A High Cholesterol Diet Ameliorates Renal Tubular Lesions in Diabetic Rats (43075)

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Abstract. **These studies examine the effect of cholesterol feeding in normal rats and in rats with streptozotocin-induced diabetes mellitus. Four groups were studied: normal rats fed either a standard rat chow or a standard rat chow supplemented with cholesterol and diabetic rats fed standard chow or standard chow plus cholesterol. Diabetic rats fed a standard diet excreted more creatinine and urea in the urine, had higher levels of blood urea nitrogen, and lower serum albumin levels than rats fed standard diet plus cholesterol. Blood glucose levels were similar in the two groups; however, diabetic rats given cholesterol had a greater body weight at the end of the study than diabetic rats eating standard chow. Urine volumes and sodium and potassium excretion in the urine were greater in diabetic rats fed a standard diet than in those fed a high cholesterol diet. Diabetic rats fed a standard diet had distinctive renal lesions characterized by swelling of tubular epithelial cells with clearing of cytoplasm. The nephron segments involved by this striking vacuolar change were the distal convoluted tubule and the thick limbs of Henle's loop. These lesions were identical to those described by Armanni-Ebstein in severely glycosuric patients. These lesions were not observed in any of the animals of the other three groups (including diabetic rats fed a high cholesterol diet). Glomeruli were normal in animals of all groups.**

Thus, cholesterol administration prevents the development of the Armanni-Ebstein lesions in diabetic rats despite persistent hyperglycemia. The mechanism by which cholesterol administration prevents the accumulation of glycogen in distal tubule cells has not been elucidated. It is suggested that glycogen accumulation in distal tubular segments may explain the greater urine volumes, natriuresis, kaliuresis, and proteinuria observed in diabetic animals fed a standard diet when compared with rats fed the same diet plus cholesterol. [P.S.E.B.M. 1990, Vol 194]

The interrelations between diabetes, hypercholesterolemia, atherosclerosis, and renal disease are well recognized. Approximately 30% of new cases of end stage renal disease in the United States are attributed to diabetic nephropathy, making it the most common cause of renal failure in the country (1). In addition, atherosclerotic disease is a leading cause of death among whites with diabetes (2, 3). Diabetes mellitus markedly increases the risk of coronary artery disease (4). It has been proposed that a persistent subclinical increase in urine albumin excretion in diabetics is associated with the later occurrence of nephropathy (5). To reduce, or prevent microalbuminuria and the occurrence of overt nephropathy in the diabetic patient, several consequences of diabetes need to be corrected. These complications include hyperglycemia (6), hypercholesterolemia (7), hypertension (8), and obesity (9). Each of these conditions can affect renal function independently, or in combination (10), and lead to end stage renal disease.

In a number of experimental animal models, it has been shown that addition of cholesterol to the diet favors the development of glomerulosclerosis (10). On the other hand, a reduction in serum cholesterol ameliorates the development of glomerulosclerosis in several experimental animal models. In the obese Zucker rat, a model of nonimmune-mediated, spontaneous focal glomerulosclerosis (FGS) (11), administration of lovastatin lowered serum cholesterol and triglyceride levels, reduced albuminuria, and ameliorated FGS, suggesting that abnormalities of lipid metabolism may be important in the pathogenesis of FGS (12). Additional evidence exists that lowering serum cholesterol levels

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ameliorates the progression not only of generalized atherosclerosis (13), but also of FGS (12, 14).

The present studies were designed to examine the role of cholesterol feeding on the progression of renal disease in a rat model of insulin-dependent diabetes mellitus.

Despite the evidence cited above on the harmful effects of feeding cholesterol and the beneficial effects of lowering serum cholesterol on renal function and histology, we found that feeding a high cholesterol diet to rats with streptozotocin-induced diabetes ameliorated not only the increased protein catabolism and decreased weight gain observed in these animals, but also decreased or prevented some of the changes associated with diabetic nephropathy, such as proteinuria and the tubular accumulation of glycogen typical of the Armanni-Ebstein lesion.

Materials and Methods

Baseline Studies. We used female Sprague-Dawley rats obtained from Sasco Inc. (Omaha, NE) and weighing 168–216 g (mean, 190.3 ± 2.0 g). Prior to study the rats were housed five or six to a cage with 12-hr light/dark cycle at 21°C. The animals were fed a standard rat chow containing 22.8% protein (Purina Lab Chow; Ralston Purina Co., St. Louis, MO) and were allowed tap water *ad libitum*. Baseline studies (Day 0) were performed in the nonfasted state several days after arrival of the animals. The studies included a 24-hr urine collection for determination of urea, creatinine, sodium, potassium, and protein excretion. After the 24-hr urine collection, the animals were weighed and under light ether anesthesia blood was collected from the tail vein in microhematocrit capillary tubes (Fisher Scientific, Pittsburgh, PA) which were centrifuged in a microhematocrit centrifuge (International Equipment Co., Meedham Heights, MA) at 3000 rpm/2.5 min. Hematocrit values were determined and the plasma was separated for chemical determinations of glucose, cholesterol, triglycerides, blood urea nitrogen (BUN), albumin, and creatinine. Blood collections were made between 7 and 9 A.M.

Induction of Diabetes. After baseline values were obtained, 15 rats received a single dose of streptozotocin (STZ) (Sigma Chemical Co., St. Louis, MO) intravenously, prepared in citrate buffer to adjust the pH to 4.5. Then 1 ml of this buffer was added to 10 ml of normal saline, final pH reading was 4.36–4.43. Then, 32.5 mg of STZ were preweighed into small tubes, using gloves, and 1 ml of the citrate buffer was added to each one just before injection to the rat. One tube was used for each animal. The STZ (50 mg/kg body wt) was administered slowly into the tail vein with a tuberculin syringe with 27-gauge needle after the animal had been lightly anesthetized with ether. No complications were observed. The animals were returned to their cages and housed two to three to a cage, with two bottles of water that were refilled twice daily. An additional 12 rats (normal controls) did not receive STZ. Normal and diabetic rats were fed the same standard rat chow for the next 12 days.

Twelve days after the induction of diabetes, a second set of 24-hr urine collections and blood samples were obtained in both normal and diabetic rats for determinations similar to those obtained at time 0.

Experimental Groups. At this time the animals were divided into four experimental groups: Group 1 consisted of six diabetic animals fed a diet containing 4% cholesterol (high cholesterol diet, HCD) and 2% cholic acid added to a basic diet 5012 (Ralston Purina). Group 2 consisted of nine diabetic animals fed a standard rat chow (basic diet 5012, Ralston Purina). Group 3 consisted of four normal rats fed a HCD and Group 4 consisted of eight normal rats fed a standard rat chow.

Another set of blood and urine determinations were obtained at Day 55. The animals had clearance studies done at Day 71, at which time blood was obtained for measurements of BUN, creatinine, albumin, cholesterol, triglycerides, and glucose.

Clearance Studies. Standard clearance studies were performed as reported previously (15). Briefly, under light ether anesthesia, a tail vein catheter (PE-50; Clay Adams, Parsippany, NJ) attached to a 25-gauge needle (Becton Dickinson, Rutherford, NJ), a femoral artery catheter (PE-10, Clay Adams), and a silicone bladder catheter (Silastic, American Scientific) were inserted. The rats were secured in plastic holders and 2 hr after recovery from anesthesia were studied in the awake state. A priming dose of chemical inulin (Sigma Chemical Co.) was infused in 0.6 ml of normal saline through the tail vein catheter over a 3-min period, followed by a sustaining infusion containing inulin delivered at 40 μl/min using a Harvard Apparatus Syringe Infusion Pump 22 (Southnatick, MA) to maintain plasma levels of inulin constant. Following an equilibration period of at least 60 min and approximately 3 hr after surgery, three consecutive urine collections were obtained. Blood was collected from the femoral artery into heparinized microhematocrit capillary tubes at the beginning, midpoint, and end of each collection period for the determination of inulin, sodium, and potassium. Urine was measured in preweighed tubes and volume was determinated by weight.

Histology. After the clearance studies were completed the animals were killed. The kidneys, heart, and aorta were harvested and weighed. Each kidney was cut sagittally and immersed in 5 ml of 10% formalin (Fisher Scientific) for subsequent histology (hematoxylin and eosin staining). Small sections of the kidney were embedded in glutaraldehyde for electron microscopy.

Analytic Determinations. Plasma glucose, cholesterol, creatinine, albumin, and triglycerides were meas-

ured using a Multistat III plus machine using reagents from Lexington, MA. Protein in the urine was determined by the BioRad protein assay dye method (BioRad, Richmond, CA). Sodium and potassium were determined using the IL 943 TM Automatic Flame Photometer (Instrumentation Laboratory, Lexington, MA). Inulin was measured using the anthrone method (16).

Calculations and Statistical Analysis. Clearances were calculated using standard formulas. For each rat, the values of three clearance periods were averaged. The same was done for chemical measurements in plasma. Results are expressed as mean ± SE and comparisons between groups were made by means of an unpaired Student's t test. Differences were considered significant when $P < 0.05$.

Results

Data for body weight and plasma chemistries in control rats and diabetic rats fed a "normal" or a high cholesterol diet at Day 0 (baseline) and at Days 13, 55, and 71 are shown in Table I. Baseline values for body weight, plasma glucose, plasma albumin, BUN, and plasma creatinine were not significantly different among the four groups of rats. The rats that received STZ, Groups 1 and 2, had significantly higher levels of plasma glucose throughout the study (Days 13, 55, and 71) than the control rats, Groups 3 and 4. There was, however, a tendency for plasma glucose to decrease toward the end of the period of observation (Day 71). This may be due to regeneration of β cells in the pancreas, a process which begins approximately 2 months after the administration of STZ.

The administration of a high cholesterol diet (Group 1) did not modify the hyperglycemia of diabetic rats. The diabetics rat fed a HCD had a greater body weight (237.5 ± 20.5 g) at the end of the study than the diabetic rats eating standard chow (201.8 ± 10.2 g). However, the difference in body weight between the two groups of rats was not significant by Day 71. Plasma albumin remained stable in all groups except in the diabetic rats fed standard chow. In these rats a significant fall in plasma albumin was seen at the end of the study period (Day 71) when compared with control rats or diabetic rats fed a HCD.

Diabetic rats fed a standard diet also had significantly higher levels of BUN by Day 13 than control rats, Groups 3 and 4. By Day 55 the levels of BUN were higher in diabetic rats than in controls and diabetic rats fed a standard diet reached their highest level of BUN by the end of the study period (Day 71). At both 55 and 71 days, the BUN levels were significantly higher in diabetic rats fed a standard diet (Group 2) than in diabetic rats fed a high cholesterol diet (Group 1). However, the values for serum creatinine were comparable between Groups 1 and 2 at Days 13 and 55.

Table I. Body Weight and Plasma Chemistries in Control Rats and Diabetic Rats Fed a Normal or a HCD[a]

	Weight (g)	Glucose (mg/dl)	Albumin (g/dl)	BUN (mg/dl)	Creatinine (mg/dl)
Day 0					
Group 1	196.8 ± 5.3	147.8 ± 2.7	3.38 ± 0.05	21.4 ± 0.8	0.57 ± 0.06
Group 2	191.4 ± 3.4	152.7 ± 4.8	3.40 ± 0.07	20.4 ± 1.1	0.50 ± 0.04
Group 3	189.5 ± 3.7	146.9 ± 2.2	3.40 ± 0.04	17.0 ± 1.8	0.65 ± 0.03
Group 4	184.8 ± 1.3	150.6 ± 1.3	3.49 ± 0.03	20.2 ± 0.2	0.58 ± 0.01
Day 13					
Group 1	194.5 ± 9.3	490.3 ± 90.7[b]	2.92 ± 0.16[b]	34.4 ± 6.4	0.70 ± 0.06
Group 2	198.9 ± 6.7	547.0 ± 26.7[c]	2.98 ± 0.10[c]	33.7 ± 3.7[c]	0.71 ± 0.03[c]
Group 3	218.0 ± 4.4	157.0 ± 3.80	3.03 ± 0.06	24.8 ± 1.0	0.58 ± 0.03
Group 4	210.3 ± 1.0	158.1 ± 1.40	3.33 ± 0.04	24.4 ± 0.2	0.63 ± 0.01
Day 55					
Group 1	222.3 ± 14.5	469.0 ± 107.7[b]	3.17 ± 0.14	24.0 ± 3.5[d]	0.73 ± 0.07
Group 2	190.2 ± 12.4[c]	423.5 ± 112.4[c]	3.01 ± 0.15	41.9 ± 5.0[c]	0.73 ± 0.02
Group 3	237.8 ± 10.1	108.6 ± 17.2	3.40 ± 0.15	27.5 ± 3.0	0.90 ± 0.07
Group 4	220.1 ± 1.90	141.4 ± 4.90	3.54 ± 0.03	24.4 ± 0.03	0.73 ± 0.03
Day 71					
Group 1	237.5 ± 20.5	351.6 ± 55.9[b]	3.00 ± 0.15[d]	29.4 ± 3.9[d]	1.17 ± 0.17[b,d]
Group 2	201.8 ± 10.2[c]	355.6 ± 55.2[c]	1.83 ± 0.19[c]	47.4 ± 8.3[c]	0.63 ± 0.08
Group 3	232.3 ± 6.30	174.9 ± 30.0	2.90 ± 0.19	29.8 ± 4.9	0.70 ± 0.14
Group 4	268.9 ± 2.28	170.0 ± 2.06	3.38 ± 0.05	26.0 ± 0.7	0.61 ± 0.02

[a] Group 1 (n = 6) was diabetic rats fed a HCD; Group 2 (n = 9) was diabetic rats fed a standard rat chow; Group 3 (n = 4) was normal rats fed a HCD; and Group 4 (n = 8) was normal rats fed a standard rat chow. Values are mean ± SE for the different groups of rats studied. A $P < 0.05$ was considered statistically significant.
[b] Group 1 versus Group 4.
[c] Group 2 versus Group 4.
[d] Group 1 versus Group 2.

Creatinine values were significantly higher at Day 71 in Group 1 rats than in Group 2 rats. This suggests that the increase in BUN noted in rats of Group 2 was not related to a greater fall in glomerular filtration rate in this group of rats when compared with those fed a HCD.

The values for plasma cholesterol and triglycerides in the four groups of rats are depicted in Figure 1. At Days 55 and 71, the diabetic rats fed a HCD had plasma cholesterol levels that were significantly greater than those seen in diabetic rats fed a standard chow diet. The normal rats fed a HCD had an initial increase (Day 55) in plasma cholesterol levels that was significantly different from the levels seen in normal animals fed a standard chow ($P < 0.001$) and comparable to the values seen in diabetic rats fed a standard diet. However, this difference in cholesterol levels between Groups 2 and 3 and Group 4 disappeared by the end of the study period (Day 71).

Rats with experimental diabetes had higher levels of plasma triglycerides than control rats at Days 13, 55, and 71. By Day 55, the diabetic rats fed a standard chow had higher levels of serum triglycerides than the diabetic rats fed a HCD. By Day 71, both groups of diabetic rats were hypertriglyceridemic when compared with control rats. However, only the difference in plasma triglyceride levels between the diabetic rats fed a HCD and the normal group fed a standard diet was significant.

Table II shows the data of 24-hr urine volumes and 24-hr urine excretion of protein, sodium, and potassium. In the basal state (Day 0) there were no differences in urine volumes, proteinuria, or electrolyte excretion in the urine among the four groups of rats studied. By Day 13, the diabetic rats had significantly greater urine volumes than the control rats. By Day 55, the diabetic rats fed a standard diet (Group 2) had significantly higher urine volume per day than the diabetic group fed a HCD (Group 1) and than the control groups (3 and 4). The diabetic rats fed a HCD excreted less sodium and potassium in the urine by Day 55 than the diabetic rats fed a standard diet. Diabetic rats fed a HCD also excreted less protein in the urine at Day 55 than the diabetic rats fed a standard chow, although the difference did not quite reach statistical significance. At Day 55, diabetic rats fed a standard chow diet excreted significantly more protein than normal rats fed a standard chow diet.

Data of urea excretion in 24-hr urine collections in the four groups of rats are shown in Figure 2. The diabetic rats excreted greater amounts of urea than the control rats. Although urea excretion between the two groups of diabetic rats was not significantly different at Day 13, by Day 55 the diabetic rats fed a HCD had a significantly lower excretion of urea than the diabetic rats fed a standard diet.

Figure 3 presents data on creatinine excretion in the urine in the four groups of rats studied. Diabetic rats fed a standard diet had the highest values of creatinine excretion among the four groups. The excretion of creatinine was significantly lower in diabetic rats fed a HCD when compared with diabetic rats fed a standard diet.

Clearance Studies. The data of clearances studies are summarized in Table III. No statistical difference in the values for glomerular filtration rate was seen between the two groups of diabetic rats. Absolute and fractional excretion of sodium were greater in the diabetic rats fed a standard diet (Group 2), but only reached statistical significance when compared with

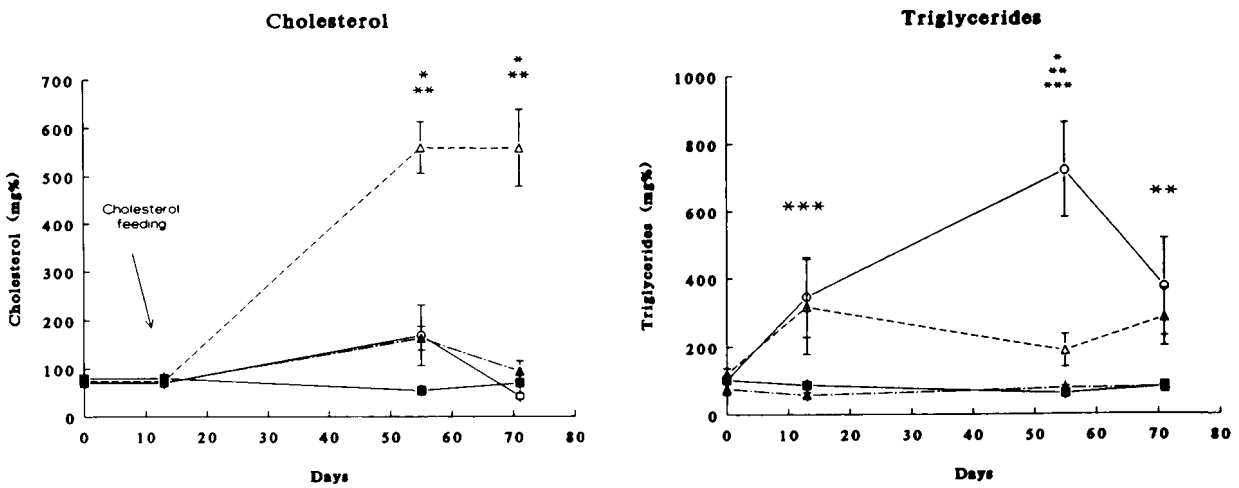

Figure 1. Concentrations of plasma cholesterol and triglycerides in the four groups of rats. The values are the mean ± SE for six diabetic rats fed a HCD (Group 1, △), nine diabetic rats fed a standard rat chow (Group 2, ○), four normal rats fed a HCD (Group 3, ▲), and eight normal rats fed a standard rat chow (Group 4, ■). Values for plasma cholesterol were significantly greater at Days 55 and 71 ($P < 0.05$) in Group 1 as compared with Group 2 (*); Group 1 versus Group 4 (**) and Group 2 versus Group 4 (***). Similar symbols for significant differences among groups were used for triglyceride levels.

Table II. Twenty-Four-Hour Urine Volume, Protein, and Electrolyte Excretion in Diabetic Rats and Control Rats Fed a HCD or a Standard Diet[a]

	Urine volume (ml/24 hr)	Proteinuria (mg/24 hr)	Urinary sodium (mEq/24 hr)	Urinary potassium (mEq/24 hr)
Day 0				
Group 1	6.50 ± 0.5	6.39 ± 1.25	0.73 ± 0.10	2.15 ± 0.09
Group 2	7.30 ± 0.9	6.43 ± 1.32	0.65 ± 0.09	1.91 ± 0.25
Group 3	10.50 ± 2.3	11.00 ± 1.52	0.97 ± 0.22	2.26 ± 0.33
Group 4	5.88 ± 0.7	9.98 ± 3.76	0.51 ± 0.03	1.23 ± 0.49
Day 13				
Group 1	62.80 ± 15.1[b]	59.10 ± 21.4	1.94 ± 0.16[b]	4.00 ± 0.36
Group 2	97.90 ± 14.3[c]	75.70 ± 19.5	1.99 ± 0.26[c]	4.32 ± 0.43
Group 3	17.80 ± 2.60	31.10 ± 5.10	1.31 ± 0.18	2.41 ± 0.76
Group 4	11.50 ± 0.55	21.90 ± 7.95	0.92 ± 0.05	4.09 ± 0.22
Day 55				
Group 1	26.70 ± 8.40[d]	26.60 ± 4.30	0.64 ± 0.15[b,d]	2.30 ± 0.23[d]
Group 2	104.70 ± 15.0[c]	59.40 ± 11.6[c]	1.83 ± 0.25[c]	4.74 ± 0.39[c]
Group 3	4.80 ± 2.20	5.11 ± 2.20	0.28 ± 0.12	0.72 ± 0.36
Group 4	18.50 ± 0.56	12.80 ± 2.27	0.11 ± 0.04	2.35 ± 0.13

[a] Group 1 ($n = 6$) was diabetic rats fed a HCD; Group 2 ($n = 9$) was diabetic rats fed a standard rat chow; Group 3 ($n = 4$) was normal rats fed a HCD; and Group 4 ($n = 8$) was normal rats fed a standard rat chow. Values are mean ± SE for the different groups of rats studied. A $P < 0.05$ was considered statistically significant.
[b] Group 1 versus Group 4.
[c] Group 2 versus Group 4.
[d] Group 1 versus Group 2.

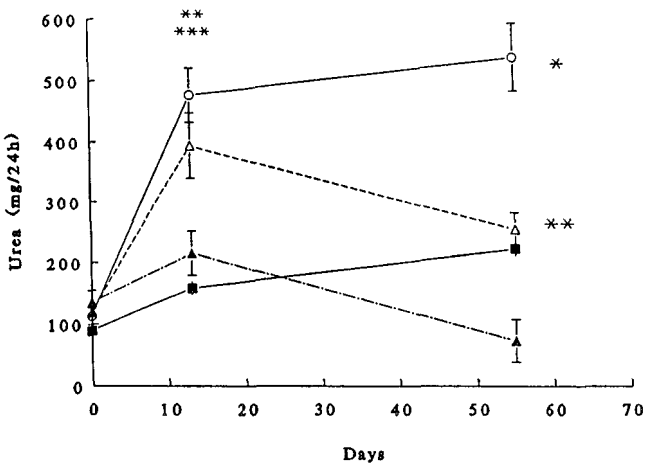

Figure 2. Urea excretion in the urine in the four groups of rats. Urea excretion is expressed as mean ± SE for nine diabetic rats fed a standard rat chow (Group 2, ○), six diabetic rats fed a HCD (Group 1, △), four normal rats fed a HCD (Group 3, ▲), and eight normal rats fed a standard rat chow (Group 4, ■). The values for urea excretion were significantly greater in rats of Group 2 ($P < 0.05$) than in those in Group 1 (*) or Group 4 (***). Rats of Group 1 also had significantly greater urea excretion ($P < 0.05$) than rats of Group 4 (**).

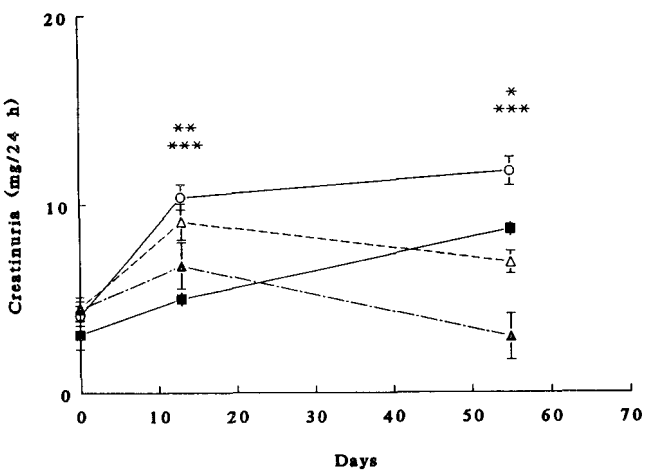

Figure 3. Creatinine excretion in the urine in the four groups of rats. Values are mean ± SE in the four groups of rats studied. Rats in Group 2 (diabetic rats fed a standard diet) had significantly greater excretion of creatinine in the urine than rats of Group 2 (diabetics fed a HCD) or Group 4 (normal rats fed a standard diet). Significance $P < 0.05$ (* Group 1 versus Group 2; ** Group 1 versus Group 4, *** Group 2 versus Group 4).

normal rats eating a standard diet. The fractional sodium excretion of the diabetic rats fed a HCD was higher than that of normal rats fed standard chow. Normal rats fed a HCD had a significantly lower glomerular filtration rate when compared with normal rats eating standard diet.

Table IV shows data for body weight, heart and kidney weights, and the ratio of heart and kidney weight to body weight. The diabetic rats fed a standard chow (Group 2) had the highest ratios of heart weight to body weight and kidney weight to body weight.

Histology. All of the diabetic animals fed a diet of standard chow showed a distinctive lesion in the kidneys examined with light microscopy in routine tissue stains (hematoxylin and eosin). This lesion consisted of

Table III. Results of Clearance Studies in the Four Groups of Rats[a]

Group	Urine flow (μl/min)	C_{in} (ml/kg/min)	$U_{Na}V$ (μEq/min)	FE_{Na} (%)	FE_K (%)
1 ($n = 6$)	28.9 ± 9.37	9.77 ± 2.16	3.49 ± 1.58[b]	0.96 ± 0.26	17.1 ± 1.8
2 ($n = 9$)	37.0 ± 3.40	11.95 ± 1.63	4.53 ± 0.93[c]	1.30 ± 0.19	17.5 ± 1.9
3 ($n = 4$)	27.1 ± 7.90	6.92 ± 1.26	2.92 ± 0.48	1.02 ± 0.25	23.4 ± 2.8
4 ($n = 8$)	44.4 ± 9.00	11.49 ± 0.91	1.62 ± 0.18	0.37 ± 0.07	19.7 ± 2.0

[a] Group 1 was diabetic rats fed a HCD; Group 2 was diabetic rats fed a standard rat chow; Group 3 was normal rats fed a HCD; and Group 4 was normal rats fed a standard rat chow. Values are mean ± SE for the different groups of rats studied. A $P < 0.05$ was considered statistically significant.
[b] Group 1 versus Group 4.
[c] Group 2 versus Group 4.

Table IV. Body Weight and Heart and Kidney Weight in Control Rats and Diabetic Rats Fed a Normal or a HCD[a]

Group	Body weight (g)	Heart weight (mg)	Kidney weight (mg)	Heart/body weight ($\times 10^{-3}$)	Kidney/body weight ($\times 10^{-3}$)
1 ($n = 6$)	237.5 ± 20.5	803 ± 33[b]	1943 ± 80	3.52 ± 0.22	8.53 ± 0.63
2 ($n = 9$)	201.8 ± 10.2	790 ± 50	1995 ± 13	4.06 ± 0.49	9.98 ± 0.71[c]
3 ($n = 4$)	232.3 ± 6.3	675 ± 26	1585 ± 80	2.91 ± 0.008	6.82 ± 0.28
4 ($n = 8$)	268.9 ± 2.28	760 ± 10	1730 ± 20	2.43 ± 0.13	7.14 ± 0.15

[a] Group 1 was diabetic rats fed a HCD; Group 2 was diabetic rats fed a standard rat chow; Group 3 was normal rats fed a HCD; and Group 4 was normal rats fed a standard rat chow. Values are mean ± SE for the different groups of rats studies. A $P < 0.05$ was considered statistically significant.
[b] Group 1 versus Group 2.
[c] Group 2 versus Group 4.

swelling of tubular epithelial cells with clearing of cytoplasm. The segment of the nephrons involved by this striking vacuolar change was obviously the distal convoluted tubule and thick limbs of Henle's loop. First, proximal tubular segments were uniformly normal. Second, the numerical distribution of hydropic segments was that of distal tubules, and normal distal tubules could not be found. Third, it was possible to find vacuolated tubular segments in relationship with afferent arterioles at the glomerular hilum characteristic of the macula densa (Fig. 4A), a distal tubular structure. Because tissue samples processed for light microscopy were fixed in aqueous fixatives (10% neutral formalin), it was not possible specifically to identify the contents of the tubular epithelial vacuoles as glycogen. (Periodic acid-Schiff stains, for example, were negative.) In spite of that, the histologic features of the lesion were those described as "Armanni Ebstein lesion" in severely glycosuric patients (or animals) with diabetes. Samples of kidneys fixed in glutaraldehyde and processed for electron microscopy showed rarifaction of cytoplasm of some tubules with several features of distal tubules. Dispersion of cytoplasmic organelles was conspicuous and accumulation of small electron-dense rosettes characteristic of glycogen was obvious (data not shown).

These changes were not observed in any of the animals of the other three groups (diabetic animals fed a high cholesterol diet, nondiabetic animals fed a high cholesterol diet, and nondiabetic animals fed a standard chow). Glomeruli were normal in animals of all groups. There was no glomerulosclerosis.

Microscopic features of the aortas in all animals in all groups were normal. Specifically, there were no features of arteriosclerosis.

Discussion

The results of this study indicate that some of the abnormalities that accompany the development of diabetes mellitus in rats given streptozotocin are ameliorated by the administration of a high cholesterol diet. Diabetic rats fed a high cholesterol diet, in the absence of insulin replacement, had better metabolic and nutritional parameters than diabetic animals fed standard chow, despite similar levels of blood glucose. Diabetic rats fed a high cholesterol diet exhibited greater growth, had higher levels of serum albumin, and excreted less creatinine, urea, and protein in the urine. In addition, urine excretion of sodium and potassium was also less in these rats than in the diabetic rats fed a standard diet. In these studies we did not observe any atherosclerosis in diabetic rats fed either a high cholesterol diet or standard chow. This may be due to the animal model used, since the Sprague-Dawley rat is not as prone as

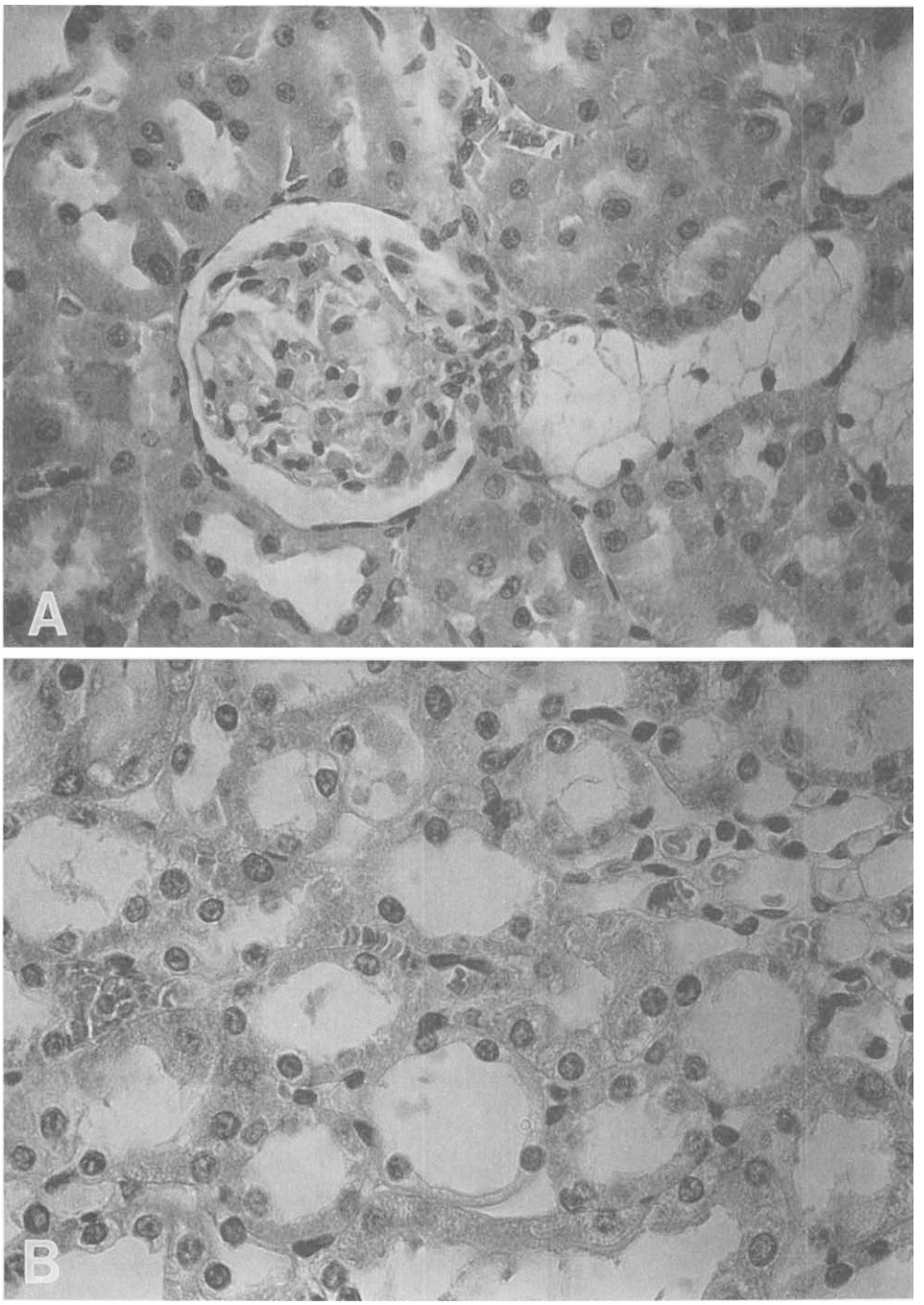

Figure 4. (A) Photomicrograph of renal cortex of a diabetic rat fed standard chow. The distal tubule that abuts on the juxtaglomerular apparatus is lined by swollen cells with clear cytoplasm (hematoxylin and eosin; original magnification ×600). (B) Photomicrograph of renal cortex of a diabetic rat fed a HCD. The distal tubules are normal (hematoxylin and eosin; original magnification ×800).

the rabbit to develop atherosclerosis and also to the duration of the experiments. The development of atherosclerosis in the rat after high cholesterol feeding may require as long as 1 year (17), whereas spontaneous atherosclerosis has been described in old rats (18). In the absence of histologic evidence of atherosclerosis in our studies, we found that other major manifestations of uncontrolled diabetes were less severe in the diabetic rats fed a high cholesterol diet. The decreased urea excretion and the lower BUN in these rats as compared with those fed a standard diet, despite comparable glomerular filtration rate values, suggests a lesser degree of protein catabolism. The decreased creatinine excretion in diabetic rats fed cholesterol as compared with diabetic animals fed a standard diet also suggest decreased muscle breakdown in the former group of rats. Weight gain was also greater in the diabetic animals fed cholesterol. Although no changes in levels of blood

glucose were discernible between the two groups of diabetic rats, the data clearly indicate that protein catabolism and muscle wasting were less marked in diabetic rats fed cholesterol. Also, these rats had higher plasma levels of albumin than rats fed a standard rat chow.

The mechanisms responsible for improved growth, decreased creatinine and urea excretion in the urine, and higher plasma levels of albumin in diabetic rats fed cholesterol as compared with those fed a standard diet cannot be elucidated from the present experiments. Cholesterol supplementation appears to decrease protein catabolism and muscle breakdown, as evidenced by decreased urea and creatinine excretion in these diabetic rats. The exact mechanism underlying these findings remains to be determined.

The most remarkable finding of this study was the prevention of the appearance of the Armanni-Ebstein lesion (19) in the kidneys of diabetic rats fed cholesterol. These lesions are mainly confined to the cells of the distal tubule to the level of the macula densa. The pathologically affected cells did not have a brush border and did not contain lysosomes and endocytic vacuoles characteristic of the cells of the proximal tubule (19). On electron microscopy these affected cells had a few rounded microvilli on the surface, but most importantly the cytoplasm of these cells contained minute diffusely distributed granules (20–35 nm) characteristic of glycogen particles. Rasch (19) reported that these lesions are confined to the cortex and the outer stripe of the outer medulla at the level of the distal tubule and in the macula densa. In man, the Armanni-Ebstein lesion was described last century in the kidneys of individuals with diabetes (20, 21). The introduction of insulin made this lesion somewhat less common, but the characteristic tubular changes of the Armani-Ebstein lesion are still seen in some insulin-treated diabetics. In man it is agreed that the lesion is most pronounced at the corticomedullary junction. Ebstein (21) suggested that these lesions were confined to the loop of Henle, and Armanni (20) identified the collecting duct as the site of the lesions. Tubular lesions have also been observed in animals with experimental diabetes.

In the present studies, these lesions were present only in the diabetic animals fed a standard diet. They were not seen in the diabetic rats fed a high cholesterol diet. The mechanism leading to the prevention of glycogen accumulation at the level of distal tubular cells in the diabetic rats fed cholesterol remains to be elucidated. It has been proposed that the high glucose concentrations to which these cells are exposed could influence the synthesis and accumulation of glycogen in the kidney tubular cells (19) and account for the development of this lesion. Since both groups of rats had comparable levels of hyperglycemia, this seems an unlikely explanation for the differences observed. In addition, it is noteworthy that insulin replacement (22) as well as HCD can prevent the formation of the Armanni-Ebstein lesion. It is possible that cholesterol supplementation in some way impairs the uptake of glucose, and the subsequent synthesis of glycogen, in cells of the distal tubule.

It is of interest that diabetic rats with distal tubular accumulation of glycogen (Group 2) had greater 24-hr urine volumes, greater sodium and potassium excretion, and somewhat greater protein excretion than diabetic animals without such lesions (Group 1). These differences in salt and water excretion occurred in the presence of similar levels of filtered glucose (both glomerular filtration rate and plasma glucose levels were not significantly different between the two groups of diabetic rats). This suggests that other mechanisms besides the osmotic effect of glucose are responsible for the differences in salt and water excretion. It is tempting to speculate that impaired tubular function, due to glycogen accumulation in rats of Group 2, is responsible for the difference in salt and water excretion observed between the two groups of diabetic rats. The differences in proteinuria between the two groups may also relate to decreased protein reabsorption by the kidney in diabetic rats fed a standard chow (Group 2).

This study demonstrates that in the STZ-induced rat model of uncontrolled insulin-dependent diabetes mellitus, high cholesterol feeding ameliorates some of the usual components of the diabetic nephropathy syndrome such as proteinuria, hypoalbuminemia, weight loss, and the typical tubular lesions of Armanni-Ebstein (glycogen deposits), despite the persistence of high levels of plasma glucose. The mechanism by which cholesterol feeding prevents the development of glycogen accumulation in distal tubular cells in diabetic rats remains to be defined.

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