Dietary Iron Deficiency Decreases Serum Osteocalcin Concentration and Bone Mineral Density in Rats

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We investigated the effects of dietary iron deficiency on bone metabolism by measuring markers of bone turnover in rats. Twelve 3-week-old male Wistar-strain rats were fed a control diet or an iron-deficient diet for 4 weeks. Dietary iron deficiency decreased hemoglobin concentration and increased heart weight. Serum osteocalcin concentration, bone mineral content, bone mineral density, and mechanical strength of the femur were significantly lower in the iron-deficient group than in the control group. These results suggested that dietary iron deficiency affected bone, which might have been due to a decrease in bone formation in rats.

Key words: dietary iron deficiency; osteocalcin; bone mineral density; rat

Iron deficiency is probably the most common nutritional deficiency in the world. Dietary iron deficiency causes several problems for health such as anemia, hyperlipidemia, lipid peroxidation and changes in vitamin metabolism.1–3) Furthermore, some reports have indicated that dietary iron deficiency affects bone metabolism in rats. Dietary iron deficiency led to decreased bone mineral density (BMD) and mechanical strength in young rats.4–6) In addition, dietary iron intake was associated with BMD in healthy postmenopausal women.7,8) Previous studies have hypothesized two mechanisms by which iron deficiency decreases BMD and mechanical strength. Iron is a cofactor for prolyl and lysyl hydroxylase in collagen synthesis, and plays an important role in collagen maturation.9) Furthermore, renal 25-hydroxyvitamin D hydroxylase, which converts 25-hydroxyvitamin D to the active form of vitamin D, is located in the mitochondria and is a three-component system involving a flavoprotein, an iron-sulfur protein, and a cytochrome P-450.10) Thus these iron-dependent enzyme activities might become lower, causing bone loss in iron-deficient anemia. Because the possibility that bone formation deteriorates by iron deficiency is no more than a guess, previous studies have not determined the cause of bone loss in iron deficiency.

This study investigated the effects of dietary iron deficiency on bone metabolism in rats. We identified the changes in bone formation and bone resorption by measuring markers of bone turnover in rats fed an iron-deficient diet.

Twelve 3-week-old male Wistar strain rats were purchased from Clea Japan (Tokyo, Japan) and housed individually in metabolic cages in a room maintained at 22°C with a 12-hour light/dark cycle. The Tokyo University of Agriculture Animal Use Committee approved the study and the animals were maintained in accordance with the guidelines for the care and use of laboratory animals of the university. All rats were fed a control diet for 3 days of acclimatization period. After 3 days, all rats were randomly divided into two experimental groups of six rats each, and fed the control diet or an iron-deficient diet respectively. The experimental diets were based on the AIN-93G diet.11) All rats were allowed to eat ad libitum and were given free access to distilled water for 4 weeks. Urine was collected for the last 24 hours for analysis. After the 4 weeks of the experimental period, all rats were sacrificed, and blood and bone samples were collected for analysis. Part of the blood sample was used for measurement of hemoglobin concentration (Hemoglobin B-test Wako, Wako Pure Chemical Industries, Osaka, Japan). Hearts were removed and weighed, because heart enlargement is one sign of iron-deficient anemia.

The blood samples were centrifuged and the supernatants were used as serum samples. Serum and urine were stored at −80°C until analysis. Serum osteocalcin was measured with Osteocalcin rat ELISA system (Amersham Bioscience, Buckinghamshire, UK). Urinary C-terminal telopeptide of type I collagen (CTX) was measured with RatLaps ELISA (Nordic Bioscience Diagnostics A/S, Herlev, Denmark). Urinary deoxypyridinoline (DPD) was measured with METRA DPD EIA kit (Quidel, San Diego, CA, USA). Urinary creatinine was measured with Creatinine-test Wako (Wako Pure Chemical Industries).

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The left femur and 4th lumbar vertebra were removed and cleansed of all soft tissues, and frozen at $-80 \, ^\circ\text{C}$ until analysis. Bone mineral content (BMC) and BMD of the femur and lumbar vertebra were measured by dual-energy X-ray absorptiometry using DCS-600EX (Aloka, Tokyo, Japan). The mineralization profiles of the specimens were stored as monitoring images, and the BMC and BMD values for the femur and lumbar vertebra were obtained.

A three-point breaking test was performed by using a load tester (Bone Strength Tester model TK-252C, Muromachi Kikai, Tokyo, Japan). The left femur was placed on a holding device with supports located at a distance of 10 mm, with the lesser trochanter proximal to and in contact with the proximal transverse bar. The midpoint served as the anterior loading point. A breaking force was applied by the crosshead at a speed of 10 mm/min until fracture occurred. The breaking force, breaking energy, and stiffness of the femur were obtained from the load-deformation curve, which was recorded continually by a computerized monitor linked to the load tester.

Results were expressed as the means $\pm$ SEM for each group of six rats. After conducting F-test to determine the homogeneity of values, Student’s T-test was used to determine significant differences between the groups. Significant differences were considered at $p$ value of less than 0.05.

Compared to the control group, lower final body weight, weight gain, and food intake were observed in the iron-deficient group (Table 1). Hemoglobin concentration was significantly lower in the iron-deficient group than in the control group. Heart weight was significantly higher in the iron-deficient group than in the control group. Serum osteocalcin concentration was significantly lower in the iron-deficient group than in the control group (Table 2). Urinary excretion of CTx was significantly lower in the iron-deficient group than in the control group. Breaking force, breaking energy, and stiffness of the femur were obtained from the load-deformation curve, which was recorded continually by a computerized monitor linked to the load tester.

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Table 2. Markers of Bone Turnover in Rats Fed a Control Diet or an Iron-Deficient Diet

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Iron-deficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum osteocalcin (ng/ml)</td>
<td>101.5 $\pm$ 4.4</td>
<td>57.1 $\pm$ 3.2*</td>
</tr>
<tr>
<td>Urine CTx (ng/mmol creatinine)</td>
<td>36.2 $\pm$ 1.1</td>
<td>85.7 $\pm$ 11.5*</td>
</tr>
<tr>
<td>Urine DPD (nM/mM creatinine)</td>
<td>592.9 $\pm$ 40.7</td>
<td>382.9 $\pm$ 11.2*</td>
</tr>
</tbody>
</table>

Data are presented as the means $\pm$ SEM for each group of six rats.
*Significant differences from the control group at $p < 0.05$.

Table 3. BMC and BMD of the Femur and the Lumbar Vertebra and Mechanical Strength of the Femur in Rats Fed a Control Diet or an Iron-Deficient Diet

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Iron-deficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Femur BMC (mg)</td>
<td>173.9 $\pm$ 3.5</td>
<td>108.8 $\pm$ 3.2*</td>
</tr>
<tr>
<td>proximal region</td>
<td>39.2 $\pm$ 0.8</td>
<td>25.3 $\pm$ 0.9*</td>
</tr>
<tr>
<td>middle region</td>
<td>68.0 $\pm$ 1.4</td>
<td>41.7 $\pm$ 1.4*</td>
</tr>
<tr>
<td>distal region</td>
<td>66.6 $\pm$ 1.5</td>
<td>41.8 $\pm$ 1.1*</td>
</tr>
<tr>
<td>Femur BMD (mg/cm$^2$)</td>
<td>103.5 $\pm$ 1.7</td>
<td>77.2 $\pm$ 1.4*</td>
</tr>
<tr>
<td>proximal region</td>
<td>101.3 $\pm$ 2.4</td>
<td>74.0 $\pm$ 1.7*</td>
</tr>
<tr>
<td>middle region</td>
<td>90.5 $\pm$ 1.1</td>
<td>66.9 $\pm$ 1.7*</td>
</tr>
<tr>
<td>distal region</td>
<td>123.0 $\pm$ 2.7</td>
<td>94.2 $\pm$ 1.2*</td>
</tr>
<tr>
<td>Femur breaking force (N)</td>
<td>63.2 $\pm$ 2.4</td>
<td>38.9 $\pm$ 2.2*</td>
</tr>
<tr>
<td>Femur breaking energy (mJ)</td>
<td>29.4 $\pm$ 1.9</td>
<td>17.7 $\pm$ 1.6*</td>
</tr>
<tr>
<td>Femur stiffness (N/mm)</td>
<td>116.6 $\pm$ 4.1</td>
<td>73.1 $\pm$ 3.9*</td>
</tr>
<tr>
<td>Lumbar vertebra BMC (mg)</td>
<td>15.3 $\pm$ 0.3</td>
<td>8.8 $\pm$ 0.3*</td>
</tr>
<tr>
<td>Lumbar vertebra BMD (mg/cm$^2$)</td>
<td>39.3 $\pm$ 1.1</td>
<td>34.3 $\pm$ 0.8*</td>
</tr>
</tbody>
</table>

Data are presented as the means $\pm$ SEM for each group of six rats.
*Significant differences from the control group at $p < 0.05$.

Some studies in human subjects have indicated that dietary iron influences BMD. In animals, dietary iron deficiency decreased BMD and mechanical strength of the femur in weanling male and female rats. However, BMC and BMD did not appear to differ by iron deficiency, while breaking force was lower in the iron-
deficient group than in the control group in one of the three studies cited above.\(^4\) In this study, dietary iron deficiency decreased BMC and BMD of the femur and the lumbar vertebra. Furthermore, breaking force, breaking energy, and stiffness of the femur were significantly lower in the iron-deficient group. We found that iron-deficient anemia decreased not only bone strength but also BMC and BMD, and changed markers of bone turnover.

In this study, food intake and final body weight were decreased in the iron-deficient rats as compared to the control rats. Therefore, it was thought that the decreases in food intake and body weight contributed to the decreases in BMC, BMD, and mechanical strength of the femur. However, Medeiros et al. reported that dietary iron deficiency decreased these parameters as compared to a pair-fed control, and suggested that iron deficiency negatively affected bone independently of energy intake and body weight.\(^6\) Thus, iron deficiency might be a dominant cause of bone loss in either status, with or without decreasing both food intake and body weight in this study.

Serum osteocalcin concentration was measured as a marker of bone formation in this study. Serum osteocalcin concentration of the iron-deficient group was greatly reduced to about 56% of the control group. This result suggested that dietary iron deficiency might decrease bone formation via a decrease in osteoblast function. Osteocalcin is a major noncollagenous protein of bone matrix and is synthesized and released from osteoblasts.\(^12\) Furthermore, osteocalcin mRNA expression is regulated by 1,25-dihydroxyvitamin D\(_3\), the active form of vitamin D.\(^13\),\(^14\) The conversion from vitamin D\(_3\) to 1,25-dihydroxyvitamin D\(_3\) requires two steps of hydroxylation in the liver and the kidney. Final hydroxylation of vitamin D is iron-dependent, because the hydroxylation requires a three-component system involving a flavoprotein, an iron-sulfur protein, and a cytochrome P-450.\(^10\) Therefore, this iron-dependent enzyme activity might be lower in dietary iron deficiency. We did not demonstrate serum 1,25-dihydroxyvitamin D\(_3\) concentration in this study, but it might be possible to estimate a reduction in serum 1,25-dihydroxyvitamin D\(_3\) concentration by the iron-deficient diet. Consequently, a decrease in serum 1,25-dihydroxyvitamin D\(_3\) concentration due to iron deficiency might reflect the result of serum osteocalcin concentration in this study.

With regard to bone resorption, two markers were used for its assessment in this study. CTx and DPD are released into the body fluids during type I collagen degradation, and urinary excretions of CTx and DPD are useful for showing changes in bone resorption.\(^15\),\(^16\) In this study, urinary excretion of CTx was significantly higher in the iron-deficient group than in the control group. In contrast, urinary excretion of DPD was significantly decreased in the iron-deficient rats. These results had a completely reverse meaning for evaluation of bone resorption. In other words, iron deficiency caused an increase in bone resorption from the result of CTx, but caused a decrease in bone resorption from the result of DPD. Iron is a cofactor in prolyl and lysyl hydroxylase in collagen synthesis, and plays an important role in collagen maturation.\(^2\) Because iron participates in collagen metabolism, markers of type I collagen degradation product such as CTx and DPD might be influenced by iron deficiency. The reasons for this discrepancy are not clear, and detailed examinations are necessary to clarify collagen metabolism in iron deficiency. Also, we could not judge bone resorption only from measurement of bone resorption markers. Therefore, there is necessity to measure the number of osteoclast by bone histomorphometry in iron deficiency.

In conclusion, we investigated the effects of dietary iron deficiency on bone metabolism by measuring markers of bone turnover in rats. Dietary iron deficiency decreased BMC, BMD, and mechanical strength of the femur. Furthermore, serum osteocalcin concentration was decreased in the iron-deficient rats. These results suggested that dietary iron deficiency caused a decrease in bone formation, which affected bone in rats.

Acknowledgments

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References


