INTRODUCTION

It is well accepted that both genetic and environmental factors contribute to the characteristics and dimensions of biological tissues. However, we do not exactly know the relative contributions of genes and environment in determining tissue properties and quantity in animals. In particular, such musculo-skeletal tissues as bone are dynamic and change their mechanical properties and structure in response to mechanical stress as a phenomenon of functional adaptation. Thus, the growth, remodeling, and disease of bone are closely related not only to genetic factors but also to environmental demands. Although the adaptation of bone to mechanical stress and the bone remodeling have been studied extensively, relatively little is known about the effects of the interaction between genes and environment on the mechanical properties and dimensions of bone.

Today, transgenic animals embody one of the most potent and exciting research tools in the biological science. Techniques for gene transfer into animals have been established over the past two decades, and various groups have produced many kinds of transgenic animals. An understanding of the developmental and tissue-specific regulation of gene expression is achieved only through in vivo whole animal studies. Therefore, the transgenic animal model is generally recognized as an essential tool for biomedical and biological research. However, there are few studies on the biomechanical properties of such musculo-skeletal tissues as bone in the transgenic animals, which will be yielding much useful information on the development and disease of these tissues.

The final goal of our studies is to elucidate the relative contributions of genetics and mechanical environment in determining the biomechanical properties of bone. For this final goal, in the present study, we studied the basic mechanical properties of cortical bone in rats whose growth hormone gene expression was suppressed by antisense RNA transgene.

MATERIALS AND METHODS

Transgenic, growth retarded rats were used for the experiment. The details of procedures for the production of these rats have been reported previously [1, 2]. Briefly, transgenic rats bearing DNA complementary to sequences in the rat growth hormone gene were generated by microinjection into rat embryos. In these animals, antisense RNA is expressed in the pituitary. The expression of the antisense RNA transgene caused a growth hormone deficiency, resulting in dwarfism in the transgenic rats. The offspring of one transgenic rat line was selected, and all transgenic offspring were obtained from brother-sister mating of each transgenic generation. We used only mature female transgenic rats (transgenic group) for the biomechanical evaluation. Normal mature female Wister rats (control group) were used to obtain control data. All the experimental animals were euthanized, and body weight of each animal was recorded. After sacrifice, the left femur in each animal was obtained from the hind limb. The length of each femur was measured with a caliper. The diaphyses of the femur, which were composed of only cortical tissues, were divided into 2 parts using a circular saw. The proximal and middle portions of the diaphyses were used for the measurement of ash fraction and compression testing, respectively. A 3 mm thick ring-like specimen was obtained from the middle portion of the femur. The cross-sectional area of the specimen was measured using an image analyzer. A compression test was performed using a conventional material tester at a crosshead speed of 1 mm/min with the specimen immersed in physiological saline solution (37°C). The bone specimens, which were obtained from the proximal portion of the femur, were dried in acetone for 3 days, in air for 1 day, and then placed in a 60°C oven for 6 hours. After determinations were made of the mass of the dry samples, the specimens were ashed in a muffle furnace at 600°C for 12 hours and weighed again. The ash fraction ($\alpha$) was calculated from dividing the weight of ash by the weight of dry bone.

RESULTS

The body weight of experimental animals and the dimensions of rat femur are shown in Table 1. The body weight of transgenic group was significantly lower than that of control group (approximately 70% of the control value). The length and cross-sectional area of the femur of transgenic group were approximately 90% and 75% of the control

---

BIOMECHANICAL PROPERTIES OF CORTICAL BONE IN RATS WHOSE GROWTH HORMONE GENE EXPRESSION WAS SUPPRESSED BY ANTISENSE RNA TRANSGENE

Ei Yamamoto (1), Kazuya Matsumoto (2), Kazuhiro Saeki (2), Yoshihiko Hosoi (2), Akira Iritani (2)

(1) Department of Mechanical Engineering
School of Biology-Oriented Science and Technology
Kinki University
Wakayama, Japan

(2) Department of Genetic Engineering
School of Biology-Oriented Science and Technology
Kinki University
Wakayama, Japan
values, respectively; these values were also significantly lower than those of control group. Table 2 shows load at break, tangent modulus, compressive strength, and strain at break of the femur, where the tangent modulus was defined by the slope of a stress-strain curve between 3 and 5% strain. The load at break of transgenic group was significantly lower than that of control group (approximately 70% of the control value). However, there were no significant differences in the tangent modulus, tensile strength, and strain at break between the two groups. Concerning the ash fraction, the values were approximately 70% for both groups (Table 2).

Table 1  Animal body weight and the dimension of rat femur.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control (n = 4)</th>
<th>Transgenic (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight W  (g)</td>
<td>324.0 ± 17.7</td>
<td>225.1 ± 31.4  *</td>
</tr>
<tr>
<td>Length of femur L₁ (mm)</td>
<td>36.4 ± 0.9</td>
<td>32.2 ± 0.1  *</td>
</tr>
<tr>
<td>Cross-sectional area of femur A₀ (mm²)</td>
<td>5.8 ± 0.4</td>
<td>4.3 ± 0.3  *</td>
</tr>
</tbody>
</table>

Mean ± S.D. * Significant difference vs. Control (p < 0.05)

Table 2  Mechanical properties and ash fraction of rat femur.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control (n = 4)</th>
<th>Transgenic (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Load at break Fᵢ (N)</td>
<td>942.8 ± 73.7</td>
<td>646.5 ± 112.0  *</td>
</tr>
<tr>
<td>Tangent modulus Eᵢ (GPa)</td>
<td>2.3 ± 1.3</td>
<td>2.8 ± 0.8</td>
</tr>
<tr>
<td>Compressive strength σₑ (MPa)</td>
<td>164.0 ± 20.3</td>
<td>149.3 ± 19.2</td>
</tr>
<tr>
<td>Strain at break εₑ (%)</td>
<td>9.2 ± 1.7</td>
<td>7.4 ± 1.7</td>
</tr>
<tr>
<td>Ash fraction α (%)</td>
<td>73.0 ± 1.9</td>
<td>72.6 ± 1.9</td>
</tr>
</tbody>
</table>

Mean ± S.D. * Significant difference vs. Control (p < 0.05)

Figure 1 shows the influence of animal body weight on the cross-sectional area, load at break, compressive strength, and ash fraction of the femur in transgenic and control groups. The cross-sectional area and load at break increased with increase in body weight; they had statistically significant linear correlations with body weight. However, body weight did not have any significant influence on the compressive strength and ash fraction.

**DISCUSSION**

The growth of transgenic rats used in the present study is significantly retarded by 30% relative to the body weight of nontransgenic, control rats. The body weight of animals is one of the most important factors which influence mechanical stresses applied to such musculo-skeletal tissues as bone. A positive linear correlation between the load at break of the femur and the body weight of animals was observed in the data obtained from both transgenic and control groups. It is assumed that the in vivo load applied to bone has relation to body weight. Therefore, we can say that cortical bone tissues of the transgenic rats change the load at break so as to adapt themselves to the decrease of in vivo load developed by the change in body weight. The cross-sectional area of the femur was also positively correlated with the body weight of animals, whereas no significant correlation was observed between compressive strength and body weight. These results indicate that the decrease in the load at break of transgenic group is related more closely to the dimensions than to the material properties of bone. The result from the present ash measurements also implies that the genetical inhibition of growth hormone affects not the characteristics but the quantity of cortical bone tissues.

In the present study, the material properties of bone were independent of the genetical inhibition of growth hormone. Jepsen et al. [3] studied the biomechanical properties of bone in transgenic mice which were genetically prevented the initiation of transcription of collagen genes, and reported that a type I collagen mutation is associated with increased brittleness of long bones from the transgenic mice. Their results are not similar to those obtained from the present study. Because antisense RNA is expressed in the pituitary of the transgenic rats used in the present study, all tissues in the rats may be influenced by the inhibition of growth hormone gene expression. In contrast, the transgenic mice used in the work done by Jepsen et al. [3] were prevented only collagen gene expression. Such a difference in the gene expression may have induced the different results between the two studies.

**ACKNOWLEDGMENTS**

The authors greatly appreciate Nippon Institute for Biological Science for providing the experimental animals. This study was financially supported in part by the Special-Grant-in-Aid for Scientific Research from School of Biology-Oriented Science and Technology, Kinki University.

**REFERENCES**