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Effects of fluoride on bone in Finland

Histomorphometry of cadaver bone from low and high fluoride areas

In three different areas of Finland, fluoride in bone and its effect on the histomorphometry of trabecular bone was studied. Bone samples were taken from cadavers from a low-fluoride area (fluoride concentration under 0.3 ppm), an area with fluoridated drinking water (1.0–1.2 ppm) and a high-fluoride area (over 1.5 ppm). The fluoride content in trabecular bone was greatly increased in the high-fluoride area, and it was also higher in the fluoridated-water area than in the low-fluoride area. Histomorphometric bone changes were markedly increased when the fluoride content in water exceeded 1.5 ppm.

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Fluoride is derived from various sources. Drinking water is often the main source of fluoride for humans. Drinking water in Finland contains 0.0–7.6 ppm fluoride in piped water and other sources of drinking water (National Board of Health, Finland, 1974). In Kuopio, drinking water has been fluoridated since 1959, up to 1.2 ppm. The daily intake of fluoride from foodstuffs in Finland has been estimated to be 0.56 mg (Varo & Koivistoinen 1980).

Fluoride is rapidly absorbed from the gastrointestinal tract (Smith 1966). Hard tissues are the main storage tissues for fluoride. In bone, fluoride is incorporated into hydroxyapatite to form fluoroapatite (Neuman & Neuman 1958). Accumulation of fluoride in bone is maximal during active growth, but the amount of fluoride in bone increases also in old age (Alhava et al. 1980). With moderate fluoride exposure, about half of the fluoride is bound by bone and the other half is mainly excreted by the kidneys (Weatherell 1966). So, skeletal storage and renal excretion of fluoride are the most important factors maintaining the fluoride balance in the body.

Histomorphometric analysis of undecalcified bone is a sensitive method for evaluating changes in trabecular bone (Bordier & Tun-Chot 1972, Melsen et al. 1978). The present paper describes a study on the fluoride content of trabecular bone and its relation to the histomorphometry of trabecular bone in three areas of Finland with different fluoride contents in drinking water.

Material and methods

Material

Low-fluoride area. In autopsies at the Kuopio University Central Hospital, the Department of Forensic Medicine in Helsinki University, the Middle Finland Central Hospital in Jyväskylä, and the North Karelian Central Hospital in Joensuu, bone specimens were taken from 50 men and 35 women. Subjects who had had any disease or medication that could have influenced metabolism of minerals in the bones were not accepted for the study. Those who had had intestinal surgery (apart from appendicectomy) were also excluded, as were institutionalised patients and alcoholics. No subject included in this study had been immobilized for more than 2 weeks. The most frequent causes of death were heart attacks and accidents. The fluoride concentration of trabecular bone was determined for 40 men and 25 women in different age groups (Table 1). These 65 patients were used as low-fluoride area controls in this study. According to the data from the Finnish National Board of Health (1974), the areas where bone specimens were collected were known to be low in fluoride (F^- concentration 0.3 mg/l or less).

Results from a histomorphometric analysis of all 85 cadavers have been published by Hoikka & Arnala (1981).

Fluoridated-water area. During autopsies in the Kuopio University Central Hospital, bone specimens were taken from 26 men and 17 women (Table 1)

Table 1. Age and sex distribution of the patients in areas with different fluoride concentrations.

Area	Sex	Age groups 20–	30–	40–	50–	60–	>70	Total
Low-F^-	F	3	3	2	5	8	4	25
	M	5	4	8	8	9	6	40
Fluoridated	F	–	2	–	4	3	8	17
	M	2	2	2	7	5	8	26
High-F^-	F	–	1	1	4	4	7	17
	M	1	–	5	10	14	10	40

who had lived at least 10 of their last years in Kuopio in the fluoridated-water area. Thirty-one of these subjects had died suddenly of cardiovascular disease without any previous long-term medical history. Some subjects with chronic diseases were also included; six of these had had slightly impaired renal function.

High-fluoride area. In a large area of south-eastern Finland, the bedrock consists of the so-called rapakivi that is rich in fluoride. As a consequence, many water supplies in this area have high fluoride levels (National Board of Health, Finland, 1974). In some places the fluoride concentration in natural wells can be eight times higher than in the fluoridated tap water of Kuopio.

Bone specimens were collected in autopsies at Kotka Central Hospital. These specimens were taken from 40 men and 17 women of different age groups (Table 1). Almost all these subjects had lived in the countryside and used their own water sources, and all had lived at least 10 of their last years in the same location. Most had lived in the same place throughout their life.

Twenty-nine had not had any disease that could affect the metabolism of minerals in bone. They died suddenly from cardiac or cerebrovascular diseases. Seven had had impaired renal function with a creatinine level between 121 and 219 $\mu mol\ l^{-1}$.

Water samples were taken from the water sources of 16 subjects.

Methods

Fluoride measurement. A piece of trabecular bone, weighing 200–500 mg, was taken from the same area of the bone sample that was measured histomorphometrically. These bone samples were reduced to ash, and the fluoride ion activity was determined potentiometrically using a fluoride selective electrode (Alhava et al. 1980).

All results were expressed as values determined from trabecular bone ash. The fluoride concentration in drinking water was measured using the same potentiometric method.

Slide preparation and stereological methods. Two bone cylinders 5 mm in diameter and 20 mm long from the iliac crest at adjacent locations about 2 cm behind the anterosuperior iliac spine were made into 5-µm sections using the hard tissue technique (Hoikka & Arnala 1981). The bone sections were stained with Masson-Goldner trichrome stain.

The specimens were evaluated histomorphometrically (Merz 1967). Using a magnification of 100×, thirty fields were measured from each section. The parameters measured were expressed as the volumetric density of trabecular bone (V_v), the volumetric density of osteoid (V_{vo}), the trabecular bone surface covered with osteoid (OS), and trabecular resorption surface (RS). V_v included both calcified and unmineralised bone. V_{vo} was the percentage of unmineralised bone in the total amount of trabecular bone.

The trabecular resorption surface was expressed as the total area of resorption cavities, with or without osteoclasts. Also the osteoid seam width (V_{vo}/OS) values were related to bone fluoride content.

Statistical methods. All parameters were tested by linear regression analysis to determine correlations between fluoride content in bone and the histomorphometric results. Regression analysis was also used to compare fluoride content in water with that in trabecular bone.

Mann-Whitney's U-test was used for comparing all parameters in the examined groups with controls from the low-fluoride area.

Results

Fluoridated-water area

The fluoride content of trabecular bone differed in fluoridated and low-fluoride areas ($p < 0.001$). Fluoride content did not differ between males and females. No significant differences were found for the histomorphometric parameters between the area with fluoridated drinking water and the low-fluoride area (Table 2).

In the area with fluoridated drinking water, linear regression analysis revealed correlations ($p < 0.05$) between fluoride content in bone and osteoid volume ($r = 0.486$) and between fluoride content and osteoid-covered tra-

Table 2. Fluoride content (F^-, ppm) in cancellous bone of the iliac crest, volumetric density of trabecular bone (V_v, percent), volumetric density of osteoid (V_{vo}, percent), trabecular bone surface covered with osteoid (OS, percent), and trabecular resorption surface (RS, percent) for females (n = 17) and males (n = 26) in the area with fluoridated drinking water and for females (n = 25) and males (n = 40) in the low-fluoride area, expressed as the mean ± SD. Mann-Whitney's U-test.

Sex	Tissue component	Fluoridated area	Low-fluoride area	p-value
F	F^-	1560±720	490±190	<0.001
	V_v	17.6±5.2	15.9±6.0	ns
	V_{vo}	1.9±1.4	2.0±1.0	ns
	OS	17.0±6.3	16.7±6.4	ns
	RS	3.6±1.1	3.7±1.3	ns
M	F^-	1220±520	460±240	>0.001
	V_v	16.1±5.0	16.3±5.4	ns
	V_{vo}	2.8±1.7	2.1±1.3	ns
	OS	20.0±8.3	19.3±7.9	ns
	RS	3.8±0.8	3.5±0.8	ns

becular bone surface (r = 0.541) in women. No other parameters, including osteoid seam width were correlated with fluoride content in the bones in either females or males. In males the highest fluoride content in bone was 2750 ppm with no histological changes. For females, the highest fluoride concentration in bone (3890 ppm) was found in a subject with impaired renal function; she had increased (Hoikka & Arnala 1981) osteoid volume (V_{vo} = 5.5), whereas the other measured parameters were normal. All subjects with slightly impaired renal function had a higher content of fluoride in bone (2090 ± 1010 ppm; mean ± SD) than did those with normal creatinine level.

Table 3. Fluoride content and histomorphometric parameters (V_v, V_{vo}, OS and RS) for females (n = 17) and males (n = 40) in the high-fluoride area and for females (n = 25) and males (n = 40) in the low-fluoride area expressed as the mean ± SD. Mann-Whitney's U-test.

Sex	Tissue component	High-fluoride area	Low-fluoride area	p-value
F	F^-	3490±2850	490±190	<0.001
	V_v	16.8±6.4	15.9±6.0	ns
	V_{vo}	7.3±8.3	2.0±1.0	<0.001
	OS	33.8±23.9	16.7±6.4	<0.001
	RS	4.1±2.1	3.7±1.3	ns
M	F^-	2830±1450	460±240	>0.001
	V_v	17.5±4.2	16.3±5.4	ns
	V_{vo}	4.4±5.6	2.1±1.3	<0.005
	OS	28.7±13.7	19.3±7.9	<0.001
	RS	3.5±0.7	3.5±0.8	ns

High-fluoride area

In the high-fluoride area there were very high fluoride contents in bone and also increased osteoid values in both sexes (Table 3). Significant differences were found in the fluoride content of trabecular bone, the volumetric density of osteoid, and osteoid-covered trabecular bone surface in both sexes between the high-fluoride area and the low-fluoride area (Table 3). The volumetric density of trabecular bone was not higher in the high-fluoride area, although osteoid volume was increased in the high-fluoride area. Resorption activity did not differ significantly in these two areas.

Regression curves revealed a correlation between fluoride content of trabecular bone and osteoid parameters (V_{vo} and OS) in both sexes (Figures 1 and 2). There was no correlation between fluoride content of bone specimens and resorption surface in either sex. In women the volumetric density of trabecular bone was correlated (r = 0.486, p < 0.005) with bone fluoride content. In men there was no such correlation. The result was the same when all high-fluoride area specimens were evaluated. Osteoid seam width was correlated with bone fluoride in both women (r = 0.462, p < 0.05) and men (r = 0.503, p < 0.001).

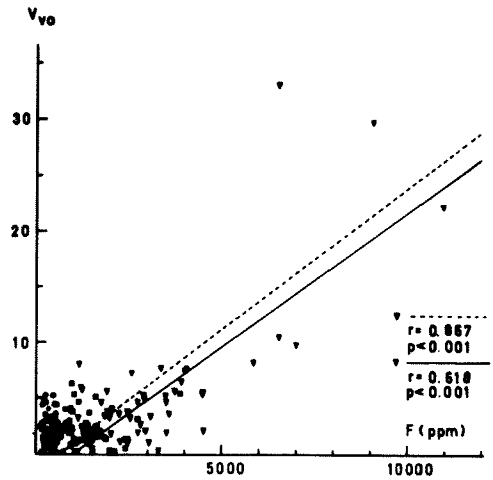

Figure 1. Linear regression analysis for fluoride content of trabecular bone and volume density of osteoid (V_{vo}). Solid signs represent men and open signs women. Circles (●, ○) are cases from the low-fluoride area, squares (■, □) are cases from the fluoridated-water area, and triangles (▲, △) are cases from the high-fluoride area.

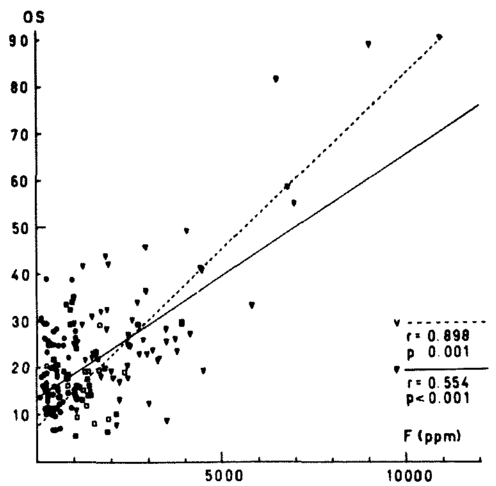

Figure 2. Linear regression analysis for fluoride content of trabecular bone and osteoid-covered trabecular bone surface. Same symbols as Figure 1.

The highest fluoride content measured in trabecular bone was 10 890 ppm in a 66-year-old female. For males the greatest fluoride content was 7090 ppm.

Four women and seven men had more than 4000 ppm fluoride in their bones. In ten of these the volumetric density of osteoid exceeded 3.4 percent, and in eight the osteoid-covered surface was over 37 percent, the upper normal limits for V_{vo} and OS (Hoikka & Arnala 1981). The volumetric density of trabecular bone and resorption surface were within normal limits. Two of these subjects had increased values for serum creatinine (121 and 219 μmol l^{-1}), and one with normal renal function had diabetes. Two females and one male had very high V_{vo} and OS values. The first female had chronic endomyocarditis of unknown origin; her renal function was normal. The other female had had chronic pyelonephritis since 1960 and impaired renal function; the cause of death in 1978 was myocardial infarction. The male had normal values for serum creatinine, and he died of cerebral thrombosis.

Water analysis

The fluoride content in drinking water (2.6 ± 1.4 ppm) was correlated ($r = 0.761, p < 0.001$) with the fluoride content of bone (4910 ± 2250 ppm). The fluoride content in drinking water was also correlated with the volume density of osteoid ($r = 0.475$, $p < 0.05$), osteoid-covered surface ($r = 0.638$, $p < 0.01$), and resorption surface ($r= 0.498$, $p < 0.05$). The fluoride content of drinking water was not correlated with the volume density of trabecular bone. There was also no correlation between the ages of the patients and the fluoride content of bone specimens.

Discussion

Fluoride analysis of the cadaver material from Kuopio revealed that fluoridation of drinking water increases the fluoride concentration in bone. In some individual cases the amount of fluoride in trabecular bone may rise to relatively high levels, notably in patients with impaired renal function. This has previously been shown for chronic renal disease (Charen et al. 1979, Hefti & Marthaler 1981), and in patients hemodialysed with fluoridated water (Cordy et al. 1974, Lough et al. 1975). In the endemic high-fluoride area, the study revealed high concentrations of fluoride in bone. Both in women and men, the amount of fluoride in the bones was several times higher than in the low-fluoride area.

It is not completely understood how fluoride acts on bone. Roholm (1937) suggested that small doses of fluoride stimulate bone growth and calcification and that large doses reduce calcification and cause bone atrophy. In animal studies, many authors (Faccini & Care 1965, Makhni et al. 1979) have shown that excess fluoride stimulates the parathyroid glands. This is thought to be responsible for the many histological findings in human skeletal fluorosis (Teotia et al. 1973, 1976). Nichols et al. (1965) assumed that activated parathyroid glands stimulate formation of new bone without any increase in bone resorption. Jowsey et al. (1968) concluded that fluoride stimulates osteoblastic activity.

The main histological change induced by fluoride is the increase of osteoid volume. This effect has been shown in studies where osteoporosis was treated using fluoride preparations (Briancon et al. 1980, Hauswaldt et al. 1977, Jowsey et al. 1972, Kuhlencordt et al. 1970, Kruse et al. 1978); bone resorption was unchanged or slightly increased (Briancon et al. 1980, Jowsey et al. 1972). In histomorphometric studies concerning the effect of drinking water fluoride on bone, there also seems to be an increase in osteoid parameters. According to earlier studies, these changes are evident at least with naturally high concentrations of fluoride in drinking water. Geever et al. (1972) found no difference when the histology of decalcified bone sections from an area with fluoridated drinking water was compared with bones from the low-fluoride areas (fluoride concentration in water was 0.1 ppm or less). This agrees with the results of our study. Very high concentrations of fluoride in drinking water (over 20 ppm) increase osteoid surface and volume abnormally and may also increase resorption (Teotia et al. 1976). This increase in osteoid parameters was observed in our study already at fluoride concentrations above 1.5 ppm. We could not find any increase in resorption.

Our study showed that concentrations around 1 ppm of fluoride in drinking water do not produce histomorphometric changes in trabecular bone. Fluoridation of drinking water up to 1.2 ppm apparently does not pose a potential risk to bone provided the renal function is normal. On the other hand, as evaluated by histomorphometry, fluoridation does not seem to protect against bone loss in old age. Thus, the only function of fluoridation may be the prevention of dental caries.

There are obviously still open questions concerning the safety of fluoridation. Especially, the effect of some diseases on susceptibility to fluoride-induced changes in bone still needs to be investigated. In the high-fluoride area, histological changes become inevitable when individuals use high-fluoride water for many years. Possible clinical manifestations, e.g. pain and increased fracture incidence, are not yet completely known with fluoride-induced disturbance in bone mineralisation at this level. The upper limit for fluoride concentration in drinking water that does not increase the amount of unmineralised bone seems to be roughly 1.5 ppm, according to the results of the drinking water analysis. We should, however, recognize that it is difficult to give a strict value for a safe fluoride concentration in drinking water, because individual susceptibility to fluoride varies.

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