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BIPHASIC SODIUM FLUORIDE EFFECTS ON BONE AND BONE MINERAL: A REVIEW

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Abstract

This paper reviews the clinical and experimental findings on the effects of sodium fluoride (NaF) on human and animal bone. NaF has been shown to cause a significant increase in axial skeletal bone mass. However, there is concern that the new bone may not provide the desired increase in bone strength. Yet, NaF remains the most commonly used agent capable of stimulating bone formation in most patients (30% non-responders). But whether NaF reduces vertebral fracture rate (VFR) remains controversial. For a given treatment duration, the effect of F on bone quality appears to depend on dose: there is a marked detrimental effect on bone strength at high dose but there tends to be a beneficial effect at low dose. This biphasic NaF effect on bone strength has also been observed in fluoridated rat femurs. Unlike a study on young female rats which shows a linear dependence of cancellous bone volume (Cn-BV/TV) on NaF dose, a short-term study on young male rats, together with studies on chicks and dogs show biphasic NaF effects. Biphasic character is also observed in the effect of NaF on the packing of canine cortical bone mineral. When taken together, the animal models that show biphasic NaF effects seem to suggest that NaF at low dose improves Cn-BV/TV and bone strength and at high dose undermines them. These findings are in agreement with the clinical observations that high NaF dose does not help reduce VFR but low dose seems to help.

Key Words: Osteoporosis, osteopenia, sodium fluoride, biphasic effects, bone fluoride content, bone histomorphometry, bone mineral, bone strength, bone formation, bone resorption.

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Introduction

In senile osteoporosis and in postmenopausal osteoporosis, the amount of bone resorbed is not fully compensated by the amount of bone formed. This dynamic imbalance has to be corrected if we want to treat these types of osteoporosis. The corrective measures taken at present are mainly experimental and involve either stimulating bone formation or inhibiting bone resorption. In normal or osteoporotic bone, the cellular processes for bone formation and bone resorption are coupled (Parfitt, 1988). As a consequence, antiresorptive regimens including estrogen, calcitonin, and bisphosphonates, will prevent further bone loss, but will not build up bone mass after one or two years. The same coupling effect also limits the formation-stimulating regimens from building up bone mass beyond the time when a new dynamic remodeling equilibrium is achieved (Ke *et al.*, 1992). However, in practice, formation-stimulating regimens including sodium fluoride (NaF), parathyroid hormone (PTH), and various growth factors, can build up bone mass more effectively, and for a longer period of time than antiresorptive regimens (Riggs, 1990). In fact, when NaF (Parfitt, 1988) or PTH (Hock *et al.*, 1989) or prostaglandin E₂ (Jee *et al.*, 1994) is the stimulating agent, there seems to be an "unbalanced coupling" in favour of bone formation, with some new bone formation taking place on inactive bone surface without a preceding resorption phase (modeling in the formation mode).

Oral NaF treatment, at 1 mg/kg/d, for postmenopausal osteoporosis has produced mixed results. It has been shown to cause a significant increase in axial skeletal bone mass (Briancon and Meunier, 1981; Harrison *et al.*, 1981; Lane *et al.*, 1984; Eriksen *et al.*, 1985; Riggs *et al.*, 1990; Kleerekoper *et al.*, 1991). However, there is concern that when NaF is given at approximately 1 mg/kg/d, the new bone may not provide the desired increase in bone strength as the new bone may be woven in nature and hyperosteoidosis may be present. At 1-1.4 mg/kg/d, a long-term study has shown that hyperosteoidosis after 7 years is not as severe as after 3 years

(Lundy *et al.*, 1989). At lower dose, it has been reported that lamellar bone without hyperosteoidosis is formed (Mamelle *et al.*, 1988). Another study reports that defective mineralization is significantly correlated to high bone fluoride content (Boivin *et al.*, 1993) and bone fluoride content is affected by several factors including the dose, the bioavailability of the compound used and the duration of treatment (Boivin *et al.*, 1988). Also, it has been suggested that NaF increases cancellous bone mineral density but decreases that of cortical bone, causing the non-vertebral fracture rate of patients to increase (Riggs *et al.*, 1990; Schnitzler *et al.*, 1990b). On the other hand, an intermittent treatment with slow-release NaF (50 mg/day; four 3-month cycles in 20 months), together with continuous vitamin D and calcium therapy, produces new bone with normal material quality (Pak *et al.*, 1989; 1994; Zerwekh *et al.*, 1992). The benefit-to-risk ratio depends on the cumulative dose. A treatment period of two years with low daily dose (50 mg in enteric-coated tablets) with calcium supplement is considered safe (Meunier and Boivin, 1993).

To date, NaF remains the most commonly used agent capable of stimulating bone formation in most patients. However, approximately 30% of patients are non-responders (Hodsman and Drost, 1989). It is the only agent that is effective in reducing vertebral fracture rate (VFR) in patients with the vertebral crush fracture syndrome (Heaney *et al.*, 1989). But whether NaF reduces vertebral fracture rate remains highly controversial. Until such time that NaF can be replaced by a more effective drug, perhaps by antiresorptive agents such as bisphosphonates (Storm *et al.*, 1990; Watts *et al.*, 1990; Reid *et al.*, 1994; Thiebaud *et al.*, 1994), or by another anabolic agent such as PTH (Reeve *et al.*, 1980; 1991), further research on the dosage, drug preparation and duration of treatment should be continued. This paper attempts to review the clinical and experimental findings on the effects of NaF on human and animal bone so as to shed light on the use of NaF to treat osteoporosis in humans.

Effects of NaF on Bone

Effects of NaF on bone quality

Long-term NaF therapy affects bone in several ways. The biology and chemistry of the bone are both affected but to a different extent. What is observed clinically is the combined result of both effects in the form of modified bone quantity and quality. The bone fluoride content varies according to exposure time and dose. While the iliac biopsies of normal subjects contain 0.05 to 0.08% by weight of F, those of NaF treated patients contain 0.24 to 0.67% and those from patients with fluorosis contain 0.56 to 1.33% (Boivin *et al.*, 1988). As

mentioned above, at the therapeutic dose of 1 mg/kg/d, NaF stimulates bone formation. New bone forms on the surface of existing trabeculae but remodelling of the thickened trabeculae is lacking (Aaron *et al.*, 1992). *In vitro* experiments also show that the fluoridated bone is more resistant to osteoclastic resorption (Okuda *et al.*, 1990) and acid dissolution (Grynepas and Cheng, 1988). Small-angle X-ray scattering of fluoridated bone shows the presence of new bone laid down on the surface of preexisting trabeculae (Fratzl *et al.*, 1994). Its mineral structure is characterized by the presence of additional large crystals, presumably located outside the collagen fibrils. These abnormal large crystals contribute to increase the bone mineral density without significantly improving the bone strength (Fratzl *et al.*, 1994). Another backscattered electron imaging study also shows that degree of mineralization increases with NaF treatment (Grynepas *et al.*, 1994). Treatment duration is another parameter in the NaF effect on bone strength. At the therapeutic dose, NaF begins to show an adverse effect on bone strength after one year, and the effect is more serious after five years (Sogaard *et al.*, 1994).

In addition to treatment duration, the effect of F on bone quality appears to depend also on dose: there is a marked detrimental effect on bone strength at high dose but there tends to be a beneficial effect at low dose (Lees and Hanson, 1992). This biphasic NaF effect on bone strength has also been observed in fluoridated rat femurs (Turner *et al.*, 1992). Clinically, it has also been shown that the effect of NaF on VFR is biphasic: VFR decreases when the effective NaF dose is low and then increases when the dose is high (Riggs *et al.*, 1994). The biphasic character of NaF effects is observed also in animal studies and will be discussed again in later sections.

Effects of NaF on bone histomorphometry

The architecture of a bone type is a major determining factor for F effect because, in addition to other pathophysiological factors including the rate of remodeling activity, the bone surface area per unit volume directly controls the fluoridation process. As a consequence, NaF is well known to affect cortical and cancellous bones differently (Cheng and Bader, 1990a; 1992; Riggs *et al.*, 1990; Zerwekh *et al.*, 1992). In a canine study, it has been shown that the cancellous F% and F/Ca increase significantly with NaF dose, whereas the cortical F% and F/Ca do not (Cheng and Bader, 1990a; 1992).

Effects of NaF on cancellous bone

Extensive work has been done on the NaF effect on human cancellous bone. Some deal with patients suffering skeletal fluorosis (Boivin *et al.*, 1989). Many deal with the effects of therapeutic NaF dose (about 1 mg/kg/d). However, most of these studies employed

other drugs such as calcium, vitamin D and phosphorus in addition to NaF (Briancon and Meunier, 1981; Vigorita and Suda, 1983; Eriksen *et al.*, 1985). So, it would be difficult to isolate the effect of NaF alone from the combined results. However, there are several animal studies involving NaF alone, and the animals employed include: pigs (Mosekilde *et al.*, 1987), sheep (Chavassieux *et al.*, 1991a; 1991b), dogs (Snow and Anderson, 1986; Cheng and Bader, 1992), rats (Turner *et al.*, 1989; Cheng and Bader, 1990b; Modrowski *et al.*, 1992; Cheng *et al.*, 1994), mice (Marie and Hott, 1986) and chicks (Lundy *et al.*, 1986). For a complete list of animal models and protocols in studies on fluoride on bone, please see the review by Chavassieux (1990). Table 1 compares some static and dynamic histomorphometric results for both humans and animals receiving non-fluorotic NaF doses. Again, one cannot compare human studies and animal studies directly as the former usually employ other drugs as well as NaF and the latter employ a wide range of NaF doses. In particular, rats are usually given NaF doses much higher than human therapeutic dose. Other factors that differ humans from animals are diet, fluoride metabolism and bone remodeling activity.

Table 1 shows that in all human studies and most animal studies NaF significantly increases cancellous bone volume (Cn-BV/TV). In fact, there are a few human studies which did not report a significant increase (Schnitzler *et al.*, 1990a; Vesterby *et al.*, 1991). However, whether or not the increase in vertebral cancellous bone volume will protect the patients from new spinal fractures is still controversial. Conflicting results have been reported. A Mayo Clinic report suggests that the increased Cn-BV/TV does not help reduce spinal fracture rate (Riggs *et al.*, 1990). But there are other reports which find a reduced spinal fracture rate associated with the increased Cn-BV/TV (Farley *et al.*, 1990; Meunier, 1990). Table 1 also shows that, in most cases, NaF increases the cancellous fractional osteoid volume (Cn-OV/BV) and osteoid surface (Cn-OS/BS). Only in two cases, BV/TV was significantly decreased: one involved 12-month-old rats treated with high dose of NaF (12 mg/kg/d) (Cheng *et al.*, 1994) and the other involved young rats with an even higher dose (equivalent to NaF at 55 mg/kg/d) (Turner *et al.*, 1989). These results suggest that high NaF dose is toxic to bone. Also, only in two studies was OS/BS reported to decrease significantly. It should be noted that one of these two studies involved dogs on a relatively low dose (0.7 mg/kg/d) for only 6 months (Snow and Anderson, 1986) and the other involved rats on high dose (8 mg/kg/d) for 3 months (Cheng and Bader, 1990b). In half of the cases, NaF also stimulates cancellous bone resorption as measured by the fractional erosion surface (ES/BS). Based

on these static morphometric observations, one can safely say that NaF at therapeutic dose stimulates bone formation but also promotes bone resorption, albeit to a lesser extent. In most of the studies listed in Table 1, dynamic histomorphometric parameters were either not measured or non-significantly changed. From the thirteen entries listed in Table 1, there are only three significant changes, an elevated mineral apposition rate in one case and two reduced adjusted apposition rates in two other cases. Hence, no conclusions can be drawn with certainty from the dynamic data.

Effects of NaF on cortical bone

Little is known about the NaF effect on human cortical bone. One study examined transiliac bone biopsies from 10 postmenopausal osteoporotic patients after 6 months and again after 5 years of NaF treatment. The treatment had no effect on the cortical bone thickness but increased the porosity by 50-75% ($p < 0.05$). Also, after 5 years, the treatment increased the fraction of osteons undergoing remodeling, showing some degree of mineralization defect and lengthened remodeling cycles (Kragstrup *et al.*, 1989a). In another study of transiliac biopsies from 29 patients suffering from skeletal fluorosis, both cortical thickness and porosity showed significant increases (38% and 120%, respectively) (Boivin *et al.*, 1989). Non-vertebral fractures, including periarticular, femoral neck and long-bone shaft, have been found to be increased by NaF treatment in some studies (Gutteridge *et al.*, 1984; Hedlund and Gallagher, 1989; Riggs *et al.*, 1990; Schnitzler *et al.*, 1990b). Non-vertebral fractures usually happen later than vertebral fractures (Schnitzler *et al.*, 1990b). However, other studies have not confirmed their findings (Riggs *et al.*, 1987; Mamelle *et al.*, 1988). In any case, there is evidence that cortical bone mineral density may decrease with NaF treatment after two years of treatment (Hodsman and Drost, 1989).

Not much more is known about the effects of NaF on animal cortical bone. F-induced increases in femoral cortical bone porosity (73%, $p < 0.05$) were observed in pigs fed 2 mg/kg/d F for 6 months. Increased osteoid density and fluorochrome label density, and reduced osteon radius, osteon wall thickness and canal radius were also observed (Kragstrup *et al.*, 1989b). Similar findings were observed in the ribs of ovariectomized beagle dams fed 0.7 mg/kg/d NaF for 6 months, except that there was a significant decrease in cortical bone porosity (Snow and Anderson, 1985).

Dose dependence of NaF effects on bone

The therapeutic dose of NaF for continuous treatment of osteoporosis is approximately 1 mg/kg/d. This dose is approximately 10-12 times larger than the equivalent dose of drinking water containing 1 mg/l of NaF.

Table 1. Effects of non-fluorotic NaF dose on static and dynamic histomorphometric results from cancellous bone.

	Briancon and Meunier (1981)	Vigorita and Suda (1983)	Eriksen <i>et al.</i> (1985)	Lundy <i>et al.</i> (1989)	Chavassieux <i>et al.</i> (1991a)	Chavassieux <i>et al.</i> (1991b)	Snow and Anderson (1986)
Animal	Human	Human	Human	Human	Sheep	Sheep	Dog
Dose mg/kg/d	1	1	1	0.6-1.4	1	3.5	0.7
Other drugs	Ca, vitD	Ca, vitD	Ca, P, vitD	Ca	-	-	-
Age	65 years	-	66 years	-	6 years	4 months	4 years
Duration	24 months	18-24 months	60 months	50 months (from 1st. bx)	45 days	120 days	6 months
Target	iliac	iliac	iliac	iliac	iliac	iliac	lumbar
Static parameters							
				(compared to 1st. bx)			
BV/TV	↑	↑	↑	↑	ns	ns	ns
OV/BV	↑	↑	-	ns	ns	↑	↓
OS/BS	↑	↑	↑	ns	↑	↑	↓
ES/BS	ns	↑	↑	-	↑	ns	↑
Tb.Th	-	-	↑	ns	-	-	↑
O.Th	ns	-	↑	ns	↑	↑	ns
Dynamic parameters							
MAR	ns	-	-	ns	ns	ns	ns
Aj.AR	-	-	↓	-	ns	↓	ns

Notes: ↑ = significant increase; ↓ = significant decrease; ns = not significant. BV = bone volume; TV = tissue volume; OV = osteoid volume; BS = bone surface; OS = osteoid surface; ES = eroded surface; Tb.Th = trabecular thickness; O.Th = osteoid thickness; MAR = mineral apposition rate; Aj.AR = adjusted apposition rate.

Recent studies on the effect of fluoridated water on bone show that such low NaF dose has little or no effect on the prevalence of fractures (Kroger *et al.*, 1994; Cauley *et al.*, 1995). At the therapeutic dose, there are side effects in some patients such as nausea, vomiting and osteoarticular pain (Briancon and Meunier, 1981; Riggs *et al.*, 1982). Also, as already mentioned above, non-vertebral fractures have been found to be more frequent in some studies. It is believed that these adverse side effects are dose related (Meunier, 1990). In fact, at high

dose, F is well known for its toxicity (Roholm, 1937). The pharmacology of high dose F-toxicity has been well studied (Caruso *et al.*, 1970). Its toxicity affects many cellular functions in many organs including bone, kidney, heart, liver, gut and others. It promotes some but inhibits many other enzymatic processes. In addition to its bio-organic toxic effects, its bio-inorganic effects on bone and teeth mineralization and demineralization could become excessive and pathologic. It is therefore highly desirable to have NaF dose as low as possible.

Biphasic effects of sodium fluoride on bone

Table 1 (continued).

Cheng and Bader (1990a, 1992)	Mosekilde <i>et al.</i> (1987)	Turner <i>et al.</i> (1989)	Cheng and Bader (1990b)	Modrowski <i>et al.</i> (1992)	Cheng <i>et al.</i> (1994)	Marie and Hott (1986)	
Dog	Pig	Rat	Rat	Rat	Rat	Mouse	Animal
1	4.4	17 55	8	1	12	0.8	Dose mg/kg/d
-	-	-	-	-	-	-	Other drugs
6 years	8 months	75 weeks	6 weeks	3 months	1 year	21 days	Age
9 months	6 months	21 days	3 months	1-6 months	4 months	1 month	Duration
femur	lumbar	tibia	femur	tibia	femur	cauda	Target
Static parameters							
↑	↑	↑ ↓	↑	↑	↓	↑	BV/TV
↑	↑	ns	ns	-	ns	-	OV/BV
↑	-	ns	↓	↑	ns	↑	OS/BS
↑	-	ns	ns	-	ns	-	ES/BS
↓	-	ns	ns	-	ns	-	Tb.Th
ns	-	-	ns	-	ns	↑	O.Th
Dynamic parameters							
↑	-	-	-	ns	-	ns	MAR
ns	-	-	-	-	-	-	Aj.AR

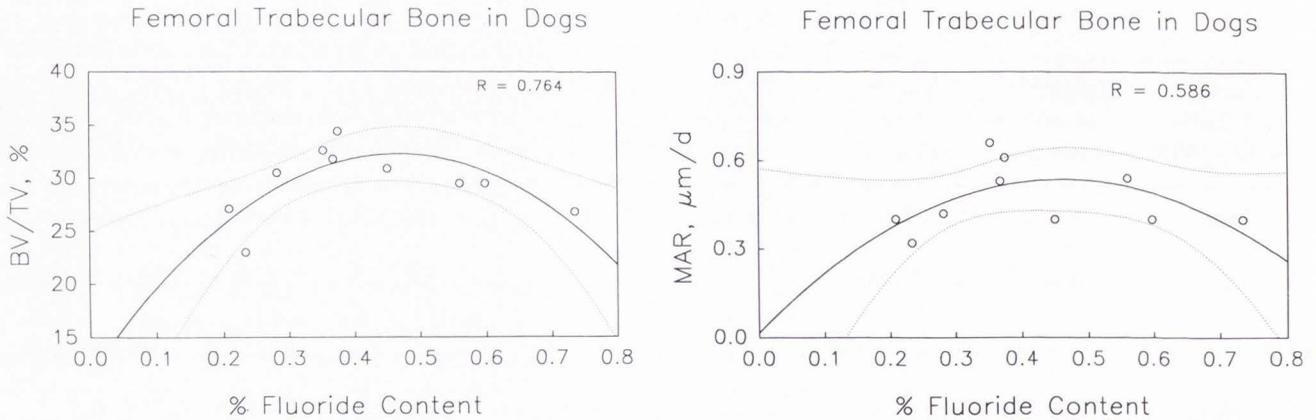
However, the dose dependence of F effects on bone has not been fully investigated. For humans, there is only one dose-response curve published, showing percent change in bone mass per year as a function of NaF dose (0 to 80 mg/day) (Kleerekoper and Balena, 1991). At 80 mg/day, the effect of NaF begins to plateau. Table 1 shows that in rats high NaF dose does decrease Cn-BV/TV (Turner *et al.*, 1989; Cheng *et al.*, 1994). Perhaps for humans, it will take a dose > 80 mg/day to show adverse effects on bone mass.

There are only six animal studies reported so far that studied the dose dependence of NaF.

Biphasic NaF effect on chick bone: Chicks were employed in the first study. A biphasic NaF effect on

Cn-OS/BS was observed when 14-day-old chicks were fed NaF solutions (0-8.4 mM) for 14 days, with OS/BS first increasing with NaF dose peaking at 5 mM and then decreasing with higher NaF doses (Lundy *et al.*, 1986).

Short-term biphasic F effect on young male rat bone: In the second study, young male rats (140 g) were fed solutions containing 2.0 mM or 4.5 mM F *ad libitum* for 21 days. Fluoride intake was 56 or 183 μmol/day (equivalent to 17 or 55 mg/kg/d NaF). Tibial metaphyseal Cn-BV/TV was significantly increased at 2.0 mM F, but was significantly decreased at 4.5 mM. With these two doses and the short treatment duration, osteoblastic and osteoclastic surfaces were not significantly affected (Turner *et al.*, 1989).



Figures 1 and 2. Analysis of canine proximal femoral cancellous bone volume (**Fig. 1 at left**) and cancellous mineral apposition rate (**Fig. 2 at right**) according to bone fluoride content.

Long-term linear NaF effect on young female rat bone: In the third study, young female Wistar rats were fed NaF solutions (0-6 mM) with different concentrations *ad libitum* for three months. The equivalent NaF dose employed ranged from 0 to 25 mg/kg/d. Although femoral metaphyseal Cn-BV/TV was shown to increase with dose, both Cn-OS/BS and Cn-ES/BS were shown to decrease with dose suggesting NaF toxicity, at least at the highest dose. Despite reduced bone formation activity, a positive bone balance was achieved at all doses and was attributed to more severely reduced bone resorption activity (Cheng and Bader, 1990b). However, when aged OVX rats (12 months old) were treated with 10-12 mg/kg/d of NaF for four months, not only was Cn-BV/TV not increased, but actually decreased when compared to untreated OVX rats. This adverse NaF effect on rat bone is probably age-related as this dose range is slightly toxic only to old rats, as evidenced by loss of body weight, but not to young rats (Cheng *et al.*, 1994). In the young rats, NaF builds up bone by inhibiting bone resorption more than bone formation (Cheng and Bader, 1990b); but in the old rats, NaF loses bone by promoting bone resorption more than bone formation (Cheng *et al.*, 1994).

Possible biphasic NaF effect on adult rat bone: In the fourth study, 3-month old female rats were treated with drinking water containing either 5.3 or 7.9 mM NaF for 90 days. Although the lumbar vertebral body ash weight was significantly increased by each dose, the vertebral trabecular bone volume was increased significantly only by the lower dose, suggesting possibly NaF had a biphasic effect in this model. Results from biomechanical testing on the vertebral bodies showed that NaF at these doses did not affect bone strength signifi-

cantly but decreased the bone quality significantly (Sogaard *et al.*, 1995).

Biphasic NaF effect on canine bone: We studied the dose dependence of NaF effects on bone in dogs. Ovariectomized beagle dams (6-7 year old) were fed NaF powders in pellets at four levels (0, 1, 3, 5 mg/kg/d) for nine months. Static and dynamic histomorphometric results as observed in ribs and femurs showed that NaF had a biphasic effect on most bone parameters (Cheng and Bader, 1990a; 1992). Unfortunately, there were only two dogs at each NaF level. In order to have a meaningful analysis of the data, we have reanalyzed the data as functions of bone F content. Figure 1 shows that the effect of NaF on proximal femoral metaphyseal Cn-BV/TV is biphasic ($R = 0.764$) with a maximum at 0.45% bone F content, which is equivalent to a NaF dose of approximately 2 mg/kg/d. Femoral cancellous mineral apposition rate (Cn-MAR) behaves similarly (Figure 2). However, Cn-OV/BV or Cn-OS/BS, which was significantly elevated by NaF at all doses, did not show a biphasic pattern. The significant hyperosteoridosis may be indicative of impaired bone mineralization.

Lack of favorable NaF effects on ewe bone: In a short-term (45 days) study on 6-year-old ewes, NaF at 1 or 5 mg/kg/d significantly decreased serum calcium and phosphorus, and non-significantly decreased iliac cancellous bone area. Both osteoid surface and eroded surface were increased significantly. Dynamic parameters showed that single and double labeled surfaces and adjusted apposition rate were non-significantly decreased while the mineralization lag time was significantly increased (Chavassieux *et al.*, 1991a).

FLUORIDE DOG MINERALIZATION PROFILE (Femoral bone)

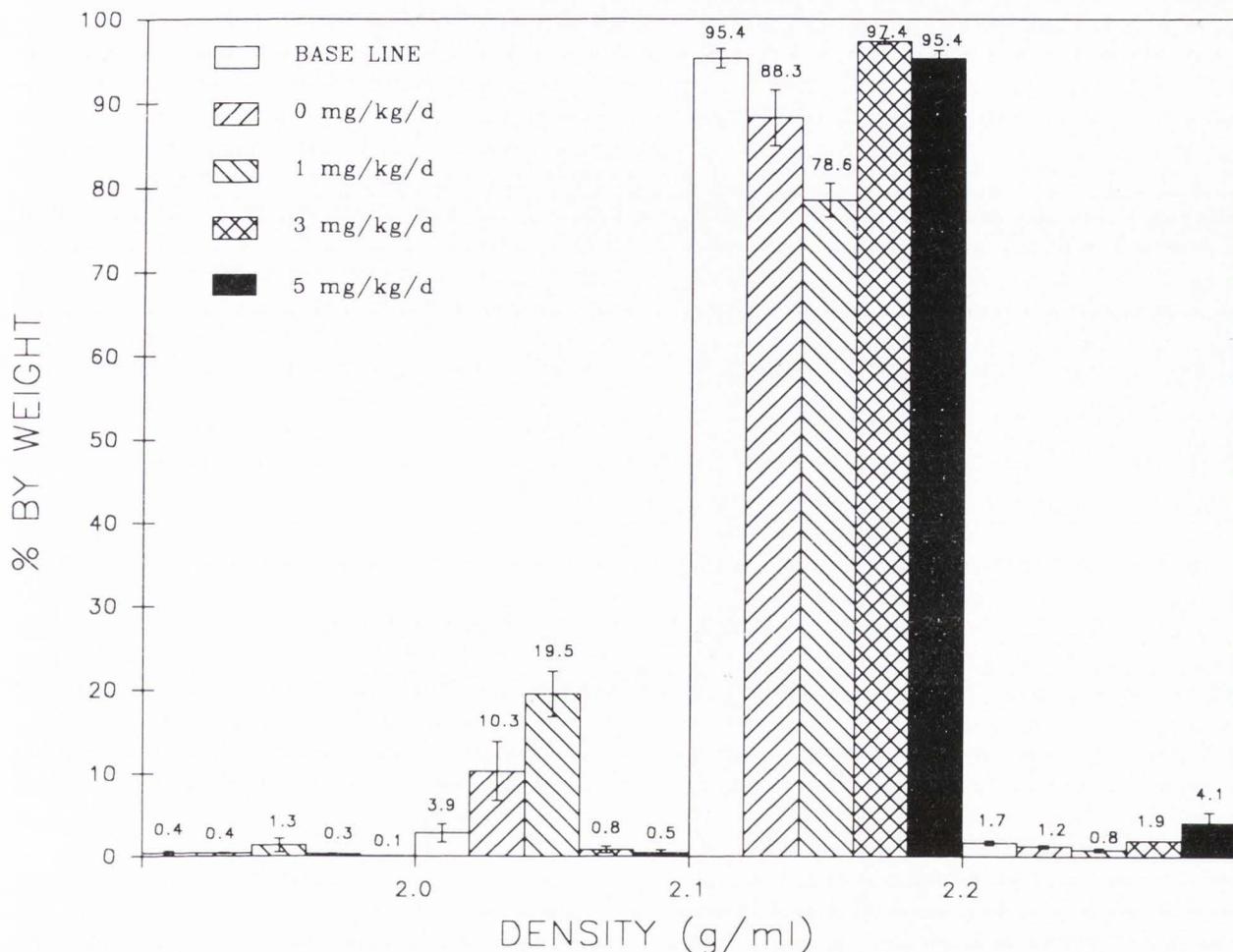


Figure 3. Effect of NaF on canine femoral cortical bone mineralization profile as determined by density fractionation. Dogs were treated with NaF for nine months. Baseline animals were killed at the beginning of the experiment.

Effects of NaF on bone mineral

Normal bone mineral is poorly crystalline hydroxyapatite [HAP, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$], with dimensions ranging from 10 to 30 nm in length and 5 to 10 nm in thickness. Besides being very small and strained, the crystallites contain many impurities such as CO_3 , Mg, Na, etc., some of which have been reported to vary with fluoride content. Fluoride substitutes for the hydroxyl ion in apatite to form the thermodynamically more stable, less soluble, albeit still poorly crystalline, fluorhydroxyapatite [FHAP, $\text{Ca}_{10}(\text{PO}_4)_6\text{F}_x(\text{OH})_{2-x}$] (Eanes and Reddi 1979; Neuman and Neuman 1958). F^- ions do not seem to diffuse into preformed HAP crystallites which are not near the bone surface, but are incorporated into FHAP crystallites during new bone minerali-

zation (Grynpsas, 1990). FHAP and HAP crystallites have very similar dimensions, except that FHAP may have slightly larger cross-sectional areas (Posner *et al.*, 1963; Grynpsas *et al.*, 1986). No change in Ca, P, or Ca/P molar ratio have been reported for fluoridated bone minerals, but significant changes in Mg, CO_3 , Na, citrate and ash weight have been reported (Zipkin *et al.*, 1960). Since bone quality and bone strength depend on the bone micro-architecture at the bone mineral level, the effects of fluoride on the physicochemical properties of FHAP crystallites are as important as the effects on bone biology. Firstly, the manner in which the minerals are formed in the bone matrix, e.g., packing density, will affect the bone strength. Secondly, the manner in which F is incorporated into bone mineral crystallites may depend on the bone microarchitecture.

The following two sections seek to relate changes in fluoridated bone mineral properties to the biphasic effects of NaF on bone as well as to the differential NaF effects on cortical and cancellous bones.

Effects of NaF on bone mineral packing density and surface area

Although FHAP and HAP crystallites have similar dimensions, bone mineral crystallites are packed closer together in fluoridated rat bone. When finely ground rat bone powders were fractionated according to their densities, the percent by weight of powders having a density greater than 2.1 g/ml was always significantly higher in fluoridated bone than in control. The F content was also highest in the same density fraction, indicating that fluoride increased bone mineral packing density (Grynpas *et al.*, 1986). This finding is supported by the observation that fluoride reduces bone mineral aggregate surface area. When rat bones were deproteinized and their bone mineral aggregate surface areas were studied by nitrogen adsorptiometry, fluoridated bone showed significantly lower aggregate surface area per gram of bone than control (Cheng and Bader, 1990b). However, this finding in rats has not been confirmed in dogs. When canine femoral cortical bone powders were analyzed by density fractionation, the results showed that there was a biphasic NaF effect on bone mineral packing. As can be seen in Figure 3, which shows the mineralization profiles of untreated and treated canine cortical bones (at four dose levels), in the density fraction between 2.0 and 2.1 g/ml, there is a peak at the dose of 1 mg/kg/d while in the density fraction between 2.1 and 2.2 g/ml, there is a trough at the same dose. These two density fractions are the more important and sensitive fractions of the four shown in the figure. Together, they show that at low dose, NaF promotes hypomineralization (favoring lower density fractions) and at high dose, it promotes hypermineralization (favoring higher density fractions). Since increased mineral packing density could lead to a more brittle bone, especially for cortical bone, this adverse NaF effect at high dose could be considered negative for bone quality.

Fluoride-lattice interactions in bone mineral

Another interesting physical phenomenon of fluoridated bone is the fluoride-lattice interactions in bone mineral crystallites. This can be measured by ^{19}F nuclear magnetic resonance (NMR) in terms of the spin-lattice relaxation time (T_1). As with neutron activation analysis, ^{19}F NMR can be used to measure the bone F content non-invasively (Code *et al.*, 1990b). We have studied ^{19}F nuclear spin-lattice relaxation rates in cortical

and cancellous bones from rats and dogs and have found that ^{19}F NMR results are species independent, but are dependent on the magnetic field strength and the bone fluoride concentration. The results suggest that there are at least two chemically inequivalent F incorporation sites in bone tissue, possibly a surface site and a bulk site (Code *et al.*, 1990a). In agreement with neutron activation analysis results, ^{19}F NMR shows that cortical bone always takes up significantly less F than cancellous bone. More important, we have observed that for a given bone F concentration, cortical and cancellous bones do not have the same T_1 values such that cortical T_1 is significantly shorter than cancellous T_1 , suggesting a stronger fluoride-lattice interaction in cortical bone mineral crystallites. For each bone type, $1/T_1$ is linearly dependent on bone F content, i.e., the higher the bone F content, the shorter the T_1 , but the regression coefficients are different for cortical and cancellous bones. This also indicates that the local environments of fluoride in cortical bone and in cancellous bone are not the same. The longer T_1 in cancellous bone is not related to the higher organic matrix content. It is probably related to the fact that cancellous bone has a higher percentage of F incorporating surface sites than cortical bone (Code *et al.*, 1992). This hypothesis can also explain the shorter T_1 values for more fluoridated bones as bone F depresses the bone mineral aggregate surface area (Cheng and Bader, 1990b).

Conclusions

Unlike the 3-month study on young female rats which shows a linear dependence of Cn-BV/TV on NaF dose, the 21-day study on young male rats and the studies on chicks and dogs show biphasic NaF effects on Cn-OS/BS in chicks and on Cn-BV/TV in dogs and in young male rats. Biphasic character is also observed in the effect of NaF on the packing of canine cortical bone mineral. When taken together, the animal models that show biphasic NaF effects seem to suggest that NaF at low dose improves Cn-BV/TV and at high dose undermines it. The results on bone strength and bone quality are more difficult to assess. At high NaF dose, bone strength suffers. But no clear verdict can be arrived at present for the effect on bone strength when low NaF dose is employed. In humans, high NaF dose does not help reduce VFR but low dose seems to help. Low NaF dose (50 mg/d) given in enteric-coated tablets with calcium supplement for 2 years is considered safe (Meunier and Boivin, 1993). But, even at therapeutic dose, long-term (5 years) NaF treatment may be detrimental to bone strength and quality, as test results on trabecular bone from iliac biopsies have shown (Sogaard *et al.*, 1994).

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Discussion with Reviewers

L. Mosekilde: How do the authors define bone strength? The authors have referred to several papers where indirect measurement of bone strength has been performed (e.g., "sonic velocity"); on the other hand, several papers where direct measurements of biomechanical strength of fluoride treated bone have been made have been omitted or mentioned only very superficially by the authors.

Authors: Ultimately, bone strength is measured by biomechanical testing. Indirect measurements should be of value too, and hence should not be overlooked.

L. Mosekilde: Do the authors consider that high-dose or long-term treatment has a negative effect on "bone biology", and on "bone chemistry"?

Authors: High-dose is definitely the ultimate culprit. In fluorosis, both bone biology and bone chemistry are abnormal. As for therapeutic doses, long-term treatment also has adverse effects on bone strength which we believe are caused by a change in bone "physical chemistry".

L. Mosekilde: What is the relationship in time between effect on "bone biology" and "bone biochemistry"?

Authors: If NaF dose is high enough to affect bone cell biology, it will not take long to affect bone biochemistry, e.g., over production of collagen matrix. If the dose is too low to affect bone cell biology significantly, only the cumulative F effects on physical chemistry (crystallography) and material science of bone mineral will be observed after prolonged exposure.

W.S.S. Jee: Does NaF have any anabolic effects on periosteal and endocortical surfaces like PTH and PGE₂?

Authors: To our knowledge, no rat data on cortical bone is available. NaF has anabolic effects on endocortical surfaces. We are not sure whether it is also true for periosteal surface.

P. Chavassieux: For a better understanding, it is essential to distinguish the effects of fluoride on bone cells and on bone mineral, both contributing to the quality of bone. Concerning the first one, it is now well estab-

lished that fluoride induces an osteoblastic proliferation probably through the osteoblast precursors. Besides this stimulatory effect, fluoride may decrease the osteoblast activity at the individual cell level. The amplitude of these effects depends on the total amount of fluoride ingested and may explain the different results in the literature. It will be important to understand the effects of fluoride in bone cells to completely understand fluoride effects on bone.

Authors: The effects of fluoride depends on two things: dose and treatment duration. For a given dose, the treatment duration counts; and for a given duration, the dose counts. The effect of fluoride on bone cells is beyond the scope of this review. We do not consider ourselves experienced enough in this area to draw conclusions from the conflicting results published.

P. Chavassieux: Concerning the effects of fluoride on bone mineral, all data reported in this paper concern only animal studies. Is there any human study after fluoride treatment or in cases of fluorosis?

Authors: Only some early human studies mentioned effects of fluoride on bone mineral, but mostly on their chemical composition.