IN VIVO MEASUREMENT OF FLUORIDE EFFECTS ON GLUCOSE HOMEOSTASIS: AN EXPLANATION FOR THE DECREASE IN INTELLIGENCE QUOTIENT AND INSULIN RESISTANCE INDUCED BY FLUORIDE

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ABSTRACT: The fluoride ion (F) is a disturbing substance for the glucose-insulin system. The effects of F have been studied in various aspects. The chronic intake of F generates hyperglycaemia with high plasma insulin levels. This effect is observed in several countries where the content of fluoride in drinking water is greater than the upper limit recommended by WHO (1.5 mg/L). The administration of a dose of F causes a decrease of insulin levels, which was attributed to a decrease in insulin secretion through *in vitro* experiments. However, measurement of insulin secretion *in vivo* has not been done so far. Moreover, in endemic fluorosis areas children had lower intelligence quotient (IQ) than children of areas with low F in drinking water. This decrease in IQ has also been observed in rats. In this work, the glucose uptake rate of insulin-independent tissues, insulin secretion, and insulin clearances were measured *in vivo* in rats that received a dose of F. A lower secretion and clearance of insulin were found in animals that received F. In addition, a decrease in glucose uptake rate from insulin-independent tissues was observed. This glucose uptake is mainly the glucose consumed by the nervous system. As a consequence, this decrease could be associated with the effect of fluoride on IQ.

Key words: Glucose homeostasis; Insulin; *In vivo* effects; IQ; Rats.

INTRODUCTION

Fluorine is the most electronegative element of periodic table and in nature it is found as the fluoride ion (F). F enters the body spontaneously or as a therapeutic resource. Despite F benefits due to its anti-cariogenic activity, particularly when applied topically as toothpaste,[1] this ion can produce dental and/or skeletal fluorosis.[2] However, the deleterious effects of F are not limited to the hard tissues and several toxic effects on the endocrine system have been reported such as disorders of glucose homeostasis.[3] The administration of a dose of F causes a decrease of insulin levels, which was attributed to a decrease in insulin secretion through *in vitro* experiments.[4,5] However, the measurement of insulin secretion *in vivo* has not been done so far. On the other hand, the chronic intake of F generates hyperglycaemia with high plasma insulin levels.[6,7,8,9] This effect is observed in several countries[10] where the content of fluoride in drinking water is greater than the upper limit recommended by WHO (1.5 mg/L) like China,[11] India,[12] and Argentina.[13] Moreover, in endemic fluorosis areas children had a lower intelligence quotient (IQ) than children of areas with low F in drinking water.[14,15] This decrease in IQ has also been observed in rats,[16] and is associated with decreases in brain weight, succinate dehydrogenase activity,[17] concentration of neurotransmitters (norepinephrine, epinephrine, and serotonin) in the spinal cord,[18] and nicotinic acetylcholine receptor expression level.[19]

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Since glucose is the main source of energy for the nervous system and F has the ability to inhibit glycolytic enzymes[20] and components of the respiratory chain,[21,22] the authors thought that the evaluation of glucose uptake by the central nervous system might help understand the F effects on IQ.

In a previous paper, a mathematical model for the *in vivo* study of glucose homeostasis was developed.[23] The model had 3 differential equations and 8 parameters that represented the different physiological processes involved in the plasma glucose homeostasis. The equation for the plasma glucose level included parameters associated to the function of liver (k_4, I_{pi}), intestine (k_a and k_0), glucose uptake rate into insulin-independent tissues (k_3), and glucose uptake rate into insulin-dependent tissues (k_2). The equation for plasma insulin level included parameters that represented insulin secretion (k_1) and clearance (k_6) of insulin from the plasma. Thus, the aim of this work was to evaluate *in vivo* the effect of F on the physiological processes involved in glucose homeostasis using the parameters of the aforementioned model.

MATERIALS AND METHODS

The experiments were carried out employing 70-day old female Sprague Dawley rats, fed with balanced food and tap water *ad libitum*. The room of the rats had a dark/light cycle of 12hr/12hr and a temperature of 25±1ºC. Blood samples were obtained from the vein of the tail in heparinized tubes. They were centrifuged and the plasma was saved at –20ºC to measure the glucose and insulin concentrations. All the experiments were performed in accordance with the international ethical guidelines of animal care[24] and the protocol was approved by the Ethics Committee, School of Medicine, Rosario National University.

Experimental groups: Control group (n=10): rats (body weight (bw): 346.6±20.4 g) that received 1 mL of distilled water by orogastric tube before the glucose administration by orogastric tube. NaF group (n=10): rats (bw: 325.2±37.4 g) that received 7.6 mg F/kg of bw in 1 mL of distilled water by orogastric tube 15 min before the glucose administration by orogastric tube.

In vivo measurement of the physiological processes involved in glucose homeostasis: In order to measure *in vivo* the different physiological processes involved in glucose homeostasis, the parameters of the mathematical model previously developed were measured. These parameters represent: insulin secretion (k_1) and its plasma clearance (k_6), the function of the liver (I_{pi}, k_4), intestine (k_a and k_0), glucose uptake rate of insulin-independent tissues (k_3), and glucose uptake rate of insulin-dependent tissues (k_2). A dose of glucose (0.06 g/ 100 g bw) was administered by orogastric tube to rats after 8 hr of fasting. Blood samples were obtained from the vein of the tail, before (0 min) and after the glucose administration (5, 10, 15, 30, 60, 90, 120, and 180 min). The plasma glucose and insulin levels were measured and used to calculate the parameters using a script developed for R. This script fits the mathematical model with values of plasma glucose and plasma insulin using the process developed in previous papers.[23]

Glucose measurement: The glucose concentration was measured with a spectrophotometer using a commercial kit (Glicemia enzimatica AA, Wiener Lab, Rosario, Argentina).

Insulin measurement: The plasma insulin levels were measured by RIA using a commercial kit (Ria kit Rat insulin, Millipore Corporation, Billerica, MA, USA) and a solid scintillation counter.

Statistic analysis: Data are shown as median and range. The results were compared with the Mann Whitney test or Wilcoxon test. Differences were considered significant when $p<0.05$.[25]

RESULTS

Effect of F in plasma insulin level: The effect of F on the plasma insulin level was verified by measuring the plasma insulin levels before and 15 min after NaF administration. The fasting plasma insulin levels after the fluoride intake (308±246.9 pmol/L) were significantly lower than before the fluoride administration (398±244.3 pmol/L), $p<0.05$ Wilcoxon test.

Effect of F on physiological processes involved in glucose homeostasis: The effect of F was evaluated in all the parameters of the model. The parameters were measured in the animals of the control and NaF groups. Statistical differences were found between the groups in: insulin clearance from plasma (k_6 parameter, Figure 1), insulin secretion rate (k_1 parameter, Figure 2), and glucose uptake rate of insulin independent tissues (k_3 parameter, Figure 3), (Mann Whitney test, $p<0.05$).

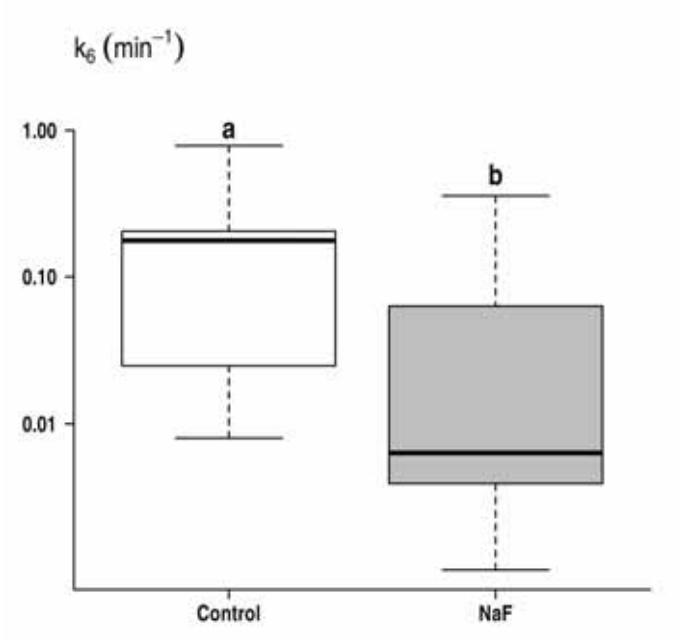

Figure 1. Insulin clearance from plasma (k_6). The graph shows the median, the 25% and 75% quartiles, and the range of the k_6 parameter in animals without F (control) and animals with an oral dose of F (NaF) before glucose administration. Different letters indicate statistically significant differences, $p<0.05$ Mann Whitney test.

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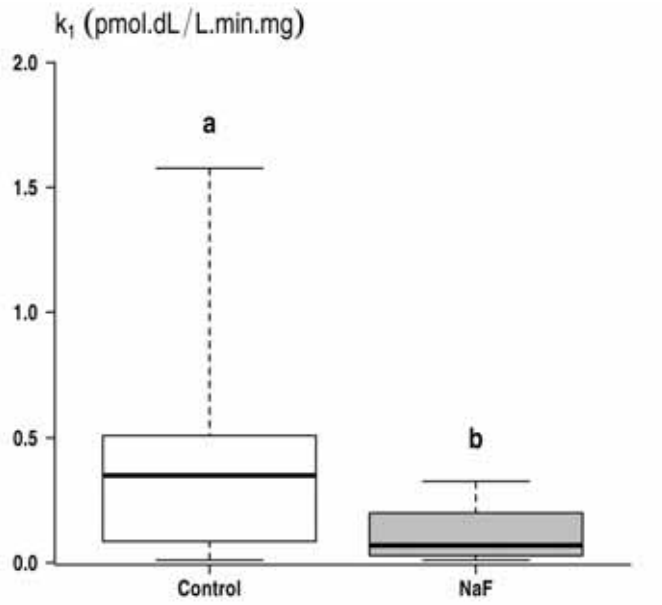

Figure 2. Insulin secretion rate (k_1) parameter in the animals treated with F. The graph shows the median, 25% and 75% quartiles, and the range of the k_1 parameter in animals without F (control) and animals with an oral dose of F (NaF) before glucose administration. Different letters indicate statistically significant differences, $p<0.05$ Mann Whitney test.

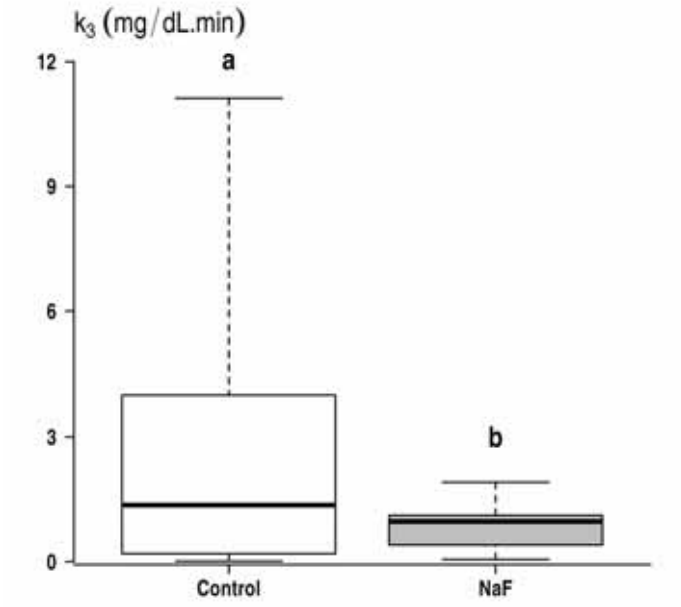

Figure 3. Glucose uptake rate by insulin independent tissues (k_3). The graph shows the median, 25% and 75% quartiles, and the range of the k_3 parameter in animals without F (control) and animals with an oral dose of F (NaF) before glucose administration. Different letters indicate statistically significant differences, $p<0.05$ Mann Whitney test.

DISCUSSION

F is a disturbing substance for the glucose-insulin system. The effects of F have been studied in various aspects. According to the plasma fluoride levels, different effects are observed. After an oral dose, F may cause a decrease in the insulin plasma levels,[4] or, with chronic intake, an increase in the concentration of this hormone.[26] More detailed studies conducted *in vitro* and *in situ* models showed that insulin secretion decreased in the presence of concentrations of fluoride higher than 5 µmol/L. The molecular mechanisms involved would be related to the operation of the signalling systems involving cAMP, protein kinase C, and intracellular calcium.[5] Nevertheless, the *in vivo* effect of fluoride on insulin secretion has not been measured yet. Moreover, high values of insulin could be associated with high insulin pancreatic production or low insulin clearance. Thus, a method for evaluating insulin secretion and clearance separately is very important and useful. For these reasons, the aim of this work was: to evaluate *in vivo* the effect of F in insulin secretion and insulin clearance using the k_1 and k_6 parameters of the model for glucose homeostasis. The parameters were measured in rats treated with F, which was administered as a single dose by a gastric tube. The plasma glucose and insulin levels were measured after an oral glucose dose (OGD) and used for the mathematical model parameter calculation. Fluoride was administered 15 min before the oral glucose administration, due to the delay of fluoride in reaching bloodstream. An oral dose of NaF before the OGD causes a decrease in the insulin secretion parameter (k_1). The decrease of this parameter *in vivo* confirms the decrease of insulin secretion previously observed in *vitro* experiments.[4] On other hand, a lower insulin clearance was found in the animals that received F and this fact could be associated with the high values of insulin plasma levels found with the chronic ingestion of F.[27] However, more experiments in animals chronically treated with F are necessary to confirm this hypothesis.

In addition, changes in the k_3 parameters were found. The k_3 parameter represents glucose uptake rate by insulin-independent tissues and this glucose uptake is mainly the glucose consumed by the nervous system. As a consequence, a decrease in k_3 would indicate a lower uptake of glucose by this system. As glucose is the main source of energy used by the nervous system in normal metabolic situations, this result could indicate an energy restriction on this system. This result could give an explanation for the decreased IQ observed in children living in fluorosis areas which has been described in previous papers. Furthermore, several studies shown an increase in the oxidative stress status of animals treated with F,[28] and that the toxic effects of F are reversed by physical exercise.[27] In previous studies carried out in our laboratory, a decrease in oxygen consumption in animals treated with F was found. This decrease was due to an inhibition of the respiratory chain which causes increased release of superoxide ion and thus increased oxidative stress.[22] Therefore we can postulate the following hypothesis: F decreases oxygen consumption because of a deficit in the respiratory chain. This could lead to a decrease in the formation of ATP and an increase in reduced NADH, which determines a cytosolic decrease in oxidized NAD and therefore an inhibition of glyceraldehyde-3-P dehydrogenase by lack of oxidized

NAD. This decrease in the availability of oxidized NAD would cause a decrease in glycolysis, generating an increase in glucose-6-phosphate that inhibits hexokinase-1. As the entry of glucose into brain is performed by the GLUT-3 glucose transporter which has a low K_m value (1–2 mM),[29] the uptake of glucose by the brain would be limited by the activity of the hexokinase-1 enzyme. This metabolic disturbance leads to decreased brain glucose consumption, which could be the cause of the decline of the IQ. In animals treated with F a decrease in the parameter k_3 (which represents the consumption of glucose independent of insulin, mainly in the nervous system) was observed and this supports this metabolic hypothesis.

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