NOTE *Laboratory Animal Science*

Bone morphological analyses in Spontaneously Diabetic Torii (SDT) fatty rats

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ABSTRACT. The Spontaneously Diabetic Torii (SDT) fatty rat, a model for obese type 2 diabetes, shows bone quantitative abnormalities, namely low bone mineral density (BMD). The objective of this study was to evaluate bone morphological changes, in particular identifying the bone qualitative abnormalities, in the SDT fatty rat. Male SDT fatty rats showed increases in total trabecular area and trabecular number and decreases in trabecular thickness in cancellous bones of the proximal tibia, indicating trabecular miniaturization. The SDT fatty rat is useful for investigation of pathophysiological changes in bone quality in diabetic osteoporosis.
KEY WORDS: bone quality, diabetic osteoporosis, SDT fatty rat

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Osteoporosis is defined as a combination of two factors, reduced bone mass and bone quality with microarchitectual abnormalities, resulting in pathological fractures that lead to significant morbidity [1, 13]. Osteoporosis is considered to be caused by the acceleration of bone resorption and/or deceleration of bone formation [1, 21]. Osteoporosis has been a significant global problem both socially and economically. In 1995, the National Osteoporosis Foundation (NOF) reported that the annual cost of treating osteoporosis fractures was $13.8 billion, which is expected to double over the next 25 years due to the increasing elderly population [15]. Therefore, novel preventive and therapeutic approaches are essential in order to regulate this disease with the current increasing aging population [1].

Osteoporosis is typically defined as a reduction in the value for bone mineral density (BMD) by the World Health Organization (WHO) [7], and various factors, such as age, sex and family history, are independent risk factors for reduction in BMD. It is a well known fact that everyone loses BMD with aging, and women are at higher risk since they rapidly lose bone mass following menopause. Recently, diabetes mellitus has been recognized as a risk factor for reductions in BMD, and osteoporosis is recognized as the most important metabolic bone disease in patients with diabetes [4, 5, 21]. Diabetic animal models are useful for elucidating the pathoetiological and pathophysiological changes in diabetic osteoporosis. In our previous studies, we reported that the Spontaneously Diabetic Torii (SDT) fatty rat, a new model for obese type 2 diabetes, showed decreases in BMD and bone mineral content (BMC) in the whole tibia and shortening of the tibia and femur at 40 weeks of age [9]. In this study, we investigated bone morphological changes in SDT fatty rats before 40 weeks of age.

Male and female SDT fatty rats at 10, 18 and 26 weeks of age from Japan Tobacco Inc. (JT) colonies were used in the study. Age-matched male and female Sprague-Dawley (SD) rats (CLEA Japan, Tokyo, Japan) were used as control animals. All animal procedures and the protocol complied with the guidelines for animal experimentation set by the Ethics Committee for Animal Use at JT and Niigata University. The rats were maintained at 23 ± 3°C on a 12 hr/12 hr light-dark cycle with *ad libitum* access to a standard diet (CRF-1; Oriental Yeast, Tokyo, Japan) and water. At each sampling day, body weights were measured, and blood samples were collected from the abdominal aorta under fed condition to determine blood chemistry parameters, such as glucose, triglyceride (TG) and total cholesterol (TC) levels. These blood levels were measured using commercial kits (Roche Diagnostics, Basel, Switzerland). Proximal tibias from each rat were collected to perform bone morphological analyses. The tibias were fixed in 10% neutral buffered formalin and, immersed in 10% EDTA solution and decalcified. After decalcification, tissues were paraffin-embedded using standard techniques and thin-sectioned (5 μm). Sections were stained with hematoxylin and eosin (HE), after which the total bone volume (BV), bone surface (BS) and total tissue volume (TV) in histological sections were measured using NIS Elements (Nikon, Tokyo, Japan). Bone volume (BV/TV), trabecular thickness (Tb. Th), trabecular number (Tb. N) and trabecular separation (Tb. Sp) were calculated using the following formula.

$$\text{Bone volume (\%)} = \text{total bone volume}/\text{total tissue volume}$$
$$\text{Trabecular thickness } (\mu\text{m}) = (\text{total bone volume} / \text{bone surface}) \times 2$$

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Table 1. Body weight and blood chemical parameters

			10 weeks of age	18 weeks of age	26 weeks of age
Body weight (g)	Male	SD rats	449.1 ± 31.7	629.2 ± 65.4	786.0 ± 91.0
		SDT fatty rats	472.4 ± 34.5	592.1 ± 59.9	576.4 ± 48.3[b)]
	Female	SD rats	282.6 ± 19.3	378.5 ± 24.6	399.0 ± 21.5
		SDT fatty rats	434.6 ± 42.2[b)]	544.0 ± 81.4[b)]	577.9 ± 80.6[b)]
Glucose (mg/d*l*)	Male	SD rats	123.4 ± 7.8	155.8 ± 12.1	140.9 ± 11.5
		SDT fatty rats	771.5 ± 35.9[b)]	845.4 ± 48.9[b)]	879.5 ± 105.3[b)]
	Female	SD rats	127.9 ± 14.1	156.8 ± 12.1	135.7 ± 11.0
		SDT fatty rats	574.5 ± 209.4[b)]	760.0 ± 103.8[b)]	736.3 ± 199.8[b)]
Triglyceride (mg/d*l*)	Male	SD rats	241.0 ± 56.5	262.3 ± 83.6	348.2 ± 190.7
		SDT fatty rats	451.2 ± 79.2[b)]	493.5 ± 55.2[b)]	548.0 ± 101.9[a)]
	Female	SD rats	136.9 ± 57.0	297.9 ± 208.3	218.2 ± 77.7
		SDT fatty rats	335.1 ± 186.9[a)]	800.3 ± 329.0[b)]	1,087.4 ± 305.7[b)]
Total cholesterol (mg/d*l*)	Male	SD rats	75.7 ± 13.6	84.0 ± 10.6	98.3 ± 12.4
		SDT fatty rats	115.5 ± 9.6[b)]	167.3 ± 12.9[b)]	154.5 ± 33.7[b)]
	Female	SD rats	82.3 ± 9.3	99.3 ± 25.4	99.1 ± 19.4
		SDT fatty rats	163.8 ± 33.1[a)]	169.3 ± 44.5[b)]	227.1 ± 120.0[b)]

Data represent mean ± standard deviation (n=7–8). a) $P<0.05$. b) $P<0.01$ vs. SD rats.

Table 2. Bone morphological parameters

			10 weeks of age	18 weeks of age	26 weeks of age
Bone volume (%)	Male	SD rats	20.51 ± 3.25	20.84 ± 2.76	22.77 ± 4.51
		SDT fatty rats	26.69 ± 5.26	21.67 ± 4.21	23.51 ± 9.83
	Female	SD rats	23.99 ± 4.14	25.47 ± 6.51	27.04 ± 5.76
		SDT fatty rats	26.94 ± 2.92	26.00 ± 3.45	21.42 ± 4.10[a)]
Bone surface (μm)	Male	SD rats	3,919.3 ± 580.4	2,994.7 ± 311.2	3,150.1 ± 371.6
		SDT fatty rats	5,929.4 ± 779.3[b)]	4,628.1 ± 1099.9[a)]	4,387.1 ± 801.0[b)]
	Female	SD rats	4,195.4 ± 871.9	4,509.9 ± 845.9	4,574.7 ± 575.1
		SDT fatty rats	4,925.5 ± 444.5	5,171.5 ± 532.2	3,977.4 ± 514.9
Trabecular thickness (μm)	Male	SD rats	55.2 ± 3.8	71.1 ± 3.8	79.4 ± 13.1
		SDT fatty rats	47.6 ± 4.6[a)]	49.2 ± 1.6[b)]	58.9 ± 22.2
	Female	SD rats	59.5 ± 3.1	60.0 ± 8.9	59.1 ± 5.7
		SDT fatty rats	58.3 ± 5.7	55.4 ± 9.1	57.3 ± 8.9
Trabecular number (1/mm)	Male	SD rats	3.67 ± 0.55	2.76 ± 0.33	2.86 ± 0.50
		SDT fatty rats	5.46 ± 0.60[b)]	4.38 ± 1.03[a)]	4.11 ± 0.75[b)]
	Female	SD rats	3.94 ± 0.82	4.23 ± 0.79	4.30 ± 0.54
		SDT fatty rats	4.63 ± 0.42	4.86 ± 0.50	3.73 ± 0.48
Trabecular separation (μm)	Male	SD rats	248.0 ± 67.8	329.8 ± 50.3	295.1 ± 98.7
		SDT fatty rats	146.9 ± 26.9[a)]	218.5 ± 103.0 [a)]	224.8 ± 101.8
	Female	SD rats	219.0 ± 61.9	204.4 ± 58.5	189.8 ± 41.3
		SDT fatty rats	166.9 ± 20.1	158.6 ± 17.8	221.9 ± 40.2

Data represent mean ± standard deviation (n=5–8). a) $P<0.05$. b) $P<0.01$ vs. SD rats.

Trabecular number (1/mm)
= (total bone volume / total tissue volume / trabecular thickness) × 1,000

Trabecular separation (μm)
=1,000 / trabecular number−trabecular thickness

Results were expressed as the mean ± standard deviation. Statistical analyses of differences between mean values were performed using an F-test, followed by a Student's *t*-test or Aspin-Welch's *t*-test. Differences were defined as significant at $P<0.05$.

In biological analyses, body weight and plasma glucose, TG and TC levels were measured (Table 1). Body weight in male SDT fatty rats significantly decreased at 26 weeks of age compared with that in male SD rats; however, the body weights in female SDT fatty rats were higher than in female SD rats during the experimental period. Blood chemistry parameters, such as glucose, TG and TC levels, in both male and female SDT fatty rats were significantly higher than in each SD rat. The results in these biological analyses were similar to those reported in previous studies [8, 9, 12], thereby showing the characteristics of SDT fatty rats.

In bone morphological analyses, BV, BS, Tb. Th, Tb. N and Tb. Sp were determined (Table 2). There were no differences in BV between SDT and SDT fatty rats throughout

the experimental period, with the exception of female rats at 26 weeks of age. Tb. Th at 10 and 18 weeks of age in male SDT fatty rats significantly decreased compared with that in SD rats, and the value at 26 weeks of age showed a tendency to decrease. Tb. Th in female SDT fatty rats was comparable to that in SD rats. BS in male SDT fatty rats significantly increased compared with that in SD rats during the experimental period; however, the values in female SDT fatty rats did not reveal significant changes. Tb. N in male SDT fatty rats significantly increased as compared with that in SD rats during the experimental period. Tb. N in female SDT fatty rats did not show significant changes compared with that in female SD rats. Tb. Sp at 10 and 18 weeks of age in male SDT fatty rats significantly decreased compared with that in SD rats; however, the value at 26 weeks of age was not significantly different from the control rats. Tb. Sp in female SDT fatty rats did not show significant changes compared with that in female SD rats.

Diabetes is associated with a risk of fractures as well as post-menopause, in women. A relationship between Type 2 diabetes and an increased risk of hip fractures in both men, with a relative risk of 2.8, and women, with a relative risk of 2.1, has been shown. The relationship between the type of diabetes and hip structure risk is higher for type 1 diabetes, with a relative risk of 6.3 [6]. Type 1 diabetes patients reportedly show low BMD at the hip, femoral neck and spine, which may lead to an increase in bone fractures [3, 11, 16, 18]. In contrast, the relationship between BMD and the incidence of fractures is controversial in type 2 diabetes. There are several reports in which investigators show an increase in BMD in patients with type 2 diabetes [14, 22]. On the other hand, several investigators reportedly found lower BMDs in type 2 diabetic patients [20, 23]. Recently, bone quality with microarchitectual abnormalities, as well as BMD, has been considered as plaing pivotal roles on the increasing incidence of bone fractures [19].

We reported that BMD in male SDT fatty rats decreased at 40 weeks of age and that trabecular bone volume and thickness of the distal femur also decreased [9, 10]. In this study, using male SDT fatty rats from 10 to 26 weeks of age, BS and Tb. N significantly increased; however, BV did not change and Tb. Th decreased, showing that the number of small and thin cancellous bones increased (Fig. 1). Trabecula in normal cancellous bone becomes thicker with bone remodeling and aging, and the number decreases [2, 17, 19]. In SDT fatty rats, which show low bone metabolism, the small and thin trabeculae are considered to be maintained with the decrease in remodeling. Sustained hyperglycemia in diabetes patients induces glycation and leads to bone fragility, showing that bone remodeling is essential to maintaining bone quality [17]. The low bone metabolism associated with hyperglycemia is considered to deteriorate bone quality in SDT fatty rats. The trabecular miniaturization observed in female rats was not significant, probably because glucose levels were lower in female rats than in male rats (p values in glucose levels between male SDT fatty rats and female SDT fatty rats, 10 weeks of age, P=0.03; 18 weeks of age, P=0.06; and 26 weeks of age, P=0.09, respectively) (Table 1).

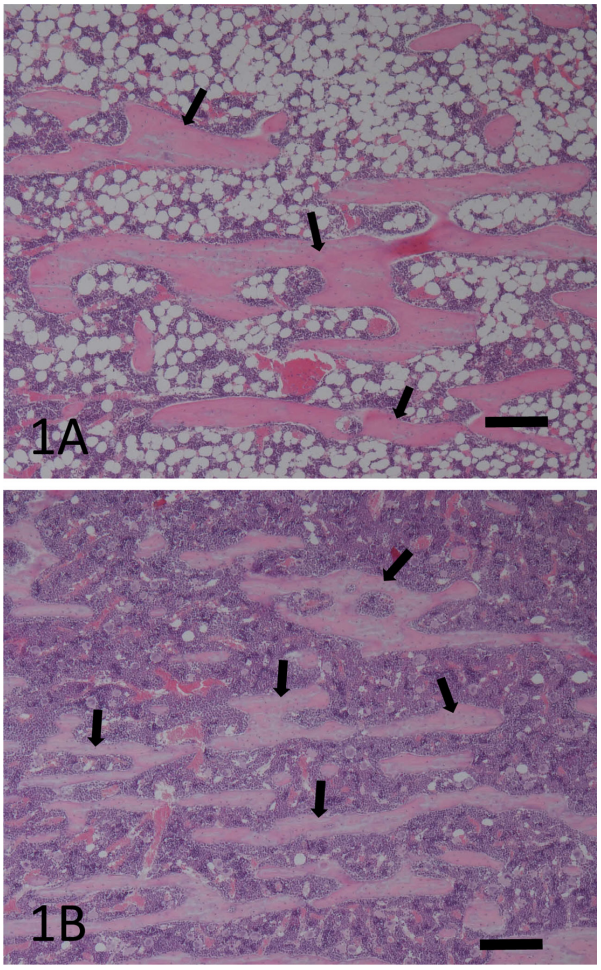

Fig. 1. Histological analysis of bone. Secondary spongiosa (arrows) of tibias at 18 weeks of age in SD (A) and SDT fatty (B) rats. The number of small and thin cancellous bones increased in SDT fatty rats. HE staining. Bar=200 μm.

There are some reports of histomorphometrical analyses in other diabetic models. In nonobese diabetic (NOD) mice, a model of type 1 diabetes, reductions in trabecular bone volume and thickness, cortical thickness and cortical strength were observed [24]. Also, in Goto-Kakizaki (GK) rats, a model of type 2 diabetes, the trabecular bone volume, trabecular thickness and number decreased [25]. Both NOD mice and GK rats showed similar changes, such as decreases in trabecular bone volume and trabecular thickness, as observed in SDT fatty rats, but the change in trabecular number in GK rats was different from that in SDT fatty rats. Male SDT fatty rats showed increases in total trabecular area and trabecular number and decreases in trabecular thickness in cancellous bones of the proximal tibia.

This study demonstrated trabecular miniaturization in cancellous bones in male SDT fatty rats. Considering our results, the SDT fatty rat is useful for investigating pathophysiological changes in bone quality as well as bone quantity in diabetic osteoporosis.

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