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Zinc deficient diet consequences for pregnancy and offsprings of wistar rats

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Adult female Wistar rats (90 days old; weight 180 to 220 grams) were submitted to different zinc deficient diets (Zn; severe = 2.6 ppm; mild = 9.0 ppm and normal diet = 81.6 ppm), during 6 weeks. After this time they were coupled with normal male Wistar rats. No differences regarding fecundity and sterility were observed between the groups. During pregnancy, part of the animals from severe and mild Zn deficient groups received the same diet and the others received normal diet. The animals from the group receiving normal diet, were divided into other 3: the first received severe, the second mild Zn deficient diet and the third normal diet. During the study we observed that animals submitted to a Zn deficient diet (acute or chronic) had tendency to lower weight gain, lower weight of the offspring, lower serum levels of Zn in maternal and newborn (pool) blood. A significant reduction in the number of alive newborns was observed in the group of animals submitted to severe Zn deficiency. These data reinforces that Zn is a very important trace element overall during pregnancy.

UNITERMS: Zinc. Pregnancy. Offspring. Deficiency. Malformations.

INTRODUCTION

Based on the studies of KEILIN & MANN (15,16), identifying the zinc (Zn) as indispensable carbonic anhydrous component of the blood red corpuscles, around 200 metalloenzymes or metalloproteins were identified as Zn dependents in all Phyla (1,29). It is believed that this number does not surpass 30 in the human beings, also being present in some aldoses, deshydrogenases, peptidases and phosphatases (17), with participation in the metabolism of the carbohydrates, lipides and proteins mainly in synthesis and degradation of nucleic acids.

Depending on the period that the Zn deficient diet instals, the clinical evaluation can be variable. During the pregnancy, effects occur in a severe way and interfering with the action of the enzymes, dependent and necessary for the cellular division and reproduction (DNA and RNA polymerases, thymidinockinase), may lead to foetus death.

In this study we have evaluated the reflection of the different types of Zn deficient diets concerning Wistar rats pregnancy and offsprings.

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MATERIAL AND METHODS

Female Wistar rats, adults, 180 - 220 grams weight, were submitted, after 97 days born, to three diets during 6 weeks. These animals were kept in individual polypropilein cages, glassed floor and covered by steel, fixed to the cage by rubber tape. Received disionized water and special diet "ad libitum". They were classified in the following groups, according to the ration received: D - Ration with severe deficiency of Zn (2.6 ppm); M - Ration with moderate Zn deficiency (9.0ppm) and C - ration without deficiency of Zn (81.6ppm). After these 6 weeks period, they were coupled with normal males during 12 hours/night, being kept only the disionized water. Female Wistar rats presenting spermatozoids in the vaginal secretion were considerd "pregnant"(G) and continued under study forming the final seven groups, according to the ration received during the 21 days pregnancy: DGD - severe Zn deficiency before and during pregnancy; DGC - severe Zn deficiency before and ration control during pregnancy; MGM - Moderate deficiency before and no deficiency during pregnancy; CGD - severe deficiency during pregnancy only; CGM - moderate deficiency during pregnancy only and CGC - no deficiency before and during pregnancy. The female that did not become pregnant in the one week deadline period, even being coupled daily, were sacrified.

After 21 days pregnancy, rats were submitted to cesarea, under ethylic ether anesthesy, and their newborn constituted the RN groups according denomination of their mothers' group. At this moment, were observed the number of concepts (RN alive, natimorti, foetus reabsorptions) and the weight of the RN (offspring average). Samples of total heparinized blood (Liquimine®) were collected from mothers through cardiac puncture and from the RN after decapitation ("pool") for the Zn levels determination, by spectrumphotometry of atomic absorption (Perkin-Elmer; wave length 213.8nm). Placentas were collected as well and, after dried in the glasshouse, 250 mg of dried tissues were submitted to liquid digestion with a nitric-perchloric acid (2) mixture and to the determination of the Zn levels through spectrumphotometry of atomic absorption.

During the research, ingestion of ration and the ponderal evolution of the animals were followed. The deficient rations were produced in the Research Laboratory of the Pediatric Department of the Faculdade de Medicina in Botucatu, presenting a basic composition of 21% of proteins (28). Zn levels were controlled by the addition of a mineral salt mixture with a known Zn concentration.

Statistical analysis was performed through: Student "T" test, Kruskal-Wallis,Wilcoxon, and participation of the qui-square establishing alpha-5%.

RESULTS

All the animals presented ponderal gain during pregnancy. However, animals submitted to the deficient diet presented moderate results (Tale 1). No difference was observed in the quantity of animals fecunded considering the initial type of ration received, D or M or C. Number of concepts (RN alive, natimorti or foetus reabsorption) was not different for all the other groups studied (Table 2). However, considering the number of RN alive, smaller values were observed between the animals of the DGB group (Table 2). The offsprings average weight in the different groups was almost the same (Table 1). About 65% of the animals whose mothers were submitted to deficient diet during pregnancy, presented paw malformations (fusion and ankles absence).

The Zn plasmic levels of the mothers pertaining to MGM group were meaningly smaller than the level of mothers pertaining to the MGC group. In the RN groups, differences were observed between the MGM < CGM groups (Table 1). No difference in the Zn placentary levels were observed (Table 1). No correspondence was observed between the maternal Zn levels, the "pool" of the RN and the placentary "pool"of the different groups studied.

DISCUSSION

The Zn nutritional deficiency has been described as associated to anorexia in a premature installation, dermatitis, alopecia and growth discontinuing. Generally, they will be more intensive according to the degree, duration and the period of life the deficiency was imposed. During pregnancy, lactation and wean, nutritional Zn needs are the maximum (18,20,24, 25,28).

We have observed in this research that, considering same weights in the begining of pregnancy, the ponderal gain of animals submitted to Zn acute deficiency (CGM and CGD) or chronic (DGD,MGM), was smaller than the informed by others (4,7,8,11,18,22-25). It is agreed that the lower ponderal gain is consequence of smaller ingestion. Our animals consumed the same ration volume, fact that did not obligate us to criate "pair fed" groups.

During the experience, we observed fur fall, diarreic occurrence and ulcerous lesions in tails, in the animals pertaining to several groups. In spite of descriptions on higher index of sterility and infecundity in females submitted to Zn diet deficiency (3,12,22,25), this has not been observed in our animals. At the end of pregnancy, the number of concepts was not different from the several other groups. However, the number of RN alive in the DGD group was significantly smaller than the verified in the others (Table 2).

HURLEY & TAO (11), studying Wistar rats during pregnancy, observed that only 68% of the animals submitted to an acute Zn diet deficiency (0.4 ppm) had RN alive and 56% of the foetus implantation in the womb of the mothers were absorbed or death and 83% of the RN presented crude malformations. These results are confirmed by others (8,18,23,24). Pregnancy itself, mainly when associated to Zn nutritional deficiency, is able to decrease the Zn serum levels and cause serious problems to foetus, since malformation till death (3,5,10,14,26,32).

RECORD et col. (22) studied the Zn deficiency imposed to "Sprange-Fawley" rats during the first 11 days pregnancy. They concluded that Zn deficiency affects the cellules in large part in what refers to cellular division, mainly the lateral portion of the cranioneural tube and the tail region, justifying a higher incidence of malformations in the SNC.

In order to avoid any eventual misunderstanding in the evaluation of the RN animals, we made use of the offspring average weight. Poor diet animals showed tendency to lower weights. Generally, diets with more deficiency are usually followed by a weight reduction of the RN. However this relation is not very clear when rations with limited deficiencies are used and that could probably be partially corrected by using maternal reserves (7,11,23-25).

Determination of the Zn plasmatic levels in the pregnant rats, at the end of this period, showed smaller values for the MGM group animals in comparison the the MGC ones (Table 1).

Zn levels in "pool" of the RN plasma (Table 1), whose mothers were submitted to any kind of deficiency (acute or chronic, intensive or slight), showed tendency to lower values when compared to the control group (CGC). However, difference was only meaning in the MGM and CGC groups. These values were significantly higher than the ones of their mothers (Table 1). Similar occurrence has been verified in the human beings (13,31).

According to PRASAD(21), maternal Zn would be transfered to foetus by active transportation through placenta. SIMMER et col. (27) by means of guinea-pigs, studied the ^{65}Zn distribution furnished to mother, through a foetusplacentary unit and observed the maintenance, in the RN, of a higher gradient between the plasmatic and the muscular Zn of the RN in comparison to the maternal values. They also got similar ^{65}Zn concentrations in the maternal liver and foetus. Capacity of the Zn concentration by the placenta, observe one hour after the unique intravenous dose of ^{65}Zn was similar to the liver concentration. Through controled perfusion of the

Table 1
Main Characteristics Evaluated in Animals Pertaining to the Different Groups Studied (Average Data)

Variable	Groups						
	DGD	DGC	MGM	MGC	CGD	CGM	CGC
Number	11	10	13	8	11	8	8
Initial weight (g)	214,30	221,60	225,10	227,80	223,90	218,60	
Final weight (g)	281,40	327,50	300,40	318,60	297,70	310,80	321,30
Nestful average weight (g)	4,27	4,73	4,37	4,57	4,30	4,33	4,50
Plasmatic Zn (mg/dl)	123,80	142,40	84,70*	151,00	113,60	139,70	137,60
Zn "pool" plasma RN (ug/dl)b	274,20	268,00	199,50*	253,30	222,70	263,20	347,00
Zn "pool" placenta (ug/g dry tissue)	51,80	50,80	41,60	52,70	44,40	44,00	45,10

Kruskal Wallis a,b - Plasmatic Zn MGM < MGC
Zn "pool" plasma RN - MGC < CGC
Wilcoxon - Plasmatic Zn < "pool" plasma RN - all groups

Table 2
Quantity of concepts, after birth, alive and not, in the different groups studied

Group	Alive	Not alive	Total	% Alive
DGD	107	20	127	84.25
DGC	117	5	116	95.65
MGM	130	8	138	94.20
MGC	77	8	85	90.58
CGD	107	6	113	94.69
CGM	84	1	85	98.82
CGC	84	3	87	96.55
TOTAL	700	50	750	93.33

Qui-square
x calculated = 22.46* x critical (6gl;5%) = 12.59
Qui-square partition
DGD x (DGC + MGM + MGC + CGD + CGM + CGC)
x calculated = 17.22* x critical (1gl;5%) = 3.84
DGD < DGC< MGM< MGC< CGD< CGM< CGC

placentary tissue, they observed that the Zn mass transfered through the placenta was closely related with the plasmatic concentrations, that vary between 0.7 and 24.1 ug/ml. Under normal conditions, daily transference of the Zn serum is of 0.12mg/day, quantity sufficient for foetus supliment and similar to the one incorporated to it in the last third part of the pregnancy. The authors conclude that the maternal foetus transfer suggests an active transport at maternal surface level, combined with slow liberation to the foetus, under a concentration gradient. Under normal conditions, 32% of plasmatic Zn circulates linked to alpha-2 macroglobuline, 66% to albumine and in less quantity to aminoacids, mainly histidine and cysteine (9). ZIMMERMANN et col. (33) observed at the end of pregnancy in human beings, similar levels of albumine and inferior to alpha-2 macroglobuline in samples of maternal serum, when compared to the obtained through the umbilical vein. However, the Zn quantity per albumine/gram was higher in the RN serum while in the one, linked to alpha-2, macroglobuline was higher in the maternal serum. This way, authors discuss the fact that the albumine should be the main protein transporter of Zn to the placenta. They consider that albumine is important for the Zn transport to tissues of rapid transference (for example intestine, liver and placenta) and that alpha-2 macroglobuline interacts with the cellules for the transport of Zn for complex metabolic requirements such as synthesis of DNA through foetus (33).

FAIRWEATHER-TAIT and col (8) in the serial of experiments with Wistar pregnant rats, submitted to a discrete Zn deficiency, observed a significant decrease in the Zn levels of placentary tissues of the deficient diet animals in comparison to those of the control group, on the 19th day of pregnancy. This lower placentary concentration of Zn resulted in a lower Zn total content in the foetus of these animals, although the placentary weight has showed higher than the one of the control group.

TERRY et col. (30) studying Zn placentary transport in pregnant female rabbits, in the third quarter of pregnancy, observed bigger ^{65}Zn passage through the placenta and a better foetus capture with the evolution of the pregnancy. The absence of ^{65}Zn in the placentary tissue made these authors define placenta as a transport organ and not a Zn reservatory. JOAQUIM (13) observed, in human beings, significantly higher levels of Zn in the placentary tissue in comparison to the maternal and RN serum, and does not believe, therefore, that placenta is a deposit organ, considering the higher Zn level found as a consequence, probably, of the growing that occurs in the placentary tissue until the end. According to METHFESSEL & SPENCER (19), the homeostasis of Zn is regulated, mainly, through its secretion. Although Zn levels in foetus and in the respective placentas of the pregnant animals submitted to deficiency are lower than the one of the control group, FAIRWEATHER-TAIT observed a bigger Zn transference to foetus. This way, they affirm that under inadequate nutrition conditions of Zn the pregnant animal is able to mobilize the endogen Zn from metabolically active deposits such as the liver, and transfer it to foetus.

RESUMO

Objetivo: Avaliar os efeitos da carência nutricional de zinco na gestação e prole de ratas Wistar.

Material e Métodos: Submetemos ratas Wistar adultas (90 dias) com peso entre 180 e 220 gramas a diferentes tipos de carência nutricional de zinco (Zn; grave: 2,6 ppm; moderada: 9,0 ppm e sem carência 81,6 ppm) durante 6 semanas. Após esse período foram acasaladas com machos normais e não observamos diferenças quanto fertilidade e esterilidade entre os animais carentes. Durante a gestação, parte dos animais continuaram recebendo o mesmo tipo de ração e parte recebeu ração sem carência. Com relação ao grupo sem carência três outros grupos foram formados: um que passou a receber dieta com carência grave, outro moderada e o terceiro continuou sem carência.

Resultados e Conclusão: Observamos entre os animais submetidos à carência aguda ou crônica tendência a menor ganho ponderal durante a gestação, menor peso da ninhada, níveis séricos de Zn materno e de "pool" de RN mais baixos. Observamos de modo significante, menor número de RN vivos entre os animais submetidos à carência grave reforçando o papel do Zn durante a gestação.

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