SUMMARY: To determine effects of fluoride on thyroid function in suckling mice, adult female mice were given 500 ppm NaF (226 ppm F⁻) in their drinking water from the 15th day of pregnancy until either the 4th or 14th day after parturition. On day 14 of full-period treatment, pups and their mothers were sacrificed. In the F⁻-exposed group the body weight of the 14-day-old pups was 15% less than the controls. This reduction in pup body weight is attributed to a defect in plasma thyroid hormone levels: free thyroxine (FT₄: –15%) and free triiodothyronine (FT₃: –6%). These reductions also correlate with a decrease in thyroid iodine content of the experimental pups (–55%) and their mothers (–68%), along with hypertrophy of their thyroid glands (+29% in the pups and +41% in the mothers), probably caused by a 1.74- and 3.15-fold increase of plasma thyroid stimulating hormone (TSH) in the pups and their mothers, respectively. These biochemical modifications also correspond histologically with an increase in thyroid follicular number and a reduction in colloid volume. When fluoride ion was eliminated from the mothers’ drinking water four days following parturition, a partial recovery occurred after ten days in body weight, thyroid gland weight, and plasma TSH level in both pups and their mothers. However, essentially complete recovery was observed in plasma levels of FT₄ and FT₃ and in thyroid iodine content. Compared with NaF-treated mice, significant amelioration in thyroid histological aspects was also observed after withdrawal of NaF treatment.

Keywords: Iodine in thyroid; Mice; Offspring mice; Pregnant mice; Sodium fluoride; Thyroid function; Thyroid hormones (FT₃; FT₄); TSH.

INTRODUCTION

Various factors such as iodine deficiency, dietary goitrigens, malnutrition, heredity, ionizing radiation, and pollution can induce non-toxic goiter.¹,² Although there is evidence to the contrary,³ it is generally believed that fluorine as used to prevent dental caries at the level of 1 ppm F⁻ in drinking water does not affect either thyroid function or its structure.⁴ On the other hand, if fluorine intake is extremely high as in an endemic fluorosis area, several parameters of thyroid activity are reported to be disturbed.⁵ In our previous studies,⁶,⁷ 500 ppm of NaF (226 ppm F⁻) given to pregnant mice in their drinking water for 20 days, decreased plasma thyroxine (FT₄) and triiodothyronine (FT₃) levels in their 14-day-old pups.

Some years ago fluoride compounds were used to treat hyperthyroidism and depress thyroid function.⁸ Numerous studies on the effects of fluoride on thyroid function have been conducted on humans,⁹,¹⁰ cattle,¹¹ calves,¹² and adult rats.¹³-¹⁵ Both adult⁵,¹⁶,¹⁷ and young mice⁶,⁷ have been shown to

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develop hypothyroidism after ingestion of fluoride. However, reports remain scarce or appear to be absent in mice after withdrawal of this pollutant.

The purpose of this study was to investigate induction and reversibility of thyroid proliferative changes in suckling mice and their mothers administered sodium fluoride in their drinking water.

**MATERIALS AND METHODS**

The same protocol was used as our previous papers.\(^6,7,18\) Thirty pregnant mice were separated into three groups of ten animals in each group. The first group (A) of animals did not receive supplemental fluoride and was considered the untreated or control group. The other two groups received sodium fluoride in their drinking water at a concentration 0.5 g/L of water (226 ppm F\(^-\)) from the 15\(^{th}\) day of pregnancy until either the 14\(^{th}\) day after parturition for the second group (B) or the 4\(^{th}\) day for the third group (C). Mice of the last group were allowed free access for the next ten days to tap water without added fluoride. This group was added in the current study in contrast to our relevant previous reports.\(^6,7,18\)

All mice were sacrificed by intra-abdominal administration of chloral hydrate, body weights were measured, and blood was collected from the brachial artery of each mouse and centrifuged at 2200 g. Plasma samples were withdrawn and kept at \(-20^\circ C\) until analysis. Plasma FT\(_3\), FT\(_4\), and TSH levels were determined by commercially available radio-immunoassay kits (Immunotech, ref: 1363, 1579 and Biocode Hycel, ref: AH R001, respectively), according to the manufacturers’ instructions. Some thyroid glands were removed from pups and dams. They were weighed and preserved at \(-20^\circ C\) until analysis for their iodine content after being made alkaline by the method of Sandell and Kolthoff.\(^19\) Other thyroid samples, destined for light microscopy, were dissected with a piece of trachea and submitted to the same protocol as reported earlier.\(^7\)

Numerical results are expressed as arithmetical means ± SEM. Statistical significance among groups was determined by using Student’s t test\(^20\) with a p value of less than 0.05.

**RESULTS**

Compared to the control group, fourteen day-old mice, whose mothers had been administered 500 ppm of NaF in their drinking water, showed a decrease in body weight (Table) and in plasma levels of FT\(_3\) and FT\(_4\) (Figure 1) as demonstrated in our earlier paper.\(^6\) Thyroid iodine content was also decreased by 68% in dams and by 55% in their offspring (Figure 2). At the same time, plasma TSH levels were increased 3.15- and 1.74-fold, respectively (Figure 1). The weights of thyroid glands were also increased by (+41% and +29%) (Table). Withdrawal of NaF treatment for 10 days resulted in a partial recovery in dams and their offspring in body weight, thyroid gland weight (Table), and plasma TSH levels (Figure 1). However, complete recovery occurred in plasma levels of FT\(_4\) and FT\(_3\) and in thyroid iodine content of pups. In dams complete recovery occurred in plasma levels of FT\(_4\) and FT\(_3\) in spite of only a partial recovery of thyroid iodine content when compared to controls (Figures 1 and 2).
Figure 1. Plasma FT₄, FT₃, and TSH levels of 14 day-old mice and their mothers: controls (A) and mothers administered NaF in their drinking water (0.5 g/L) from the 15th day of pregnancy until either the 14th day (B) or the 4th day (C) after parturition.
Significant differences: Treated (B) vs Controls (A): * p ≤ 0.05; † p ≤ 0.01; ‡ p ≤ 0.001.
Treated (C) vs Treated (B): * p ≤ 0.05; † p ≤ 0.01; ‡ p ≤ 0.001.
Table. Body and thyroid weights of 14-day-old mice and of their mothers: controls (A) and mothers administered NaF in their drinking water (0.5 g/L) from the 15th day of pregnancy until either the 14th day (B) or the 4th day (C) after parturition.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls (A)</td>
<td>Treated (B)</td>
</tr>
<tr>
<td></td>
<td>Mean±SEM</td>
<td>Mean±SEM</td>
</tr>
<tr>
<td>Body weight</td>
<td>Offspring</td>
<td>8.99±0.44</td>
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<tr>
<td>(g)</td>
<td>(n = 40)</td>
<td></td>
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<tr>
<td></td>
<td>Mothers</td>
<td>41.44±1.68</td>
</tr>
<tr>
<td></td>
<td>(n = 10)</td>
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<tr>
<td>Thyroid weight</td>
<td>Offspring</td>
<td>1.04±0.18</td>
</tr>
<tr>
<td>(mg)</td>
<td>(n = 40)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mothers</td>
<td>3.67±0.41</td>
</tr>
<tr>
<td></td>
<td>(n = 10)</td>
<td></td>
</tr>
</tbody>
</table>

Significant differences: Treated (B) vs. Controls (A): *p ≤ 0.05; †p ≤ 0.001. Treated (C) vs. Treated (B): *p ≤ 0.05; †p ≤ 0.001.

Figure 2. Thyroid content of 14 day-old mice and their mothers: controls (A) and mothers administered NaF in their drinking water (0.5 g/L) from the 15th day of pregnancy until either the 14th day (B) or the 4th day (C) after parturition. Significant differences: Treated (B) vs Controls (A): *p ≤ 0.001. Treated (C) vs Treated (B): *p ≤ 0.001.

Light microscopy revealed changes in the thyroid glands of pups of NaF-treated mice (group B) compared to untreated animals (group A) (Figures 3B and 3A). Ten days after discontinuing treatment in group C, a partial recovery of thyroid histological aspect was obtained (Figure 3C).
Figure 3. Histology of thyroid sections of 14-day-old mice: controls (A) and pups of mothers administered NaF in their drinking water (0.5 g/L) from the 15\textsuperscript{th} day of pregnancy until either the 14\textsuperscript{th} day (B) or the 4\textsuperscript{th} day (C) after parturition. Optical microscopy HE (x 400).

Arrows: \rightarrow indicate thyroid follicles; \longrightarrow indicate blood vessels.
DISCUSSION

Our results showed, after NaF treatment of mothers, a decrease in plasma thyroid hormone levels of nursing pups as also reported in our earlier paper.\textsuperscript{6} This may be explained either by transplacental transfer of fluoride from the mothers to their fetuses as demonstrated by Shen and Taves,\textsuperscript{21} or by inhibition of sodium iodide coupled symport transport abolishing prolactin effects on iodide accumulation and incorporation in the mammary gland.\textsuperscript{22} A low total iodide pool may impose a reduction of thyroid iodine content and a critical limitation on hormonal synthesis,\textsuperscript{8} since the iodine is necessary for thyroid hormone biosynthesis. This last hypothesis is confirmed by data of Jentzer,\textsuperscript{23} who found that rabbits fed with 0.05 mg F\textsuperscript{−} per day had their thyroid iodine content reduced by 25%. Other previous findings of Galletti and Joyet\textsuperscript{8} also suggested that fluoride inhibits thyroid iodide concentration. Likewise, Kahl and Bobek\textsuperscript{24} found a significant reduction of protein-bound iodine as well as an overall reduction of iodide and a reduction of iodine uptake by the thyroid gland in fluoride-treated rats. Other reports in the literature suggest that tyrosine and its metabolite levels are also influenced by fluoride. In fact, increased urinary loss of tyrosine is known to occur in men living in high-fluoride areas\textsuperscript{25} and in monkeys receiving low daily doses of fluoride.\textsuperscript{26}

Still other concepts of fluoride interference with thyroid hormone synthesis have been proposed and documented. Thus, Tezelman et al.\textsuperscript{27} reported that fluoride, by increasing the intracellular cAMP concentration, causes desensitization of the thyroid stimulating hormone receptor. On the other hand, Willems et al.\textsuperscript{28} demonstrated that NaF depresses the endocytosis of colloid and thyroid secretion by inhibiting aerobic glycolysis in the follicular cells. We therefore suggest that administration of this pollutant provokes thyroid hyperplasia in mice. Indeed, the weight of the thyroid gland was increased. The mechanism for this response is an increase in TSH levels as seen in the present study due to a decrease in production of thyroid hormones as reported in our previous papers.\textsuperscript{6,7} The changes in the thyroid weight in our study are also similar to those described in other studies. Several investigators found that fluoride administration to experimental rats may cause an enlargement of the thyroid gland.\textsuperscript{15} Similarly, a close relationship was found between fluoride intake and the incidence of goiter in areas containing high levels of fluoride in water.\textsuperscript{29-32} Histological changes seen in the thyroid gland of mice treated with NaF are characterized by follicular cell hyperplasia, an increase of follicular number and vascularity, and a decrease of colloid.

In our study, when fluoride ion was removed from the dams’ drinking water on day four after delivery, body growth rate increased and thyroid weight decreased, but they did not return to control values after ten days. Reversibility of sodium fluoride effects was thus observed with a return of thyroid hormone production and of thyroid iodine content, although plasma TSH level was only partially restored. Restoring iodide, by removing sodium fluoride from the dams’ drinking water on day four after delivery, reduced the hypertrophy of follicle epithelial
cells and increased the colloidal volume. Some thyroid follicles became larger than those of treated mice in group B. In human pathologies, goiter size has been found to be reduced either by application of an iodide treatment,\textsuperscript{33} which results in a decrease in the size of cells and follicles\textsuperscript{34} or in a reduction of plasma TSH levels as demonstrated by our results. In fact, Li et al.\textsuperscript{33} reported that iodide uptake by the thyroid gland is competitively inhibited by fluoride, especially in high-fluoride areas and is alleviated by the provision of iodized salt causing a decrease in the occurrence of goiter. This reversibility evidently involves the return to normal aspect of thyroid gland in which the phenomena of proliferation and cell death must be involved.

In conclusion, this study has shown that NaF administered in drinking water (0.5 g/L) provoked hypothyroidism in female mice and in their nursing pups, which could be largely reversed by withdrawing NaF treatment for a period of ten days.

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