Fetal and postnatal zinc restriction: sex differences in metabolic alterations in adult rats

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Highlights

- Male adult zinc-deficient rats show hyperglycemia and hypertriglyceridemia
- An increase in hepatic lipid peroxidation was observed in zinc deficient males
- Zinc-deficient male rats show adipocyte hyperthrophy and increased oxidative stress
- Female rats were less sensitive to the metabolic effects of zinc restriction
- Adequate zinc diet after weaning prevent most of the metabolic alterations

Fetal and postnatal zinc restriction: sex differences in metabolic alterations in adult rats

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Author contributions

Serum metabolic profile was determined by Diego Lucero and Laura Schreier. Oral glucose tolerance test was performed by Facundo Mendes Garrido Abregú, María Natalia Gobetto, Rosana Elesgaray, Analía Lorena Tomat. Hepatic histology was analyzed by Facundo Mendes Garrido Abregú and Agustina Castañón. RPAT histology was analyzed by Facundo Mendes Garrido Abregú, Carolina Caniffi and Rosana Elesgaray. Hepatic and RPAT oxidative stress was determined by Facundo Mendes Garrido Abregú and Agustina Castañón. Experimental design, analysis of results and manuscript drafting were performed by Facundo Mendes Garrido Abregú, Cristina Arranz and Analia Tomat. Scientific and technical supervision was performed by Cristina Arranz and Analia Tomat.

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Disclosure

The authors declare no conflict of interest.

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Abstract

Objective: Intrauterine and postnatal micronutrient malnutrition may program metabolic diseases in adulthood. We examined whether moderate zinc restriction in male and female rats throughout fetal life, lactation and/or post-weaning growth induces alterations in liver, adipose tissue and intermediate metabolism.

Methods: Female Wistar rats were fed low or control zinc diets from pregnancy to offspring weaning. After weaning, male and female offspring were fed either a low or a control zinc diet. At 74 days of life, oral glucose tolerance tests were performed and serum metabolic profiles were evaluated. Systolic blood pressure as well as oxidative stress and morphology of liver and retroperitoneal adipose tissue were evaluated in 81 day-old offspring.

Results: Zinc restriction during prenatal and postnatal life induced an increase in systolic blood pressure, hyperglycemia, hypertriglyceridemia, higher serum glucose levels at 180 minutes after glucose overload, and greater insulin resistance indexes in males. Hepatic histological studies revealed no morphological alterations, but an increase in lipid peroxidation and catalase activity were observed in zinc deficient males. Adipose tissue from zinc-deficient male rats showed adipocytes hypertrophy, an increase in lipid peroxidation and a reduction in catalase and glutathione peroxidase activity. Adequate dietary zinc content during post-weaning growth reversed basal hyperglycemia, hypertriglyceridemia, insulin resistance indexes, hepatic oxidative stress and adipocyte hypertrophy. Female rats were less sensitive to the metabolic effects of zinc restriction.
Conclusions: This study strengthens the importance of a balanced intake of zinc during growth to ensure adequate lipid and carbohydrate metabolism in adult life.

Keywords: Zinc deficiency; Metabolism; Oxidative stress; Adipose tissue; Liver

Abbreviations

AT: adipose tissue, BW: body weight, CAT: catalase, GLUT: glutathione, GPx: glutathione peroxidase, IR: insulin resistance, OGTT: oral glucose tolerance test, RPAT: retroperitoneal adipose tissue, SBP: systolic blood pressure, SOD: superoxide dismutase, TBARS: 2-thiobarbituric acid reactive substances, TG: triglycerides, TL: tibia length

Introduction

Numerous epidemiological and experimental studies demonstrate a correlation between an adverse intrauterine environment and increased risk of cardiovascular and metabolic diseases in adulthood **[1]**. People exposed to famine in utero show a more atherogenic lipid profile, impaired glucose tolerance and higher prevalence of hypertension and diabetes **[2,3]**. In addition, animal models have shown that maternal suboptimal nutrition programs metabolic alterations that promote liver steatosis and obesity **[4]**. Moreover, it has been reported that there would be sex differences in the metabolic alterations programmed by prenatal nutritional injuries **[5]**.

Micronutrient malnutrition affects over 2 billion people worldwide and it is now estimated that 17.3% of the world's population does not reach the recommended dietary requirements of zinc **[6]**. Moderate zinc restriction during pregnancy could be a nutritional injury for fetal and postnatal development since it is an essential micronutrient for cell growth, development and differentiation **[7,8]**. Zinc has anti-oxidant, anti-apoptotic and

anti-inflammatory properties **[9].** It is involved in the regulation of triglycerides and fatty acid synthesis and degradation **[10]**. Zinc plays a key role in insulin production and secretion since this hormone is stored as insulin-zinc crystals that protect it from degradation in pancreatic beta cells. Moreover, zinc may contribute to adequate insulin signaling pathway and tissue glucose uptake **[11]**.

In previous studies we demonstrated that dietary zinc restriction during prenatal and postnatal growth programs an increase in systolic blood pressure (SBP) and impaired renal and cardiac development and function in adult male rats. These alterations are related to higher renal oxidative stress and reduced renal and cardiac nitric oxide synthase activity **[12-14]**. Zinc deficiency during early life also programs vascular alterations in both male and female adult rats. However, females are less sensitive to the cardiovascular effects of zinc deficiency **[15]**.

We hypothesize that prenatal and postnatal moderate zinc restriction in male and female rats induces alterations in liver, adipose tissue (AT) and lipid and glucose metabolism that can, in turn, increase cardiovascular risk in adult life. These metabolic alterations could not be completely reversed by adequate zinc intake during postnatal life. The objective of this study was to evaluate liver and retroperitoneal adipose tissue (RPAT) morphology and oxidative stress as well as serum metabolic profile and glucose tolerance in adult male and female rats exposed to zinc deficiency during fetal life, lactation and/or postnatal growth.

Materials and methods

Animals and study design

Female Wistar rats weighing 280±10 g obtained from the breeding laboratories of Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Argentina, were mated by exposure to Wistar males for one week. Immediately afterwards, female rats were randomly fed either a moderately zinc-deficient (L, 8ppm, n=10) or a control zinc diet (C, 30ppm, n=5) during pregnancy and lactation periods. Eight rat pups remained with each mother until 21 days of life (weaning) by random culling of pups at birth and retaining a 1:1 male-to-female ratio. After weaning, male (m) and female (f) offspring of L mothers were fed either a low (8ppm; Llm and Llf groups, n=20/group) or a control (30ppm; Lcm and Lcf groups, n=20/group) zinc diet for 60 days, and offspring of C mothers were fed a control zinc diet (30ppm; Ccm and Ccf groups, n=20/group) (Fig. 1).

Both diets included all the necessary nutrients, except zinc content, to meet rat requirements for the pregnancy and lactation periods according to AIN-93

recommendations **[16]**. Mothers and their offspring were housed in plastic cages in a humidity- and temperature-controlled environment with a 12-hour light-dark cycle. Animals were allowed food and deionized water *ad libitum*. At 74 days of life, part of the offspring from each experimental group were fasted for 6 hours to perform the oral glucose tolerance test (OGTT) and the rest were fasted for 12 hours to evaluate serum metabolic profile. Blood was obtained from the tail vein and serum samples were stored at -20 °C until analysis.

At 81 days of life, SBP was measured indirectly in awake animals by the tail-cuff method (PowerLab 8/30, LabChart 6 Pro software, ADInstruments, Australia), as described previously **[15]**. Afterwards rats were weighed and euthanized by cervical decapitation. Blood was collected to determine serum zinc concentration using atomic absorption spectrometry (spectrometer SpectrAA-20, air acetylene flame, 0.5nm slit, wavelength 213.9nm, Varian, Australia) **[17]**. Liver and RPAT, perigonadal and mesenteric AT were removed and weighted. In order to evaluate hepatic and RPAT histology, samples were fixed in 4% phosphate buffered formalin for 24 hours and transferred to 70% ethanol, trimmed and embedded in paraffin. Liver samples were frozen in liquid nitrogen to perform oil red O staining. Tissue samples were frozen in liquid nitrogen and stored at -80°C to evaluate oxidative stress. Right tibia length (TL) was measured.

Animals were cared for according to Argentina's National Drug, Food and Medical Technology Administration standards (Regulation No.6344/96) and the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No.85-23, Revised 1996). Experimental procedures were approved by the ethics committee for the care and use of laboratory animals of Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Argentina (Resolution No.3191).

Serum metabolic profile

Glycemia, triglycerides (TG), total cholesterol, high-density lipoprotein (HDL) cholesterol and activity of transaminase enzymes –aspartate transaminase (AST) and alanine transaminase (ALT)– were measured by standardized enzymatic methods in a Cobas 6000 analyzer (Roche Diagnostics, Germany). Non-HDL cholesterol, as an indicator of apoB-containing lipoproteins, was calculated as the difference between total and HDL cholesterol **[18]**. Castelli's risk index (total/HDL cholesterol) was calculated for cardiovascular risk assessment. TG/HDL cholesterol index and TyG index (ln (TG*glycemia/2)) were estimated to evaluate insulin resistance (IR) **[19,20]**.

Oral glucose tolerance test

A load of 0.2 g of glucose/100g body weight (BW) was administered orally to fasted rats. Blood was sampled before loading (t=0) and at 30, 60, 120 and 180 minutes after glucose administration. Glycemia was measured using test strips and a glucometer (Accu-Chek Performa, Roche Diagnostics, Germany). Integrated area under the curve (AUC) was obtained from the plotting of glucose concentration as a function of time **[21]**.

Histological evaluation

Liver sections (5µm thick) were stained with hematoxylin-eosin to evaluate tissue organization **[22]** and with Picrosirius Red to assess interstitial collagen levels **[23]**. Oil red O staining was performed to detect lipid droplets (9µm thick) **[24]**. RPAT sections (6µm thick) were stained with hematoxylin-eosin for determination of size and density of adipocytes **[25]**.

Histological studies were performed using an Olympus BX51 light microscope equipped with a digital camera (Qcolor 3 Olympus America) and connected to Image-Pro Plus 4.5.1.29 software (Media Cybernetics, LP, Silver Spring, MD). Histological examination was performed in a blinded manner, analyzing 20 fields at x400 per animal.

Hepatic and retroperitoneal adipose tissue oxidative stress

Lipid oxidative damage was assessed measuring the extent of formation of 2-thiobarbituric acid reactive substances (TBARS) [26]. Glutathione content (GLUT) was determined using the Ellman reagent (5,5'-dithiobis-(2-nitrobenzoic acid or DTNB) [27]. Superoxide dismutase (SOD) activity was assessed by measuring the ability of the homogenate to inhibit autoxidation of epinephrine [28]. Catalase (CAT) activity was determined by the conversion of hydrogen peroxide to oxygen and water [29]. The assay described by Flohé and Gunzler was used to measure glutathione peroxidase (GPx) activity [30]. Protein concentration was determined by the method of Bradford [31].

Statistical analysis

All values are expressed as mean ± SEM. A two-way analysis of variance (ANOVA) followed by a Bonferroni post hoc test for multiple comparisons was performed (Graph Pad Prism 5.0 Software, San Diego, CA). One factor was diet and the other was sex. $P<0.05$ was considered a significant difference.

Results

Body and tissue weight, serum zinc concentration and systolic blood pressure

Llm, Lcm, Llf and Lcf offspring showed reduced BW and TL compared with Ccm and Ccf, respectively. These growth markers were higher in male than in female groups.

Llm rats showed a reduced RPAT weight compared with Ccm and Lcm. However, zinc restriction did not induce changes in liver, perigonadal AT or mesenteric AT weight. As previously reported, female rats exhibited higher perigonadal AT and lower RPAT weight than males **[32]**. Serum zinc concentration was lower in Llm and Llf compared with male and female Lc and Cc rats. Llm and Lcm offspring showed an increase in SBP compared with Ccm. However, no differences in SBP levels were observed among female groups (Table 1).

Serum metabolic profile

Llm rats showed an increase in glycemia, TG, TG/HDL cholesterol and TyG index compared with Ccm and Lcm. No differences were observed among females. Zinc restriction did not induce changes in transaminases or in total, HDL or non-HDL cholesterol. However, HDL cholesterol levels were higher in females, and Castelli's risk index was lower in Llf and Lcf rats compared with Llm and Lcm, respectively (Table 2).

Oral glucose tolerance test

No differences were found in basal glycemia or at 30, 60 or 120 minutes after glucose overload. However, Llm and Lcm rats had higher serum glucose levels at 180 minutes post-overload compared with Ccm. No differences were observed in AUC (Ccm: 27.5±0.6; Llm: 27.5±0.8; Lcm: 28±1; Ccf: 26.3±0.7; Llf: 25.5±0.8; Lcf: 27±1 min.mg/dl/1000; n=6-8/group) (Fig. 2).

Hepatic histological evaluation

Hematoxylin-eosin staining revealed no alterations in hepatic parenchyma organization, steatosis or infiltration of inflammatory cells in the liver of the different groups (Fig. 3). Sirius red showed no differences in hepatic collagen deposition (Fig. 4). Zinc restriction did not induce changes in hepatic lipid deposition according to Oil red O staining (Fig. 5).

Hepatic oxidative stress

Llm rats showed an increase in TBARS levels and CAT activity compared with Ccm and Lcm. Zinc restriction did not alter TBARS levels or CAT activity among female rats. Female offspring showed lower CAT activity compared with male offspring. No differences were observed in GLUT content or in SOD or GPx activity (Table 3).

Retroperitoneal adipose tissue histological evaluation

Llm rats showed larger adipocytes and a decrease in adipocytes density compared with Ccm and Lcm. Zinc restriction did not induce changes in these parameters among females (Fig. 6, Fig. 7).

Retroperitoneal adipose tissue oxidative stress

Llm and Lcm rats showed an increase in lipid peroxidation and a reduction in CAT and GPx activity. Moreover, Llf and Lcf rats showed lower GPx activity compared with Ccf. Zinc restriction did not induce changes in GLUT levels or in SOD activity (Table 4).

Discussion

The results of the present study show that moderate zinc deficiency during fetal and postnatal development leads to cardiovascular and metabolic alterations in adult life.

Zinc restriction during prenatal and postnatal life induced an increase in SBP only in adult males and a growth delay in offspring of both sexes. Moreover, an adequate zinc diet during post-weaning life could not normalize either growth markers in male and female offspring or SBP in male rats. Several studies showed that zinc stimulates cell proliferation by up-regulating gene expression of enzymes involved in DNA synthesis as deoxythymidine kinase **[33]** and by stimulating production of growth hormone and insulin-like growth factors **[8]**. Moreover, our group has demonstrated that this nutritional injury programs morphological and functional alterations in cardiovascular and renal tissues, that are greater in adult male rats than in females **[13-15]**. These changes would contribute to SBP increase only in males. Furthermore, our results are in agreement with different developmental programming animal models that show that female offspring exhibit a protected status compared with male offspring **[5]**.

In the present study, we observed that chronic zinc restriction during life induced an increase in glycemia after a 12-hour fasting, as well as, in IR indexes. In this regard, it has been reported that zinc plays an important role in blood glucose control since it is crucial for insulin biosynthesis, storage and release. Moreover, zinc favors the actions of insulin in target tissues increasing phosphorylation of its receptor and proteins involved in the insulin signaling pathway, as protein-kinase B **[11]**. Furthermore, our results are supported by human studies reporting an inverse correlation between serum zinc levels and fasting blood glucose **[34]**. Likewise, it has been shown that zinc supplementation reduces glycemia in diabetic patients **[35]**.

When OGTT was performed, no changes in basal glycemia were observed among experimental groups. We suggest that the greater sensitivity of Llm rats to stressing stimuli could explain why a prolonged 12-hour food restriction, but not a 6-hour fasting, increased basal glycemia. However, Llm and Lcm offspring showed higher glycemia at 180 minutes

post-overload compared with Ccm. This alteration would reflect a lower glucose tolerance programmed by zinc restriction during fetal life and lactation in male rats. Moreover, changes in fasting glycemia and glucose intolerance could be considered early signs of type 2 diabetes **[36]**.

Chronic zinc restriction also induced a rise in serum TG concentration in Llm rats. This result is relevant since TG increase contributes significantly to cardiovascular risk and the associated mortality, independent of cholesterol levels **[37]**. Ranasinghe P et al. described that zinc reduces IR and inhibits lipolysis in AT. Consequently, this micronutrient could reduce the release of free fatty acids to the circulation and their flow to the liver, preventing the excessive synthesis of hepatic lipoproteins and the elevation of blood TG **[38]**. In addition, zinc favors fatty acids utilization in hepatocytes mitochondria, thus regulating the hepatic synthesis of lipids **[39]**. Therefore, we postulate that the rise in TG observed in Llm rats could be due to alterations in lipid metabolism in AT and liver induced by zinc restriction. Further studies in these tissues should be conducted to confirm our hypothesis.

In our experimental model, metabolic alterations induced by zinc restriction were not associated with changes in hepatic morphology or in serum transaminases activity. However, Llm rats showed an increase in hepatic lipid peroxidation that was accompanied by a rise in CAT activity, probably to compensate a tissue pro-oxidant state. Changes in hepatic oxidative stress in Llm, as well as alterations in serum metabolic profile, would be an effect of chronic zinc restriction. In accordance with our results, previous studies have demonstrated that low hepatic zinc bioavailability induces an increase in oxidative stress and apoptosis in rodents **[40]**. In addition, hepatic oxidative stress is associated with IR development **[41]**.

Alterations in abdominal AT, including RPAT, are associated with dyslipidemia, IR and higher cardiovascular risk **[42]**. In the present study, Llm rats showed an increase in the size of RPAT adipocytes accompanied by a reduction in adipocyte density and in RPAT mass. In this regard, previous studies have shown a correlation between adipocyte hypertrophy and their dysfunction, IR and a greater release of pro-inflammatory factors **[42]**. Therefore, we postulate that morphological RPAT changes would be related to alterations in the serum metabolic profile observed in Llm rats. It has been shown that adipocytes hypertrophy is not necessarily associated with an increase in AT mass **[43]**. Thus, we suggest that chronic zinc deficiency in male rats would affect adipogenesis

leading to a reduced RPAT adipocyte number since several transcription factors involved in this process have zinc finger motifs **[44]**.

There is ample evidence that AT oxidative stress not only correlates with IR but also precedes it and is involved in its development. Zinc acts as an antioxidant inducing the generation of metallothioneins and activating GPx gene expression by nuclear factor (erythroid-derived 2)-like 2 **[45]**. In the present study, Llm and Lcm rats showed an increase in RPAT lipid peroxidation and a decrease in CAT and GPx activities. Similar results were previously observed in kidney of zinc-deficient male rats and also in AT of other fetal programming animal models **[12,46]**.

Our results demonstrated that female rats are less sensitive to zinc deficiency since they showed lesser metabolic effects than male offspring. Moreover, females showed higher levels of HDL cholesterol and lower values of Castelli's risk index, suggesting a reduced cardiovascular risk compared with males. Even though we have not evaluated the zinc-related mechanisms associated with these sex differences, previous studies have shown that estrogen exerts multiple protective effects by regulating insulin secretion in pancreatic beta cells **[47],** playing antioxidant actions on hepatic and adipose tissues **[48]**, increasing insulin sensitivity and preventing inflammation and lipid accumulation on skeletal muscle, AT and liver **[49]**. Other nutritional injuries such as a high-fat diet induced later development of AT oxidative damage, IR and obesity in female mice compared with males **[50]**. Moreover, Stubbins RE et al. demonstrated that 17β-estradiol administration improves glucose tolerance and the serum lipid profile and reverts adipocyte hypertrophy and oxidative stress in AT of ovariectomized mice exposed to high-fat diet **[51]**.

Conclusion

Our findings suggest that dietary zinc restriction during fetal life, lactation and post-weaning growth induces an increase in IR indexes and in serum glucose and TG in male rats, which could be related to alterations in liver and RPAT. Consequently, these metabolic disturbances could increase cardiovascular risk in adult male rats exposed to zinc deficiency during vulnerable periods of life. Moreover, we demonstrated that an adequate zinc diet during post-weaning life could revert most of these metabolic alterations. Finally, female rats were less sensitive to the metabolic effects of this nutritional injury.

Figure legends

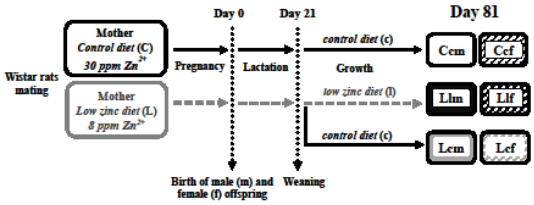

Fig. 1. Experimental animal model of moderate zinc deficiency during fetal life, lactation, and/or postnatal life. Female rats were randomly fed either a moderately zinc deficient diet (L, 8ppm) or a control zinc diet (C, 30ppm) during the pregnancy and lactation periods. Rat pups remained with each mother until weaning (21 days of life). Female (f) and male (m) offspring born from zinc deficient mothers were fed a low- (Llm and Llf, 8ppm) or a control (Lcm and Lcf, 30ppm) zinc diet for 60 days after weaning, and m and f offspring born from control mothers were fed control zinc diet (Ccm and Ccf, 30ppm).

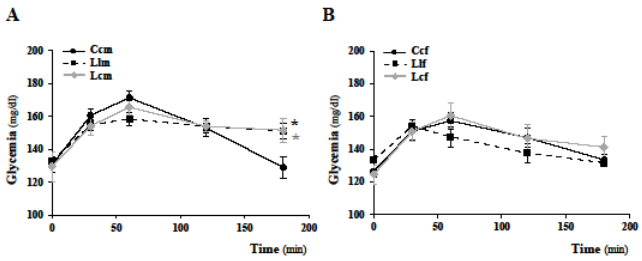

Fig. 2. Oral glucose tolerance test in male (A) and female (B) rats at 74 days of life. Female (f) and male (m) offspring born from zinc deficient mothers were fed a low- (Llm and Llf, 8ppm) or a control (Lcm and Lcf, 30ppm) zinc diet after weaning, and m and f offspring born from control mothers were fed control zinc diet (Ccm and Ccf, 30ppm).*P<0.05 vs. Ccm 180min. (n=6-8/group)

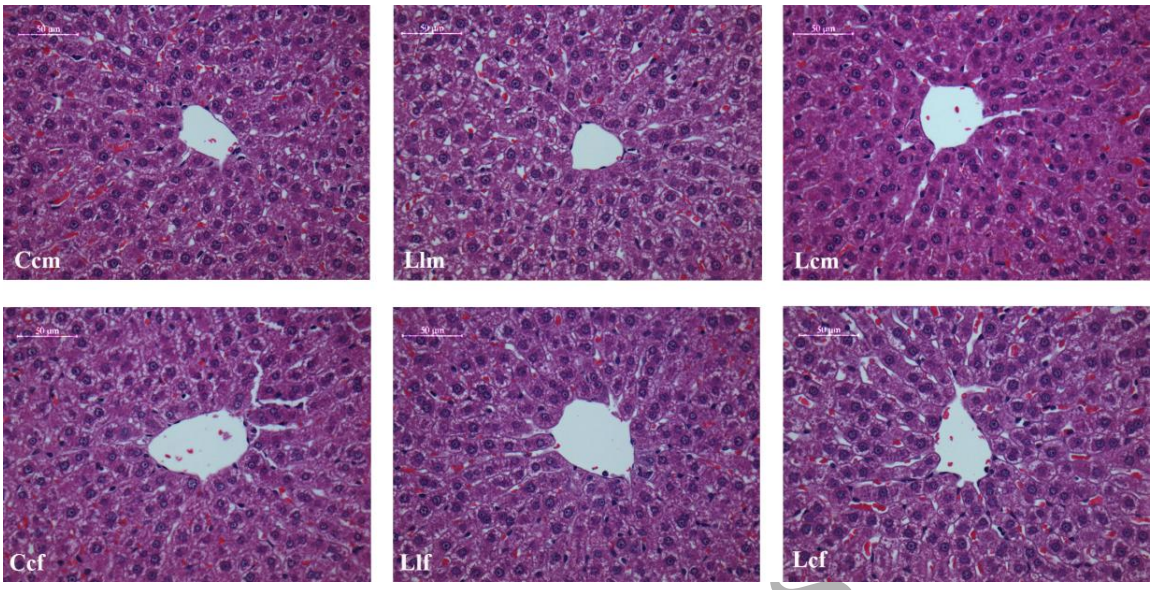

Fig. 3. Hematoxylin-eosin staining in liver of 81-day-old rats. Female (f) and male (m) offspring born from zinc deficient mothers were fed a low- (Llm and Llf, 8ppm) or a control (Lcm and Lcf, 30ppm) zinc diet for 60 days after weaning, and m and f offspring born from control mothers were fed control zinc diet (Ccm and Ccf, 30ppm). 400x; scale bar = 50μm. (n=6/group)

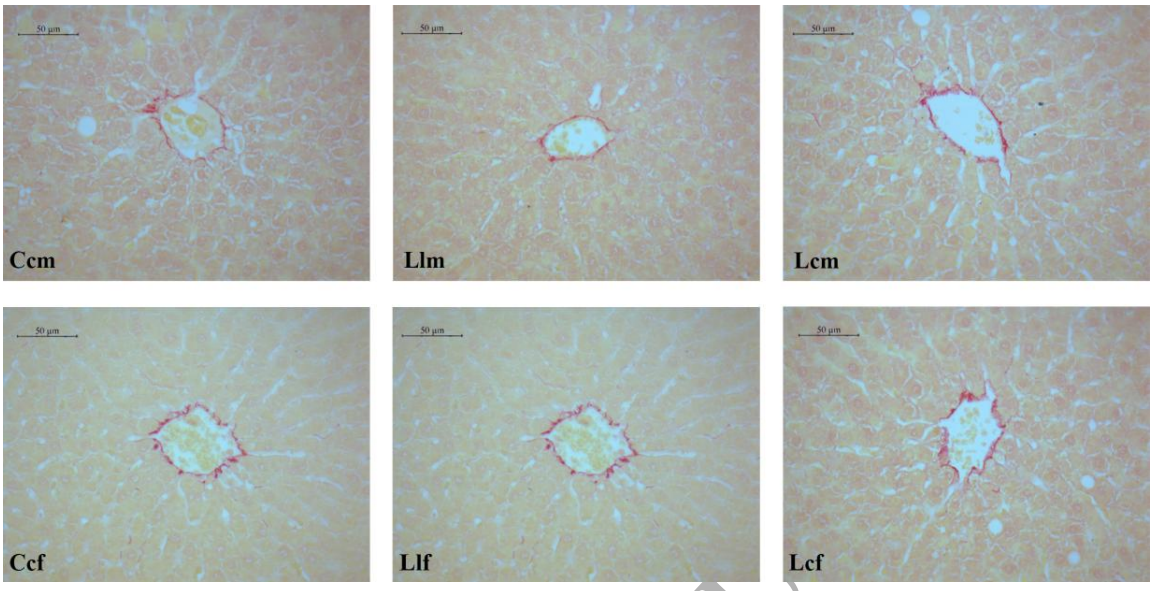

Fig. 4. Picrosirius Red staining in liver of 81-day-old rats. Female (f) and male (m) offspring born from zinc deficient mothers were fed a low- (Llm and Llf, 8ppm) or a control (Lcm and Lcf, 30ppm) zinc diet for 60 days after weaning, and m and f offspring born from control mothers were fed control zinc diet (Ccm and Ccf, 30ppm). Collagen fibers are stained red; 400x; scale bar = 50μm. (n=6/group)

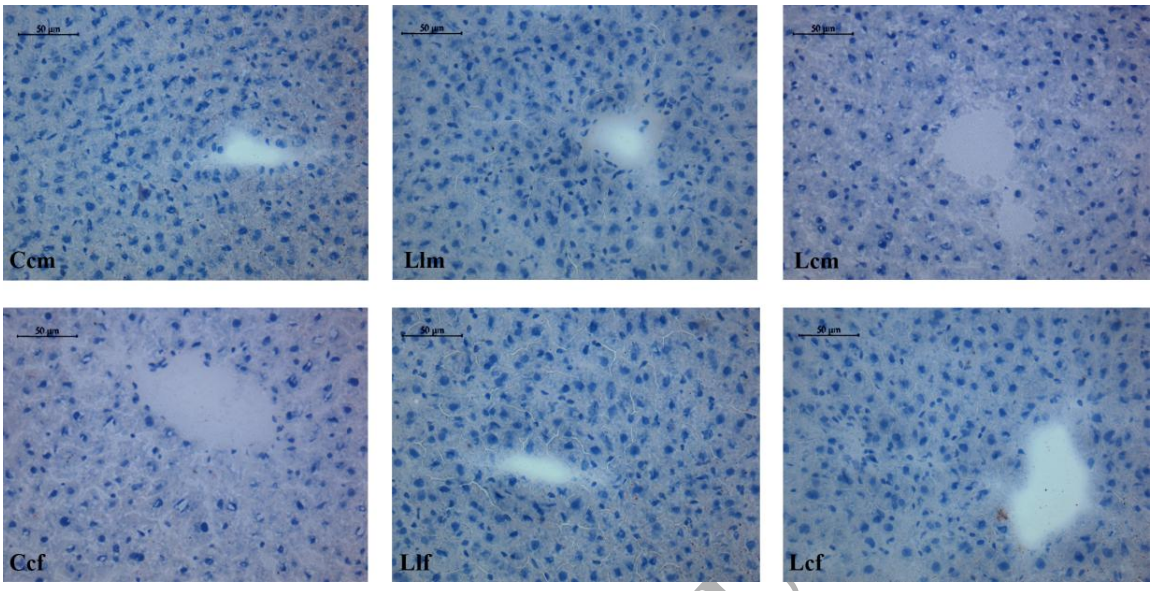

Fig. 5. Oil red O staining in liver of 81-day-old rats. Female (f) and male (m) offspring born from zinc deficient mothers were fed a low- (Llm and Llf, 8ppm) or a control (Lcm and Lcf, 30ppm) zinc diet for 60 days after weaning, and m and f offspring born from control mothers were fed control zinc diet (Ccm and Ccf, 30ppm). 400x; scale bar = 50μm. (n=6/group)

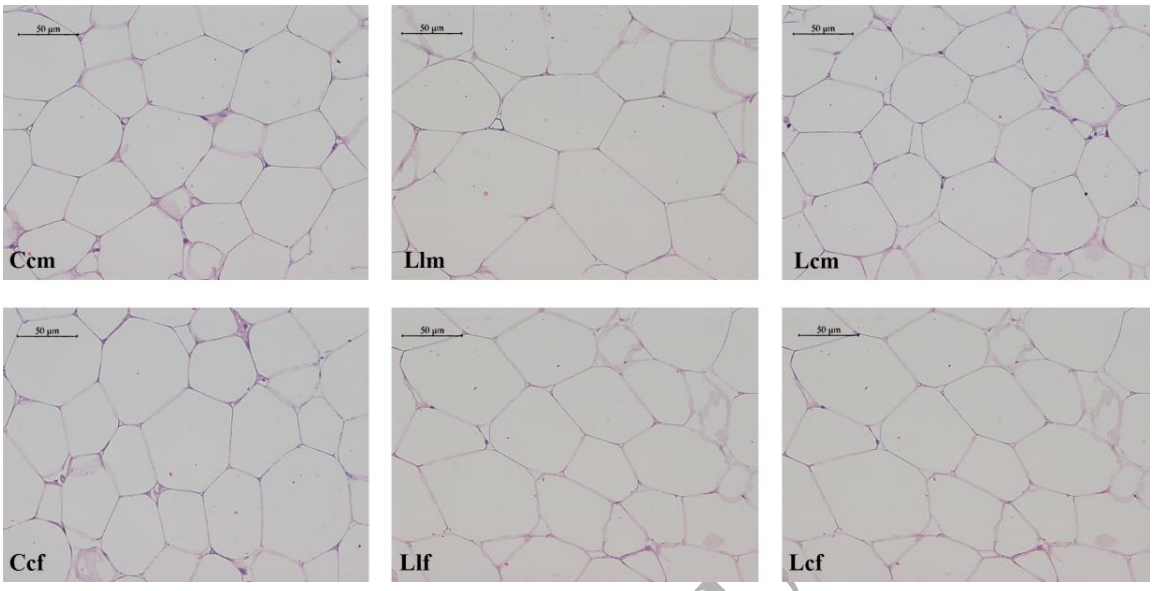

Fig. 6. Hematoxylin-eosin staining in retroperitoneal adipose tissue of 81-day-old rats. Female (f) and male (m) offspring born from zinc deficient mothers were fed a low- (Llm and Llf, 8ppm) or a control (Lcm and Lcf, 30ppm) zinc diet for 60 days after weaning, and m and f offspring born from control mothers were fed control zinc diet (Ccm and Ccf, 30ppm). 400x, scale bar = 50µm. (n=6/group)

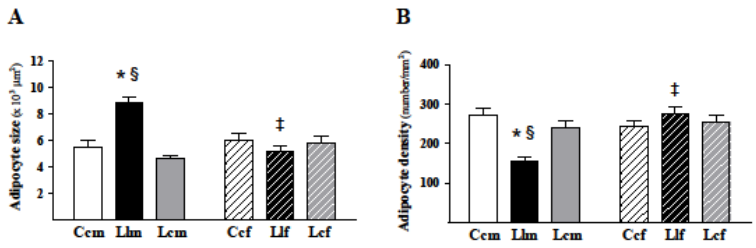

Fig. 7. Adipocyte size (A) and density (B) in retroperitoneal adipose tissue of 81-day-old rats. Female (f) and male (m) offspring born from zinc deficient mothers were fed a low- (Llm and Llf, 8ppm) or a control (Lcm and Lcf, 30ppm) zinc diet for 60 days after weaning, and m and f offspring born from control mothers were fed control zinc diet (Ccm and Ccf, 30ppm). *P<0.05 vs. Ccm; ‡P<0.05 vs. Llm; §P<0.05 vs. Lcm. Sex x diet interaction was considered significant (P<0.05). (n=6/group)

Table 1. Body and tissue weight, tibia length, serum zinc concentration and systolic blood pressure at 81 days of life.

	Ccm	Llm	Lcm	Ccf	Llf	Lcf
BW (g)	389±6	312±7*	346±6*,‡	249±4*	215±5†,‡	221±4†,§
Liver/BW (g/kg)	30.7±0.7	32.1±0.6	31.6±0.9	30.1±0.4	31.5±0.7	29.7±0.6
TL (cm)	3.91±0.04	3.62±0.03*	3.70±0.03*	3.56±0.03*	3.38±0.02†,‡	3.39±0.02†,§
RPAT/BW (g/kg)	12.2±0.9	9±1*,§	12±1	8.3±0.6*	6.3±0.5‡	6.9±0.5§
Perigonadal AT/BW (g/kg)	11.0±0.6	10.5±0.7	10.9±0.6	18.1±0.9*	17±1‡	16±1§
Mesenteric AT/BW (g/kg)	8.3±0.3	8.5±0.5	8.2±0.4	9.6±0.4	8.7±0.3	8.6±0.5
Serum zinc concentration (μg/dl)	163±8	118±5*,§	153±9	159±5	103±6†,a	153±5
SBP (mmHg)	123±1	144±2*	145±2*	121±3	119±4‡	122±4§

BW: body weight, TL: tibia length, RPAT: retroperitoneal adipose tissue, AT: adipose tissue, SBP: systolic blood pressure. Female (f) and male (m) offspring born from zinc deficient mothers were fed a low- (Llm and Llf, 8ppm) or a control (Lcm and Lcf, 30ppm) zinc diet for 60 days after weaning, and m and f offspring born from control mothers were fed control zinc diet (Ccm and Ccf, 30ppm). *P<0.05 vs. Ccm; ‡P<0.05 vs. Llm; §P<0.05 vs. Lcm; †P<0.05 vs. Ccf; aP<0.05 vs. Lcf. Sex x diet interaction: significant (P<.05) for RPAT/BW and SBP. (n=12/group)

Table 2. Serum metabolic profile at 74 days of life.

	Ccm	Llm	Lcm	Ccf	Llf	Lcf
Glycemia (mg/dl)	130±6	154±6*,§	128±5	136±5	135±5‡	131±5
TG (mg/dl)	85±4	112±6*,§	80±5	75±4	76±6‡	82±6
Total cholesterol (mg/dl)	67±3	68±3	70±3	76±2	74±2	73±3
HDL cholesterol (mg/dl)	47±2	46±2	49±2	61±1*	62±2‡	60±3§
Non-HDL cholesterol (mg/dl)	17±1	16±1	18±1	16±1	14±1	13±1
Total-cholesterol/ HDL-cholesterol	1.32±0.02	1.35±0.02	1.35±0.02	1.23±0.02	1.20±0.02‡	1.22±0.02§
TG/HDL cholesterol	1.8±0.1	2.4±0.1*,§	1.6±0.1	1.2±0.1*	1.2±0.1‡	1.4±0.2
TyG index	8.6±0.1	9.1±0.1*,§	8.5±0.1	8.5±0.1	8.5±0.1‡	8.6±0.1
AST (IU/l)	86±2	82±4	89±5	87±2	80±3	79±4
ALT (IU/l)	28±2	25±1	28±2	22±2	20±1	22±2

TG: triglycerides, AST: aspartate transaminase, ALT: alanine transaminase. Female (f) and male (m) offspring born from zinc deficient mothers were fed a low- (Llm and Llf, 8ppm) or a control (Lcm and Lcf, 30ppm) zinc diet after weaning, and m and f offspring born from control mothers were fed control zinc diet (Ccm and Ccf, 30ppm). *P<0.05 vs. Ccm; ‡P<0.05 vs. Llm; §P<0.05 vs. Lcm. Sex x diet interaction: significant (P<0.05) for glycemia, TG, TG/HDL cholesterol and TyG index. (n=10-12/group)

Table 3. Liver oxidative state at 81 days of life.

	Ccm	Llm	Lcm	Ccf	Llf	Lcf
TBARS (pmol/mg protein)	24.4±0.3	37.1±0.4*,§	22.1±0.2	26.8±0.2	26.2±0.3‡	23.8±0.3
GLUT (μg/mg protein)	6.7±0.4	5.8±0.3	5.9±0.5	4.3±0.5	4.6±0.3	4.2±0.3
SOD (U/mg protein)	3.5±0.4	3.3±0.5	3.2±0.4	3.4±0.5	3.3±0.4	3.4±0.3
CAT (pmol/s.mg protein)	2.1±0.1	3.1±0.3*,§	2.3±0.1	1.4±0.1*	1.4±0.1‡	1.3±0.1§
GPx (μmol/min.mg protein)	164±13	154±9	154±8	164±6	161±13	146±6

TBARS: 2-thiobarbituric acid reactive substances, GLUT: glutathione, SOD: superoxide dismutase, CAT: catalase, GPx: glutathione peroxidase. Female (f) and male (m) offspring born from zinc deficient mothers were fed a low- (Llm and Llf, 8ppm) or a control (Lcm and Lcf, 30ppm) zinc diet for 60 days after weaning, and m and f offspring born from control mothers were fed control zinc diet (Ccm and Ccf, 30ppm). *P<0.05 vs. Ccm; ‡P<0.05 vs. Llm; §P<0.05 vs. Lcm. Sex x diet interaction: significant (P<.05) for TBARS and CAT. (n=6/group)

Table 4. Retroperitoneal adipose tissue oxidative state at 81 days of life.

	Ccm	Llm	Lcm	Ccf	Llf	Lcf
TBARS (nmol/mg protein)	0.35±0.04	0.55±0.03*	0.82±0.04*,‡	0.63±0.03*	0.61±0.03	0.66±0.06
GLUT (μg/mg protein)	6.8±0.7	6.4±0.6	6.3±0.7	4.7±0.7	4.6±0.7	4.2±0.7
SOD (U/mg protein)	2.4±0.2	2.7±0.2	2.3±0.2	2.8±0.2	2.8±0.2	2.9±0.3
CAT (pmol/s.mg protein)	1.44±0.08	1.03±0.08*	1.12±0.04*	1.17±0.06	0.89±0.08	0.97±0.07
GPx (μmol/min.mg protein)	89±7	58±3*	66±7*	85±5	64±4†	67±3†

TBARS: 2-thiobarbituric acid reactive substances, GLUT: glutathione, SOD: superoxide dismutase, CAT: catalase, GPx: glutathione peroxidase. Female (f) and male (m) offspring born from zinc deficient mothers were fed a low- (Llm and Llf, 8ppm) or a control (Lcm and Lcf, 30ppm) zinc diet for 60 days after weaning, and m and f offspring born from control mothers were fed control zinc diet (Ccm and Ccf, 30ppm). *P<0.05 vs. Ccm; ‡P<0.05 vs. Llm; †P<0.05 vs. Ccf. Sex x diet interaction: significant (P<0.05) for TBARS and CAT. (n=6/group)

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