Korean J Nutrition 23(6) : 451~458, 1990

Effect of Estrone Treatment on Proteinuria in Maturing Lean and Obese Male SHR/Mcc-cp Rats

Park, Sonhee · McCune, Sylvia A.
Department of Food Science & Technology, The Ohio State University, U.S.A.

ABSTRACT

Lean and obese male spontaneously hypertensive(SHR/Mcc-cp) rats were fed a ground rat chow diet with or without 0.001% estrone added from 6 to 18weeks of age. Urine samples were collected weekly with 24hour fasting. Both control lean and obese rats showed significantly higher urinary protein than their estrone treated counterparts. Treatment with 0.001% estrone diet was found to reduce the proteinuria in the maturing lean and obese SHR/Mcc-cp rats. Peaks in urinary protein level were noted at 16weeks of age in both lean and obese control rats. Both control and estrone treated lean rats showed higher proteinuria than the obese rats. Therefore, obesity does not appear to be a contributing factor to the proteinuria in young male SHR/Mcc-cp rats.

KEY WORDS : proteinuria · estrone · obesity.

INTRODUCTION

Sexual dimorphism in the development of diabetes and diabetic complications has been reported in many studies[1-9]. Renal disease is one of the common problems in diabetes[10-12]. Proteinuria reflects changes in renal function and morphology[9-11)13)14)].

Sexual dimorphism was also shown in renal disease in some genetically hypertensive rat strains[12)15)]. Abraham and Michaelis[12)] reported that spontaneously hypertensive and obese male(SHR/N-cp) rats showed more severe renal disease state than obese female rats. Our preliminary aging study also revealed that both obese and lean male SHR/Mcc-cp rats excreted more urinary protein than both types of female rats at the various ages [15)].

There were several studies with female sex hormone treatment, which resulted in a decrease in diabetic lesions[16-19)]. Hoversland et al[6)]. reported that obese SHR/Mcc-cp females treated with tamoxifen, an anti-estrogen, showed a more impaired glucose tolerance pattern compared to the untreated rats. Therefore, female sex hormone may play a role in the suppression of diabetic renal lesions.

Several studies found that genetically obese rats showed significantly higher proteinuria than their lean littermates, representing abnormal renal function[10)20)]. Spontaneously hypertensive SHR/Mcc-cp rats also showed marked proteinuria in both obese and lean males in the previous study[15)]. Therefore, this study has investigated the effect of estrone treatment and age of animal on proteinuria in the obese and lean male SHR/Mcc-cp rats.

Accepted November 23, 1990.

MATERIALS AND METHODS

Experimental animals

Six week old lean and obese male SHR/Mcc-cp(Mcc originated from the name of Dr. McCune who developed this strain) rats were used. Each of 12 lean and 8 obese male rats were divided into control and estrone treatment group by systematized block design according to the initial body weight. The average initial body weights in control lean and estrone treated lean male rats were 131± 8 g and 133± 6g respectively. The average initial body weights in control obese and estrone treated obese male rats were 159± 14g and 162± 8g respectively. Lean and obese male rats were from two families respectively. Animals were caged in a temperature-controlled room with a 12 hour light cycle from 6 a.m. to 6 p.m.

Diet and experimental period

Animals were fed water and diet *ad libitum*. Control rats were fed regular Agway Prolab rat chow, and estrone treated rats were fed ground Agway Prolab rat chow containing 0.001% estrone from 6 to 18 weeks of age. To make 0.005% estrone diet, 50mg of estrone(Sigma, St. Louis, MO) was solubilized in 200ml acetone, mixed with 1kg ground rat chow, air dried, and then kept at room temperature. 0.001% estrone diet was made by diluting 0.005% estrone diet with ground diet before feeding to rats.

Urine collection and analysis

a 24hour urine sample was collected in an erlenmeyer flask while the rats were in metabolic cages with *ad libitum* access to water but no food. Sodium fluoride(0.3ml of 25mg/ml) was added into the flask to prevent bacterial growth in the urine. Urine volume was measured with a graduated cylinder. The urine sample was centrifuged at 1900 ×G for 10minutes. Urinary protein was determined by the method of Bradford[21]. The amount of urinary protein was expressed as mg protein/total urine collected for 24hours(mg/24h).

Statistical analysis

Statistical differences between treatment and control groups and between lean and obese groups were assessed by unpaired Student t-test. The level of significant probability was $p<0.05$[22].

RESULTS

No significant difference was found in the average initial concentration of urinary protein between control and estrone treated lean male rats (Fig. 1). After one week of estrone treatment, lean rats showed a significantly lower urinary protein than the control leans for the entire experiment. Control lean rats exhibited a very high peak (47.0± 9.9mg/24h) in urinary protein at 2weeks of treatment(8weeks of age) ; the urinary protein levelled off and around 30mg/24h throughout the remainder of the experiment except for another peak at the 10th week(16weeks of age). Estrone treated lean male rats did not show the high peak at 2weeks and showed a peak in protein excretion at 10weeks of treatment. Estrone treated lean rats, from 9th week to the end of experiment, seemed to be less responsive to the treatment although their urinary protein was significantly lower than that of control lean rats at all times.

The urinary protein content of control obese male rats was much higher than that of estrone treated rats(Fig. 2). After 5weeks of treatment, estrone treated obese rats exhibited significantly lower urinary protein than the controls. The urinary protein in obese controls increased at 10weeks like lean rats. Although the urinary protein in control obese males declined at 11 and 12weeks, it

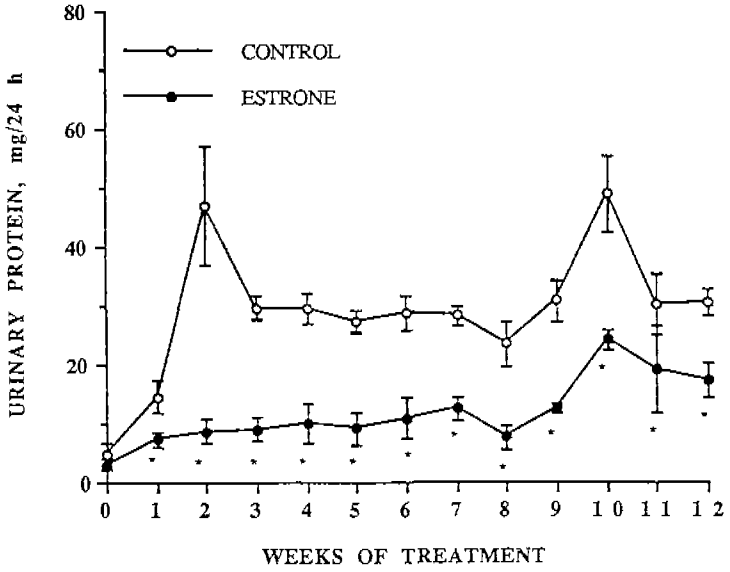

Fig. 1. Estrone effect on urinary protein in lean male rats Each value is mean± SE of 6 rats. "The level of significant probability is $p<0.05$.

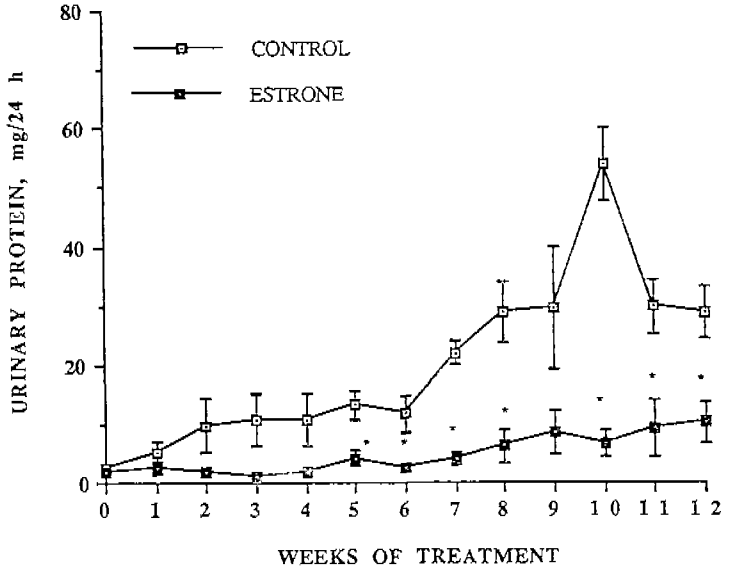

Fig. 2. Estrone effect on urinary protein in obese male rats Each value is mean± SE of 4 rats. "The level of significant probability is $p<0.05$.

was significantly higher compared to that of estrone treated obese males.

In control groups, lean rats showed significantly higher urinary protein excretion than obese rats from 7 to 13weeks, and no significant difference was found from 14 to 18weeks of age(Table 1). From the beginning to the end of the experiment, in estrone treated rats, the urinary protein of obese

Table 1. Urinary protein of lean and obese control rats

Weeks of treatment	Lean(n=6)	Obese(n=4)
	mg/24h	
0[1)]	4.9± 1.9	2.6± 0.5
1	14.6± 2.7	5.1± 1.9*
2	47.0± 9.9	9.7± 4.6*
3	29.7± 2.1	10.6± 4.5*
4	29.7± 2.5	10.7± 4.3*
5	27.4± 1.9	13.1± 2.5*
6	28.9± 2.8	11.7± 3.1*
7	28.4± 1.7	21.9± 2.1*
8	23.6± 3.9	28.7± 5.2
9	31.0± 3.6	29.5± 10.2
10	49.2± 6.5	53.9± 6.3
11	30.3± 5.2	29.8± 4.6
12	30.8± 2.2	28.8± 4.3

All values are mean± SE
1) 6weeks of age at the beginning of experiment
*The level of significant probability is $p<0.05$.

Table 2. Urinary protein of lean and obese estrone treated rats

Weeks of treatment	Lean(n=6)	Obese(n=4)
	mg/24h	
0[1)]	3.1± 0.9	2.0± 0.6
1	7.3± 1.4	2.6± 1.2*
2	8.7± 2.1	2.0± 0.2*
3	9.0± 2.0	1.0± 0.3*
4	9.9± 3.4	1.8± 0.4
5	9.1± 2.8	4.2± 1.3
6	10.9± 3.6	2.5± 0.6
7	12.5± 2.1	4.0± 1.0*
8	7.6± 1.9	6.1± 2.7
9	12.6± 0.7	8.4± 3.7
10	24.3± 1.6	6.7± 2.3*
11	19.3± 7.4	9.2± 4.8
12	17.4± 2.9	10.2± 3.4

All values are mean± SE
1) 6weeks of age at the beginning of experiment
*The level of significant probability is $p<0.05$.

males tended to be lower than that of lean males (Table 2). Significant differences were found at 1~3, 7, and 10weeks of treatment. The difference in urinary protein between lean and obese rats was greater in the control rats compared to that of the estrone treated rats during the earlier stage of experiment. Estrone seemed to be more effective in decreasing urinary protein in the obese rats in the last few weeks of the experiment.

DISCUSSION

Proteinuria is one of the common indications of renal injury. Quantitative and qualitative analysis of the urinary protein reflects the severity and type of glomerular injury[14]. Urinary proteins are mainly derived from the blood plasma by glomerular filtration followed by an escape from reabsorption[23]. Estrone treatment may protect kidneys from the morphologic or functional deterioration in male SHR/Mcc-cp rats since estrone treated rats showed significantly lower urinary protein than the control animals in both lean and obese groups. The exact mechanism of estrone treatment that lowered urinary protein in the male rats can not be elucidated from the present study. However, one of the possible reasons why there seemed to be more effect in the obese male may be related to change in glycosylation of protein. Estrone treated obese rats showed lower glycosylated hemoglobin than the control rats in our previous study[15]. Glycosylation of proteins including albumin, immunoglobulin, or hemoglobin is enhanced in diabetic subjects[21-26]. Microalbuminuric diabetic patients excreted more glycosylated albumin compared to nonglycosylated albumin in the urine, reflecting increased permeability and urinary clearance of protein by glycosylation[24]. Kennedy et al[25]. implied that nonenzymatically glycosylated plasma

protein may develop glomerular basement membrane thickening, resulting in diabetic nephropathy.

Another alternative hypothesis may be related to levels of atrial natriuretic factor(ANF). The ANF level was lower in estrone treated lean male rats than control rats in our preliminary studies(S. McCune : unpublished data). Suenaga et al[27]. demonstrated that ANF plays an important role in the regulation of urinary protein excretion in the essential hypertensive patients. They suggested that ANF seems to change the permeability of glomerular capillary wall to plasma protein, to reduce renal vascular resistance, or to inhibit reabsorption of protein filtered through the glomerulus. ANF is an important peptide in vasodilation and may increase protein filtration in the kidney[28].

When considering the fact that protein excretion was greater in the lean rats than in the obese rats, it seems that obesity *per se* does not affect proteinuria in the male SHR/Mcc-cp rats aged 6~18weeks. In contrast to our findings, male obese SHR/N-cp rats at 19weeks of age excreted more protein than their lean counterparts[20] : however, these rats were on refined high starch diet and our rats were only on regular chow. Lean SHR/N-cp male rats also showed lower urinary protein than our SHR/Mcc-cp lean male rats[10]. Velasquez et al[10]. found that their obese rats showed significantly lower glomerular filtration rate than lean rats as measured by creatinine clearance, representing lowered kidney function. However, our non-diabetic lean male rats were more prone to severe proteinuria at an early age compared to the obese male rats.

Another possible reason that estrone treated males excreted less protein than non-treated males in the urine may be due to alpha-2-globulin which is a male specific, low molecular weight, and major urinary protein in the mature male rat[23][29-31]. Hepatic synthesis of alpha-2-globulin is regulated by androgen, glucocorticoids, thyroxine, growth hormone, and insulin[30][31]. In some preliminary studies, male obese rats had lower testosterone levels compared to the lean males(R. Hoversland : unpublished data). Therefore, since the testosterone level can regulate alpha-2-globulin synthesis and excretion, this might be an explanation of why young obese male rats excreted less urinary protein than young lean male rats. Estrone may decrease the proteinuria by affecting the synthesis of alpha-2-globulin by opposing testosterone action ; therefore, it may be part of the reason that estrone treated rats exhibited less proteinuria. Female SHR/Mcc-cp rats did not show proteinuria when they were under 18weeks of age, and older female rats showed much lower proteinuria than males at the same age[15]. Abraham and Michaelis[12] also found that proteinuria was milder in the female SHR/N-cp rats with same renal alterations compared to the male rats.

In conclusion, estrone treatment decreased proteinuria in both lean and obese rats, and obesity *per se* is not a factor to increase proteinuria in growing male SHR/Mcc-cp rats. In both lean and obese control rats, peaks in urinary protein level were observed at 8 and 16weeks of age. To understand the exact mechanisms of estrone action on urinary protein excretion in relation to glycosylated protein, ANF, alpha-2-globulin, and plasma lipid level, further studies are in progress.

Literature cited

1) Clark JB, Palmer CJ, Shaw WN. The diabetic Zucker fatty rat. *Proc Soc Exp Biol Med 173* : 68-75, 1982
2) Ikeda H, Shino A, Matsuo T, Iwatsuka H, Suzuoki Z. A new genetically obese-hyperglycemic rat(Wistar fatty). *Diabetes 30* : 1045-1050, 1981
3) Leiter EH, Coleman DL, Hummel KP. The influence of genetic background on the expression of

mutations at the diabetes locus in the mouse.-III. Effect of H-2 haplotype and sex. *Diabetes 30* : 1029-1035, 1981

4) Kava RA, West DB, Lukasik VA, Greenwood MRC. Sexual dimorphism of hyperglycemia and glucose tolerance in Wistar fatty rats. *Diabetes 38* : 159-163, 1989
5) Leiter EH, Coleman DL, Eisenstein AB, Strack I. Dietary control of pathogenesis in C57BL/Ksj db/db diabetes mice. *Metabolism 30* : 554-562, 1981
6) Hoversland R, McCune SA, Simmons A, Prewitt Z, Rimell F, Ramirez S, Jurin RR. Sex differences in the diabetic characteristics of the SHR/N : Mcc-cp obese rats. In : Hansen CT, Michaelis IV OE, eds. New Models of Genetically Obese Rats for Studies in Diabetes, Heart Disease, and Complications of Obesity. *NIH Workshop : NIH Puble, pp*105-108, 1988
7) Leiter EH. The genetics of diabetes susceptibility in mice. *FASEB J*3 : 2231-2241, 1989
8) Kava R, West DB, Lukasik VA, Savard R, Greenwood MRC. Sexual dimorphism in the Wistar fatty rat. In : Hansen CT, Michaelis IV OE, eds. New Models of Genetically Obese Rats for Studies in Diabetes, Heart Disease, and Complications of Obesity. *NIH Workshop : NIH Puble, pp*121-130, 1988
9) McCune SA, Jurin RR, Hansen T, Dunn RB, Chedid A, Fox E, Summers B, Wei TT. SHR : N-Mcc-cp substrain : General characteristics and congestive heart failure syndrome. In : Hansen CT, Michaelis IV OE, eds. New Models of Genetically Obese Rats for Studies in Diabetes, Heart Disease, and Complications of Obesity. *NIH Workshop : NIH Puble, pp*95-105, 1988
10) Velasquez MT, Kimmel PL, Michaelis IV OE, Carswell N, Bosch JP. Influence of dietary carbohydrate on renal function in the SHR/N-cp rat. In : Hansen CT, Michaelis IV OE, eds. New Models of Genetically Obese Rats for Studies in Diabetes, Heart Disease, and Complications of Obesity. *NIH Workshop : NIH Puble, pp*81-84, 1988
11) Coronel F, Hortal L, Naranjo P, Cruceyra A, Barrientos A. Captopril, proteinuria and peritoneal leakage in diabetic patients. *Nephron 51* : 443, 1989
12) Abraham AA, Michaelis IV OE : Histopathology of renal and adrenal gland lesions in SHR/N-cp rats. In : Hansen CT, Michaelis IV OE, eds. New Models of Genetically Obese Rats for Studies in Diabetes, Heart Disease, and Complications of Obesity. *NIH Workshop : NIH Puble, pp*85-92, 1988
13) Goldstein RS, Tarloff JB, Hook JB. Age-related nephropathy in laboratory rats. *FASEB J 2* : 2241-2251, 1988
14) Morioka T, Sugano H, Matsui K, Kagami S, Shimizu F, Oite T. The electrophoretic pattern of urinary protein in in situ immune complex glomerulonephritis. *Nephron 50* : 116-120, 1988
15) Park S. Studies on the effect of estrone treatment in obese and lean SHR/Mcc-cp rats. Dissertation. *The Ohio State University*, 1989
16) Leiter EH. The influence of genetic background on the expression of mutations at the diabetes locus in the mouse. -IV. Male lethal syndrome in CBA/Lt mice. *Diabetes 30* : 1036-44, 1981
17) Prochazka M, Premdas FH, Leiter EH, Lipson LG. Estrone treatment dissociates primary versus secondary consequences of "Diabetes"(db) gene expression in mice. *Diabetes 35* : 725-28, 1986
18) Paik SG, Michelis MA, Kim YT, Shin S. Introduction of insulin-dependent diabetes by streptozotocin : inhibition by estrogens and potentiation by androgens. *Diabetes 31* : 724-29, 1981
19) Schemmel RA, Teague RJ, Bray GA. Obesity in Osborne-Mendel and S5B/P1 rats : effects of sucrose solutions, castration, and treatment with estradiol or insulin. *Am J Physiol 243* : R347-53, 1982
20) Michaelis IV OE, Carswell N, Ellwood KC. Metabolic characteristics of the LA/N-corpulent and SHR/N-corpulent rat strains. In : Hansen CT, Michaelis IV OE, eds. New Models of Genetically Obese Rats for Studies in Diabetes, Heart Disease, and Complications of Obesity. *NIH Workshop : NIH Puble, pp*13-15, 1988
21) Bradford MM. A rapid and sensitive method for

the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Analytical Biochemistry 72*, pp248-254, 1976

22) Steel RGD, Torrie JH. Principles and procedure of statistics : A biometrical approach. 2nd ed. McGraw-Hill International Editions, 1980

23) Neuhaus OW. Renal reabsorption of low molecular weigh proteins in adult male rats : alpha-2μ-globulin(42376). *Proc Soc Exp Biol Med 182* : 531-539, 1986

24) Nakamura M, Myers BD. Charge selectivity of proteinuria in diabetic glomerulopathy. *Diabetes 37* : 1202-1211, 1988

25) Kennedy L, Mehl TD, Elder E, Varghese M, Merimee TJ. Nonenzymatic glycosylation of serum and plasma proteins. *Diabetes 31(suppl 3)* : 52-56, 1982

26) Means GE, Chang MK. Nonenzymatic glycosylation of proteins : structure and function changes. *Diabetes 31(suppl 3)* : 1-4, 1982

27) Suenaga K, Mizuno K, Niimura S, Mori K, Sato M, Tani M, Yabe R, Yatabe Y, Fukuchi S. Atrial natriuretic factor(ANF) increases urinary protein excretion in patients with essential hypertension : A possible role of ANF for renal handling of protein. *Biochem Biophysic Res Com 158* : 3 : 936-942, 1989

28) Vesely DL, Winters CJ, Sallman AL. Atrial natriuretic factor in the pleural fluid of congestrive heart failure patients. *Chest 95* : 5 : 1107-1109, 1989

29) Goldsworthy TL, Lyght O, Burnett VL, Popp JA. Potential role of alpha-2μ-globulin, protein droplet accumulation, and cell replication in the renal carcinogenicity of rats exposed to trichloroethylene, perchloroethylene, and pentachloroethane. *Toxicol Appl Pharm 96* : 367-179, 1988

30) Murty CVR, Sarkar FH, Mancini MA, Roy AK. Sex-independent synthesis of alpha-2μ-globulin and its messenger ribonucleic acid in the rat preputial gland : Biochemical and immunocytochemical analysis. *Endocrinology 121* : 1000-1005, 1987

31) Swenberg JA, Short B, Borghoff S, Josef S, Charbonneau M. The comparative pathobiology of alpha-2μ-globulin nephropathy. *Toxicol Appl Pharm 97* : 35-46, 1989

Estrone 투여가 SHR/Mcc-cp 계통의 정상체중과 비만한 성장기 숫쥐들에서 단백뇨 증상에 미치는 영향

박 선 희 · McCune, Sylvia A.
Department of Food Science & Technology, The Ohio State University, U.S.A.

국문초록

고혈압 증세를 나타내는 정상체중과 비만의 SHR/Mcc-cp 숫쥐들에게 0.001% estrone 식이를 생후 6주부터 18주까지 투여하고, 매주 일회 24시간 금식 시키는 동안 뇨를 채취하여 뇨단백량을 측정하였다. Estronc 식이를 시작하기 직전에는 정상체중의 쥐와 비만의 쥐 모두에게서, estronc 식이군과 대조군 사이에 뇨단백량에 있어서, 유의한 차이가 없었다. 그러나, 정상체중의 쥐에게서, estrone 투여후 1주부터 estronc 식이군의 뇨단백량이 대조군에 비해 유의적으로 낮았다($p<0.05$). 비만인 쥐도 estrone 식이후 1주부터 cstrone 식이군의 뇨단백량이 대조군에 비해 현저하게 낮아지기 시작해서 제 5주부터는 유의한 차이가 보였다. 그러므로, 0.001% estrone 식이가 성장기의 정상체중과 비만의 SHR/Mcc-cp 숫쥐들의 단백뇨 상태를 호전시킴을 발견했다. 또한, SHR/Mcc-cp 쥐들이 13주령 이전에는 정상체중의 쥐들이 비만인 쥐들보다, 더 높은 뇨단백량을 보였으나, 그 이후부터 18주 사이의 주령에는 정상체중의 쥐와 비만의 쥐들 사이에는 유의적 차이가 없었다. 그러므로, SHR/Mcc-cp 쥐에게 있어서, 비만증 자체가 단백뇨 증세를 악화시키는 요인이 아님을 알 수 있었다.