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# EFFECTS OF HIGH FLUORIDE INTAKE ON CHILD MENTAL WORK CAPACITY: PRELIMINARY INVESTIGATION INTO THE MECHANISMS INVOLVED

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**SUMMARY: A study was carried out on 157 children, age 12–13, from a coal-burning fluorosis endemic area together with an experiment looking into the effect of high fluoride intake in animals. The results showed that early, prolonged high fluoride intake causes a decrease in a child's mental work capacity and that prolonged high uptake of fluoride causes a child's levels of hair zinc to drop. A multifactoral correlative analysis demonstrated a direct correlation between hair zinc and mental work capacity. The decrease of 5-hydroxyindoleacetic acid and the increase of norepinephrine in animal brains exposed to high levels of fluoride suggest a possible mechanism for mental work capacity deficits in children. However, further research is necessary.**

Keywords: Child mental ability; Coal-burning fluorosis; Hair zinc; 5-Hydroxyindoleacetic acid; Mental work capacity; Norepinephrine.

## INTRODUCTION

In recent years, research on the damaging effect of excess fluoride on children's soft tissues has steadily expanded, but studies related to the effects on the central nervous system, particularly the effects on mental work capacity, have been few, and the conclusions are varied. One study[1] of children from an area with fluoride-contaminated drinking water showed that high levels (0.5-7.3 mg/L) had no major effect on brain function. Other studies[2,3] have indicated that high fluoride intake has a negative effect on the brains of animals and humans, and that a brain system in the process of development is one of the most susceptible targets for the toxic effects of fluoride. At present, however, there is very little research on the mechanisms involved in brain damage caused by fluoride poisoning. Through observation of the effects of different levels of fluoride intake on child metal work capacity (MWC) in connection with hair zinc levels, along with experiments to determine the effects of high fluoride intake on 5-hydroxyindoleacetic acid (5-HIAA) and norepinephrine (NE) levels in the brain, the aim here is to expand on previous work showing the effects of excess fluoride on brain function, and do a preliminary investigation into the mechanisms involved as they relate to trace elements and neurotransmitters.

## SUBJECTS AND METHODS

*Subjects:* Children, aged 12–13, from centralized middle schools of two neighboring townships (administrative unit between the village and county level) in a coal-burning, endemic fluorosis area. After the group was screened to remove children who had other acute or chronic diseases not related to fluoride, 157 subjects remained for the study. The two townships had identical fluoride levels in their drinking water (0.3 mg/L), and the fluoride content of the air was 0.02–0.51 mg/m$^3$. Excess fluoride exposure was primarily a result of the burning of high-

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fluoride coal to cook grains. Since some villages use firewood for fuel, and the method of cooking varies, the level of fluoride contamination also shows variation. However, from the perspective of economic and cultural status, lifestyle, dietary habits, and the basic constituents of their food, the villages in the study were essentially the same. Sub-groups within the subject group were formed based on the presence of dental fluorosis and the fluoride content of their grain, as follows:

In the control group: no dental fluorosis, average fluoride content of grain = 0.5 mg/kg. High fluoride group I (HiF I): no dental fluorosis, average fluoride content of grain = 4.7 mg/kg. High fluoride group II (HiF II): dental fluorosis present (grade 3), average fluoride content of grain = 5.2 mg/kg. High fluoride group III (HiF III): dental fluorosis present (grade 3) average fluoride content of grain = 31.6 mg/kg. The age, gender, and grade level composition of each group were kept as constant as possible.

*Methods and content:* Dental fluorosis was classified according to the three-grade system. For the food-related part of the study, a combination of questionnaires and weighing was used, and the fluoride content of the food was measured using the acid-immersion electrode test. The zinc hair test was conducted according to requirements that asked all subjects in a particular village to provide a hair sample, and from them 2–3 samples were chosen randomly. Atomic absorption spectrometry was used to determine the zinc content. A hemoglobin ferricyanide test was used to determine the hemoglobin count. A proofing test was used to determine the number of letters found (NLF), the rate of error (RE), and the index of mental capacity (IMC). A task involving twenty groups of three-digit numbers to be written in reverse order from memory was used to determine short-term memory capacity (SMC). An automated testing apparatus measured visual reaction time (RT).

In the long-term laboratory experiment, Wistar rats with an average weight of 72.8±4.2 g were used. The rats were randomly selected to be part of the control (14 rats eating their usual diet), test group 1 (14 rats, 45 days of exposure), or test group 2 (24 rats, 12 weeks of exposure). The test groups had fluoride mixed into their regular feed at a concentration of (300 mg/kg). The rats were free to consume food and water (0.6 mg/L fluoride) as they chose. At the end of the experimental period the brains of the rats were removed and examined, and the 5-HIAA and NE content measured by means of a Daojin RT-510 fluorospectrophotometer.

## RESULTS

*Relationship between child fluoride intake and mental work capacity (MWC):* In Table 1 below, the HiF II and HiF III groups of children showed a lower NLF and IMC score when compared to the HiF I and the control groups. HiF II showed a lower SMC score compared to the HiF I and the control groups, and the SMC of HiF III was lower than HiF I. Although there were no statistically significant differences between the four groups in regard to reaction time, if subjects without dental fluorosis (the control and HiF I) are grouped (average RT 466.4 ms), and the subjects with grade 3 dental fluorosis (HiF II and HiF III) are grouped (average

RT 495.0 ms), the time difference becomes fairly significant ($P$<0.5). There were no significant differences between the control and HiF I, or between HiF II and HiF III for any of the indices.

**Table 1.** Comparison of mental work capacity (MWC) between different groups of children

| Group | RT[a] (msec) | | SMC[b] (No.) | | NLF[c] (letters/ 2 min) | | RE[d] (%) | | IMC[e] | |
|---|---|---|---|---|---|---|---|---|---|---|
| | n | mean±SD | n | mean±SD | n | mean±SD | n | mean±SD | n | mean±SD |
| Control | 51 | 463.0±93.9 | 49 | 17.2±2.9 | 49 | 555.2±79.5 | 49 | 0.5±0.4 | 49 | 267.2±39.5 |
| HiF I | 33 | 471.7±77.4 | 31 | 18.6±2.0 | 33 | 569.1±96.6 | 33 | 0.5±0.4 | 33 | 273.2±50.2 |
| HiF II | 37 | 494.5±68.4 | 38 | 15.6±4.1*‡ | 38 | 507.4±66.3†§ | 38 | 0.6±0.6 | 38 | 243.2±36.2†§ |
| HiF III | 36 | 495.6±99.2 | 36 | 16.6±4.0‡ | 36 | 500.0±73.7†§ | 36 | 0.5±0.4 | 36 | 240.0±30.8†§ |

[a]RT: reaction time, [b]SMC: short-term memory capacity, [c]NLF: number of letters found, [d]RE: rate of error, [e] IMC: index of mental capacity. Compared with control, *P<0.05, † P <0.01; compared with HiF I group, ‡P<0.05 §P<0.01.

*Relationship between child fluoride intake and hair zinc:* In Table 2 below, the average hair zinc of HiF II and HiF III is markedly lower than the average of HiF I and the control. However, when any of the high fluoride groups is compared with the control, the difference is not statistically significant.

The levels of zinc in the soil of the two townships were tested to be 96.3± 37.7 mg/kg and 104.6±57.7 mg/kg; the difference is not significant. Each group's diet was simple and essentially the same, with the daily intake of nutrition approximately the same, and there were no significant differences in hemoglobin levels. Therefore, any difference in the level of hair zinc was not due to the soil or the diet.

**Table 2.** Comparison of hair zinc in different groups(μg/g)

| Group | n | Hair zinc ( mean±SD ) |
|---|---|---|
| Control | 49 | 174.8 ± 30.7 |
| HiF I | 31 | 181.5 ± 34.1 |
| HiF II | 36 | 142.6 ± 41.8* |
| HiF III | 34 | 152.9 ± 35.2* |

F= 9.85, P <0.01, Comparison with control and HiF 1 group, *P<0.01.

*Relationship between hair zinc and fluoride content of food with MWC:* A multifactoral correlation analysis yielded the following results: When the fluoride content of the food (FF) is held constant, high zinc is directly correlated with SMC, NLF, and IMC, and if zinc is held constant, the various indices of FF and MWC show no linear relation. This demonstrates that hair zinc and MWC are directly correlated, so if hair zinc levels increase, so does MWC, while levels of FF have no direct relation to MWC.

*Relationship between high fluoride intake and neurotransmitters:*As seen in Table 4, rat test group 2 had reduced levels of 5-HIAA when compared to both test group 1 and the control, and NE levels in test group 2 were elevated compared to test group 1 and the control. The differences between test group 1 and the control for either index were not significant.

**Table 3.** Correlation analysis of hair zinc (X1), FF (X2), and MWC(Y)

| MWC | n | R | R (Y1.2) | R (Y2.1) |
|---|---|---|---|---|
| RT | 142 | 0.1763* | 0.1708* | 0.0236 |
| SMC | 142 | 0.3317* | 0.3263* | 0.1092 |
| NLF | 142 | 0.2459* | 0.2378* | 0.0355 |
| IMC | 142 | 0.2478* | 0.2381* | 0.0419 |

*P<0.01

**Table 4.** Comparison of neurotransmitters in different groups of rats (ng/g)

| Group | 5-HIAA[a] | | NE[b] | |
|---|---|---|---|---|
| | n | Mean±SD | n | Mean±SD |
| Control | 14 | 656.6 ± 126.7 | 13 | 245.0 ± 148.1 |
| Test 1 | 14 | 655.9 ± 114.0 | 13 | 209.4 ± 103.1 |
| Test 2 | 14 | 570.8 ± 109.8*† | 22 | 344.0 ± 137.2*‡ |

[a]5-HIAA = 5-hydroxyindole acetic acid, [b]NE = norepenephrine; $F_{5\text{-HIAA}} = 3.53$; $P<0.05$, $F_{NE}=4.89$ $P<0.05$; compared with control group, * $P<0.05$; compared with test group 1, †$P<0.05$, ‡$P<0.01$.

## DISCUSSION

Dental fluorosis is universally acknowledged as the earliest clinical manifestation of chronic endemic fluoride poisoning, and is thought generally to result from high fluoride uptake before the age of 7 or 8 (i.e. early childhood). The subjects of this study were 12–13 year-old children, so those with dental fluorosis can be regarded as having a history of early, excessive fluoride intake, and because the fluoride poisoning of this region is primarily a result of using coal fires to roast grains, the fluoride content of those grains will generally reflect the relative proportion of recent intake. Therefore having dental fluorosis indicates prolonged high fluoride intake, and eating high fluoride food indicates recent high-fluoride intake.

As shown in this study, the mental work capacity (MWC) of the two groups of children with grade 3 dental fluorosis was lower than the two groups with no dental fluorosis. Although the high-fluoride food eaten by both HiF I and HiF II groups suggested recent fluoride intake, only HiF II had grade 3 dental fluorosis and showed a corresponding drop in MWC compared to HiF I. This indicates that early, long-term exposure to excess fluoride causes deficits in memory, attention, and reaction time, but 12–13 year-old children with only recent exposure show no major effects. Studies [on human fetuses][3] have already shown that the developing brain is one of the ripest targets for disruption by fluoride poisoning. Given that before six years of age the human brain is in its fastest stage of development, and that around seven and eight basic structural development is completed, therefore the brain is most vulnerable to damage from excess fluoride intake before this age.

This study showed that the hair level of zinc in subjects with grade 3 dental fluorosis was lower than in those without dental fluorosis. Krishnamachari[4] has reported the serum zinc of fluorosis patients was significantly reduced when compared to a control. This indicates that long-term high fluoride intake can reduce the body levels of zinc, likely a case of absorption antagonism between the two trace elements. Further analysis reveals that the relation of MWC to hair zinc and to recent intake from contaminated grain is almost identical. However, the

results of a multifactoral correlative analysis show that in fact MWC is correlated directly with hair zinc, thereby indicating no significant correlation with the fluoride content of subject's food. This indicates that long-term high fluoride intake interferes with zinc metabolism, and it is only after zinc is reduced that MWC is negatively affected. One report[5] indicates that hair zinc and IQ are directly correlated. On the whole, this suggests that inference with zinc metabolism caused by prolonged intake of excess fluoride leading to lowered levels of zinc in the body is perhaps one of the mechanisms by which fluoride affects MWC.

Similarly, the results of the animal experiments show that high fluoride intake over long periods lead to decreased levels of 5-HIAA and elevated levels of NE, while rats exposed to fluoride over shorter periods showed no corresponding changes. 5-HIAA is the primary product of 5-hydroxytryptamine (5-HT) metabolism, so it can reflect the metabolic status of 5-HT. Under normal circumstances, cerebral 5-HT and NE are deactivated by monoamine oxidase. Zavoronkov[6] pointed out that high fluoride can cause 5-HT and NE to become inactive, leading to a decrease in 5-HIAA and an increase of NE. Geeraerts et al.[7] observed that in rats with high fluoride intake, both 5-HT and 5-HIAA are reduced, indicating that fluoride can become a barrier to the production of 5-HT in the brain. Others[8] report that in animal experiments a lack of zinc can lead to an increase in NE. This connects directly with what was observed in the present study, i.e., long-term fluoride intake correlated with a drop in hair zinc, and is quite possibly the reason for the increase in NE. Since 5-HT and NE are important neurotransmitters, vital in maintaining the balance between excitation and inhibition during high-level central nervous function, the elevation of 5-HT together with the reduction of NE could lead to over-stimulated brains showing poor performance in tasks that involve attention and memory. Pratusevich et al.[9] reported a similar finding. Therefore, high fluoride intake interfering with the metabolism of 5-HT and NE is another possible mechanism for diminished MWC. Further experimental research should be done to investigate this.

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