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A high-fat diet aggravates tubulointerstitial but not glomerular lesions in obese Zucker rats

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Background. Despite a large body of evidence that manipulation of dietary fat alters glomerular lesions, reports regarding the effects of dietary fat on tubulointerstitial lesions are limited. Obese Zucker rats (OZR) spontaneously develop glomerular and tubulointerstitial lesions in association with hyperlipidemia. We sought to elucidate the effects of dietary fat on glomerular and tubulointerstitial lesions in OZR versus lean Zucker rats (LZR) and to assess the involvement of macrophages in the development of these lesions.

Methods. We fed LZR and OZR either a low- (1%) or high-fat (20%) diet. After 30 weeks of the specified diet, the creatinine clearance (C_{Cr}) and renal histology as well as plasma lipid concentrations were examined. For morphological evaluation, glomerular sclerosis (GSI) and tubulointerstitial indices (TII) were each determined by a point-counting method. Infiltrating macrophages were stained immunohistochemically using an avidin-biotin complex technique.

Results. The high-fat diet increased the plasma low-density lipoprotein concentration in OZR. Both low- and high-fat OZR groups had higher GSI and TII than LZR receiving either diet. The high-fat diet aggravated TII but not GSI or C_{Cr} in OZR; conversely, high fat intake worsened GSI and C_{Cr} but not TII in LZR. Tubulointerstitial macrophages were most prominent in the high-fat OZR group, followed by the low-fat OZR group. Glomerular macrophages were similar in number in all groups.

Conclusions. The manipulation of dietary fat has diverse effects on the kidney. A high-fat diet aggravated macrophage-mediated tubulointerstitial lesions in OZR, whereas in LZR, the diet induced glomerulosclerosis.

Obese Zucker rats (OZR) spontaneously develop hyperlipidemia and renal injury characterized by tubulointerstitial monocytic infiltration and focal segmental glomerulosclerosis. Manipulation of dietary fat alters the course of renal disease. The administration of a high-cholesterol diet to rats with puromycin aminonucleoside (PA) nephrosis results in an increase in plasma total cholesterol (TC) concentration and augments glomerulosclerosis in association with increases in the renal content of lipid peroxidation products and oxidized low-density lipoprotein (LDL) [1]. Although many studies have evaluated the effects of dietary fat on glomerular lesions, limited information is available regarding its effects on tubulointerstitial lesions. To examine whether a high-fat diet aggravates glomerular and tubulointerstitial lesions, we administered a high (20%)- or low (1%)-fat diet to OZR and lean Zucker rats (LZR) for 30 weeks.

In this study, the high-fat diet aggravated tubulointerstitial but not glomerular injury in OZR, whereas the opposite occurred in LZR. We also evaluated whether macrophages are involved in the development of dietary fat-induced tubulointerstitial and glomerular lesions in these rats.

METHODS

Five-week-old male LZR and OZR were purchased from Charles River Laboratories (Kingston, NY, USA). Rats had free access to food. LZR and OZR were each divided into two groups according to dietary fat content: low-fat LZR ($N = 9$), high-fat LZR ($N = 7$), low-fat OZR ($N = 6$), and high-fat OZR ($N = 8$). Low-fat rats were given a daily diet with 1% fat (corn oil, 0.5%; lard, 0.5%). The high-fat diet included 20% fat (corn oil, 10%; lard, 10%). The rats' body weight was measured every three weeks. Daily food intake was determined twice weekly. After collecting 24-hour urine samples at 30 weeks of dietary treatment, the rats were killed. The abdomen was incised under sodium pentobarbital anesthesia (100 mg/kg). Blood samples were obtained from the aorta to determine plasma concentrations of TC, triglyceride (TG), LDL, lipid peroxides. Then both kidneys were perfused with phosphate-buffered saline, removed, and weighed.

Coronal sections of the kidney were fixed in 4% paraformaldehyde and embedded in paraffin. Sections were stained with periodic acid-silver methenamine. Glomerular volume (GV), a glomerular sclerosis index (GSI), and a tubulointerstitial index (TII) were calculated using the point-counting method described previously [2]. Monocytes/macrophages were stained by an avidin-biotin com-

Key words: dietary fat, hyperlipidemia, macrophages.

Table 1. Body weight at 5 and 35 weeks of age, mean daily food intake, kidney weight, plasma lipid concentrations, and plasma TBARS concentrations

Rats	Lean Zucker (LZR)		Obese Zucker (OZR)		Statistical analysis
Dietary fat	low	high	low	high	
N	9	7	6	8	
Body weight *g*					
5 weeks of age	120 ± 11	120 ± 10	172 ± 21	174 ± 15	a
35 weeks of age	488 ± 54	624 ± 57	776 ± 86	837 ± 105	a, b
Mean daily food intake					
Calorie intake *kcal/day*	91.0 ± 6.7	99.0 ± 6.5	120.0 ± 8.0	131.0 ± 15.5	a, b, c
Fat intake *g/day*	0.22 ± 0.04	4.31 ± 0.26	0.30 ± 0.02	5.61 ± 0.43	b, c
Protein intake *g/day*	5.8 ± 0.5	5.4 ± 0.4	7.7 ± 0.5	7.3 ± 0.6	a
Bilateral kidney weight *g*	3.24 ± 0.53	3.93 ± 0.61	6.11 ± 0.54	7.38 ± 3.18	a
TG *mg/dl*	121 ± 57	177 ± 41	481 ± 175	540 ± 300	a
TC *mg/dl*	112 ± 63	126 ± 29	476 ± 97	517 ± 158	a
LDL *mg/dl*	199 ± 56	310 ± 86	564 ± 93	1368 ± 422	a, c
Plasma TBARS *nmol MDA/ml*	5.9 ± 4.5	9.5 ± 3.7	44.2 ± 21.9	125.4 ± 57.7	a, c

Lean and obese Zucker rats were treated with a low or high fat diet from 5 to 35 weeks of age. Mean daily food intake during the experimental period was obtained from daily food consumption determined at each week. Bilateral kidney weight, plasma concentrations of lipids and TBARS were measured at 35 weeks of age. Data are expressed as the mean ± SD. Analysis of variance was performed, followed by Fisher's PLSD.

Abbreviations are: TBARS, thiobarbituric acid-reactive substances; TG, triglyceride; TC, total cholesterol; LDL, low density lipoprotein; MDA, malondialdehyde.

[a] $P < 0.05$, both LZR groups vs. both OZR groups

[b] $P < 0.05$, low vs. high fat LZR groups

[c] $P < 0.05$, low vs. high fat OZR groups

plex method using antibodies for ED1, as described previously [2]. ED1-positive cells were quantitated as the number per glomerulus and per tubulointerstitial field observed at a magnification of ×400. Thirty glomerular and tubulointerstitial regions were evaluated.

Urinary albumin excretion was measured by an immunodiffusion method described previously [2]. Proteinuria was measured by a biuret method. Plasma concentrations of creatinine, TC, TG, and LDL were determined using commercial kits (Wako, Osaka, Japan). The degree of plasma and renal lipid peroxidation was determined by measuring thiobarbituric acid-reactive substances (TBARS) using a commercial kit (Wako). Malondialdehyde (MDA) was used as a standard.

RESULTS

Body weight was greater in both OZR groups than in either LZR group and also was greater in high-fat LZR than in low-fat LZR (Table 1). The weight of both kidneys was greater in both OZR groups than in either LZR group, with no difference between low- and high-fat rats in either LZR or OZR group (Table 1). Protein and calorie intake of both OZR groups exceeded that of both LZR groups; additionally, a significant difference was present in both OZR and LZR with respect to caloric intake, but not protein intake, between low- and high-fat rats (Table 1). Both OZR groups had higher plasma TG and TC concentrations than both LZR groups, but these concentrations were similar between low- and high-fat rats. Plasma concentrations of LDL and TBARS were higher in both OZR groups than in either LZR group and were higher in high-fat OZR than in low-fat OZR (Table 1).

Daily urinary albumin and protein excretion were greater in both OZR groups than in either LZR group, with no difference between low- and high-fat rats (Table 2). Both OZR groups had greater GSI and GV and lower C_{Cr} than either LZR group (Table 2). The treatment of LZR with the high-fat diet aggravated GSI and C_{Cr} compared with low-fat LZR, but such effects of the high-fat diet were not noted in OZR (Table 2). In contrast, OZR fed a high-fat diet had a higher TII than did low-fat OZR, whereas TII were similar between low- and high-fat LZR. The renal content of TBARS was similar in the four groups (Table 2). Tubulointerstitial macrophages were most prominent in high-fat OZR, followed in order by low-fat OZR and both LZR groups (Table 2). Glomerular macrophages were similar in number in the four groups (Table 2).

DISCUSSION

Our study showed that manipulation of dietary fat has diverse effects on renal function and histology. In OZR, the high-fat diet resulted in an increase of plasma LDL concentration, and the diet aggravated tubulointerstitial lesions in association with an increase in tubulointerstitial macrophages; in LZR, the high-fat diet augmented glomerulosclerosis.

Macrophages are capable of producing a variety of proteases and growth factors, as well as significant quantities of reactive oxygen species (ROS) that can mediate renal injury [3]. Macrophage-derived ROS oxidize LDL;

Table 2. Parameters of renal function and histology at 5 and 35 weeks of age

Rats	Lean Zucker (LZR)		Obese Zucker (OZR)		Statistical analysis
Dietary fat	low	high	low	high	
N	9	7	6	8	
Proteinuria *mg/day*					
5 weeks of age	19.6 ± 4.8	17.0 ± 6.5	20.0 ± 8.9	13.6 ± 4.5	NS
35 weeks of age	87 ± 56	75 ± 37	380 ± 83	330 ± 160	a
Albuminuria *mg/day*					
5 weeks of age	0.06 ± 0.04	0.06 ± 0.03	0.81 ± 0.52	0.61 ± 0.66	a
35 weeks of age	16.0 ± 18.9	19.1 ± 15.2	165.2 ± 54.2	96.0 ± 48.1	a
Renal content of TBARS					
nmol MDA/mg of protein	0.64 ± 0.18	0.68 ± 0.26	0.52 ± 0.12	0.79 ± 0.25	NS
C_{Cr} *ml/min*	0.90 ± 0.16	0.50 ± 0.08	0.34 ± 0.10	0.38 ± 0.02	a, b
N	5	5	5	5	
GV ($\times 10^6$ μm^3)	1.39 ± 0.33	1.40 ± 0.32	1.89 ± 0.24	1.78 ± 0.48	a
GSI %	26.3 ± 2.2	34.8 ± 4.8	42.6 ± 5.8	38.4 ± 5.3	a, b
TII %	10.42 ± 1.2	10.8 ± 1.8	33.1 ± 3.7	46.8 ± 4.6	a, c
ED1-positive cells per:					
glomerulus	0.41 ± 0.20	0.87 ± 0.36	0.78 ± 0.33	1.02 ± 0.47	NS
tubulointerstitial field	3.02 ± 2.24	3.02 ± 2.60	5.96 ± 1.97	10.10 ± 1.58	a, c

Lean and obese Zucker rats were treated with low or high fat diet from 5 to 35 weeks of age. Renal content of TBARS, C_{Cr}, and morphological evaluation were performed at 35 weeks of age.

Data are expressed as the mean ± SD. Analysis of variance was performed, followed by Fisher's PLSD.

Abbreviations are: TBARS, thiobarbituric acid-reactive substances; MDA, malondialdehyde; C_{Cr}, creatinine clearance; GV, glomerular volume; GSI, glomerulosclerosis index; TII, tubulointerstitial index; NS, not significant.

[a] $P < 0.05$, both LZR vs. both OZR groups

[b] $P < 0.05$, low vs. high fat LZR groups

[c] $P < 0.05$, low vs. high fat OZR groups

the oxidized LDL has been shown to be injurious to renal tissue [4]. However, given the similar renal content of TBARS in all groups of our study, our working assumption that macrophage-mediated tubulointerstitial lesions in OZR were attributable to increases in lipid peroxides was not verified.

Magil [5] observed a close association of tubulointerstitial damage with the numbers of tubulointerstitial monocytes and cytotoxic T lymphocytes, suggesting that tubulointerstitial damage in OZR is mediated directly by mononuclear cell infiltrates. In hyperlipidemia, however, albuminuria also is likely to contribute to tubulointerstitial damage. A monocyte-specific chemotactic substance can be detected in urine from albumin-overloaded rats and is likely to be derived from proximal tubule [6]. Production of this chemoattractant is considered a consequence of the metabolism of lipid-associated albumin endocytosed by the proximal tubules, whereas lipid-depleted albumin do not induce this chemotactic factor [6]. These results suggest that abnormal lipid metabolism and hyperlipidemia may cause interstitial recruitment of monocytes and macrophages, regardless of amount of albuminuria.

The administration of the high-fat diet to LZR aggravated GSI and C_{Cr}. These findings are in agreement with previous observations that feeding rats a fat-rich diet induces mesangial expansion and hypercellularity and a modest degree of glomerulosclerosis [7] and that the increased mesangial cellularity is in part a result of increased macrophage influx [8]. This study showed a marginal increase of macrophages in high-fat LZR ($P = 0.052$).

In summary, dietary fat had diverse effects on the kidney. A diet high in fat induced tubulointerstitial lesions in OZR but aggravated glomerulosclerosis in LZR.

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