Fetal Programming of Hypertension Induced by Moderate Zinc Restriction during Prenatal Life and Lactation: Early Morphological and Functional Alterations in Cardiovascular System in Both Sexes

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SUMMARY

Objective

Several studies suggest that metabolic disorders and nutrition imbalance during prenatal life may induce adaptations that program cardiovascular diseases and hypertension. We have previously shown that moderate zinc restriction during prenatal life, lactation and/or growth leads to the development of hypertension and renal dysfunction in adulthood.

Objectives

To evaluate the presence of early cardiovascular alterations in rats exposed to a moderate zinc deficient diet during prenatal life and lactation, and to determine whether there are differences between males and females.

Material and Methods

Female Wistar rats received low zinc diet or control diet from the beginning of pregnancy up to weaning. Four experimental groups were established at birth: males and females born from low-diet mothers, and males and females born from control-diet mothers. Male and female offspring were sacrificed at 6 and 21 days of life to evaluate body weight, heart weight, cardiovascular morphometric parameters and nitric oxide synthase activity in the cardiovascular system and cardiac oxidative status.

Results

The insufficient zinc intake during prenatal life and lactation induced a remodeling process of the cardiomyocyte which was different in males and females, increased cardiac oxidative stress, produced a hypotrophic remodeling of the thoracic aorta and reduced nitric oxide synthase activity in the cardiovascular system. The insufficient zinc intake during prenatal life and lactation induced a remodeling process of the cardiomyocyte which was different in males and females, increased cardiac oxidative stress, produced a hypotrophic remodeling of the thoracic aorta and reduced nitric oxide synthase activity in the cardiovascular system.

Conclusions

This study shows that zinc deficiency induces cardiovascular abnormalities in early stages of development, which are different in males and females that may contribute to programming of diseases in adulthood.

Key words > Blood pressure - Myocytes – Aorta - Nitric oxide - Oxidative stress - Zinc

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BACKGROUND
During the last years, many epidemiological and experimental studies have provided enough evidence suggesting that metabolic disorders and nutritional imbalances during critical periods of development result in permanent effects on offspring’s health. In addition, they may be responsible for diseases of the adulthood that are programmed in the uterus, such as obesity, diabetes and hypertension. (1,2)

Micronutrient malnutrition during critical periods of development, known as hidden hunger, is an important public health problem in developed and developing countries, mostly affecting pregnant women, infants and children taking an unbalanced diet. This condition includes deficiency of minerals and vitamins, such as zinc, iron, calcium, vitamin A and vitamin D. (3)

Zinc is an essential trace element required by all living organisms for many physiological functions, including growth and reproduction. Zinc deficiency may affect the development of many organs, including the brain, lungs, skeleton, kidneys and the heart. 1 Subcellular location of zinc deposits is essential for the structural and functional integrity of cells, by maintaining the physiological barrier function, reducing oxidative stress and inhibiting apoptosis. (4)

Recent studies published by the Lancet about maternal and child undernutrition reported that zinc deficiency accounts for 4% of child deaths. (5) In addition, according to the Food and Agricultural Organization (FAO) approximately 20.5% of the world’s population is estimated to be at risk of inadequate zinc intake. (6) Nowadays, cases of severe zinc deficiency are uncommon, while moderate deficiencies due to inadequate zinc intake or absorption, increased losses of zinc from the body, or increased requirements for zinc are frequent. (7)

We have previously demonstrated that moderate zinc deficiency in rats during prenatal and postnatal growth is a model of fetal programming of cardiovascular and renal diseases during adulthood. Zinc restriction during prenatal life and lactation increases blood pressure and induces the development of abnormalities in renal function in adulthood. The rats exposed to zinc-deficient diet presented reduction of abnormalities in renal function in adulthood. The increases blood pressure and induces the development of cardiovascular and renal diseases during adulthood.

MATERIAL AND METHODS
Female Wistar rats (weight 271 g ± 7 g) from the laboratories of the School of Pharmacy and Biochemistry (University of Buenos Aires, Argentina) were mated by exposure to Wistar males during 1 week. Immediately afterwards, female rats were randomly fed either a low zinc-deficient diet (L; 8 ppm zinc, n = 9) or a control zinc diet (C; 30 ppm zinc, n = 9) during pregnancy and lactation periods. After birth, no more than 10 offspring remained with each mother until they were 6 to 21 days old. Therefore, four experimental groups were studied: male offspring of control-diet mother (Cm); female offspring of control-diet mother (Cf); male offspring of low-diet mother (Lm) and female offspring of low-diet mother (Lf). All animals received AIN-93 diet that fulfilled the nutrient requirements for pregnancy, lactation and 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.

Animal care was in accordance with the 6344/96 regulation of the Administración Nacional de Medicamentos Alimentos y Tecnología Médica (ANMAT, National Drug Food and Medical Technology Administration) and with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, Revised 1996). Mothers and their offspring were housed in plastic cages with a humidity and temperature controlled environment, illuminated with a 12:12 hours light-dark cycle.

Animals were allowed food and deionized water at will. Mothers and offspring food intake was monitored every two days during the experiment.

Body weight was determined in males and females offspring at days 6 and 21. The animals were killed by decapitation and the heart and the thoracic aorta were removed. The heart weight was expressed per 100 g body weight.

Plasma zinc concentrations in male and female offspring were measured at 21 days using Varian Spectra AA-20 atomic absorption spectrophotometer, air acetylene flame, 0.5 nm slit, at the wave length of 213.9 nm (Perkin Elmer Corp, Norwalk CT, USA) (12)

Histological evaluation
Cross-sections of cardiac tissue and thoracic aorta (3µm) underwent hematoxylin and eosin stain to determine the morphometric parameters: major and minor diameters of cardiomyocytes, and thickness of the intima an media. The studies were performed on ten areas from two sections of cardiac and vascular tissues, and 100 measurements of these parameters were obtained per animal. Morphometric parameters were evaluated in a double-blind fashion with a power magnification 1 x 400 using Nikon E400 light microscope (Nikon Instrument Group, Melville, NY). Images were analyzed using Image-Pro Plus 4.5.1.29 software (Media Cybernetics, LP Silver Spring, MD).

Cardiac oxidative state
Lipid peroxidation was evaluated by measuring the
formation of 2-thiobarbituric acid reactive substances (TBARS, nmol/mg protein). (13) We also measured reduced glutathione content which was expressed as mg/mg protein. (14) Superoxide dismutase (SOD) activity was evaluated by measuring in the homogenates the inhibition of epinephrine autoxidation, and was expressed as units of SOD/ mg protein. (15) Catalase activity (CAT) was determined by the autoxidation, and was expressed as units of SOD/ mg protein. (16) Glutathione peroxidase activity (GPx) was measured using the assay described by Flohé and Gunzler, and was expressed in pmol/min/mg protein. (17) The protein concentration was determined using the Lowry assay. (18)

Nitric oxide synthase activity
Nitric oxide synthase activity was determined by the formation of [14C] L-citrulline from [14C] L-arginine (specific activity: 360 mCi / mmol, Perkin Elmer Life and Analytical Sciences, Boston, USA), in the thoracic aorta and in the heart, and expressed per gram of tissue per minute. (19)

Statistical Analysis
All values are expressed as mean ± SEM. The GraphPad Prism software was used for statistical analysis. Data were analyzed using two-way analysis of the variance (ANOVA), followed by the Bonferroni test for multiple comparisons. A p value < 0.05 was considered statistically significant.

RESULTS
At 6 and 21 days of life, body weight and heart weight (corrected by body weight) of male and female offspring of mothers with low zinc intake during pregnancy and early after postnatal life, Lm and Lf, were lower compared to their respective controls (Tables 2 and 3). There were no significant differences between both sexes in these parameters.

At day 6, cardiomyocyte major diameter was longer in males and females with zinc deficiency compared to the control group. Minor diameter was reduced only in males. Diameters in offspring Lf were longer compared to offspring Lm. At day 21, major and minor diameters were longer in animals Lm compared to the control group and to Lf. In addition, minor diameter in zinc deficient diet females was longer compared to their respective control group.

At 6 days of life, intima thickness was lower in both male and female offspring of mothers with low zinc intake, and media thickness was reduced in animals Lm and was greater in the group Lf compared to control group (Table 2). Intima thickness was also lower in male and female animals with zinc deficiency at 21 days. There were no significant differences in media thickness (Table 3).

Table 4 shows the indicators of oxidative stress in the groups studied at 21 days of life. GPx activity was lower in groups Lm and Lf compared to the control group without differences between both sexes. There were no differences in the activities of CAT and SOD. Reduced glutathione was lower in groups Lm and Lf compared to groups Cm and Cf, respectively. The concentration of TBARS was lower in animals Cf and Lf compared to animals Cm and Lm, respectively.

Figure 1 illustrates the results of NOS activity in the heart at 6 and 21 days of life, and in the thoracic aorta at day 21, in the four experimental groups. Moderate zinc restriction during prenatal life and lactation decreased NOS activity in male and female animals with zinc deficiency with no differences between sexes.

DISCUSSION
Nutrient deficiency during early stages of life is associated with the development of diseases in the adult life. The hypothesis by Barker et al. relates an adverse intrauterine environment to abnormalities in fetal growth and organ function during adult life. (1) We have demonstrated that the abnormalities in the morphology of cardiovascular tissue induced by zinc restriction during prenatal life and lactation might be one of the mechanisms responsible for programming increased blood pressure in the adult life of these animals, as we have previously observed.
Male and female offspring exposed to zinc deficiency presented slow growth, evidenced by low body weight and heart weight during early postnatal life. Low heart weight was accompanied by hypertrophic remodelling of myocytes at 6 and 21 days of life. Zinc is a micronutrient involved in the regulation of cell proliferation and programmed cell death, and, thus, the morphological changes observed may be due to the fact that zinc deficiency alters the processes of cardiac hyperplasia and hypertrophy that take place during the development of the heart and its further maturation. It is well-known that the accumulation of binucleated cardiomyocytes in rodents occurs between days 4 and 12 of postnatal life. After this period, myocardial cells lose the ability to divide, and the heart grows due to myocyte hypertrophy and hyperplasia of other cells. (, , ,)

We have also observed that these changes were

### Table 2. Body weight, heart weight and morphometric parameters of cardiomyocytes and thoracic aorta at 6 days of life

<table>
<thead>
<tr>
<th></th>
<th>Cm</th>
<th>Lm</th>
<th>Cf</th>
<th>Lf</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>11.4 ± 0.5</td>
<td>8.5 ± 0.3*</td>
<td>10.5 ± 0.6</td>
<td>8.1 ± 0.3†</td>
</tr>
<tr>
<td>Heart weight (g/100 g body weight)</td>
<td>0.73 ± 0.01</td>
<td>0.63 ± 0.02*</td>
<td>0.75 ± 0.02</td>
<td>0.65 ± 0.02†</td>
</tr>
<tr>
<td><strong>Cardiomyocytes: morphometric parameters</strong></td>
<td></td>
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<tr>
<td>Major diameter (µm)</td>
<td>9.0 ± 0.2</td>
<td>13.3 ± 0.6*</td>
<td>8.2 ± 0.4</td>
<td>15.7 ± 0.8†, †</td>
</tr>
<tr>
<td>Minor diameter (µm)</td>
<td>8.8 ± 0.2</td>
<td>7.0 ± 0.2*</td>
<td>8.2 ± 0.2</td>
<td>8.6 ± 0.4†</td>
</tr>
<tr>
<td><strong>Thoracic aorta: morphometric parameters</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intimal thickness (µm)</td>
<td>21.9 ± 0.3</td>
<td>18.3 ± 0.2*</td>
<td>21.9 ± 0.4</td>
<td>18.9 ± 0.2†</td>
</tr>
<tr>
<td>Media thickness (µm)</td>
<td>246 ± 2</td>
<td>233 ± 3*</td>
<td>257 ± 3</td>
<td>263 ± 3†</td>
</tr>
</tbody>
</table>

Cm: Male offspring of control-diet mother. Lm: Male offspring of low-diet mother. Cf: Female offspring of control-diet mother. Lf: Female offspring of low-diet mother. Diet-related factor: * p < 0.05 vs. Cm, † p < 0.05 vs. Cf; sex-related factor: ‡ p < 0.05 vs. Lm; interaction diet x sex: non-significant. Weight; n = 10 for each group Morphometric parameters: n = 6 for each group.

### Table 3. Body weight, heart weight and morphometric parameters of cardiomyocytes and thoracic aorta at 21 days of life

<table>
<thead>
<tr>
<th></th>
<th>Cm</th>
<th>Lm</th>
<th>Cf</th>
<th>Lf</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>39.4 ± 1.3</td>
<td>33.9 ± 0.7*</td>
<td>38.4 ± 1.3</td>
<td>30.1 ± 1.1†</td>
</tr>
<tr>
<td>Heart weight (g/100 g body weight)</td>
<td>0.79 ± 0.09</td>
<td>0.57 ± 0.01*</td>
<td>0.79 ± 0.08</td>
<td>0.56 ± 0.02†</td>
</tr>
<tr>
<td><strong>Cardiomyocytes: morphometric parameters</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Major diameter (µm)</td>
<td>4.0 ± 0.3</td>
<td>18.2 ± 0.4*</td>
<td>15.4 ± 0.4</td>
<td>15.1 ± 0.3†</td>
</tr>
<tr>
<td>Minor diameter (µm)</td>
<td>8.6 ± 0.2</td>
<td>10.7 ± 0.2*</td>
<td>9.4 ± 0.2§</td>
<td>10.1 ± 0.2†, †</td>
</tr>
<tr>
<td><strong>Thoracic aorta: morphometric parameters</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Intimal thickness (µm)</td>
<td>23.3 ± 0.2</td>
<td>18.3 ± 0.1*</td>
<td>23.9 ± 0.2</td>
<td>18.9 ± 0.2†</td>
</tr>
<tr>
<td>Media thickness (µm)</td>
<td>280 ± 2</td>
<td>298 ± 2</td>
<td>287 ± 3</td>
<td>280 ± 2</td>
</tr>
</tbody>
</table>

Cm: Male offspring of control-diet mother. Lm: Male offspring of low-diet mother. Cf: Female offspring of control-diet mother. Lf: Female offspring of low-diet mother. Diet-related factor: * p < 0.05 vs. Cm, † p < 0.05 vs. Cf; sex-related factor: ‡ p < 0.05 vs. Lm; § p < 0.01 vs. Cm; interaction diet x sex: non-significant. Weight; n = 20 for each group Morphometric parameters: n = 6 for each group.

### Table 4. Postoperative complications and mortality by groups of risk.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cm</th>
<th>Lm</th>
<th>Cf</th>
<th>Lf</th>
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<tbody>
<tr>
<td>Antioxidant enzymes activity</td>
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<td></td>
</tr>
<tr>
<td>GPx (pmol/mg protein.min)</td>
<td>81 ± 8</td>
<td>65 ± 6*</td>
<td>86 ± 6</td>
<td>69 ± 8†</td>
</tr>
<tr>
<td>SOD (USOD/mg protein)</td>
<td>6.0 ± 0.9</td>
<td>5.2 ± 0.3</td>
<td>4.5 ± 0.5</td>
<td>3.9 ± 0.2</td>
</tr>
<tr>
<td>CAT (pmol/mg protein)</td>
<td>0.35 ± 0.02</td>
<td>0.30 ± 0.03</td>
<td>0.35 ± 0.03</td>
<td>0.34 ± 0.03</td>
</tr>
<tr>
<td>Glutathione (mg/mg protein)</td>
<td>8.6 ± 0.8</td>
<td>2.7 ± 1.1*</td>
<td>10.1 ± 1.3</td>
<td>5.8 ± 0.3†</td>
</tr>
<tr>
<td>TBARS (nmol/mg protein)</td>
<td>0.40 ± 0.05</td>
<td>0.32 ± 0.02</td>
<td>0.22 ± 0.04§</td>
<td>0.18 ± 0.02‡</td>
</tr>
</tbody>
</table>

Cm: Male offspring of control-diet mother. Lm: Male offspring of low-diet mother. Cf: Female offspring of control-diet mother. Lf: Female offspring of low-diet mother. Diet-related factor: * p < 0.05 vs. Cm, † p < 0.05 vs. Cf; sex-related factor: ‡ p < 0.05 vs. Lm, § p < 0.01 vs. Cm; interaction diet x sex: non-significant. n = 10 for each group.
different and more evident in males than in females. Thus, we may ask ourselves which mechanisms are involved, and whether estrogens play a protective role in the development of diseases in the adult life induced by injury during prenatal life.

The animals subjected to moderate zinc restriction also showed less antioxidant activity in cardiac tissue, as they presented lower GPx activity and reduced glutathione content. There were no differences between both sexes in the activity of antioxidant enzymes (SOD, GPx and CAT). However, females presented lower oxidative damage, evidenced by lower levels of lipid peroxidation suggestive of a greater antioxidant protection. These results are coincidental with several papers indicating that zinc protects cells against oxidative damage through its effects on glutathione, the main intracellular antioxidant. On the other hand, zinc can bind to membrane sites that might otherwise join metals involved in redox reactions (Cu, Fe) and is an essential component of metallothioneins. ( )

Our results show that low zinc intake during fetal life and early postnatal life promote a reduction in the activity of vascular and cardiac NOS activity that might be related with less availability of zinc for an adequate formation of zinc/thiolate clusters which are essential to maintain the catalytic activity.( ) In addition, increased oxygen reactive species might produce NOS uncoupling and decrease nitric oxide synthesis.

The thoracic aorta presented hypotrophic remodelling of the intima and a reduction in the NOS activity in males and females, suggestive of endothelial dysfunction. In this way, conducting vessels would not respond adequately to changes in blood flow and shear stress.

Our results are consistent with the hypothesis of fetal programming that proposes that exposure of the fetus to an adverse environment in uterus alters intrauterine growth and results in lower birth weight. Subsequently, adaptive responses lead to changes in the normal development of the heart and cardiovascular system. (20)

Zinc deficiency during critical periods of development constitutes a nutritional risk factor related to the development of cardiovascular diseases as hypertension in adult life.

**Acknowledgments**

This prospective and multicenter registry analyzed the outcomes of a small cohort of patients undergoing percutaneous Corew-up: 7 months) and without

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**RESUMEN**

Programación de hipertensión arterial por restricción moderada de cinc durante la vida fetal y la lactancia. Alteraciones morfológicas y funcionales tempranas del sistema cardiovascular en ratas de ambos sexos

**Introducción**

Numerosos estudios sugieren que trastornos metabólicos y desequilibrios nutricionales durante la vida intrauterina pueden inducir adaptaciones que programen enfermedades cardiovasculares e hipertensión arterial. En trabajos previos mostramos que la restricción moderada de cinc durante la vida fetal, la lactancia y/o el crecimiento conduce al desarrollo de hipertensión arterial y disfunción renal en la adultez.

**Objetivo**

Evaluar la presencia de alteraciones cardiovasculares tempranas en ratas sometidas a una deficiencia moderada de cinc durante la vida fetal y la lactancia y si existen diferencias respecto del sexo.

**Material y métodos**

Ratas Wistar hembras recibieron durante la preñez hasta el destete una dieta control o baja en cinc. En el momento del nacimiento se conformaron cuatro grupos experimentales: machos y hembras nacidos de madres bajas y machos y hembras nacidos de madres controles. A los 6 y a los 21 días de vida se sacrificaron y se determinaron el peso corporal, el peso del corazón, parámetros morfométricos cardiovasculares, la actividad de la óxido nítrico sintasa en el sistema cardiovascular y el estado oxidativo cardiaco.

**Resultados**

El aporte insuficiente de cinc durante la vida fetal y la lactancia indujo un proceso de remodelación del cardiómocito, diferente en machos que en hembras, un aumento del estrés oxidativo cardiaco, una remodelación hipotrófica de la aorta torácica y una disminución de la actividad de la óxido nítrico sintasa en el sistema cardiovascular.
Conclusiones
Este trabajo demuestra que la deficiencia de cinc induce alteraciones cardiovasculares, distintas en machos que en hembras, tempranas en el desarrollo, que podrían contribuir a la programación de enfermedades en la vida adulta.

Palabras clave > Presión arterial - Miocitos - Aorta - Óxido nítrico - Estrés oxidativo - Cinc

BIBLIOGRAPHY

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