¹Effects of zinc deficiency in male mice on glucose metabolism of male offspring

Yang LI¹, LingLing LI¹, Wenjie YANG^{2*}, Zengli YU^{1*}

¹ Department of Nutrition and Food Hygiene, College of Public Health, Zhengzhou University, Zhengzhou 450001

² Department of Epidemiology and Health Statistics, College of Public Health, Zhengzhou University, Zhengzhou 450001

¹ * Corresponding authors.

E-mail addresses: ywjie@zzu.edu.cn (YANG Wenjie), zly@zzu.edu.cn (YU Zengli).

Abstract: As an essential metal, zinc is central to insulin biosynthesis and energy metabolism. Zinc can not only maintain the activity of insulin, but also has insulin-like effect. When zinc is sufficient, the body needs less insulin. Zinc can correct abnormal glucose tolerance and even replace insulin to improve glucose metabolism disorder in diabetic rats. However, the effect of paternal zinc deficiency on glucose metabolism of offspring is still unclear. In the present study, sixteen 8-week-old male mice were randomly allocated into low-zinc group and control group (8 mice in each group), which were fed with low zinc and standard diet for 6 weeks, respectively. The mice were mated with female mice fed with standard diet to get the first generation of mice (F1) to explore the effect zinc deficiency on the glucose metabolism of offspring. Glucose tolerance, insulin sensitivity and insulin secretion in mice were determined by oral glucose tolerance test (OGTT), insulin tolerance test (ITT) and glucose stimulated insulin secretion test (GSIS), respectively. Compared with the control group, the fasting blood glucose levels of F1 generation male mice in the low zinc group increased at 15min and 30min after glucose injection. The blood glucose of F1 generation male mice in the low zinc group of mice decreased at 60min, 90min, 120min, 180min, 240 min after insulin injection. Compared with the control group, serum insulin of F1 generation male mice in the low zinc group decreased at 15min after glucose injection (all *P values* <0.05). However F1 female mice in the low zinc group did not show abnormal glucose metabolism. Marginal zinc deficiency in male mice can cause abnormal glucose homeostasis in male offspring, but not in female offspring.

Keywords: Zinc deficiency, Glucose homeostasis, Insulin, Offspring

Introduction

Zinc is an essential trace element of life, which regulates the expression and activation of various biomolecules, such as transcription factors, enzymes, aptamers, biological channels, growth factors and their receptors^{1, 2)}. Zinc is a key structural or catalytic component of more than 300 enzymes involved in all aspects of cell signal transduction³⁾. Zinc also regulates cell signal transduction, proliferation, differentiation and survival^{4, 5)}. As a result, almost all important physiological processes in cells depend on proper function of zinc.

Although zinc is the second most abundant trace element in the human body, which is next only to iron⁶⁾. Human body cannot store zinc, and needs continuously supply from diet⁷⁾. Zinc deficiency occurs due to inadequate intake, reduced absorption or excretion loss, which is one of the major nutritional imbalance challenges for global population, ranking 11th among the world's important health risk factors and 5th among developing countries⁸⁾. Zinc deficiency is associated with a variety of diseases, and could cause systemic health problems, including growth and development, neurological dysfunction, reproductive and immune system damage etc^{9, 10)}. Marginal zinc deficiency refers to zinc deficiency with zinc ion level reduced in blood circulation in the body, but no signs of skin injury, hair loss and other signs¹¹⁾, which is the most common type of zinc deficiency in population, especially in children.

Recently, the relationship between zinc deficiency and diabetes mellitus has aroused people's attention. Zinc maintains the activity of insulin, and has insulin-like effect itself^{12, 13)}. Body needs less insulin when zinc is sufficient. Zinc can also correct abnormal glucose tolerance and even replace insulin to improve glucose metabolism disorder in diabetic rats¹⁴⁾. Zinc deficiency can induce insulin resistance and even diabetes. Recent study founded that diabetic patients are generally in a state of zinc deficiency, and diabetic complication also is concerned with the decrease of cellular zinc or zinc-dependent antioxidant oxidase, such as diabetic retinopathy and diabetic peripheral neuropathy¹⁵⁾. In addition, studies founded that zinc supplementation can significantly reduce some key blood glucose indicators^{16, 17)}.

Other studies have shown that zinc deficiency during pregnancy and lactation in Wistar female rats can lead to insulin secretion dysfunction in offspring¹⁸⁾. However, the effect of paternal zinc deficiency on glucose metabolism of offspring is still unclear. In this study, C57BL/6J male mice were used to establish a mouse model of marginal zinc deficiency to explore its effect on glucose metabolism of offspring.

Methods and Materials

Animals and reagents

C57BL/6J mice (6 weeks of age, both males and females) were purchased from Vital River Laboratory Animal Technology Co., Ltd., Beijing, China. D-glucose and bovine insulin were purchased from Sigma-Aldrich. Ultra Sensitive Mouse Insulin Assay ELISA kit was purchased from Crystal Chem Inc. General tissue fixative liquid was purchased from Wuhan Servicebio Biotechnology Co., LTD. Hematoxylin eosin (HE) staining kit was purchased from Beijing Solaibao Biotechnology Co., LTD. Blood glucose meter and blood glucose test paper were purchased from ROCHE. Serum interleukin-6 (IL-6) and Superoxide Dismutase (SOD) activity detection kits were purchased from NanJing JianCheng Bioengineering Research Institute Co., LTD. The Diets used in this experiment were standard diet (D08080401, zinc content: 29mg/kg) and zinc deficient diet (D08080403, zinc content: 0mg/kg) customized by Research Diets Inc. The protocol for the use of animals was approved by the Animal Ethics Board of Zhengzhou University.

Preparation of low zinc and control diets Firstly, the diet was stirred into powder in a blender, and the zinc deficiency diet(827.6g) and standard diet (172.4g) were fully mixed, and an appropriate amount of ultra-pure water was added to mix together, and then the Low Zinc diet (5 mg/kg) was made and dried at room temperature^[7]. In order to maintain consistency, 1 000 g standard diet was processed in the same way, which was used as control diet. All diet was stored at -20°C.

Establishment and experimental grouping of male mice with marginal zinc deficiency Sixteen male mice were randomly divided into two groups, as low zinc group and the control group (8 for each group), which were the Parental mice (F0

generation male mice), fed with low-zinc and standard diet for 6 weeks respectively. Then they were mated with female mice (fed with standard diet) in the cage from 8 PM to 6 AM in the next morning, the mice had no diet but water during the cage. The female mice were checked every morning and be separated with male when vaginal plug was found, to obtain the first generation of mice (F1 generation mice), the weight and survival rate of F1 offspring mice were recorded. Female and F1 generation mice were given control diet throughout the research.

Measurement of zinc levels in serum and other tissues Zinc ion level was detected by inductively coupled plasma mass spectrometry (ICP-MS) in Guangzhou Jinyu Medical Laboratory Group Co., LTD.

Detection of interleukin-6 (IL-6) in serum Serum samples were taken, and serum IL-6 level was detected according to the instructions of ELISA kit.

Determination of SOD activity SOD activity in serum was measured using the xanthine oxidase method. The procedures were conducted in accordance with kit instructions.

Histological observation of pancreas The pancreas tissues were taken and placed in 4% paraformaldehyde fixation solution for more than 24 h. The sections were routinely embedded in paraffin and stained with hematoxylin-eosin. The pathological changes of the tissue structure were observed under light microscope.

Oral Glucose tolerance Test (OGTT) After fasting for 16h, 20% D-glucose solution was prepared and gavaged to the mice at a dose of 2g/kg. Blood was collected from tail vein, and fasting blood glucose was recorded at 0min, 15min, 30min, 60min, 90min and 120min after gavage.

Insulin tolerance Test (ITT) After fasting for 2 h, mice were injected with 50 U/L insulin saline at a dose of 0.5 U/kg, and blood was collected from tail vein. Blood glucose values were recorded at 0min (before injection), 15 min, 30 min, 60 min, 120 min, 180 min, and 240 min after injection.

Glucose stimulated insulin secretion Test (GSIS) After fasting for 16 h, inner canthal blood was extracted from mice, and about 50 μ L (2-3 drops) was placed in EP tube at room temperature. 20% D-glucose solution was prepared and the mice were

given intragastric administration at a dose of 2g/kg. Blood was taken in the same way after intragastric administration 15min and 30min, respectively. The movement should be gentle during the whole process to avoid stress reaction of the mice.

Statistical Analysis

SPSS 25.0 was used for statistical analysis, and data were obtained from three independent experiments and presented as Means $\pm$ S.D. Comparisons were performed using one-way ANOVA with Tukey's post hoc test following appropriate transformation of raw data to achieve a normal distribution and equalized variance when necessary, and the Student's *t*-test was used to compare the difference between two groups. $P < 0.05$ was considered statistical significant.

Results

Low zinc diet induced zinc decreased in whole body tissues of F0 generation male mice As shown in Fig. 1A, the zinc content in control and low zinc diet were 28.36 ± 1.13 and 5.01 ± 0.34 mg/kg, respectively. Serum zinc in control group was 1.93 ± 0.15 ng / μ L, serum zinc in low zinc group was decreased 32.6% compared with the control group (Fig.1B), and the zinc content in tissues of low zinc group mice decreased to varying degrees ($P < 0.05$) (Fig. 1C).

Low zinc diet caused spleen enlarged and IL-6 increased in F0 generation male mice As shown in Fig. 2A, In F0 generation, there was no statistical difference in liver/body weight coefficient between the low zinc group and control group ($P > 0.05$). The spleen/body weight ratio increased, and the difference was statistically statistical compared with the control group ($P < 0.05$) (Fig. 2B). Serum IL-6 level in low zinc group was increased 36.5%, and the difference was statistically statistical ($P < 0.05$) (Fig. 2C), while SOD activity was no statistical difference from that in control group ($P > 0.05$) (Fig. 2D). After 6 weeks of low zinc diet, there was no statistical change in OGTT in the low zinc group compared with the control group ($P > 0.05$) (Fig. 2E). These results suggest that marginal zinc deficiency had a slight effect on F0 generation mice.

Low zinc diet reduced survival rate of F1 offspring mice The survival rate of F1 offspring mice in the zinc deficient group was 75.1%, which was significantly lower than that in the control group ($P<0.05$) (Fig. 3A). The weight of surviving F1 generation mice was recorded, and the results showed that compared with the control group, the weight of female and male F1 generation mice did not change significantly ($P>0.05$), as shown in Fig. 3B and Fig. 3C.

Low zinc diet attenuates glucose metabolism in F1 male offspring As shown in figure 4A, compared with the control group, the fasting blood glucose of F1 generation male mice in the low zinc group increased 15min and 30min after glucose injection, with statistical significance ($P<0.05$). Compared with the control group, the glucose levels of F1 generation male mice in the low zinc group decreased at 60 min, 90 min, 120min, 180 min and 240 min after insulin injection, with statistically statistical differences ($P<0.05$), which as shown in figure 4B. Insulin secretion of F1 generation male mice in the low zinc group decreased 23.2% after glucose injection 15min, compared with the control group ($P<0.05$), as shown in figure 4C. There was no difference in pancreatic tissue morphology of F1 generation male mice in the low zinc group compared with the control group (Figure 4D and 4E).

Low zinc diet had no effect on glucose metabolism in F1 female offspring As shown in fig. 5A, there was no statistical difference in OGTT of F1 generation female mice in low zinc group compared with the control group ($P>0.05$). ITT of F1 generation female mice in low zinc group was not significantly different from that in control group ($P>0.05$) (Fig. 5B). There was no statistical difference in GSIS of F1 generation female mice in low zinc group compared with the control group ($P>0.05$) (Fig. 5C). There was no difference in pancreatic tissue morphology of F1 generation male mice in the low zinc group compared with the control group (Figure 5D and 5E).

Discussion

The relationship between nutrients and nutrient sensing pathway and diabetes has also attracted great attention, and the role of nutrients in diabetes is most concerned with macronutrients, such as carbohydrates and fat. While micronutrients, such as iron

and zinc, which are easy to be ignored, are also closely related to diabetes¹⁹⁻²¹). Studies have reported that zinc deficiency can induce insulin resistance and even diabetes in the body^{22, 23}).

Zinc deficiency not only affects individual health, but also adversely affects the health of their offspring. We used zinc deficient diet and control diet (5mg/kg and 30mg/kg, respectively) to intervene F0 generation mice²⁴), and the serum zinc in low zinc group decreased by about 32.6%, and the zinc content in tissues of low zinc group mice decreased to varying degrees. However, there were no signs of severe zinc deficiency such as skin ulceration and hair loss, indicating that the mouse model of marginal zinc deficiency was successfully constructed. The serum inflammatory factor IL-6 in male zinc-deficient mice of F0 generation increased, and the organ coefficient of spleen increased compared with the control group, indicating splenomegaly, which is consistent with previous reports that zinc deficiency has been confirmed to cause damage to human immune function²⁵). The activity of SOD enzyme in serum of female Zinc-deficient mice of F0 generation decreased, which confirmed that zinc deficiency would affect SOD enzyme activity^{26, 27}). Previous literatures have reported that zinc ion can improve the catalytic efficiency and stability of SOD enzyme^{28, 29}). F0 generation male mice did not show glucose tolerance impairment after the low zinc diet intervention, which may be due to the marginal zinc deficiency of mice, which is relatively mild and does not cause the disorder of glucose metabolism.

The survival rate of F1 generation mice in the low zinc group reduced, indicating that zinc deficiency in male mice affected the growth and development of their offspring. However, most of the died mice died soon after birth, which were too young to accurately identify their sex, which is a limitation of our study. The growth curve of the surviving F1 generation mice showed no abnormality, which maybe because the normal diet was restored in the later stage, which alleviated the influence of zinc deficiency in early life on offspring to some extent. Consistent with our study, some studies have shown that zinc deficiency will affect the growth and development of offspring, but zinc supplementation can recover to a certain extent^{18, 30}).

F1 generation of male mice impaired glucose tolerance and insulin secretion in damage, but the body of insulin sensitivity enhancement, speculation may be when the mice blood glucose, insulin secretion from the body reflective. Whereas, as a result of mice impaired insulin secretion, insulin levels, body compensatory to adjust the insulin sensitivity. Compensatory regulation could not completely compensate for the functional impairment, and finally resulted in dyshomeostasis of glucose in mice. There was no tissue damage in the pancreas of F1 generation male mice, and this phenotype and sex difference may be involved in epigenetic mechanism. Zinc ion is an essential metal element in the epigenome and is necessary for various epigenetic enzymes to exert their activities. Sperm has specific epigenetic characteristics, including DNA methylation, DNA-related proteins, post-translational histone modification and so on³¹⁻³⁴⁾ Dysregulation of zinc homeostasis can lead to changes in epigenetic characteristics²⁴⁾. Population studies have shown that men with normal zinc intake have higher sperm quality and less DNA damage than men with low zinc intake³⁵⁾.

Female mice of F1 generation did not show abnormal glucose metabolism. Previous literature also pointed out that female mice are less sensitive to metabolic effects caused by zinc deficiency compared with males, while males are more sensitive to malnutrition^{36, 37)}. Gender differences in the effects of zinc deficiency on offspring have been reported^{38, 39)}, and epidemiological studies have shown that the risk of diabetes in males is higher than that in females^{40, 41)}.

This study is demonstrated that the effects of paternal zinc deficiency on offspring. The results presented interesting results that low zinc diet increased serum IL-6 level, paternal marginal zinc deficiency lowered offspring survival rate, and attenuated glucose metabolism through insulin decrease in F1 male offspring but not in F1 female. This study provides a theoretical basis for gender differences in diabetes patients, and provides the most direct evidence that parental zinc deficiency causes abnormal glucose metabolism in male offspring.

In conclusion, marginal zinc deficiency in male C57/BL6J mice can lead to abnormal glucose metabolism in male offspring, but has no effect on female offspring,

and the exact mechanism needs to be further explored.

Acknowledgement This study was supported by the National Natural Science Foundation of China (Grant No. 31750110463, 21577119 and 81801547).

Conflict of Interest The authors declare no conflict of interest.

References

- 1) Cuajungco M P, Ramirez M S, Tolmasky M E. Zinc: Multidimensional Effects on Living Organisms [J]. *Biomedicines*, 9, (2021).
- 2) Takatani-Nakase T. Zinc Transporters and the Progression of Breast Cancers [J]. *Biol. Pharm. Bull.*, 41, 1517-1522 (2018).
- 3) Jansen J, Karges W, Rink L. Zinc and diabetes--clinical links and molecular mechanisms [J]. *J. Nutr. Biochem.*, 20, 399-417 (2009).
- 4) Bonaventura P, Benedetti G, Albareda F, Miossec P. Zinc and its role in immunity and inflammation [J]. *Autoimmun. Rev.*, 14, 277-285 (2015).
- 5) Choi B Y, Suh S W. Antimicrotubule Agent-Induced Zinc Neurotoxicity [J]. *Biol. Pharm. Bull.*, 41, 1001-1005 (2018).
- 6) Duan M, Li T, Liu B, Yin S, Zang J, Lv C, Zhao G, Zhang T. Zinc nutrition and dietary zinc supplements [J]. *Crit. Rev. Food Sci. Nutr.*, 1-16 (2021).
- 7) Sanna A, Firinu D, Zavattari P, Valera P. Zinc Status and Autoimmunity: A Systematic Review and Meta-Analysis [J]. *Nutrients*, 10, (2018).
- 8) Guilbert J J. The world health report 2002 - reducing risks, promoting healthy life [J]. *Educ. Health (Abingdon)*, 16, 230 (2003).
- 9) Elitt C M, Fahrni C J, Rosenberg P A. Zinc homeostasis and zinc signaling in white matter development and injury [J]. *Neurosci. Lett.*, 707, 134247 (2019).
- 10) Ueda H, Nakai T, Konishi T, Tanaka K, Sakazaki F, Min K S. Effects of zinc deficiency and supplementation on leptin and leptin receptor expression in pregnant mice [J]. *Biol. Pharm. Bull.*, 37, 581-587 (2014).
- 11) Waalkes M P. Effect of dietary zinc deficiency on the accumulation of cadmium and metallothionein in selected tissues of the rat [J]. *J. Toxicol. Environ. Health.*, 18, 301-313 (1986).
- 12) Zhang H, Cai L. Zinc homeostasis plays an important role in the prevention of obesity-induced cardiac inflammation, remodeling and dysfunction [J]. *J. Trace. Elem. Med. Biol.*, 62, 126615 (2020).
- 13) Naito Y, Yoshikawa Y, Shintani M, Kamoshida S, Kajiwaru N, Yasui H. Anti-hyperglycemic Effect of Long-Term Bis(hinokitiolato)zinc Complex ([Zn(hkt)₂]) Ingestion on Insulin Resistance and Pancreatic Islet Cells Protection in Type 2 Diabetic KK-A(y) Mice [J]. *Biol. Pharm. Bull.*, 40, 318-326 (2017).
- 14) Sprietsma J E, Schuitemaker G E. Diabetes can be prevented by reducing insulin production [J]. *Med. Hypotheses*, 42, 15-23 (1994).
- 15) Maremanda K P, Srivalliputturu S B, Jena G. Zinc deficient diet exacerbates the testicular and epididymal damage in type 2 diabetic rat: Studies on oxidative stress-related mechanisms [J]. *Reprod. Biol.*, 20, 191-201 (2020).
- 16) Wang X, Wu W, Zheng W, Fang X, Chen L, Rink L, Min J, Wang F. Zinc supplementation improves glycemic control for diabetes prevention and management: a systematic review and meta-analysis of randomized controlled trials [J]. *Am. J. Clin. Nutr.*, 110, 76-90 (2019).
- 17) Maret W. Zinc in Pancreatic Islet Biology, Insulin Sensitivity, and Diabetes [J]. *Prev. Nutr. Food Sci.*, 22, 1-8 (2017).
- 18) Padmavathi I J, Kishore Y D, Venu L, Ganeshan M, Harishankar N, Giridharan N V, Raghunath M. Prenatal and perinatal zinc restriction: effects on body composition, glucose tolerance and insulin response in rat offspring [J]. *Exp. Physiol.*, 94, 761-769 (2009).

- 19) Zhang Y, Xu S, Zhong C, Li Q, Wu M, Zhang G, Chen R, Liu C, Wu J, Huang L, Chen X, Gao Q, Sun G, Xiong G, Yang X, Hao L, Jin Z, Yang H, Yang N. Periconceptional iron supplementation and risk of gestational diabetes mellitus: A prospective cohort study [J]. *Diabetes Res. Clin. Pract.*, 176, 108853 (2021).
- 20) Feng J, Wang H, Jing Z, Wang Y, Wang W, Jiang Y, Sun W. Relationships of the Trace Elements Zinc and Magnesium With Diabetic Nephropathy-Associated Renal Functional Damage in Patients With Type 2 Diabetes Mellitus [J]. *Front. Med. (Lausanne)*, 8, 626909 (2021).
- 21) Al-Ali K, Abdel Fatah H S, El-Badry Y A. Dual Effect of Curcumin-Zinc Complex in Controlling Diabetes Mellitus in Experimentally Induced Diabetic Rats [J]. *Biol. Pharm. Bull.*, 39, 1774-1780 (2016).
- 22) Sisnande T, Lima C K, Da Silva D C, Beninato T M, Alves N L, Amaral M J, Miranda-Alves L, Lima L. Dietary zinc restriction promotes degeneration of the endocrine pancreas in mice [J]. *Biochim. Biophys. Acta. Mol. Basis. Dis.*, 1866, 165675 (2020).
- 23) Minami T, Sasaki H, Okazaki Y, Inoue T, Yamada Y, Fushimi H. Effects of insulin and 1 alpha-hydroxy vitamin D3 on streptozotocin-induced diabetic rats fed a low zinc diet [J]. *Biol. Pharm. Bull.*, 16, 468-470 (1993).
- 24) To P K, Do M H, Cho J H, Jung C. Growth Modulatory Role of Zinc in Prostate Cancer and Application to Cancer Therapeutics [J]. *Int. J. Mol. Sci.*, 21, (2020).
- 25) Hojyo S, Fukada T. Roles of Zinc Signaling in the Immune System [J]. *J. Immunol. Res.*, 2016, 6762343 (2016).
- 26) Zhang L, Yan M, Ren Y, Chen Y, Zhang S. Zinc regulates the hydraulic response of maize root under water stress conditions [J]. *Plant Physiol. Biochem.*, 159, 123-134 (2021).
- 27) Peng C, Cheng Q, Liu Y, Zhang Z, Wang Z, Ma H, Liu D, Wang L, Wang C. Marginal Zinc Deficiency in Mice Increased the Number of Abnormal Sperm and Altered the Expression Level of Spermatogenesis-Related Genes [J]. *Biol. Trace Elem. Res.*, (2021).
- 28) Nazem M R, Asadi M, Jabbari N, Allameh A. Effects of zinc supplementation on superoxide dismutase activity and gene expression, and metabolic parameters in overweight type 2 diabetes patients: A randomized, double-blind, controlled trial [J]. *Clin. Biochem.*, 69, 15-20 (2019).
- 29) Tomas-Sanchez C, Blanco-Alvarez V M, Martinez-Fong D, Gonzalez-Barrios J A, Gonzalez-Vazquez A, Aguilar-Peralta A K, Torres-Soto M, Soto-Rodriguez G, Limon I D, Brambila E, Millan-Perez-Pena L, Cebada J, Orozco-Barrios C E, Leon-Chavez B A. Prophylactic Zinc and Therapeutic Selenium Administration Increases the Antioxidant Enzyme Activity in the Rat Temporoparietal Cortex and Improves Memory after a Transient Hypoxia-Ischemia [J]. *Oxid. Med. Cell. Longev.*, 2018, 9416432 (2018).
- 30) Abdollahi M, Ajami M, Abdollahi Z, Kalantari N, Houshiarrad A, Fozouni F, Fallahrokni A, Mazandarani F S. Zinc supplementation is an effective and feasible strategy to prevent growth retardation in 6 to 24 month children: A pragmatic double blind, randomized trial [J]. *Heliyon*, 5, e02581 (2019).
- 31) Warner M J, Ozanne S E. Mechanisms involved in the developmental programming of adulthood disease [J]. *Biochem. J.*, 427, 333-347 (2010).
- 32) Xia W, Xie W. Rebooting the Epigenomes during Mammalian Early Embryogenesis [J]. *Stem. Cell Reports*, 15, 1158-1175 (2020).
- 33) Hemberger M, Dean W, Reik W. Epigenetic dynamics of stem cells and cell lineage commitment: digging Waddington's canal [J]. *Nat. Rev. Mol. Cell Biol.*, 10, 526-537 (2009).

- 34) Brito S, Lee M G, Bin B H, Lee J S. Zinc and Its Transporters in Epigenetics [J]. *Mol. Cells*, 43, 323-330 (2020).
- 35) Schmid T E, Eskenazi B, Marchetti F, Young S, Weldon R H, Baumgartner A, Anderson D, Wyrobek A J. Micronutrients intake is associated with improved sperm DNA quality in older men [J]. *Fertil. Steril.*, 98, 1130-1137 (2012).
- 36) Mendes Garrido Abregu F, Gobetto M N, Castanon A, Lucero D, Caniffi C, Elesgaray R, Schreier L, Arranz C, Tomat A L. Fetal and postnatal zinc restriction: Sex differences in metabolic alterations in adult rats [J]. *Nutrition*, 65, 18-26 (2019).
- 37) Hoyenga K B, Hoyenga K T. Gender and energy balance: sex differences in adaptations for feast and famine [J]. *Physiol. Behav.*, 28, 545-563 (1982).
- 38) Tomat A L, Veiras L C, Aguirre S, Fasoli H, Elesgaray R, Caniffi C, Costa M A, Arranz C T. Mild zinc deficiency in male and female rats: early postnatal alterations in renal nitric oxide system and morphology [J]. *Nutrition*, 29, 568-573 (2013).
- 39) Jou M Y, Philipps A F, Lonnerdal B. Maternal zinc deficiency in rats affects growth and glucose metabolism in the offspring by inducing insulin resistance postnatally [J]. *J. Nutr.*, 140, 1621-1627 (2010).
- 40) Phillips D I, Barker D J, Hales C N, Hirst S, Osmond C. Thinness at birth and insulin resistance in adult life [J]. *Diabetologia*, 37, 150-154 (1994).
- 41) Pena J E, Rascon-Pacheco R A, Ascencio-Montiel I J, Gonzalez-Figueroa E, Fernandez-Garate J E, Medina-Gomez O S, Borja-Bustamante P, Santillan-Oropeza J A, Borja-Aburto V H. Hypertension, Diabetes and Obesity, Major Risk Factors for Death in Patients with COVID-19 in Mexico [J]. *Arch. Med. Res.*, 52, 443-449 (2021).

Fig. 1. Low zinc diet induced low zinc state in tissues of F0 generation male mice.

(A) Zinc content in diet, (B) Serum Zinc, (C) Zinc content in tissues of F0 generation male mice (Control group: n=5; Low zinc group n=6). Bars represent the Means±S.D.

* represents $P<0.05$, ** represents $P<0.01$, compared with the control group.

Fig. 2. Low zinc diet caused spleen enlarged and IL-6 increased in F0 generation male mice. Organ coefficient(A and B), inflammatory factors(C), oxidative stress levels (D) and OGTT (E) of F0 generation male mice. Bars represent the Means $\pm$ S.D. * represents $P<0.05$ compared with the control group.

Fig. 3. Low zinc diet reduced survival rate and had no effect on body weight of F1 offspring. The Survival rate (A) and body weight of F1 female offspring mice (B) and F1 female offspring mice (C) (n=15 mice/group). Bars represent the Means $\pm$ S.D. * represents $P<0.05$, ** represents $P<0.01$, compared with the control group.

Fig. 4. *Low zinc diet attenuates glucose metabolism in F1 male offspring.* OGTT in mice following with 2 g/kg glucose challenge(A), ITT in mice post 0.5 IU/kg insulin injection(B), insulin secretion function(C), Pancreatic histomorphology of Control group (D) and Low zinc group (E) of F1 male offspring (100×). (Control group: n=5; Low zinc group n=6). Bars represent the Means±S.D. * represents $P<0.05$, ** represents $P<0.01$, compared with the control group.

Fig. 5. *Low zinc diet had no effect on glucose metabolism in F1 female offspring.* OGTT in mice following with 2 g/kg glucose challenge(A), ITT in mice post 0.5 IU/kg insulin injection(B), insulin secretion function(C), Pancreatic histomorphology of Control group (D) and Low zinc group (E) of F1 female offspring(100×), (Control group: n=5; Low zinc group n=6). Bars represent the Means±S.D.