CHRONIC TREATMENT WITH A MILD DOSE OF NaF PROMOTES DYSLIPIDEMIA IN RATS

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SUMMARY: Excessive fluoride ion (F) intake promotes systemic metabolic alterations, such as a decrease in insulin secretion, hyperglycemia, and insulin resistance, similar to those observed in diabetes. Cardiovascular disease is responsible for a large degree of morbidity and mortality in individuals with diabetes, and dyslipidemia is considered to be one of the most important risk factors for the development of cardiovascular disease. This study aimed to evaluate the chronic effect of NaF on insulin signal transduction in liver and in muscle, and to determine the plasma concentrations of triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and very low-density lipoprotein cholesterol (VLDL-C). Seven-week-old castrated male Wistar rats were randomly distributed into a control group, which received 76.4 mg/L NaCl in their drinking water, and a fluoride group, which received 54.9 mg/L NaF in their drinking water and F in their food pellets (estimated total F intake: 4.0 mg/kg body weight (bw)/day). After 42 days, the insulin receptor substrate-1 (IRS-1) serine phosphorylation status in the liver and muscle tissues and plasma concentrations of triglycerides, total cholesterol, HDL-C, LDL-C, VLDL-C and F were evaluated. The chronic treatment with F promoted an increase in the plasma concentrations of TG, TC, VLDL-C and F but no alteration in the plasma concentrations of HDL-C and LDL-C or in the IRS-1 serine phosphorylation status in liver or muscle tissue. These results demonstrate that chronic treatment with NaF (4.0 mg/kg bw/day) promotes dyslipidemia in castrated rats but does not interfere with the IRS-1 serine phosphorylation status, one of the inhibitory pathways of the insulin signal in muscle and liver tissues.

Keywords: Cholesterol; Diabetes mellitus; Dyslipidemia; Insulin resistance; Insulin receptor substrate-1; IRS-1 serine phosphorylation status; Triglycerides.

INTRODUCTION

The consumption of the fluoride ion (F) must be controlled and maintained within the recommended parameters, because excessive F intake can promote toxic effects on the endocrine system, resulting in disorders and alterations in carbohydrate metabolism, glucose tolerance, and insulin signaling.[1,2]

Lupo et al. demonstrated that plasma insulin levels increased as the F concentration of the drinking water increased, producing insulin resistance.[3] Another study showed that F can reduce insulin signaling.[1]

Chronic F intake promotes a decrease in the insulin receptor substrate (pp185 - IRS-1/2) tyrosine phosphorylation status of muscle and white adipose tissue and an increase in the IRS-1 serine phosphorylation status of white adipose tissue, resulting in insulin resistance.[4,5]

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Type 2 diabetic subjects normally show changes in lipid profile, and evidence suggests that insulin resistance plays a central role in the development of dyslipidemia, since it causes an increase in free fatty acid release by insulin-resistant adipose cells.[6] Dyslipidemia is known to promote an increased risk of cardiovascular disease.[7]

Recognizing that F can promote an alteration of the secretion of insulin and, in consequence, in the metabolism of lipids and carbohydrates, the current study aimed to evaluate the chronic effect of NaF on insulin signal transduction in liver and in muscle, and on plasma concentrations of TG, TC, HDL-C, LDL-C, VLDL-C, and F in rats.

MATERIALS AND METHODS

Animal model: All experimental procedures were approved by the Institutional Committee on Animal Research and Ethics (Protocol No. 2006-08802). Four-week-old male Wistar rats were maintained on a 12-hr light–dark cycle (lights on at 07:00) at a temperature of 23ºC±2ºC, with free access to a regular laboratory rat diet (LABINA Indústria de Rações do Brasil LTDA, Paulínia, Brazil) and water. The rats were castrated to prevent any influence of testosterone. After 21 days, the 32 seven-week-old castrated rats were distributed into two groups that were treated with drinking water containing NaCl or NaF. The control group (n = 16) received an average of 76.4 mg/L NaCl in their drinking water throughout the experiment; the F group (n = 16) received an average of 54.9 mg/L NaF in their drinking water throughout the experiment, plus an F supplement in their food pellets (total estimated F intake = 4.0 mg F/kg bw/day, from 3.1 mg F/kg bw/day in their drinking water and 0.9 mg/kg per day in their diet). For 42 days, body weight, amount of feed, and daily volume of water consumed per rat were measured at 2- or 3-day intervals. At the end of this period, the control and F group rats were starved for 14 hr, and the F group rats were deprived of the NaF solution for 4 hr prior to the day of the experimental measurements to avoid an acute F effect. The F and control groups were then anesthetized with sodium thiopental (Thiopental® 3%, 5 mg/100 g bw, i.p.). Each group was divided into two subgroups for determination of the insulin receptor substrate-1 (IRS-1) serine phosphorylation status and the quantification of plasma concentrations of TG, TC, HDL-C, LDL-C, VLDL-C, and F. Six control and six F-treated rats were used to quantify the IRS-1 serine phosphorylation status in the liver and muscle tissue before and after the administration of regular human insulin (1.5 U, i.v.). A median laparotomy was performed in 10 control and 10 F-treated rats, and blood samples were collected from the inferior vena cava. The plasma was stored at –70ºC until biochemical analysis.

Assessment of the IRS-1 serine phosphorylation status: The IRS-1 serine phosphorylation status was quantified according to the method described by Carvalho et al.[8]

Determination of triglycerides, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and very low-density lipoprotein cholesterol levels: Blood samples were collected from the inferior vena cava in heparinized tubes to measure the plasma concentrations of TG, TC, and HDL-C. After centrifugation, the supernatant was collected and stored at –70ºC until use. Plasma concentrations of triglycerides, total cholesterol, and HDL-C were measured using an enzymatic colorimetric method (Labtest Diagnostica S.A. Lagoa Santa, Minas Gerais, Brazil) using a spectrophotometer at wavelengths of 505, 500, and 500 nm, respectively.

Levels of LDL-C and VLDL-C were determined from the values for triglycerides, total cholesterol, and HDL-C, using the Friedewald equation.[9]

Determination of F ion concentration in blood plasma: The F concentration in plasma was determined after 12 hr of diffusion facilitated by the hexamethyldisiloxane[10] using a F ion selective electrode (Orion Research, Cambridge, Mass., USA, model 9409).

Statistical analysis: Two analyses were performed: 1) ANALYSIS OF INSULIN SIGNALING: The normality of the data set was verified. Data were analyzed by analysis of variance. 2) ANALYSIS OF PLASMA CONCENTRATIONS OF TG, TC, HDL-C, LDL-C, VLDL-C AND F: Data were analyzed using the Student's *t*-test for non-paired samples.

All numerical values are presented as the mean ± standard error of the mean (SEM), and differences among groups were considered significant when $p < 0.05$.

RESULTS

Table 1 shows the means of the control and F group rats: 1) the body weight; 2) the daily volume of water consumed per rat, on the first day, the twenty-first day and the forty-second day of F treatment.

Table 1. Parameters of the control and fluoride groups of rats (mean±SEM; n=10)

Parameter	1st day		21st day		42nd day	
	Control group	Fluoride group	Control group	Fluoride group	Control group	Fluoride group
Body weight (g)	260.2 ±2.9	259.5 ±2.0	326 ±6.2	328.4 ±3.4	353.8 ±3.3	351.6 ±2.8
Daily volume of water consumed per rat (mL)	43.5 ±0.5	43.9 ±0.3	43.75 ±0.4	44.5 ±0.3	44.0 ±0.3	43.5 ±0.1

Table 2 shows the plasma concentrations of F, TG, TC, HDL-C, LDL-C, and VLDL-C in the control and F groups. The group treated with sodium fluoride showed a significant increase in plasma concentrations of TG, TC, and VLDL-C compared to the control group. No significant difference in plasma concentrations of HDL-C and LDL-C were observed between the groups. The rats treated with

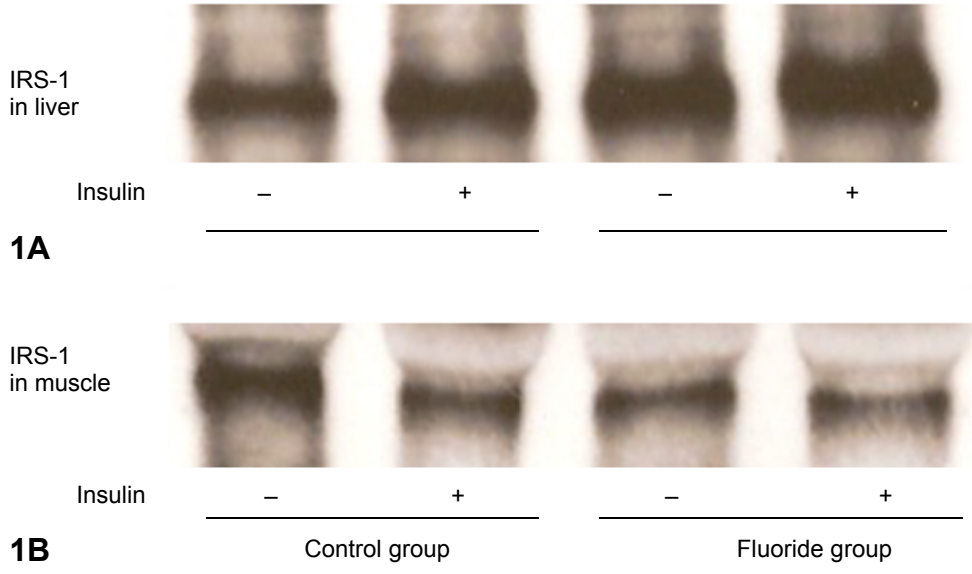
NaF presented a highly significant increase in plasma F in comparison with the control group.

Table 2. Plasma concentrations of TG, TC, HDL-C, LDL-C, VLDL-C, and serum F concentration in the control and fluoride groups (mean ± SEM, n=10)

Parameter	Control group	Fluoride group
TG (mg/dL)	30.65±3.6	46.93±2.7*
TC (mg/dL)	76.15±2.11	87.02±4.164*
HDL-C (mg/dL)	35.4±0.85	35.6±0.5
LDL-C (mg/dL)	36.35±2.84	33.13±4.19
VLDL-C (mg/dL)	6.13±0.72	9.39±0.54*
Fluoremia (µg F/mL)	0.06±0.01	0.12±0.01*

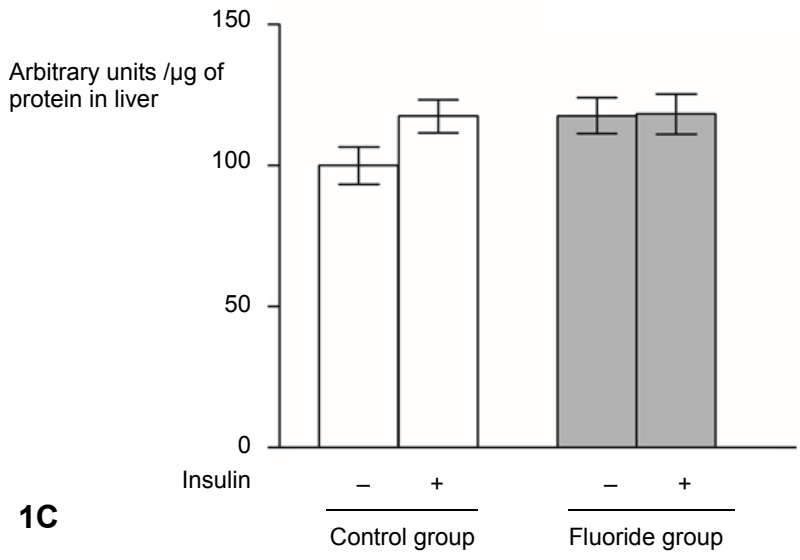
*p<0.05 compared to control group

Figures 1A and 1B show typical autoradiograms of the insulin-stimulated serine phosphorylation status of IRS-1 in liver and muscle tissues, respectively.



Figures 1A and 1B. Insulin-stimulated serine phosphorylation status of IRS-1 in the liver and the muscle of the control and F groups, before (–) and after (+) insulin injection. In 1A and 1C are the typical autoradiograms of the insulin-stimulated serine phosphorylation status of IRS-1 in the liver and the muscle, respectively.

was observed after the insulin stimulus in relation to the baseline in both groups and tissue types (intragroup comparison). In the baseline and post-insulin infusion measurements, the F group also showed no alteration in the IRS-1 serine phosphorylation status in the liver and muscle tissues compared to the control group (intergroup comparison).



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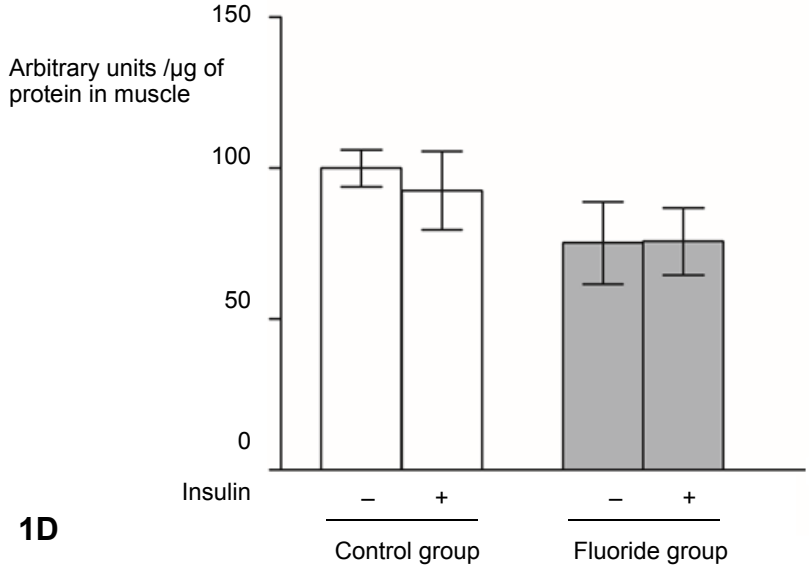

1D

Figures 1C and 1D. Insulin-stimulated serine phosphorylation status of IRS-1 in the liver and the muscle of the control and F groups, before (–) and after (+) insulin injection. 1C and 1D show the data, of the autoradiograms of the insulin-stimulated serine phosphorylation status of IRS-1, expressed in arbitrary units per µg of protein in the liver and the muscle, respectively. The bars represent the mean±SEM of 6 samples from 6 separate experiments.

DISCUSSION

Chronic treatment with NaF (4.0 mg F/kg bw/day) promoted an increase in the plasma concentrations of TG, TC, and VLDL-C compared to the control group (Table 2). These findings are in agreement with several studies that have suggested that F caused changes in the lipid profile, which is a major risk factor for the development of cardiovascular diseases.[11-13] Afolabi et al.[11] performed a study on male rats exposed to 50 mg/L and 100 mg/L of F through drinking water for seven weeks and observed that both concentrations promoted hypercholesterolemia and decreased HDL-C levels, and the 100 mg/L concentration induced hypertriglyceridaemia. Similarly, in the present study, an increase in plasma concentrations of TC and TG was observed in the group treated with F. It should be noted that in the present study, the rats were exposed to a dose of 20 mg/L of F through drinking water. Additionally, another study performed by Sanjay et al.[14] showed that when animals fed on a high cholesterol diet were exposed to F (221.043 mg/L of NaF) through drinking water, the plasma lipid profiles were further elevated.

The changes observed in the lipid profile of the animals chronically treated with NaF are also in accord with the results obtained by Czerny et al.[12] In a study performed in rats, the authors showed that exposure to ammonium fluoride at a concentration of 2 mg/m^3, administered in aerosol form over 3 months, 6 hr per day and 5 days per week in a toxicological chamber, promoted changes in the lipid metabolism of the animals, which exhibited an increase in serum levels of TC (29%) and TG (26%).[12] These alterations in lipid metabolism may be related to changes in the activities of enzymes responsible for lipid transformation, such as triglyceride lipase, nonspecific esterase, and pyrophosphatase.[15-19]

Excessive serine phosphorylation of insulin receptor substrate proteins promotes a decrease in insulin signal transmission by reducing the capacity of the receptor to phosphorylate tyrosine after insulin stimulation, causing insulin resistance.[20] The present study demonstrated that chronic treatment with NaF does not promote alterations in the IRS-1 serine phosphorylation status after insulin stimulation, in either liver or muscle tissue. There was also no significant difference in IRS-1 serine phosphorylation status after insulin stimulus in relation to the baseline in liver tissue. The absence of an alteration of the IRS-1 serine phosphorylation status in hepatic tissue is in agreement with the study published by Chiba et al., that detected no alteration in the pp185 (IRS-1/2) tyrosine phosphorylation status after insulin stimulation in the liver of castrated rats chronically treated with NaF (4.0 mg F/kg bw/day).[4] Cho et al.[21] suggest that the liver can play an important role to compensate for the insulin resistance observed in the adipose tissue to prevent hyperglycemia.

The chronic treatment of rats with NaF did not promote any alteration in the IRS-1 serine phosphorylation status in the muscle tissue after insulin stimulation. However, previous studies using the same experimental model as in the present study verified that chronic NaF intake induced a decrease in the pp185 (IRS-1/2) tyrosine phosphorylation status in muscle tissue after insulin stimulation.[4]

Although no change was observed in the IRS-1 serine phosphorylation status, the decrease in insulin signal in muscle tissue may have been caused by other factors, such as increased activity of protein tyrosine phosphatases such as PTP1B.[22] This protein tyrosine phosphatase is responsible for negatively regulating insulin signaling by directly promoting dephosphorylation of the phosphotyrosine residues of the insulin receptor kinase activation segment.[23]

In the analysis of the plasma F ion concentration, a statistically significant increase in the F group compared to the control group was observed (Table 2), thereby confirming the relation between F ingestion and absorption by the body.

The F dose used in this study was based on studies that evaluated the amount that a child can ingest daily during tooth brushing with a fluoridated dentifrice (0.155 mg F/kg bw/day) and through diet (0.045 mg F/kg bw/day).[24] This dose was increased 20-fold, based on studies performed in humans and in rats in which the researchers observed that it is necessary to increase the F dose in rats by this proportion to reach a similar plasma level in the two models.[25] The more rapid rate of bone turnover in rats, compared to humans, can explain the necessity for the higher dose in rats. It has been well established that physical activity can decrease the incidence of mortality from cardiovascular disease and enhance lipid lipoprotein profiles. It should be noted that a recent study indicated that the performance of daily physical activity could reduce the negative effects of chronic ingestion of NaF on glucose homeostasis.[26]

Based on the present results, we can conclude that chronic treatment with NaF promoted: 1) an increase in plasma concentrations of TG, TC, and VLDL-C; 2) no alteration in plasma concentrations of HDL-C and LDL-C; 3) no alteration in the IRS-1 serine phosphorylation status in liver and muscle tissue. Therefore, knowing that chronic NaF intake is capable of promoting dyslipidemia, improved control of exposure to fluoridated products becomes very important, especially for diabetic people, for whom excessive F consumption may increase the risk of developing cardiovascular disease, which is responsible for high rates of morbidity and mortality in people with diabetes.

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