Revista de la Facultad de Ciencias Médicas de Córdoba

Effect of training on bone quality in rats with insulin resistance induce by fluoride consumption

Efecto del ejercicio físico sobre la calidad ósea en ratas con resistencia a la insulina inducida por el consumo de fluoruro

Efeito do ejercicio físico sobre la calidad ósea en ratas com resistência à insulina inducida pelo consumo de fluoruro

Mercedes Lombarte[1], Brenda Lorena Fina[2], Victoria Inés García[3], Maela Lupo[4], Alfredo Rigalli[5].

DATOS DE AUTORES

1. PhD Bone Biology Laboratory, School of Medicine, Rosario National University, Argentina. E-mail de contacto: mercedes_lombarte@yahoo.com.ar. ORCID: https://orcid.org/0000-0003-1114-319X.
2. PhD Grupo de Descargas Eléctricas, Universidad Tecnológica Nacional Facultad Regional de Venado Tuerto, Argentina. E-mail de contacto: brendafina@gmail.com. ORCID: https://orcid.org/0000-0003-3708-0756.
3. PhD Bone Biology Laboratory, School of Medicine, Rosario National University, Argentina. E-mail de contacto: garciavictoriaines@hotmail.com.
4. University Centre for Environmental Studies, School of Medicine, Rosario National University, Argentina. E-mail de contacto: maela_lupo@hotmail.com. ORCID: https://orcid.org/0000-0002-1331-1591.
5. PhD University Centre for Environmental Studies, School of Medicine, Rosario National University, Argentina. E-mail de contacto: arigalli@conicet.gov.ar. ORCID: https://orcid.org/0000-0002-3953-278X.

Recibido: 2023-09-20 **Aceptado:** 2024-05-02

DOI: http://dx.doi.org/10.31053/1853.0605.v81.n2.42505

Effect of training on bone quality in rats with insulin resistance induce by fluoride consumption

CONCEPTOS CLAVE:

Qué se sabe sobre el tema.

El flúor es un elemento que se encuentran naturalmente en algunas fuentes de agua y cuando su ingesta es elevada tiene numerosas consecuencias para la salud. Afectando a huesos, dientes y también al sistema endócrino. Se sabe que la realización de ejercicio físico puede prevenir el efecto negativo de una ingesta moderada de flúor sobre la resistencia a la insulina.

Qué aporta este trabajo.

Este trabajo muestra que la mayor captación ósea de flúor estimulada por el ejercicio no trae consecuencias negativas para el hueso pero si ayuda a revertir la resistencia a la insulina.

Divulgación

El Flúor comúnmente presente en las pastas dentales es un elemento que ayuda a prevenir las caries y se encuentra naturalmente en algunas fuentes de agua de consumo. Sin embargo, una ingesta elevada puede producir resistencia a la insulina. La realización de ejercicio físico puede revertir las consecuencias nocivas para la salud al estimular la captación de este elemento en los huesos sin causar daño en los mismos.

Effect of training on bone quality in rats with insulin resistance induce by fluoride consumption

Abstract

Palabras clave: fluorine; bone and bones; insulin resistance.

When large amounts of Fluoride are consumed produces insulin resistance, but exercise can reverse insulin resistance in rats, because of a high fluoride uptake by bone tissue. However, bone quality has not been studied in those experiments. Therefore, the aim of this work was to evaluate bone quality in rats treated with fluoride when performing exercise. Sprague-Dawley rats were divided into 3 groups (n=6 per group): Control (drinking water without fluoride), Fluoride (drinking water with fluoride 15 mg/L for 30 days) and Exercise (daily running on a treadmill and drinking water with fluoride 15 mg/L for 30 days). Then, bone mineral density, mechanical and histological properties and bone fluoride level were measured. No effect of treatment on any bone parameters were observed. These results indicate that exercise normalizes glucose metabolism in insulin-resistant rats by bone fluoride uptake; however, this increase in bone fluoride does not manifest in bone deterioration

Efecto del ejercicio físico sobre la calidad ósea en ratas con resistencia a la insulina inducida por el consumo de fluoruro

Resumen

Palabras clave:
ejercicio físico; flúor; huesos; resistencia a la insulina.

Introduction: Cuando se consumen grandes cantidades de fluoruro se produce resistencia a la insulina, pero la realización de ejercicio puede revertir dicho efecto en ratas, debido a una alta captación de fluoruro por el tejido óseo. Sin embargo, la calidad ósea no ha sido estudiada. Por ello, el objetivo de este trabajo fue evaluar la calidad ósea en ratas tratadas con flúor que realizan ejercicio. Se trabajó con ratas Sprague-Dawley que se dividieron en 3 grupos (n=6 por grupo): Control (recibieron agua sin flúor), Flúor (recibieron agua con flúor 15 mg/L durante 30 días) y Ejercicio (realizaron ejercicio diariamente en cinta ergométrica y recibieron agua con fluoruro 15 mg/L por 30 días). Luego, se midieron la densidad mineral ósea, las propiedades biomecánicas e histológicas y el nivel de fluoruro óseo. No se observó ningún efecto del tratamiento sobre ningún parámetro óseo. Estos resultados indican que el ejercicio normaliza el metabolismo de la glucosa en ratas resistentes a la insulina mediante la captación ósea de fluoruro; sin embargo, este aumento del fluoruro óseo no se manifiesta en deterioro óseo.

Efeito do ejercicio físico sobre la calidad ósea en ratas com resistência à insulina inducida pelo consumo de fluoruro

Resumo

Palavras-chave:
exercício físico; flúor; osso e ossos; resistência a insulina

Quando grandes quantidades de flúor são consumidas, a resistência à insulina é produzida, mas a realização do exercício pode reverter esse efeito em ratos, devido a uma alta absorção de flúor pelo tecido ósseo. No entanto, a qualidade óssea não foi estudada. Por isso, o objetivo deste trabalho foi avaliar a qualidade óssea em ratos tratados com flúor que realiza exercício. Foi trabalhado com ratos Sprague-Dawley que foram divididos em 3 grupos (n=6 por grupo): Controle (recebeu água sem flúor), Flúor (recebeu água com flúor 15 mg/L durante 30 dias) e Ejercicio (realizou exercício diariamente em cinta ergométrica e recepção de água com flúor 15 mg/L por 30 dias). Luego, se mediu a densidade mineral ósea, as propriedades biomecânicas e histológicas e o nível de fluoruro óseo. Nenhum efeito do tratamento foi observado em nenhum parâmetro ósseo. Esses resultados indicam que o exercício normaliza o metabolismo da glicose em ratos resistentes à insulina por meio da captação óssea de fluoruro; Contudo, este aumento do fluoruro óseo não se manifesta na deterioração do óseo.

Introduction

Fluoride (F-) is a substance that has been used in the prevention and treatment of dental caries for many years. However, when large amounts are ingested, fluoride gives rise to a condition known as fluorosis[1]. Fluoride enters the body spontaneously or as a therapeutic resource. The main route of F- entry into the organism is drinking water. Several studies have documented the relationship between the concentration of F- in drinking water and fluorosis. Fluorosis is a public health problem that affect millions of people in the word[2]. For this reason, the World Health Organization (WHO) recommends a maximum concentration of 1.5 mg/L of F- in drinking water[9].

Deleterious effects of F- are not limited to bones and teeth, several toxic effects on the endocrine system have been also reported[4]. The chronic ingestion of F- produces insulin resistance[5-6], which was also observed in large parts of the world[7-8] where F- content in drinking water is higher than the recommended by the WHO[9]. Conversely, physical activity improves the effect of insulin on target tissues[10]. Moreover, the performance of daily physical activity could reduce negative effects of chronic ingestion of F- on glucose homeostasis[11-12]. A decrease in insulin resistance was observed in rats that did physical activity and consumed water with 15 mg/L of F-. Normalization of insulin resistance by exercise could be the consequence of the decrease in plasma F- levels and increase in bone F- content due to stimulated bone formation by exercise[13]. A lower plasma F- level and higher bone F- content observed in trained fluoride-treated rats supports the previous hypothesis. On the other hand, harmful effects of F- on bone structure and quality have been reported when fluoride is consumed[14,15]. Therefore, the aim of this study was to evaluate bone quality and strength in rats that reversed fluoride-induced insulin resistance when performing exercise

Materials and methods

Experimental groups

Animals were kept in collective cages with water and balanced food (Gepsa, Pilar, Córdoba, Argentina) *ad libitum*, in a temperature-controlled environment of 23-25ºC, with a 12h-12h light-dark cycle and filtered airflow. Experiments were conducted under international standard rules for animal care[16] and has been approved by the Ethical Committee of the School of Medicine of Rosario National University (N° 6739/2014, November 27 of 2014).

Eighteen 70-day-old female Sprague-Dawley rats (body weight 266.6±7.4g) were randomly assigned to treatments constituting three experimental groups (n=6 per group): Control (drinking water without F^-), Fluoride (drinking water with F^- 15 mg/L) and Exercise (daily running on a treadmill during 60 min at 2.25 m/min and drinking water with F^- 15 mg/L)[12]. A group that realized exercise but drink water without fluoride was not include because the aim of this work is study the bone quality in rats that have insulin resistance consequence of drink water with fluoride. The concentration of 15 mg/L of $F^-$was chosen to simulate the ingestion of artificially fluoridated water by humans, considering that rodents

metabolize F faster than humans[17]. After 30 days of treatment, rats were euthanized by CO_2 inhalation after isofluorane anesthesia 24 h after the last exercise session and with 12 h of fast. Femurs and tibias were obtained for mechanical testing, bone mineral density (BMD), histological analysis, and bone fluoride level measurements. Blood samples were obtained by heart puncture into heparinized tubes and plasma was processed for glucose, insulin, and fluoride measurement[18].

Biochemistry measurement

Plasma glucose levels were measured with colorimetric technique using a spectrophotometer (Perkin Elmer lambda 11, Norwalk, CT. USA) and with a commercial kit (Wiener Laboratorios, Rosario, Argentina).

Plasma insulin levels were measured by RIA (radio inmuno assay) using a commercial kit (Ria kit Rat insulin, Millipore Corporation, Billerica, MA, USA) and with a solid scintillation counter Alfanuclear Cmos (Buenos Aires, Argentina).

Plasma glucose and insulin levels were used to calculate HOMA-IR index (homoeostasis model assessment - insulin resistance) according to:

HOMA-IR = fasting glucose level (mmol/L) x fasting insulin level (µUI/mL)/22.5

This index allows to evaluate insulin resistance, a high value of HOMA-IR index indicates insulin resistance.

Plasma fluoride levels were measured by direct potentiometry using an ion selective electrode ORION 94-09, Orion Research (MS, USA) after isothermal distillation; and for bone fluoride measurement, bone tissue was incinerated for 6 h at 550°C before distillation process[12].

Mechanical testing

The cortical and trabecular bone strength of femurs was determined with a three-point bending test at midshaft, and a compression test in distal epiphysis[19]. The mechanical tests were performed on a mechanical testing machine designed by the engineering department of the Bone Biology Laboratory with a 300 N load cell with 0.01 N of discrimination and an accuracy of 10 µm in displacements. The support span in three-point bending test was 11 mm. The area of the circular compression platen was 7 mm^2 and a 2.5 mm thickness transversal section of distal epiphysis of femurs was used. In both tests, the speed was 0.01 mm/s and was monitored on a computer. Load versus displacement plots were recorded by the software Biomedical Data Acquisition Suite 1.0, (Argentina, 2011) to determine bone properties. Ultimate load (N) was defined as the highest load and the fracture load (N) was recorded as the load when bone fractured. The stiffness (N/mm) was determined as the slope of the linear portion of the load versus displacement curve.

BMD measurement by X-ray absorptiometry

At the end of the experiment, bone mineral density (BMD, mg Ca/cm^2) was measured by an Xray equipment (70 KV) simultaneously with an aluminium step wedge which was previously calibrated with known Ca concentrations[18].

Measurements were done on digital images with ImageJ 1.40 software. The BMD was determined in the same places where biomechanical tests were performed. Trabecular BMD was measure in the epiphysis of femurs and cortical BMD were measure in diaphysis of femur. All measurements were blinded.

Bone Histomorphometry

The proximal epiphysis of the left tibiae was fixed in 10% phosphate buffered formaldehyde and decalcified in 10% EDTA before embedded in paraffin. Five-mm longitudinal sections were stained with hematoxylin & eosin. Bone histomorphometric analyses were performed in digital images obtained at a 40x magnification of proximal epiphysis. The following measurements were performed with ImageJ 1.40 software (NIH, Maryland, USA): (1) total tissue volume, TV (μm^2); (2) trabecular bone volume, BV (μm^2); and 3) trabecular bone surface, BS (µm). With these values, histomorphometrical variables were calculated: 1) bone volume, BV/ TV (%) = [BV100/TV]; (3) trabecular thickness, Tb.Th (µm) =[2/(BS/ BV)]; (4) trabecular number, Tb.N (1/mm) = [(BV/TV)/(Tb.Th)]; and (5) trabecular separation, Tb.Sp (µm)= [(1/Tb.N)-Tb.Th]. The 3D parameters from 2D measurements were assessed using standard stereology theory.

The analysis of trabecular interconnectivity was performed as previously published. The following parameters were measured: total number of nodes (Nd) and number of terminals (Tm). With these parameters, we proceeded to calculate an interconnectivity parameter known as node to termini ratio (R = Nd/Tm). The greater the value of R, the more connectivity the trabecular bone has[19].

Statistical analysis

Results are shown as media ± Standard Error (SE). The comparisons between groups were made using one-way ANOVA, and LSD post-test. Differences were considered significant when $p<0.05$. Statistical analyses were performed with R 2.14.1 software.

Resuls

Insulin resistance increased after 30 days of fluoride consumption in the same way as previous work[12] (Figure 1).

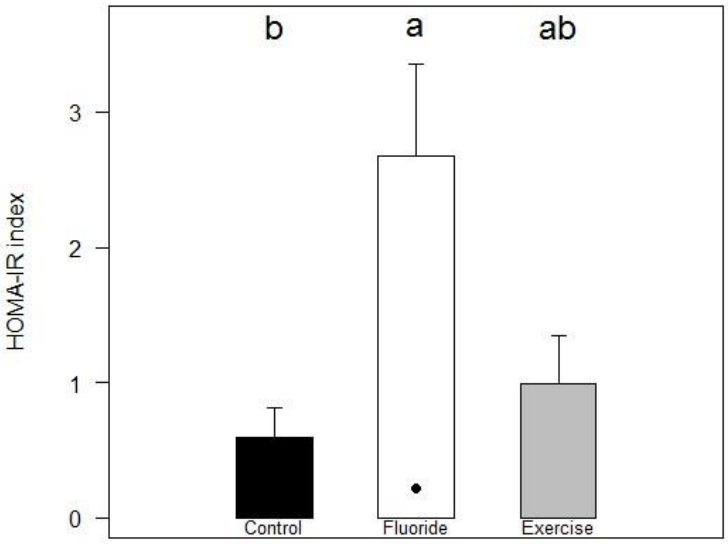

Figure N° 1. HOMA-IR of rats that received the different treatments. Data are shown as mean ± SE, Control, Fluoride and Exercise. Different letters indicates that the group are significantly different. p<0.05 one way ANOVA, and LSD post-test.

However, insulin sensitivity did not differ fluoride group might be due to lower plasma fluoride between Control and Exercise group. The decrease level (Figure 2) and higher bone fluoride level of HOMA-IR index in exercise group respect to. (Figure 3).

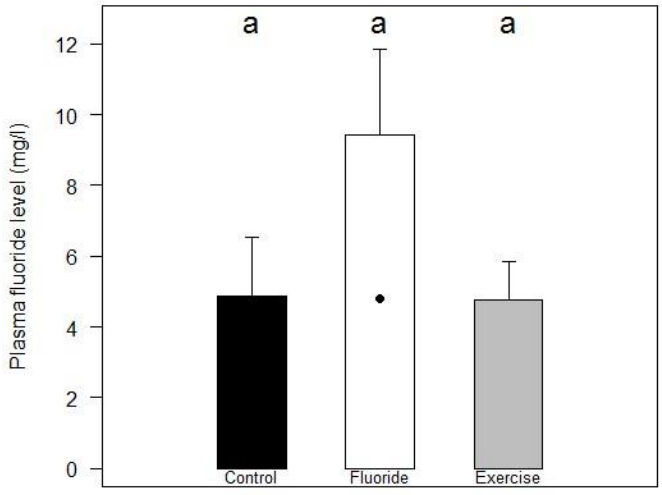

Figure N° 2. Plasma fluoride levels of rats that received the different treatments. Data are shown as mean ± SE, Control (drinking water without F-), Fluoride (drinking water with F- 15 mg/L) and Exercise (daily running on a treadmill during 60 min at 2.25 m/min and drinking water with F- 15 mg/L). Different letters indicates that the group are significantly different. p<0.05 one way ANOVA, and LSD post-test.

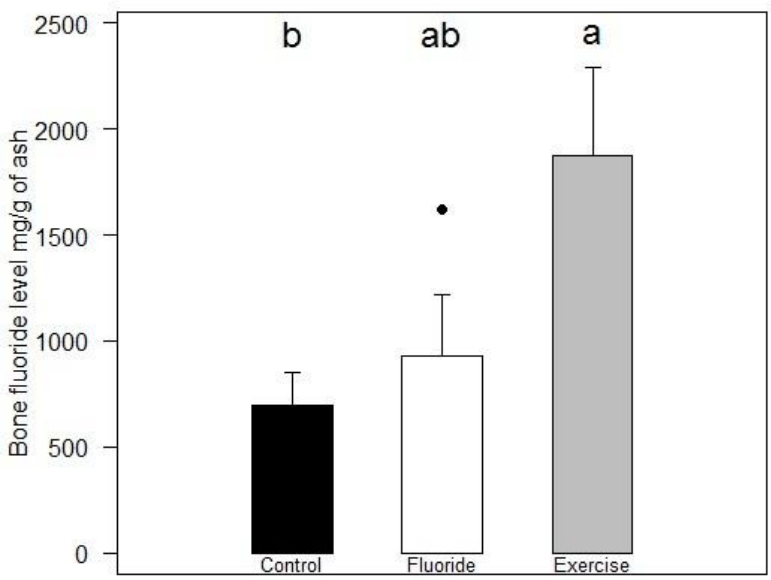

Figure N°3. Bone fluoride levels of rats that received the different treatments. Data are shown as mean ± SE, Control (drinking water without F-), Fluoride (drinking water with F- 15 mg/L) and Exercise (daily running on a treadmill during 60 min at 2.25 m/min and drinking water with F- 15 mg/L). Different letters indicates that the group are significantly different. $p<0.05$ oneway ANOVA, and LSD post-test.

Plasma fluoride level in fluoride group is two times higher than plasma fluoride level in exercise group; although the statistical test showed no significant difference, this difference may be biologically relevant and has been reported in previous work[12]. The lack of statistically significant differences may be the result of the great variation observed in the Control group. This variation could be the consequence of plasma fluoride level not being controlled by a homeostatic system and was reported in others works[20].

Table 1 show the effect of treatments on bone properties. Bone quality was studied through the biomechanical properties of the bones. Both through two parameters, fracture strength and stiffness in two tests, the 3-point bending test which allows us to evaluate the properties of the diaphysis of a long bone which is mainly composed of cortical bone. In addition, the compression test of a slice of the proximal epiphysis of the femur was used to evaluate the biomechanical properties of the trabecular bone. In addition, the study was complemented with measurements of bone mineral density and a histomorphological analysis with parameters that evaluate the amount of trabecular bone (BV/TV, TbN, TbSp, TbTh) and a trabecular connectivity index (R). The variables were compared with a oneway ANOVA and F^- consumption did not affect any of bone properties. These results indicate that the treatments did not modify the quality or quantity of bone.

Table N° 1: Effect of treatment on bone variables of rats.

Variable/Factor	Control	Fluoride	Exercise
Three-point test Ultimate load (N)	100.10±5.08	100.11±2.70	97.11±1.16
Three points test Stiffness (N/mm)	238.98±27.08	259.87±7.05	271.73±34.3
Compression test Ultimate load (N)	76.08±16.21	72.29±11.66	79.19±9.60
Compression test Stiffness (N/mm)	422.72±55.45	440.06±87.92	334.37±55.64
Cortical BMD (mg Ca/cm^2)	16.8±0.69	14.3±0.42	15.9±1.33
Trabecular BMD (mg Ca/cm^2)	28.1±2.13	27.4±1.44	26.9±0.72
%BV/TV	33.62±3.5	35.46±13.2	24.93±5.3
TbN	7.66±0.4	10.24±4.4	9.60±4.07
TbSp	0.082±0.004	0.064±0.03	0.085±0.04
TbTh	0.044±0.005	0.042±0.03	0.028±0.005
R	0.58±0.06	0.49±0.08	0.49±0.05

The table shows the mean±SE of groups. Significant differences were not found. (One way ANOVA, p>0.05). BMD: bone mineral density, %BV/TV: bone volume, TbN: trabecular number, TbSp: trabecular separation, TbTh: trabecular thickness, R: interconnectivity parameter known as node to termini ratio.

Discussion and conclusions

Drinking water is the main source of fluoride; it contains varying amounts of fluoride depending on the region[21]. The WHO has set an upper limit value of 1.5 mg/L[3]. In large parts of the world, such as India, Iran, Pakistan, Tanzania, China, and the southwest of Buenos Aires and Santa Fe provinces of Argentina, the amount of fluoride in drinking water exceeds this limit[2]. The products used in the prevention of tooth decay constitute another major source of chronic ingestion of fluoride[22].

The results presented in this paper come from rats that develop insulin resistance due to the ingestion of 15 mg/L F^- in drinking water for 30 days[12]. Moreover, previous works demonstrated that 15 mg/L of F^- for rats would be equivalent to 3 mg/L for humans because rats metabolize fluoride faster[16]. Consequently, the results found in this work could be extrapolated to people consuming water with 3 mg/L or more of fluoride, which consequently produces an average intake of 5-10 mg of F^- per day.

Physical activity and chronic fluoride administration have antagonistic effects on insulin action[4,10]. Previous work had demonstrated that controlled exercise could be a tool to counteract insulin resistance induced by fluoride. However, normalization of the insulin resistance was accompanied by a high fluoride uptake by the bone[12].

Physical exercise stimulates bone formation[13] which can be beneficial for bone, but fluoride have deleterious effects on it[14,15]. Therefore, in this work we evaluate bone properties of rats that received simultaneously F^- in drinking water and daily physical training.

Bone biomechanical, histologic properties and BMD were studied, and no effect of treatment was found despite of the high content of F^-. These results indicate that the increased uptake of F^- by bone tissue in rats that perform exercise is not accompanied by loss of bone strength. Other works found decrease in trabecular bone area of the tibia, changes in bone metabolism markers, poorer connectivity, and less trabecular bone network in cancellous bone of rats treated with fluoride[15]. In this work we do not found change in bone properties. However, work that found effect in bone used high doses of fluoride 50mg/l[23] or administrated the fluoride by orogastric tube[15]. In previous work of our group[24], we found that the bone effect of fluoride is different when fluoride is intake by orogastric tube or in drinking water. This may explain the difference between the results of this work and other studies. It is important to highlight that future studies that analyse longer periods of time are required to complete this study.

Exercise was effective in reduce the toxic effect of fluoride in brain[25], liver[26], kidney[27] and intestine[28]. In glucose homeostasis were found a reduction of plasma insulin level[12], changes in protein expression in muscle and liver[11,29]. Moreover, changes induce for exercise in pharmacokinetics of fluoride were reported[30]. Exercise is a recommended practice for improve bone health[13] and reduce de insulin resistance[10]. Thus, an improvement in bone quality induced by exercise would be expected. Although this work does not report a bone benefit from exercise, other authors have found such an effect in mice treated with 100mg/l of fluoride[31]. It is important to note that no deleterious effects of fluoride ingestion were observed either. It is likely that the dose of fluoride used, or the exposure time was not sufficient to produce bone effects, although they were observed at the level of glucose homeostasis.

In summary, this work proposes that exercise is a useful mechanism for reduce the toxic effect of fluoride. Although the result of this work does not confirm this hypothesis other work found positive results[11-12,25-28,31].

Physical activity is clearly not a solution for people who consume water with high concentrations of fluoride. The appropriate solution for these people is to change the source of drinking water, for instance having access to a reverse osmosis system supply or a new water source with an appropriate concentration of fluoride. However, without these possibilities, physical exercise would be very useful to reduce the toxic effects caused by fluoridated drinking water consumption.

References

1. Gazzano E, Bergandi L, Riganti C, Aldieri E, Doublier S, Costamagna C, Bosia A, Ghigo D. Fluoride effects: the two faces of janus. Curr Med Chem. 2010;17(22):2431-41. doi: 10.2174/092986710791698503.
2. Srivastava S, Flora SJS. Fluoride in Drinking Water and Skeletal Fluorosis: a Review of the Global Impact. Curr Environ Health Rep. 2020 Jun;7(2):140-146. doi: 10.1007/s40572-020-00270-9.
3. Adler P, World Health Organization. Fluoride and human health. World Health Organization. Ginebra, Suiza; 1970. Available from: https://iris.who.int/handle/10665/41784.
4. Skórka-Majewicz M, Goschorska M, Żwierełło W, Baranowska-Bosiacka I, Styburski D, Kapczuk P, Gutowska I. Effect of fluoride on endocrine tissues and their secretory functions -- review. Chemosphere. 2020 Dec;260:127565. doi: 10.1016/j.chemosphere.2020.127565.
5. Lombarte M, Rigalli A, Chiba FY, Sumida DH. Chapter 17: Effect of Fluoride on the Sensitivity and Secretion of Insulin. In: Preedy VR editor. Fluorine: Chemistry, Analysis, Function and Effects. Food and Nutritional Components in Focus. 2015. p. 292–307. doi: 10.1039/9781782628507-00292.
6. de Cássia Alves Nunes R, Chiba FY, Pereira AG, Pereira RF, de Lima Coutinho Mattera MS, Ervolino E, Louzada MJ, Buzalaf MA, Silva CA, Sumida DH. Effect of Sodium Fluoride on Bone Biomechanical and Histomorphometric Parameters and on Insulin Signaling and Insulin Sensitivity in Ovariectomized Rats. Biol Trace Elem Res. 2016 Sep;173(1):144-53. doi: 10.1007/s12011-016-0642-2.
7. Shashidhar KN, Uppalamethi M, Ram Mohan SD, Mendez D, Anjanappa R. Changes in diabetic and renal profile of people exposed to fluoride in south India. Bioinformation. 2022 Sep 30;18(9):820-824. doi: 10.6026/97320630018820.
8. Itai K, Onoda T, Nohara M, Kuribayashi T, Tanno K, Ohsawa M, Mori M, Okayama A. Slightly Elevated Serum Ionic Fluoride Levels Inhibit Insulin Secretion and Increase Glucose Levels in a General Japanese Population: a Cross-sectional Study. Biol Trace Elem Res. 2021 Aug;199(8):2819-2825. doi: 10.1007/s12011-020-02415-1.
9. World Health Organization. Preventing disease through health environments. Inadequate or excess fluoride: A major public health concern. Geneva, Switzerland; 2019. Available from: https://www.who.int/publications/i/item/WHO-CED-PHE-EPE-19.4.5.
10. Richter EA, Sylow L, Hargreaves M. Interactions between insulin and exercise. Biochem J. 2021 Nov 12;478(21):3827-3846. doi: 10.1042/BCJ20210185.
11. Fernandes MS, Sabino-Arias IT, Dionizio A, Fabricio MF, Trevizol JS, Martini T, Azevedo LB, Valentine RA, Maguire A, Zohoori FV, L Amaral S, Buzalaf MAR. Effect of Physical Exercise and Genetic Background on Glucose Homeostasis and Liver/Muscle Proteomes in Mice. Metabolites. 2022 Jan 25;12(2):117. doi: 10.3390/metabo12020117.

12. Lombarte M, Fina BL, Lupo M, Buzalaf MA, Rigalli A. Physical exercise ameliorates the toxic effect of fluoride on the insulin-glucose system. J Endocrinol. 2013 Jun 1;218(1):99-103. doi: 10.1530/JOE-13-0067.
13. Chang X, Xu S, Zhang H. Regulation of bone health through physical exercise: Mechanisms and types. Front Endocrinol (Lausanne). 2022 Dec 7;13:1029475. doi: 10.3389/fendo.2022.1029475.
14. Fina BL, Rigalli A. Chapter 12: Effect of Fluoride on Bone Metabolism, Structure and Remodeling. In: Preedy VR editor. Fluorine: Chemistry, Analysis, Function and Effects. Food and Nutritional Components in Focus. 2015. p. 200–16. doi: 10.1039/9781782628507-00200.
15. Li H, Chen X, Zhang Z, Zhang J, Xu H. Microstructural Analysis of Cancellous Bone in Fluorosis Rats. Biol Trace Elem Res. 2023 Oct;201(10):4827-4833. doi: 10.1007/s12011-023-03564-9.
16. Olfert ED, Cross BM, McWilliam AA. Guide to the care and use of experimental animals. Ottawa: Canadian Council on Animal Care; 1993. (vol. 1).
17. Dunipace AJ, Brizendine EJ, Zhang W, Wilson ME, Miller LL, Katz BP, Warrick JM, Stookey GK. Effect of aging on animal response to chronic fluoride exposure. J Dent Res. 1995 Jan;74(1):358-68. doi: 10.1177/00220345950740011201.
18. Rigalli A, Di Loreto VE. Experimental Surgical Models in the Laboratory rat. 1st ed. Rigalli A, Di Loreto VE, editors. Boca Raton, USA. Taylor & Francis Group; 2009.
19. Fina BL, Lupo M, Da Ros ER, Lombarte M, Rigalli A. Bone Strength in Growing Rats Treated with Fluoride: a Multi-dose Histomorphometric, Biomechanical and Densitometric Study. Biol Trace Elem Res. 2018 Oct;185(2):375-383. doi: 10.1007/s12011-017-1229-2.
20. Rigalli A, Ballina JC, Beinlich AD, Alloatti R, Puche RC. Pharmacokinetic differences between sodium fluoride and sodium monofluorophosphate and comparative bone mass increasing activity of both compounds in the rat. Arzneimittelforschung. 1994 Jun;44(6):762-6.
21. Podgorski J, Berg M. Global analysis and prediction of fluoride in groundwater. Nat Commun. 2022 Aug 1;13(1):4232. doi: 10.1038/s41467-022-31940-x.
22. Stephen KW. Fluoride toothpastes, rinses, and tablets. Adv Dent Res. 1994 Jul;8(2):185-9. doi: 10.1177/08959374940080020901.
23. Pereira AG, Chiba FY, de Lima Coutinho Mattera MS, Pereira RF, de Cássia Alves Nunes R, Tsosura TVS, Okamoto R, Sumida DH. Effects of fluoride on insulin signaling and bone metabolism in ovariectomized rats. J Trace Elem Med Biol. 2017 Jan;39:140-146. doi: 10.1016/j.jtemb.2016.09.007.
24. Fina BL. Estudio de las propiedades óseas de ratas tratadas con fluoruro de sodio y su relación en el proceso inflamatorio. [Doctoral thesis]. Universidad Nacional de Rosario; 2015.
25. Chai L, Cao Q, Liu K, Zhu R, Li H, Yu Y, Wang J, Niu R, Zhang D, Yang B, Ommati MM, Sun Z. Exercise Alleviates Fluoride-Induced Learning and Memory Impairment in Mice: Role of miR-206-3p and PREG. Biol Trace Elem Res. 2024 Jan 20. doi: 10.1007/s12011-024-04068-w.
26. Liu K, Chai L, Zhao T, Zhang S, Wang J, Yu Y, Niu R, Sun Z. Effects of Treadmill Exercise on Liver Apoptosis in Fluoride-Exposed Mice. Biol Trace Elem Res. 2023 Dec;201(12):5734-5746. doi: 10.1007/s12011-023-03619-x.
27. Yu Y, Niu R, Zhao F, Zhao Y, Wang J, Wang J, Cao Q, Fu R, Nateghahmadi MH, Sun Z. Moderate exercise relieves fluoride-induced liver and kidney inflammatory responses through the IKKβ/NFκB pathway. Environ Sci Pollut Res Int.

2022 Nov;29(52):78429-78443. doi: 10.1007/s11356-022-21360-1.

28. Fu R, Niu R, Zhao F, Wang J, Cao Q, Yu Y, Liu C, Zhang D, Sun Z. Exercise alleviated intestinal damage and microbial disturbances in mice exposed to fluoride. Chemosphere. 2022 Feb;288(Pt 3):132658. doi: 10.1016/j.chemosphere.2021.132658.
29. Lombarte M, Fina BL, Lima AL, Lupo M, Rabelo Buzalaf MA. Differential Expression of Protein in Muscle of Rats Treated With Fluoride and Exercise. Fluoride. 2022;55(4):343–51. http://hdl.handle.net/11336/156972.
30. Mahmood M, Azevedo LB, Maguire A, Buzalaf M, Zohoori FV. Pharmacokinetics of fluoride in human adults: The effect of exercise. Chemosphere. 2021 Jan;262:127796. doi: 10.1016/j.chemosphere.2020.127796.
31. Li R, Gong Z, Yu Y, Niu R, Bian S, Sun Z. Alleviative Effects of Exercise on Bone Remodeling in Fluorosis Mice. Biol Trace Elem Res. 2022 Mar;200(3):1248-1261. doi: 10.1007/s12011-021-02741-y.

Agradecimientos:

We thank Ornela N Acciarri and Hilda S Moreno for technical assistance. We thank Wiener Lab for providing reactive kits.

Limitaciones de responsabilidad:

La responsabilidad del trabajo es exclusivamente de quienes colaboraron en la elaboración del mismo.

Conflicto de interés:

Ninguno.

Fuentes de apoyo:

This work was supported by Consejo Nacional de Investigaciones Científica y Técnicas, CONICET, (Grant PIP 122-200801-00462) and Agencia Nacional de Promoción Científica y Técnica, ANPCyT, (Grant PICT-PRH 50).

Originalidad:

Este artículo es original y no ha sido enviado para su publicación a otro medio de difusión científica en forma completa ni parcialmente.

Cesión de derechos:

Quienes participaron en la elaboración de este artículo, ceden los derechos de autor a la Universidad Nacional de Córdoba para publicar en la Revista de la Facultad de Ciencias Médicas de Córdoba y realizar las traducciones necesarias al idioma inglés.