

# Effects of Acute Sodium Fluoride Exposure on Kidney Function, Water Homeostasis, and Renal Handling of Calcium and Inorganic Phosphate

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**Abstract** Fluoride compounds are abundant and widely distributed in the environment at a variety of concentrations. Further, fluoride induces toxic effects in target organs such as the liver and kidney. In this study, we performed an early analysis of renal function using a clearance technique in Wistar rats acutely exposed to fluoride at a plasma concentration of 0.625  $\mu\text{g}/\text{ml}$ . Our results revealed that fluoride, at a concentration close to the concentration present in the serum after environmental exposure, induced a significant tubular dysfunction, resulting in diluted urine, impaired protein reabsorption, and increased calcium and phosphate urinary excretion. Our work demonstrates that even acute exposures to low concentrations of NaF may induce renal damage and confirms that, after exposure, the kidney participates directly in the calcium and phosphate deficiencies observed in fluoride-exposed populations.

**Keywords** Fluoride · Kidney · Acute exposure · Calcium · Phosphate · Proteinuria · Diuresis

## Introduction

Fluorine is a highly electronegative and reactive element and therefore never naturally occurs in a free state. Fluoride compounds are abundant and widely distributed in the environment at a variety of concentrations. The main source of fluoride exposure is through the intake of ground-water contaminated with the inorganic form of this mineral (reaching concentrations of 30–50 mg/l), mainly from geological sources. Currently, there is no consensus about the role of fluoride as an essential element. However, low doses of fluoride prevent dental caries, thus justifying water fluoridation programs (at 1.0 mg/l) and topical applications through toothpastes and gels. The negative effects associated with fluoride exposure include dental fluorosis in populations chronically exposed to relatively low doses of inorganic fluoride in potable water (1.0–2.0 mg/l) and skeletal fluorosis upon exposure to higher levels; these effects are related to an alteration in calcium homeostasis [1]. Further, fluoride induces toxic effects in other target organs such as the liver and kidney. It is known that approximately 50–80 % of the fluoride absorbed is excreted through the urine, primarily by glomerular filtration. The results of some previous studies have suggested that environmental or occupational fluoride exposure is associated with renal damage [2]. However, it is known that renal toxicity could be a consequence of acute or chronic fluoride exposure in both animals and humans [3]. Subsequent studies have highlighted the presence of fluoride in drinking water as a possible contributor to the etiology of chronic kidney disease [4]. In the nephron, the main target of fluoride is the proximal tubule epithelial cell [5, 6]. Recently, two proximal tubule-

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specific biomarkers of nephrotoxicity, *N*-acetyl- $\beta$ -glucosaminidase and  $\gamma$ -glutamyl transpeptidase, have been reported to be increased in children chronically exposed to over 2.0 mg/L of fluoride in the drinking water. The serum fluoride levels in this population were approximately 0.25 mg/L [7]. In experimental models, chronic fluoride administration induces hypocalcaemia, and it has been suggested that these effects are associated with decreases in renal tubular transport and a reduction in intestinal calcium absorption [8, 9].

Fluoride renal toxicity has been clearly demonstrated in experimental models and industry-related exposures to high doses of this mineral, mainly in the form of NaF [10]. Interestingly, the role of low doses of fluoride in renal toxicity remains unclear. It has been suggested that fluoride does not act independently in the renal toxicity observed at low doses. Mixtures with other salts may play an important role due to the increased sodium/calcium ratio associated with high fluoride levels and their relationship to some renal implications [11]. Epidemiological and environmental studies have provided some evidence establishing the relationship between fluoride exposure and renal damage. However, the effects of acute fluoride exposure at low doses on renal function have not been fully elucidated.

In the present study, we evaluated the early effects of a single low dose of sodium fluoride (close to the concentration present in the serum after environmental exposure) on renal function, calcium and phosphate handling, and renal water management.

## Material and Methods

### Animals

Female Wistar rats (198.8 $\pm$ 2.63 g body weight) were housed with 12/12-h light/dark cycles at 22 $\pm$ 1 °C and 50 $\pm$ 5 % humidity. They received standard chow (PMI, 5008, Purina, Alief City, TX, USA) and purified water ad libitum. The use of animals was in accordance with the ILAR Guide for Care and Use of Laboratory Animals and was approved by the Institutional Committee of Ethics of Cinvestav, CICUAL (Comité Interno para el Cuidado y Uso de los Animales de Laboratorio; number 391-07).

### Surgery

Animals had free access to water until the start of the experiment and were starved for 18 h prior to the surgical procedure. Anesthesia was induced by injection of pentobarbital sodium (Nembutal, 5 mg/100 g bw, i.p.) and maintained by additional 1-mg doses administered when necessary to ensure muscular relaxation and the absence of

pedal and corneal reflexes. The animals were then placed on a heated table to maintain the body temperature between 37 and 39 °C. The temperature was monitored using a rectal microprobe thermometer (BAT-10, World Precision Instruments). A tracheotomy was performed, leaving the thyroid gland untouched. One catheter (PE-20) was inserted into the right jugular vein for the perfusion of experimental solutions, and another catheter (PE-10) was inserted into the left ureter for urine collection. For clearance experiments, a third catheter (PE-50) was inserted into the right femoral artery for sampling blood and recording the arterial blood pressure (Research BP Transducer, Harvard Apparatus). The surgery duration was 15 min and a surgical recovery time of 30 min was allowed before performing the clearance experiments.

### Clearance Experiments

The clearance experiments were performed to analyze the effects of an acute NaF load on whole kidney function. Clearance experiments were carried out in rats intravenously infused with a 0.9 % NaCl solution at a rate of 20  $\mu$ l/min. Creatinine clearance was used to estimate the glomerular filtration rate (GFR). Urine samples were serially collected every 20 min and three urine samples were collected from each animal during the control period. Blood samples were taken halfway through each urine collection and three blood samples were collected from each animal during the control period. Urine collection began 1 h after the end of the surgery. After three control clearance periods, a priming dose of NaF (250  $\mu$ l of 100 mg/L NaF solution diluted in 0.9 % NaCl) was initially added, followed by a continuous infusion of 0.315 mg/L NaF solution diluted in 0.9 % NaCl at a rate of 20  $\mu$ l/min to maintain a plasma fluoride concentration near 0.625  $\mu$ g/mL. After a 40-min equilibration period, urine was collected during three additional 10-min clearance periods (three urine samples were collected per animal during the fluoride exposure). The respective blood samples were taken halfway through each urine collection (three blood samples were collected per animal during the fluoride exposure). At the end of the experiment, rats were sacrificed by an overdose of Nembutal, and the kidneys were removed to determine the weights and calculate the renal parameters (1.42 $\pm$ 0.05 g kidney weight). Between the blood collections, the arterial catheter was connected to a pressure transducer to collect arterial blood pressure measurements.

### Analytical Procedures

The creatinine (cr), phosphate (PO<sub>4</sub><sup>2-</sup>), and calcium (Ca<sup>2+</sup>) concentrations, as well as the osmolality and proteinuria were determined into plasma and urine samples. Creatinine

levels in the serum and urine were measured by the Jaffe colorimetric method, calcium was measured by the O-cresolphthalein complexone colorimetric method and phosphate was measured by the Chen colorimetric method using the Randox laboratories colorimetric kits CR510, CA590 and PH7965, respectively, and a microplate reader (Infinite 200, Tecan). The total urine protein concentration was measured by the Bradford colorimetric method (Bio-Rad Protein Assay Kit II).

The functional parameters were calculated using conventional equations as follows:

$$\text{GFR} = C_{\text{cr}} = (U_{\text{cr}}/P_{\text{cr}}) \times J_v$$

where GFR is the glomerular filtration rate,  $C_{\text{cr}}$  is the clearance value of creatinine (in millimole per minute/100 g bw),  $U_{\text{cr}}$  and  $P_{\text{cr}}$  are the concentration values of creatinine in the urine and plasma, respectively (in millimole per liter), and  $J_v$  is the urinary flow rate (in microliter per minute per 100 g bw).

$$\begin{aligned} C_{\text{Ca}} &= (U_{\text{Ca}}/P_{\text{Ca}}) \times J_v & \text{and} & & \text{FeCa} &= (C_{\text{Ca}}/C_{\text{cr}}) \times 100 \\ \text{FaCa} &= P_{\text{Ca}} \times C_{\text{cr}} & \text{and} & & \text{EaCa} &= U_{\text{Ca}} \times J_v \end{aligned}$$

where  $C_{\text{Ca}}$  is the calcium clearance (in milliliter per minute),  $U_{\text{Ca}}$  and  $P_{\text{Ca}}$  are the concentration values of calcium in the urine and plasma, respectively (in millimole per liter),  $J_v$  is the urinary flow rate (in microliter per minute per 100 g bw), FeCa is the calcium fractional excretion (in percent), FaCa is the calcium filtrated amount (in micromole per minute per 100 g bw), and EaCa is the calcium excreted amount (in micromole per minute per 100 g bw).

The same procedures were used to calculate the fractional excretion, filtrated amount, and excreted amount of phosphate. Osmolality was measured in the serum and urine by a vapor pressure method (Wescor 5500, Portland, OR, USA). Osmolar and free-water clearances were calculated using the following conventional equations:

$$\begin{aligned} C_{\text{osm}} &= (U_{\text{osm}}/P_{\text{osm}}) \times J_v \\ C_{\text{H}_2\text{O}} &= J_v - C_{\text{osm}} \end{aligned}$$

where  $C_{\text{osm}}$  is the osmolar clearance (in microliter per minute per 100 g bw),  $U_{\text{osm}}$  and  $P_{\text{osm}}$  are the osmolality values in the urine and plasma, respectively (in milliosmole per liter),  $J_v$  is the urinary flow rate (in microliter per minute per 100 g bw), and  $C_{\text{H}_2\text{O}}$  is the water clearance (in microliter per minute per 100 g bw).

### Statistical Analyses

Unpaired Student's *t* tests were used to compare quantitative variables between the control and experimental groups. The data are expressed as mean  $\pm$  SE. Differences were considered significant for *p* values of  $<0.05$  (SigmaPlot/SigmaStat software).

## Results

### Arterial Blood Pressure

Arterial pressure measurements obtained during the control and fluoride treatment periods were not different, indicating that fluoride did not modify the hemodynamic parameters in our experimental model (Table 1).

### The Effect of Fluoride on the Urinary Flow Rate and Creatinine Clearance

To determine whether fluoride altered general renal function, we measured the  $J_v$  and estimated the GFR by determining creatinine clearance (Table 1) before and after acute exposure to fluoride in the same animals. Exposure to the halogenated element significantly increased the  $J_v$ , demonstrating an important diuretic effect. In addition, the GFR remained unchanged, indicating that fluoride did not alter glomerular function.

### The Effect of Fluoride on Osmolar and Free Water Clearances

The  $C_{\text{osm}}$  values obtained during the control and fluoride treatment periods are shown in Table 1. Fluoride induced a significant threefold increase in the  $C_{\text{osm}}$ . The free-water clearance was significantly decreased, indicating that the renal reabsorption of water increased in the presence of fluoride.

### The Effect of Fluoride on Proteinuria

The total protein concentration decreased in the presence of fluoride, most likely due to an increase of  $J_v$  and the general dilution of the urine. The proteinuria, corrected by  $J_v$ , showed a significant increase, indicating that a protein waste was elicited by fluoride treatment (Table 1).

### The Effect of Fluoride on the Renal Handling of Calcium

Figure 1 shows the principal parameters used to characterize the renal handling of calcium. The urinary concentration of calcium increased (Fig. 1a) during fluoride treatment whereas the plasma calcium (Fig. 1b,  $P_{\text{Ca}}$ ) decreased. This change in  $P_{\text{Ca}}$  did not modify the amount of filtered calcium (Fig. 1d). However, the fractional excretion (Fig. 1c) and excreted amount (Fig. 1e) of calcium significantly increased, indicating diminished calcium reabsorption in the presence of fluoride.

### The Effect of Fluoride on the Renal Handling of Inorganic Phosphate

Data describing the renal handling of inorganic phosphate are shown in Fig. 2. Contrary to  $P_{\text{Ca}}$ , the plasma concentration of

**Table 1** Changes in renal function parameters and renal handling of calcium and inorganic phosphate elicited by acute exposure to fluoride (values are means±SEM)

Parameters	Control group (n=5)	Fluoride group (n=5)
Urinary flow rate (μl/min/100 g)	1.17±0.07	10.68±2.02*
Glomerular filtration rate (μl/min/100 g)	360.44±28.54	386.62±25.78
Osmolar clearance (μl/min/100 g)	6.12±0.26	19.43±2.59*
Free water clearance (μl/min/100 g)	-4.94±0.26	-8.75±0.81*
Proteinuria (μg/min/100 g)	1.93±0.20	6.94±1.29**
Arterial blood pressure (mmHg)	97.00±1.73	101.80±3.87
Calcium handling		
Filtrated amount (μmol/min/100 g)	0.52±0.04	0.54±0.04
Excreted amount (nmol/min/100 g)	0.20±0.01	4.50±1.10**
Fractional excretion (%)	0.05±0.01	0.90±0.25***
Inorganic phosphate handling		
Filtrated amount (μmol/min/100 g)	0.65±0.04	0.83±0.06***
Excreted amount (nmol/min/100 g)	9.00±1.00	34.70±3.60*
Fractional excretion (%)	1.43±0.10	43.66±5.39*

\* $p < 0.0001$ , \*\* $p < 0.001$ , and \*\*\* $p < 0.01$  compared with the control (Student's *t* test)

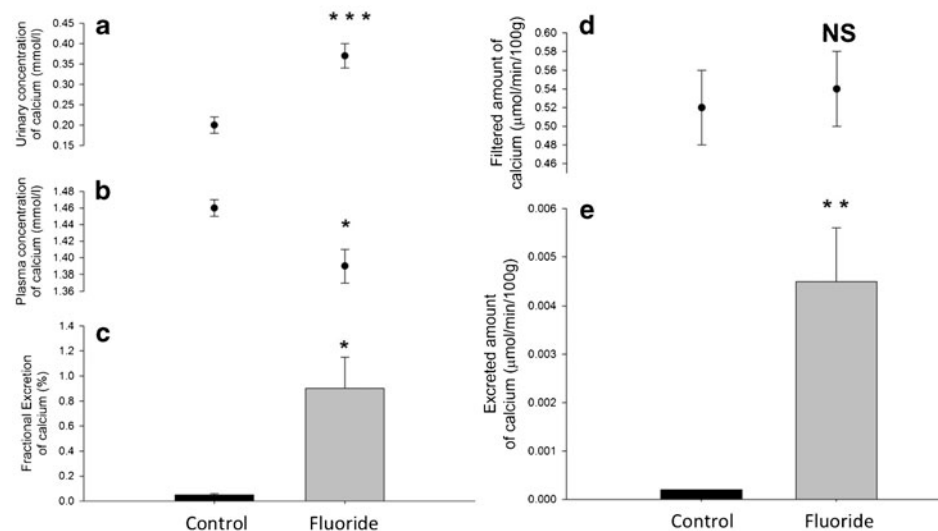
inorganic phosphate increased (Fig. 2b) with fluoride exposure, and consequently, the filtered amount also increased significantly. Further, the urinary concentration (Fig. 2a), excreted amount (Fig. 2e) and fractional excretion (Fig. 2c) of phosphate showed significant increases that were elicited by fluoride. These changes suggested that fluoride not only modified the body content/distribution but also decreased the renal reabsorption of inorganic phosphate.

## Discussion

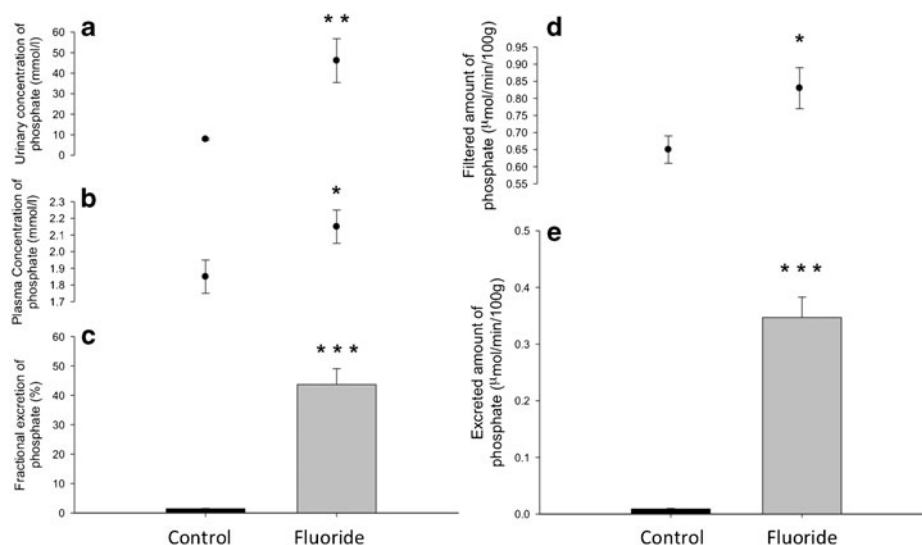
In an attempt to better understand the role of fluoride in the physiopathology of kidney disease, renal function was evaluated after intravenous administration of an acute load of sodium fluoride. This was calculated to reach plasma concentrations close to those reported in environmentally

exposed populations. The GFR was estimated by the endogenous creatinine clearance. To avoid errors introduced by the tubular secretion of creatinine, only female animals, in which no secretion occurs, were used [12]. There have been controversial results regarding the effects of fluoride on the GFR, possibly because there is a dramatic decrease at high doses (8.57 mg/kg), whereas the GFR is apparently unaffected at low doses [3]. However, the histopathology of the kidneys of rats exposed both acutely [13] and chronically to relatively high fluoride doses revealed vascular congestion in the glomerular capillaries [14]. Similar to previous reports, creatinine clearance was unaffected. Further, our results showed that fluoride administration induced a critical increase in the urinary flow rate, similar to results reported by other authors [6, 15]. It has been suggested that the increase in urinary volume is not caused by glomerular alterations but rather by the inhibition of salt and water

**Fig. 1** The effects of fluoride on the physiological parameters describing the plasma, urine, and renal handling of calcium. Fluoride significantly decreased the plasma concentration of calcium (a) but increased the urine concentration (b) and fractional excretion (c). The excreted amount also increased (e), whereas the filtered amount remained unchanged (d). The mean±SEM is shown. \* $p < 0.01$ , \*\* $p < 0.001$ , \*\*\* $p < 0.0001$  (Student's *t* test,  $n = 5$  for each experimental condition)



**Fig. 2** The effects of fluoride on the physiological parameters describing the plasma, urine and renal handling of inorganic phosphate. Fluoride significantly increased the plasma (a) and urine concentrations (b) and the fractional excretion (c) of inorganic phosphate. The filtered (d) and excreted amounts (e) also increased. The mean  $\pm$  SEM is shown. \* $p < 0.01$ , \*\* $p < 0.001$ , \*\*\* $p < 0.0001$  (Student's *t* test,  $n = 5$  for each experimental condition)



reabsorption in the proximal tubule [16] and the thick ascending limb of the Henle loop [17]. Therefore, the polyuria induced by fluoride could be attributed to the impairment of tubular water management.

Tubular dysfunction induced by fluoride administration was confirmed by the substantial increase in the elimination of isotonic urine, which was evident from the increase in osmolar clearance (from 6.12 to 19.43  $\mu\text{l}/\text{min}/100\text{ g}$ ). Consequently, the elimination of solute-free urine was also significantly diminished (free water clearance from  $-4.94$  to  $-8.75\ \mu\text{l}/\text{min}/100\text{ g}$ ). The osmolar clearance and free water clearance are highly dependent on sodium management in the Henle loop and the distal and collector tubule segments. It has been suggested that fluoride could inhibit sodium and water reabsorption along the nephron [17]. However, this is the first time that the alteration in fine tubular water regulation has been confirmed by evaluation in a model of acute low-dose administration of fluoride.

Chemical interactions between fluoride and other ions, such as magnesium, aluminum, and calcium, in biological systems are widely known, and these interactions result in several effects on metabolism [10]. Renal calcium handling occurs primarily in the Henle loop. Authors previously reported that an acute administration of high fluoride doses increased urinary calcium [18] and decreased serum calcium [19]. Our results showed that fluoride administration induced a clear increase in the fractional excretion of calcium and a decrease in the plasma concentration, whereas the calcium urinary concentration increased. The tubular origin was confirmed when the amount of filtered calcium was calculated. These results showed that the increase in urine calcium was not caused by augmented filtration but rather by a decrease in the reabsorption of this ion.

Calcium and phosphate are the primary mineral constituents of bone tissues. Therefore, their metabolism is closely related to the preservation of homeostasis. The increase in

serum calcium and phosphate after fluoride administration has been previously reported by Nabavi et al. [20]. As expected, the fractional excretion of phosphate was increased, as determined by a high urinary concentration. In contrast to calcium, the plasma concentration of phosphate was also elevated. The increase in plasma concentration matched the filtered amount accordingly. Because of the diminished calcium reabsorption, it is highly probable that the decrease in calcium plasma levels induced a redistribution of calcium and phosphate from bone tissues.

We also evaluated the presence of proteins in the urine. Normally, high molecular weight proteins are not present in the urine, whereas primarily small peptides and low molecular weight proteins are could be present in the urine. As we previously described, fluoride induced the excretion of diluted urine, and therefore, the  $J_v$  corrected for proteinuria. Our results also showed a significant increase in total urinary proteins. Because the Bradford method is a highly sensitive but nonspecific method, it is not possible to determine the precise origin of these proteins. However, as the glomerular filtration rate was not affected by fluoride administration, our results suggest that low molecular weight proteins were not reabsorbed by the tubular segment.

Recent epidemiologic studies suggest that fluoride could be an environmental factor in the development of chronic kidney disease of unknown etiology [7, 11]. Previous studies have shown a relationship between fluoride exposure and oxidative stress in renal tissue [21]. However, the role of fluoride remains unclear because the toxicological effects of low fluoride doses on renal function have not been fully elucidated. Herein, we report the earliest effects of acute low-dose fluoride administration (similar to the levels reached in environmentally exposed populations) on renal function and the renal handling of calcium and phosphate. These findings highlight the role of water tubular management and explain the polyuria, which has been previously

described in similar models. Both beneficial and deleterious effects have been attributed to fluoride administration. Water fluoridation programs, topical applications and therapeutic administration have been and will most likely continue to be seriously questioned. Our results confirm that the administration of low fluoride doses quickly induces deleterious renal effects, primarily tubular dysfunction, as demonstrated by polyuria, diluted urine, impaired protein reabsorption, and increased calcium and phosphate urinary excretion.

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