Key Engineering Materials Vols. 529-530 (2013) pp 341-344

doi:10.4028/www.scientific.net/KEM.529-530.341

Changes in Bone Quality of the Femoral Diaphysis Induced by High-Level Fluorine Ingestion in Ovariectomized Rats

Hiroshi Nakada[1,a], Toshiro Sakae[2,b], Mari Teranishi[3,c], Takao Kato[3,d], Takehiro Watanabe[1,e], Takahiro Takahashi[1,f], Yasuhiko Kawai[1,g] and Racquel Z. LeGeros[5,h]

[1] Department of Removable Prosthodontics, Nihon University School of Dentistry at Matsudo
[2] Department of Histology, Nihon University School of Dentistry at Matsudo
[3] Department of Oral Implantology, Nihon University School of Dentistry at Matsudo
2-870-1 Sakaecho-Nishi, Matsudo, Chiba, 271-8587, Japan.
[4] Department of Biomaterials & Biomimetics, New York University College of Dentistry
345 East 24th Street, New York, NY, 10010, U.S.A.
[a]nakada.hiroshi@nihon-u.ac.jp, [b]sakae.toshiro@nihon-u.ac.jp, [c]mama11223@g.nihon-u.ac.jp, [d]katou.takao@nihon-u.ac.jp, [e]mata11017@g.nihon-u.ac.jp, [f]mata12015@g.nihon-u.ac.jp, [g]kawai.yasuhiko@nihon-u.ac.jp, [h]rzl1@nyu.edu

Keywords: ovariectomized rats, bone, fluorine, micro-CT, bone mineral density

Abstract. Ovariectomized rats were fed a diet containing minerals at high concentrations, such as Ca, P, and F (high-mineral diet), and changes in the femoral diaphysis were investigated after 24 weeks. The femur was mainly red and partially orange on the color scale of the 3D-map in Groups A and B, showing a high BMD. The region adjacent to the marrow cavity was yellow, showing a lower BMD than that in the outer region of the femur. In Group C, the red area was small in the outer region and the inner region was mainly yellow and green on the color scale. The inner region adjacent to the marrow cavity showed a view of unevenly resorbed bone, and the BMD was lower than those in Groups A and B. Incorporation of F into the body influences the apatite crystal structure and crystal growth, which subsequently influences adsorption of F to crystals and structural changes. Therefore, it is important to ingest F at the optimum concentration.

Introduction

The main component of teeth and bone, hydroxyapatite (HAp), is not only used in the medical and dental care fields but also in cosmetics and industrial products. HAp is basic calcium phosphate with the chemical formula of $Ca_{10}(PO_4)_6(OH)_2$. It is present in bone and teeth as the main component and minerals in nature and has high biocompatibility, being applied in various biological materials. HAp has superior ion exchangeability. HAp is converted to various compounds through substitution of calcium by iron and magnesium and the hydroxyl group by fluorine (F) and carbonate ions [1,2]. The molecule with substitution of the hydroxyl groups by F is added to dentifrices as fluoroapatite ($Ca_{10}(PO_4)_6F_2$) for caries prevention. F is generally ingested into the body through food and drinking water, absorbed

Table 1 Compositions of normal diet (AIN-93M) for Group A and B, and modified high-mineral diet for Group C.

Composition	AIN-93M	Modified high-mineral	
α-Cornstarch	40.00	40.00	(%)
β-Cornstarch	22.07	7.17	
Casein	14.00	14.00	
L-Cystine	0.18	0.18	
Sucrose	10.00	7.00	
Soybean Oil	4.00	4.00	
Cellulose Powder	5.00	5.00	
Mineral Mixture	3.50	3.50	
Vitamin Mixture	1.00	1.00	
Choline Bitartrate	0.25	0.25	
Tert-Butylhydroquinone	0.00	0.00	
Additives			
Calcium citrate tetrahydrate Calcium phosphate, Dibasic Mg, Zn, F		7.90	
Fructooligosaccharides		10.00	
Mineral content			
Ca (%)	0.51	2.00	
P (%)	0.30	1.20	
Mg (%)	0.05	0.14	
Zn (ppm)	3.01	90.70	
F (ppm)	1.01	822.61	

from the intestine, and partially accumulated in bone tissue [3]. It is used as a therapeutic drug for osteoporosis through increasing the bone mineral density and strength. However, F ingestion has both positive and negative influences on bone tissue depending on the ingested amount. It was reported that ingestion of an appropriate amount of F promoted bone formation through osteoblast proliferation [4]. However, excess F ingestion for a prolonged period caused fluorosis of the teeth [5] and osteosclerosis [6]. In fluorosis of the teeth, it is considered that excess fluoride ingestion in the tooth calcification period causes enamel hypocalcification at an F level of about 1-2 ppm. Osteosclerosis occurs when the F concentration in drinking water is about 8 ppm, and ligaments and tendons are calcified with an increase in the F level, manifesting arthralgia and motor disturbance. Many studies on the appropriate intake and concentration of F have been performed, but many points are unclear with regard to bone remodeling induced by changes in the F level. In this study, a rat ovariectomy-induced osteoporosis model (ovariectomized rats; OVX) was fed a diet with high contents of minerals, such as Ca, P, and F (high-mineral diet), and changes in the femoral diaphysis after 24 weeks were investigated.

Materials and Methods

Experimental animals: Eighteen female Wistar rats (Sankyo Labo Service Co., Japan), aged 19 weeks, were used for the experiment. The experimental protocol was approved by an animal experiment ethics committee (No. AP10MD006-2). All experiments were conducted according to the Guidelines for the Treatment of Animals, Nihon University, Tokyo, Japan. The 18 rats were randomly assigned to 3 groups (n = 6 per group). Group A sham operated and provided with a normal diet (American Institute of Nutrition (AIN)-93M, Nosan Co., Japan). Group B consisted of OVX rats on a normal diet. Group C consisted of OVX rats on a high-mineral diet (AIN-93M with increased mineral content; Nosan Co.) (Table 1).

Measurement of body weight: The body weights of all rats were measured at the following times: 1 week before surgery (age, 19 weeks), at the time of surgery (20 weeks), 1 week after surgery (21 weeks), and 24 weeks after surgery (44 weeks). All animals were then euthanized with carbon dioxide at 24 weeks after surgery, and bilateral femora were removed.

Micro-CT settings: Femoral micro-CT images were acquired setting the measurement range at the middle point region of the full femoral length measured using calipers (4.0 mm × 4.0 mm × 2.0 mm). The micro-CT (R-mCT; Rigaku Co., Japan) imaging conditions were as follows: tube voltage, 90 kV; tube current, 88 μA; magnification, ×6.7; measurement time, 17 s.

1) Measurement of bone mineral density and bone mineral content: The micro-CT images were converted to a 16-bit gray scale TIFF format using the Atlas TIFF Convertor software (Rigaku Co., Japan), and were observed using TRI/3D-Bon BMD software (TRI/3D; Ratoc System Engineering Co., Japan). For bone mineral density (BMD) and bone mineral content (BMC) measurements, a hydroxyapatite calibration curve was prepared from images of phantoms (hydroxyapatite content: 200-1,550 mg/cm^3), and the femur was measured using the TRI/3D trabecular structure analysis routine (auto-detection mode) employing the obtained CT values.

2) Observation of CT image and 3D color map: Bone situation (inferred from BMD values) was determined from CT image and 3D color map (3D-map) showing BMD distributions obtained by micro-CT, represented in pseudocolors (High: red and orange, Middle: yellow and green, Low: light blue and purple). Analysis of the 3D-map was conducted using TRI/3D image analysis software from BMD values.

Measurement of bone strength: Bone strength was measured using an Instron type testing machine system (TCM500CR, Minebea Co., Japan). The femur was supported using a 3-point bending jig with a between-fulcrum distance of 10 mm, and a bending force was loaded on the middle point of the bone length at 5.0 mm/min.

Observation of cecum: The cecum was decalcified in formic acid-formaldehyde solution. The maximum cross-section containing the ileocecum and cecocolic junction was cut in half, and paraffin-embedded sections of the cross-sectional surface were prepared. The sections were stained with H.E. and observed by a microscope.

Statistical analysis: The mean values and standard deviations of body weight, BMD, BMC, and bone strength were calculated for each group, and compared using one-way analysis of variance and Tukey's multiple comparison test with IBM SPSS software (SPSS Inc, USA). Values of $P < 0.05$ were considered significant.

Results

Body weight: The body weight slightly decreased in the week following sham operation or ovariectomy (Table 2). At 24 weeks, the body weight was increased in Groups A and B, but no increase was noted in Group C.

CT image and 3D-map: The CT images (upper stage) and 3D-maps (lower stage) of the 3 groups at 24 weeks are shown in Fig. 1. In the CT images, a circumferential non-penetrated region was present around the bone in Groups A and B. Round penetrated regions in the inner region of the femur were observed in Group C. In the 3D- maps, the femur was mainly colored red and partially orange on the color scale in Group A, showing a high BMD. The region adjacent to the marrow cavity was yellow, showing a lower BMD than that in the outer region. The colors observed in Group B were similar to those in Group A. In Group C, there were small red areas in the outer femur, and the inner region was mainly yellow and green. The inner region adjacent to the marrow cavity showed uneven bone resorption and the BMD was lower than that in Group B.

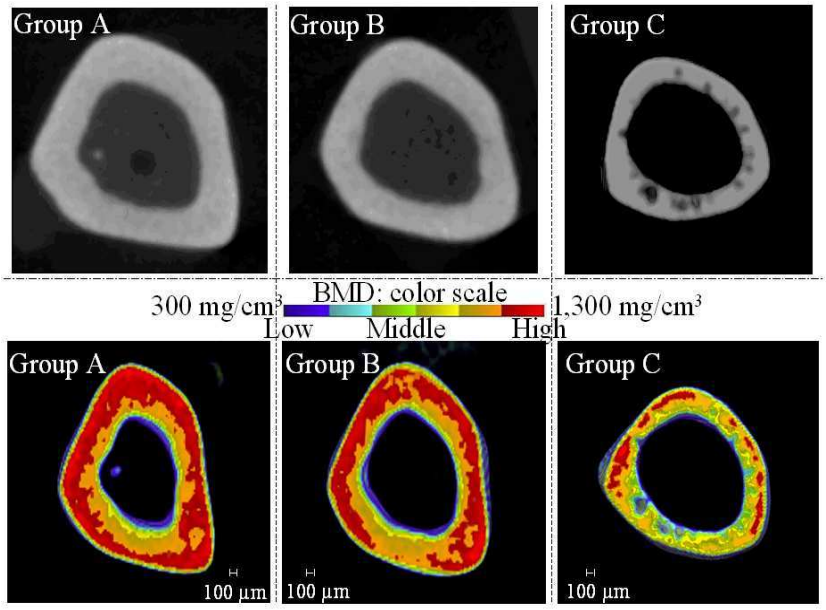

Fig. 1 Micro-CT image (upper stage) and 3D bone mineral density distribution maps.

Table 3 Bone mineral density, bone mineral content and bone strength of femoral mid-shaft for the 3 groups 24 weeks after the surgery. * $P < 0.05$, ** $P < 0.001$

	Bone mineral density	Bone mineral content	Bone strength
Group A	1228.4±6.0	13.0±0.5	165.0±5.9
Group B	1200.5±7.1	10.4±0.6	144.0±5.9
Group C	1016.7±15.2	7.38±0.5	56.0±4.4

BMD and BMC measurements: The results of femoral BMD and BMC measurements after 24 weeks of ingestion are shown in Table 3. Both BMD and BMC significantly decreased in Group B compared to those in Group A. In Group C, both parameters significantly decreased compared to those in Groups A and B.

Fracture strength measurement: The results of femoral fracture strength measurement after 24 weeks of ingestion are shown in Table 3. The strength significantly decreased in Group B compared to that in Group A. In Group C, the strength significantly decreased compared to those in Groups A and B.

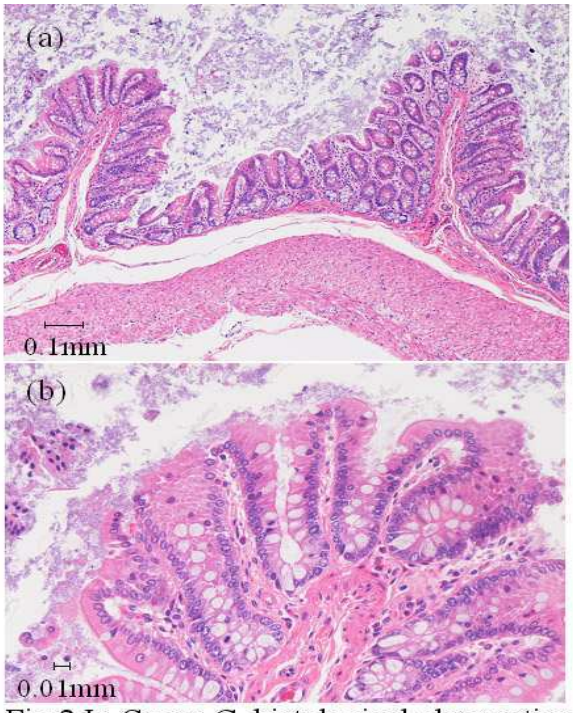

Fig. 2 In Group C, histological observation of cecum (H.E. stain) at (a) low magnification and (b) high magnification.

Histopathological examination: In Group C, regarding the presence or absence of inflammation, only a small number of lymphocytes and polymorphonuclear leukocytes (neutrophils) were present in the lamina propria mucosae and submucosa, and no abnormal finding was noted (Fig. 2).

Discussion and Conclusion

Since F absorbed in the body promotes osteoblast proliferation and subsequent bone formation, it is an essential element for the body. F ingestion has been reported to reduce the lumbar vertebra fracture rate and increase the lumbar BMD, showing its usefulness to prevent osteoporosis [7]. F administered to rats through drinking water, food, or a gastric tube inhibited body weight gain or reduced the body weight in many reports, and weight loss with an increase in the dose of F [8] is consistent with our finding. F promotes bone formation and inhibits bone resorption, but it was clarified that ingestion of an appropriate amount is important, and a high intake reduced the BMD, BMC, and fracture strength of bone.

Perkins et al. reported that the influence of F on hard tissue involves the function of blood [9], and Narita et al. reported that F is absorbed into the femur through not only blood in bone tissue but also tissue fluid through the periosteum and endosteum [10]. Since nutrients are supplied over the femur through blood vessels in the haversian canal present in the lumen of osteon, a high level of F may have accumulated on the inner side, i.e., the bone marrow side, rather than the outer side, resulting in the lower BMD on the inner than the outer side in the 3D-map. Regarding incorporation of F into the body, Ream et al. reported that active femoral bone resorption was observed in pregnant rats given drinking water containing 150 ppm of F [11], showing a similar finding to ours.

Since dietary F is absorbed and metabolized in the stomach and small intestine, we pathologically examined the cecum, but no inflammatory change was noted, suggesting that a high level of F was absorbed through blood vessels in the stomach and intestine and diffused over the femur from the inner side through the periosteum and bone marrow, which reduced the bone quality and led to bone resorption. Since F ingestion influences the apatite crystal structure and growth of crystal particles in bone tissue, and adsorption of F to crystals and structural changes may be influenced, it is important to ingest F at the optimum level.

Acknowledgements

This study was supported in part by Grants-in-Aid for Scientific Young Scientists (B) (22791942) and A Grant for Supporting Project for Strategic Research (S0801032) 2008-2012 from The Ministry of Education, Culture, Sports, Science and Technology, Japan.

References

[1] R.Z. LeGeros, A. Ito, K. Ishikawa, T. Sakae, J.P. LeGeros: Advanced Biomaterials. John Wiley & Sons, (2009), 19-52.
[2] M. Inoue, H. Nagatsuka, H. Tsujigiwa, M. Inoue, R.Z. LeGeros, T. Yamamoto, N. Nagai, Dent. Mater. J. 24 (2005) 398-402.
[3] G.E. Shambaugh Jr, A. Prtrovic, J.A.M.A. 204 (1968) 111-115.
[4] H.E. Gruber, D.J. Baylink, Clin. Orthop. Relat. Res. 267 (1991) 264-277.
[5] T. Aoba, O. Fejerskov, Crit. Rev. Oral Biol. Med. 13 (2002) 155-170.
[6] P.F. Møller, S.V. Gudjonsson, Acta Radiol. 13 (1937) 269-294.
[7] D. Haguenauer, V. Welch, B. Shea, P. Tugwell, J.D. Adachi, G. Wells, Osteoporos Int. 11 (2000) 727-738.
[8] M. Mizohata, Y. Kameyama, Histological Acta Anat. 133 (1988) 134-139.
[9] F.M. Parkins, N. Tinanoff, M. Moutinho, M.B. Anstey, M.H. Waziri, Calcif. Tissue Res. 16 (1974) 335-338.
[10] N. Narita, K. Kato, H. Nakagawa, Y. Sakakibara, Aichi-Gakuin J. Dent. Sci. (in Japanese) 26 (1988) 213-221.