Nutrition 29 (2013) 568–573

Contents lists available at ScienceDirect

Nutrition

journal homepage: www.nutritionjrnl.com

Basic nutritional investigation

Mild zinc deficiency in male and female rats: Early postnatal alterations in renal nitric oxide system and morphology

Analia Lorena Tomat Ph.D. [a,*], Luciana Cecilia Veiras Miss. [a], Sofía Aguirre Miss. [a], Héctor Fasoli Ph.D. [b], Rosana Elesgaray Ph.D. [a], Carolina Caniffi Miss. [a], María Ángeles Costa Ph.D. [a], Cristina Teresa Arranz Ph.D. [a]

[a] Cátedra de Fisiología, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, IQUIMEFA-CONICET, Buenos Aires, Argentina
[b] Laboratorio de Química y Ciencia Ambiental, Facultad de Ciencias Fisicomatemáticas e Ingeniería, Universidad Católica Argentina, Buenos Aires, Argentina

ARTICLE INFO

Article history:
Received 29 May 2012
Accepted 20 September 2012

Keywords:
Zinc restriction
Fetal life and lactation
Kidney
Glomeruli
Nitric oxide synthase
Apoptosis
Oxidative stress

ABSTRACT

Objective: Fetal and postnatal zinc deficiencies induce an increase in arterial blood pressure and impair renal function in male adult rats. We therefore hypothesized that these renal alterations are present in early stages of life and that there are sexual differences in the adaptations to this nutritional injury. The aim was to study the effects of moderate zinc deficiency during fetal life and lactation on renal morphology, oxidative stress, apoptosis, and the nitric oxide system in male and female rats at 21 d of life.
Methods: Female Wistar rats received low (8 ppm) or control (30 ppm) zinc diets from the beginning of pregnancy to weaning. Glomerulus number, morphology, oxidative stress, apoptotic cells, nitric oxide synthase activity, and protein expression were evaluated in the kidneys of offspring at 21 d.
Results: Zinc deficiency decreased the nephron number, induced glomerular hypertrophy, increased oxidative damage, and decreased nitric oxide synthase activity in the male and female rat kidneys. Nitric oxide synthase activity was not affected by inhibitors of the neuronal or inducible isoforms, so nitric oxide was mainly generated by the endothelial isoenzyme. Gender differences were observed in glomerular areas and antioxidant enzyme activities.
Conclusion: Zinc deficiency during fetal life and lactation induces an early decrease in renal functional units, associated with a decrease in nitric oxide activity and an increase in oxidative stress, which would contribute to increased arterial blood pressure and renal dysfunction in adulthood. The sexual differences observed in this model may explain the dissimilar development of hypertension and renal diseases in adult life.

© 2013 Elsevier Inc. All rights reserved.

Introduction

Human and experimental studies have suggested that a nutritional imbalance during critical developmental periods has persistent effects on the health of offspring and may be responsible for in utero programming of common disorders such as hypertension in adult life. Moreover, these studies of fetal programming have shown a sexual dichotomy in the expression of renal and cardiovascular diseases owing to intrinsic sexual differences and the influence of sex steroids [1–3].

Moderate and marginal zinc deficiency observed in pregnant women could be a nutritional insult to fetal and postnatal development [4,5]. The Food and Agricultural Organization of the United Nations has estimated the prevalence of inadequate zinc intake to be as high as 20.5% worldwide [6]. Zinc deficiency is usually due to an inadequate zinc intake or absorption, increased losses of zinc from the body, or increased zinc requirements [7–9].

Zinc is involved in the decrease of oxidative stress and the inhibition of apoptosis [10,11]. Moreover, nitric oxide synthase (NOS), a family of metalloenzymes that catalyzes the synthesis of nitric oxide (NO) and L-citrulline from L-arginine, uses zinc as a cofactor and is involved in blood pressure regulation and

This work was supported by the University of Buenos Aires (2008/2011, grant B 008; 2010/2012, Young Investigator grant 20020090300036; 2011/2014, grant 20020100100370), the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) and Instituto de Química y Metabolismo del Fármaco–CONICET (grant PIP-112-200801-01121), and the Agencia Nacional de Promoción Científica y Tecnológica (Proyecto Joven PICT-2008 FONCYT-132 no. 1321).

* Corresponding author. Tel./fax: +5411-4964-8280.
E-mail address: atomat@ffyb.uba.ar (A. L. Tomat).

0899-9007/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.nut.2012.09.008

renal function. The NOS family consists of three isoforms: neuronal, endothelial (eNOS), and inducible [12–15].

The kidney may play a key role in the association of maternal undernutrition and intrauterine programming of hypertension, and it is known that renal structure, specifically nephron number, is a major determinant of blood pressure and renal function [16,17].

In previous studies we showed that dietary zinc restriction during prenatal and postnatal growth induces an increase in arterial blood pressure and impairs renal function in male adult rats [18,19]. Therefore, we hypothesized that the renal alterations observed in adult life were present in the early stages of life and that there were sexual differences in the adaptations to this nutritional injury. Therefore, to delve deeper into the early mechanisms leading to these renal alterations, we evaluated the effects of moderate zinc deficiency during fetal life and lactation on renal morphology, oxidative stress, apoptosis, and the NO system in male and female rats at 21 d of life.

Materials and methods

Animals and study design

Female Wistar rats weighing 271 ± 7 g obtained from the breeding laboratories of Facultad de Farmacia y Bioquímica (Universidad de Buenos Aires, Buenos Aires, Argentina) were mated by exposure to Wistar males for 1 wk. Immediately afterward, female rats were randomly fed a moderately zinc-deficient diet (L; 8 ppm, $n = 10$) or a control zinc diet (C; 30 ppm, $n = 10$) during the pregnancy and lactation periods. Eight rat pups remained with each dam until 21 d of life (weaning) by a random culling of pups at birth and retaining a 1:1 male-to-female ratio. The experimental groups were the male offspring of C mothers (Cm), the female offspring of C mothers (Cf), the male offspring of L mothers (Lm), and the female offspring of L mothers (Lf). The two diets had all necessary nutrients, except for zinc content, to meet the rat requirements for the pregnancy and lactation periods according to AIN-93 recommendations [19,20].

The animals were cared for according to Argentina's National Drug, Food and Medical Technology Administration Standards (Regulation 6344/96) and the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH publication 85–23, revised 1996), and the experimental procedures were approved by the ethics committee of the School of Pharmacy and Biochemistry, Universidad de Buenos Aires.

The mothers and their offspring were housed in plastic cages in a humidity- and temperature-controlled environment with a 12-h light/dark cycle. The animals were allowed food and deionized water ad libitum.

The offspring were weighed at 6 and 21 d of life and the body weight gain was calculated. At 21 d of life, the offspring were sacrificed by cervical decapitation, and the two kidneys were immediately removed and weighed. Blood from the offspring and mothers was collected to determine the zinc concentration in serum using atomic absorption spectrophotometry (air acetylene flame, 0.5-nm slit, 213.9-nm wavelength; Varian Spectrophotometer Spectr AA-20, Perkin Elmer Corp, Norwalk, CT, USA) [21,22].

Morphology, cortical apoptotic cells, total glomerulus number, NOS activity, eNOS protein expression, and antioxidant and oxidant systems were evaluated in offspring kidneys.

Histologic evaluation, total number of glomeruli, and terminal deoxynucleotidyl transferase dUTP nick end labeling assay

The renal morphometric parameters were determined in 10 consecutive cortical and juxtamedullary areas from two renal sections stained with hematoxylin and eosin. Each field corresponded to an area of 1.367 mm^2 (magnification 400×).

Kidney sections were subjected to collagen-specific staining with picrosirius red to determine the presence of fibrosis in the renal parenchyma [19,22]. Staining was scored as 0 (normal and slight staining surrounding the tubular, glomerular, and vascular structures), 1 (weak staining that doubles the normal label surrounding the tubular, glomerular, and vascular structures), 2 (moderate staining in the peritubular interstitium and inside the glomeruli), 3 (strong staining that replaces the glomerular and tubular structures, compromising <25% of the cortical area), or 4 (strong staining that replaces the glomerular and tubular structures, compromising >25% of the cortical area).

The number of apoptotic cells per cortical area was determined in 20 visual fields (magnification 400×) from two renal sections per rat using the DeadEnd colorimetric terminal deoxynucleotidyl transferase dUTP nick end labeling system kit (Promega Corp., Madison, WI, USA) [19,22].

The total number of glomeruli per kidney was determined by the modified maceration method [23]. The kidney was cut into small pieces in 1% NH_4Cl and gently agitated in 30 mL of 50% HCl for 90 min at 37°C. After slow-speed centrifugation, the pellet containing the glomeruli was suspended in 25 mL of distilled water. Twenty 20-μL aliquots were pipetted onto slides and all glomeruli were counted at 100× magnification. Although some experts favor stereology over maceration, the latter technique was chosen because it is simple and rapid and allows for the detection of differences between groups.

The histologic studies were analyzed using an Olympus BX51 light microscope equipped with a digital camera (Qcolor 3, Olympus America, Inc., Richmond Hill, Ontario, Canada) connected to Image-Pro Plus 4.5.1.29 software (Media Cybernetics, LP, Silver Spring, MD, USA). All determinations were performed blindly and under similar light, gain, and offset conditions by the same researcher.

Renal oxidative stress evaluation

Lipid oxidative damage was assessed by measuring the extent of 2-thiobarbituric acid-reactive substances formation [24]. Glutathione content [25], superoxide dismutase (SOD) [26], catalase [27], and glutathione peroxidase (GPx) [28] activities were also measured. Protein concentration was determined by the method of Lowry et al. [29].

Renal NOS activity

Tissue slices (2–3 mm thick) were incubated for 30 min at 37°C in Krebs solution with [^{14}C] L-arginine 0.5 μCi/mL (specific activity 360 mCi/mmol; Perkin Elmer Life and Analytical Sciences, Boston, MA, USA). Antagonists were added at the beginning of the incubation period according to the protocol described in Figure 1. All drugs were purchased from Sigma (St. Louis, MO, USA). Tissue samples were homogenized in the stop solution (ethylene glycol bis[2-aminoetyl ether]-N,N,N′,N′-tetraacetic acid 0.5 mmol/L, ethylenediaminetetraacetic acid 0.5 mmol/L, 2,4-hydroxyethyl-l-piperazineethane sulfonate 20 mmol/L, pH 5.5) and the homogenates were centrifuged at 12 000 × *g* for 20 min. The supernatants were applied to a 1-mL Dowex AG 50W-X8 column (Na^+ form; Bio-Rad laboratories, Hercules, CA, USA) and eluted with 2 mL of distilled water, and the amount of [^{14}C] L-citrulline was determined with a liquid scintillation counter (Wallac 1414 WinSpectral, EG&G Company, Turku, Finland). NOS activity was expressed as picomoles of [^{14}C] L-citrulline per gram of wet weight per minute [30,31].

Renal eNOS protein expression by western blot analysis

Rabbit polyclonal anti-eNOS antibody (dilution 1:500, epitope at the amino terminus; Santa Cruz Biotechnology, Santa Cruz, CA, USA) was used and a secondary immunoreaction with a goat anti-rabbit antibody conjugated with horseradish peroxidase was performed (dilution 1:5000; Amersham Pharmacia Biotech, Uppsala, Sweden). All experiments were performed in triplicate [18].

Statistical analysis

All values are expressed as mean ± standard error of the mean. GraphPad Prism 5 software (La Jolla, CA, USA) was used for statistical analysis. Data were analyzed using Student's *t* test, one-way analysis of variance, and two-way analysis of variance followed by a Bonferroni multiple-comparison post hoc test. $P < 0.05$ was considered a statistically significant difference.

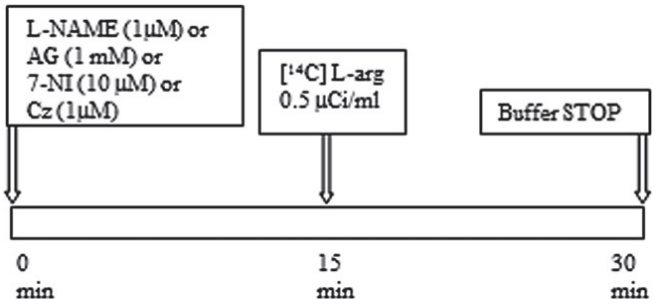

Fig. 1. Renal nitric oxide synthase activity protocol. AG, aminoguanidine (inducible nitric oxide synthase inhibitor); Cz, calmidazolium (Ca^{2+}-calmodulin complex antagonist); L-Arg, L-arginine; L-NAME, L-nitro-arginine-methyl-ester (nitric oxide synthase inhibitor); 7-NI, 7-nitroindazole (neuronal nitric oxide synthase inhibitor).

Table 1
Renal morphology at 21 d

	Cm ($n = 10$)	Lm ($n = 10$)	Cf ($n = 10$)	Lf ($n = 10$)
Kidney weight (g/100 g body weight)	0.60 ± 0.03	0.58 ± 0.02	0.56 ± 0.03	0.53 ± 0.02
Glomeruli per kidney	18 110 ± 413	15 500 ± 437*	17 750 ± 429	15 700 ± 418†
Total glomerular area (μm^2)				
Cortical area	3098 ± 61	3717 ± 72*	2638 ± 67*	3221 ± 130†
Juxtamedullary area	4628 ± 90	5018 ± 86*	4046 ± 96*	4697 ± 99†
Glomerular capillary area (μm^2)				
Cortical area	2284 ± 84	2642 ± 63*	1992 ± 69*	2427 ± 109†
Juxtamedullary area	3472 ± 99	3551 ± 120	3093 ± 102*	3627 ± 100†

Cf, female offspring with adequate zinc in the maternal diet; Cm, male offspring with adequate zinc in the maternal diet; Lf, female offspring with inadequate zinc in the maternal diet; Lm, male offspring with inadequate zinc in the maternal diet

Data were analyzed using two-way analysis of variance followed by a Bonferroni post hoc test. Values are presented as mean ± SEM. The interaction between sex and diet was considered non-significant.

* $P < 0.01$ versus Cm.

† $P < 0.01$ versus Cf; diet and sex were considered significant.

Results

The Lm and Lf offspring exhibited a lower body weight at 21 d compared with the C offspring and there were no differences between male and female rats (Cm 39.4 ± 1.3 g, Lm 33.8 ± 0.7 g, $P < 0.01$ versus Cm for diet; Cf 38.4 ± 1.3 g, Lf 30.1 ± 1.1 g, $P < 0.01$ versus Cf for diet; sex, not significant; interaction of sex and diet, not significant).

Moreover, the body weight gain from 6 to 21 d was lower in the Lm and Lf rats than in the Cm and Cf rats (Cm 27.5 ± 0.8 g, Lm 23.8 ± 0.7 g, $P < 0.05$ versus Cm for diet; Cf 27.2 ± 1.8 g; Lf 20.2 ± 1.3 g, $P < 0.01$ versus Cf for diet; sex, not significant; interaction of sex and diet, not significant).

The Lm and Lf offspring exhibited a lower body weight compared with the Cm and Cf offspring (Cm 39.4 ± 1.3 g, Lm 33.8 ± 0.7 g, $P < 0.01$ versus Cm for diet; Cf 38.4 ± 1.3 g, Lf 30.1 ± 1.1 g, $P < 0.01$ versus Cf for diet). There were no differences in body weight between male and female offspring.

At weaning, the L mothers showed a lower serum zinc concentration than C mothers (L 28 ± 7 versus C 66 ± 7 μg/dL, $P < 0.0001$, $n = 10$ per group). The Lm and Lf offspring exhibited lower serum zinc concentrations compared with the Cm and Cf offspring (Cm 87 ± 8 μg/dL, Lm 48 ± 4 μg/dL, $P < 0.01$ versus Cm for diet; Cf 86 ± 7 μg/dL, Lf 54 ± 8 μg/dL, $P < 0.01$ versus Cf for diet, $n = 10$ per group). There were no differences in plasma zinc concentration between male and female offspring.

Kidney weight was similar in all the experimental groups. The Lm and Lf offspring showed fewer glomeruli per kidney compared with the Cm and Cf offspring, respectively. No sexual differences were observed. The total glomerular areas of the Lm and Lf kidneys were larger than the Cm and Cf kidneys. The Lm and Lf kidneys also showed increased glomerular capillary areas in the cortical zones compared with the Cm and Cf kidneys. However, in the juxtamedullary regions, the capillary area was increased only in the Lf kidneys. The Cf kidneys showed smaller glomerular areas than the Cm kidneys in both regions of the kidney (Table 1).

Sirius Red staining showed no signs of early fibrosis in the glomeruli, tubules, and peritubular interstitium of the renal cortex of the Lm, Cm, Lf, and Cf kidneys (scores: Cm = 0.4 ± 0.2, Lm = 0.5 ± 0.1, Cf = 0.5 ± 0.1, Lf = 0.6 ± 0.1, $n = 10$ per group). Areas of positive Sirius Red staining were confined only to the smooth muscle cells of blood vessels.

All the experimental groups showed similar numbers of apoptotic cells in the renal cortex (Fig. 2).

There were no differences in glutathione content among the experimental groups. The Lm and Lf groups exhibited higher 2-thiobarbituric acid-reactive substances levels compared with the Cm and Cf groups. The Lf animals showed lower levels of renal SOD, catalase, and GPx activity compared with the Cf animals. Renal GPx activity was lower in Lm animals compared with Cm animals. There were no differences in SOD or catalase activity between the Lm and Cm animals. The Cf kidneys exhibited higher SOD and catalase enzyme activities than the Cm kidneys (Table 2).

Renal tissues from the Lm and Lf groups showed decreased basal NOS activity compared with the Cm and Cf groups. No sexual differences were observed. Basal NOS activity was blunted when L-nitro-arginine-methyl-ester was added previously, thus verifying that the activity measured was linked specifically to NOS. Neither neuronal NOS inhibition nor inducible NOS blockade modified the basal NOS activity. However,

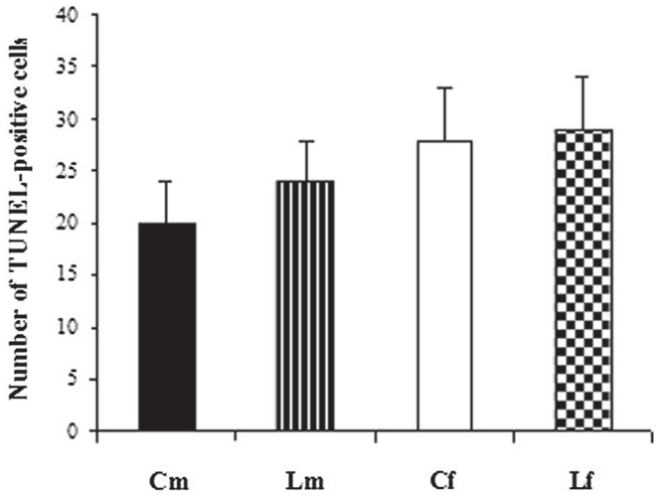

Fig. 2. Number of TUNEL-positive cells per renal cortical area in the Cm ($n = 10$), Lm ($n = 10$), Cf ($n = 10$), and Lf ($n = 10$) groups at 21 d of life. Values are presented as mean ± SEM. Data were analyzed using two-way analysis of variance followed by the Bonferroni post hoc test. The factors diet and sex and their interaction were considered not significant. All images are at the same magnification of 400×. Scale bar = 30 μm. Cf, female offspring with adequate zinc in the maternal diet; Cm, male offspring with adequate zinc in the maternal diet; Lf, female offspring with inadequate zinc in the maternal diet; Lm, male offspring with inadequate zinc in the maternal diet; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling.

Table 2
Antioxidant and oxidant stress systems at 21 d

	Cm ($n = 10$)	Lm ($n = 10$)	Cf ($n = 10$)	Lf ($n = 10$)
GLUT (mg/mg protein)	7.2 ± 0.6	6.2 ± 0.5	7.5 ± 0.9	6.8 ± 0.3
TBARS (nmol/mg protein)	0.13 ± 0.02	0.35 ± 0.09*	0.14 ± 0.01	0.45 ± 0.07†
SOD activity (U/mg protein)	13.2 ± 0.9	12.2 ± 0.9	19.1 ± 1.0*	12.1 ± 0.9†
CAT activity (pmol/mg protein)	6.1 ± 0.8	5.6 ± 0.5	10.3 ± 0.3*	4.1 ± 0.7†
GPx activity (pmol · min^{-1} · mg^{-1} protein)	252 ± 27	166 ± 10*	264 ± 26	177 ± 17†

CAT, catalase; Cf, female offspring with adequate zinc in the maternal diet; Cm, male offspring with adequate zinc in the maternal diet; GLUT, glutathione; GPx, glutathione peroxidase; Lf, female offspring with inadequate zinc in the maternal diet; Lm, male offspring with inadequate zinc in the maternal diet; SOD, superoxide dismutase; TBARS, 2-thiobarbituric acid-reactive substances
Values are presented as mean ± SEM. Data were analyzed using two-way analysis of variance followed by the Bonferroni post hoc test. The interaction between sex and diet was considered non-significant.
* $P < 0.01$ versus Cm.
† $P < 0.01$ versus Cf, sex and diet were considered significant.

Ca^{2+}-calmodulin inhibition provoked a decrease in basal NOS activity in all groups studied (Fig. 3).

Figure 4 shows that eNOS protein expression was increased in Lm kidneys compared with Cm kidneys, but there was no difference in eNOS protein abundance between the Cf and Lf groups.

Discussion

The present study demonstrates that the renal morphologic alterations induced by zinc deficiency during fetal life and lactation could be one of the mechanisms involved in the increase in arterial blood pressure and renal dysfunction in adult life described previously [18,19].

In accordance with other models of fetal programming, male and female offspring exposed to zinc deficiency showed a growth delay in early postnatal life. Moreover, the smaller number of glomeruli observed in the Lm and Lf offspring allows us to assume that zinc restriction during fetal life and lactation impairs intrauterine and postnatal nephrogenesis. A small nephron number is frequently associated with low weight and has been recognized as a powerful risk factor for cardiovascular and renal diseases [16,17]. The loss of nephrons within the developing kidney can confer an immediate adaptive benefit for survival but results in the programming of the organ's decreased functional capacity for life. In our experimental model, the morphologic renal abnormalities appeared before the onset of arterial blood pressure increase and glomerular filtration rate decrease, which occur at 50 d of life [18,19,21,22].

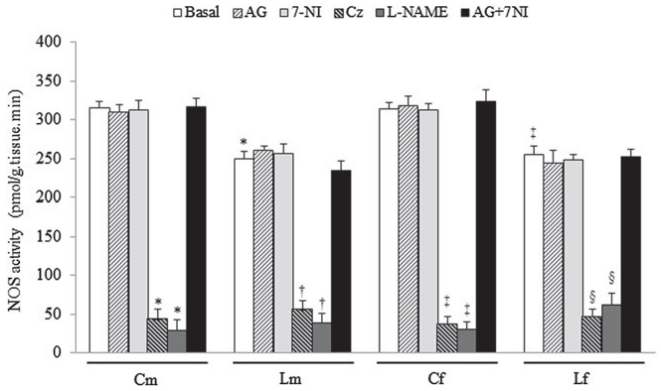

Fig. 3. Basal renal NOS activity and changes provoked by AG (inducible NOS inhibitor), 7-NI (neuronal NOS specific inhibitor), Cz (Ca^{2+}-calmodulin complex antagonist), and L-NAME (NOS inhibitor) in the Cm ($n = 10$), Lm ($n = 10$), Cf ($n = 10$), and Lf ($n = 10$) groups at 21 days of life. Values are presented as mean ± SEM. NOS activity changes induced by the antagonists in each group were analyzed using one-way analysis of variance followed by the Bonferroni post hoc test: $^{*}P < 0.01$ versus basal Cm; $^{\dagger}P < 0.01$ versus basal Lm; $^{\ddagger}P < 0.01$ versus basal Cf; $^{\S}P < 0.01$ versus basal Lf. Basal renal NOS activity in all groups was analyzed using two-way analysis of variance followed by the Bonferroni post hoc test. Diet: $^{*}P < 0.01$ versus basal Cm, $^{\ddagger}P < 0.01$ versus basal Cf; sex, not significant; interaction between sex and diet, not significant. AG, aminoguanidine; Cf, female offspring with adequate zinc in the maternal diet; Cm, male offspring with adequate zinc in the maternal diet; Cz, calmidazolium; Lf, female offspring with inadequate zinc in the maternal diet; Lm, male offspring with inadequate zinc in the maternal diet; L-NAME, L-nitro-arginine-methyl-ester; 7-NI, 7-nitroindazole; NOS, nitric oxide synthase.

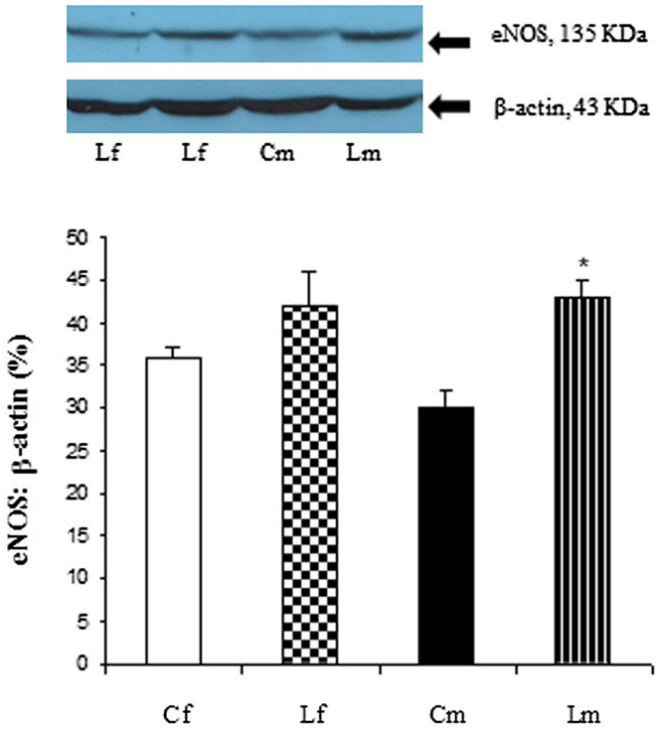

Fig. 4. Western blots showing the protein expression of eNOS in the kidneys of the Cf, Lf, Cm, and Lm diet groups at 21 days of life. Values are presented as mean ± SEM ($n = 6$ rats per group). All experiments were performed in triplicate. Each blot was normalized to the expression of the β-actin marker from the same gel. Data were analyzed using two-way analysis of variance followed by the Bonferroni post hoc test. Diet, $^{*}P < 0.01$ versus Cm; sex, not significant; interaction between sex and diet, not significant. Cf, female offspring with adequate zinc in the maternal diet; Cm, male offspring with adequate zinc in the maternal diet; eNOS, endothelial nitric oxide synthase; Lf, female offspring with inadequate zinc in the maternal diet; Lm, male offspring with inadequate zinc in the maternal diet.

The glomerular hypertrophy observed in the Lm and Lf rats is an adaptive mechanism that allows the maintenance of an adequate glomerular filtration function. These processes of glomerular hypertrophy would increase the intraglomerular pressure and glomerular filtration rate and, as a consequence, damage in the functioning glomeruli, and a progressive loss of other nephrons may occur [32,33].

In the Lm group, filtration surface hypertrophy was observed in the more immature regions of the renal cortex, whereas in the Lf group, glomerular hypertrophy was observed in the cortical and juxtamedullary regions, where more developed nephrons are found [34]. In this stage of life, morphologic changes in the renal cortex of zinc-deficient animals were not accompanied by an activation of apoptotic processes.

In this study, no signs of parenchymal fibrosis were observed in the early periods of life. Nevertheless, in previous studies, we demonstrated the presence of fibrosis in the renal tissue of adult animals exposed to zinc deficiency during fetal life, lactation, and growth [19]. Therefore, we assume that chronic zinc deficiency throughout life is necessary to induce the development of renal fibrosis.

Our study has demonstrated that moderate zinc deficiency in the early stages of renal development induces a renal imbalance between the oxidant and antioxidant systems in the male and female offspring. In male offspring, zinc deficiency induced a decrease in GPx activity, most likely generating an increase in reactive oxygen species, because GPx is not efficiently metabolizing hydrogen peroxide. Therefore, we posit that these effects may have contributed to the increase in renal lipid peroxidation end products in the kidney.

In contrast, the Cf rats exhibited greater antioxidant activity than Cm rats, showing higher SOD and catalase activity. However, the female rats would be more sensitive to an inadequate zinc intake because the Lf offspring showed more evident alterations in the antioxidant systems. The decrease in SOD activity observed would increase the superoxide anion, whereas the decrease in catalase and GPx activity would decrease hydrogen peroxide clearance. Therefore, the higher production of reactive oxygen species may induce lipid peroxidation in the kidney.

These results show that male and female offspring adapt differently to developmental stressors. Male and female sex steroids have a profound influence on the development and progression of programmed disease states. However, because sexual differences are apparent quite early in embryonic development and are independent of sex hormones, we posit that innate sexual differences in the renal system may play a role in the different responses of antioxidant enzymes and in morphologic changes to zinc deficiency.

In addition, this study confirms that renal NO impairment in adult zinc-deficient rats is initiated during the nephrogenic and renal maturation periods of life. The decrease in the renal NO system activity during the early periods of life could alter the normal development of the kidney because it has been reported that the progressive increase in NO production would contribute to decrease renal vascular resistance and increase renal blood flow during renal postnatal maturation [35].

Furthermore, our findings suggest that, because NOS activity was not affected by specific inhibitors of the neuronal and inducible isoforms but was decreased by a calcium-calmodulin antagonist, NO is mainly generated by the eNOS isoform at this stage of renal development. Therefore, the decrease in renal NOS activity would be due mainly to lower eNOS activity but would not be associated with a lower expression of eNOS protein. On the contrary, eNOS expression was increased in male zinc-deficient rats, probably as a compensatory mechanism to maintain NOS activity.

Therefore, we posit that the increased oxidative stress condition probably contributes to decreased NO bioavailability and NOS activity because oxygen free radicals could trigger the uncoupling of the enzyme [15]. We further postulate that disturbances in zinc homeostasis would affect other NOS regulation mechanisms, including alterations in the dimeric enzyme structure, in substrate and cofactor synthesis, and in transport [13,36].

Contrasting information on eNOS isoform expression in different animal tissues exposed to zinc deficiency has been found in the literature. In our previous study, no effects of zinc deficiency on renal eNOS protein expression in adult rats were observed [18]. Conversely, Sato et al. found that severe zinc deficiency during adult life does not change NOS activity and aortic eNOS expression in normotensive rats [37], but increases the eNOS mRNA and protein expression in the thoracic aorta of spontaneously hypertensive rats [38].

Zinc deficiency during fetal life and lactation might alter the normal trajectory of renal development in male and female offspring by decreasing nephron number, inducing glomerular hypertrophy, increasing renal oxidative damage, and decreasing renal NO system activity. The sexual differences observed in the glomerular areas and antioxidant enzyme activities may induce a dissimilar development of hypertension and renal diseases in adult male and female animals exposed to zinc deficiency during fetal and early postnatal life.

Conclusion

This study highlights the importance of adequate zinc intake during fetal life and lactation to prevent the development of early and irreversible renal alterations that can predispose to adult diseases. Therefore, zinc-supplementation programs and changes in nutritional lifestyle for zinc-deficient women during pregnancy and lactation should be considered.

Acknowledgments

The authors thank D. Cardelli Alcalde, R. Sanchez, F. Mendes Garrido Abregú, L. Juriol, and M. N. Gobetto for technical assistance and Ana Borthwick for proofreading and language assistance.

References

[1] Christian P, Stewart CP. Maternal micronutrient deficiency, fetal development, and the risk of chronic disease. J Nutr 2010;140:437–45.
[2] Geelhoed JJ, Jaddoe VW. Early influences on cardiovascular and renal development. Eur J Epidemiol 2010;25:677–92.
[3] Rogers LK, Velten M. Maternal inflammation, growth retardation, and preterm birth: insights into adult cardiovascular disease. Life Sci 2011;89:417–21.
[4] Sandstead HH. Is zinc deficiency a public health problem? Nutrition 1995;11:87–92.
[5] Vallee BL, Falchuk KH. The biochemical basis of zinc physiology. Physiol Rev 1993;73:79–118.
[6] Wuehler SE, Peerson JM, Brown KH. Use of national food balance data to estimate the adequacy of zinc in national food supplies: methodology and regional estimates. Public Health Nutr 2005;8:812–9.
[7] de Benoist B, Darnton-Hill I, Davidsson L, Fontaine O, Hotz C. Conclusions of the joint WHO/UNICEF/IAEA/IZiNCG interagency meeting on zinc status indicators. Food Nutr Bull 2007;28:S480–4.

[8] Gibson RS. Zinc: the missing link in combating micronutrient malnutrition in developing countries. Proc Nutr Soc 2006;65:51–60.
[9] Maret W, Sandstead HH. Zinc requirements and the risks and benefits of zinc supplementation. J Trace Elem Med Biol 2006;20:3–18.
[10] Truong-Tran AQ, Ho LH, Chai F, Zalewski PD. Cellular zinc fluxes and the regulation of apoptosis/gene-directed cell death. J Nutr 2000;130: 1459S–66S.
[11] Yousef MI, El-Hendy HA, El-Demerdash FM, Elagamy EI. Dietary zinc deficiency induced-changes in the activity of enzymes and the levels of free radicals, lipids and protein electrophoretic behavior in growing rats. Toxicology 2002;175:223–34.
[12] Baylis C. Nitric oxide deficiency in chronic kidney disease. Am J Physiol Renal Physiol 2008;294:F1–9.
[13] Mount PF, Power DA. Nitric oxide in the kidney: functions and regulation of synthesis. Acta Physiol (Oxf) 2006;187:433–46.
[14] Zhou L, Zhu DY. Neuronal nitric oxide synthase: structure, subcellular localization, regulation, and clinical implications. Nitric Oxide Biol Chem 2009;20:223–30.
[15] Zou MH, Shi C, Cohen RA. Oxidation of the zinc–thiolate complex and uncoupling of endothelial nitric oxide synthase by peroxynitrite. J Clin Invest 2002;109:817–26.
[16] Benz K, Amann K. Maternal nutrition, low nephron number and arterial hypertension in later life. Biochim Biophys Acta 2010;1802:1309–17.
[17] Ritz E, Amann K, Koleganova N, Benz K. Prenatal programming-effects on blood pressure and renal function. Nature reviews. Nephrology 2011; 7:137–44.
[18] Tomat A, Elesgaray R, Zago V, Fasoli H, Fellet A, Balaszczuk AM, et al. Exposure to zinc deficiency in fetal and postnatal life determines nitric oxide system activity and arterial blood pressure levels in adult rats. Br J Nutr 2010;104:382–9.
[19] Tomat AL, Inserra F, Veiras L, Vallone MC, Balaszczuk AM, Costa MA, Arranz C. Moderate zinc restriction during fetal and postnatal growth of rats: effects on adult arterial blood pressure and kidney. Am J Physiol Regul Integr Comp Physiol 2008;295:R543–9.
[20] Reeves PG, Nielsen FH, Fahey GC Jr. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J Nutr 1993;123:1939–51.
[21] Tomat AL, Weisstaub AR, Jauregui A, Pineiro A, Balaszczuk AM, Costa MA, Arranz CT. Moderate zinc deficiency influences arterial blood pressure and vascular nitric oxide pathway in growing rats. Pediatr Res 2005;58:672–6.
[22] Tomat AL, Costa MA, Girgulsky LC, Veiras L, Weisstaub AR, Inserra F, et al. Zinc deficiency during growth: influence on renal function and morphology. Life Sci 2007;80:1292–302.
[23] Welham SJ, Wade A, Woolf AS. Protein restriction in pregnancy is associated with increased apoptosis of mesenchymal cells at the start of rat meta-nephrogenesis. Kidney Int 2002;61:1231–42.
[24] Buege JA, Aust SD. Microsomal lipid peroxidation. Methods Enzymol 1978;52:302–10.
[25] Tietze F. Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione: applications to mammalian blood and other tissues. Anal Biochem 1969;27:502–22.
[26] Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem 1972;247:3170–5.
[27] Maehly AC, Chance B. The assay of catalases and peroxidases. Methods Biochem Anal 1954;1:357–424.
[28] Flohe L, Gunzler WA. Assays of glutathione peroxidase. Methods Enzymol 1984;105:114–21.
[29] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265–75.
[30] Costa MA, Loria A, Elesgaray R, Balaszczuk AM, Arranz C. Role of nitric oxide pathway in hypotensive and renal effects of furosemide during extracellular volume expansion. J Hypertens 2004;22:1561–9.
[31] Costa MA, Elesgaray R, Caniffi C, Fellet A, MacLaughlin M, Arranz C. Role of nitric oxide as a key mediator on cardiovascular actions of atrial natriuretic peptide in spontaneously hypertensive rats. Am J Physiol Heart Circ Physiol 2010;298:H778–86.
[32] Black MJ, Briscoe TA, Constantinou M, Kett MM, Bertram JF. Is there an association between level of adult blood pressure and nephron number or renal filtration surface area? Kidney Int 2004;65:582–8.
[33] Zandi-Nejad K, Luyckx VA, Brenner BM. Adult hypertension and kidney disease: the role of fetal programming. Hypertension 2006;47:502–8.
[34] Larsson L, Aperia A, Wilton P. Effect of normal development on compensatory renal growth. Kidney Int 1980;18:29–35.
[35] Ishii N, Fujiwara K, Lane PH, Patel KP, Carmines PK. Renal cortical nitric oxide synthase activity during maturational growth in the rat. Pediatr Nephrol 2002;17:591–6.
[36] Herrera M, Garvin JL. Recent advances in the regulation of nitric oxide in the kidney. Hypertension 2005;45:1062–7.
[37] Sato M, Kurihara N, Moridaira K, Sakamoto H, Tamura J, Wada O, Yanagisawa H. Dietary Zn deficiency does not influence systemic blood pressure and vascular nitric oxide signaling in normotensive rats. Biol Trace Elem Res 2003;91:157–72.
[38] Sato M, Yanagisawa H, Nojima Y, Tamura J, Wada O. Zn deficiency aggravates hypertension in spontaneously hypertensive rats: possible role of Cu/Zn-superoxide dismutase. Clin Exp Hypertens 2002;24: 355–70.