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Araştırma / *Research Article*

The Renal Structural and Functional Changes in Rats with Intrauterine Growth Retardation and its Correlation to Serum Leptin Level

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İntrauterin Büyüme Gerilikli Ratlarda Böbrekte Yapısal/Fonksiyonel Değişiklikler ve Serum Leptin Seviyesiyle İlişkisi

ABSTRACT

Objective: *Our aim was to determine the effects of intrauterine growth retardation (IUGR) on the kidney and whether serum leptin level was associated with these changes.*
Method: *After feeding with standard diet for 7 days pregnant rats were divided into 2 groups on the 8th day. While the control group continued to be fed with normal diet during pregnancy, the study group was fed with only half of their normal needs. Structural evaluations were performed in fetuses at 21. gestational days and offsprings at 3, 6, 16 weeks of age The kidneys were investigated according to their weight, light microscopy findings, nephron counts and glomerular volume. Renal excretory function was determined as for functional evaluation. Fetuses and 3 week- old rat kidneys were investigated only structurally. Serum leptin levels were measured at 3, 6, 16 weeks of age, while direct blood pressure and renal blood flows were measured at 16. week of age.*
Results: *Renal weights were lower in the IUGR groups. In IUGR group, decreased number of nephrons were found in fetuses and 16-week-old offsprings. Urine flow rate, renal blood flow, GFR, Na, K and, Mg excretions were lower in the 16-week offspring group compared to the control group, but protein excretion was higher and blood pressure was at normal level. The IUGR rats had significantly elevated serum leptin levels at 3-week-old rats. The leptin levels in 6-week-old females were almost 60% higher than controls. For all ages there was a negative correlation between leptin levels and fractional kidney weights. Leptin level and urine volume were correlated in the 6-week-old rats. There were positive correlations between leptin and blood pressure, excretions of creatinin, Na and K in 16-week-old rats.*
Conclusion: *In conclusion, temporary increase of serum leptin concentration during weaning may not be associated with the deleterious effect of kidney structure and function at least until early adulthood in IUGR.*

Keywords: *IUGR, kidney, leptin*

ÖZ

Amaç: *Amacımız, intrauterin büyüme geriliğinin (İUBG) böbrekler üzerine etkilerini ve serum leptin düzeylerinin bu değişikliklerle ilişkili olup olmadığını belirlemektir.*
Yöntem: *Standart diyetle 7 gün besledikten sonra gebe ratlar 8.gün iki gruba ayrılmıştır. Kontrol grubu gebelik sırasında normal diyetle beslenmeye devam ederken, çalışma grubuna normal gereksinmelerinin yarısı kadar besin verilmiştir. Fetüsler 21 günlükken, yavrular ise 3., 6., 16. haftalarda değerlendirilmiştir. Böbrekler yapısal olarak ağırlıkları, ışık mikroskopisi bulguları, nefron sayısı ve glomerül hacmine göre incelenmiş ve işlevsel açıdan renal ekskretuar fonksiyon değerlendirilmiştir. Fetüsler ve 3 haftalık rat böbrekleri yalnızca yapısal açıdan incelenmiştir. Üç, 6 ve 16 haftalık ratların serum leptin düzeyleri 16 haftalıkların ise kan basınçları ve böbrek kan akımları ölçülmüştür.*
Bulgular: *İUBG gruplarının böbrekleri daha hafifti. İUBG grubundaki fetüslerde ve 16 haftalık yavrularda daha az sayıda nefron saptanmıştır. Kontrollerle karşılaştırıldığında idrar akış hızı, renal kan akımı, GFH, Na, K ve Mg klirensleri 16 haftalık yavrularda daha düşük, ancak protein klirensi daha yüksek ve kan basıncı normal düzeylerdeydi. Üç haftalık İUBG olan ratlarda serum leptin düzeyleri anlamlı derecede yükselmişti. Altı haftalık dişilerde leptin düzeyleri kontrollere göre hemen hemen %60 oranında yükselmişti. Tüm yaşlar için leptin düzeyleriyle fraksiyonel böbrek ağırlıkları arasında negatif bir korelasyon mevcuttu. Altı haftalık ratlarda leptin düzeyi ile idrar hacmi koreleydi. On altı haftalık ratlarda leptin ve kan basıncı, kreatinin, Na, K klirensleri arasında pozitif korelasyonlar mevcuttu.*
Sonuç: *İUBG'de sütten kesildikten sonra serum leptin konsantrasyonunda geçici artış en azından erken erişkin döneme kadar böbreklerde yapısal ve işlevsel bozulma ile ilişkili olmayabilir.*

Anahtar kelimeler: *İUBG, böbrek, leptin*

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INTRODUCTION

Intrauterine growth retardation leads to adverse health outcomes in childhood and adulthood. Epidemiological and experimental studies have shown that IUGR predisposes to chronic diseases such as hypertension, cardiovascular, metabolic, and renal diseases [1-3]. It has been demonstrated that reduction in food intake during pregnancy can program later-life hypertension in animal models [4-6]. A number of mechanisms have been proposed to explain how IUGR may affect offspring blood pressure, including reprogrammed hypothalamic-pituitary-adrenal axis, altered sympathetic activity and renin angiotensin aldosterone system [7,8]. IUGR could affect renal development. It has been documented that human infants with IUGR have reduced number of nephrons and increased glomerular volume [9,10]. New nephrons are not produced after birth. Consequently, reduced renal reserve at birth leads to an increased risk for the development progressive renal disease and hypertension [11,12].

Leptin is a hormone produced mainly by white adipose tissue, and acts to regulate body weight. In children, as in adults, serum leptin concentrations positively correlate with body weight and amount of body fat mass [13]. In addition, leptin levels in human umbilical cord blood positively correlate with birth weight and adiposity at delivery. Because of this, SGA newborns have reduced serum leptin concentrations. On the contrary, it has been demonstrated that infants born with IUGR have high leptin levels during catch-up growth [14]. A long-term effect of high leptin level in IUGR in early life on health problem in adulthood is not known. The purpose of the study was to determine the effects of experimental intrauterine growth retardation on the structure and function of the kidney and whether serum leptin level was associated with these changes.

MATERIALS and METHODS

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 .

Animals and study design

Wistar rats were kept in a room with controlled temperature (22±1˚C) and with artificial dark-light cycle (12L: 12D). Virgin female Wistar rats (n=18) were mated with male rats for one night. The next day was considered to be day 1 of pregnancy if spermatozoa were found in the vaginal smears. Pregnant females were then transferred into individual cages with free access to food until day 8 of pregnancy. Female rats were randomly assigned to one of two diets at the day 8 of pregnancy. One group (n=10) was fed a normal chow diet. Their offspring constituted the control group. The second group (n=8) received 50% of the needs from day 8 of pregnancy until parturition. Their offspring composed the IUGR group. Water was always available ad libitum for all experimental groups. Maternal weight gains were evaluated during pregnancy.

For studies some offsprings from control and food restricted mothers (one from each group) were delivered by cesarean section at day 21 of gestation. Other offsprings were spontaneously delivered through the vagina. After giving birth, all dams were given free access to food. Weights of pups were recorded at birth. The offsprings were weaned at 3 week of age and separated into male and female subgroups. All the offsprings received a regular diet. At age of 6 and 16 weeks the rats were placed in metabolic cages for 24 hours for the purpose of measuring their food and water consumption, and urine output 1 day before their sacrification. Urine samples were stored at -20˚C until assayed.

Anesthesia

The 3 week old rats were anesthetized with ketamine (100 mg/kg, ip) and the 6 and 16 week old rats with a mixture (0.4 ml/kg, ip) of fentanyl (5 ml) and midazolom (2 ml).

Measurement of blood pressure and renal blood flow

Blood pressure and renal blood flow (RBF) were measured at 16 weeks of age.

The left femoral artery of the 16 weeks old rats was cannulated with a PE-50 polyethylene tubing, and left renal artery and vein were visualized by dissection of the surrounding tissues after anesthesia. Systemic blood pressures and RBF were recorded after 1 hour after anesthesia. Systolic blood pressure (SBP), diastolic blood pressure (DBP) were recorded with a polygraphic system (MP 100, BIOPAC Systems) connected to a pressure transducer. Mean arterial blood pressure (MABP) was calculated from SBP and DBP. RBF was recorded with a laser Doppler Flow module (ISO-140, BIOPAC Systems), indirectly [15]. Following the record of RBF, kidneys were removed.

Kidney weight

Kidney weights were measured in fetuses at day 21 of gestation and 3, 6, 16 week-pups. Wet and dry weights of kidneys and the fractional weights (adjusted to total body weight) were determined. The left kidney was weighed and dried to constant weight, and dry weight was determined.

Histological examination

The right kidneys were fixed in formalin and embedded in paraffin. Five-micrometer-thick sections were stained with hematoxylin and eosin. Histological sections were evaluated as for number of nephrons, glomerular volume and development of glomerulosclerosis.

Measurement of glomerular volume: Measurements of glomerular size were performed in all groups except for fetuses. In each kidney specimen, 50 glomeruli were selected randomly in serial sections. The diameters were calculated as the means of the longest and shortest diameters. The glomerular volume was determined from the mean glomerular diameter (d[G]), using the formula $4\pi(d[G]/2)3/3$, and expressed as $10^4 x \mu m^3$ [16].

Determination of the number of nephrons: Nephron number is expressed as nephron numbers of 3 cortex fields at 100x magnification in the light microscopy.

Blood samples

Blood samples were taken from the vena cava inferior of the 3 and 6-week-old rats and from the femoral artery of the 16-week old rats.

Blood samples were placed in serum separator tubes, centrifuged to obtain serum, which was stored at -70˚C until analyzed. Serum samples were used to measure leptin in the 3-week-old rats and leptin, creatinine (Cr), sodium (Na), potassium (K), calcium (Ca), phosphorus (P) and magnesium (Mg) in the 6 and 16-week old rats. Serum concentrations of Cr, Na, K, Ca, P and Mg were measured using an automated analytical system (Konelab60i, Intelligent Diagnostic Systems, Espoo, Finland). Urine Cr, Na, K, Ca, P, Mg and protein levels were measured by the same analytical system. Serum leptin levels were measured using a commercial kit (mouse Leptin TiterZyme EIA kit).

Renal clearance studies

It was performed in 6 and 16 weeks old rats.

Urine flow rate (UF) was calculated as the volume of collected urine divided by the duration of the collection. The excretion rate of a substance was the urine concentration multiplied by UF. Glomerular filtration rate (GFR) was calculated as creatinine clearance (Cr excretion rate divided by serum concentration).

Statistical analysis

Statistical analyses were carried out using the program SPSS 11.5 for Windows (SPSS Inc., Chicago, IL, USA). Results were expressed as the mean±SD or as median values and ranges for continuous variables. Differences between the control and the experimental groups were compared using the Student's T Test or Mann-Whitney U test. The Spearman correlation was used for testing correlations between leptin levels and other variables. P-values < 0.05 were considered significant.

RESULTS

Maternal weight gain during pregnancy

Maternal weight gain of food restricted mothers was significantly lower than that of control mothers from 8. gestational day until parturition (Fig. 1).

Body and kidney weights

Birth weights were significantly lower in the offspring of food restricted mothers (IUGR: 4,15±0,42 g, vs. control: 5,09±0,53 g, P=0,0001, n=40 IUGR litters and 54 control litters).

In the fetuses and in 16 weeks offsprings, body weight was significantly lower in the IUGR group relative to the control group. The body weights of 3 week old offsprings did not differ between IUGR and control rats. Body weights of 6 week old male rats with IUGR were lower than the age matched controls. Body weights of 6 week old female rats with IUGR was not different from those of age-matched controls (Table 1).

Weights of kidney tended to be lower in the IUGR groups than in controls at all ages studied (Table 1).

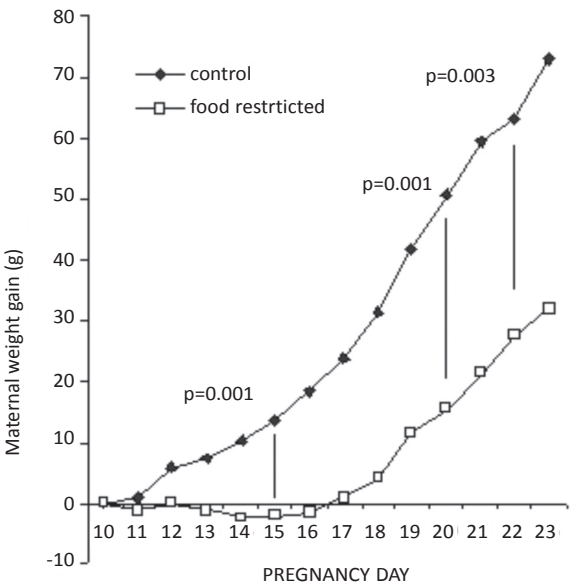

Figure 1. The food restricted rats gained significantly less weight than the control rats during pregnancy.

Nephron number, glomerular volume and glomerulosclerosis

Number of nephrons significantly decreased in fetuses, 3 week old males and 16 week old female offsprings of food restricted mothers than in controls. Glomerular volume was not significantly different between IUGR and control groups. However, glomerular volume was elevated in 6 week offsprings with IUGR compared with the controls, without any statistically significant difference (Table 1). In all groups, glomerulosclerosis was not observed.

Serum leptin level

Serum leptin concentrations significantly increased in 3 week old offsprings with IUGR compared with the level in control rats (Fig. 2). The leptin levels in 6 week old IUGR females were higher than controls (Table 2).

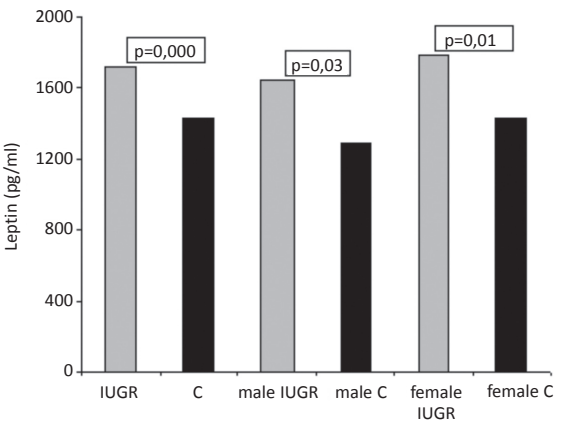

Figure 2. Serum leptin levels in IUGR and controls group at 3 week.

Serum electrolyte and renal clearance studies

There were no significant differences in serum concentrations of Cr, Na, K, Ca, P, and Mg between IUGR and control groups (data not shown).

Urine output did not differ between IUGR and control groups (data not shown). Urine flow rate, excretions of Na, K and, Mg were lower in the 16-week IUGR group (n=13) compared to controls (n=12) (5,65±2,84 vs. 8,05±1,88, P=0,022; 0,53 (0,37-1,05) vs. 0,73 (0,53-1,07), p=0,019; 0,99±0,34 vs 1,27±0,25, p=0,031; 0,031 (0,010-0,073) vs. 0,054 (0,014-0,070), p=0,034, respectively). and UVP were significantly lower in the 6 week

Tablo 1. Body and kidney weights, nephron number and glomerular volume.

	Body weight (g)	Wet kidney wt (mg)	Dry kidney wt (mg)	Wet kidney wt (%)	Dry kidney wt (%)	Nephron number	Glomerular volume (10^4x µm^3)
Fetuses							
Control (n=11)	2.83 (2,47-3,38)	12.2 (10,7-16,7)	1,10 (0,8-2,2)	0,41 (0,38-0,59)	0,041 (0,027-0,078)	22 (14-33)	-
IUGR (n=10)	2.58 (2,25-2,97) **	10.2 (5,3-12,2) **	0,95 (0,3-1,2)	0,36 (0,24-0,45)	0,035 (0,013-0,044)	10 (6-17) **	-
Week 3							
Male Control (n=5)	28,88 (24,58-30,06)	160,7 (127,7-172,2)	33,5 (27,5-37,3)	0,57 (0,52-0,57)	0,122 (0,112-0,127)	119,0 (65-122)	4,58 (3,58-5,55)
Male IUGR (n=12)	27,06 (22,55-31,71)	133,0 (115,6-172,1)	29,1 (25,7-37,9)	0,51 (0,49-0,56)	0,114 (0,106-0,123) **	77,5 (57-98) *	4,86 (3,13-6,21)
Female Control (n=16)	27,15 (19,19-31,68)	155,0 (110,4-194,4)	34,7 (23,8-46,2)	0,58 (0,52-0,63)	0,128 (0,114-0,152)	78 (54-140)	4,45 (3,29-5,88)
Female IUGR (n=6)	24,07 (22,75-29,01)	128,7 (119,3-162,9) *	27,3 (26,1-34,7) *	0,54 (0,52-0,56) **	0,117 (0,111-0,129) **	75 (50-110)	4,58 (3,38-4,91)
Week 6							
Male Control (n=5)	129,2 (125,1-137,6)	603 (590-631)	146.(139-152)	0,47 (0,44-0,50)	0,112 (0,105-0,121)	62,7 (37-91)	12,92 (10,59-27,68)
Male IUGR (n=5)	112,8 (83,0-116,2) **	541 (388-604) *	135 (91-137) **	0,49 (0,46-0,52)	0,118 (0,110-0,121)	60,0 (35-77)	21,80 (14,27-71,57)
Female Control (n=9)	104,7 (94,4-115,7)	516 (484-650)	127 (112-142)	0,50 (0,44-0,50)	0,115 (0,107-0,137)	48 (36-90)	23,68 (11,00-34,33)
Female IUGR (n=7)	96,9 (87,2-128,3)	474 (413-561)	121 (102-130)	0,47 (0,42-0,53)	0,117 (0,101-0,127)	56 (52-63)	27,88 (15,84-53,38)
Week 16							
Male Control (n=6)	298,5 (284-327)	1035 (995-1131)	255 (241-285)	0,35 (0,33-0,37)	0,084 (0,082-0,094)	42,5 (28-58)	43,61 (29,28-54,03)
Male IUGR (n=6)	275,5 (252-286) **	969 (843-1016) *	242 (211-268)	0,34 (0,33-0,38)	0,088 (0,084-0,096)	34,5 (22-40)	40,55 (28,35-53,33)
Female Control (n=6)	196 (164-212)	732 (550-792)	168.5 (131-194)	0,37 (0,34-0,39)	0,084 (0,080-0,095)	56,5 (50-63)	35,64 (21,71-55,15)
Female IUGR (n=6)	175 (157-186) *	641 (567-840)	151.5 (139-179)	0.36 (0.33-0.50)	0,090 (0,080-0,107)	47 (21-53) *	33,83 (28,46-60,51)

*Values are median (range). Statistical comparisons between C and IUGR. *p<0.05, **p<0.01 vs sex-matched controls.*

Table 2. Serum leptin levels and renal excretory functions at 6 weeks.

	MALE		FEMALE	
	C	IUGR	C	IUGR
Leptin (pg/ml)	1040 (256-1528)	1024 (784-1520)	648 (200-1552)	1040 (400-1440)
UF rate (µl/min)	5,90 (3,96-7,85)	3,96 (2,36-5,56)	4,93 (1,81-6,6)	5,69 (2,92-9,31)
GFR (ml/min)	0,71 (0,51-0,80)	0,48 (0,30-0,76)	0,35 (0,24-0,68)	0,48 (0,26-0,58)
UVNa (µmol/min)	0,64 (0,54-0,73)	0,54 (0,29-0,70)	0,38 (0,28-0,68)	0,35 (0,25-0,61)
UVK (µmol/min)	0,73 (0,54-0,73)	0,52 (0,32-0,73) *	0,67 (0,19-0,83)	0,52 (0,42-0,94)
UVPr (µg/min)	2,59 (1,42-2,89)	1,61 (0,57-2,59)	1,38 (0,70-2,54)	1,64 (0,89-3,54)
UVCa (µg/min)	0,80 (0,41-1,86)	0,99 (0,54-2,03)	0,65 (0,25-0,94)	0,93 (0,51-1,54)
UVP (µg/min)	2,16 (1,18-4,59)	1,38 (0,34-1,88) *	1,24 (0,38-2,15)	0,77 (0,26-3,45)
UVMg (µmol/min)	0,005 (0,003-0,033)	0,017 (0,001-0,041)	0,004 (0,001-0,033)	0,007 (0,001-0,027)

*Values are given as median (range). Statistical comparisons between C and IUGR. *p<0,05 vs sex-matched controls. (n= 3-9 per group). C= control*

Table 3. Serum leptin levels and renal excretory functions at 16 weeks.

	MALE		FEMALE	
	C (n=6)	IUGR (n=6)	C (n=6)	IUGR (n=6)
Leptin (pg/ml)	2280 (1048-3760)	2208 (1400-2680)	908 (800-1120)	928 (560-1920)
UF rate (µl/min)	9,03 (6,60-12,15)	6,36 (3,40-10,62)	6.77 (5,83-8,68)	4,16 (1,32-7,71)
GFR (ml/min)	1,03 (0,63-1,28)	0,87 (0,30-0,89)	0.69 (0,52-0,84) *	0,51 (0,40-0,57)
UVNa (µmol/min)	0,90 (0,71-1,07)	0,70 (0,48-1,05)	0.61 (0,53-0,82) *	0,45 (0,37-0,55)
UVK (µmol/min)	1,47 (1,17-1,74)	1,27 (0,72-1,51)	1.12 (0,87-1,28)	0,93 (0,44-1,20)
UVPr (µg/min)	10,26 (7,82-12,91)	9,23 (7,40-15,10)	8.77 (0,22-26,51)	20,27 (6,60-36,84)
UVCa (µg/min)	1,00 (0,70-1,41)	0,80 (0,22-1,46)	1.06 (0,19-2,20)	0,87 (0,21-1,63)
UVP (µg/min)	2,45 (0,84-3,91)	1,66 (1,33-3,96)	2.04 (0,20-2,83)	2,26 (1,24-3,86)
UVMg (µmol/min)	0,059 (0,049-0,070)	0,037 (0,010-0,073)	0.053 (0,014-0,061)	0,027 (0,011-0,056)

*Values are given as median (range). Statistical comparisons between C and IUGR. *p<0,05 vs sex-matched controls.*

Table 4. Arterial blood pressures and renal blood flow of control and IUGR offspring.

	C	IUGR	p
SBP (mmHg)	119,4±14,2 (12)	114,2±12,5 (12)	0,35
DBP (mmHg)	88,3±13,5 (12)	81,0±10,6 (12)	0,15
MABP (mmHg)	103,9±13,4 (12)	97,9±11,2 (12)	0,25
RBF (ppm)	348,8±48,5 (11)	281,4±48,7 (11)	0,004

Values are given as mean±SD. The number of animals given in parentheses

Table 5. Correlation of serum leptin levels and fractional kidney weight.

	n	r	P
Week 3			
Wet kidney wt	36	-0,320	0,057
Week 6			
Wet kidney wt	26	-0,652	0,0001
Dry kidney wt	26	-0,486	0,014
Week 16			
Wet kidney wt	23	-0,538	0,008

r: correlation coefficient

old IUGR males compared with the control males (Table 2). In 16 week old IUGR female offsprings GFR and Na excretion were significantly less compared with the control rats. Protein excretion was higher in the 16 week female group than control group (Table 3).

Food and water intake

Food intake was significantly less in female IUGR than control rats (median 9,97 vs. 12,63 g/day; p=0,021 at 6 weeks and median 12,3 vs. 14,9 g/day; p=0,021 at 16 weeks) whereas it was similar in males. Water intake was significantly less in the 6-week-old IUGR males versus controls (median 20 vs. 33 ml/day; p=0.008) and in the 16-week-old IUGR females versus controls (median 19,5 vs. 27,0 ml/day; p=0.036).

Blood pressure and Renal blood flow

No differences in SBP, DBP, and MABP were observed in IUGR rats compared with controls. RBF was significantly decreased in the IUGR group versus controls (Table 4). Sex-related differences in RBF were observed. RBF was significantly lower in the IUGR females versus controls (median 252.7 vs. 331.6 ppm, p= 0.028). In contrast, RBF in the IUGR males was similar to thre controls (median 313.4 vs. 338.5 ppm, p= 0.14).

Table 6. Correlation of serum leptin levels and arterial blood pressures.

	n	r	P
SBP	22	0,448	0,037
DBP	22	0,558	0,007
MABP	22	0,513	0,015

r: correlation coefficient

Relationships between serum leptin levels and other variables

For all ages there was a negative correlation between with leptin levels and fractional kidney weights (Table 5). Urine output (r= 0,561, p= 0,003), and UF (r= 0,550, p= 0,004) were positively correlated with the serum leptin concentration at 6 weeks of age. Urine output (r= 0,472, p= 0,026), UF (r= 0,472, p= 0,026), UVCr (r= 0,571, p= 0,005), UVNa (r= 0,466, p= 0,029), and (r= 0,484, p= 0,023) were positively correlated with the serum leptin concentration at 16 weeks of age. No significant relationship was found between UVPr and leptin. There was a positive correlation between BP and serum leptin concentration (Table 6). No correlation was found between serum leptin concentrations and RBF rates.

DISCUSSION

Low birth weight due to intrauterine growth restriction (IUGR) leads to various diseases in adult life (17). In this study, we have investigated the effects of IUGR on kidney.

In the adverse intrauterine environment, development and growth of the brain and the heart are preserved at the expense of the kidney and other organs and general somatic growth (18). In the food-restricted groups, renal weight was lower relative to the control groups for overall ages in our study.

We have observed that the number of nephrons in the IUGR groups tends to catch up the controls at week 3, although renal weight and number of nephrons in the IUGR group were significantly lower than the control group in fetuses. The reason for this

may be that the rat nephrogenesis extends some days after birth. However, the number of nephrons in male food-restricted group was still low at week 3. Number of nephons significantly decreased in IUGR group than in control in adulthood while they did not differ between IUGR and control rats at week 6. This data may be an indicator of the maintaining kidney damage in the later life. Hence, renal size and absolute or relative number of nephrons were significantly lower, even though catch-up growth was achieved with postnatal normal nutrition; this exactly reflects impaired development of kidneys [19].

After a precocious postnatal period, progressive age-related changes occur in the glomerular compartment, characterized by increased glomerular volume and a progressive reduction in the number of glomeruli [4]. In several intrauterine undernutrition models with reduced number of nephrons, glomeruli hypertrophy has been interpreted as a manifestation of compensatory mechanism [4,20,21]. In the study groups, glomerular volume tended to be elevated only at 6 weeks of age. This tendency was clear particularly in male offsprings. Our findings are consistent with the findings of Lucas et al. [19].

In this study, we could not demonstrate glomerulosclerosis. We think that glomerulosclerosis could be seen if a long- term study were performed. Because Regina et al. [22] reported that in histological evaluation of kidneys of rats which underwent intrauterine food restriction by 50%, 18-month-old rats presented with glomerulosclerosis, unlike 3-month-old rats, and age-related structural changes in kidney may aggravate in rats which subjected to intrauterine food restriction.

No significant differences were observed between IUGR and controls rats in terms of blood pressure. Many previous studies have shown that blood pressure is high in IUGR -induced rats [4,20,23-28]. In these studies blood pressure was determined in conscious rats using an indirect method. It has been also noted that blood pressure is elevated in conscious and anesthetized rats as determined using a direct method [6,29]. However, Holemans et al. [30] could not detect significant differences in blood pressures which were measured in conscious rats using direct method between control offsprings and offsprings subjected to prenatal food restriction. The authors have interpreted that balanced food restriction during pregnancy has a lesser effect on blood pressure in the offspring than the restriction of specific nutrients. Discrepancy between our findings about blood pressure and other various published literature may have resulted from our measurements performed in anesthetized and unconscious rats. It was demonstrated that juvenile lambs which were exposed to undernutrition in early and mid gestation exhibited lower resting blood pressure but an increased sensitivity to cortisol and stressor challenges [31]. Another explanation for this lower blood pressure also may be that sufficiently longer time was not allowed for the development of hypertension. However, it seems that this disparity is not an overt explanation apart from different measurement methods. Since renal blood flow and GFR were lower compared to controls among 16-week-old rats, it is presumed that serious renal damage has started in young adults. Furthermore, number of nephrons significantly decreased, and protein excretion was higher in addition to decreased GFR and RBF in the 16-week-old IUGR rats.

Some differences in biochemical measurements of body composition and urine findings which reflect renal status were observed in food restricted groups compared with controls. Urine flow rate, excretions of Na, K and, Mg were found to be lower in the 16-week food restricted group compared to controls, except for serum biochemical test results. Protein excretion was found to higher in this group, without any significant intergroup differences. These findings indicate that progressive deterioration exists with aging in the kidney of the rats subjected to intrauterine food restriction. Battista et al. [25] found lower sodium excretions in 12-week-old offsprings which were subjected to intrauterine low-sodium diet. The authors emphasized that urinary sodium is lower in

females compared with males and this difference was attributed to less food intake in females. This explanation may be true. Similarly, in our study also sodium excretion and food intake were lower in females. Decreased sodium excretion is probably due to the efforts to regulate serum sodium level. Lower potassium excretion was also found in our study group. This difference was attributed to lesser food intake in IUGR rats. In our study, lower Mg excretion was observed in 16-week-old IUGR group. Diminished Mg excretion may be the result of lesser food consumption by offspring with IUGR.

Painter R.C. et al. [32] describes that individuals who were subject to undernutrition in mid-gestation had higher rates of microalbuminuria at age 50 compared to control individuals. Other studies also found that there was a close correlation between low-birthweight and albuminuria level. The experimental studies also reported that proteinuria was higher in the rats exposed to intrauterine food restriction [4,20,22]. In our study protein excretion was higher in 16 week -old offsprings with IUGR relative to controls without any statistically significant differences. This condition was associated with small sampling size and very large distribution range of protein excretion. It was observed that tendency to proteinuria was higher in female rats. Some studies have reported that effect of birth weight was more pronounced in females rather than males [33-35].

A major goal of the present study was to investigate whether the leptin contribute to IUGR-related alterations in kidneys. Our findings which are in agreement with the literature indicate that serum leptin levels augment in rats with IUGR with normal postnatal nutrition in infancy which is catch-up growth period. Therefore, increased serum leptin levels in the weaning may be a programming factor for the endocrine changes which were observed in adulthood [36]. Moreover, there was a positive correlation between body weight and serum leptin concentration at 6 and 16 weeks old offsprings. This condition suggests that these rats possess a relative resistance against leptin [37]. Thus the increasing amount of data have indicated that individuals who were born with IUGR had an augmented risk of obesity [36].

For all ages, there was a negative correlation between leptin levels and fractional kidney weight. It means that the less the weight of the kidney is in proportion to body weight, the higher are the serum leptin levels, or vice versa. We were not able to find any comparative studies in the literature. These findings can be interpreted in that the relationship between the leptin levels and the fractional kidney weight do not have anything to do with the kidney but results from the relationship between leptin levels and the body weight. These higher leptin plasma levels could be related to the changes of the body weight detected in IUGR.

We determined that there was a positively significant correlation between blood pressures and serum leptin concentrations, which was in concordance with the literature.

Intravenous, intraperitoneal or local administration of leptin into the renal artery causes natriuresis and diuresis without affecting blood pressure, GFR and potassium excretion, suggesting that the natriuretic effect of leptin results from inhibition of tubular sodium reabsorption [40]. Gunduz et al. [15] found that long-term leptin infusion increased creatinine excretion in addition to increased sodium excretion. In the present study, we have demonstrated that serum leptin level and urine volume were correlated in the 6-week-old rat group while there were positive correlations between leptin and blood pressure; excretions of creatinine, Na and, K at 16-week-old rats. Our findings are in line with the literature data, except for potassium excretion. Quantitatively sodium excretion was lower in rats with IUGR compared to controls, despite rising effect of leptin on the sodium excretion. This may be because of diminished GFR in this group.

The present study supports the assertion that the leptin hormone which is an important factor in the

protection of body weight balance assumes a physiological role in the protection of balance between body fluid and sodium. However, it is not clear whether hyperleptinemia is a cause or outcome in these children and rat pups with IUGR during catch-up growth period. Hence, there is a need for future studies to elucidate the effect of rising endogen leptin on the renal function.

In conclusion, considerable morphofunctional changes have appeared in the kidney of rat pups that developed IUGR by intrauterine food restriction. Temporary increase in serum leptin concentrations during weaning may not be associated with the deleterious structural, and functional effects of IUGR on kidney at least until early adulthood. Further studies are necessary to determine precisely the role of increased endogenous leptin in the renal function and hemodynamic changes in catch-up growth period in those with IUGR.

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REFERENCES

1. Lau C, Rogers JM. Embryonic and fetal programming of physiological disorders in adulthood. Birth Defects Res C Embryo Today 2004;72:300-12. https://doi.org/10.1002/bdrc.20029
2. Fowden AL, Forhead AJ. Endocrine mechanisms of intrauterine programming. Reproduction 2004;127: 515-26. https://doi.org/10.1530/rep.1.00033
3. Wu G, Bazer FW, Cudd TA, Meininger CJ, Spencer TE. Maternal nutrition and fetal development. J Nutr. 2004;134:2169-72. https://doi.org/10.1093/jn/134.9.2169
4. Almeida JR, Mandarim-de-Lacerda CA. Maternal gestational protein-calorie restriction decreases the number of glomeruli and causes glomerular hypertrophy in adult hypertensive rats. Am J Obstet Gynecol. 2005;192:945-51. https://doi.org/10.1016/j.ajog.2004.09.010
5. Gilbert JS, Lang AL, Grant AR. Maternal nutrient restriction in sheep: hypertension and decreased nephron number in offspring at 9 months of age. J Physiol. 2005;565:137-47. https://doi.org/10.1113/jphysiol.2005.084202
6. Perez H, Ruiz S, Soto-Moyano R. Prenatal malnutrition-induced hypertension in young rats is prevented by neonatal capsaicin treatment. Neurosci Lett 2002;328:253-6. https://doi.org/10.1016/S0304-3940(02)00526-8
7. Vehaskari VM, Woods LL. Prenatal programming of hypertension: lessons from experimental models. J Am Soc Nephrol. 2005;16:2545-56. https://doi.org/10.1681/ASN.2005030300
8. Ashton N. Perinatal development and adult blood pressure. Braz J Med Biol Res. 2000;33:731-40. https://doi.org/10.1590/S0100-879X2000000700002
9. Hinchliffe SA, Lynch MR, Sargent PH, Howard CV, Van Velzen D. The effect of intrauterine growth retardation on the development of renal nephrons. Br J Obstet Gynaecol. 1992;99:296-301. https://doi.org/10.1111/j.1471-0528.1992.tb13726.x
10. Ma-alich R, Reyes L, Herrera M, Melendi C, Fundora I. Relationship between weight at birth and the number and size of renal glomeruli in humans: a histomorphometric study. Kidney Int. 2000;58:770-3. https://doi.org/10.1046/j.1523-1755.2000.00225.x
11. Marchand MC, Langley-Evans SC. Intrauterine programming of nephron number: the fetal flaw revisited. Nephrol. 2001;14:327-31.
12. McMillen IC, Robinson JS. Developmental origins of the metabolic syndrome: prediction, plasticity, and programming. Physiol Rev. 2005;85:571-633. https://doi.org/10.1152/physrev.00053.2003
13. Jaquet D, Leger J, Levy-Marchal C, Oury JF, Czernichow P. Ontogeny of leptin in human fetuses and newborns: effect of intrauterine growth retardation on serum leptin concentrations. J Clin Endocrinol Metab. 1998;83:1243-6. https://doi.org/10.1210/jcem.83.4.4731
14. Jaquet D, Leger J, Tabone MD, Czernichow P, Levy-Marchal C. High serum leptin concentrations during catch-up growth of children born with intrauterine growth retardation. J Clin Endocrinol Metab. 1999;84:1949-53. https://doi.org/10.1210/jc.84.6.1949
15. Gunduz Z, Dursun N, Akgun H, Ozturk F, Okur H, Koc N. Renal effects of long-term leptin infusion and preventive role of losartan treatment in rats. Regul Pept. 2005;132:59-66.

https://doi.org/10.1016/j.regpep.2005.09.017
16. Gunduz Z, Canoz O, Per H, Dusunsel R, Poyrazoglu MH, Tez C, Saraymen R. The effects of pentoxifylline on diabetic renal changes in streptozotocin-induced diabetes mellitus. Ren Fail 2004;26:597-605. https://doi.org/10.1081/JDI-200038329
17. Schreuder M, Waal HDV, Wijk AV. Consequences of intrauterine growth restriction for the kidney. Kidney Blood Pres Res. 2006;29:108-25. https://doi.org/10.1159/000094538
18. Hoy WE, Hughson MD, Bertram JF, Douglas-Denton R, Amann K. Nephron number, hypertension, renal disease, and renal failure. J Am Soc Nephrol. 2005;16:2557-64. https://doi.org/10.1681/ASN.2005020172
19. Lucas SR, Costa Silva VL, Miraglia SM, Zaladek Gil F. Functional and morphometric evaluation of offspring kidney after intrauterine undernutrition. Pediatr Nephrol. 1997;11:719-23. https://doi.org/10.1007/s004670050374
20. Gil FZ, Lucas SR, Gomes GN, Cavanal Mde F, Coimbra TM. Effects of intrauterine food restriction and long-term dietary supplementation with L-arginine on age-related changes in renal function and structure of rats. Pediatr Res. 2005;57:724-31. https://doi.org/10.1203/01.PDR.0000159514.06939.7E
21. do Carmo Pinho Franco M, Nigro D, Fortes ZB, Tostes RC, Carvalho MH, Lucas SR, Gomes GN, Coimbra TM, Gil FZ. Intrauterine undernutrition-renal and vascular origin of hypertension. Cardiovasc Res. 2003;60:228-34. https://doi.org/10.1016/S0008-6363(03)00541-8
22. Regina S, Lucas R, Miraglia SM, Zaladek Gil F, Machado Coimbra T. Intrauterine food restriction as a determinant of nephrosclerosis. Am J Kidney Dis. 2001;37:467-76. https://doi.org/10.1053/ajkd.2001.22088
23. Kwong WY, Wild AE, Roberts P, Willis AC, Fleming TP. Maternal undernutrition during the preimplantation period of rat development causes blastocyst abnormalities and programming of postnatal hypertension. Development 2000;127:4195-202.
24. Langley-Evans SC, Welham SJ, Jackson AA. Fetal exposure to a maternal low protein diet impairs nephrogenesis and promotes hypertension in the rat. Life Sci. 1999;64:965-74 https://doi.org/10.1016/S0024-3205(99)00022-3
25. Battista MC, Oligny LL, St-Louis J, Brochu M. Intrauterine growth restriction in rats is associated with hypertension and renal dysfunction in adulthood. Am J Physiol Endocrinol Metab. 2002;283:124-31. https://doi.org/10.1152/ajpendo.00004.2001
26. Brawley L, Itoh S, Torrens C, Barker A, Bertram C, Poston L, Hanson M. Dietary protein restriction in pregnancy induces hypertension and vascular defects in rat male offspring. Pediatr Res. 2003;54:83-90. https://doi.org/10.1203/01.PDR.0000065731.00639.02
27. Nwagwu MO, Cook A, Langley-Evans SC. Evidence of progressive deterioration of renal function in rats exposed to a maternal low-protein diet in utero. Br J Nutr. 2000;83:79-85.
28. Franco Mdo C, Arruda RM, Dantas AP, Kawamoto EM, Fortes ZB, Scavone C, Carvalho MH, Tostes RC, Nigro D. Intrauterine undernutrition: expression and activity of the endothelial nitric oxide synthase in male and female adult offspring. Cardiovasc Res. 2002;56:145-53. https://doi.org/10.1016/S0008-6363(02)00508-4
29. Sahajpal V, Ashton N. Renal function and angiotensin AT1 receptor expression in young rats following intrauterine exposure to a maternal low-protein diet. Clin Sci. 2003;104:607-14. https://doi.org/10.1042/CS20020355
30. Holemans K, Gerber R, Meurrens K, De Clerck F, Poston L, Van Assche FA. Maternal food restriction in the second half of pregnancy affects vascular function but not blood pressure of rat female offspring. Br J Nutr. 1999;81:73-9.
31. Symonds ME, Gopalakrishnan G, Bispham J, Pearce S, Dandrea J, Mostyn A, et al. Maternal nutrient restriction during placental growth, programming of fetal adiposity and juvenile blood pressure control. Arch Physiol Biochem. 2003;111:45-52. https://doi.org/10.1076/apab.111.1.45.15141
32. Painter RC, Roseboom TJ, van Montfrans GA, Bossuyt PM, Krediet RT, Osmond C, et al. Microalbuminuria in adults after prenatal exposure to the Dutch famine. J Am Soc Nephrol. 2005;16:189-94. https://doi.org/10.1681/ASN.2004060474
33. Jacobsen P, Rossing P, Tarnow L, Hovind P, Parving HH. Birth weight- a risk factor for progression in diabetic nephropathy? J Intern Med. 2003;253:343-50. https://doi.org/10.1046/j.1365-2796.2003.01109.x
34. Hoy WE, Rees M, Kile E, Mathews JD, McCredie DA, Pugsley DJ, Wang Z. Low birthweight and renal disease in Australian aborigines. Lancet 1998;352:1826-7. https://doi.org/10.1016/S0140-6736(05)79888-3
35. Zidar N, Cavić MA, Kenda RB, Koselj M, Ferluga D. Effect of intrauterine growth retardation on the clinical course and prognosis of IgA glomerulonephritis in children. Nephron 1998;79:28-32. https://doi.org/10.1159/000044987
36. Passos MC, da Fonte Ramos C, Potente Dutra SC, Gaspar de Moura E. Transfer of iodine through the milk in protein-restricted lactating rats. J Nutr Biochem. 2001;12:300-3. https://doi.org/10.1016/S0955-2863(01)00142-5
37. Desai M, Gayle D, Babu J, Ross MG. Programmed obesity in intrauterine growth-restricted newborns: modulation by newborn nutrition. Am J Physiol Regul Integr Comp Physiol. 2005;288:91-6. https://doi.org/10.1152/ajpregu.00340.2004