

## **SKELETAL METABOLISM AND BONE MINERAL DENSITY IN FLUORIDE-EXPOSED RATS**

Beata Urbańska, Wojciech Czarnowski, Jerzy Krechniak,<sup>a</sup>  
Iwona Inkielewicz, Katarzyna Stolarska  
Gdańsk, Poland

**SUMMARY:** As part of a two-stage investigation, adult male Wistar rats were exposed to above-normal fluoride intake for 6 months: a) to HF by inhalation for 2 hr/day at a concentration of  $8.7 \pm 5.7 \text{ mg/m}^3$  and b) to NaF in drinking water containing 20 mg F<sup>-</sup>/L. The fluoride content in urine, vertebrae L<sub>2</sub>–L<sub>4</sub>, tibia, and incisors was significantly increased in the exposed animals. More fluoride was excreted in urine by animals exposed by the inhalatory than by the oral route. Bones of control and exposed animals were of similar mechanical resistance. No differences in bone mineral density (BMD) were found between exposed and control animals. Moreover, no significant differences were found in alkaline phosphatase activity, calcium and magnesium concentration in serum, and hydroxyproline concentration in urine between control and exposed animals. The results indicate that even a threefold increase in bone fluoride does not cause a distinct change in metabolism, mineral density, and mechanical resistance of bones of exposed animals.

Keywords: Airborne fluoride, Bone fluoride, Bone metabolism, Bone mineral density, Fluoride exposure, Rat exposure, Urine fluoride.

### **INTRODUCTION**

Approximately 99% of the fluoride in the body is associated with calcified tissues. Fluoride is incorporated into bones and teeth where, as a result of similarities in size and charge, it replaces the hydroxyl ion in the crystal lattice of apatite. It can be released from bone by ion exchange at the crystal surface and by the dissolution of bone crystals through osteoclastic activity. Fluoroapatite is less soluble, more compact, and slower to undergo remodeling in bone.

Excessive intake of fluoride may lead to pathological changes in teeth and bones: dental and skeletal fluorosis. Fluorosis may be caused by occupational exposure or by excessive intake of fluoride from drinking water.<sup>1-4</sup> Fluoride is still used in therapy for certain bone disorders (e.g. osteoporosis) characterized by impairment of bone microstructure and decline of bone tissue. Except in their earliest stages, most bone disorders, including fluorosis, can be ascertained by radiological methods.

In a previous study conducted on a human population exposed to an elevated fluoride level (about 3 mg/L) in drinking water, we found a significant increase in bone mineral density (BMD) compared to controls.<sup>2</sup> The aim of this study was to assess whether a correlation exists between bone fluoride and the metabolic status, mechanical resistance, and mineral density of

---

<sup>a</sup>For correspondence: J. Krechniak, Department of Toxicology, Medical University of Gdańsk, Al. Gen. Hallera 107, 80-416 Gdańsk-Wrzeszcz, Poland. E-mail: wojtekc@pf.pl

bones. This work is part of an investigation on rats exposed to fluorine compounds by oral or inhalatory routes.<sup>5</sup> The intensity of exposure was selected in order to imitate a high but probable level of occupational or environmental exposure of humans.

#### MATERIALS AND METHODS

*Experimental design:* As described earlier,<sup>5</sup> eight-week-old, male Wistar rats each weighing about 200 g, were divided into three groups of 10 animals each: Group I, controls; Group II, animals exposed to HF by inhalatory route; Group III, animals given NaF in their drinking water at a concentration of 20 mg F<sup>-</sup>/L. Rats in groups I and II were given tap water containing 0.3 mg F<sup>-</sup>/L. All animals were fed a standard laboratory pellet diet containing 0.7 ppm F<sup>-</sup>.

Rats from Group II were put in pairs into a whole-body exposure chamber of about 5 L volume with dynamic exposure conditions to inhale hydrogen fluoride for 2 hr daily at a concentration of  $8.7 \pm 5.7$  mg/m<sup>3</sup>. This concentration was obtained by passage of air through a solution of 48 % reagent-grade hydrofluoric acid diluted with water 1:3 (v/v) at a rate of 50 L of air/hr.<sup>5</sup>

Every month rats were put into metabolic chambers, and samples of urine were collected. Also at the same interval samples of blood were collected. After six months of treatment the animals were sacrificed by ether anesthesia and material was collected for fluoride determination. Vertebrae L<sub>2</sub> – L<sub>4</sub> and femurs were used as bone samples, incisors as tooth samples.

Bone mineral density was measured by means of quantitative computer tomography (QCT) using a Tomoscan apparatus (Philips) in the Department of Radiology, Medical University of Gdańsk. The fluoride level in urine samples was determined directly after dilution with equal volumes of TISAB buffer. The fluoride level in bone and teeth was determined as described by Stolarska *et al.*<sup>5</sup>

Fluoride concentrations were measured by a fluoride ion specific electrode and an Ag/AgCl reference electrode with a double jacket. Calculations were based on a response factor from a standard curve prepared daily.

Recovery of F from analyzed materials amounted to  $100 \pm 5\%$ . The coefficient of variation in samples of urine was 1.89%, in bones and teeth, 2.3%. The mechanical resistance of femur bone was measured with a FTS 107 M(SPAIS) dynamometer by a three-point bending test. A support with two loading points 12 mm apart from each other was placed on the stage of the testing machine and charged with increasing force.

The metabolic status of the bones was assessed by determination of hydroxyproline content in urine,<sup>6</sup> activity of alkaline phosphatase (Cormay diagnostic kit), and calcium and magnesium concentration vs blood serum.<sup>7</sup>

The accuracy of the determinations was tested with Nycomed-Seronorm™ Trace Elements reference urine.

Statistical analysis of results was performed with a Microsoft Excel 98 spreadsheet program.

### RESULTS AND DISCUSSION

The results of this study are presented in Tables 1-3 and in Figures 1-5. The experiments were conducted on rats over a period of 6 months under conditions that simulate an elevated but not improbably high level of occupational (HF in air) or environmental ( $F^-$  in drinking water) exposure of humans. Essentially no differences were observed in appearance, behavior, and weight gain between the two exposed groups and the controls nor in the mean weight of femur bones (Table 1). These bones were also similar in mechanical resistance and did not differ significantly in the exposed and control animals (Table 2). In the exposed animals there were also no signs of dental fluorosis. However, urinary fluoride levels in both groups of exposed rats were much higher during the exposure period than in the controls. They were also higher in the animals exposed by the inhalatory route than by the oral route (Figure 1). Similar findings have already been reported for the bones and teeth of these rats.<sup>5</sup> In rats treated orally with NaF the fluoride content of the bones and teeth was twice that of the controls, and in rats that inhaled HF it was about three times higher.<sup>5</sup>

**Table 1.** Weights of rats and femur bones after six months

Group of animals	A	B	B/A x 100 %
	Mean body wt (g)	Mean femur wt (g)	
I (Controls), N = 9, ± SD	436.6 ± 68.7	1.489 ± 0.208	0.341 ± 0.018
II (HF), N = 8, ± SD	428.7 ± 41.9	1.464 ± 0.124	0.341 ± 0.035
III (NaF), N = 10, ± SD	423.0 ± 34.9	1.465 ± 0.221	0.346 ± 0.032

Group I – controls; Group II - rats exposed to HF by inhalatory route;  
Group III - rats given as drinking water containing 20 mg  $F^-$ / L.

**Table 2.** Mechanical properties of the femoral shaft after six months

	Group I (Controls)	Group II (HF)	Group II (NaF)
Ultimate deflection strength (kN)	0.16	0.16	0.15
Elastic deflection (mm)	0.02	0.02	0.02
Ultimate deformity (mm)	0.2	0.19	0.24
Maximal deflection strength (kN)	0.24	0.23	0.26
Bone fracture energy (J)	0.038	0.035	0.047

*Webmaster's note: figures not shown on the internet.  
Please see our [membership page](#) for subscription information*

**Figure 1.** Mean ratio of fluoride concentration to creatinine excretion in urine.

**Figure 2.** Mean hydroxyproline concentration in urine.

Despite these distinct, statistically significant increases in bone fluoride, the radiological images did not appear to be affected; and no signs of osteofluorosis could be seen in the radiograms, nor were there any changes in

bone density (Table 3). Thus radiological measurement of bone mineral density in these F-exposed rats was not a sensitive indicator of changes in skeletal tissue caused by fluoride. Similar results were reported by Jiang *et al*<sup>8</sup> in their investigation of the effect of NaF on bone mass and mineral density in rats. Moreover, no differences in mechanical resistance of the bones of exposed animals compared with controls could be detected (Table 2). These findings are in agreement with data reported by a subcommittee of the US National Research Council.<sup>9</sup>

**Figure 3.** Mean alkaline phosphatase activity in serum.

**Figure 4.** Mean calcium concentration in serum.

**Figure 5.** Mean magnesium concentration in serum.**Table 3.** Mean bone mineral density (BMD) of vertebrae L<sub>2</sub> – L<sub>4</sub> in rats after 6 months

Group	BMD (mg/cm <sup>3</sup> )	± SD
I (Controls), N = 9	552.22	63.25
II (HF), N = 8	548.17	26.97
III (NaF), N = 10	537.75	18.34

In contrast to our results, Søgaaard *et al*<sup>10</sup> observed a decline in bone quality of rats exposed to much higher levels of fluoride (100-150 ppm) in their drinking water. Similarly, Bohatyrewicz has found decreased bending strength in the femoral shaft of rats ingesting levels of only 30 or 60 ppm F<sup>-</sup> in their drinking water after six weeks, starting at six weeks of age.<sup>11</sup> In another study, Søgaaard *et al*<sup>12</sup> proved that treatment with fluoride had little effect on the osteopenic rat skeleton and failed to restore the ovariectomy induced loss of bone strength. Bely *et al*<sup>13</sup> found in rats receiving intraperitoneally NaF (0.5-5 mg daily) for a period of 3 months that the quantity of bone tissue increased and the quality diminished with increase in NaF levels administered. Further evidence for a possible detrimental effect of fluoride on bone quality gives Søgaaard<sup>14</sup> indicating that the anabolic action of fluoride is not reflected in a concomitant increase in bone strength.

According to Turner *et al.*,<sup>15</sup> humans incorporate fluoride into the skeleton about 18 times more readily than rats, indicating that human exposure to elevated fluoride levels causes greater skeletal accumulation of fluoride than in rats. This fact may in part account for the differences in BMD found by us in our human<sup>2</sup> and animal<sup>5</sup> studies.

The metabolic status of bones is reflected in alkaline phosphatase activity,<sup>16</sup> calcium and magnesium concentrations in serum, and hydroxyproline level in urine. The results obtained here, however, were not unequivocal. The concentration of hydroxyproline in urine (Figure 2) and the activity of alkaline phosphatase in serum (Figure 3) decreased during the course of the experiment in all animals, which may indicate age-dependent effects. Slightly lower values were found in the exposed rats than in the controls, but the differences were not statistically significant. Moreover, no changes were found in serum calcium (Figure 4) or magnesium (Figure 5). Thus the results of our study indicate that even a threefold increase in bone fluoride in rats did not cause a distinct change in bone metabolism.

This paper was presented at the XXIIIrd Conference of the International Society for Fluoride Research, Szczecin, Poland, June 11-14, 2000.

#### ACKNOWLEDGEMENT

This study was supported by grants W-40 and W-53 from the Medical University of Gdańsk.

#### REFERENCES

- 1 Czarnowski W, Wrześniowska K, Krechniak J. Fluoride in drinking water and human urine in Northern and Central Poland. *Sci Total Env* 1996;191:177-84.
- 2 Czarnowski W, Krechniak J, Urbańska B, Stolarska K, Taraszewska-Czarnowska M, Muraszko-Klaudiel A. The impact of water-borne fluoride on bone density. *Fluoride* 1999;32:91-5.
- 3 Kahama RW, Kariuki DN, Njenga LW. Fluorosis in children and sources of fluoride around lake Elementaita region of Kenya. *Fluoride* 1997;30:19-25.
- 4 Wu RQ, WuDQ, Xu RY. Relations between environment and endemic fluorosis in Hohhot region Inner Mongolia. *Fluoride* 1997;30:26-8.
- 5 Stolarska K, Czarnowski W, Urbańska B, Krechniak J. Fluoride in hair as an indicator of exposure to fluorine compounds. *Fluoride* 2000;33:174-81.
- 6 Czarnowski W, Krechniak J. Hydroxyproline and urinary fluoride in rats repeatedly exposed to inhaled phosphorites. *Fluoride* 1989;22:24-8.
- 7 Gochman N, Givelber H. Automated, simultaneous microdetermination of calcium and magnesium by atomic absorption. *Clin Chem* 1970;16:229-34.
- 8 Jiang Y, Zhao J, Vanaudekercke R, Dequeker J, Geusens P. Effects of slow-dose long-term sodium fluoride preventive treatment on the bone mass and biomechanical properties. *Calcified Tissue Int* 1996;58:30-9.
- 9 National Research Council Subcommittee on Health Effects of Ingested Fluoride. Washington, DC: US National Academy of Sciences; 1993. p. 63-8.

- 10 Søgaard CH, Mosekilde L, Schwartz W, Leidig G, Minne H W, Zeigler R. Effects of fluoride on vertebral body biochemical competence and bone mass. *Bone* 1995;16:163-9.
- 11 Bohatyrowicz A. Effects of fluoride on mechanical properties of femoral bone in growing rats. *Fluoride* 1999;32 :47-54.
- 12 Søgaard CH, Mosekilde L, Thomsen JS, Richards A, Mcosker JE. A comparison of the effects of two anabolic agents (fluoride and parathyroid hormone) on ash density and bone strength assessed in a osteopenic rat model. *Bone* 1997;20:439-49.
- 13 Bely M, Ferencz G, Itai K, Tsunoda H. Experimental osteofluorosis and arthrofluorosis in rats. Proceedings of the XXI Conference of the International Society for Fluoride Research, 1996 Aug 25-28; Budapest, Hungary. Abstract in *Fluoride* 1997;30:113-4.
- 14 Søgaard CH. Antiresorptive therapy, anabolic therapy, and exercise effects on bone mass, structure, and strength assessed in a rat model. PhD thesis, University of Aarhus, Denmark, 1994. Abstract in *Fluoride* 1997;30:119-21.
- 15 Turner C H, Akhten M P, Heaney R P. The effects of fluoridated water on bone strength. *J Orthop Res* 1992;10:581-7.
- 16 Krook L, Minor RL. Fluoride and alkaline phosphatase. *Fluoride* 1998;31: 177-82.