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Influence of Dietary Fluoride Restriction on Regulation of Plasma and Soft Tissue Fluoride Contents (39274)

L. SINGER, R. H. OPHAUG, AND W. D. ARMSTRONG

Department of Biochemistry, University of Minnesota Medical School, Minneapolis, Minnesota 55455

Wide variations in skeletal fluoride load, administration of parathyroid hormone, or food deprivation for a period of 7 days were shown by us (1) not significantly to alter the mean total plasma fluoride level of experimental animals. Also, no difference between the fluoride contents of the blood of pregnant and nonpregnant rats was found (2), and the constancy of total fluoride content of plasma of humans using communal waters in the range of 0.15–2.5 ppm has been reported (3); however, persons using communal water containing 5.4 ppm had a distinct elevation of total fluoride contents.

The role of the skeleton in regulation of plasma and tissue fluoride contents of rats given large doses of fluoride has been described (4). The concentration of fluoride in the plasma of rats can be raised by large increases in dietary fluoride intake (2, 5). Under such circumstances the capacity of the mechanism(s) regulating the plasma fluoride concentration is exceeded. Likens *et al.* in a study in humans found some indirect indication from observations of fluoride levels in urine following defluoridation of a communal water supply that fluoride was being mobilized from the skeleton (6). However, it is not known whether there is adaptation by restoration to a lower plasma fluoride content when animals, having been on a regimen of elevated fluoride intake, are given a diet very low in fluoride content for a period of time or how quickly the adjustment occurs. Likewise, it is not known whether the soft-tissue fluoride concentrations under these conditions are supported at higher than normal values by mobilization of fluoride from the large skeletal fluoride pool. For these reasons, an investigation was designed to determine the effects of a prolonged sharp restriction in fluoride intake on the fluoride contents of plasma, humeri, and soft tissues of rats which had been maintained for a period of high-fluoride intake.

Methods. One-hundred-and-twenty-six male Holtzman rats with a mean weight of approximately 180 g were maintained on Purina Laboratory Chow (34 ppm F) and municipal water (1 ppm F) for 1 week after receipt. Twenty-two animals were then sacrificed to obtain baseline data, and the remaining animals were continued on the diet and provided distilled water to which 50 ppm F, as sodium fluoride, was added. The animals at this time had a mean weight of 232 ± 3.7 (SEM) g and at the end of 4 weeks of increased fluoride intake, 20 animals were weighed (373 ± 4.3 g) and sacrificed. The remaining animals (379 ± 2.7 g) were transferred to the low-fluoride diet of Taylor *et al.* (7), containing 0.21 ppm F, and provided distilled water. Groups of 20 or more animals were killed at 3, 7, 14, or 21 days after being placed on the low-fluoride dietary.

The experimental animals were given *ad libitum* access to food and water until sacrificed, at which time they were bled by heart puncture with special fluoride-free syringes containing heparin as the anticoagulant. Plasma from each animal was harvested and stored at 0° until analyzed. The humeri, liver, and a sample of muscle tissue were obtained from each animal. The soft tissues were dried at 105°, thus giving their dry weights and water contents, and ground to a fine powder. The humeri were cleaned of soft tissue and marrow and rendered fat-free by Soxhlet extraction with a 50:50 mixture of absolute alcohol and anydrous ethyl ether for 48 hr. The right humerus of each animal was divided into the epiphyses and diaphyses for separate analyses, whereas the entire left humerus was analyzed as a single sample. All bone samples were ashed for 16 hr at 500°, prior to analysis. The total fluor-

ide content of each sample was determined colormetrically after isolation by microdiffusion (8). Fluoride in liver, muscle and plasma was determined by the same method after perchloric acid extractions of unashed specimens.

Results and discussion. The weights of all animals were approximately the same when they were placed on the high-fluoride diet, and the animals, within a group, had approximately equal weights when sacrificed. The animals gained approximately 130–150 g during the 4-week period of high-fluoride intake prior to being transferred to the low-fluoride diet. No gain in body weight was noted during the depletion period. This is difficult to explain since an increase in weight of animals of this age is expected to occur. Bone growth and/or mineralization, however, occurred during the fluoride depletion period since the total ash weights of the humeri increased by 10%.

The means and standard errors of the means and number of animals for the ash contents of the dry, fat-free humeri epiphyses, diaphyses, and entire humeri were 63.5 ± 0.72 (22), 73.8 ± 0.83 (24), and 69.3 ± 0.59% (23), respectively, at the end of the 21-day period of low-fluoride intake as compared with values of 55.6 ± 1.12 (20), 69.0 ± 1.17 (21), and 63.4 ± 0.76% (21), respectively, at the beginning of the depletion period. The increase in ash contents of the bones in 20 days is a reflection of an enhanced degree of calcification which occurred with aging.

The results obtained for the fluoride contents of the ash of the epiphyses, diaphyses, and whole humeri are given in Table I. These results demonstrate dramatically the increase in fluoride contents of the entire humeri and the epiphyseal and diaphyseal regions which occurred during the 4-week period of high-fluoride intake. This is seen by comparing the results of the baseline animals with those of the "0"-day animals which marked the end of the period of high-fluoride intake. The diaphyses, which are largely composed of dense cortical bone with a lower fluoride turnover then epiphyseal bone had, as expected, somewhat lower fluoride increments of fluoride concentration than the epiphyseal portions of the bones.

The tabulated values of relative fluoride concentrations are based on those found in the "0"-day specimen. The loss of fluoride from the humeri and their components during the 21-day period of fluoride restriction reflects in some degree the differences in compactness and access of circulating fluids to the diaphyseal and epiphyseal regions of the bones. However, the decrease in bone fluoride concentrations shown in the columns headed "% F" in Tables I and II during the fluoride depletion period are in a large measure due to dilution of fluoride originally present in the bones by bone formed during this period with a lower fluoride content compared with that present at "0"-day. This is made evident by noting (Table II) the close approximation of the percentage decrease in absolute amount of fluoride in the entire humeri (7.7%) with the percentage grain (10%) in bone mineral mass between Days 0 and 21.

The results in the right hand column of Table I indicate an increment of 12-fold in

TABLE I. FLUORIDE IN BONE ASH (MEAN ± SEM).

Experimental period	Epiphyses		Diaphyses		Whole humerus		
	F (%)	Relative concentration[a]	F (%)	Relative concentration	F (%)	Relative concentration	F (μg)
Baseline:							
High-F Intake	0.047 ± 0.0026		0.032 ± 0.0019		0.038 ± 0.0029		34.3 ± 0.66
Low-F Intake							
Day 0	0.275 ± 0.0206	100	0.217 ± 0.0202	100	0.239 ± 0.0199	100	424 ± 10.2
3	0.273 ± 0.0267	99.3	0.219 ± 0.0201	100.9	0.243 ± 0.0232	101.7	436 ± 9.5
7	0.252 ± 0.0156	91.6	0.210 ± 0.0203	96.8	0.220 ± 0.0209	92.1	410 ± 11.3
14	0.226 ± 0.0204	82.2	0.196 ± 0.0223	90.3	0.207 ± 0.0208	86.6	381 ± 10.2
21	0.220 ± 0.0218	80.6	0.185 ± 0.0172	85.3	0.203 ± 0.0201	84.9	391 ± 9.5

[a] Fluoride content of 1st-day animals set at 100%.

TABLE II. TOTAL FLUORIDE CONTENTS OF PLASMA, MUSCLE, AND LIVER (MEAN ± SEM).

Experimental period	Tissue water (mg F/Kg)			Ratio mg of F/kg of tissue water	
	Plasma	Muscle	Liver	Muscle/plasma	Liver/plasma
Baseline:					
4 weeks high-F intake	0.17 ± 0.007	0.16 ± 0.009	0.23 ± 0.012	0.95 ± 0.061	1.42 ± 0.016
Low-F intake:					
Day 0	0.26 ± 0.013	0.21 ± 0.016	0.23 ± 0.023	0.81 ± 0.073	0.86 ± 0.097
3	0.17 ± 0.006	0.19 ± 0.017	0.22 ± 0.011	1.07 ± 0.093	1.40 ± 0.092
7	0.14 ± 0.006	0.14 ± 0.0012	0.29 ± 0.0012	1.11 ± 0.100	2.39 ± 0.120
14	0.13 ± 0.005	0.18 ± 0.009	0.20 ± 0.009	1.39 ± 0.109	1.60 ± 0.116
21	0.13 ± 0.005	0.07 ± 0.010	0.17 ± 0.016	0.65 ± 0.072	1.38 ± 0.132
Radiofluoride (^{18}F) 220 min				0.38 ± 0.05[a]	0.80 ± 0.03[a]

[a] These results of distribution of $^{18}F^-$ between the waters of muscle and liver and plasma water were obtained in another investigation that has been presented only in a preliminary form (11). Mature rats received injection of carrier-free $^{18}F^-$ in isotonic saline that was labeled with $^{36}Cl^-$. It was demonstrated that the ratios of distribution of $^{36}Cl^-$ between plasma and tissue waters and the specific activities of chloride in these situations reached constant values by 80 min indicating complete mixing of $^{36}Cl^-$ through the chloride space. It is thus assumed that $^{18}F^-$ had also, in this time and at 220 min, reached an equilibrium of distribution throughout the ionic and exchangeable fluoride compartments of plasma and tissues.

the total fluoride content of the entire humeri during the 4-week period of high-fluoride consumption. However, during the fluoride depletion period only 7.7% of the fluoride was mobilized and removed from the humeri. It has been shown that 2.2% of the entire skeletal fluoride is present in a single rat humerus (9). By use of this factor it can be calculated that the total skeletal fluoride of the base-line animals was 15 mg, which increased to 193 mg during the 4 weeks of high-fluoride intake and, of the latter quantity, only 15 mg fluoride was removed from the skeleton during the period of fluoride depletion. In this calculation the undoubtedly small uptake of fluoride from the diet containing 0.21 ppm of fluoride was neglected. These results indicate a high degree of intrabone retention or reincorporation of fluoride in the processes of bone remodeling and growth.

Savchuck and Armstrong (9) in 1951 studied the acquisition and elimination of fluoride by bone, and found that withdrawal of fluoride supplement from both young and mature rats resulted in the elimination of approximately 10 to 15% of the skeletal fluoride in a period of 40 days. Their results with animals the same age as those used in this study indicated that approximately 10% of the skeletal-fluoride load was removed in 20 days, a value not dissimilar to that found in this study, even though dietary fluoride intake was more severely curtailed in the present study. The findings of Likens *et al.*, that fluoride was being mobilized and excreted when the communal water was defluoridated (8 to 1.3 ppm), is not inconsistent with our findings that a decrease of 7.7% in the skeletal load occurred during the depletion period. The fluoride eliminated was transported through the plasma without an apparent increase in the plasma fluoride content. This may have also been the case in the population surveyed by Likens *et al.* Plasma fluoride contents were not measured, however, by these investigators. Also, Spencer, Kramer, Osis, and Wiatrowski found that human subjects, after being withdrawn from a high fluoride intake, excreted excess fluoride for only 6–12 days and accomplished, in this period, the excretion of only 9–14% of the fluoride that had been retained during the high-fluoride intake period.

The results for total fluoride contents of plasma, muscle, and liver in terms of milligrams of F per kg of water are given in Table II. They indicate small increases in fluoride contents of the tissues and plasma to result from 4 weeks feeding of the high-fluoride dietary to animals that had previously received a moderate fluoride intake. This can be seen by comparison of the results obtained with the specimens from the base-line animals with those from the "0"-day animals. During the period of very low fluoride intake, following that of high fluor-

ide consumption, the plasma and tissue fluoride contents returned to initial values in a few days. In the case of plasma and muscle this situation was reached in the 3–7 days of the depletion period and the same was probably true of liver, although the high value obtained at 7 days is anomalous. There is a suggestion, most marked in the instance of muscle, that the fluoride contents of plasma, muscle, and liver, by the end of the depletion period, had decreased to values lower than those obtained for the base-line animals which had received a moderate fluoride dietary. In an earlier paper (1) rats raised on a commercial laboratory diet (60–70 ppm F) had a considerably higher mean plasma and bone fluoride levels than those in this study. When these animals were placed under a variety of stresses, they did not show a significant alteration in the mean total fluoride level, whereas in the present study there was clearly a significant relationship between plasma fluoride levels and fluoride intake. This difference in results may be a reflection of the total amount of fluoride available for mobilization and transfer to plasma by animals with varying skeletal loads even though there is a demonstrated low order of mobilization of fluoride from the skeleton.

In order to allow comparisons of distribution of fluoride between plasma and tissue, the results are also presented in Table II as ratios of milligrams of total F per kg of tissue water to milligrams of total F per kg of plasma water. All of these ratios, with three exceptions, are near to or distinctly above 1.0. The exceptions relate to the "0"-day animals and these low ratios result primarily from the elevated plasma fluoride contents of the animals at the end of the period of high-fluoride intake when the animals had been directly removed from access to the high-fluoride containing water so that intestinal absorption of fluoride was probably still a factor. The other exception is muscle at 21 days of the deletion period when, as already pointed out, muscle total fluoride was considerably lower.

The interpretation of the ratios of distribution of total fluoride between tissue water and plasma water is aided by comparison of these ratios with similar ratios of distribution of radioactive fluoride ($^{18}F^-$), since the latter was present in the ionized state. Results of distribution of $^{18}F^-$ between plasma water and that of muscle and liver waters (11) are also presented in Table II. The markedly lower ratios of $^{18}F^-$ distribution compared with total fluoride distribution, except for "0"-day with liver, indicate that tissues contain a component of bound and un-ionized fluoride which, furthermore, does not exchange completely with ionic fluoride. It can also be inferred that the bound fluoride component of liver is larger than that of muscle. Also, the results with $^{18}F^-$ indicate that the intracellular ionic fluoride concentration of liver is larger than that of muscle or, alternatively, a larger fraction of bound fluoride in liver is exchangeable than in muscle.

Summary The adjustments in total fluoride concentration in plasma, bones, liver, and muscle were examined when rats were given a diet of very low fluoride content following a dietary regimen of elevated fluoride intake. The animals received a diet containing 34 ppm of fluoride and water with 50 ppm added fluoride in the 28-day initial period and in the depletion period they were given a diet containing only 0.21 ppm of fluoride and distilled water. The findings indicated a 12-fold increase in the fluoride content of the humeri after 28 days of high-fluoride intake with a greater increment by the epiphyses than by the diaphyses. During 21 days of the depletion period the skeletal fluoride was reduced by only 7.7% indicating a marked retention of fluoride during processes of bone remodeling and growth. The plasma, muscle, and liver total fluoride contents were significantly increased at the end of the period of high-fluoride intake, but these concentrations were found to be restored to base-line levels in 3–7 days of the depletion period. By comparison of the distribution of total fluoride with injected radiofluoride between tissue and plasma waters, it was concluded that muscle and liver contain bound fluoride that does not exchange completely with ionic fluoride.

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