur in terms of the mineral/matrix ratio. In this study, the crystallinity of mineral was not assessed. Further studies are needed to clarify this point.

As for the biomechanical parameters measured by the three point bending test, the strength and stiffness of the femoral shaft showed a significant difference between USN-OP and CONT. Previous studies have established that the mechanical strength and stiffness are positively correlated with the bone ash and mineral content (24, 25). Carter *et al*.(22) reported that the bone strength and stiffness might be predicted from BMD. However, in the present study to measure a three-dimensional density with pQCT, USN reduced cortical BMC accompanied with a decrease of the cortical bone area, but did not cause a significant change in cortical BMD during the development of the femur. Therefore, the difference in the strength of femoral shaft after USN did not correspond to cortical BMD. The results suggested that USN affected the cortical BMC and cortical bone area to decrease the bone strength of the developing femur.

Based on these facts, we conclude that USN leads to the inhibition of periosteal bone formation in cortical bone of the developing femur without inducing a significant change in the mineral/matrix ratio, and that USN reduces the bone strength of the femoral shaft, resulting from a decrease of the cortical BMC and bone area.

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