

## ABSTRACTS

### ACCELERATED DEATH OF RAT ERYTHROCYTES BY FLUORIDE

#### EXCESSIVE FLUORIDE CONSUMPTION LEADS TO ACCELERATED DEATH OF ERYTHROCYTES AND ANEMIA IN RATS

The present study was performed to evaluate an overall effect of long-term consumption of excessive amounts of fluoride (F) by rats on their erythrocytes. The animals were administered regular drinking water (0.4 ppm F) or the same water supplemented with 2, 10, or 20 ppm F (as NaF) for 12 months. Chronic exposure of the rats to increasing dosages of F induced a progressive rise of the plasma F concentration accompanied by a dose-dependent fall of hematocrit and decrease in the mean erythrocyte volume. Consumption of 10 and 20 ppm F resulted in appearance of morphologically abnormal cells (stomatocytes and echinocytes) in the peripheral blood. The water F concentration of 20 ppm F led to a significant increase in the number of phosphatidylserine-exposing erythrocytes, although suppression of cell viability occurred in all three groups of F-poisoned rats. A compensatory enhanced release of reticulocytes was not sufficient to compensate for erythrocyte loss. Dose-dependent accumulation of free cytosolic  $\text{Ca}^{2+}$  appears to be a major pathophysiological process underlying the development of F-induced death processes in rat erythrocytes. In addition, 10 and 20 ppm F induced ATP depletion and generation of peroxides in erythrocytes, whereas superoxide and glutathione levels were not altered. Thus, long-term intoxication of the rats with F triggers premature death of their erythrocytes due to intrinsic death-associated biochemical defects and development of anemia.

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Keywords: Anemia; Intracellular calcium; Premature death; Rat erythrocytes; Reticulocyte production.

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### BONE EFFECTS OF FLUORIDE WITH LOW VS. HIGH DIETARY CALCIUM

#### EFFECT OF FLUORIDE AND LOW VERSUS HIGH LEVELS OF DIETARY CALCIUM ON mRNA EXPRESSION OF OSTEOPROTEGERIN AND OSTEOPROTEGERIN LIGAND IN THE BONE OF RATS

The ratio of osteoprotegerin ligand (OPGL) to osteoprotegerin (OPG) determines the delicate balance between bone resorption and bone synthesis. The main objective of the present study is to investigate the possible role of OPGL and OPG in the bone metabolism of rats exposed to fluoride and the protective or aggravating effect of calcium (Ca). In a 6-month study, 270 weanling male Sprague-Dawley rats weighing between 70 and 90 g were divided randomly into six groups of 45 rats in each group. Three groups (groups I, III, and V) served as controls. They received deionized water and were fed purified rodent diets containing either 1,000 mg Ca/kg (low Ca), 5,000 mg Ca/kg (normal Ca), or 20,000 mg Ca/kg (high Ca). The three experimental groups (groups II, IV, and VI) were given the same diets but they drank water containing 100 mg/L fluoride (as NaF). Every 2 months 15 rats were randomly selected from each group and

sacrificed for the study. The ratio of OPGL mRNA to OPG mRNA was significantly increased by the sixth month in the distal femur joints of the F-exposed rats. Serum tartrate-resistant acid phosphatase activity and serum calcitonin activity in the F-exposed groups were increased, although changes were not apparent in the serum alkaline phosphatase or Gla-containing proteins, especially in the low calcium and high calcium diet F-exposed groups. The results indicated that OPG and OPGL may play important roles in skeletal fluorosis, and that fluoride may enhance osteoclast formation and induce osteoclastic bone destruction. A high Ca diet did not play a protective role, but rather may aggravate the fluoride-induced damage.

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Keywords: Bone metabolism; Dietary calcium; Osteoprotegerin; Osteoprotegerin ligand.

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## **IMPAIRMENT OF HEART RECOVERY IN ENDEMIC FLUOROSIS PATIENTS**

### **IMPAIRED HEART RATE RECOVERY IN PATIENTS WITH ENDEMIC FLUOROSIS**

The objective of this study was to determine the heart rate recovery index (HRRI), a marker of autonomic nervous system function, in patients with endemic fluorosis. Forty patients with endemic fluorosis (16 men/24 women) and 40 age-, sex-, and body mass index-matched healthy controls (16 men/24 women) with normal fluoride intake were enrolled in this study. HRRI was calculated by subtracting the heart rate values at the first, second, and third minutes of the recovery phase from the peak heart rate (HRRI 1, HRRI 2, HRRI 3). As expected, urine fluoride levels of fluorosis patients were significantly ( $p < 0.001$ ) higher than the control subjects. HRRI 2 was significantly lower in fluorosis patients than in the controls, while the incidence of abnormal HRRI 1 was significantly higher ( $p < 0.05$ ). Our observations indicated that HRRI, a marker of autonomic nervous system function, is impaired in patients with chronic fluorosis.

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Keywords: Autonomic nervous system; Endemic fluorosis; Heart rate recovery index.

Source: Biol Trace Elem Res 2013;152:310-5.

## **BIOCHEMICAL PARAMETERS OF MYELOPEROXIDASE IN ENDEMIC FLUOROSIS FROM COAL BURNING**

### **MYELOPEROXIDASE ACTIVITY AND ITS CORRESPONDING mRNA EXPRESSION AS WELL AS GENE POLYMORPHISM IN THE POPULATION LIVING IN THE COAL-BURNING ENDEMIC FLUOROSIS AREA IN GUIZHOU OF CHINA**

The myeloperoxidase (MPO) activity and its corresponding mRNA expression as well as gene polymorphism were investigated in the population of an endemic fluorosis area. In the study, 150 people were selected from the coal-burning endemic fluorosis area and 150 normal persons from the non-fluorosis area in Guizhou province of China. The blood samples were collected from these people. The activity of MPO in the plasma was determined by spectrophotometer; the expression of MPO mRNA was measured by real-time polymerase chain reaction;

DNAs were extracted from the leucocytes in blood and five SNP genotypes of MPO promoter gene detected by a multiplex genotyping method, adapterligation-mediated allele-specific amplification. The results showed that the MPO activity and its corresponding mRNA in blood were significantly increased in the population living in the area of fluorosis. The different genotype frequencies of MPO, including -1228G/A, -585T/C, -463G/A, and -163C/T, and the three haplotypes with higher frequencies, including -163C-463G-585T-1228G-1276T, -163C-463G-585T-1228G-1276C, and -163C-463G-585T-1228A-1276T, were significantly associated with fluorosis. The results indicated that the elevated activity of MPO induced by endemic fluorosis may be connected to the stimulated expression of MPO mRNA and the changed gene polymorphism.

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Keywords: Coal burning fluorosis; Endemic fluorosis; Genotype changes; Guizhou, China; mRNA; Myeloperoxidase; Polymorphism.

Source: Biol Trace Elem Res 2013;152:379-86.

## EFFECT OF FLUORIDE ON ENAMEL ADHESION OF ORAL BACTERIA

### REDUCED ADHESION OF ORAL BACTERIA ON HYDROXYAPATITE BY FLUORIDE TREATMENT

The mechanisms of the caries preventive action of fluoride have been discussed controversially for decades. The effect on the teeth is often traced back to demineralization. However, an effect on bacterial adhesion was indicated by indirect macroscopic studies. To characterize adhesion to fluoridated samples of hydroxyapatite on a single bacterial level, we used force spectroscopy with bacterial probes to measure adhesion forces directly. We tested the adhesion of *Streptococcus mutans*, *Streptococcus oralis*, and *Staphylococcus carnosus* on smooth, high-density hydroxyapatite surfaces, pristine, and after treatment with fluoride solution. All bacteria species exhibit lower adhesion forces after fluoride treatment of the surfaces. These findings suggest that the decrease of adhesion properties is a further key factor for the cariostatic effect of fluoride besides the decrease of demineralization.

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Keywords: Bacterial adhesion; Cariostatic fluoride effect; Hydroxyapatite; Oral bacteria.

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## FIELD STUDY ON FLUORIDATED SALT FOR CARIES PREVENTION

### FLUORIDATED SALT FOR CARIES PREVENTION AND CONTROL —A 2-YEAR FIELD STUDY IN A DISADVANTAGED COMMUNITY

Salt fluoridation is widely considered to be a cost-effective community strategy for reducing caries. The present study was conducted to evaluate the effect of school-based and domestic distribution of F-salt to schoolchildren residing in a disadvantaged community. Seven hundred and thirty-three schoolchildren (12–14 years of age) attending two public schools in an urban area of Malmö, Sweden were enrolled. One school was assigned to the intervention study (IS), whereas the other served as a reference study (RS). Subjects in IS were given access to F-salt

(250 ppm F) in marked jars at school lunch and through free supply for domestic use. The 2-year caries increment and progression rate, assessed from bite-wing radiographs, was scored. Information on diet, oral hygiene, and fluoride exposure was collected through a baseline questionnaire. The dropout rate was high (IS 27%; RS 18%). At baseline, the IS children displayed more unfavourable risk factors and a higher caries experience than RS children. There were no significant differences in total caries increment or proximal progression rate between the two schools. However, a negative correlation ( $r = -0.29$ ;  $p < 0.05$ ) between the amount of delivered salt and the caries progression rate was noted. No side effects were reported. Although F-salt was not effective in this setting, the findings indicate that salt may be a beneficial source of fluoride in schoolchildren provided that compliance can be secured.

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**Editorial Comment:** Some of the problems reported in this study (such as unknown overall fluoride exposure and ingestion compliance) could have been avoided by following a protocol presented in a paper published eight years ago in *Fluoride*,<sup>1</sup> which, however, was evidently not consulted for this research.

#### REFERENCE

- 1 Tóth Z, Gintner Z, Bánóczy J. The effect of ingested fluoride administered in salt, milk, and tablets on salivary and urinary fluoride concentrations. *Fluoride* 2005;38(3):199-204.