Evaluation of bone mineral density and bone strength in autochthonous transgenic model mice for diabetes mellitus (Akita mice)

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Abstract

Objectives: Diabetes mellitus (DM) causes secondary osteoporosis, which reduces bone mineral density (BMD) and bone strength. Akita mice (AM) are DM model mice used to evaluate glucose metabolism. However, bone metabolism in AM remains unclear. The purpose of this study was to evaluate BMD, bone strength, and serum sclerostin levels in AM.

Methods: Female AM and control mice (C57/BL/6NCrSlc; CM) were divided into four groups: (1) a CM group sacrificed at 14 (CM-14w; n = 8) or (2) 18 weeks of age (CM-18w; n = 6); and (3) an AM group sacrificed at 14 (AM-14w; n = 9) or (4) 18 weeks of age (AM-18w; n = 6). Blood glucose level, serum sclerostin level, total tibial BMD, and femoral shaft bone strength were evaluated at each time point.

Results: Blood glucose levels were significantly higher in AM than in CM (p < 0.001). Serum sclerostin levels were significantly lower in AM-18w than in CM-18w (p < 0.001). BMD was significantly lower in AM-14w than in CM-14w (p = 0.004). Stiffness of the femoral shaft was significantly lower in AM-18w than in CM-14w (p = 0.04). Body weight (r = 0.608, p < 0.01) and maximum load (r = 0.438, p < 0.05) were significantly positively correlated with serum sclerostin levels, while blood glucose levels showed a significant negative correlation (r = -0.708, p < 0.01).

Conclusions: AM showed decreased BMD and bone strength with lower levels of serum sclerostin than CM.

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Keywords: Diabetes mellitus; Akita mice; Sclerostin

1. Introduction

Osteoporosis is a systemic disease characterized by low bone strength resulting in low bone mineral density (BMD) and bone quality, and it is known to increase the risk of fractures. There are many diseases related to osteoporosis, and diabetes mellitus (DM) is especially important as a cause of secondary osteoporosis. Understanding abnormal bone metabolism in DM is necessary for those treating osteoporosis.

DM is divided into two types: type 1 and type 2. A meta-analysis has demonstrated that both type 1 and 2 DM are risk factors for proximal femoral fractures compared with non-DM patients [1]. In type 1 DM, insulin secretion is decreased. Insulin facilitates the differentiation of osteoblasts, so the loss of BMD in type 1 DM is believed to be caused by insulin deficiency, which leads to decreased bone formation [2]. However, the risk of fracture in type 1 DM is much higher than that due to loss of BMD [3]. On the other hand, the BMD is not necessarily low in type 2 DM compared with non-DM, even though the risk of fracture is higher [4,5]. These studies suggest that both type 1 and 2 DM accelerate bone fragility independent of the loss of BMD.

Various animal models have been used to study diabetic osteoporosis. Streptozotocin-induced diabetic rats (STZ rats)
have been used to evaluate bone metabolism in DM [6]. Several other DM model animals, such as WBN/Kob rats and Goto-Kakizaki rats, were evaluated in previous reports [7,8]. In the present study, Akita mice (AM) were evaluated as a DM model to analyze the bone in DM. AM are autochthonous transgenic model mice for DM, and they show a strong lack of glucose tolerance [9]. The AM model has been used to evaluate the complications of DM and therapeutic effects, but there have been no studies of bone metabolism in AM. To analyze bone metabolism in osteoporotic animal models, serum bone alkaline phosphatase (BAP) and cross-linked N-telopeptide of type I collagen (NTX) are commonly used as markers. In the present study, serum sclerostin levels were measured in AM. An increase in sclerostin is associated with a high fracture risk, regardless of bone metabolism turnover, but the mechanism remains unclear [10]; thus, it is important to investigate the relationship between hyperglycemia and serum sclerostin to understand bone metabolism in DM. The purpose of this study was to evaluate BMD and bone strength in AM and to study bone metabolism in DM.

2. Materials and methods

2.1. Animals

Twelve-week-old female AM (Japan SLC, Inc., Shizuoka, Japan) and C57/BL/6NCrSlc mice (CM; Japan SLC, Inc.) as a control were used. They were housed in a controlled environment at 22 °C with a 12-h light/dark cycle. They were pair-fed and allowed free access to water and standard food (CE-2; CLEA Japan, Tokyo, Japan) containing 1.14% calcium, 1.06% phosphorus, and 250 IU of vitamin D3 per 100 g.

2.2. Experimental design

The mice were divided into four groups: (1) a CM group sacrificed after 2 weeks of feeding (CM-14w: n = 8); (2) an AM group sacrificed after 2 weeks of feeding (AM-14w: n = 9); (3) a CM group sacrificed after 6 weeks of feeding (CM-18w: n = 6); and (4) an AM group sacrificed after 6 weeks of feeding (AM-18w: n = 6). The mice were euthanized under anesthesia with an intra-abdominal injection of ketamine (Sankyo, Tokyo, Japan) and xylazine (Zenoaq, Fukushima, Japan). After sacrificing, the blood and bilateral femora and tibiae were harvested. The left tibiae were soaked in 10% formalin and used for BMD measurement. The right femora and serum were stored at −20 °C. The right femora were used for three-point bending tests to evaluate bone strength, and the serum was used for biochemical parameter measurements. The animal experimentation protocols were approved by the Animal Committee of Akita University Graduate School of Medicine. All animal experiments conformed to the Guidelines for Animal Experimentation of Akita University.

2.3. Biochemical parameters

The blood was collected from the inferior vena cava. After measuring the blood glucose level (mg/dl) (Antsense III; Horiba, Kyoto, Japan), the remaining blood samples were centrifuged at 15,000 rpm for 20 min to separate the serum. Serum sclerostin (pg/ml) was measured using an enzyme-linked immunosorbent assay (ELISA) kit (Mouse Sclerostin ELISA; ALPCO Diagnostics, Salem, NH, USA).

2.4. Bone mineral density (BMD)

Total BMD (mg/cm²) of the left tibia was measured by dual energy X-ray absorptiometry (QDR-4500; Hologic Inc., Waltham, MA, USA).

2.5. Biomechanical analysis

Bone strength of the right femur was evaluated by a three-point bending device using a mechanical testing machine (MZ-500S; Maruto, Tokyo, Japan). The femur was placed horizontally on a two-point holder (6-mm span) with the anterior aspect facing up, and a load was applied on the midshaft with a crosshead speed of 10 mm/min until fracture occurred. The biomechanical analysis was performed to calculate the maximum load (N) and stiffness (N/mm).

2.6. Statistical analyses

All values are presented as means ± standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA). Statistical differences among all groups were compared using Scheffe’s method for multiple comparisons. Spearman’s rank correlation coefficient method was used to evaluate the correlations between serum sclerostin level and body weight, blood glucose level, BMD, and biomechanical data. All statistical analyses were performed using Statistical Package for the Biosciences software (SPBS v9.6) [11]. Values of p less than 0.05 were considered significant.

3. Results

3.1. Body weight (Table 1)

Body weight was significantly lower for AM-18w than for AM-14w (p = 0.004) and CM-18w (p < 0.001). There was no significant difference in body weight between CM-14w and CM-18w.

3.2. Blood glucose and serum sclerostin (Table 1)

Blood glucose levels were significantly higher in the AM groups than in the CM groups both at 14 weeks (p < 0.001) and at 18 weeks (p < 0.001) of age. The serum sclerostin level was significantly lower for AM-18w than for CM-18w (p < 0.001).
3.3. BMD and biomechanical data (Table 2)

The total tibial BMD of AM-14w, but not that of AM-18w, was significantly lower than the total tibial BMD of CM-14w ($p = 0.004$). There were no significant differences in maximum load on the three-point bending test between the AM groups and the CM groups both at 14 weeks and at 18 weeks of age. Stiffness on the three-point bending test also showed similar tendencies between the two groups, with stiffness significantly smaller for AM-18w than for CM-14w ($p = 0.04$).

3.4. Correlation between serum sclerostin and other parameters (Table 3)

On Spearman’s rank correlation analysis, body weight ($r = 0.608, p < 0.01$) and maximum load ($r = 0.438, p < 0.05$) were significantly positively correlated with serum sclerostin. BMD had a similar tendency, but it was not significant. In contrast, blood glucose showed a significant negative correlation with serum sclerostin ($r = -0.708, p < 0.01$). Stiffness and serum sclerostin had no significant correlation.

4. Discussion

AM, discovered by Koizumi in 1997, are non-obese diabetes model mice originating from C57/BL/6N mice [12]. AM develop severe DM associated with hypoinsulinemia. They develop obvious polydipsia and polyuria at approximately 10 weeks of age and then develop hydronephrosis until 40 weeks of age. Young AM were used in this study because end-stage DM can only be used to evaluate completed osteoporosis, while early-stage DM has been considered useful for evaluating the pathogenetic mechanism of diabetic osteoporosis.

AM have been used as a model of type 1 DM [13], and they have been used to evaluate the mechanisms of DM complications and therapeutic effects [9,14]. Kakoki et al. evaluated BMD in AM and reported that BMD was significantly lower in AM than in wild-type mice [15]. However, no advanced study involving AM has yet been performed. Thus, BMD, bone strength and bone metabolism in DM were evaluated in AM in the present study.

The BMD tended to be lower in AM than in CM, but the difference was not significant. In general, BMD is low in type 1 DM, while BMD is normal or higher in type 2 DM than in normal subjects [3]. There are some previous studies that evaluated BMD in DM model rats [16,17]. Matthew et al. reported that rats with type 1 DM had a progressive loss of trabecular BMD compared with control rats [18]. On the other hand, Armas et al. reported that there was no significant difference in total hip BMD, which reflects cortical BMD, between type 1 DM and a control group [19]. In the present study, total tibial BMD, which reflects cortical BMD, was evaluated, so we similarly found no significant difference in BMD between AM and CM. Alternatively, the AM were young, so the effect of hyperglycemia might not have been

Table 1

<table>
<thead>
<tr>
<th></th>
<th>CM-14w (n = 8)</th>
<th>AM-14w (n = 9)</th>
<th>CM-18w (n = 6)</th>
<th>AM-18w (n = 6)</th>
<th>ANOVA</th>
</tr>
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<tbody>
<tr>
<td>Body weight (g)</td>
<td>23.4 ± 0.5</td>
<td>22.5 ± 0.8</td>
<td>24.4 ± 1.3</td>
<td>20.7 ± 0.7\textsuperscript{a}</td>
<td>$p &lt; 0.001$</td>
</tr>
<tr>
<td>Blood glucose (mg/dl)</td>
<td>172.6 ± 16.9</td>
<td>302.8 ± 61.5</td>
<td>159.3 ± 21.8</td>
<td>392.7 ± 65.3\textsuperscript{b}</td>
<td>$p &lt; 0.001$</td>
</tr>
<tr>
<td>Serum sclerostin (pg/ml)</td>
<td>307.2 ± 44.8</td>
<td>274.6 ± 42.7</td>
<td>367.2 ± 46.3</td>
<td>249.6 ± 30.8\textsuperscript{c}</td>
<td>$p &lt; 0.001$</td>
</tr>
</tbody>
</table>

All values are means ± SD.

\textsuperscript{a} $p = 0.004$ versus AM-14w.
\textsuperscript{b} $p = 0.04$ versus CM-14w.
\textsuperscript{c} $p = 0.04$ versus CM-18w by Scheffe’s multiple comparison method.

Table 2

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<th></th>
<th>CM-14w (n = 8)</th>
<th>AM-14w (n = 9)</th>
<th>CM-18w (n = 6)</th>
<th>AM-18w (n = 6)</th>
<th>ANOVA</th>
</tr>
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<tbody>
<tr>
<td>Total BMD (g/cm\textsuperscript{2})</td>
<td>0.056 ± 0.005</td>
<td>0.049 ± 0.002\textsuperscript{d}</td>
<td>0.053 ± 0.002</td>
<td>0.051 ± 0.003</td>
<td>$p = 0.003$</td>
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<tr>
<td>Biomechanical data</td>
<td></td>
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<td>Maximum load (N)</td>
<td>19.0 ± 2.9</td>
<td>14.4 ± 4.6</td>
<td>17.6 ± 1.6</td>
<td>14.4 ± 4.4</td>
<td>$p = 0.04$</td>
</tr>
<tr>
<td>Stiffness (N/mm)</td>
<td>90.4 ± 20.6</td>
<td>57.3 ± 29.8</td>
<td>74.0 ± 23.8</td>
<td>50.1 ± 17.9\textsuperscript{e}</td>
<td>$p = 0.02$</td>
</tr>
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</table>

All values are means ± SD.

\textsuperscript{d} $p = 0.004$ versus CM-14w.
\textsuperscript{e} $p = 0.04$ versus CM-14w by Scheffe’s multiple comparison method.

Table 3

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<th>Serum sclerostin</th>
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<tbody>
<tr>
<td></td>
<td>$r$</td>
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<tr>
<td>Body weight</td>
<td>0.608</td>
</tr>
<tr>
<td>Blood glucose</td>
<td>−0.708</td>
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<tr>
<td>BMD</td>
<td>0.308</td>
</tr>
<tr>
<td>Maximum load</td>
<td>0.438</td>
</tr>
<tr>
<td>Stiffness</td>
<td>0.256</td>
</tr>
</tbody>
</table>

BMD: bone mineral density, n.s.: not significant.

On Spearman’s rank correlation analysis, body weight ($r = 0.608, p < 0.01$) and maximum load ($r = 0.438, p < 0.05$) were significantly positively correlated with serum sclerostin. BMD had a similar tendency, but it was not significant. In contrast, blood glucose showed a significant negative correlation with serum sclerostin ($r = -0.708, p < 0.01$). Stiffness and serum sclerostin had no significant correlation.
reflected. Further evaluation of the cancellous BMD of older AM would be needed to determine the effect of type 1 DM on BMD.

In the three-point bending test, the maximum load was not significantly different between AM and CM, while stiffness was significantly lower in AM than in CM. There have been only a few reports of the three-point bending test of long bones in DM model rats [20]. Suzuki et al. reported that the ultimate strength and stiffness were significantly lower in STZ rats than in control rats [6]. On the other hand, Fowlkes et al. reported that both peak force and stiffness were similar in STZ rats and control rats [21]. Regarding the differences in maximum load and stiffness, Saito et al. reported that bone volume was the most important factor related to maximum load, while stiffness was mostly related to enzymatic mature or immature crosslinks, which reflect bone quality [22]. The present results may reflect an earlier deterioration of bone quality rather than a decrease of bone volume in AM.

One of the indicators of bone quality evaluated in AM in the present study was the serum sclerostin level, which was measured as a parameter of bone formation. Sclerostin is a glycoprotein secreted from osteocytes. Sclerostin binds to the low-density lipoprotein receptor-related proteins 5 and 6 (LRP5 and LRP 6) and inhibits bone formation by inhibiting the canonical Wnt/β-catenin signaling pathway [23]. In the present study, the serum sclerostin level was lower in AM than in CM, and there was no positive correlation between serum sclerostin and BMD. In contrast, serum sclerostin was significantly negatively correlated with blood glucose. These results were not consistent with those of previous reports that showed the serum level of sclerostin was increased in patients with type 2 DM and was associated with an increased risk of vertebral fractures, independently of BMD and bone turnover [10,24]. The relationship between DM and the serum sclerostin level has not been clearly elucidated. Gennari et al. reported that serum sclerostin levels were significantly higher in the type 2 DM group than in the type 1 DM group or the normal group [25]. It is known that sclerostin is secreted from more mature osteocytes, which are deeply embedded osteocytes [26]. In addition, hyperglycemia or deficiency of insulin induces cell apoptosis and decreases bone formation [27]. In view of these mechanisms, the present results suggest that the serum sclerostin level was decreased in AM as a result of acceleration of dysfunction or apoptosis of osteoblasts or osteocytes.

The present study has several limitations. First, since only total tibial BMD was evaluated, the results might not reflect trabecular BMD, which is more affected in type 1 DM. Further examinations, such as those using peripheral quantitative computed tomography (pQCT), which can evaluate both trabecular and cortical BMD, may be useful. Second, the AM were young, especially at 14 weeks. There was a significant difference in serum sclerostin levels between AM and CM only at 18 weeks of age. The serum sclerostin level was shown to increase with age in a previous report [28]. Thus, older AM should be studied to evaluate the effect of type 1 DM. The lifespan of AM is long, so it is easy to evaluate the complications of long-term hyperglycemia in these mice. Third, histological examinations were not performed. Evaluations of the bone microarchitecture in DM are needed with bone morphometry or pQCT.

In conclusion, BMD, bone strength, and the serum sclerostin level were lower in AM. The serum sclerostin level had a significant negative correlation with the blood glucose level, but there was no significant relationship between the serum sclerostin level and total tibial BMD or bone strength on the three-point bending test of the femur.

Conflicts of interest

Drs. Ohuchi, Miyakoshi, Kasukawa, segawa, Kinoshita, and Shimada have no conflicts of interest.

Acknowledgments

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References


