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FLUORIDE AND ADRENAL GLAND FUNCTION IN RABBITS

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SUMMARY: In an investigation of the effect of sodium fluoride (NaF) on adrenal gland function, male and female albino rabbits were administered NaF subcutaneously in 1 mL of double distilled water at dosages of 5, 10, 20, and 50 mg/kg bw/day for 15 weeks. The controls were given 1 mL of double distilled water/kg bw/day for the same period. Slight to large decreases in body weight gain were noted in the NaF exposed animals. Biochemical analysis of the adrenal gland revealed significant (P<0.001) decline in the DNA and RNA content of fluoridated animals of both sexes compared to the control. Accumulation of glycogen indicated reduction in the activities of glycolytic pathway enzymes. A significant decrease (P<0.001) in acidic, basic, and total proteins in test animals suggested inhibition of protein synthesis by fluoride and increased proteolysis. Significantly (P<0.001) enhanced levels of free amino acids indicated reduced incorporation of amino acids into proteins. Hyperlipidemia and hypertriglyceridemia in the adrenal gland of fluorotic animals reflected a disturbance of lipoprotein metabolism. In males, phospholipids exhibited significant declines (P<0.001) in groups II and III, but an increase in group IV. In females, the amount of phospholipids was significantly (P<0.001) reduced in groups II, III, and IV. In group V, the level of phospholipids in both sexes returned to the control values. An inhibitory effect of fluoride was also observed on the levels of adrenal free fatty acids and cholesterol.

Keywords: Acidic proteins; Adrenal gland function; Albino rabbits; Basic proteins; Cholesterol; DNA synthesis; Fluorosis; Free amino acids; Free fatty acids; Glycogen; Phospholipids; Protein synthesis; RNA synthesis; Sodium fluoride; Total lipids; Triglycerides.

INTRODUCTION

Fluoride is toxic to various organs including endocrine glands. In fluorosis various types of skeletal lesions occur, such as osteoporosis, osteomalacia, and osteopetrosis. Earlier work in this laboratory revealed that sodium fluoride (NaF) induces hepatotoxicity,[1] myocardial damages,[2] impaired renal function,[3] gastroduodenal ulcers, and biochemical alterations in brain,[5] reproductive organs,[6,7] and thyroid gland.[8,9] Fluoride inhibits numerous phosphatases and kinases as well as ATPases which are associated with cell energy processes.[10] The fall in ATP levels in cells induced by fluoride has a detrimental effect on many metabolic processes connected with the action of ATP such as metabolism of carbohydrates, proteins, nucleic acids, lipids, and active transport.[11] Das and Susheela[12] observed adrenal insufficiency due to suppressed ACTH release system in chronic fluoride toxicity. Hypocortisolemia in fluorosis patients and in experimental animals subjected to long-term fluoride administration has also been reported.[13]

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The present study was undertaken to evaluate biochemical changes in the adrenal gland of rabbits exposed to varying levels of sodium fluoride.

MATERIALS AND METHODS

Design of experiments: Sixty young albino rabbits of both sexes weighing 400-600 g were divided into five equal groups, each with six males and six females. Groups II, III, IV, and V were subcutaneously injected with NaF, dissolved in 1 mL of double distilled water, at the doses of 5, 10, 20, and 50 mg/kg bw/day, respectively, for 15 weeks. Group I served as the control and received only the vehicle. All the animals had *ad libitum* access to normal low-fluoride tap water and were maintained on a standard pellet diet.

After 15 weeks, the control and experimental animals were sacrificed under ether anaesthesia. The adrenal gland was dissected out, washed in normal saline, and processed for biochemical estimations.

BIOCHEMICAL ASSAYS:

Nucleic acids: Nucleic acids were extracted by the method of Webb and Levy.[14]

DNA: DNA was assayed by Dische's diphenylamine reaction as modified by Burton.[15]

RNA: RNA was determined by the Orcinol method described by Markham.[16]

Glycogen: Glycogen was extracted by the method of Heatley[17] and estimated according to the method of Montgomery.[18]

Proteins: Proteins were assayed by the method of Lowry *et al*.[19] using bovine serum as standard.

Free amino acids: Free amino acids were estimated using the method of Troll and Cannon.[20]

Total lipids: Extraction of total lipids was done by the method of Folch *et al*.[21]

Phospholipids: After treatment with 10% aqueous magnesium nitrate, the samples were ashed by heating in a test tube over a strong flame until the brown fumes disappeared and the residue turned white. The tubes were then allowed to cool, 0.5 M HCl was added, and the tubes were heated in a boiling water bath to hydrolyze any pyrophosphates formed during ashing. After the solutions had cooled the tubes were incubated for 20 min at 45ºC, phosphate (and hence the phospholipids content) was determined as described by Ames.[22]

DETERMINATION OF LIPID CONSTITUENTS

Separation of Neutral lipids: Silica Gel G thin layer plates were prepared for their layer chromatography.[23]

Triglycerides: Triglycerides were determined by the method of VanHandle and Zilversmit.[24]

Free fatty acids: Quantitative analysis of free fatty acids was done by the method of Chakrabarty *et al*.[25]

Cholesterol: The cholesterol content was assessed by the method of Stadtman.[26]

DATA ANALYSIS

All the data were expressed as mean ±S.D. The significance of differences was evaluated by Student's t test for paired or unpaired data and probability values lower than 0.05 were considered statistically significant.

RESULTS

Table 1 reveals the DNA, RNA, and glycogen content in the adrenal gland of control and fluoride treated rabbits of both sexes.

Table 1. DNA, RNA, and glycogen in the adrenal gland of rabbits after 15 weeks in control and fluoridated groups (12 animals per group; each value is the mean ± SD of nine observations)

Biochemical parameter mg/g tissue weight	Sex	Group (NaF dose mg/kg-bw/day)				
		I Control	II 5 mg NaF	III 10 mg NaF	IV 20 mg NaF	V 50 mg NaF
DNA	M	5.54 ± 0.43	4.65 ± 1.25 –16.1	3.77 ± 0.32 –31.9	1.80 ± 0.16 –67.5	1.05 ± 0.05 –81.0
	F	5.87 ± 0.11	2.96 ± 0.04 –49.6	2.11 ± 0.06 –64.1	1.87 ± 0.02 –68.1	0.73 ± 0.02 –87.6
RNA	M	6.47 ± 0.20	5.96 ± 0.05 –7.9	4.30 ± 1.68 –33.5	4.07 ± 0.10 –37.1	3.83 ± 0.29 –40.8
	F	8.13 ± 0.22	5.73 ± 0.10 –29.5	5.23 ± 0.21 –35.7	5.05 ± 0.87 –37.9	4.05 ± 0.04 –50.2
Glycogen	M	1.12 ± 0.02	2.47 ± 0.46 +120.5	8.25 ± 0.87 +636.6	11.43 ± 0.49 +920.5	41.00 ± 1.73 +3560.7
	F	1.05 ± 0.03	2.77 ± 0.22 +163.8	8.76 ± 0.16 +734.3	13.67 ± 0.58 +1201.9	53.33 ± 1.15 +4979.0

+ indicates % increase over control; – indicates % decrease over control.

DNA: The DNA content in the adrenal gland of both sexes showed a significant decline P<0.001 in animals of groups III, IV, and V in comparison to group I. In males, the DNA level did not change significantly in the animals of group II. In females, however, there was a significant decline (P<0.001) of DNA in the adrenal gland in this group. In both sexes, the DNA content decreased significantly (P<0.05–0.001) in group III vs IV and group IV vs V.

RNA: In both sexes, the RNA content fell significantly (P<0.02–0.001) in the adrenal gland of all fluoridated groups of animals compared to the control. In both sexes, the RNA content declined significantly in group II vs III (P<0.05–0.001) and group IV vs V (P<0.02–0.001).

Glycogen: The concentration of glycogen in the adrenal gland exhibited a significant (P<0.001) rise in all experimental groups of both sexes compared to the control. The elevation in glycogen content in group II vs III, group III vs IV, and group IV vs V was highly significant (P<0.001) in rabbits of both sexes.

Table 2 records the effect of NaF on protein and amino acid synthesis.

Table 2. Levels of protein and free amino acids in the adrenal gland of rabbits after 15 weeks in the control and fluoride treated groups (12 animals in each group; each value is the ± SD of nine observations)

Biochemical parameter mg/g tissue weight	Sex	Group (NaF dose mg/kg-bw/day)				
		I Control	II 5 mg NaF	III 10 mg NaF	IV 20 mg NaF	V 50 mg NaF
Acidic protein	M	31.33 ± 0.94	17.42 ± 0.86 –44.4	14.31 ± 4.40 –54.3	11.66 ± 1.44 –62.8	6.47 ± 1.15 –79.3
	F	31.87 ± 3.61	26.86 ± 0.68 –15.21	12.69 ± 0.54 –60.2	10.21 ± 0.73 –68.0	4.15 ± 0.22 –87.0
Basic protein	M	11.18 ± 1.02	10.59 ± 0.93 –5.3	7.61 ± 0.40 –31.9	6.88 ± 1.12 –38.5	3.91 ± 0.38 –65.0
	F	11.25 ± 2.17	11.11 ± 4.81 –1.24	6.42 ± 0.29 –42.9	4.04 ± 0.37 –64.1	1.81 ± 0.21 –83.9
Total protein	M	42.51 ± 1.96	28.01 ± 1.79 –34.1	21.92 ± 4.80 –48.4	18.54 ± 2.56 –56.4	10.38±1.53 –75.6
	F	43.12 ± 5.78	37.97 ± 5.49 –11.94	19.11 ± 0.83 –55.7	14.25 ± 1.10 –66.6	5.96 ± 0.43 –86.2
Free amino acids	M	1.17 ± 0.06	1.98 ± 0.9 +69.2	2.07 ± 0.19 +76.9	16.68 ± 0.19 +1325.6	69.37 ± 0.43 +5829.1
	F	1.13 ± 0.11	1.37 ± 0.27 +21.2	1.94 ± 0.05 +71.7	44.64 ± 0.34 +3850.4	60.08 ± 1.78 +5216.8

+ indicates % increase over control; – indicates % decrease over control.

Protein: Acidic proteins exhibited a significant decline (P<0.01–0.001) in fluorotic animals of both sexes compared to the control. In males, the depletion in acidic proteins was significant (P<0.001) in group IV vs V. In females, a significant decrease (P<0.001) was recorded in group II vs III and group IV vs V.

Basic proteins showed no significant differences in male and female rabbit treated with 5 mg NaF. In the remaining experimental groups, basic proteins exhibited a significant decline (P<0.001). In males, there was a decrease in basic proteins in group II vs III and group IV vs V (P<0.001). In females, they decreased in group II vs III (P<0.05), group III vs IV (P<0.001), and group IV vs V (P<0.001).

Total proteins in females had a higher percent decrease (86.2%) than in males (75.6%).

Free amino acids: In males, the adrenal free amino acids were significantly elevated (P<0.001) in all fluoridated groups of animals. These remained at statistically constant levels in group II vs III and rose in group III vs IV and group IV vs V (P<0.001). In females, free amino acids showed no statistically significant differences in animals treated with 5 mg NaF/kg bw. In the other test groups, the adrenal free amino acids in females registered a significant increase in group II vs III (P<0.01), group III vs IV (P<0.001), and group IV vs V (P<0.001). In group V the males showed higher percent increase (5829.1%) in free amino acids in comparison to the females (5216.8%).

Table 3 summarizes the effects of sodium fluoride on lipid biosynthesis in the adrenal gland.

Total lipids: Differences in the level of total lipids in the adrenal gland of fluoridated and control rabbits of both sexes were significant (p<0.001). Males showed higher percent elevation (398.3%) in total lipids compared to females (365.4%).

Phospholipids: In males, the concentration of phospholipids in the adrenal gland exhibited a significant (P<0.001) fall in animals treated with 5 and 10 mg NaF/kg bw, followed by sudden rise (P<0.001) in animals treated with 20 mg NaF/kg bw. In females, the level of phospholipids fell significantly (P<0.001) in animals administered 5, 10, and 20 mg NaF/kg bw. In both sexes, the level of phospholipids returned to the control value in animals given 50 mg NaF/kg bw. In both sexes, quantity of phospholipids showed an increase in group II vs III (P<0.001) and group III vs IV (P< 0.001). In males, the phospholipids decreased in group IV vs V (P<0.001), whereas in females they increased (P<0.001).

Table 3. Lipid parameters in the adrenal gland of rabbits after 15 weeks in the control and fluoride treated groups (12 animals in each group; each value is the mean ± SD of nine observations)

Biochemical parameter mg/g tissue weight	Sex	Group (NaF dose mg/kg-bw/day)				
		I Control	II 5 mg NaF	III 10 mg NaF	IV 20 mg NaF	V 50 mg NaF
Total lipids	M	160.64 ± 13.37	221.16 ± 6.10 +37.7	260.81 ± 16.00 +62.4	388.39 ± 27.99 +141.8	800.45 ± 71.35 +398.3
	F	89.04 ± 8.80	154.45 ± 17.01 +73.5	248.32 ± 21.03 +178.9	276.29 ± 4.90 +210.3	414.37 ± 8.06 +365.4
Phospholipids	M	63.07 ± 3.76	36.52 ± 5.98 –42.1	57.41 ± 0.88 –8.9	145.83 ± 18.04 +131.2	63.57 ± 2.47 +0.8
	F	86.90 ± 2.06	32.32 ± 1.15 –62.8	40.78 ± 0.31 –53.1	48.60 ± 1.22 –44.1	88.05 ± 0.21 +1.3
Triglycerides	M	39.53 ± 5.43	44.24 ± 14.57 +11.9	74.95 ± 0.28 +89.6	156.19 ± 3.30 +295.1	750.00 ± 20.00 +1797.3
	F	25.95 ± 3.61	35.09 ± 12.39 +35.2	74.39 ± 0.64 +186.7	89.82 ± 2.43 +246.1	200.95 ± 13.30 +674.4
Free fatty acids	M	47.69 ± 0.53	26.33 ± 4.61 –44.8	23.01 ± 0.11 –51.8	18.76 ± 0.95 –60.7	15.04 ± 1.32 –68.5
	F	34.67 ± 0.66	25.50 ± 0.53 –26.4	19.43 ± 0.61 –44.0	18.08 ± 1.97 –47.9	11.09 ± 0.97 –68.0
Cholesterol	M	33.53 ± 1.23	72.87 ± 10.99 +117.3	21.46 ± 0.16 –35.9	22.28 ± 0.99 –33.5	16.00 ± 3.46 –52.3
	F	25.90 ± 1.65	24.69 ± 0.64 –46.7	15.10 ± 1.40 –41.7	11.65 ± 0.48 –55.0	6.10 ± 0.45 –76.4

+ indicates % increase over control; – indicates % decrease over control.

Triglycerides: The levels of triglycerides in the adrenal gland were highly elevated ($P<0.001$) in fluorotic animals of both sexes except in animals given 5 mg NaF/kg bw. In both sexes, triglycerides increased significantly ($P<0.001$) in group II vs III, group III vs IV, and group IV vs V. A much higher percent increase was seen in males of group V (1797.3%) compared to the females (674.4%).

Free fatty acids: Fluoride caused a significant ($P<0.001$) decrease in adrenal free fatty acids at each dose in the experimental groups of both sexes compared to the control. In male rabbits, there was a significant fall of contents in group II vs III ($P<0.05$), group III vs IV ($P<0.001$), and group IV vs V ($P<0.001$). In females, the decrease in free fatty acids of adrenal gland was significant ($P<0.001$) only in group II vs III and group IV vs V.

Cholesterol: The cholesterol content of adrenal gland displayed a sudden rise ($P<0.001$) in male animals treated with 5 mg NaF/kg bw followed by rapid decline ($P<0.001$) in subsequent groups. This decline was highly significant in group II vs III ($P<0.001$) and group IV vs V ($P<0.01$). In females, the level of cholesterol was unchanged in group II compared to the control, but it decreased significantly in group II vs III ($P<0.001$), group III vs IV ($P<0.01$), and group IV vs V ($P<0.001$).

DISCUSSION

The significant decline of DNA and RNA in the adrenal gland of fluoridated rabbits in this investigation may be due to alterations in DNA polymerase activity and changes in enzymes involved in nucleic acid synthesis.[7,9] Some enzymes, such as peptidases, alpha amylases, phosphatases, and ATPases, are activated by calcium ions and are inhibited by fluoride due to calcium binding to fluoride in the catalytic centre.[27] Fluoride also inhibits protein synthesis *in vitro* and *in vivo*.[28] In the present study, the level of proteins in adrenal gland exhibited a significant decline in the NaF treated rabbits. This decrease in the protein synthesis may be due to impairment of peptide chain initiation,[29] decrease in mRNA transcription,[30] and inhibition of DNA synthesis.[31] Fluoride inhibits key enzymes of the glycolytic pathway and thus reduces energy metabolism and protein synthetis.[32] Earlier studies from our laboratory revealed a dose-dependent decline in protein levels in skeletal muscle[33] and brain[34] in experimental fluorosis in rabbits.

Fluoride also inhibits amino acid uptake by cells and reduces protein synthesis.[35] In addition, it affects the action of Na-K-ATPase in the cell membrane which may influence the transport of amino acids into the cell.[36] Moreover, it has a very strong ability to form a hydrogen bond with the phenolic hydroxyl group of tyrosine in proteins to disrupt the normal spatial conformation of various proteins.[37]

Likewise, fluoride disturbs normal carbohydrate metabolism, depresses glycogen turnover, and decreases the level of glucose-1-phosphate dehydrogenase, thereby elevating glycogen in muscles and reproductive organs.[38] Significant accumulation of glycogen in the adrenal gland of rabbits of groups II, III, IV, and V in the present study could therefore be due to inhibition of glycolysis by fluoride, decrease in isocitrate dehydrogenase, and reduction of phosphorylase activity, an enzyme which catalyses the conversion of glycogen into glucose-1-phosphate. Increase in catecholamines, the hormones secreted by the adrenal medulla to regulate carbohydrate metabolism, may also be responsible for the increase in glycogen and disturbed carbohydrate metabolism.[38] In agreement with this view, Chinoy *et al*[39] found higher levels of ascorbic acid in the adrenal gland male rats as a consequence of stress imposed by the fluoride.

According to Das and Susheela,[12] fluorotic human populations and NaF treated rabbits suffer from adrenal hypofunction. In patients with osteofluorosis, they thus found hypocortisolemia. Suketa and Terui[40] observed lower serum sodium levels in rats after fluoride administration, while potassium levels, on the other hand, increased significantly. They attributed these changes to adrenal hypofunction and cell deterioration. Serum catecholamines increased significantly in fluorotic individuals. Changes in serum calcium, sodium, and potassium levels indicated electrolyte imbalance in fluorotic individuals.[41]

In the present study, hyperlipidemia and hypertriglyceridemia in the adrenal gland of fluoridated animals were observed. These effects have also been found in other soft organs such as brain,[4] testis,[6] and thyroid gland[8] in rabbits following fluoride administration. Distinct hypercholesterolemic effects in the serum were observed in animals after exposure to fluoride.[42] The level of serum testosterone and hepatic cholesterol decreased in rats given 100 and 200 mg NaF/L in drinking water.[43] In acute fluoride poisoning stimulation of epinephrine secretion from adrenal medulla has been recorded.[44]

Rao and Susheela[45] noticed decreased activity of Δ^5-3β-hydroxysteroid dehydrogenase in chronic fluoride intoxication, thereby suggesting impaired steroid production. In agreement with this view, fluoride was found to have an inhibitory effect on hepatic cholesterol and free fatty acid synthesis in fluoride treated rabbits.[28] In male rats, however, NaF treatment did not cause any alteration in the levels of serum cholesterol.[39] On the other hand, Saralakumari *et al*[46] recorded a decrease in plasma free fatty acids as well as total lipids, along with an increase in serum cholesterol in rats exposed to fluoride in drinking water for 60 days.

Fluoride is also known to damage cell membranes. The weakening of lipid metabolism by fluoride may be due to repression of enzymes responsible for lipid transformation, *e.g.*, lipases, nonspecific esterases,[27] and the complete blockage of pyrophosphate activity,[47] thereby repressing oxidation of fatty acids. In the present study, administration of 50 mg NaF/kg bw to rabbits restored the content of phospholipids to their initial values in the adrenal gland.

The present data clearly indicate metabolic and functional imbalance in the adrenal gland in fluorosis, leading to adrenal dysfunction.

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