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Evaluation of the Renal Function in the Intrauterine Growth Restricted Rats and the Effect of Maternal Glucocorticoids

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Abstract

The purpose of the study was to determine the effect of maternal glucocorticoids on experimental growth retarded rats and the effect of maternal undernutrition in different gestation periods for function of the kidney. This study had two sections. In the first section, 5 groups were formed. 10g/d diet was given in the 1st, 2nd, and 3rd trimester and throughout the pregnancy period. The control group was fed a normal diet. In the second section, 3 groups formed and all the rats take 10 g/d diet throughout pregnancy period. To determine the effect of endogenous glucocorticoids first group was treated with metyrapone, second group metyrapone+dexamethasone and the placebo injected saline until 14 days of pregnancy. The offsprings body and kidney weights were detected in the 0, 3rd and 20th weeks of age. Renal extraction functions and blood pressures from tail detected in the 20th week of age. Urinary excretion and glomerular filtration rate were low in rats that had dietary restriction in the last trimester. The glomerular filtration rates were found to be low in the group that had diet restriction during the whole pregnancy. Blood pressure values were found to be lower in the group that had diet restriction during their pregnancy compared to the control group. Kidney weights were similar in all groups in the first phase. It was observed that renal excretion functions were preserved in the group receiving metyrapone treatment, but there was no statistically significant difference between the results. Low blood pressures were normalized with metyrapone treatment. The kidney sizes at the 20th week of the rats which receiving metyrapone treatment were found to be smaller than those receiving physiological saline solution. Food restriction destroys renal functions but no effects with high blood pressure in adulthood. Glucocortiod exposure in pregnancy may reduce renal development.

Keywords: IUGR, Maternal glucocorticoids, renal excretion functions

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Introduction

Intrauterine growth retardation (IUGR) is a decrease in fetal growth rate due to genetic and environmental effects and the inability of the fetus to reach its genetic potential growth.[1] Maternal undernutrition during pregnancy reduces fetal and placental growth in animals and humans. It has been shown in clinical and experimental studies that maternal malnutrition may cause permanent structural changes in the baby's kidneys, which in turn increases the tendency to adult heart and kidney diseases and may lead to hypertension.[2-5] In fetuses exposed to endogenous and exogenous excessive glucocorticoids during the intrauterine period, HT and many metabolic changes can be observed in advanced ages, in addition to IUGR.[6] This study, it was aimed to evaluate the effects of maternal balanced nutrient restriction on kidney growth, renal excretion functions and blood pressure, and to evaluate the role of excessive maternal glucocorticoid exposure in the intrauterine period.

Highlights

- Intrauterine growth retardation (IUGR) may result in permanent structural changes in kidneys, which in turn increases the tendency to adult heart and kidney diseases and may lead to hypertension
- The effects of maternal balanced nutrient restriction on kidney growth, renal excretion functions, blood pressure the role of excessive maternal glucocorticoid exposure in the intrauterine period remains unclear
- Nutritional restriction during pregnancy, especially during pregnancy and in the last trimester, causes intrauterine growth retardation and deterioration in kidney functions.
- IUGR adversely affects kidney functions, but this effect is independent of the increase in endogenous glucocorticoids.
- Total restriction of nutrients during pregnancy adversely affects intrauterine growth, kidney growth, and kidney excretion functions,
- Total restriction of nutrients during pregnancy does not cause adult blood pressure elevation

Material and Method

All procedures were approved by the local Ethics Committee. All rats received care in compliance with the Principles of the guide for the Care and Use of Laboratory animals. In the study, 45 virgin female Wistar Albino rats were kept in a room with controlled temperature (22±1ºC) and with artificial dark-light cycle (12L:12D). Female rats were mated with male rats for one night. The first day of pregnancy was determined by the demonstration of spermatozoa in the vaginal smears. Pregnant female rats were randomly divided into two groups for the stages of the study and then transferred into individual cages. Maternal weight gains were evaluated during pregnancy.

First Stage

Restricted diet group rats (n=21) were randomly divided into 5 groups. The first group was determined as the control group and fed a normal chow diet. The other groups had a half-restricted diet (10 gr/day feed) at different periods of their pregnancy period. Half restricted diet was given between 0-7th days of pregnancy (first trimester) at the second group of rats. The diet was given between the 8-14th days of pregnancy (second trimester) at the third group of rats and the diet was given between 15-22th days of gregnancy (third trimester) at the fourth group of rats. Fifth group of rats was fed with half restricted diet throughout the pregnancy period. Water was always available ad libitum for all experimental groups. Pregnant mothers gave birth by vaginal delivery on an average of 22 days. Baby rats were evaluated at the 0th, 3rd, and 20th weeks. The weights of the 0-week-old puppies were recorded. After anesthesia (100 mg/g intraperitoneal ketamine) was given, the kidneys were removed and the kidney weights were recorded. Surviving baby rats were allowed to stay with their mothers until they were three weeks old and their weekly weights were recorded. After anesthesia that was given in the 3rd week, the kidneys were removed and their weights were recorded. The remaining baby rats were separated from their mothers from this period onwards and were kept alive until the 20th week by allowing normal food and water intake. In the 20th week, the blood pressures of the baby rats were measured by the tail blood pressure measurement method. One day before the renal hemodynamic evaluation, they were placed in the metabolic cage and their 24-hour urine was collected. A blood sample was taken for biochemical evaluation. After anesthesia was given, the kidneys were removed and their weights were recorded.

Second Stage

The diets of the rats (n=18) in the metyrapone group were restricted by approximately 50% during their pregnancy and were given 10 gr of feed daily. Rats were randomly divided into 3 groups. In the first group, 0,5 mg/ml dose of metyrapone was added to the drinking water and water was available ad libitum between the 1-14th days of pregnancy. In the second group, 0,2 mg/kg dexamethasone (to replace the suppressed maternal glucocorticoids) was injected intraperitoneally at the same time in the morning between the 1-14th days of pregnancy besides metyrapone treatment. In the third group, between the 1-14th days of pregnancy physiological saline solution was injected intraperitoneally. Pregnant rats gave birth by normal vaginal delivery. Postpartum mothers were given a standard laboratory diet. The body weights of some of the baby rats were measured on the first day and after they were anesthetized, their kidneys were removed and kidney weights were measured. When the remaining baby rats were three weeks old, some of the baby rats were anesthetized after their body weights were recorded, then their kidneys were removed and kidney weights were recorded. The remaining group was separated from their mothers and given a standard laboratory rat diet and tap water ad libitum. Weekly body weights were recorded. In the twentieth week, the blood pressures of the baby rats were measured by the tail blood pressure measurement method. One day before the renal hemodynamic evaluation, they were placed in the metabolic cage and 24-hour urine was collected. A blood sample was taken for biochemical evaluation. After anesthesia was given, the kidneys were removed and kidney weights were recorded.

Serum Cr and urine Cr, Na, K levels were studied in the biochemistry laboratory of our hospital using the Konelab60i autoanalyzer device and Thermo Clinical Labsystem (Finland) kits.

Kidney Weight

When the kidneys of the offspring in both the restricted diet group and the metyrapone group were removed at the 1st day, 3rd, and 20th weeks, right and left kidney weights were recorded separately. Fractional kidney weight (FKW) was obtained by dividing the right kidney weight of the offspring by body weight and multiplying by 100. It was expressed as %.

Blood Pressure Measurement

Systolic blood pressure (SBP) and diastolic blood pressure (DBP) of conscious subjects were measured by indirect tail-cuff method (MAY BPHR 9610-PC TAIL-CUFF indirect blood pressure recorder). The blood pressure values obtained were recorded on the computer. The average of the 3 blood pressure values measured from each subject was taken.

Clearance Calculations

Clearance calculations were made using the following formulas.

Urine flow rate (UF) = urine volume/time (µl/min)

Excretion of a substance = urine concentration of this substance x urine flow rate

Sodium excretion (UVNa) was expressed as µmol/min.

Potassium excretion (UVK) was expressed as µmol/min.

Creatinine clearance (ClCr) = creatinine excretion/serum Cr concentration (expressed as ml/min).

Glomerular filtration rate (GFR) was calculated as creatinine clearance (Cr excretion rate divided by serum concentration).

Statistical Analysis

Data were evaluated in Statistical Package for Social Sciences (SPSS) 15.0 and SigmaStat 3.5 Statistical Package Programs. Mean, standard deviation (SD), median, minimum (min), and maximum (max) values were given as descriptive statistics. Kolmogorov-Smirnov and Shapiro Wilk normality tests were used to determine whether the data showed normal distribution. In comparisons involving three or more groups, One-Way Analysis of Variance (One-Way ANOVA) was used for normally distributed variables, and Kruskal Wallis Analysis was used for non-normally distributed variables. Tukey test was used to compare the groups with differences in One Way Analysis of Variance and Dunn Method was used for comparison of groups with differences in Kruskal Wallis Analysis. A p-value of <0.05 was considered statistically significant in all statistical analyses.

RESULTS

Body Weights

When the body weights of the offspring of the rats exposed to food restriction during different gestational periods were compared, it was seen that the lowest body weight was in the rats that were exposed to the restricted diet during pregnancy. Although the first-day body weight was found to be low in the group that received a restricted diet in the last trimester, there was no statistically significant difference when compared with the other groups (**Table 1**). In the comparison of the 3rd-week weights of the restricted diet group rats, the lowest body weight was found in the rats exposed to the restricted diet in the last trimester (**Table 2**). In the 20th week rats, the lowest body weight was found in the group that received a restricted diet in the second trimester. Although the 20th-week body weight of the group that had diet restriction during pregnancy was low, no statistically significant decrease was found (**Table 3**).

In the metyrapone group, which was created to evaluate the effects of endogenous and exogenous steroids, when the body weights of the 1st day, 3rd week, and 20th week were compared, no statistically significant difference was found between the groups (data not shown).

Table 1
1st-day body weights of rats in the restricted diet group.

	Control		0-7th days		8-14th days		15-22nd days		during pregnancy		
	n	mean±SS	n	mean±SS	n	mean±SS	n	mean±SS	n	mean±SS	p value
BW 1st day (gr)	19	5.09±0.41	34	5.26±0.46	22	5.32±0.53	27	4.97±0.46	25	4.64±0.51[a,b,c]	<0.05

[a] p<0.005; compared to the control group, [b] p<0.005; compared to the group that received a restricted diet between the 0-7th days, [c] p<0.005; compared to the group that received a restricted diet between the 8-14th days

Table 2
Body weights of the restricted diet group rats at the 3rd week.

	Control		0-7th days		8-14th days		15-22nd days		during pregnancy		
	n	mean±SS	n	mean±SS	n	mean±SS	n	mean±SS	n	mean±SS	p value
BW 3rd week (gr)	13	30.11±3.33[a,b,c]	23	25.18±6.24[b]	15	21.11±2.02	16	19.07±2.51	14	22.13±1.84	<0.05

[a] p<0.005; compared to the group that received a restricted diet between the 8-14th days, [b] p<0.005; compared to the group that received a restricted diet between the 15-22nd days, [c] p<0.005; compared to the group that received a restricted diet during pregnancy.

Table 3
Body weights of rats in the restricted diet group at the 20th week.

	Control		0-7th days		8-14th days		15-22nd days		during pregnancy		
	n	mean±SS	n	mean±SS	n	mean±SS	n	mean±SS	n	mean±SS	p value
BW 20th week (gr)	7	225.63±59.78	13	233.76±58.23	7	170.58±32.57[a]	8	214.75±44.8	6	190.28±41.47	<0.05

[a] p<0.005; compared to the group that received a restricted diet between the 0-7th days

Kidney Weight

There was no difference between the groups in terms of kidney weights of rats at the 0th, 3rd, and 20th weeks in all groups that in the first stage. The kidney sizes of the offspring of the rats receiving metyrapone treatment at the 20th week were found to be smaller than those receiving physiological saline solution (data not shown).

Renal Excretion Functions

Urinary excretion and glomerular filtration rate (GFR) were low in rats that had a dietary restriction in the last trimester. The glomerular filtration rate was found to be low in the group that had a diet restriction during the whole pregnancy (**Table 4**). In rats with intrauterine growth retardation, renal excretion functions were preserved in those treated with metyrapone, but no statistically significant difference was found between the results (**Table 5**).

Blood Pressure Values

Blood pressure values were found to be lower in the group that had a diet restriction during their pregnancy compared to the control group (**Figure 1**). Low blood pressures were normalized with metyrapone treatment (**Figure 2**).

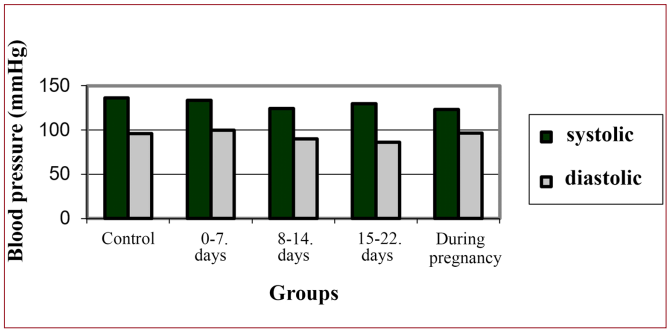

Figure 1. 20th week blood pressures of the restricted diet group rats

Discussion

Many epidemiological pieces of evidences show that the risk of hypertension and coronary heart disease in adults is not only determined by adult lifestyle and environment but also associated with fetal life. Fetal growth pattern (low birth weight) related to malnutrition during pregnancy is associated with elevated blood pressure and increased cardiovascular mortality in childhood.[7-9] Although the growth path of the fetus is primarily determined by genetic factors, it is also guided by the maternal environment.[9] Among the methods that cause fetal growth retardation, the most commonly used method is to reduce the amount of food for the mother, which can be either by reducing the total intake or by reducing the nutritional content such as protein, vitamin A, sodium or iron. In addition, IUGR is created by placental embolization, surgical reduction of placental blood flow, or the use of steroids.[10] In our study, we reduced the amount of maternal nutrition by 50% (10 g/day) to create IUGR. We applied half-nutrition restriction throughout pregnancy or at different periods of pregnancy for one-week periods in order to determine which nutritional deficiency period is more effective on the renal function. No dietary restriction was applied during pregnancy in the control group rats. With this method, the 1st-day body weights of the offsprings in the group that received a restricted diet during pregnancy were significantly lower than the other groups. The offspring of mothers which were fed

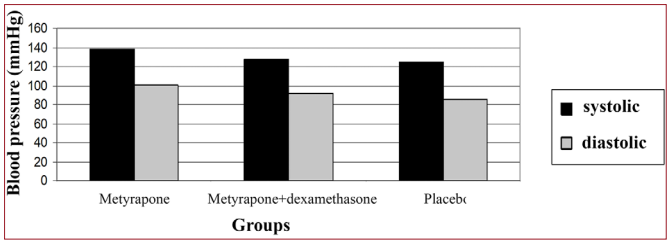

Figure 2: 20th week blood pressures of the metyrapone group rats

Table 4
Renal excretion functions of rats in the restricted diet group at the 20th week.

	Control		0-7th days		8-14th days		15-22nd days		during pregnancy		p value
	n	mean±SS	n	mean±SS	n	mean±SS	n	mean±SS	n	mean±SS	
UF (µl/min)	7	5.32±2.36	13	5.40±5.75	7	3.28±0.86	8	2.67±0.63[a,b]	6	3.03±0.94	<0.05
UVNa (µmol/min)	7	0.42±0.2	13	0.4±0.15	7	0.38±0.053	8	0.39±0.1	6	0.36±0.1	>0.05
UVK (µmol/min)	7	1.30±0.48	13	1.28±0.33	7	1.41±0.21	8	1.29±0.29	6	1.08±0.28	>0.05
GFR (ml/min)	7	1.69±1.01	13	1.16±0.43	7	1.11±0.48	8	0.76±0.30[a]	6	0.84±0.25[a]	<0.05

[a] p<0.005; compared to the control group, [b] p<0.005; compared to the group that received a restricted diet between the 0-7th days

Table 5
Renal excretion functions of 20th week rats in the metyrapone group.

	Metyrapone		Metyrapone+Dexamethasone		Plasebo		p value
	n	mean±SS	n	mean±SS	n	mean±SS	
UF (µl/min)	6	3.99±0.3	11	3.29±1.66	11	3.41±1.9	>0.05
UVNa (µmol/min	6	0.49±0.08	11	0.35±0.16	11	0.34±0.13	>0.05
UVK (µmol/min	6	1.5±0.14	11	1.26±0.45	11	1.18±0.46	>0.05
GFR (ml/min)	6	1.06±0.3	11	0.89±0.35	11	1.02±0.41	>0.05

a restricted diet in the third trimester of pregnancy also tended to have low body weight. Perez et al.[5] also found low weight of the offspring of pregnant rats, which they fed with a diet reduced by 50% in the last two trimesters of pregnancy. Langley-Evans et al.[11] reported that intrauterine growth retardation occurred in the offspring of pregnant rats who underwent protein restriction both during pregnancy and in the third trimester in the intrauterine growth retardation model they created with protein restriction during pregnancy and in 3 different trimesters of pregnancy. Our findings are consistent with the findings of these reports.

It is known that the availability of food influences the rhythm of the HPA axis. Indeed, fasting and food restriction increase HPA axis activity in both humans and rats, leading to secondary adrenal hypertrophy and an increase endogenous steroids.[12] In the metyrapone group, which was created to evaluate the effects of endogenous and exogenous steroids, 50% dietary restriction was applied throughout their pregnancy. There was no statistically significant difference between the groups in the 1st day, 3rd week, and 20th week body weights. This finding suggested that metyrapone treatment had no effect on the weight of baby rats exposed to a restricted diet during the intrauterine period. This result can be interpreted as maternal steroid synthesis, which occurs in response to dietary restriction, does not have a direct effect on the body weights of the offspring. However, Smith and Waddell[13] reported that administration of dexamethasone and carbenoxolone decreased birth weight in rats fed normally during pregnancy, while metyrapone treatment increased birth weight.

In the study by Langley-Evans et al.[11] birth kidney weights of rats exposed to protein restriction between the 8-14th, 15-22nd, and 0-22nd days were detected lower than the control group since it was not statistically significant, so they emphasized that the diet has no negative effect on kidneys. However, in the same study between the 0-7th days, the relative kidney weight of the group that received 9% casein was found to be statistically significantly higher than the control group. In our study, the 1st day kidney weights of rats exposed to an intrauterine restricted diet were lower than the control value; this decrease was more evident in those which were exposed to a restricted diet during pregnancy and in the first trimester. This decrease in kidney weight persisted when corrected for body weight. Our findings are similar to the findings of the study by Langley-Evans et al.[11]

In our study, kidney weight in the 3rd week was found to be low in rats exposed to a restricted diet in the 3rd trimester of pregnancy. However, when corrected for body weight (fractional kidney weight) was higher in these baby rats than in control baby rats. Fractional kidney weights were higher in offspring exposed to restricted diet in the 1st and 2nd trimesters of pregnancy than controls. There might be two reasons for this result. The first is the low body weight of the offspring during this period; the ratio of kidney weight to body weight might be increased. Second, the initially small kidneys might be hypertrophied as a result of a possible decrease in the number of nephrons. We think that these two reasons together contribute to this result.

Intrauterine nutritional deficiency appears to impair kidney development by several mechanisms. It is known that the renin-angiotensin system plays an important role in the normal morphological development of the kidney. Previous studies support that renal renin and angiotensin II mRNA levels are significantly lower in newborns born with intrauterine nutritional deficiency and that suppression of intrarenal RAS may affect nephrogenesis.[14]

Intrauterine nutritional deficiency causes an increase in maternal glucocorticoids and ultimately increases the exposure of the fetus to glucocorticoids. Metyrapone is a potent inhibitor of 11-β hydroxylation of corticosteroids; inhibits corticosterone synthesis from maternal and fetal adrenal glands. In the second part of our study, it was aimed to investigate the effects of maternal glucocorticoids on fetal kidney development in intrauterine nutritional deficiency. For this purpose, endogenous glucocorticoid synthesis was suppressed by giving metyrapone to pregnant rats which received 50% reduced food during pregnancy, and dexamethasone was given externally to another group which glucocorticoid synthesis was suppressed to bring out the effect of suppressed glucocorticoids again. In our study, it was found that metyrapone treatment did not prevent low birth weight in pregnant rats given a restricted diet. In contrast, 1st day absolute kidney weights were better preserved in the offspring of mothers treated with metyrapone and metyrapone+dexamethasone. However, this effect did not persist in advancing periods of life. Since we could not determine the number of nephrons, we cannot comment on the effect of metyrapone treatment on the number of nephrons.[15,16]

Decreased birth weight and kidney weight, high blood pressure, increased albuminuria, low GFR, low Na excretion, decreased fractional sodium excretion and high tissue sodium content were found in fetal programming models that were given both prenatal dexamethasone and maternal protein restriction.[17,18] Although it was not statistically significant, sodium excretion of baby rats exposed to restricted diet during pregnancy tended to be low in our study. Urinary sodium excretion increased with metyrapone therapy (not statistically significant). This finding supports that maternal glucocorticoids reduce sodium excretion.

Systolic blood pressures of puppies born with IUBG tended to be lower than control rats. No difference was found in diastolic blood pressures. In the studies conducted by Langley-Evans et al.[11,19] they found high blood pressure in the offspring of rats fed low protein. While specific nutrient (protein) restriction was applied in these studies, we applied global energy restriction. The difference in our study from these data can be explained by the difference in method. In the study of Holemans et al.[20] in which they performed food restriction, blood pressure was found to be normal. Our findings and those of Holemans K. may indicate that balanced nutrient restriction during pregnancy has less effect on offspring blood pressure than specific nutrient deficiency.

The diastolic blood pressure of the rats treated with metyrapone were higher than the control IUGR rats. This finding can be interpreted as normalization of blood pressure. A possible explanation of this finding may

be metyrapone treatment increased intrarenal renin-angiotensin system (RAS) activity. In studies investigating the interaction between glucocorticoid and intrarenal RAS, there are data that glucocorticoid excess has negative effects on the RAS components of the fetus.[1]

In our study, 24-hour urine of 20-week-old baby rats was collected in metabolic cage and creatinine clearance was calculated. It was determined that the GFR values of the offspring born to mothers which were given a half-restricted diet on the 15-22nd days of pregnancy and during pregnancy (0-22nd days) were statistically significantly lower than the control group. In the second stage of the study, when the rats were evaluated in terms of kidney functions, GFR reduction was more pronounced in the group that received exogenous glucocorticoid therapy in addition to metyrapone. There was no significant effect of metyrapone treatment on GFR. This finding supports that the negative effect of intrauterine growth retardation induced by the dietary restriction on kidney functions is through mechanisms different from the increase in endogenous glucocorticoids.

When evaluated in terms of renal excretion functions, sodium and potassium excretion of baby rats born to mothers which had dietary restrictions during pregnancy were low, although there was no statistically significant difference. It was observed that sodium and potassium excretion returned to normal with metyrapone treatment. We can say that this positive effect of metyrapone treatment on renal sodium and potassium excretion is independent of GFR. Based on this finding, we can conclude that the increase in endogenous glucocorticoids has negative effects on kidney structure as well as negative effects on sodium and potassium transport mechanisms of the kidney. We think that studies at the molecular level are needed to clarify this situation.

Conclusion

In this study, we found that total restriction of nutrients during pregnancy adversely affects intrauterine growth, kidney growth, and kidney excretion functions, but does not cause adult blood pressure elevation. We can say that some of these negative effects are due to the increase in maternal endogenous glucocorticoids that occur in intrauterine nutritional deficiency. Further studies are needed to show the effect of maternal endogenous glucocorticoid increase on kidney structure and functions.

Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

Conflict of Interest: There are no conflicts of interest in connection with this paper, and the material described is not under publication or consideration for publication elsewhere.

Ethics Committee Approval: The study was carried out with the permission of Erciyes University Faculty of Medicine Ethics Committee (Date: 2005, Decision No: 05/159)

Financial Disclosure: The authors have no conflicts of interest to declare.

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