Bone Density of the Femur and Fiber Cross-Sectional Area and Oxidative Enzyme Activity of the Tibialis Anterior Muscle in Type II Collagen-Induced Arthritic Mice

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Abstract: Femur bone densities and tibialis anterior muscle properties of type II collagen-induced arthritic mice were determined. Furthermore, voluntary running activities of arthritic mice were compared with those of controls. Arthritis was induced by an intradermal injection of type II collagen in the adjuvant. Body and muscle weights were lower in arthritic mice than in controls. Cortical and trabecular bone densities and muscle fiber cross-sectional areas were decreased by arthritis. After classifying the arthritic severity into slight, intermediate, and severe levels based on the degree of knuckle swelling, cortical and trabecular bone densities, fiber cross-sectional areas, and fiber succinate dehydrogenase activities were lowest when arthritis was most severe. Furthermore, arthritic mice, especially those with intermediate and severe levels, showed lower voluntary running activities. These findings indicate that lower bone density and muscle atrophy of type II collagen-induced arthritic mice are related to arthritic severity and decreased motor activity.

Key words: bone density, cross-sectional area, motor activity, mouse, muscle fiber, succinate dehydrogenase activity, type II collagen-induced arthritis.

Degeneration in the musculoskeletal system, including muscle atrophy and bone osteoporosis, is widely accepted as being induced by aging [1–5], hind-limb suspension [6–8], or exposure to microgravity [9–12]. These changes are considered to result from decreased motor activity.

Previous studies observed that animal models [13–16] and patients [17] with arthritis showed decreased spontaneous motor activities because of severe chronic pain in the joints. Type II collagen-induced arthritis is a model of polyarthritis induced in susceptible rodents by immunization with type II collagen [18, 19]. Muscle atrophy and decreased bone density occur in type II collagen-induced arthritic rodents because these animals may have lower motor activities as a result of their distal joint pain. Therefore this study investigated cortical and trabecular bone densities of the femur and fiber cross-sectional areas and succinate dehydrogenase (SDH) activities of the tibialis anterior muscle in type II collagen-induced arthritic mice. Furthermore, voluntary running activities of arthritic mice were compared with those of controls.

METHODS

All experimental procedures and animal care were conducted in accordance with the Guide for the Care and Use of Laboratory Animals prepared by the Physiological Society of Japan. This study was also approved by the Institutional Animal Care and Use Committee of Kyoto University.

Antigens and immunizations. Sixty-day-old DBA/1J male mice were randomly divided into control (n = 5) and arthritic (n = 13) groups. All mice were individually housed in cages of the same size. The mice in the arthritic group were immunized from 60 to 81 days after birth. Type II collagen was solubilized from fetal bovine articular cartilage by limited proteolysis with pepsin [20]. Type II collagen was dissolved in 0.01 M acetic acid at 4°C prior to use. The mice were immunized by intradermal injection of 400 µg of type II collagen emulsified in FCA (Freund complete adjuvant) at 4 mg/ml. Twenty-one days after primary immunization, they were immunized by an intradermal injection of 400 µg of type II collagen emulsified in FIA (Freund incomplete adjuvant) at 4 mg/ml. Arthritic severity was classified into three levels based on
the degree of knuckle swelling in at least one hind-limb; slight (one digit or mild swelling), intermediate (two digit swelling or swelling of the tarsus and ankle), and severe (hard swelling or bone deformity) [20].

All mice were kept in a controlled environment with fixed 12:12 h light:dark cycles (lights off from 20:00 to 08:00), and room temperature was maintained at 22 ± 2°C. Food and water were provided ad libitum.

Tissue procedures. At 126 days after birth, the mice were weighed and anesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg/kg body weight). The femur bone was removed and cleaned of connective and soft tissues. The tibialis anterior muscle was also removed, and it was wet-weighed.

The femur bone was analyzed using peripheral quantitative computed tomography [5]. The distal site of the bone was chosen for an analysis of bone density.

The muscle was pinned on a cork at its in vivo length, immediately frozen in isopentane cooled in a mixture of dry ice and acetone, and stored at −80°C until analyses. The midportion of the muscle was mounted on a specimen chuck using compound. Serial transverse sections, 10-μm thick, of the muscle on chuck were cut in a cryostat at −20°C. The sections were brought to room temperature, air-dried for 30 min, and stained for SDH activity, an indicator of mitochondrial oxidative capacity [21–23]. The SDH activity was rendered visible by incubating the sections in 0.1 M phosphate buffer (pH 7.6) containing 0.9 mM sodium azide, 0.9 mM 1-methoxyphenazine methylsulfate, 1.5 mM nitroblue tetrazolium, 5.6 mM EDTA-disodium salt, and 48 mM succinate disodium salt. The reaction was terminated by multiple washings in distilled water, dehydration in graded ethanol, and being passed through xylene. For histochemical controls, either succinate disodium salt or nitroblue tetrazolium was excluded from the incubation medium. Tissue sections were digitized as gray scale images, and the value of the SDH activity was expressed as an optical density (OD) value on a computer-assisted image processing system (Neuroimaging System, Kyoto, Japan) [24–26].

Each pixel was quantified as one of 256 gray levels. A gray value of zero was equivalent to 100% transmission of light, and that of 255 was equivalent to 0% transmission of light. The fiber cross-sectional area and OD value were measured by tracing the outline of each fiber in the section. The OD values of all pixels within a fiber were converted to a mean OD value using a calibration photographic tablet with 21-step gradient density ranges of diffused density values. The fiber cross-sectional area and SDH activity of fibers in the deep (close to the bone), middle (between the deep and surface regions), and surface (away from the bone) regions of the muscle were examined. Approximately 100 fibers were sampled from each region in the muscle.

Voluntary running activities. Eighty-four-day-old DBA/1J male control (n = 10) and arthritic (n = 19) mice were used to measure voluntary running activity for 6 weeks. Arthritic mice were immunized from 60 to 81 days after birth, and the arthritic severities were classified into slight (n = 6), intermediate (n = 6), and severe (n = 7) levels. Each mouse was housed in a cage with a wheel [27] and allowed to run voluntarily in the wheel for 24 h. Voluntary running distances of individual mice were continuously monitored and data were stored in a personal computer connected to the wheels [28]. All mice were kept in a controlled environment with fixed 12:12 h light:dark cycles (lights off from 20:00 to 08:00), and room temperature was maintained at 22 ± 2°C. Food and water were provided ad libitum.

Statistics. Means, standard deviations, and correlation coefficients were calculated from individual values using standard procedures. A Student’s t-test was used to determine significant differences between the control and arthritic groups. A one-way analysis of variance (ANOVA) was used to evaluate significant differences among the three levels of arthritic severity: slight, intermediate, and severe. When the differences were significant based on ANOVA analyses, further comparisons were made using post hoc tests. A probability value of 0.05 was considered significant.

RESULTS

Body and muscle weights

The body weight of the arthritic group was lower than that of the control group (Fig. 1A). The absolute and relative weights of the tibialis anterior muscle in the arthritic group were lower than those in the control group (Fig. 1, B and C).

Bone densities

The cortical and trabecular bone densities of the femur in the arthritic group were lower than those in the control group (Fig. 2, A and B).

The arthritic severities were classified into three levels: slight, intermediate, and severe (Fig. 3). The cortical bone density of the femur was greatest for animals with slight arthritis and lowest for those with severe arthritis (Fig. 4A). The trabecular bone density of the femur was greater for animals with slight arthritis than for those with intermediate and severe arthritis (Fig. 4B). A relationship between cortical and trabecular bone densities of the femur in the arthritic group was observed (Fig. 5).

Muscle fiber properties

The fiber cross-sectional areas of the tibialis anterior muscle in the arthritic group were lower than those in the control group, regardless of the muscle region (Fig. 6A). In contrast, there were no differences in the fiber SDH activity of the tibialis anterior muscle between the control and...
Fig. 1. Body weights (A) and absolute (B) and relative (C) weights of the tibialis anterior muscle in control and arthritic mice. Values are means and standard deviations from 5 control and 13 arthritic mice. BW, body weight. *p < 0.05 compared with control.

Fig. 2. Cortical (A) and trabecular (B) bone densities of the femur in control and arthritic mice. Values are means and standard deviations from 5 control and 13 arthritic mice. *p < 0.05 compared with control.

Fig. 3. Arthritic severity was classified into three levels based on the degree of knuckle swelling. A, control; B1 and B2, slight arthritis; C1–C3, intermediate arthritis; D1–D3, severe arthritis. The bar on D3 indicates 5 mm.
Fig. 4. Cortical (A) and trabecular (B) bone densities of the femur in mice with slight, intermediate, and severe arthritis. Values are means and standard deviations from 4 mice with slight arthritis, 5 with intermediate arthritis, and 4 with severe arthritis. *p < 0.05 compared with slight; †p < 0.05 compared with slight and intermediate.

Fig. 5. The relationship between cortical and trabecular bone densities of the femur in control (white circles, n = 5) and arthritic (black circles, n = 13) mice. A, B1 and B2, C1–C3, and D1–D3 correspond to the photos in Fig. 3. In arthritic mice (n = 13), r = 0.82, p < 0.01.

Fig. 6. Fiber cross-sectional areas (A) and succinate dehydrogenase activities (B) of the tibialis anterior muscle in control and arthritic mice. Values are means and standard deviations from 5 control and 13 arthritic mice. CSA, cross-sectional area; SDH, succinate dehydrogenase; OD, optical density. *p < 0.05 compared with control in the same region.

Arthritic groups, regardless of the muscle region (Fig. 6B). The fiber cross-sectional areas of the tibialis anterior muscle were greatest for animals with slight arthritis and lowest for those with severe arthritis, regardless of the muscle region (Fig. 7A). Fiber SDH activities in the deep and surface regions of the tibialis anterior muscle were greatest for animals with slight arthritis and lowest for those with severe arthritis. The fiber SDH activity in the middle region of the tibialis anterior muscle was lower for animals with severe arthritis than for those with slight and intermediate arthritis (Fig. 7B).

There were no pathological changes in the tibialis anterior muscle fibers of the arthritic group, regardless of the muscle region or arthritic severity.
Voluntary running activities

The mean voluntary running distance in the control group during 6 weeks of exercise was 1,778 m·day⁻¹, and in the arthritic group it was 299 m·day⁻¹. The mean voluntary running distances of animals with slight, intermediate, and severe arthritis were 655, 188, and 88 m·day⁻¹, respectively. The voluntary running distance was lower in the arthritic groups than in the control group, regardless of the arthritic severity (Fig. 8). Furthermore, the voluntary running distances of animals with intermediate and severe arthritis were less than those of animals with slight arthritis.

DISCUSSION

Increased and decreased neuromuscular activities induce fiber hypertrophy and atrophy in skeletal muscles, respectively. In general, strength exercise elicits fiber hypertrophy and enhances fiber glycolytic enzyme activities in skeletal muscles [29, 30], but endurance exercise enhances fiber oxidative enzyme activities and capillary densities [30, 31]. In contrast, unloading induces decreased bone density and muscle fiber atrophy in the musculoskeletal system [7, 8]. Fiber atrophy and a type shift of fibers from a slow to fast phenotype in skeletal muscles, especially in antigravity muscles, along with decreased oxidative enzyme activity in spinal motoneurons innervating skeletal muscles, presumably antigravity muscles, were observed following exposure to microgravity [9–12].

Immunizations with type II collagen induce polyarthritis in genetically susceptible mice and rats [18, 19]. Type II collagen-induced arthritis is an autoimmune disease initiated by the binding of antibodies to autologous type II collagen in the joint. The arthritic mouse model based on the DBA/1 strain shows degenerative changes in the cartilage and bone with severe joint pain.

This study investigated bone densities of the femur and fiber cross-sectional areas and SDH activities of the tibialis anterior muscle in type II collagen-induced arthritic mice because these animals may have decreased motor activity levels resulting from distal joint pain. Therefore a lower bone density and muscle fiber atrophy would be induced by decreased motor activities. Indeed,
Interleukin-6 is expressed at high levels in several inflammatory and anti-inflammatory effects of interleukin-6 influences the development of chronic inflammation and disease [37]. Furthermore, interleukin-6 levels correlate with markers of disease activity, including sedimentation rate and C-reactive protein [38, 39], and with clinical symptoms, including morning stiffness and number of inflamed joints [38, 40]. Therefore it is suggested that decreased fiber SDH activities in the tibialis anterior muscle of arthritic mice are associated with the increased levels of some cytokines.

We have concluded that type II collagen-induced arthritis induces lower bone density, muscle atrophy, and decreased fiber SDH activity. We further believe that lower bone density and muscle atrophy are related to the levels of arthritic severity and motor activity.

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