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Renal Function and Hemodynamic Study in Obese Zucker Rats

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Objectives : *To investigate the renal function and hemodynamic changes in obesity and hyperinsulinemia which are characteristics of type II diabetes.*

Methods : *Studies were carried out in two groups of female Zucker rats. Group 1 rats were obese Zucker rats with hereditary insulin resistance. Group 2 rats were lean Zucker rats and served as controls. In comparison with lean Zucker rats, obese Zucker rats exhibited hyperinsulinemia but normoglycemia. Micropuncture studies and morphologic studies were performed in these rats.*

Results : *Functional studies showed that obese Zucker rats exhibited increases in kidney weight and GFR(obese Zucker, 1.23±.07)ml/min; lean Zucker, 0.93±.03ml/min). Micropuncture studies revealed that the increase in GFR in obese Zucker rats was attributable to the increases in the single nephron plasma flow rate and glomerular transcapillary hydraulic pressure. The glomerular ultrafiltration coefficient was the same in both groups. Morphologic studies revealed that the increase in GFR in obese Zucker rats was associated with an increase in glomerular volume.*

Conclusions : *These results suggest that obesity and hyperinsulinemia, which are the characteristics of type II diabetes, can be associated with glomerular hyperfiltration and glomerular capillary hypertension.*

Key Words : *Hyperinsulinemia, Obesity, Diabetes mellitus, GFR, Kidney*

INTRODUCTION

Kidney disease of diabetes mellitus is the most common cause of end-stage renal failure in the world[1]. Renal hemodynamics play a critical role in the progression of diabetic nephropathy and likely even contribute to its initiation. Alterations of the renal circulation are apparent early in type I diabetes before the onset of nephropathy. Mogensen[2] described a 40% increment in GFR in 11 young adult patients with newly diagnosed juvenile diabetes compared with values in normal, nondiabetic subjects of similar age. Similar increases in GFR have been also observed in rats made diabetic with streptozotocin, β cell toxins[3]. Studies in these rats demonstrated that increases in GFR in type I diabetes were caused by increases in single nephron plasma flow and in the glomerular transcapillary hydraulic pressure difference[3].

Renal function and hemodynamics have been studied less extensively in type 2 diabetes than in type I diabetes. The aim of the current study was to examine renal function and hemodynamics in a rat model of obesity and hyperinsulinemia which are the characteristics of type II diabetes. The model selected for study was the Zucker rat. Obesity in these rats is an autosomal recessive trait and resembles early onset human obesity in several ways. Obese Zucker rats, for example, exhibit hyperphagia, hyperlipidemia, hyperinsulinemia and peripheral insulin resistance [4,5]. Lean littermates have normal renal structure and function, and are ideal for use as experimen-

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tal controls.

Micropuncture studies were performed to assess the determinants of GFR in this setting.

MATERIALS AND METHODS

Experiments were carried out in female Zucker rats(Charles River, Cambridge, MA, USA). The average body weight of obese Zucker rats(n=7) was 474g and age 18 weeks. Lean Zucker rats(n=6) with body weight 226g and age 17 weeks served as controls. Samples of tail blood for measurement of plasma insulin and blood glucose levels were obtained.

Functional studies were carried out in each group. A tail blood sample was first obtained for determaination of blood glucose concentration. Rats were then anesthetized with Inactin, 130mg /kg i.p. in obese Zucker rats and 100mg/kg i.p. in lean Zucker rats and placed on a temperature-regulated table. Following anesthesia, a PE-50 tubing catheter was inserted in the right femoral artery and used for blood sampling and estimation of mean arterial pressure(AP). (AP) was continuously monitored with an electronic transducer connected to a direct writing recorder. After tracheostomy, PE-50 catheters were inserted in the right and left jugular veins for infusion of rat plasma, saline and radiolabeled inulin. Plasma was infused in an amount equal to 0.66% body weight over 40-45 minutes, followed by a reducton of the infusion rate to 0.4ml/h for the duration of the study. Saline was infused at 2.4ml/h throughout the study. A PE-10 catheter was installed into the left ureter for collection of urine. After -120 minutes, tritiated methoxy-inulin was added to the saline to achieve an infusion rate of -120mCi/h following a loading dose of -60mCi.

Clearance measurements were carried out over two three 30 to 40 minute periods. In each period, a 200ml arterial blood sample was obtained for determination of blood glucose, hematocrit, plasma inulin and protein concentrations. A renal vein blood sample was obtained with each arterial blood sample for determination of filtration fraction by renal vein inulin extraction. Timed(4 minute) samples of tubule fluid were collected from surface proximal convolutions of 3 to 5 nephrons for determination of single nephron glomerular filtration rate(SNGFR). Stop-flow pressure was measured in 2 to 3 proximal tubules as described by Allison et al[6] using a continuous recording servonull micropipette transducer system(Model V, Instrumentation for Physiology and Medicine, San Diego, CA, USA). Stop-flow pressure was measured in portions of proximal tubules in which at least 4 surface convolutions distal to the site of measurement could be identified by initial injection of a small droplet of oil. Free-flow pressures were measured in additional proximal tubules(3 to 6 determinations) and in efferent arterioles(2 to 5 determinations.).

Following functional studies, kidneys were fixed by retrograde aortic perfusion with 1.25% glutaraldehyde in 0.1 M cacodylate buffer(pH 7.4). Mid-coronal sections of kidney tissue were dehydrated in 90% ethanol, embedded in methacrylate, and stained with the periodic acid-Schiff technique. The mean glomerular tuft volume(VG) for each animal was determined from mean glomerular cross-sectional area(AG) as assessed by light microscopy. AG was determined on 100 consecutive glomerular profiles from each animal using a computer assisted morphometric unit(Bioquant II software). VG was then calculated as:

$$VG = b/k \cdot (AG)^{3/2}$$

Where b=1.38 is the shape coefficinent for spheres(the idealized shape of glomeruli) and k=1.1 is a size distribution coefficient[7,8].

Blood glucose concentration was determined with a refractance meter(Ames Division, Miles Laboratory, Elkhart, IN, USA). Plasma insulin concentraion was determined in duplicate using a commercially available kit(Incstar, Stillwater, MN, USA) and rat inulin standards. Plasma protein concentration was determined using a Lowry method modified to avoid interference by plasma lipids[9].

Efferent arteriolar protein concentration was calculated from the relation:

$$CE = \frac{CA}{1-FF}$$

Oncotic pressure(p) of efferent and afferent arteriolar plasma was estimated from plasma protein concentration(C)as:

$$p = 1.629C + 0.294C^2$$

Glomerular capillary pressure(P_{GC}) was estimated from measured stop-flow pressure(P_{SF}) and calculated afferent arteriolar oncotic pressure (PA) as:

$$P_{GC} = P_{SF} + PA$$

A standard mathematical model was used to derive the glomerular ultrafiltration coefficient (Kf)[10]. The statistical significance of differences

Table 1. Metabolic Parmaeters of Obese and Lean Zucker Rats at the Time of Micropuncture Study

	BG mg/dl	[Ins] μU/ml	BWMP
Obese Zucker (n=7)	64 ±2	656* ±107	474* ±15
Lean Zucker (n=6)	73 ±6	75 ±11	226 ±6

Mean values ± SEM

* $p<0.05$ obese Zucker vs lean Zucker

Abbreviations: BG, blood glucose; [Ins], plasma insulin; BW_{MP}, body weight at end of study.

between obese Zucker rats and lean Zucker rats was assessed using the unpaired t-test with significance defined as $p<0.05$. Values are expressed as means ± SE throughout.

RESULTS

Values for blood glucose and insulin in awake rats are depicted in Table 1. Obese Zucker rats exhibited severe hyperinsulinemia([insulin]plasma: obese Zucker, 656 ± 107 μU/ml; lean Zucker, 75 ± 11 μU/ml) but exhibited normoglycemia([glucose]blood: obese Zucker, 64 ± 2mg/dl; lean Zucker, 73 ± 6mg/dl). These findings reflect insulin resistance associated with hereditary obesity in the Zucker rat[4,5,11].

Studies of glomerular function under anesthesia are summarized in Table 2. Values for mean arterial pressure, hematocrit and arterial plasma protein concentration were not different between obese Zucker and lean Zucker rats. However, the average GFR value of 1.23 ± .07ml/min in obese Zucker rats was significantly greater than the average GFR value of 0.93 ± .03ml/min in lean Zucker rats. Similarly, the average kidney weight(LKW) of 1.46 ± .08g in obese Zucker rats was significantly greater than the average kidney weight of 1.06 ± .02g in lean Zucker rats. Micropuncture studies showed that the average value for SNGFR of 44 ± 4nl/min in obese Zucker rats was greater than the average value for SNGFR of 31 ± 1nl/min in lean Zucker rats. Values for filtration fraction were similar in the two groups, so that the average value for single nephron plasmal flow(QA) of 131 ± 13nl/min in obese Zucker rats was greater than the value for QA of 96 ± 4nl/min in lean Zucker rats. The value for the ultrafiltration coefficient(Kf) of 3.2 ± 2nl/min/mm Hg in obese Zucker rats was not different from the value for Kf 3.0 ± 2nl/min/mm Hg in lean Zucker rats. The value for the glomerular capillary pressure in obese Zucker rats was higher than that of lean Zucker rats(P_{GC}: obese Zucker, 60 ± 1mm Hg: lean Zucker, 54 ± 1mm Hg).

Morphologic studies revealed that the increase in GFR in obese Zucker rats was associated with an increase in glomerular volume(VG: obese Zucker, $2.02 \pm .09 \times 10^6 \mu^3$; lean Zucker, $1.30 \pm .09 \times 10^6 \mu^3$). ($p<0.05$)

DISCUSSION

Diabetic nephropathy is the leading cause of end stage renal failure in the world[1]. Recently there have been developments in understanding the risk factors and pathogenetic mechanisms such as glycemic control, familial, genetic and ethnic predisposition and hemodynamic disturbances including systemic and glomerular hypertension[12~15]. Studies in type I diabetes suggested that hemodynamic factor played a substantial role in the pathogenesis of diabetic nephropathy[3]. Increases in glomerular filtration-rate and kidney size have been regularly docu-

Table 2. Systemic Parameters and Renal Function of Obese and Lean Zucker Rats at the Time of Micropuncture Study

	BGMP mg/dl	AP mm Hg	Hct %	CA g/dl	GFR ml/min	LKW g
Obese Zucker (n=7)	109 ±6	118 ±3	35 ±1	5.0 ±.1	1.23* ±.07	1.46* ±.08
Lean Zucker (n=6)	101 ±9	110 ±3	34 ±1	5.2 ±.1	0.93* ±.03	1.06* ±.02

Mean values ± SEM

* $p<0.05$ obese Zucker vs lean Zucker

Abbreviations: BG_{MP}, blood glucose during micropuncture study; AP, mean arterial pressure; Hct, hematocrit; CA, afferent arteriolar plasma protein concentration; LKW, left kidney weight.

Table 3. Glomerular Hemodynamic Parameters of Obese and Lean Zucker Rats at the Time of Micropuncture Study

	SNGFR nl/min	FF	QA nl/min	Kf nl/min/mmHg	PGC	PT	DP mmHg	PE
Obese Zucker (n=7)	44* ±4	0.33 ±.02	131* ±13	3.2 ±.2	60* ±1	16 ±1	44* ±2	17 ±1
Lean Zucker (n=6)	31 ±4	0.32 ±.01	96 ±4	3.0 ±.2	54 ±1	15 ±1	39 ±2	15 ±1

Mean values ± SEM

* $p<0.05$ obese Zucker vs lean Zucker

Abbreviations: SNGFR, single nephron glomerular filtration rate; FF, filtration fraction; QA, glomerular plasma flow rate; Kf, glomerular capillary ultrafiltration coefficient; PGC, mean glomerular capillary hydraulic pressure; PT, proximal tubule hydraulic pressure; DP, transcapillary hydraulic pressure gradient; PE, efferent arteriolar hydraulic pressure.

mented in type I diabets[16]. Studies by Hostetter et al[3]. of streptozotocin-induced diabetes in the rat have demonstrated that the increments in GFR were caused by changes in two of the hemodynamic factors governing filtration rate. First, incresaed single nephron plasma flow accounted for a substantial portion of the increase in GFR. This increase in single nephron plasma flow was the result of reductions in both afferent and efferent arterial resistances. Second, increments in the glomerular transcapillary hydraulic pressure difference also contributed to the increase in GFR. As in the studies of human diabetic patients, no changes occurred in systemic oncotic pressure. The calculated gomerular ultrafiltation coefficient did not account for the increased filtration rate. Studies of streptozotocin-induced diabetes in the rat and other kidney disease models have demonstrated that the glomerular hypertension may in turn contribute to development of glomerular injury[12].

Contribution of hemodynamic changes to development of kidney injury in type II diabetes has been harder to evaluate. Patients with type II diabetes develop glomerular injury indistinguishable from glomerular injury seen in type I diabetes[17]. Some but not all studies suggest that type II diabetes is associated with an increase in GFR[17-19]. But renal function and hemodynamic studies have been performed less extensively in type II diabetes than in type I diabetes because of lack of suitable animal model. Human studies on diabetic nephropathy have some limitation in performance because it takes 10-20 years for diabetic nephropathy to develop in prospective studies and it is difficult to get clinical kidney samples in diabetic patients. Adequate methods for determination of glomerular hemodynamic parameters in humans are not yet available. So, many studies were performed on experimental animals. The aim the current study was to assess renal function and hemodynamics in rats with obesity and hyperinsulinemia which are the characteristics of type II diabetes. Experiments were carried out in obese Zucker rats with hereditary insulin resistance[4,5,11]. They are not perfect models for type II diabetes, due to lack of hyperglycemia, but they are close to type II diabetes because of obesity, hyperinsulinemia and hyperlipidemia. Lean Zucker rats served as controls.

In comparison with lean Zucker rats, obese Zucker rats showed an increase in GFR and glomerular hydrostatic pressure. The value for the ultrafiltration coefficient(Kf) in obese Zucker rats was not different from that in lean Zucker rats. These findings suggest that obesity and hyperinsulinemia, which are the characteristics of type II diabetes, like type I diabetes, can cause glomerular hyperfiltration and glomerular capillary hypertension. But it can't be sure whether changes we obesrve are due to insulin resistance or increased food consumption and obesity or both. Previous studies have shown that glomerular hyperfiltration in type I diabetes is associated with both renal and glomerular hypertrophy[20,21]. Morphologic features in the current study revealed that the increase in GFR in obese Zucker rats was associated with an increase in glomerular volume. This shows that similar alterations in renal structure are associated with glomerular hyperfiltration in obesity and hyperinsulinemia which are the characteristics of type II diabetes.

The obese male Zucker rat develops proteinuria and progressive focal segmental glomer-

ulosclerosis spontaneously in old age[22,23]. The current study was performed in female rats and before that age. Previous studies showed that obese Zucker rats demonstrated small increases in superficial nephron glomerular filtration rate and plasma flow but no difference between obese and lean Zucker rats. The factors important in the development of focal segmental glomerulosclerosis in obese rats are unknown. But it is hypothesized that hemodynamic factors including glomerular hyperfiltation and glomerular capillary hypertension and metabolic factors including hyperlipidemia may cause focal segmental glomerulosclerosis[23].

Micropuncture studies by Zatz et al[24] showed that capillary hypertension is the hemodynamic derangement most closely associated with development of glomerular sclerosis in the experimental type I diabetes model. They observed that increased dietary protein intakes led to elevated glomerular capillary pressures in diabetic rats and were associated with greater amount of proteinuria and glomerular injury. They have further linked elevated glomerular pressure to subsequent injury in diabetes by by demonstrating taht a reduction in glomerular capillary pressure by administration of a converting enzyme inhibitor lowered capillary pressure with a subsequent diminution in glomerular pathology[25]. In both of these studies, glycemic control was not affected by the dietary protein or antihypertensive drug, thus emphasizing the potentially critical contribution of hemodynamic stresses to glomerular damage. These findings have prompted the suggestion that glomerular capillary hypertension causes glomerular injury in humans with type I diabetes[26-29]. Although the glomerular lesions in experimental diabetic animals are different from those of human diabetic nephropathy[30], results of the current study suggest that capillary hypertension may initiate glomerular injury in obesity and hyperinsulinemia which are the characteristics of type II diabetes.

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