ORIGINAL ARTICLE

Effects of zinc supplementation on pancreatic islet morphometry in pregnant Wistar rats with moderate diabetes

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Received: 03/20/2022 - Approved: 09/04/2022

ABSTRACT

Introduction: in the study of diabetes mellitus it is essential to use experimental models that help to understand the particularities of the disease in humans.
Objective: to describe the effects of zinc supplementation on pancreatic islet morphometry in pregnant Wistar rats with moderate diabetes.
Methods: a descriptive, cross-sectional, experimental study with quantitative approach was performed from January 2016 to December 2018. Histological slides of pancreas were analyzed from 32 rats randomly distributed in four experimental groups which were induced diabetes mellitus with streptozotocin and treated with zinc sulfate. The ANOVA parametric test was applied and a 95% confidence interval was used.
Results: an increase in the mean number of islets was observed in all groups with respect to the control group. The highest mean value for both the largest and smallest islet diameter was in the diabetic-Zn and control-Zn groups, respectively. The highest mean area was in the control-Zn group and the highest circularity was shared by the diabetic and diabetic-Zn groups.
Conclusions: during the study there were no significant differences in the number, the largest diameter and the circularity of the pancreatic islets between the study groups. No significant effects of zinc supplementation on any of the morphometric parameters studied were found.
Key words: islets of langerhans; diabetes mellitus; morphometry; Wistar rats; zinc

RESUMEN

Introducción: en el estudio de la diabetes mellitus es fundamental la utilización de modelos experimentales que ayuden a comprender las particularidades de la enfermedad en humanos.
Objetivo: describir los efectos de un suplemento con zinc sobre la morfometría de los islotes pancreáticos en ratas Wistar gestantes con diabetes moderada.
Métodos: se realizó un estudio descriptivo, transversal, experimental con enfoque cuantitativo, de enero de 2016 a diciembre de 2018. Se analizaron láminas histológicas de páncreas a 32 ratas distribuidas de forma aleatoria en cuatro grupos experimentales a las que se les indujo la diabetes mellitus con estreptozotocina y se

trataron con sulfato de zinc. Se aplicó la prueba paramétrica ANOVA y se trabajó con un intervalo de confianza del 95%.
Resultados: se apreció un incremento en la media del número de islotes en todos los grupos con respecto al grupo control. El valor más alto de la media, tanto del diámetro mayor como del diámetro menor de los islotes, fue la del grupo diabético-Zn y control-Zn, respectivamente. La media más elevada del área fue en el grupo control-Zn y la circularidad más alta estuvo compartida por los grupos diabéticas y diabéticas-Zn.
Conclusiones: durante el estudio no se evidenciaron diferencias significativas en cuanto al número, el diámetro mayor y la circularidad de los islotes pancreáticos entre los grupos de estudio. En el trabajo no se evidenciaron efectos significativos del suplemento de zinc sobre ninguno de los parámetros morfométricos estudiados.
Palabras clave: islotes pancreáticos; diabetes mellitus; morfometría; ratas Wistar; zinc

INTRODUCTION

Diabetes mellitus (DM) comprises a range of common metabolic disorders resulting from various pathogenic mechanisms leading to hyperglycemia. Its pathogenesis, influenced by genetic and environmental factors, includes insufficient insulin secretion, reduced response to endogenous or exogenous insulin, increased glucose production, or abnormalities in fat and protein metabolism.[1,2]
The number of individuals with diabetes is increasing rapidly worldwide, so that already in 2011 the International Diabetes Federation reported about 336 million people with this condition. This disease, in addition to causing approximately 4.6 million deaths each year, considerably reduces the quality of life and life expectancy of patients, not to mention the high costs of treatment.[3]
The World Health Organization estimates that there are currently more than 250 million people with diabetes worldwide, 90% of whom are diagnosed as type 2 diabetes mellitus (DM2); it is considered likely that this figure will more than double by the year 2030 and it is further estimated that up to 30% of the actual population with DM2 is still undiagnosed.[4,5]
The use of animals as experimental models in the study of the pathophysiology of diabetes has allowed understanding some of its causes and consequences, as well as obtaining advances in its treatment and control, despite the fact that its signs and alterations are not exactly reproduced in the animal model. The literature reports the use of experimental models of diabetes in different species ranging from the dog, cat, rabbit, pig and sheep to the rat. These models allow the simulation of some phenomena observed in the clinic and contribute to the knowledge of the fisiological, biochemical and environmental factors that predispose to the disease.[4]
Specifically in the rat, diabetes can be produced spontaneously or experimentally induced by different methods. Historically, the most important was pancreatectomy, which revealed the importance of the islets of Langerhans for the regulation of glycemia. Another widely used method to experimentally induce DM2 is the chemical destruction of islets with alloxan or streptozotocin (STZ).[1,4,6,7,8]

Zinc (Zn) is an essential and fundamental micronutrient for humans and other organisms that is essential for the synthesis and transcription of deoxyribonucleic acid (DNA), protein stabilization and the activity of more than 300 enzymes directly linked to cellular processes such as cell division and apoptosis. In patients with diabetes, a significant increase in urinary Zn excretion is observed, and in DM2 a significant decrease in plasma levels of this micronutrient has also been observed, suggesting a deterioration of its levels directly associated with the disease. In contrast to DM2, plasma Zn levels tend to increase in DM1, probably as a result of the destruction of the beta cells of the pancreas, which leads to an increase in the supply of this micronutrient to the blood. The cause of this phenomenon lies in the high concentrations of Zn present in the pancreatic islets, in which it participates in the conversion of proinsulin into insulin and in the crystallization of insulin for its subsequent release, which clearly demonstrates its importance in the metabolism of this hormone.[9,10,11,12]

Zn is secreted together with insulin and, in fact, insulin hypersecretion can deplete the reserves of this element in islet beta cells. In 1938 it was first reported that Zn levels in cadavers of diabetic patients were 50% lower compared to cadavers of non-diabetic patients, suggesting an association between Zn concentration and diabetes, which prompted this research.[9,13,14,15]

METHODS

The present work responded to a research project of Basic Biomedical Sciences developed at the Biomedical Research Unit of the University of Medical Sciences of Villa Clara, Cuba, in which a model of diabetes with moderate hyperglycemia and other clinical conditions of type 2 diabetes mellitus was reproduced through neonatal induction with streptozotocin (STZ) in female Wistar rats in the period from January 2016 to December 2018.

A descriptive, cross-sectional, experimental study with quantitative approach using a morphometric method system was performed. Female Wistar rats from the National Center for Laboratory Animal Production were used, subjected to conventional all-purpose feeding conditions, CMO 1000 formula and free access to water, as well as 12/12 light/dark cycle, room temperature of 25°C and controlled humidity of 50 to 70%. The sample consisted of 32 pregnant female rats (diabetic: n=16 and healthy: n=16). Four experimental groups were formed randomly from diabetic and healthy pregnant rats (control group, control-Zn group, diabetic group and diabetic-Zn group), of eight animals each; the control and control-Zn groups were administered vehicle (water) or the dose of zinc sulfate (50 mg/kg), respectively, the induction of diabetes was performed in the diabetic and diabetic-Zn groups, to which a subcutaneous injection of STZ was applied on the second day after birth. Each of the groups provided five morphometric parameters (number of islets per lamella, largest islet diameter, smallest islet diameter, islet area and islet circularity).

The morphometric study of pancreatic islets was performed on pancreatic samples fixed in 10% neutral formalin, processed by the classic technique of inclusion in kerosene from the study. Biopsy sections were fixed for 72 hours in 10% formalin and washed in distilled water for one hour, processed by the

kerosene embedding technique due to their small size and followed the scheme: dehydration, absolute alcohol at 50, 60, 70, 80, 86 and 100% for 10 minutes each, clarification, alcohol-Xylol in equal parts for five minutes, alcohol-Xylol until clarification and pure alcohol-Xylol for five minutes, inclusion, kerosene 10 minutes, kerosene 20 minutes and inclusion kerosene 20 minutes, all at 56°C. In this way solidified blocks were obtained, cooled in ice and cut at 5 micrometers, in a vertical microtome, which allowed obtaining serial slices of uniform thickness, which were placed on histological slides stained with hematoxylin-eosin under pH control.

The process for applying hematoxylin-eosin was as follows:

- The slides are placed in the rack
- Alcohol-Xylol for dewaxing (five minutes in each alcohol-Xylol, there must be four alcohol-Xylol)
- Alcohol. Three washing alcohols of decreasing degradations are used
- Water
- Hematoxylin, from one to three minutes
- Water, drain well and apply 1% acid alcohol for one minute and then water for one to two minutes (pH change)
- Eosin, one to three minutes
- Dehydrating alcohols of increasing gradation (six alcohols)
- Alcohols-Xylol.

The morphometric examination of the sample was carried out starting with the separation, classification and arrangement of the slides. Images were captured with a semi-professional Olympus G11 digital camera, coupled to an Olympus BH-2 CCD Scion binocular microscope (4x and 20x objective lens and 10x eyepiece lens).

Measurements were determined using the public domain image analysis and processing program ImageJ®, version 1.44p (National Institutes of Health, United States). Analyses were performed on photographs at 100% of their size. A scale of 10 µm (434 pixels) was used to perform the measurements.

Measurements were executed with subsequent checking to minimize errors and were backed up with their traces and annotations for later consultation. The calculation options were area and count. Pancreatic islets were counted and then the largest diameter, smallest diameter, area and circularity of the islets were determined. The data were saved in a Microsoft Excel 2016 spreadsheet, designed for this purpose, which was subsequently imported into the statistical software package SPSS (Statistical Package for the Social Sciences) version 20.0 for Windows, for statistical processing of the information.

Operationalization of variables

The variables corresponded to the histological parameters analyzed: major diameter, minor diameter, area and circularity of the islets. In addition, the number of islets per slice was counted. The variables were operationalized as follows:

- Number of islets: it is obtained by counting the number of pancreatic islets per slice
- Largest islet diameter: it is obtained by carefully marking from the outer edge of one end of the pancreatic islet to the outer edge of the opposite

end, it is a flat image. The computer provides its value automatically in micrometers
- Smaller islet diameter: it is achieved by carefully marking from the inner edge of one end of the pancreatic islet to the inner edge of the opposite end, it is a flat image. The computer provides its value automatically in micrometers
- Islet area: it is achieved by carefully marking the contour of the pancreatic islet until it is completely surrounded, it is a flat two-dimensional image. The computer provides its value automatically in square micrometers
- Circularity of the islet: it is achieved by carefully marking the contour of the pancreatic islet until it is completely surrounded; it is a flat two-dimensional image. The computer provides its value automatically. It describes the degree of circularity of an object, such that it has a value of one when it is a perfect circle and zero when it is a line. As this value increases towards one, it indicates a more circular shape.

Methods of data analysis and processing

The data collected were summarized and statistically processed using the SPSS package version 20.0 for Windows, which are shown in tables and graphs that made possible the adequate interpretation of the information collected. For the construction of these graphs, the Microsoft Office 2016 version software package was used. Descriptive statistical techniques were used to characterize the sample by the different variables studied, as well as the use of statistical inference to obtain confidence intervals and contrasts between variables according to the objectives set, which made it possible to obtain the population point behavior of the mean (M)±standard error of the mean (SEM). The parametric test (one-factor ANOVA for analysis of variance) was applied in the study for group comparisons with a significance level of 0.05.

Statistical characterization was performed using descriptive statistics:

a) Arithmetic mean

b) Standard error of the mean.

The one-factor ANOVA parametric statistical test was used for analysis of variance. The following statistical significance values were considered:

- If p is less than 0.01 there are highly significant differences.
- If p is greater than or equal to 0.01 and less than 0.05 there are significant differences.
- If p is greater than and equal to 0.05 there are no significant differences.

A T-test was performed for comparison of the means of the different groups, as well as the standard error of the mean in cases where significance was obtained by performing the one-factor ANOVA statistical test.

RESULTS

Table 1 shows that the mean number of islets was lower in the control group with 9.88 islets per lamina, while it was higher (14.80) in the control-Zn group. The ANOVA test was applied and a bilateral significance value of 0.380 was obtained, indicating that this variable does not show significant differences between the groups.

Table 1. Distribution of the sample according to the number of pancreatic islets per slice

Groups	Number of islets	
	Mean	Standard error of the mean
Control	9.88	± 1.540
Control-Zn	14.80	± 2.898
Diabetic	10.88	± 2.216
Diabetic-Zn	10.00	± 2.211

Data are presented as M±EEM. No statistical significance ANOVA p>0.05

In the control group, the mean islet diameter was 205.45 µm. This mean was exceeded in the Zn-diabetic group, while in the rest of the groups it was lower than in the control group. The ANOVA test gave a significance value of 0.654, indicating that this variable did not show significant differences between the groups (Table 2).

Table 2. Sample distribution according to the largest diameter of pancreatic islets

Groups	Largest diameter of the islet	
	Mean	Standard error of the mean
Control	205.45	± 15.962
Control-Zn	191.50	± 8.086
Diabetic	121.41	± 8.610
Diabetic-Zn	256.85	± 139.396

Data are presented as M±EEM. No statistical significance ANOVA p>0.05

The mean of the smallest islet diameter in the control group was 135.81 µm. This value was exceeded in the control-Zn group and in the rest of the groups it was smaller than in the control group and the smallest of all was found in the diabetic group, with 82.03 µm. The ANOVA test gave a significance value of 0.000, suggesting highly significant differences between the groups in this variable (Table 3).

Table 3. Sample distribution according to the minor diameter of pancreatic islets

Groups	Minor diameter of the islet	
	Mean	Standard error of the mean
Control	135.81	± 13.435
Control-Zn	141.02	± 11.369
Diabetic	82.03	± 4.157(*)
Diabetic-Zn	83.61	± 2.802(**)

Data are presented as M±EEM. One-factor ANOVA p= 0.000. T-test (*) p<0.05 with respect to the control group, (**) p<0.05 with respect to the control-Zn group

With respect to the area of the pancreatic islets, the mean in the control group was 19523.55 µm^2. This measurement was higher in the control-Zn group and in the other two groups it was much lower than that of the control group. When a contrast test was performed by means of ANOVA, a bilateral significance value of 0.000 was obtained, which shows highly significant differences between the groups (Table 4).

Table 4. Distribution of the sample according to the area of the pancreatic islets

Groups	Islet area	
	Mean	Standard error of the mean
Control	19523.55	± 1323.748
Control-Zn	20987.84	± 1297.872
Diabetic	9098.03	± 1104.258(*)
Diabetic-Zn	8148.71	± 520.450(**)

Data are presented as M±EEM. One-factor ANOVA p= 0.000. T-test (*) p<0.05 with respect to the control group, (**) p<0.05 with respect to the control-Zn group

The mean islet circularity was similar in the control group and in the control-Zn group with 0.86, slightly exceeding the value of the control in the other two groups (0.88). The ANOVA test yielded a bilateral significance of 0.176, indicating that this variable showed no significant differences between groups (Table 5).

Table 5. Distribution of the sample according to the circularity of the pancreatic islets

Groups	Circularity of the islet	
	Mean	Standard error of the mean
Control	0.86	± 0.008
Control-Zn	0.86	± 0.010
Diabetic	0.88	± 0.008
Diabetic-Zn	0.88	± 0.008

Data are presented as M±EEM. No statistical significance ANOVA p>0.05

DISCUSSION

In the present study, an animal model was used in which streptozotocin was used as the induction agent for diabetes and in which characteristics of the human diabetic syndrome were evident.

During the study of the number of pancreatic islets, no significant differences were observed between the groups for this variable. According to these results, there was no evidence of a positive effect of zinc supplementation on the number of islets. The small effect of the induction of type 2 diabetes in rats on the number of pancreatic islets in diabetic animals differs from that observed in type 1 diabetes, in which a marked decrease in the number of islets has been observed. For example, a study conducted by Al-Asadi et al. in 2016 analyzed the morphometry of pancreatic islets in diabetic rats and reported that the number of islets was even higher in the study group than in the control group;[16] however, other research consulted differs from the above results and reports a lower number of islets in diabetic rats and even in their offspring. A study conducted at the University of Medical Sciences of Villa Clara in 2016 on the action of streptozotocin in an experimental model of neonatal induction of diabetes showed a significant decrease in the mean number of islets in the groups of diabetic rats.[3]

Similar results were obtained in 2016 in studies investigating the diabetogenic action of streptozotocin in an experimental model of neonatal induction.[17,18] In a work performed in the same year, the effect of zinc supplementation in diabetic rats was studied and it was reported that the difference in mean islet number between groups was almost imperceptible.[13]

Regarding the analysis of the mean islet diameters, the most striking results corresponded to the smallest diameter. When comparing the diabetic group with the control group, a significant decrease in this diameter was observed in the diabetic group, a result that coincides with what was expected in the experimental model; however, a decrease in this value was also observed in the Zn-diabetic group with respect to the Zn-control, which was not the result expected for the experimental model. In both cases the differences between the groups were highly significant. The smaller diameter was also slightly larger in the diabetic-Zn group than in the diabetic group, but this difference was not significant. In the case of the largest islet diameter, no significant differences were found between the study groups.

Equally interesting was the analysis of the mean pancreatic islet area. The comparison of the diabetic group with the control group showed that the area of the islets was smaller in the diabetic group, as expected in the experimental model, since the presence of smaller islets is to be expected in diabetic animals. When the same comparison was made between the diabetic-Zn group and the control-Zn group, it was observed that the mean of the diabetic-Zn group was much lower. In both cases the differences between the groups were highly significant; however, no significant differences were found between the diabetic-Zn group and the diabetic group. As can be seen, the induction of type 2 diabetes in the pregnant rats corresponded to a decrease in islet area, but it was not possible to observe any influence of zinc supplementation on this parameter. An analogous result regarding the effect of zinc on islet size had already been reported.[13]

In a 2016 study at Al-Nahrain University in Iraq they analyzed pregnancy-induced histomorphometric changes in pancreatic islets and noted that gestation was associated with an increase in islet area.[16] In another study, no significant differences in pancreatic islet area between diabetic and healthy rats were reported.[17] In a human research conducted in 2019 in Sweden, no significant differences in islet area of diabetic and healthy patients were also found.[19]

With respect to the islet circularity variable, the mean value was similar in the control group and in the Zn-control group, while it was slightly higher in the rest of the groups; no significant differences between the groups were demonstrated. Taking into account that circularity allows us to evaluate the shape of an object, these results suggest that there were no significant deformations of the islets in the different study groups. This finding seems to indicate that beta-cell destruction in diabetes is not necessarily always accompanied by visible alterations in islet shape. A remarkable loss of beta cells without significant alterations in islet shape in diabetic rats had already been reported in previous research.[20]

A comparable result was offered in another work in which also no significant differences in pancreatic islet shape were reported in healthy patients and patients with type 2 diabetes.[19]

CONCLUSIONS

The present investigation was aimed to describe the effects of zinc supplementation on pancreatic islet morphometry in pregnant Wistar rats with

moderate diabetes. During the study, no significant differences in the number, major diameter and circularity of pancreatic islets were evident between the study groups. Both the area and the smallest diameter of the islets were significantly lower in the diabetic group than in the control group. These parameters behaved similarly in the Zn-diabetic group compared to the control. No significant effects of zinc supplementation on any of the morphometric parameters studied were found.

BIBLIOGRAPHIC REFERENCES

1. Amaya-Chávez A, Dolores-Ledezma E, Álvarez-Sánchez P, Ferreira-Rubio G, Gómez-Oliván LM, Galar-Martínez M. Evaluación de un modelo de diabetes tipo 2 para estudiar la actividad hipoglicemieante de la glibenclamida. Rev Mex Cienc Farm [Internet]. 2007 [cited 03/15/2022];38(3):5-11.Available at: https://www.redalyc.org/articulo.oa?id=57938302
2. Sánchez Martínez B, Vega Falcón V, Gómez Martínez N, Vilema Vizuete GE. Estudio de casos y controles sobre factores de riesgo de diabetes mellitus tipo 2 en adultos mayores. Universidad y Sociedad [Internet]. 2020 [cited 03/15/2022];12(4):156-164. Available at: http://scielo.sld.cu/pdf/rus/v12n4/2218-3620-rus-12-04-156.pdf
3. Bequer L, Gómez T, Molina JL, Artiles D, Bermúdez R, Clapés S. Acción de la estreptozotocina en un modelo experimental de inducción neonatal de la diabetes. Biomédica [Internet]. 2016 [cited 03/15/2022];36(2):230-38. Available at: https://revistabiomedica.org/index.php/biomedica/article/view/2686. https://doi.org/10.7705/biomedica.v36i2.2686
4. Figueroa MC, Pérez IH, Mejía R. Caracterización de un modelo de diabetes tipo 2 en ratas Wistar hembra. Rev MVZ Córdoba [Internet]. 2013 [cited 03/15/2022];18(Supl):3699-3707. Available at: http://www.scielo.org.co/pdf/mvz/v18s1/v18supla14.pdf
5. Asbaghi O, Sadeghian M, Fouladvand F, Panahande B, Nasiri M, Khodadost M, et al. Effects of zinc supplementation on lipid profile in patients with type 2 diabetes mellitus: A systematic review and meta-analysis of randomized controlled trials. Nutr Metab Cardiovasc Dis [Internet]. 2020 [cited 03/15/2022];30(8):1260-1271. Available at: https://pubmed.ncbi.nlm.nih.gov/32451277/. https://doi.org/10.1016/j.numecd.2020.03.021
6. Cabrejos C, Ipanaqué K, Tejada E, Fupuy J, Vásquez A. Efecto glicemiante de Pellaea ternifolia "cuti – cuti" sobre Mus musculus (ratón) cepa Balb/c con hiperglicemia inducida con estreptozotocina. Rev Cienc Tecnol Humanid [Internet]. 2016 [cited 03/15/2022];7(1):99–108. Available at: http://revistas.unprg.edu.pe/openjournal/index.php/revistacientifica/article/view/48/32
7. Aro P, Acosta Chalcatana M, Chacon Terrazas S, Aguilar Olano J. P33 Modelo animal basado en una dieta alta en carbohidratos y dosis bajas de estreptozotocina en la inducción de diabetes mellitus tipo 2. Rev Soc Argent Diabetes [Internet]. 2020 [cited 03/15/2022];54(3 Sup):138. Available at: https://www.revistasad.com/index.php/diabetes/article/viewFile/418/384. http://dx.doi.org/10.47196/diab.v54i3Sup.418
8. Bequer L, Quintero M, Gómez T, Guerra I, Molina JL, Freire C, et al. Efectos de la inyección neonatal de estreptozotocina sobre indicadores bioquímicos y de estrés oxidativo en ratas. Rev Cuba Investig Bioméd [Internet]. 2021 [cited 03/15/2022];40(3):e804. Available at: http://scielo.sld.cu/pdf/ibi/v40n3/1561-3011-ibi-40-03-e804.pdf

9. Valenzuela R, Pérez F, Ruz M. Efecto del estimulo con zinc sobre la liberación de insulina en el modelo celular min-6. Rev Chil Endocrinol Diabetes [Internet]. 2012 [cited 03/15/2022];5(4):172-174. Available at: http://www.revistasoched.cl/4_2012/6_4-2012.pdf
10. Pérez Bazán AF. Efecto de la suplementación con zinc sobre la secreción de glucagón en diabetes tipo 2 [doctoral thesis]. Santiago de Chile: Universidad de Chile; 2017 [cited 03/15/2022]. Available at: https://repositorio.uchile.cl/bitstream/handle/2250/167744/Efecto-de-la-suplementacion-con-zinc-sobre-la-secrecion-de-glucagon-en-diabetes-tipo-2.pdf
11. Tang KS. The current and future perspectives of zinc oxide nanoparticles in the treatment of diabetes mellitus. Life Sci [Internet]. 2019 [cited 03/15/2022];239:117011. Available at: https://pubmed.ncbi.nlm.nih.gov/31669241/. https://doi.org/10.1016/j.lfs.2019.117011
12. Samadi A, Isikhan SY, Tinkov AA, Lay I, Doşa MD, Skalny AV, et al. Zinc, copper, and oxysterol levels in patients with type 1 and type 2 diabetes mellitus. Clin Nutr [Internet]. 2019 [cited 03/15/2022];39(6):1849-1856. Available at: https://pubmed.ncbi.nlm.nih.gov/31427180/. https://doi.org/10.1016/j.clnu.2019.07.026
13. Barman S, Srinivasan K. Zinc supplementation alleviates hyperglycemia and associated metabolic abnormalities in streptozotocin-induced diabetic rats. Can J Physiol Pharmacol [Internet]. 2016 [cited 03/15/2022];94(12):1356–1365. Available at: https://pubmed.ncbi.nlm.nih.gov/27782759/. https://doi.org/10.1139/cjpp-2016-0084
14. Gómez T, Bequer L, Molineda A, Molina JL, Álvarez AO, Lavastida M, et al. Beneficios de la suplementación con zinc sobre el estado metabólico, redox y de elementos traza en un modelo diabetes moderada en ratas. J Pharm Pharmacogn Res [Internet]. 2019 [cited 03/15/2022];7(2):144-155. Available at: https://jppres.com/jppres/pdf/vol7/jppres18.515_7.2.144.pdf
15. MacKenzie S, Bergdahl A. Zinc Homeostasis inDiabetesMellitus and Vascular Complications. Biomedicines [Internet]. 2022 [cited 03/15/2022];10(1):139. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8773686/. https://doi.org/10.3390/biomedicines10010139
16. Al-Asadi AK, Al-Habib MF, Al-Kawaz UM. Histomorphometrical Study Of Pregnancy Induced Changes In The Islets Of Langerhans. World J Pharm Res [Internet]. 2016 [cited 03/15/2022];5(11):340-350. Available at: https://www.wjpr.net/abstract_file/6017
17. Moharerri A, Ghafari S, Golalipour MJ. Gestational Diabetes Reduces Pancreatic Beta Cells in Rat Offspring. Int J Morphol [Internet]. 2016 [cited 03/15/2022];34(4):1386-1391. Available at: https://www.scielo.cl/pdf/ijmorphol/v34n4/art34.pdf. http://dx.doi.org/10.4067/S0717-95022016000400034
18. Bequer L, Gómez T, Molina JL, Artiles D, Bermúdez R, Clapés S. Acción de la estreptozotocina en un modelo experimental de inducción neonatal de la diabetes. Biomédica [Internet]. 2016 [cited 03/15/2022];36(2):230-238. Available at: https://revistabiomedica.org/index.php/biomedica/article/view/2686. https://doi.org/10.7705/biomedica.v36i2.2686
19. Seiron P, Wiberg A, Kuric E, Krogvold L, Jahnsen FL, Dahl-Jørgensen K, et al. Characterisation of the endocrine pancreas in type 1 diabetes: ilet size is maintained but islet number is markedly reduced. J Pathol Clin Res [Internet]. 2019 [cited 03/15/2022];5(4):248-255. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6817830/. https://doi.org/10.1002/cjp2.140

20. Riccillo FL, Bracamonte MI, Montenegro S, Martínez SM, Ronderos JR. Progressive histopathological changes and β-cell loss in the pancreas of a new spontaneous rat model of type 2 diabetes. Tissue Cell [Internet]. 2012 [cited 03/15/2022];44(2):101–10. Available at: https://pubmed.ncbi.nlm.nih.gov/22244242/. https://doi.org/10.1016/j.tice.2011.12.002

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

CONTRIBUTION OF THE AUTHORS

RPO: conceptualization, research, formal analysis, methodology, data curation and validation.
MGA, MGA, ITP: data curation, writing the initial draft and final version of the manuscript.
RLP: data curation, validation, writing the initial draft and final version of the manuscript.