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Maternal food restriction during pregnancy induces oxidative stress, vascular defects, cardimyocytes pathology in male rats offspring.

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ABSTRACT

Background: Intrauterine nutrition status is reported to correlate with risk of cardiovascular diseases in adulthood. These effects could be mainly attributed to the participation of reactive oxygen species (ROS) generated by dietary restriction..**Objective**: This study aims to determine the influence of maternal food restriction on myocardial and aortal histology and histochemistry beside oxidative stress markers, blood pressure and kidney function of offspring. **Study design** : In the present study, female rats were divided into two equal groups: control, fed standard chow ad libitum and restricted group fed 50% of the *ad libitum* intake. At 6 weeks of age, males offspring from both control and restricted groups were used for the experimental investigations. **Results**: The pups of food restricted dams showed a significant increase in plasma concentrations of hydroperoxide, creatinine phosphokinase, lactate dehydrogenase, creatinine, and uric acid. Moreover, cardiac thiobarbituric acid reactive substances(TBARS) and blood pressure of offspring elevated significantly. On the otherhand, cardiac reduced glutathione (GSH) ,Glomular filtration rate and body weight of pups exhibited a significant decrease. Cardiomyocytes and aorta of offspring showed some histological and histochemical changes. **Conclusion**: The results of the present study suggest that food restriction in pregnant rats provoked alterations in cardiovascular system that might be pronounced by an increase in blood pressure ,oxidative stress and histopathological changes in cardiomyocytes and aorta, which suggest involvement of lipid peroxidation in these alterations.

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INTRODUCTION

The fetal environment is considered a key factor in the etiology of cardiovascular disease later in life. However, Barker *et al*. (1989) first noted the inverse relationship between weight at birth and risk environment as a new component in the etiology of cardiovascular disease. Based on their observations, that developmental programming of adult disease occurs in response to an imbalance during fetal life between fetal demands and nutrient supply resulting in fetal undernutrition. Impairment in fetal development, which can be marked by intrauterine growth restriction and low birth weight, results from these fetal adaptations to an adverse fetal environment leading to molecular and physiological adaptive changes .Although these fetal adaptations allow fetal survival, they also result in long-term consequences such as marked alterations in the physiology and structure of the cardiovascular system (Barker, 1995). Food restriction during pregnancy produced growth-restricted newborns that exhibited catch-up growth that resulted in markedly heavier adult offspring, although with relatively decreased weights of heart, kidney, lung, and brain as compared with controls (Desai *et al*., 2005).

The participation of reactive oxygen species (ROS) generated by dietary restriction, leading to oxidative stress (Robinson *et al*., 1997) was reported. It was demonstrated that maternal protein restriction is associated with oxidative stress and fibrosis in pancreatic islets of male offspring at 3 and 15 month of age. Islet xanthine oxidase expression was increased in offspring, which suggests increased oxidative-stress and lipid peroxidation. Superoxide dismutase and heme oxygenase-1 were reduced significantly in offspring which indicated impairment of oxidative defense (Tarry-Adkins *et al*., 2010). It is well recognized that oxidative stress can cause direct damage to macromolecules within cells, including nucleic acids, proteins, and lipids (Valko *et al*., 2007). Under pathophysiological conditions, there is an excess production of ROS which prevail over the antioxidant

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defenses leading to oxidative stress. Oxidative stress is deleterious as the excess ROS is responsible for causing injury of cellular components (Valko *et al*., 2007 Bartosz, 2009). Substantial evidence has accumulated over the past few decades from cell, animal and human studies establishing oxidative stress as a major contributor to the development of heart disease and its progression into heart failure (Sugamura and Keaney, 2011). Oxidative stress contributes to vascular damage by promoting cell growth, extracellular matrix protein deposition, endothelial dysfunction and increased vascular tone; characteristic features of the vascular phenotype in hypertension (Paravicini and Touyz, 2008). The increased production of superoxide anion and hydrogen peroxide, reduced nitric oxide synthesis, and decreased bioavailability of antioxidants have been demonstrated in both experimental and human hypertension (Vaziri *et al*.,2000, Champlain *et al*., 2004; Touyz, 2004) .Glutathione, an important water-soluble antioxidant, directly quenches ROS such as lipid peroxides, and also plays a major role in xenobiotic metabolism. GSH depletion leads to cell death,and has been documented in many degenerative diseases (Kidd, 1997). Several studies about generation of oxidative stress by food restriction suggest that dietary restriction modifies the process of glutathione (GSH) metabolism and alters the status of antioxidant defense systems (Fetouia *et al*., 2007). The present study was designed to investigate the effects of food restriction during maternal pregnancy on histological and histochemical changes in cardiomyocytes and aorta ,oxidative stress markers, blood pressure, and kidney fuction of offspring. Moreover, distribution of cardiomyocytes DNA of offspring was assessed.

MATERIAL AND METHODS

Animals and diet:

Adult Wistar rats, used throughout the experiment, were made available from animal house in College of Pharmacy (King Saud University, KSU). They were kept in an air-conditioned room. (temperature, 22°C ± 3°C; relative humidity, 40%) with a12-hour light/dark cycle. A commercial rodent diet (food pellet) and tap water were available ad libitum. After 1 week of acclimatization to the laboratory conditions, male and female rats were kept in pairs in each cage. Ten adult virgin female Wistar rats were used to generate 16 males offspring for the present study. Pregnant female rats were inspected daily by the presence of the vaginal plug, which indicated day zero of pregnancy. Because IUGR produced by placental insufficiency results in the development of hypertension only in male offspring (Ojeda *et al*., 2007). the present study was performed only on male offspring. This study was conducted in Zoology department ,Faculty of Science,King Saud university. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory animals of ethical committee of King Saud University, College of Science.

Dietary protocol:

Following confirmation that mating had occurred, the females were housed individually in standard rat cages and randomly they were divided into two groups: Control(n=5), fed standard chow ad libitum with an approximate composition of (g/kg): 220 protein, 435 carbohydrates,40 fat, 80cellulose, 100 minerals, 125 water plus salt and vitamin mixtures and Restricted group (n=5), fed 50% of the ad libitum intake which determined by the amount of food consumed by the control group from day 1 of pregnancy until parturition (Chou *et al*., 2008). Their food consumption was measured daily by weighing the container after carefully collecting spilled chow. After confirmation of delivery, the dams were switched to standard chow, and the pups were weighed weekly. The offspring were nursed by their mothers until being weaned at 4weeks of age,and then they were switched to standard chow ad libitum. At 6 weeks of age ,offspring males from both control (8rats) and restricted (8rats) groups were used randomly for the studies detailed below.

Collection of samples:

Offspring rats were housed in metabolic cages (Each animal in one cage) and received free access water during the 24 hour collection period. Urine volume was determined and urine was centrifuged and kept in refrigerator. The blood was drawn from the animals by puncturing the retroorbital venous sinus with capillary tubes under anesthesia. The blood was collected in heparin-coated centrifuge tubes, centrifuged at 200 × g for 10 minutes, then plasma was separated in eppendorf tubes and stored at −40°C. Whole blood was used for the determination of the level of hydroperoxide, whereas separated plasma was used to determine biochemical parameters. After collection of blood samples,the animals from all groups were killed by cervical dislocation and autopsied. The heart and aorta of different groups were removed, rinsed with cold saline and fixed in 10% formol saline. Some heart samples were kept frozen.

The frozen heart tissue samples were weighed and homogenized (Automated homogenizer, IKA, T25D, Germany) (1:10, w/v) in 10 mM KCl in 1.15% phosphate buffer and ethylenediamine tetraacetic acid (EDTA; pH 7.4) and centrifuged at 5000 × g for 10 min. The supernatant was used to assay the level of thiobarbituric acid reactive substances (TBARS) and reduced glutathione (GSH).

2.4. Body and organs weight:

Body weight of offspring was recorded weekly until 6 weeks of age, while weight of heart, liver and kidneys was recorded at 6th week only.

Biochemical studies:

Blood hydroperoxide level was evaluated using an analytical system (Iram, Parma, Italy). The test is a colorimetric test that takes advantage of the ability of hydroperoxide to generate free radicals after reacting with transitional metals. When buffered chromogenic substance is added, a colored complex appears. This complex was measured spectrophotometrically. Lipid peroxidation level in the cardiomyocytes was measured by method of Ohkawa, *et al* (1979) as thiobarbituric acid reactive substances (TBARS).The absorbance was read at 532 nm. using 1,1,3,3-tetramethoxypropane as standard. We also measured reduced glutathione content in heart tissues of pups which was expressed as mg/mg protein (Tietze, 1969). All the chemicals were purchased from Merk Company, Darmstadt, Germany. Creatinine phosphokinase, lactate dehydrogenase, total protein, creatinine and uric acid in plasma of Offspring were determined colourimetrically at wave lengths 340nm, 340nm, 540nm 492nm and 520nm respectively by using kits from Bio Merieux, France. The intensity of the coloration was measured by using spectrophotometer, UV/visible-Model- 80-2106-00, Pharmacia Biotech. Cambridge, England. Glomular filtration rate(GFR) was calculated from the following formula:

$$\text{GFR (ml/min)} = \frac{\text{mg creatinine/dl urine} \times \text{ml urine/24}}{\text{mg creatinine/dl plasma} \times 1440}$$

2.6. Measurement of blood pressure:

The blood pressure was determined in conscious rats from both groups by an indirect tail-cuff method (pneumatic transducer, PowerLab 4/S, AD Instruments Pty Ltd). Rats were kept at 27 °C for 5 min. The animal placed in the holder at 10 minutes prior to obtaining pressure measurements and then three stable consecutive measurements of blood pressure were averaged. Care was taken in selecting an appropriate cuff size for each animal. The ideal small animal holder should comfortably restrain the animal, create a low-stress environment. It is very beneficial to incorporate a darkened nose cone into the rodent holder to limit the animal's view and reduce the level of animal stress. The tail of the animal should be fully extended and exit through the opening of the holder.

Histological and histochemical studies:

The heart and aorta of different groups were removed and fixed in 10% formol saline. Paraffin sections (5μm thick) underwent haematoxylin and eosin to investigate the histological changes using Nikon light microscope with a power magnification 1x 200. Other sections were stained by Masson trichrome to demonstrate the collagen fibers.

Further sections of heart were stained for DNA (Feulgen and Rosen-beck, 1942) and counterstained with light green. DNA analysis was performed by Lecia Qwin 500 image cytometry in the Department of pathology, National Research Center, Egypt. For each section, 100-120 cells were randomly measured. The threshold values were defined by measuring control cells. The results are presented in a table4 which demonstrates the percentage of the diploid cells (2C), the triploid cells (3C), the tetroploid cells (4C) and the aneuploid cells (>5C). The DNA was classified according to Danque *et al*. (1993). Sections of heart and aorta stained for Protein (Mazia *et al*., 1953) and mucopolysaccharides (Mac-Manus and Cason,1950) were subjected to cytophotometric measurments using a Leitz MPV compact cytophotometer and the optical density(O.D) values were considered to represent the concentration of intracellular material according to Lambert law.

Statistical analyses:

Data were analyzed by ANOVA single factor using EXCEL Microsoft office 2007, and presented as mean±S.E.

Results:

Body and organs weight:

Offspring body weight is shown in Table 1. The pups from food-restricted dams had45%,27%, 21% ,21% and 9% significant lower body weights compared with pups from control dams at 1,2,3,4,5 weeks of age, while it was higher in food restriced group at 6th week of age,however, the difference was non-significant.

Offspring of food restricted dams at 6 weeks old, showed a significant decrease ($p \leq 0.05$) in the wet weight of heart as compared with the control. However, the remaining organs (namely, liver and kidneys) had almost similar weights as the controls (Table 2).

3.2. Biochemical assays and blood pressure:

Lipid peroxidation measured as hydroperoxide in blood and as thiobarbituric acid reactive substance in heart tissues was significantly ($p \leq 0.01$) increased in offspring of food restricted dams as compared with control ones (Table 3). On the otherhand, cardiac GSH content in offspring of food restricted dams decreased significantly($p \leq 0.01$).

Both systolic and diastolic blood pressure of pups of food restricted dams were significantly($p \leq 0.01$) higher than those of control ones.

Data presented in the Table 3 demonstrated a significant increase ($p \leq 0.01$) in concentrations of creatinine phosphokinase, lactate dehydrogenase, creatinine and uric acid of offspring born from food restricted dams. On the otherhand, plasma protein and glomerular filtration rate decreased significantly in these rats.

Histological examination:

Examination of Cardiac tissue obtained from offspring of malnourished female rats showed that, most of the nuclei were deeply stained pyknotic. Scattered focal areas of mycocardial damage in the form of necrosis and karyolysis were also detected with dilated and congested coronaries. Few vacuolar degeneration in cardiomyocytes could be observed (Fig1 B&C). Dilation and Congestion of capillaries in cardiac muscle fiber were also seen. Wide separation of muscle bundles occupied with loose connective tissue could be observed (Fig1.D).

Aortic sections obtained from offsprings of malnourished rats showed that thinning of aorta as compared to control (Fig.2 B). Focal areas of intima appeared necrotic and more irregularity. The elastic fiber appeared more wavey with prominent smooth muscle cell. Gap areas were also noticed in subintimal layer and in tunica media and filled with few congestion. Vacuolar degeneration in smooth muscle fiber of tunica media is observed (Fig. 2 C).

Sections stained with Masson trichrome of pups of maternal malnourished rats showed an increase in collagenous fibers in between cardiac or aortal muscles (Figs3B,3D).

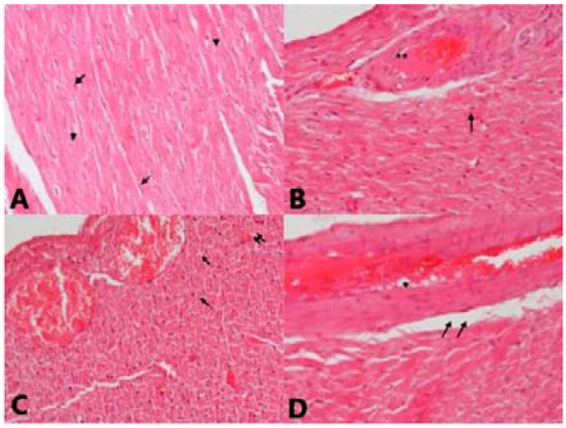

Fig. 1: A) Section of the cardiac muscle of a normal rat. muscle fibers appear as long cylindrical structure with multiple large oval nuclei (arrow head). B)Section in the heart obtained form offspring of maternal malnutrition showing most of nuclei were deeply stained and pyknotic(arrow). Dilated and congested coronaries (star) lined by endothelial cells were also observed .C)): Another field from previous group showing signs of degeneration in the form of karyolysis (arrow) and focal area of necrosis(double arrows). D) Another field of a section in the heart of rat obtained from offspring of maternal malnutrition showing dilatation congestion and vacuolation of capillaries (arrow head) in between cardiac muscle fiber. Wide separation of the muscle bundles occupied with loose connective tissue could be observed (arrow) (HX&E200)

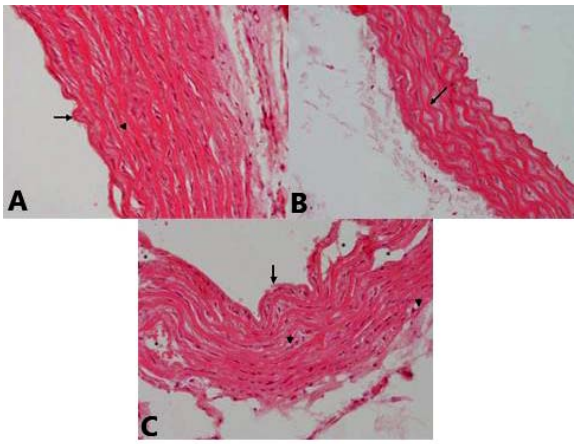

Fig. 2: A) Section in the aorta of a normal rat showing its normal structure, where the tunica media occupies most of the thickness of the wall and appears full of a wavy elastic fibers (arrow head).B) Section in the aorta of rat obtained from offspring of maternal malnutrition showing decrease in thickness of tunica media. The elastic fiber appeared more wavy (arrow). C) Anther field of a section in the aorta of the later group showing focal area of tunica initma appeared necrotic (arrow) and more irregularity. Gap areas were also noticed in supintimal layer and in tunica media. Vacuolar degeneration is also observed in tunica media(arrow head) (HXEx20)

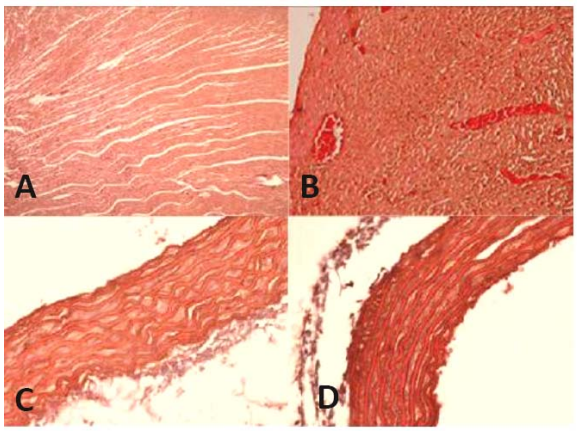

Fig. 3: Section in the heart of rats of offspring showing fine collagenous fibers in between the cardia cmuscle fibers and around the blood vessels (A): control cardiac muscle(B): group of restricted food showing increase in the collagenous fibers in between the cardiac muscle. (C): Section in the aorta of control rats .(D): group of restricted food showing increase collagen content in muscle fiber (Masson trichrome x200)

Table 1: Body weight(g) of male offspring rats of control and food restricted dams.

Group / Age in week	Control	Food restricted
1	15.32 ± 0.17	8.44 ± 0.30**
2	23.10 ± 0.80	16.90 ± 0.40**
3	30.90 ± 2.10	24.40 ± .80**
4	44.80 ± 1.20	35.40 ± 0.95**
5	63.40 ± 2.12	57.40 ± 1.90**
6	82.00 ± 5.10	91.20 ± 6.00

Each value is the mean ± S.E, n=8
Values marked with asterisks differ significantly from control value at P**<0.01.

Histochemical Studies:

Normal distribution of DNA content in the heart of the control group showed that 30.84% of the examined

cells contained DNA (<1.5C), 42.99 % of the examined cells contained diploid DNA value (2c), 17.75 % of the examined cells contained (3c) DNA value (medium proliferation index) and 7.47 % of the examined cells at (4c) area (table4). The group of puups of malnourished dams showed that 2.63% of the examined cells were more than 5c(aneuploidy), 42.982% of the examined cells contained DNA (1.5c), 31.57% of the examined cells contained diploid DNA value (2c), 18.42% of the examined cells contained (3c) DNA value (medium proliferation index) and 3.50% of the examined cells at 4c are tetrapoloidy.

The offspring of maternal malnourished rats showed a significant decrease in PAS positive material and protein content inside the cytoplasm of the cardiac or aortal muscles (Table 3).

Table 2: Wet weight of organs(g) in 6 weeks old male offspring rats of control and food restricted dams

Group / Organ	Control	Food restricted
Heart	0.74 ± 0.01	0.68 ± 0.02*
Liver	6.44 ± 0.22	6.50 ± 0.46
Kidney	1.11 ± 0.03	1.15 ± 0.08

Each value is the mean ± S.E, n=8
Values marked with asterisks differ significantly from control value at P*<0.05.

Table 3: Lipid peroxidation markers, blood pressure ,some biochemical parameters and Glomular filtration rate (GFR) of 6 weeks old male offspring of control and food restricted dams.

parameter		treatment	
		control	Food restricted
Blood hydroperoxide (mg /100ml)		26.15 ± 0.92	49.56 ± 2.34 **
Cardiac malondialdehyde (µ mol/ l)		0.40 ± 0.01	0.54 ± 0.02**
Cardiac GSH (mg/mg protein)		7.30 ± 0.20	3.11 ± 0.08**
Cardiac mucopolysaccharides (O.D)		1.87 ± 0.03	0.74 ± 0.02**
Aortal mucopolysaccharides (O.D)		1.19 ± 0.07	0.84 ± 0.02**
Cardiac protein (O.D)		0.93 ± 0.04	0.33 ± 0.01**
Aortal protein (O.D)		0.88 ± 0.03	0.20 ± 0.01**
Plasma Protein (g /100ml)		6.80 ± 0.25	6.02 ±0.15 *
Blood pressure (mm Hg)	systolic	107.00 ± 2.50	134.00 ± 1.50 **
	Diastolic	76.00 ± 2.11	114.00 ± 3.45 **
Plasma Creatine phosphokinase (U/l)		207.96 ± 19.05	515.71 ± 35.82**
Plasma Lactate dehydrogenase (U/l)		223.97 ± 18.83	450.64 ± 28.08 **
Plasma Creatinine (mg/100ml)		0.43 ± 0.02	0.69 ± 0.04 **
Plasma Uric acid (mg/100ml)		1.56 ± 0.10	2.79 ± 0.12 **
GFR(ml/min/100g bw)		1.36 ± 0.05	0.86 ± 0.02**

Each value is the mean ± S.E., n=8, values marked with asterisks differ significantly from control values at p*< 0.05, **p <0.01

Table 4: DNA Ploidy of the heart of pups of control and food restricted dams(FRD)

Range	Total Cells		% Cells		DNA Index	
	control	Pups of FRD	control	Pups of FRD	control	Pups of FRD
All	107	114	100.0%	100.0%	1.000	0.948
5cER	0	3	0.0%	2.632%	0	2.827
< 1.5c	33	49	30.841%	42.982%	0.529	0.571
1.5c-2.5c	46	36	42.991%	31.579%	0.996	0.988
2.5c-3.5c	19	21	17.757%	18.421%	1.390	1.461
3.5c-4.5c	8	4	7.477%	3.509%	1.862	1.921
> 4.5c	1	4	0.935%	3.509%	2.401	2.693

Discussion:

The current study showed that pups, whose mothers were food restricted during pregnancy, exhibited increase in markers of lipid peroxidation(blood hydroperoxide and cardiac TBARS) and a decrease in cardiac GSH content indicating an increase of oxidative stress. GSH is an important intracellular antioxidant that spontaneously neutralizes several reactive oxygen species (Lu, 1999). Fetoui *et al*. (2007) concluded that the progeny of food restricted female rats showed an increase in TBARS and a decrease of reduced glutathione in blood. It was demonstrated that nutritional restriction of female rats is associated with oxidative stress, fibrosis in pancreatic islets and impairment of oxidative defense of their pups (Ojeda *et al*., 2007). Oxidative stress is responsible for causing injury of cellular components (Vaziri *et al*., 2000, de Champlain *et al*., 2004) and it is considered as a major contributor to the development of heart diseases (Touyz, 2004). Examination of heart sections obtained from offspring of malnourished female rats fed on restricted food revealed degenerative changes in the form of necrosis, karyolysis and vacuolation with congested coronaries. Most of cardiomycocyte appeared pyknotic. It was reported that free radical particularly reactive oxygen species, play a cardinal role in pathogenesis of oxidative myocardial damage and in killing cells by necrosis or apoptosis leading to further myocardial injury (Lowenstein, 2004). The maternal malnutrition can lead to low birth weight of offspring with reduced number of cardiomyocytes in newborn rats pups which can amplify age related vascular and structure changes and increase cardiac fibrosis and capillarization in adulthood (Corstius *et al*., 2005; Skilton *et al*., 2006)

.However, reduced heart cell mass and altered heart morphology during development due to adverse effects of maternal malnutrition were noticed (Cheema *et al*., 2005; Buznicov *et al*., 2001). The changes in heart morphology in pups of malnourished rats may be due to increase level of norepinephrine (Buznicov *et al*., 2001) .The increase of the concentrations of creatinine phosphokinase and lactate dehydrogenase in pups of malnourished dams in the present study may indicate acute myocardial injury and myocarditis (Karjalainen and Heikkilä, 1986). Concerning ploidy results, the cardiac sections from offspring resulting from malnourished rats showed 2.63% of the examined cells at (>5c), 31.57% of the examined cells contained diploid DNA value (2c) and medium proliferation index. Maternal malnutrition influenced DNA damage and cause DNA single strand break (Tarry-Adkins *et al*., 2008). Moreover, increase of reactive oxygen species production in malnourished rats, caused oxidative damage to cellular macromolecules including DNA (Franco *et al*., 2003).

Oxidative stress may play a critical role in the pathogenesis of hypertension, as well as other cardiovascular disorders (Halliwell and Gutteridge, 1989). GSH depletion induced oxidative stress which resulted in perturbation of the nitric oxide system and severe hypertension in normal animals (Vaziri *et al*, 2000). The blood pressure of the present offspring of maternal malnutrition increased significantly while, thickness of aorta was decreasesd with focal areas of necrosis in intima. The development of hypertension in intrauterine undernourished rats may result from overactivity of chymase and increased intrarenal Angiotensin II production (Chou *et al*., 2008). Among the proposed mechanisms to explain the effects of maternal malnutrition on the arterial blood pressure in adult offspring, one could underline a decrease in hypothalamus –pituitary-adrenal sensitiveness, a decrease of placental activity of 11-b steroids, and changes in renal development (Langley-Evans, 1997; Langley-Evans *et al*., 2003). As a consequence of elevated blood pressure, arterial elasticity is reduced and wall damage appears, which can lead to cholesterol and fat deposition on those lesions and eventually to obstruction of the vessels (Safar, and London, 1994). The deficiency of elastin in fetus whose growth is impaired would lead to permanent changes in the mechanical properties of aorta (Martyn and. Greenwald, 1997). Results of this work were in agreement with other studies concluded that low birth weight offspring, resulting from maternal protein restriction, demonstrated cardiac structure abnormalities, vascular dysfunction and reduction in aortic wall thickness and elastin content (Brawley *et al*., 2003; Lim *et al*., 2006). The aortic intima-media thickness is the best non-invasive anatomical marker of atherosclerosis disease progression. It was shown that reduced fetal growth (a marker of intrauterine nutrition) is associated with increased aortic wall thickness in newborn infants (Skilton *et al*., 2005). However, we observed a decrease in aortic wall thickness in those rats exposed to *in utero* food restriction. These contrasting findings are most probably attributable to the different causes of fetal undernutrition, suggesting that a broad-spectrum intrauterine undernutrition due to placental insufficiency is involved in the pathophysiology of increased aortic wall thickness, rather than the maternal food restriction used in the rat mode. It is possible, however, that a thinner aortic wall may predispose to future hypertension and cardiovascular disease, via alterations to both arterial compliance and elastic properties. Indeed, in the present study, we observed reductions in aortic elastin content accompanying *in utero* food restriction. The impact of a reduction in aortic elastin on future cardiovascular events was beyond the scope of the present study, but is a potential area for future research.

Collagen content around cardiomyocytes and in aorta muscles of the offspring born from malnutrition female rats showed an increase. Collagen is a very stiff protein and has the role to limit vessel distension. Therefore, excessive collagen in the vascular wall leads to vessel fibrosis and increased stiffness. This is a characteristic feature of hypertensive vascular remodeling (Safar *et al*., 2001). An inversely association between glomular filtration rate and cardiovascular mortality, and stroke in hypertensive patients have established (Zhang *et al*. ,2011). Hypertension in low birth weight individuals also appears to be mediated in part through a reduction in nephron number (Zandi-Nejad *et al*., 2006). In the present investigation, a significant increase in plasma creatinine and uric acid concentrations associated with a reduction in GFR were noticed in pups of food restricted dams. High blood pressure over time, can damage blood vessels throughout the body. If the blood vessels in the kidneys are damaged, they may delay removing wastes from the body result in raise blood creatinine and uric acid.

Concerning histochemical results the heart and aorta of rats offspring obtained from malnourished rats showed a decrease in protein content. Moreover, plasma protein also decreased. In previous some investigators suggested that the decrease in protein content of cardiomyocytes may be due to reduced synthesis of myofibril components leading to myofibril reduction, and increased myofibrillar protein catabolism as a result of heightened liposmic protinase activity (Widenthal, 1975; Ramirez-de-Marten *et al*., 1998). In the present experiment the heart and aorta of pups of malnourished rats showed decrease in glycogen content. It was suggested that food restriction induced depressed bioenergetic function which may contribute to abnormal glycolytic function (Ayaz *et al*., 2002). The decrease in glycogen content may be due to distributing metabolic requirement of the myocardium, or increased demands for glucose (John Sutton *et al*, 1985).

In conclusion, food restriction to rats during pregnancy increases lipid peroxidation which may alter cardiovascular system, blood pressure and kidney functions in their progeny.

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REFERENCES

Ayaz, M., B. Can, S. Ozdemir and B. Turan, 2002. Protective effect of selenium treatment on diabetic induced myocardial structural alterations. Biol. Traco. Elem. Res., 89: 215-226.

Barker, D.J., P.D. Winter, C. Osmond, *et al*., 1989. Weight in infancy and death from ischaemic heart disease. Lancet, 2: 577-580.

Barker, D.J., 1995. Intrauterine programming of adult disease. Mol Med Today, 1: 418-423.

Bartosz, G., 2009. Reactive oxygen species: destroyers or messengers? Biochem. Pharmacol, 77(8): 1303-15.

Brawley, L., L. Poston and M.A. Hanson, 2003. Mechanism underlying the programming of small artery dysfunction: review of the model using low protein diet in pregnancy in the rat. Arch. Physiol. Biochem, 111: 23-35.

Buznicov, G.A., H.W. Lambert and J.M. Lauder, 2001. Serotonin and serotonin like substances as regulatory of early embryogenesis and morphogenesis. Cell and Tissue Research, 305: 177-186.

Cheema, K.K., M.R. Dent, H.K. Saini, N. Arovtiounova and A. Tappia, 2005 .Prenatal exposure to maternal under nutrition induced adult cardiac dysfunction. Br. J. Nutr., 93: 471-477.

Chou, H.C., L.F. Wang, K.S., C.M. Lu, X. Chen, 2008. Effects of maternal undernutrition on renal angiotensin II and chymase in hypertensive offspring. Acta histochemica, 110: 497-504.

Corstius, H.B., M.A. Zimanya, N. Maka, T. Herath, W. Thomas *et al*, 2005. Effect of intrauterine growth restriction on the number of cardiomtyocyte in rat heart. Pediatr. Res., 57: 796-800.

Danque, P.O., H.B. Chen, J. Patil, J. Jagirdar, G. Orsatti and F. Paronetto,1993. Image analysis versus flow cytometry for DNA ploidy quantiation of solid tumors: A comparison of six methods of sample preparation. Mod. Pathol., 6: 270-275.

De Champlain, J., R. Wu, H. Girouard, M. Karas, A.E. Midaoui and M.W.L. Laplante, 2004. Oxidative stress in hypertension. Clin Exp Hypertens, 26: 593-601.

Desai, M., D. Gayle, J. Babu and M.G. Ross, 2005. Permanent reduction in heart and kidney organ growth in offspring of undernourished rat dams. Amer J. of Obstetrics and Gynecology, 193: 1224-32.

Fetouia, H., A. Mahjoubi-Sameta, K. Jamoussib, F. Ayadib, F. Ellouzeb and N. Zeghala, 2007. Food restriction in pregnant and lactating rats induces anemia and increases plasma lipid peroxidation in their progeny. Nutrition Research, 27: 788-793.

Feulgen, R. and H.C. Rosen-beck, 1942. Manual of histological demonstration technique. Butterworth and co (publishers) LTd. London, Ford, Harverhill.

Franco, M.C.P., E.H. Akamine, G.S. DiMarco, D.E. Casarini, B. Fortes, R.C.A. Tostes, M.H. Carvalho and D. Nigro, 2003. NADPH oxidase and enhanced superoxide generation in intrauterine undernourished rats involvement of the rennin-angiotensin system. Cardiovasc. Res., 59: 767-775.

Halliwell, B. and J.M. Gutteridge, 1989. Protection against oxidants in biological systems: the superoxide theory of oxygen toxicity, in Oxford Science Publications (ed): Free Radicals in Biology and Medicine, 2nd ed. Oxford, UK, Clarendon, pp: 87-187.

John Sutton, M.G. St, T. Plappert, P. Grosby, J. Mullen and N. Peickek, 1985. Effect of reduced left ventricular mass on chamber architecture and function : a study of anorexia nervosa, Circulation, 72: 991-1000.

Karjalainen, J. and J. Heikkilä1, 1986. Acute pericarditis": Myocardial enzyme release as evidence for myocarditis . American heart J., 111(3): 546-552.

Kidd, P.M., 1997. Glutathione: Systemic protection against oxidative and free radical damage.Alt Med Rev., 2(3): 155-176.

Langley-Evans, S.C., 1997. Hypertension induced by fetal exposure to a maternal low-protein diet, in the rat, is prevented by pharmacological blockade of maternal glucocorticoid synthesis. *J Hypertens*,15: 537-44.

Langley-Evans, S.C., A. Langley-Evans and M. Marchand, 2003. Nutritional programming of blood pressure and renal morphology. Arch Physiol Biochem, 111: 8-16.

Lim, K., Y.A. Zmanyi and M.J. Black, 2006. Effect of maternal protein restriction on rats on cardiac fibrosis and capillarzation in adult hood. Pediatr Res., 60: 83-87.

Lowenstein, C., 2004. Exogenous thiore-doxin reduce inflammation in autoimmune myocarditis. Circulation, 110: 1178-1179.

Lu, S.C., 1999. Regulation of hepatic glutathione synthesis: current concepts and controversies. FASEB J, 1: 1169-1175.

Mac-Manus, J.P.A., J.E. Cason, 1950. Carbohydrate histochemistry studies by a cetylation technique-periodic acid method. J. Exp. Med., 91(6): 651-654.

Martyn, C.N and S.E. Greenwald, 1997. Impaired synthesis of elastin in walls of aorta and large conduit arteries during early development as an initiating event in pathogenesis of systemic hypertension. Hypothesis, 350(Issue 9082): 953-955.

Mazia, D., P.A. Drewer and M .Affert, 1953. The Cytochemical staining and measurement of protein with mercuric bromophenol blue. Biol.Bull., 104: 57-67.

Ohkawa, H., N. Ohishi and K. Yagi, 1979. Assay of lipid peroxides in animal tissues by barbituric acid reaction. Analyt Biochem, 95(2): 351-358.

Ojeda, N.B., D. Grigore, L.L. Yanes, R. Iliescu, E.B. Robertson, H. Zhang, *et al*, 2007. Testosterone contributes to marked elevations in mean arterial pressure in adult male intrauterine growth restricted offspring. Amer J Physiol, 292: R758-63.

Paravicini, T.M. and R.M. Touyz, 2008. NADPH oxidases, reactive oxygen species, and hypertension. Clinical implications and therapeutic possibilities. Diabetes Care, 31(Suppl 2): S170-80.

Ramirez-de-Marten, L.M., J.E. Martens Cook and E.l. Palacios-Pru, 1998. Protein energy malnutiriton and hypobaric stress effect on ultrastructure of the heart muscle in rats. Acta Cientifica Venezolana, 349: 31-37.

Robinson, M.K., R.R. Rustum, E.A. Chambers, J.D. Rounds, D.W. Wilmore and D.O. Jacobs, 1997. Starvation enhances hepatic free radical release following endotoxemia. J Surg Res., 69: 325-325.

Safar, M., and G.M. London, 1994. The arterial system in human hypertension.; Oxford' Blackwell Scientific Publishers.

Safar, M.E., P. Chamiot-Clerc, G. Dagher, and J.F. Renaud, 2001. Pulse pressure, endothelium function. and arterial stiffness in spontaneously hypertensive rats. Hypertension, 38: 1416-1421.

Skilton, M.R., N. Evans, K.A. Griffiths, J.A. Harmer and D.S. Celermajer, 2005. Aortic wall thickness in newborns with intrauterine growth restriction. Lancet., 365: 1484-148.

Sugamura, K. and J.F. Keaney Jr., 2011. Reactive oxygen species in cardiovascular disease. Free Radic Biol Med., 51(5): 978-92.

Tarry-Adkins, J.L., M.S. Martin, J.H. Chen, R.L. Cripps and S.E. Ozanne, 2008. Maternal diet influences DNA damage, arotic telomere length, oxidative stress, and antioxidant defense capacity in rats. FASEB J., 22: 2037-2044.

Tarry-Adkins, J.L., J.H. Chen, R.H. Jones, N.H. Smitha and S.E. Ozanne, 2010. Poor maternal nutrition leads to alterations in oxidative stress, antioxidant defense capacity, and markers of for development of the diabetic phenotype in later life. FASEB J, 24: 2762–2771.

Tietze, F., 1969. Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione: applications to mammalian blood and other tissues. Anal Biochem, 27: 502-22.

Touyz, R.M., 2004. Reactive oxygen species, vascular oxidative stress, and redox signaling in hypertension: what is the clinical significance? Hypertension, 44: 248-52.

Valko, V., D. Leibfritz, J .Moncol, M.T.D. Cronin, M. Mazur and J. Telser, 2007. Free radicals and antioxidants in normal physiological functions and human disease. Int. J. Biochem. Cell Biol, 39: 44-84.

Vaziri, N.D., X.Q. Wang, F. Oveisi and B. Rad, 2000. Induction of oxidative stress by glutathione depletion causes severe hypertension in normal rats. Hypertension, 36: 142-6.

Widenthal, K., 1975. Dietary control of cardiac lysosomal enzyme activities. Recent Advances in studies on cardiac structure and metabolism, 8: 519-589.

Zandi-Nejad, K., V.A. Luyckx and B.M. Brenner, 2006. Adult hypertension and kidney disease. The Role of fetal programming. Hypertension, 47(2): 502-508.

Zhang, R, L. Zheng, Z. Sun, X. Zhang, J. Li, D. Hu, Y. Sun, 2011. Decreased glomerular filtration rate is associated with mortality and cardiovascular events in patients with hypertension: A Prospective Study.PLOS one, 6(11): e27359- e27359.