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Evaluation of velocity anisotropy of cortical bone of hyperglycemic rat using a micro-Brillouin scattering technique

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1. Introduction

Decrease in bone strength is one of the factors contributing to bone fracture risk. The National Institutes of Health (NIH) reported that bone strength depended not only on bone mineral density (BMD) but also on bone quality [1]. Bone quality is a vague term that describes bone characteristics such as micro to macro structure, bone turnover, and material properties that affect bone elasticity. Diabetic patients often have a high risk of bone fracture despite of the normal BMD. One reason may be the deterioration of collagen, which accounts for 50% of bone volume [2]. In diabetes, insulin levels are reduced and Advanced Glycation End Products (AGEs) cross-linking is more likely to occur. This abnormal collagen cross-linking in bone would be expected to affect bone elasticity. However, BMD cannot assess collagen elasticity. In contrast, noninvasive, inexpensive, and safe ultrasonographic methods can provide information on elasticity as well as BMD. Yasui et al. reported a decrease in bone elastic modulus in spontaneously diabetic Trii (SDT) rats by a micro-Brillouin scattering technique [3].

In this study we have investigated the effects of hyperglycemia on longitudinal wave velocity in the Sprague-Dawley-rats using the micro-Brillouin scattering technique.

2. Material and methods

2.1. Specimen

Figure 1 shows the procedure of the specimen preparation. Fourteen male 10-week-old rats were used. Seven rats were injected with 100 mg/kg streptozotocin (STZ) dissolved in physiological saline solution to induce hyperglycemia. The other seven rats (control) were injected with an equivalent volume of physiological saline solution. One to four weeks after injection, thin dry bone specimens (thickness 70 mm) were fabricated from tibia for the micro-Brillouin scattering (μ-BR) measurements. Longitudinal wave velocities in the directions parallel and perpendicular to the bone axis were measured. The wave velocities were the averaged values of nine different measurement area in one specimen.

2.2. Brillouin scattering technique

Brillouin scattering measurements were carried out with a six-pass tandem Fabry-Pérot interferometer using a laser (532 nm). The actual spot diameter of the focused laser beam on the specimen was approximately 10 μm.

A scattering geometry, called Reflection Induced θ Angle scattering geometry, was used [4]. This geometry enables to observe ultrasound waves propagating in two directions ($q^{\theta A}$ and q^{180}). Here, we focused on the in-plane $q^{\theta A}$ direction. From following equation, we can obtain wave velocity.

$$v^{\theta A} = f^{\theta A} \frac{\lambda_0}{2\sin(\frac{\theta}{2})}$$

Here, $v^{\theta A}$ is the wave velocity, $f^{\theta A}$ is the measured shift frequency and λ_0 is the incident light wavelength.

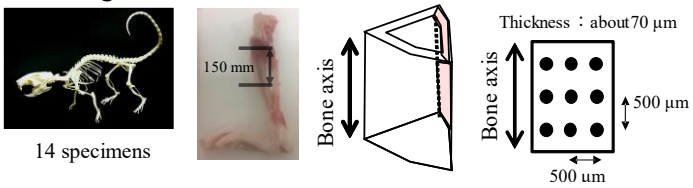

Figure 1 Measurement preparation

3. Results and discussion

3.1. BR scattering spectrum

Figure 2 shows a typical Brillouin spectrum obtained from the sample of a 12-week Sprague-Dawley rat. From this shift frequency, the longitudinal wave velocity was estimated as 4.74 × 10^3 m/s.

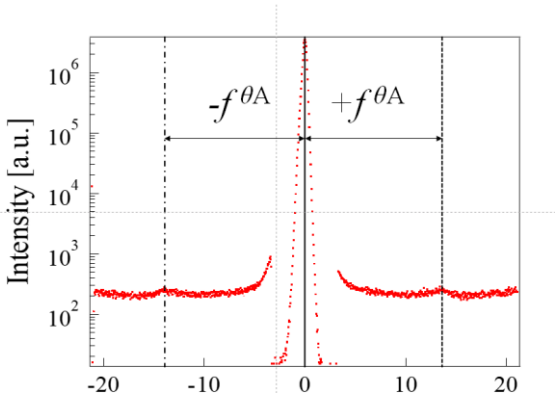

Figure 2 A typical Brillouin spectrum

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3.2. Changes of Wave velocities

Seven rats showed hyperglycemia in 4 days after injection. Figures 3 and 4 show the averaged wave velocities of tibia bones. In the bone axis (Fig. 3), velocities of the STZ specimens were 1.2% and 2.0% lower than those of the control specimens after one and two weeks (11 and 12 weeks after birth) of injection. After four weeks (14 weeks after birth), velocities in the STZ specimen were significantly lower (3.5%) than those in the control specimens. In the vertical direction (Fig. 4), velocities of the STZ specimens were 0.8%, 1.2%, and 2.8% lower than those of the control specimens after one, two, and four weeks (11, 12, and 14 weeks after birth) of injection. In the vertical direction, there were no significant differences after one week of injection.

11- to 12-week-old rats are about 10 to 12 years old in terms of human age. The rate of decrease in wave velocities seemed larger in the direction of the bone axis than in the vertical, suggesting the possible small effects of AGEs cross-linking on the bone anisotropy.

Figure 5 shows the ratio of wave velocities in the bone axial and vertical directions. The denominator of this ratio is the velocity in the direction vertical to the bone axis and the numerator is the velocity in the bone axis direction. The ratios of the STZ samples were smaller at 1, 2, and 4 weeks (11, 12 and 14 weeks) after STZ injection. However, there were no significant differences statistically.

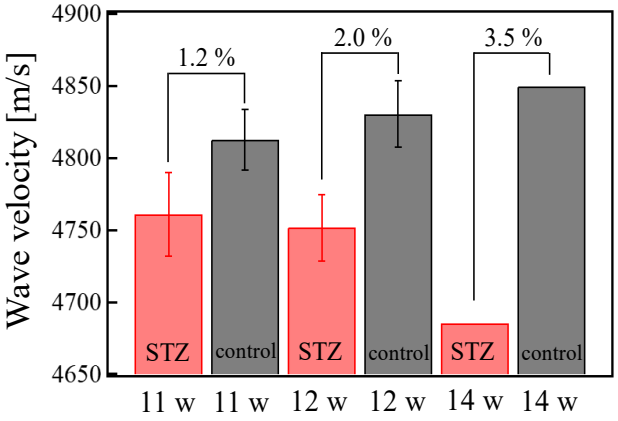

Figure 3 Averaged wave velocities in the axial in STZ and control specimens

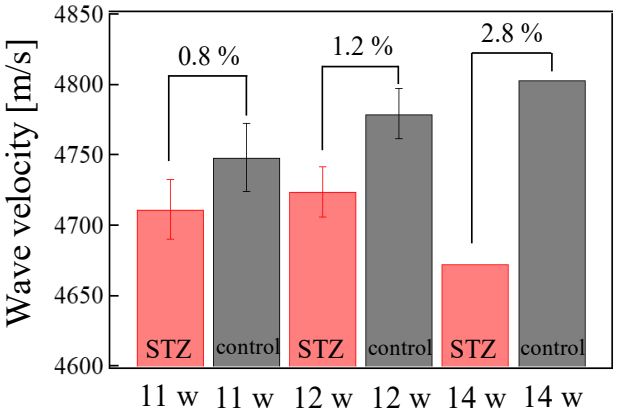

Figure 4 Averaged wave velocities in the vertical in STZ and control specimens

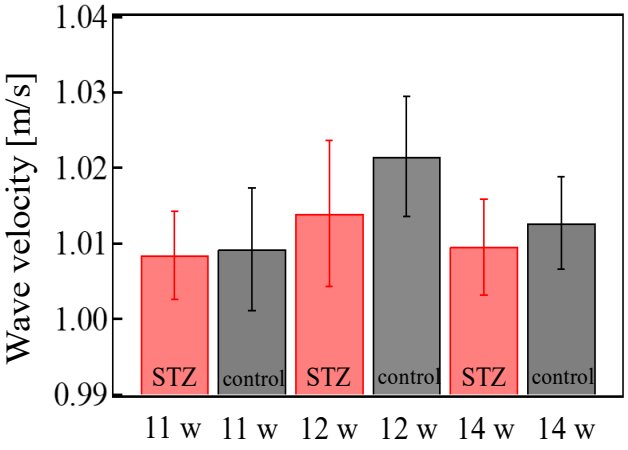

Figure 5 The ratio of wave velocity in the direction of the bone axis to vertical

4. Summary

We found a decrease in wave velocities of rat bones due to hyperglycemia during young growth periods. It is necessary to examine whether similar results can be obtained in higher-week-old rats. The anisotropy of wave velocity was not significantly affected in four weeks after becoming hyperglycemia.

References

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