EFFECTS OF FLUORIDE INTAKE ON INSULIN SENSITIVITY AND INSULIN SIGNAL TRANSDUCTION

Keila Aziz Chehoud,[a,b] Fernando Yamamoto Chiba,[a,b] Kikue Takebayashi Sassaki,[a]
Cléa Adas Saliba Garbin,[b] Doris Hissako Sumida[a]
Araçatuba, Brazil

SUMMARY: Forty seven-week-old male castrated Wistar rats were randomly divided evenly into a control group and a fluoride (F) group. The latter was given a single dose of NaF from a solution containing 1.0 mg F ion/kg bw, administered by gavage. After 30 min, the following experiments were performed: intravenous insulin tolerance test (0.75 U/kg bw) and determination of the insulin receptor substrate (pp 185–IRS-1/IRS/2) tyrosine phosphorylation status. The acute treatment with NaF promoted increased blood glycemia, but there were no significant changes in the insulin sensitivity and in the pp185 tyrosine phosphorylation status in the muscular or in the white adipose tissues.

Keywords: Insulin receptor substrate; Insulin sensitivity; Insulin signal; Plasma fluoride; Rats and insulin.

INTRODUCTION

Excessive ingestion of fluoride (F) causes chronic or acute intoxication such as dental fluorosis[1] and alterations in carbohydrate metabolism.[2,3,4] Trivedi et al. showed that 40% of patients with endemic fluorosis had impaired glucose tolerance, but this anomaly was reversed by removing the excess F from drinking water.[2] Rigalli et al. found diminished insulin secretion both in patients and in rats that ingested high doses of NaF.[3] In later studies Rigalli et al., showed that when NaF disseminated in the plasma is above 5 μmol/L, it affects glucose homeostasis.[4] Furthermore, NaF causes glycolysis inhibition,[5] and depletion of hepatic and muscular glycogen.[5,6] Knowing that F can interfere with carbohydrate metabolism, we felt it was important and fundamental to undertake a study to examine the acute effect of NaF on insulin sensitivity and insulin signal transduction.

MATERIALS AND METHODS

Animals: Four-week-old Wistar rats, obtained from the Araçatuba Dental School Central Vivarium (Paulista State University, Araçatuba – SP) were kept under a 12/12 hr light/dark cycle (light on at 0700) and temperature of 23±2ºC, with free access to regular diet (Guabi, 26.3 mg F/kg) and tap water (0.3 mg F/L). The rats were castrated at 4 weeks of age to avoid any influence of testosterone. After 21 days the experiments were performed to allow the animals to have a sufficient amount of periepididymal white adipose tissue. Forty of the seven-week-old castrated rats were divided into two groups: the control group (n=20) received NaCl solution by gavage (3.2 mg Na/kg bw), and the F group (n=20) received NaF solution by gavage (1 mg F/kg bw). Sodium thiopental (Thiopentax® 3%, 5 mg/

[a]Department of Basic Sciences, Dental School of Araçatuba, São Paulo State University – UNESP, Brazil. [b]Department of Child and Social Dentistry, Dental School of Araçatuba, São Paulo State University – UNESP, Brazil. For Correspondence: DH Sumida, Dental School of Araçatuba, São Paulo State University –UNESP, Brazil, R. José Bonifácio, 1193, CEP: 16015-050, Araçatuba-SP, Brazil. E-mail: dorishs@foa.unesp.br

100 g bw, i.p.) was used for anesthetization. Thirty minutes after the NaF and NaCl administration, each group was subdivided into three groups in order to measure plasma fluoride levels, to perform insulin tolerance test and to measure the phosphorylation status of the insulin receptor substrate (IRS). Median laparotomy was performed in 10 control and 10 F-treated rats, and blood was collected from the inferior cava vein. The plasma was stored at –20ºC until the F was determined. Five control and 5 F-treated rats were submitted to the intravenous insulin tolerance test (0.75 U insulin/kg bw). Five control and 5 F-treated rats were also used to quantify the IRS tyrosine phosphorylation status in insulin-sensitive tissues: gastrocnemius muscle and periepididymal white adipose tissue (WAT), after insulin (1.5 U, i.v.) stimulation. The design of this research conforms to the Ethical Principles in Animal Experimentation adopted by the Brazilian College of Animal Experimentation and was approved by the Ethics Commission on Animal Experimentation at a meeting on 09/21/2004, in accordance with Protocol No. 49/04.

Determination of F ion concentration in blood plasma: To analyze F in plasma, pre-diffusion with heated hexamethyldisiloxane (HMDS)-H_2SO_4 was done to remove CO_2. The F concentrations in plasma were determined after 12 hr of HMDS-facilitated by diffusion[7] modified by Whitford[8] using a F ion selective electrode (Orion Research, Cambridge, Mass., USA, model 9409) and a calomel reference micro–electrode (Accumet, #13-620-79) joined and coupled to a potentiometer (Orion Research, model EA 940), as described by Buzalaf et al.[9]

Short intravenous insulin tolerance test (ITT): The tests were performed in the morning after a 12-hr fast. The animals were anesthetized and the right hand jugular vein was cannulated under anesthesia by introducing a "Sylastic" cannula close to the atrium (superior cava vein). This cannula served to inject the 0.75 U/kg bw dose of insulin. Blood samples were collected (from the tail section) at 0 (before insulin injection), 4, 8, 12 and 16 min (after insulin injection) and glycemia was measured using a glycemia monitor (One Touch Ultra; Johnson & Johnson, USA). Glucose disappearance speed (K_{itt}/min) is the rate constant of the one-phase exponential decay function that fits the values of glucose after 4 min.[10]

Assessment of the insulin receptor substrate (pp185–IRS-1/IRS-2) tyrosine phosphorylation status: The tests were performed in accordance with the method described by Carvalho et al.[11] The samples of tissues were submitted to the Western Blotting method to quantify the tyrosine phosphorylation status of pp185, after insulin stimulation, using the antiphosphotyrosine antibody (Santa Cruz Technology, Santa Cruz, CA).

Statistical Analysis: All values were presented as mean±SEM. Statistical analysis was done by the Student t test for non-paired samples, and the differences between the two groups were considered significant when $p<0.05$.

RESULTS

As seen in Table 1, the F-treated rats presented a highly significant increase in plasma F in comparison with the control group.

Table 1. Concentration of plasma fluoride µg F^-/mL) in control and fluoride groups (mean±SEM, n=10)

Control group	Fluoride group
0.0362±0.0017	0.4136±0.0870*

*p<0.0005 compared to control.

In the glycemia comparison (Figure 1) at each period of time, the group treated with NaF showed significantly higher glycemia compared with the control group except after 8 min.

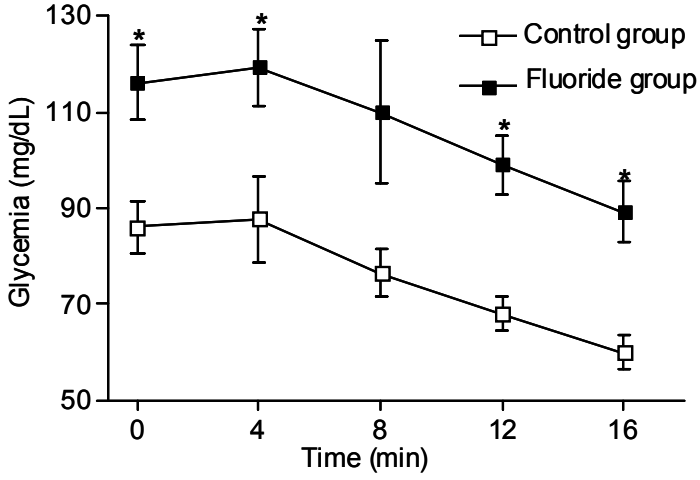

Figure 1. Intravenous insulin tolerance test in control and F groups (mean±SEM, n=5). The samples were collected at the times of 0 (basal), 4, 8, 12, 16 minutes, after insulin injection. *p<0.05 compared to control.

Table 2 shows the glucose disappearance rate (K_{itt}), during the insulin tolerance test, performed in the first 16 min after the hormone infusion in the control and F groups. No statistically significant inter-group differences were found in the K_{itt} values.

Table 2. Glucose disappearance rate (Kitt) in control and fluoride groups (mean±SEM, n=5)

Control group	Fluoride group
2.3±0.36	1.77±0.25*

*p<0.05 compared to control.

Figures 2A and 2C show a typical autoradiogram with reference to the insulin-stimulated tyrosine phosphorylation status of pp185 in the muscle and white adipose tissue (WAT), respectively. Figures 2B and 2D show the results, expressed in arbitrary units per µg of protein. No alteration was found in the phosphorylation of this substrate between control and F groups in both tissues.

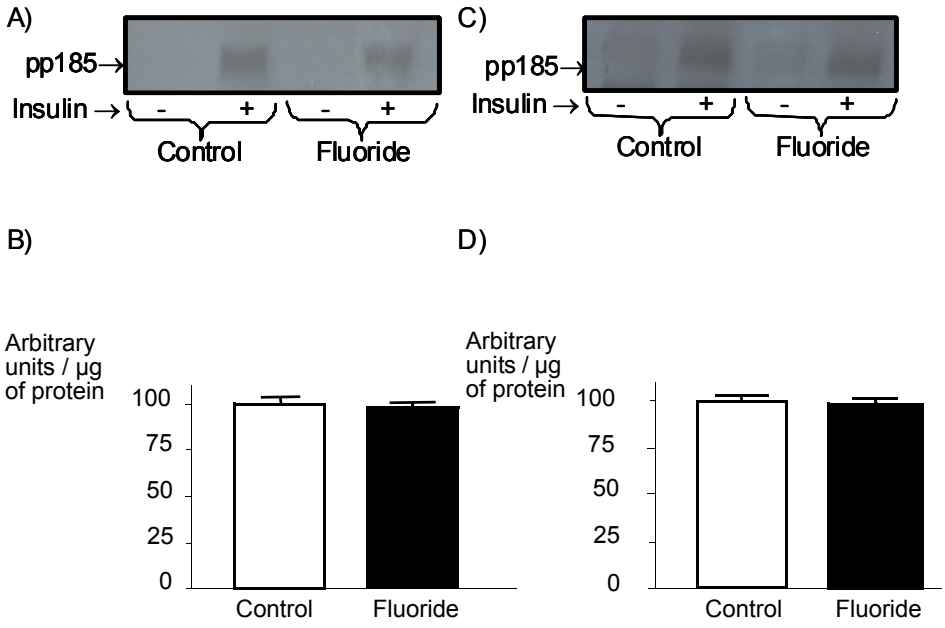

Figure 2. Insulin-stimulated tyrosine phosphorylation status of pp185 (IRS-1/IRS-2) in the muscle and in the WAT of control and F groups, before (-) and after (+) insulin infusion. In A and C, typical autoradiogram of muscle and WAT, respectively. In B and D, the results of the insulin-stimulated tyrosine phosphorylation status of pp185 in the muscle and in the WAT, respectively, are expressed in arbitrary units per µg of protein (mean±SEM, n=5).

DISCUSSION

Acute treatment with high doses of NaF induces hyperglycemia, thereby indicating that glucose homeostasis is affected by F.[3,4,12,13] As seen in Figure 1, the results of the present work, in agreement with that others, confirm that NaF-treated rats presented hyperglycemia 30 min after gavage with NaF. Moreover, hyperglycemia also occurred after insulin infusion (except at the time of 8 min). The mechanism by which NaF induces hyperglycemia may involve one or more factors such as:

a) increased glycogenolysis due to an increase in AMPc[14]

b) increased epinephrine release[14,15]

c) activation or inhibition of certain enzymes[14]

d) reduction in insulin secretion which is likely to increase the rate of glycogenolysis and gluconeogenesis and to decrease the rate of glycolysis and glycogenogenesis.[3]

However, acute treatment with NaF did not alter sensitivity to insulin, shown by the unaltered K_{itt} during the ITT.

When analyzing the plasma fluoride ion concentrations, a statistically significant increase was noted in the F group compared to the control group. This confirms the relation between F ingestion and absorption by the body. In the present study,

the experiments started 30 min after gavage with NaF. Roldi and Cury[16] also noted a significant increase in blood plasma F in humans 30 min after a fluoridated dentifrice was ingested.

In the present study, the F dose used was 20 times higher than the maximum value a child can ingest (0.155 mg/F/day/kg) during one tooth brushing session with fluoridated dentifrice.[17] This dose was used because Rigalli et al., conducting studies in humans and in rats, observed that they needed to increase the fluoride dose by this proportion, administered in rats in relation to the dose in humans, to attain a similar plasmatic peak in the two models. This is probably due to the faster bone turnover rate in rats compared to humans.[3]

As seen in Figure 2, the acute treatment with NaF did not induce any alteration in the pp185 tyrosine phosphorylation status after insulin stimulus in either the gastrocnemius or the WAT. These results may have favored the unaltered sensitivity to insulin between the control and F groups. On the other hand, the *in vitro* studies of Viñals et al. on insulin receptors isolated from rat muscular tissue and human placenta, showed that F induced a reduction in the insulin-induced auto-phosphorylation, and phosphorylation of the exogenous substrates used.[18] This reduction can at least be partly explained by the difference in methodology used among these studies and the present study. It is also relevant that a higher fluoride concentration was used by Viñals et al.[18] The present study showed that the amount of fluoride administered increased glycemia, but did not interfere in pp185 phosphorylation. However, the alterations in phosphorylation of other insulin substrates involved in glycemia homeostasis cannot be overlooked.

In view of this disagreement of results, further studies must be conducted to explain the interference of NaF in the initial stage of insulin action more clearly, as a reduction in insulin receptor phosphorylation has been well correlated with situations of resistance to insulin.[19]

Based on the results obtained here, one can conclude that acute treatment with NaF promoted a significant increase in glycemia, but did not alter sensitivity to insulin and pp185 phosphorylation. Therefore, knowing that a high dose of NaF is capable of altering carbohydrate metabolism, the use of dentifrices with a lower fluoride content is recommended, especially for diabetic children, in whom excessive F consumption may lead to worsening the condition of these children's health.

ACKNOWLEDGEMENTS

This work was supported by grants (05/53301-1) from São Paulo Foundation for Research (FAPESP). We are also thank Mrs Margery Galbraith (native English speaker) and Noemia Akamine Kajimoto for their technical assistance in the preparation of this manuscript.

REFERENCES

1 Burt BA. The changing patterns of systemic fluoride intake. J Dent Res 1992;71(5):1228-37.
2 Trivedi N, Mithal A, Gupta SK, Godbole MM. Reversible impairment of glucose tolerance in patients with endemic fluorosis. Fluoride Collaborative Study Group. Diabetologia 1993;36(9):826-8.
3 Rigalli A, Ballina JC, Roveri E, Puche RC. Inhibitory effect of fluoride on the secretion of insulin. Calcified Tissue International 1990;46:333-8.
4 Rigalli A, Alloatti R, Menoyo I, Puche RC. Comparative study of effect of sodium fluoride and sodium monofluorophosphate on glucose homeostasis in the rat. Arzneimittelforschung 1995;45(3):189-92.
5 Dost FN, Knaus RM, Johnson DE, Wang CH. Fluoride impairment of glucose utilization: nature of effect in rats during and after continuous NaF infusion. Toxicol Appl Pharmacol 1977;41:451-8.
6 Shashi JP, Singh, Thapar SP. Changes in glycogen content in some tissues during fluorosis – an experimental study on rabbits. Fluoride 1988;21:82-6.
7 Taves DR. Separation of fluoride by rapid diffusion using hexamethyldisiloxane. Talanta 1968;15: 969-74.
8 Whitford GM. The metabolism and toxicity of fluoride. New York: Karger; 1996.
9 Buzalaf MAR, Fuhushima R, Granjeiro JM, Cury JA. Correlation between plasma and nail fluoride concentrations in rats given different levels of fluoride in water. Fluoride 2002;35(3)185-92.
10 Bonora E, Moghetti P, Zancanaro C, Cigolini M, Querena M, Cacciatori V, et al. Estimates of *in vivo* insulin action in man: comparison of insulin tolerance tests with euglycemic and hyperglycemic glucose clamp studies. J Clin Endocrinol Metab 1989;68(2):374-8.
11 Carvalho CR, Brenelli SL, Silva AC, Nunes AL, Velloso LA, Saad MJ. Effect of aging on insulin receptor, insulin receptor substrate-1, and phosphatidylinositol 3-kinase in liver and muscle of rats. Endocrinology 1996;137(1):151-9.
12 Sakurai T, Suzuki K, Taki T, Suketa Y. The mechanism of changes in metabolism and transport of glucose caused by fluoride administration to rats [abstract]. Fluoride 1993;26(3):210.
13 Grucka-Mamczar E, Birkner E, Kasperczyk S, Kasperczyk A, Chlubek D, Samujlo D, et al. Lipid balance in rats with fluoride-induced hyperglycemia. Fluoride 2004;37(3):195-200.
14 Allmann DW, Kleiner HS. Effect of NaF on rat tissue cAMP levels *in vivo*. Pharmacol Ther Dent 1980;5:73-78.
15 McGown EL, Suttie JW. Mechanism of fluoride induced hyperglycemia in the rat. Toxicol Appl Pharmacol 1977;40:83-90.
16 Roldi CR, Cury JA. Fluoride metabolism after dentifrice intake. RGO 1986;34(5):425-7.
17 Pessan JP, Silva SMB, Buzalaf MAR. Evaluation of the total fluoride intake of 4-7-year-old children from diet and dentifrice. J Appl Oral Sci 2003;11 (2):150-6.
18 Viñals F, Testar X, Palacin M, Zorzano, A. Inhibitory effect of fluoride on insulin receptor autophosphorylation and tyrosine kinase activity. Biochem J 1993;291:615-22.
19 White MF, Kahn CR. The insulin signaling system. J Biol Chem 1994;269:1-4.