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Basic nutritional investigation

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

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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, or postweaning growth induces alterations in liver, adipose tissue, and intermediate metabolism.
Methods: Female Wistar rats were fed low-zinc or control zinc diets from pregnancy to offspring weaning. After weaning, male and female offspring were fed either a low-zinc or a control zinc diet. At 74 d of life, oral glucose tolerance tests were performed and serum metabolic profiles were evaluated. Systolic blood pressure and oxidative stress and morphology of liver and retroperitoneal adipose tissue were evaluated in 81 d 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 min after glucose overload, and greater insulin resistance indexes in male rats. Hepatic histologic studies revealed no morphologic alterations, but an increase in lipid peroxidation and catalase activity were identified in zinc-deficient male rats. Adipose tissue from zinc-deficient male rats had adipocyte hypertrophy, an increase in lipid peroxidation, and a reduction in catalase and glutathione peroxidase activity. Adequate dietary zinc content during postweaning 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.

Introduction

Numerous epidemiologic and experimental studies have reported a correlation between an adverse intrauterine environment and increased risk of cardiovascular and metabolic diseases in adulthood [1]. People exposed to famine in utero have a more atherogenic lipid profile, impaired glucose tolerance, and higher prevalence of hypertension and diabetes [2,3]. In addition, animal models have indicated 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 more than 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 because it is an essential micronutrient for cell growth, development, and differentiation [7,8]. Zinc has antioxidant, antiapoptotic, and antiinflammatory properties [9]. It is involved in the regulation of

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triglycerides and fatty acid synthesis and degradation [10]. Zinc plays a key role in insulin production and secretion because 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 found 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, female rats 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 and serum metabolic profile and glucose tolerance in adult male and female rats exposed to zinc deficiency during fetal life, lactation, 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 male Wistar rats for 1 wk. Immediately afterward, female rats were randomly fed either a moderately zinc-deficient (L, 8 ppm, $n = 10$) or a control zinc diet (C, 30 ppm, $n = 5$) during pregnancy and lactation periods. Eight rat pups remained with each mother until 21 d 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 (8 ppm; Llm and Llf groups, $n = 20$/group) or a control (30 ppm; Lcm and Lcf groups, $n = 20$/group) zinc diet for 60 d, and offspring of C mothers were fed a control zinc diet (30 ppm; 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-h light-dark cycle. Animals were allowed food and deionized water ad libitum. At 74 d of life, part of the offspring from each experimental group were fasted for 6 h to perform the oral glucose tolerance test, and the rest were fasted for 12 h to evaluate serum metabolic profile. Blood was obtained from the tail vein, and serum samples were stored at −20°C until analysis.

At 81 d of life, SBP was measured indirectly in awake animals by the tail-cuff method (PowerLab 8/30, LabChart 6 Pro Software, ADInstruments, Bella Vista, NSW, Australia), as described previously [15]. Afterward, 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, 0.5-nm slit, wavelength 213.9 nm, Varian, Australia) [17]. Liver and RPAT and perigonadal and mesenteric AT were removed and weighted. To evaluate hepatic and RPAT histologic characteristics, samples were fixed in 4% phosphate buffered formalin for 24 h 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 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 and alanine transaminase—were measured by standardized enzymatic methods in a Cobas 6000 analyzer (Roche Diagnostics, Mannheim, 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 Triglyceride glucose index (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 glucose/100 g body weight (BW) was administered orally to fasted rats. Blood was sampled before loading ($t = 0$) and at 30, 60, 120, and 180 min after glucose administration. Glycemia was measured using test strips and a glucometer (Accu-Chek Performa, Roche Diagnostics). Integrated area under the curve was obtained from the plotting of glucose concentration as a function of time [21].

Histologic evaluation

Liver sections (5 μm 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 (9 μm thick) [24]. RPAT sections (6 μm thick) were stained with hematoxylin-eosin for determination of size and density of adipocytes [25].

Histologic 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, USA). Histologic examination was performed in a blinded manner, analyzing 20 fields at 400 × per animal.

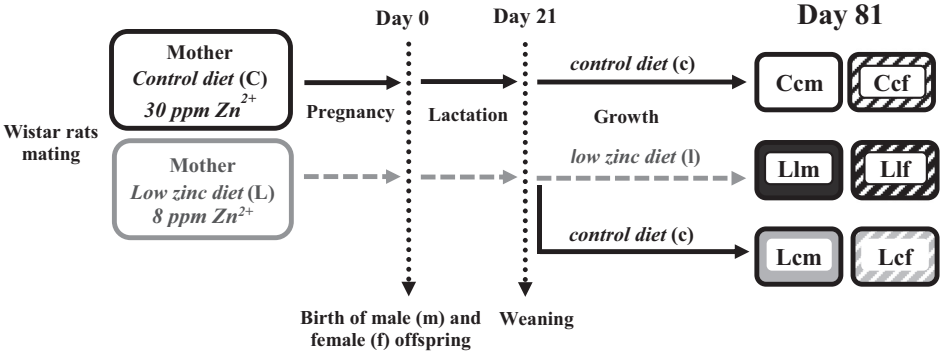

Fig. 1. Experimental animal model of moderate zinc deficiency during fetal life, lactation, or postnatal life. Female rats were randomly fed either a moderately zinc-deficient diet (L, 8 ppm) or a control zinc diet (C, 30 ppm) during the pregnancy and lactation periods. Rat pups remained with each mother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

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 [30] was used to measure glutathione peroxidase (GPx) activity. Protein concentration was determined by the method described by Bradford [31].

Statistical analysis

All values are expressed as mean ± SEM. A two-way analysis of variance followed by a Bonferroni post hoc test for multiple comparisons was performed (GraphPad Prism 5.0 Software, San Diego, CA, USA). 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 had reduced BW and tibia length compared with Ccm and Ccf, respectively. These growth markers were higher in male than in female groups. Llm rats had 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 male rats [32]. Serum zinc concentration was lower in Llm and Llf compared with male and female Lc and Cc rats. Llm and Lcm offspring had an increase in SBP compared with Ccm. However, no differences in SBP levels were identified among female groups (Table 1).

Serum metabolic profile

Llm rats had an increase in glycemia, TG, TG and HDL cholesterol, and TyG index compared with Ccm and Lcm. No differences were identified among female rats. Zinc restriction did not induce changes in transaminases or in total, HDL, or non-HDL cholesterol. However, HDL cholesterol levels were higher in female rats, 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 min after glucose overload. However, Llm and Lcm rats had higher serum glucose levels at 180 min postoverload compared with Ccm. No differences were identified in the area under the curve (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 per 1000; $n = 6–8$/group) (Fig. 2).

Hepatic histologic 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 indicated 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 had increased 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 had lower CAT activity compared with male offspring. No differences were identified in GLUT content or in SOD or GPx activity (Table 3).

Retroperitoneal adipose tissue histologic evaluation

Llm rats had larger adipocytes and decreased adipocyte density compared with Ccm and Lcm. Zinc restriction did not induce changes in these parameters among female rats (Figs. 6 and 7).

Retroperitoneal adipose tissue oxidative stress

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

Table 1
Body and tissue weight, tibia length, serum zinc concentration, and systolic blood pressure at 81 d 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‖	153 ± 5
SBP (mm Hg)	123 ± 1	144 ± 2*	145 ± 2*	121 ± 3	119 ± 4†	122 ± 4§

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

Table 2
Serum metabolic profile at 74 d 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

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

Discussion

The results of the present study indicate 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 male rats and a growth delay in offspring of both sexes. Moreover, an adequate zinc diet during postweaning life could not normalize either growth markers in male and female offspring or SBP in male rats. Several studies have found that zinc stimulates cell proliferation by upregulating gene expression of enzymes involved in DNA synthesis such as deoxythymidine kinase [33] and by stimulating production of growth hormone and insulinlike growth factors [8]. Moreover, our group has found that this nutritional injury programs morphologic and functional alterations in cardiovascular and renal tissues that are greater in adult male rats than in female rats [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 indicate that female offspring exhibit a protected status compared with male offspring [5].

In the present study we found that chronic zinc restriction during life induced an increase in glycemia after a 12-h fasting, as well as in IR indexes. In this regard, it has been reported that zinc plays an important role in blood glucose control because 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, such 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 found that zinc supplementation reduces glycemia in diabetic patients [35].

When an oral glucose tolerance test was performed, no changes in basal glycemia were identified among experimental groups. We suggest that the greater sensitivity of Llm rats to stressing stimuli could explain why a prolonged 12-h food restriction, but not a 6-h fasting, increased basal glycemia. However, Llm and Lcm offspring had higher glycemia at 180 min postoverload 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].

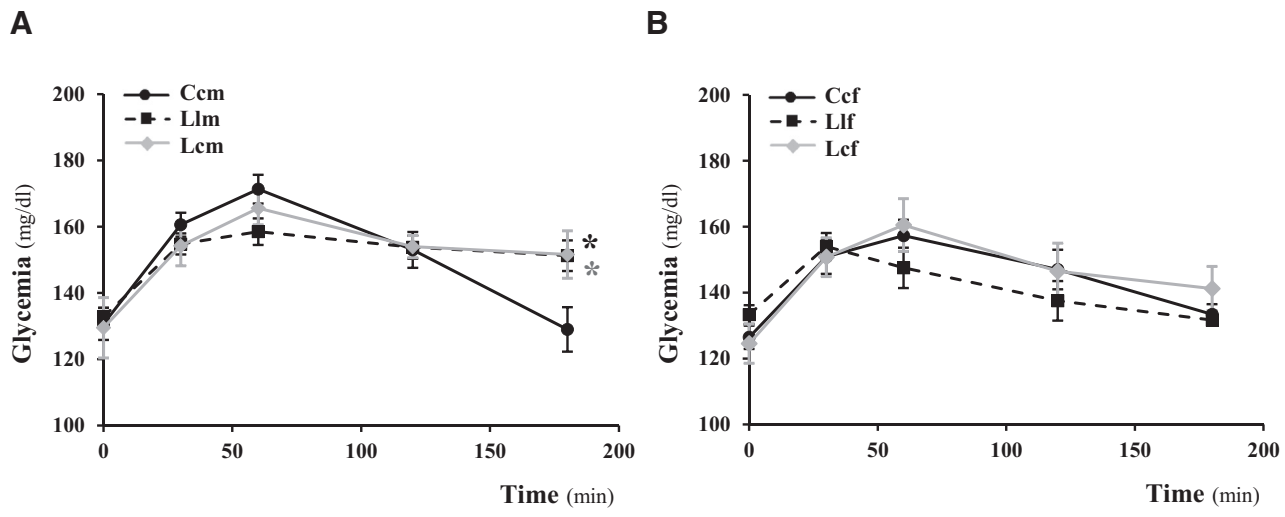

Fig. 2. Oral glucose tolerance test in male (A) and female (B) rats at 74 d of life. Female (f) and male (m) offspring born from zinc-deficient mothers were fed a low-zinc (Llm and Llf, 8 ppm) or a control zinc diet (Lcm and Lcf, 30 ppm) after weaning, and male and female offspring born from control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

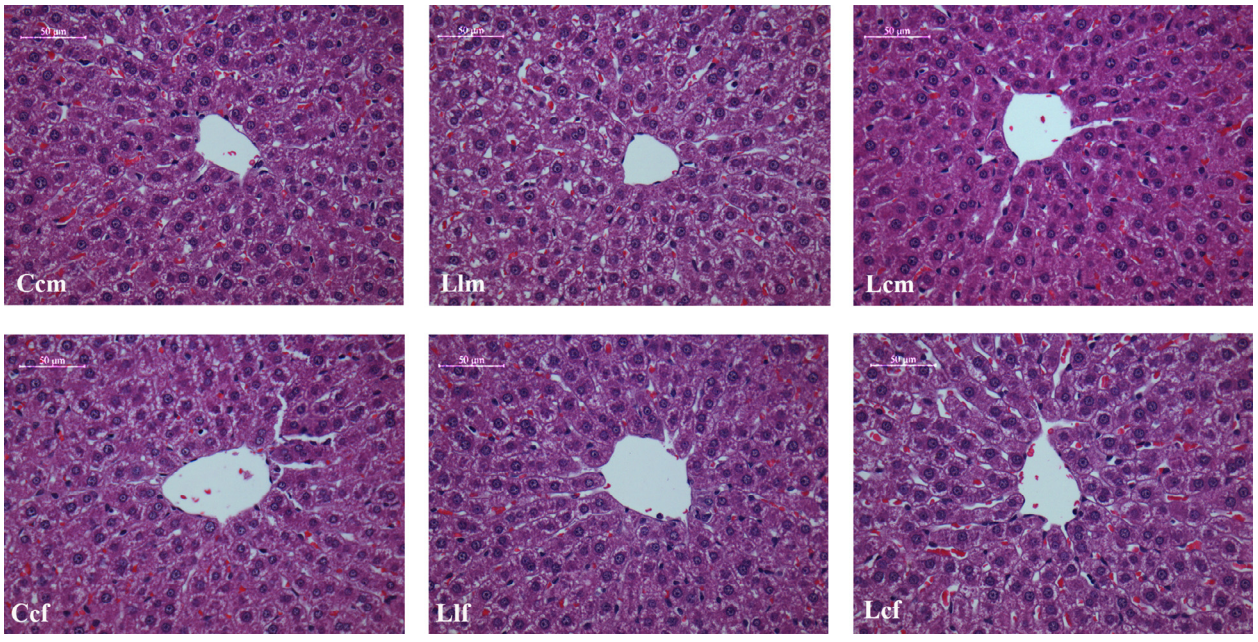

Fig. 3. Hematoxylin-eosin staining in liver of 81-d-old rats. Female (f) and male (m) offspring born from zinc-deficient mothers were fed a low-zinc (Llm and Llf, 8 ppm) or a control zinc diet (Lcm and Lcf, 30 ppm) for 60 d after weaning, and male and female offspring born from control mothers were fed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

Chronic zinc restriction also induced a rise in serum TG concentration in Llm rats. This result is relevant because TG increase contributes significantly to cardiovascular risk and the associated mortality, independent of cholesterol levels [37]. Ranasinghe et al. [38] found that zinc reduced IR and inhibited 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. 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 found 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

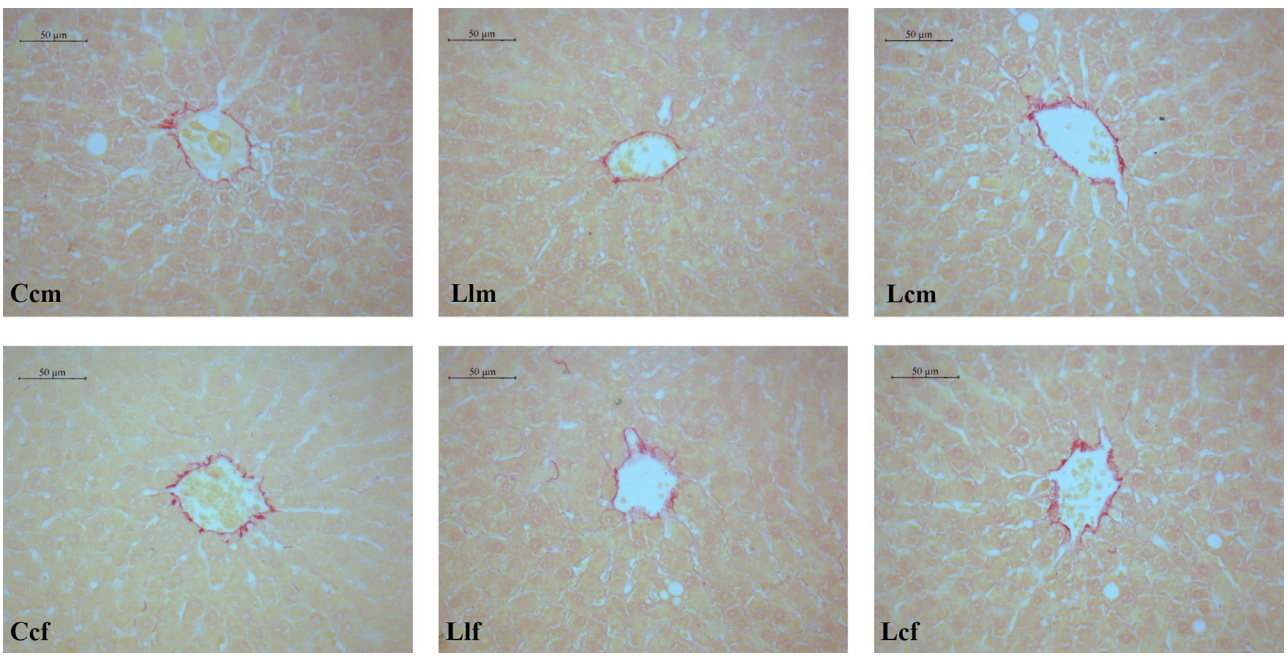

Fig. 4. Picrosirius red staining in liver of 81 d old rats. Female (f) and male (m) offspring born from zinc-deficient mothers were fed a low-zinc (Llm and Llf, 8 ppm) or a control zinc diet (Lcm and Lcf, 30 ppm) for 60 d after weaning, and male and female offspring born from control mothers were fed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

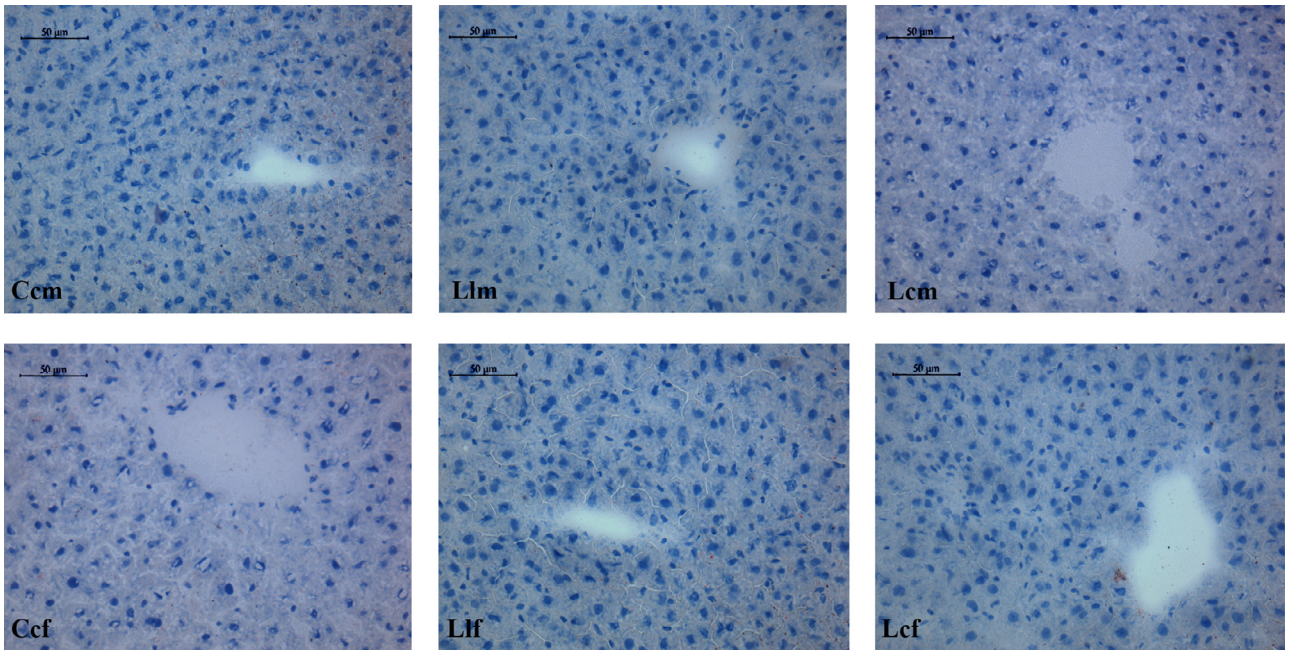

Fig. 5. Oil red O staining in liver of 81 d old rats. Female (f) and male (m) offspring born from zinc-deficient mothers were fed a low-zinc (Llm and Llf, 8 ppm) or a control zinc diet (Lcm and Lcf, 30 ppm) for 60 d after weaning, and male and female offspring born from control mothers were fed a control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

morphology or in serum transaminases activity. However, Llm rats had an increase in hepatic lipid peroxidation that was accompanied by a rise in CAT activity, probably to compensate a tissue prooxidant state. Changes in hepatic oxidative stress in Llm, and alterations in serum metabolic profile, would be an effect of chronic zinc restriction. In accordance with our results, previous studies have found 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 had 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 found a correlation between adipocyte hypertrophy and their dysfunction, IR, and a greater release of proinflammatory factors [42]. Therefore we postulate that morphologic RPAT changes would be related to alterations in the serum metabolic profile identified in Llm rats. It has been found that adipocyte 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, because 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 had an increase in RPAT lipid peroxidation and a decrease in CAT and GPx activities. Similar results were previously reported in kidneys of zinc-deficient male rats and also in AT of other fetal programming animal models [12,46].

Our results indicate that female rats are less sensitive to zinc deficiency because they had lesser metabolic effects than male offspring. Moreover, female rats had higher levels of HDL cholesterol and lower values of Castelli's risk index, suggesting a reduced cardiovascular risk compared with males. Although we have not evaluated the zinc-related mechanisms associated with these sex differences, previous studies have found that estrogen exerts multiple protective effects by regulating insulin secretion in pancreatic beta cells [47], producing antioxidant actions on hepatic and

Table 3
Liver oxidative state at 81 d 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

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

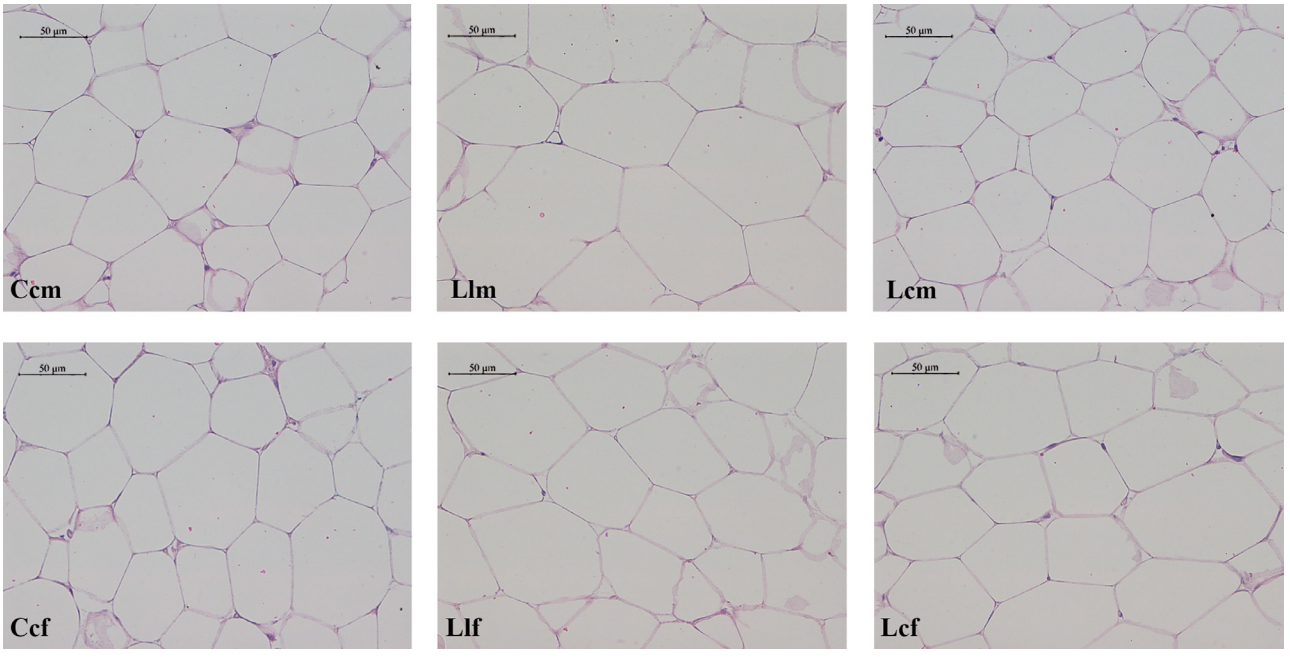

Fig. 6. Hematoxylin-eosin staining in retroperitoneal adipose tissue of 81 d old rats. Female (f) and male (m) offspring born from zinc-deficient mothers were fed a low-zinc (Llm and Llf, 8 ppm) or a control zinc diet (Lcm and Lcf, 30 ppm) for 60 d after weaning, and male and female offspring born from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

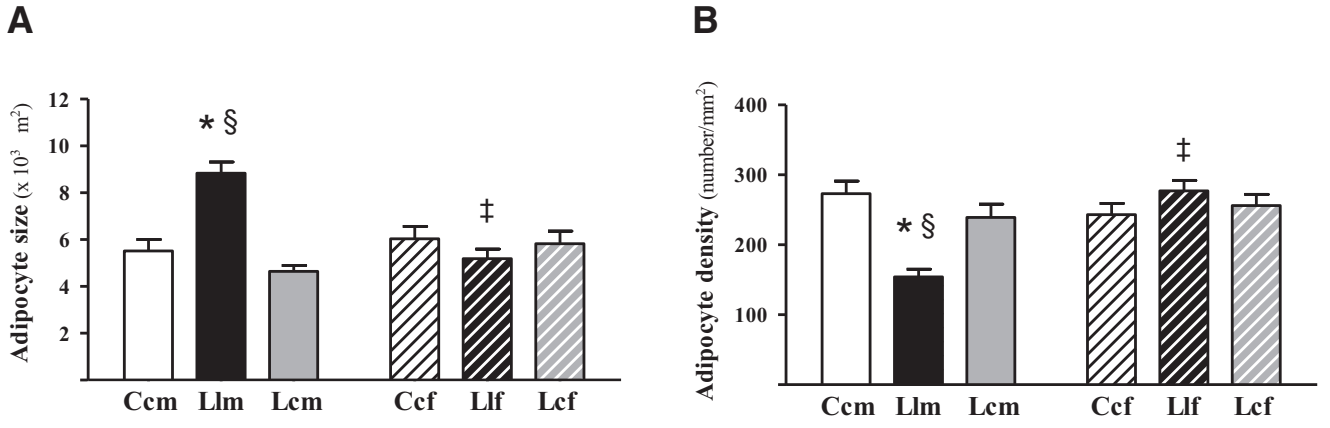

Fig. 7. Adipocyte size (A) and density (B) in retroperitoneal adipose tissue of 81 d old rats. Female (f) and male (m) offspring born from zinc-deficient mothers were fed a low-zinc (Llm and Llf, 8 ppm) or a control zinc diet (Lcm and Lcf, 30 ppm) for 60 d after weaning, and male and female offspring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

Table 4
Retroperitoneal adipose tissue oxidative state at 81 d 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‡

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

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 male mice [50]. Moreover, Stubbins et al. [51] found that 17β-estradiol administration improved glucose tolerance and the serum lipid profile and reverted adipocyte hypertrophy and oxidative stress in AT of ovariectomized mice exposed to high-fat diet.

Conclusions

Our findings suggest that dietary zinc restriction during fetal life, lactation, and postweaning 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 found that an adequate zinc diet during postweaning life could revert most of these metabolic alterations. Finally, female rats were less sensitive to the metabolic effects of this nutritional injury.

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