Early Programming of Alterations in Renal and Vascular Nitric Oxide System Induced by Zinc-Related Deficiencies

MARIELA M. PLODER[1], ANALÍA L. TOMAT[2], ROXANA ELESGARAY[3], ANDREA L. FELLET[4], ANA M. BALASZCZUK[5], MARÍA A. COSTA[6], CRISTINA T. ARRANZ[7]

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Address for reprints:
Cristina Arranz
Cátedra de Fisiología, Facultad de Farmacia y Bioquímica
Junín 956 - Piso 7
(1113) Ciudad Autónoma de Buenos Aires, Argentina
Phone number: (5411) 4964-8280
Fax: (5411) 4508-3645
e-mail: carranz@ffyb.uba.ar

SUMMARY

Background
Several studies have reported that nutritional deficiencies during fetal and postnatal life predispose to the development of diseases such as hypertension and renal disorders during adulthood. The ubiquitous distribution of zinc and its chemical properties determine their essentiality in the biological systems.

Objectives
To assess whether renal and cardiovascular alterations induced by moderate zinc restriction during fetal life, lactation period and/or growth are associated with changes in the nitric oxide system.

Material and Methods
Female Wistar rats received low zinc diet or control diet from the beginning of pregnancy up to weaning. After weaning, male offspring were randomly fed with low zinc diet or control diet for 60 days.

Results
Zinc deficiency through pre-weaning and post-weaning growth induced increase in blood pressure and reduced glomerular filtration volume in adult life; these findings were associated with reductions in renal and vascular nitric oxide system. In addition, low zinc intake during intrauterine life induced low birth weight offspring which had a negative correlation with blood pressure in adulthood.

Conclusions
Zinc deficiency during prenatal and postnatal growth constitutes a risk factor for cardiovascular and kidney diseases as it induces alterations in blood pressure and renal function regulation in adult life.

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Key words > Blood Pressure - Nitric Oxide - Kidney - Blood Vessels - Zinc

Abbreviations >

Ll	Low-diet mother, low-diet offspring	**NOS**	Nitric oxide synthase
Lc	Low-diet mother, control-diet offspring	**WHO**	World Health Organization
Cl	Control-diet mother, low-diet offspring	**SBP**	Systolic blood pressure
Cc	Control-diet mother, control-diet offspring	**UNICEF**	United Nations Children's Fund
NO	Nitric oxide	**GFR**	Glomerular filtration rate

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[1] Bachelor of Science in Life Sciences
[2] PhD, University of Buenos Aires, Physiology Area. Head of Practical Assignments, Chair of Physiology, School of Pharmacy and Biochemistry, University of Buenos Aires.
[3] Assistant Professional of the CONICET. Assistant Professor, Chair of Physiology, School of Pharmacy and Biochemistry, University of Buenos Aires.
[4] PhD, University of Buenos Aires, Physiology Area. Assistant Researcher of the CONICET. Head of Practical Assignments, Chair of Physiology, School of Pharmacy and Biochemistry, University of Buenos Aires.
[5] PhD, University of Buenos Aires, Physiology Area. Adjunct Professor, Chair of Physiology, School of Pharmacy and Biochemistry, University of Buenos Aires.
[6] PhD, University of Buenos Aires, Physiology Area. Adjunct Researcher of the CONICET Adjunct Professor, Chair of Physiology, School of Pharmacy and Biochemistry, University of Buenos Aires.
[7] Independent Researcher of the CONICET Associate Professor, Chair of Physiology. School of Pharmacy and Biochemistry, University of Buenos Aires.

BACKGROUND

During the last years, many epidemiological and experimental studies have proposed that hypertension and cardiovascular diseases may be programmed in the uterus. Nutritional deficiencies and insufficient oxygen supply during fetal life limit fetal growth and might induce adaptation mechanisms that produce permanent disorders in the different systems and predispose to the development of endocrine, metabolic, cardiovascular and renal diseases during adulthood. (1-3)

This nutritional problem, known as hidden hunger, has been well described by the WHO and the UNICEF as micronutrient malnutrition, a public health problem mostly affecting children and pregnant women that results primarily from diets deficient in zinc. (4, 5)

Severe zinc deficiency is currently uncommon; however, a moderate level of zinc deficiency has been reported in a variety of conditions. (6) This micronutrient is widely distributed in a variety of foods such as meat, fish, shellfish, legume and cereal grains. Nevertheless, zinc deficiencies mostly occur due nutritional imbalance during certain stages of life, such a growth, pregnancy and breastfeeding, when the body's requirement for zinc increases. In addition, diets based only on cereal, vegetables and fruit can generate chronic diseases as high concentrations of dietary fiber and phytic acid, present in these foods, inhibit zinc absorption. (7-12)

Previous studies have demonstrated that low zinc diet during critical periods of fetal and postnatal development produces an increase in blood pressure and renal function impairment in adult life that are associated with reduction in the glomerular filtration surface area, activation of the apoptosis process, and increase in renal oxidative stress. (13-15)

In previous studies, we demonstrated that zinc deficiency during postweaning growth induced alterations in the activity of the vascular and renal nitric oxide (NO) system. (13, 14) It is well-known that NO is a highly diffusible gas synthesized from the amino acid L-arginine by nitric oxide synthases (NOS). (16, 17) The three isoforms of NOS (neuronal , inducible and endothelial) have a zinc thiolate cluster at the dimer interface, and NOS is catalytically active only in dimeric form. (18) This family of enzymes are expressed in many tissues, including blood vessels and the kidney. The production of NO by endothelial cells constitutes a key factor for the regulation of blood flow and blood pressure in mammals due to smooth muscle relaxation. In the kidney, NO plays an important role in glomerular, vascular and tubular homeostasis. (19-21) Therefore, any reduction in the production of nitric oxide and/or its availability might be involved in vascular and renal alterations previously observed in animals exposed to zinc deficiency during growth.

Therefore, the aim of the present study was to determine whether zinc deficiency during fetal life and lactation modified the activity of vascular and renal NOS in the adult life and to evaluate if adequate zinc intake during postweaning growth might reverse these alterations of the NO system.

MATERIAL AND METHODS

Female Wistar rats were mated by exposure to sexually mature Wistar males during 1 week. Immediately afterwards, female rats were randomly fed either a moderately zinc-deficient diet (L; mg zinc/kg diet) or a control zinc diet (C; 30 mg zinc/kg diets) during pregnancy and lactation periods.

Offspring were weaned 21 days after birth. After weaning, male offspring from each mother were randomly assigned to control (groups Cc and Lc) or low (groups Cl and Ll) zinc diets during 60 days (adult life).

Animals were allowed food and deionized water at will. All animals received AIN-93 diet that fulfilled the nutrient requirements for the growth phase of rodents, except in the content of zinc. Table 1 describes the composition of the experimental diet AIN-93 and the nutritional recommendations for the growth phase of rodents.

Animals were housed separately in acrylic cages in a humidity and temperature controlled environment, illuminated with a 12:12 hours light-dark cycle in the Animal Care Facility from the Chair of Nutrition and Food Sciences, School of Pharmacy and Biochemistry, University of Buenos Aires. Animal care was in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, the American Physiological Society "Guiding Principles in the Care and Use of Animals," and with the 6344/96 regulation of the *Administración Nacional de Medicamentos Alimentos y Tecnología Médica* (ANMAT, National Drug Food and Medical Technology Administration).

Body weight was determined in mothers during pregnancy and lactation and in offspring at day 1 and 60 after birth.

Food was weighed before filling the feeder and two days after during the whole experiment; mothers and offspring food intake was determined by difference between both values and was expressed as the average daily intake of food registered during the study periods.

Systolic blood pressure (SBP)in offspring was measured indirectly using a Grass polygraph (model 79H, Grass Instrument Co., Quincy, MA, USA) at 60 days. Then the animals were placed in acrylic metabolic cages to collect 24-hrs urine samples. The following day, the animals were removed from the metabolic cages and blood samples were collected from the rats' tails. Blood samples were heparinized and plasma was obtained after centrifugation.

The animals were killed by decapitation, and both kidneys and the thoracic aorta were removed to determine NOS activity using L-[U14C]-arginine as substrate.

Urine volume was determined gravimetrically. Plasmatic and urinary creatinine levels were measured by colorimetry (Wiener Laboratory) and creatinine clearance was determined to estimate glomerular filtration rate.

Concentrations of zinc in food, plasma and renal tissue were measured using Varian Spectra AA-20 atomic absorption spectrophotometer at the wave length of 213.9 nm (22). Reference material NIST RM 8435 (whole milk powder) underwent a similar analysis to verify the accuracy and re-

Table 1. Nutritional recommendations for the growth phase of rodents and composition of experimental diets (g/kg diet)

Diet	AIN-93	Control	Low
Potassium caseinate*	200	200	200
Soybean oil	70	70	70
Mineral mix†	35	35	-
Mineral mix without zinc ‡	-	-	35
Vitamin mix§	10	10	10
Choline	1	1	1
Dextrin	684	684	684
Zinc&	0.03	0.03	0.008

*Potassium caseinate, content/100g: 85.1 g of proteins.
†Ingredient (g/kg/mix): Calcium carbonate (357), potassium phosphate, monobasic (196), sodium chloride (74), magnesium sulphate, 7 hydrate (146,9), ammonium ferric citrate (6.06), zinc chloride (1.79), manganous sulphate, 1 hydrate (0.92), cupric sulphate, 5 hydrate (0.63), potassium iodate (0.0078), sodium selenate anhydrous (0.1025), ammonium paramolybdate, 4 hydrate (0.008), powdered sucrose (162)
‡ Ingredient: Identical to mineral mix with absence of zinc chloride.
§Ingredient (g/kg/mix): Nicotinic acid (3), calcium panthotenate (1.6), pyridoxine HCl (0.7), thiamine HCl (0.6), riboflavin (0.6), folic acid (0.2), vitamin B12, cyanocobalamin (2.5), vitamin E, *all-rac*-alpha-tocopheryl acetate, 500 IU/g (15), vitamin A *all-trans*-retinyl palmitate, 500 IU/g, (0.8), vitamin D3, cholecalciferol, 400 IU/g (0.25), vitamin K, phylloquinone (0.075), powdered sucrose (974.655).
&Zinc content in low diet results from the presence of traces of zinc in the other components of the diet

producibility of the analytic method. All the laboratory material was previously washed with nitric acid (20%) and deionized water.

NOS activity was determined by the formation of [^{14}C] L-citrulline from [^{14}C] L-arginine in the thoracic aorta and in renal cortex and medulla, and expressed per gram per minute, as previously described. (23, 24)

Statistical Analysis

Results are expressed as mean ± SE (standard error) statistical software package Prism (Graph Pad Software, Inc., San Diego CA, USA) was used for statistical analysis. Data were analyzed using ANOVA followed by the Bonferroni correction for multiple comparisons. Linear regression analysis was used to determine the relationships between birth weight and SBP at 60 days in groups Cc and Lc, NOS activity in renal cortex and GFR at 60 days, and SBP and GFR at 60 days in all animals. A p value < 0.05 was considered statistically significant.

RESULTS

Body weight of mothers fed control diet or low zinc intake diet was similar before delivery (C: 407 ± 16 versus L: 380 ± 23 g) and at the moment of offspring weaning (C: 299 ± 10 versus L: 298 ± 7 g).

There were no significant differences in daily intake of food among mothers during pregnancy and lactation periods in both groups (pregnancy: C: 22 ± 2 versus L: 19 ± 2 g/day; lactation: C: 43 ± 9 versus L: 40 ± 2 g/day); thus, it was not necessary to pair feed control rats (mothers that received an amount of control diet equal to mothers with low zinc intake diet).

Male offspring of mothers with low zinc intake during pregnancy and lactation periods exhibited lower body weight at day 1 after birth compared with male offspring of control mothers (C: 7.7 ± 0.1 versus L: 6.8 ± 0.2 g; $p < 0.01$). However, at the end of the dietary period (60 days), body weight of animals exposed to moderate zinc deficiency during growth, before and after weaning, was similar to control group (Cc: 400 ± 10 g; Cl: 379 ± 11 g; Lc: 390 ± 19 g; Ll: 373 ± 12 g).

There were no significant differences in daily intake of food among the different experimental groups (Cc: 22.2 ± 0.9 g/day; Cl: 21.3 ± 0.8 g/day; Lc: 22.9 ± 0.9 g/day; Ll: 23.0 ± 0.9 g/day); thus, it was not necessary to pair feed control rats.

Details of zinc concentrations in plasma and renal tissue during diet treatment are shown in Table 2. Offspring of mothers fed low zinc diet during pregnancy and lactation presented lower plasmatic levels of zinc at the moment of weaning compared to offspring of controls. Sixty days after weaning, the animals fed low zinc diet during growth period (groups Cl and Ll) had lower plasmatic levels of zinc than animals fed control diet (groups Cc and Lc). Moderate deficiency of this micronutrient induced a reduction in zinc concentrations in the kidneys of animals Ll and Cl compared to rats CC and Lc.

Animals exposed to a moderately zinc-deficient diet during pregnancy, lactation and/or postweaning growth exhibited higher values of SBP at 60 days compared to control group (Figure 1). However, these parameter was similar in animals in groups Cl, Lc and Ll In addition, 60 days after weaning, a negative correlation was observed between birth weight and SBP in animals Cc and Lc ($r = 0.8305$; $p < 0001$).

Figure 2 illustrates the results of NOS activity in the thoracic aorta and kidneys of animals Cc, Lc and Ll at the end of the experimental period. Moderate zinc deficiency during prenatal life, lactation and/or postweaning growth induced a reduction in NOS activity in the thoracic aorta, renal cortex and medulla with no significant differences in enzyme activity in animals Cl, Lc and Ll in the tissues studied. In addition, animals Cl, Lc and Ll showed lower GFR compared to group Cc (Figure 3); a positive correlation was observed between NOS activity in the renal cortex and GFR ($r = 0.6131$; $p < 0.001$); yet, 60 days after weaning, a negative correlation was observed between SBP and GFR ($r = 0,7639$; $p < 0,01$).

DISCUSSION

This study demonstrates that moderate zinc deficiency during fetal life, lactation and/or growth produces an increase in blood pressure and renal function impairment in adulthood that are associated

	Cc	I	LC	LI
Plasma levels of zinc (mg/dl)				
Weaning	128 ± 8	128 ± 7	90 ± 7*†	83 ± 3*†
Day 60	168 ± 13	110 ± 7*‡	140 ± 10	98 ± 8*‡
Zinc in renal tissue (mg/dl)				
Day 60	27 ± 1	19 ± 1*‡	25 ± 2	18 ± 1*‡

Cc: Control-diet mother, control-diet offspring. Cl: Control-diet mother, low-diet offspring. Lc: Low-diet mother, control-diet offspring. Ll: Low-diet mother, low-diet offspring. * p < 0.05 versus Cc; † p < 0.05 versus Cl; ‡ p < 0.05 versus Lc; n = 10 for each group.

Table 2. Zinc concentrations in plasma and renal tissue.

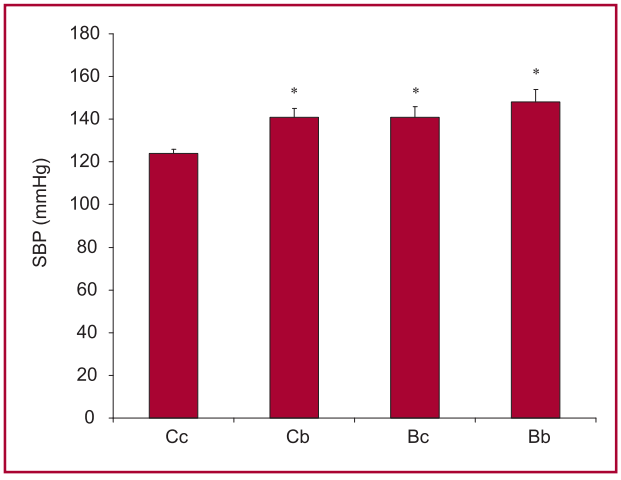

Fig. 1. Systolic blood pressure (SBP) after 60 days of diet. Cc: Control-diet mother, control-diet offspring. Cl: Control-diet mother, low-diet offspring. Lc: Low-diet mother, control-diet offspring. Ll: Low-diet mother, low-diet offspring. * p < 0,05 versus Cc; n = 10 for each group.

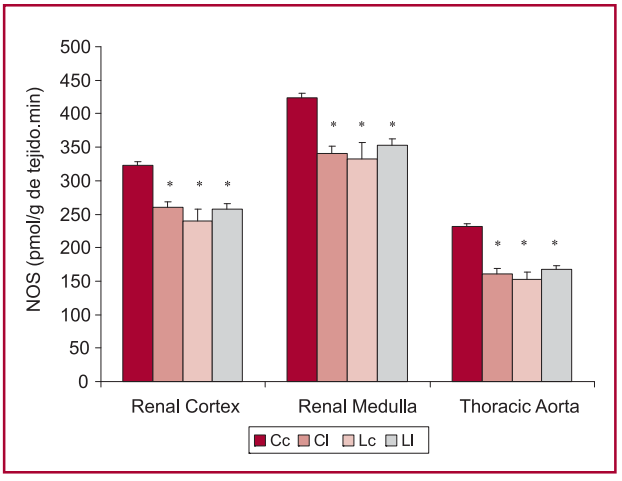

Fig. 2. NOS activity in the thoracic aorta and kidneys of animals after 60 days of diet. Cc: Control-diet mother, control-diet offspring. Cl: Control-diet mother, low-diet offspring. Lc: Low-diet mother, control-diet offspring. Ll: Low-diet mother, low-diet offspring. * p < 0.001 versus Cc; n = 10 for each group.

with reduction in the activity of the vascular and renal NO system.

Our experimental model showed that moderate restriction of this nutrient during fetal life and lactation induced low birth rate and increased blood pressure during adult life. The negative correlation observed between birth rate and SBP at 60 days has been previously reported in several studies of fetal programming in human beings as well as in animals. (25) Although the mechanisms involved have not been elucidated yet, intrauterine growth restriction produces not only in low birth weight but also compromises the development of organs, involved in blood pressure regulation, such as the kidney and the endothelium. (1, 26) We have already reported a reduction in the number of glomeruli and in glomerular area. (15)

Although zinc deficiency during pregnancy was associated with low birth weight, these animals reached body weights during adult life that were simi-

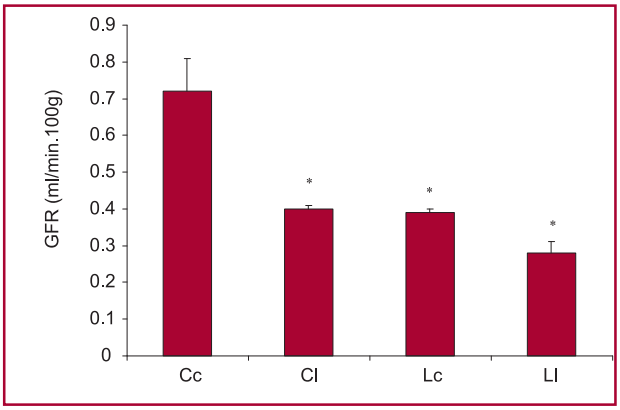

Fig. 3. Glomerular filtration rate (GFR) after 60 days of diet. Cc: Control-diet mother, control-diet offspring. Cl: Control-diet mother, low-diet offspring. Lc: Low-diet mother, control-diet offspring. Ll: Low-diet mother, low-diet offspring. * p < 0.01 versus Cc; n = 10 for each group.

lar to offspring of control mothers. Our results are consistent with previous studies that reported that accelerated postnatal growth also constitutes a risk factor for cardiovascular disease in human adults. (27-30)

The mechanisms responsible for blood pressure increase in animals with zinc deficiency during any period of their lives seem to be different and to depend on the adaptations generated during critical periods of development. The negative correlation between glomerular filtration rate and blood pressure in adult life suggests that blood pressure increase might be related to disorders in renal function.

Our results show that low zinc intake during fetal life and early postnatal life promote a reduction in the activity of vascular and renal NO system during adult life, even if adequate amounts of zinc are supplied after weaning.

Reduction of vascular and renal NOS activity might be related to lower availability of zinc for zinc-thiolate cluster formation which is necessary for its catalytic activity. (31)

Increase in systemic and renal oxidative stress has been previously reported with this model and it might be one of the factors responsible for reducing vascular and renal NO availability. (13-15) Increase in reactive oxygen species may produce NOS uncoupling and reduce NO synthesis. (32-34)

In addition, these results strengthen the hypothesis that low birth weight and adult hypertension are associated with disorders of endothelial function. Other models of fetal programming presented disturbances in endothelium-dependent relaxation and lower NOS activity in the aorta. (35, 36)

In the present study, the reduction in NOS activity in the thoracic aorta might suggest endothelial dysfunction which might contribute to the development of hypertension and alter the capacity of conducting arteries to regulate changes in blood flow and shear stress.

In turn, the decrease in renal NO system activity might play a key role in the hemodynamic anomalies observed in this experimental model. The positive correlation between GFR and NO production in the renal cortex suggests that zinc deficiency at different stages of growth might reduce NOS activity at the level of glomeruli and thus decrease glomerular filtration.

Adequate zinc intake after weaning normalized zinc concentrations in the kidney; however this improvement was not enough to normalize SBP and GFR levels, probably due to the persistence of intrauterine alterations, such as the number of nephrons and vascular and renal NO system activity.

This study demonstrates that moderate zinc deficiency during critical periods of development constitutes a nutritional deprivation that, associated with other factors, might contribute to determine blood pressure levels and renal function in adult life.

RESUMEN

Programación temprana de alteraciones en el sistema del óxido nítrico renal y vascular inducidas por la deficiencia de cinc

Introducción
Numerosos estudios mostraron que la deficiencia nutricional durante la vida fetal y posnatal predisponen al desarrollo de patologías en la vida adulta, como la hipertensión arterial y las enfermedades renales. La distribución ubicua del cinc y sus propiedades químicas determinan su esencialidad en los sistemas biológicos.

Objetivos
Evaluar si las alteraciones renales y cardiovasculares en la vida adulta inducidas por la restricción moderada de cinc durante la vida fetal, la lactancia y/o el crecimiento se asocian con cambios en el sistema del óxido nítrico.

Material y métodos
Ratas Wistar hembra recibieron durante la preñez hasta el destete de las crías una dieta control o una baja en cinc. Luego del destete, las crías macho se asignaron al azar a dos grupos que recibieron una dieta control o una baja en cinc durante 60 días.

Resultados
Los resultados mostraron que el aporte insuficiente de cinc durante el crecimiento previo y/o posterior al destete indujo un aumento de la presión arterial y una disminución del volumen de filtrado glomerular en la vida adulta, asociados con una disminución del sistema del óxido nítrico renal y vascular. Además, el bajo aporte de este mineral durante la vida fetal indujo un peso menor al nacer, que se correlacionó en forma negativa con la presión arterial en la vida adulta.

Conclusiones
Este trabajo brinda evidencias importantes que sugieren que el aporte inadecuado de cinc durante el crecimiento prenatal y posnatal constituye un factor de riesgo cardiovascular y renal, dado que induce alteraciones en la regulación de la presión arterial y en la función renal en el individuo adulto.

Palabras clave > Presión arterial - Óxido nítrico - Riñón - Vasos sanguíneos - Cinc

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