

The Effect of Dietary Protein and Sucrose on the Diabetic Rat Kidney

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Summary

The effect of 3 months feeding with diets of different protein and sucrose content (9 % casein + 70 % sucrose vs. 18 % casein + 61 % sucrose) on the development of diabetic nephropathy and changes in serum lipid spectrum was investigated in rats with insulin-dependent diabetes (streptozotocin 45 mg.kg⁻¹). Metabolism of diabetic animals (before the nutritional regimen) was characterized by hyperglycaemia, moderate hyperlipidaemia, lipid accumulation in the liver and elevated creatinine concentration in the blood. Kidney weight and protein content were not significantly changed. Histological picture of kidneys showed initial changes of glomerular structure. After three months hyperlipoproteinaemia was more accentuated in animals given either of the two diets, the kidneys were hypertrophic with a higher protein content and displayed morphological changes of diabetic nephropathy. Animals given the low-protein diet developed smaller morphological changes both in glomeruli and tubuli. The study indicates that dietary protein and not hyperlipoproteinaemia is the major factor, which may significantly influence the progress of diabetic nephropathy.

Key words

Insulin-dependent diabetes – Rats – Hyperlipoproteinaemia – Kidneys – Low protein diet – Sucrose

Introduction

Diabetic nephropathy is a serious complication of diabetes, affecting 30–40 % of young diabetics. It is characterized by proteinuria, hypertension and a progressive decline of renal function. Following factors are considered to play a role in the pathogenesis: 1. haemodynamic changes, 2. morphological and functional disorders caused by non-enzymatic protein glycosylation, 3. increased glucose utilization *via* the polyol pathway, 4. disorders in collagen and proteoglycan synthesis and metabolism, and 5. abnormalities in lipid metabolism (Cohen 1989). Attention has been directed not only to the pathogenesis or effective therapy, but also to nutritional factors which can considerably influence these changes and kidney function. Several experimental studies showed that long-term feeding a diet with a higher sucrose content provokes albuminuria, kidney hypertrophy and diffuse

glomerulosclerosis (Cohen and Rosenmann 1971, Kang *et al.* 1982). Some authors observed an additive effect of the diet in diabetes (Kang *et al.* 1982). However, others failed to confirm this (Taylor *et al.* 1980). Diet with added cholesterol not only elevates systolic blood pressure and albuminuria but also causes focal glomerulosclerosis in experimental animals (Kasiske *et al.* 1990). An increased protein content in the diet is also considered to be a factor supporting the development of glomerulosclerosis and alterations of renal function (Masoro 1989). On the contrary, it was repeatedly shown that a low-protein diet slows down the progress of nephropathy and improves kidney function in patients (Viberti and Keen 1984, Zeller *et al.* 1991), as well as in experimental diabetic animals (Wen *et al.* 1985). A similar protective effect was exerted by fish oil (Ito *et al.* 1985) and ethylesters of eicosapentanoic acid (Hamazaki *et al.* 1990).

Based on this knowledge, we decided to study the effects of the diet on the development of diabetic nephropathy and changes of serum lipid spectrum in rats with insulin-dependent diabetes given a low-protein diet combined with sucrose.

Material and Methods

Male rats of the Wistar strain (Dobrá Voda) with body weight 319 ± 5 g were used for the experiments. Diabetes was induced by an i.v. injection of streptozotocin (45 mg.kg^{-1} body weight) in citrate buffer, pH 4.5. Control animals were given the buffer only. Detailed characteristics of the model were presented elsewhere (Chorváthová *et al.* 1981). Diabetic animals were given Superdep insulin (6 U.kg^{-1}) s.c. every day between 1300 and 1400 h in the course of the experiment (7 days or 3 months, respectively). On the 8th day, 12 control animals and 21 diabetic animals were decapitated to assess the acute metabolic and morphological changes evoked by the diabetes. The remaining diabetic animals, divided into 2 subgroups, were given semisynthetic diets for three months with a different protein and carbohydrate content ($\text{g}/100 \text{ g}$): sucrose 70 or 61, and casein 9 or 18, lard 10, mineral and vitamin mixture 4 or 1, cellulose 6. Content of other nutrients, phosphate, calcium and energy (1608 and 1624 kJ, respectively) was the same.

Satiated animals (without restricted food intake) were decapitated after 12 weeks.

Levels of blood glucose, triglycerides, total cholesterol, protein and serum creatinine were assessed with BIO-LA-Tests, the kidney protein content was measured by the method of Lowry *et al.* (1951). Cholesterol concentration in Folch's extract was determined according to Lieberman-Buchard, levels of triglycerides (phospholipids were removed with silicagel) using the BIO-LA-Test kits. Lipoproteins were isolated by gradual flotation on a preparative ultracentrifuge from pooled samples of two animals. Their respective densities were: very low density lipoproteins (VLDL, $d < 1.006$), intermediate density lipoproteins (IDL, $d < 1.019$), low density lipoproteins (LDL, $d < 1.063$) and high density lipoproteins (HDL, $d < 1.21 \text{ kg.m}^{-1}$) (Havel *et al.* 1955). Cholesterol in isolated lipoproteins was determined by the method of Zlatkis *et al.* (1953) and triglycerides according to Carlson (1963).

Material for histological evaluation was fixed in 10 % formol containing a phosphate buffer. Histological and histochemical methods used were: haematoxylin-eosin, PAS, PAS after digestion with diastase, Mallory's phosphowolfram haematoxylin and Oil Red for evaluation of cryostat sections (Pearse 1960). Statistical significance of results was analyzed by Student's t-test. Linear regression was used for correlation of kidney weight with selected indicators.

Table 1
Effect of diabetes and diets on selected characteristics of rats

	Controls	Diabetes (7 days)	Diabetes (3 months)	
			9 % protein	18 % protein
Number of animals	(12)	(21)	(18)	(21)
Body weight (g)	312.5 \pm 6.6	273.8 \pm 4.8**	341.6 \pm 13.0##	344.7 \pm 13.0##
Kidney				
Weight (g)	0.91 \pm 0.02	0.93 \pm 0.02	1.46 \pm 0.09##	1.42 \pm 0.06##
Weight (%)	0.28 \pm 0.01	0.33 \pm 0.01**	0.44 \pm 0.03##	0.42 \pm 0.02##
Protein content (mg)	133.8 \pm 3.0	135.7 \pm 3.2	186.7 \pm 5.3##	177.6 \pm 5.25##
Serum				
Glucose (mmol.l^{-1})	5.07 \pm 0.16	21.05 \pm 0.54**	22.38 \pm 1.67	19.64 \pm 1.25
Creatinine ($\mu\text{mol.l}^{-1}$)	77.3 \pm 2.7	115.3 \pm 6.6**	100.2 \pm 3.8	99.8 \pm 5.3+###
Total protein (g.l^{-1})	—	—	71.1 \pm 2.2	72.8 \pm 2.2

Significant differences: *** $P < 0.05$, 0.001 controls vs diabetes #, ## $P < 0.05$, 0.001 diabetes (7 days vs 3 months); +, ++ $P < 0.05$ 0.001 diabetes (3 months) (9 % vs 18 % protein)

Results

Significantly higher glycaemia and creatinine concentrations were observed in diabetic animals in compared to the controls. Kidney weight and protein content were the same as in the controls (Table 1). The histological sections revealed enlarged glomeruli with capillary dilatation and slightly thickened mesangium in glomerular hili (Fig. 1). Basement membranes were more accentuated than in the controls. These changes were found in 90 % of animals with acute diabetes.

Cholesterol and triglycerides were significantly influenced by diabetes, the levels of both indicators being elevated in the serum and liver (Table 2). An increase in cholesterolaemia was reflected by changes in the lipoprotein spectrum, resulting in elevated cholesterol concentrations in VLDL, LDL and HDL (Table 3). VLDL and its final metabolite LDL participated considerably in the triglycerides increase in diabetic animals (Table 4). Body weight of diabetic animals declined by 10.5 % compared to the controls.

Table 2
Effect of diabetes and diets on serum lipids and liver lipids in rats

	Controls	Diabetes (7 days)	Diabetes (3 months)	
			9 % protein	18 % protein
Number of animals	(12)	(21)	(18)	(21)
Serum				
Triglycerides (mmol.l ⁻¹)	1.21±0.11	2.60±0.31*	28.04±8.61#	14.70±3.13##
Cholesterol (mmol.l ⁻¹)	1.83±0.06	2.70±0.15**	5.00±0.87#	5.02±0.57##
Liver				
Triglycerides (mmol.l ⁻¹)	13.47±1.39	19.19±1.84*	39.80±3.62##	35.34±3.07##
Cholesterol (mmol.l ⁻¹)	6.28±0.25	6.15±0.16	7.72±0.45#	9.61±0.45+##

Significant differences: *,** P<0.05, 0.001 controls vs diabetes #, ## P<0.05, 0.001 diabetes (7 days vs 3 months); +, ++ P<0.05 0.001 diabetes (3 months) (9 % vs 18 % protein)

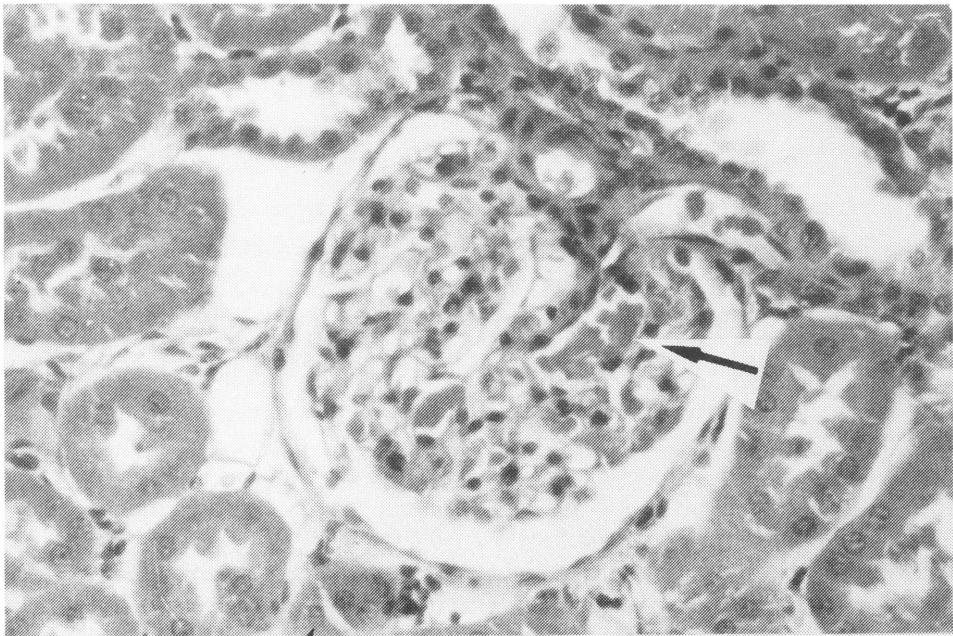


Fig. 1
Glomerulus with dilatation of capillaries and erythrocyte accumulation (arrow) in acute diabetes (haematoxylin-eosin, x400)

Table 3
Cholesterol concentrations in lipoproteins of diabetic rats and controls

	Controls	Diabetes (7 days)	Diabeters (3 months)	
			9 % protein	18 % protein
Number of animals	(7)	(9)	(9)	(9)
VLDL	0.19±0.01	0.46±0.04**	1.36±0.47#	1.77±0.52#
%	10.7 ±0.7	17.8 ±1.4*	22.1 ±4.1	30.5 ±0.05#
IDL	–	–	0.61±0.33	0.46±0.19
%	–	–	8.0 ±3.1	7.7 ±1.80
LDL	0.28±0.03	0.37±0.02*	1.14±0.33#	1.11±0.27#
%	15.8 ±1.3	14.3 ±0.6	19.5 ±2.8	20.8 ±1.60#
HDL	1.3 ±0.06	1.76±0.13*	1.82±0.14	1.46±0.11
%	73.5 ±1.4	67.9 ±1.8*	50.4 ±8.8	41.0 ±0.69#

Significant differences: *,** P<0.05, 0.001 controls vs diabetes #, ## P<0.05, 0.001 diabetes (7 days vs 3 months); +, ++ P<0.05 0.001 diabetes (3 months) (9 % vs 18 % protein)

Table 4
Triglyceride concentrations in lipoproteins of diabetic rats and controls

	Controls	Diabetes (7 days)	Diabetes (3 months)	
			9 % protein	18 % protein
Number of animals	(7)	(9)	(9)	(9)
VLDL	0.85±0.09	1.32±0.15*	14.55±4.21#	7.81±2.70#
%	74.9 ±1.8	63.6 ±2.9*	69.6 ±2.9	68.9 ±1.3
IDL	–	–	3.93±1.33	2.75±0.95
%	–	–	21.4 ±2.2	18.4 ±1.9
LDL	0.23±0.04	0.60±0.07*	1.36±0.53	0.82±0.16
%	19.4 ±1.9	28.6 ±0.01*	6.5 ±0.7	10.0 ±1.5+
HDL	0.06±0.01	0.17±0.02**	0.43±0.13	0.22±0.04
%	5.7 ±0.7	8.4 ±0.7*	2.4 ±0.3##	2.7 ±0.3##

Significant differences: *,** P<0.05, 0.001 controls vs diabetes #, ## P<0.05, 0.001 diabetes (7 days vs 3 months) +, ++ P<0.05 0.001 diabetes (3 months) (9 % vs 18 % protein)

After three months of diabetes and the respective diets, glycaemia and creatinine level remained the same as those found in animals with acute diabetes. Kidney weight increased by 50 % and the kidney protein content rose by 30 % in both groups, despite the different protein content of the diet (Table 1). A positive correlation between kidney weight and glycaemia in both groups of diabetic animals was observed (r=0.73, P<0.001 and r=0.57, P<0.02). Positive correlation was also found between the weight and protein content of the kidney (r=0.88, P<0.001 and r=0.91; P<0.001). Proteinuria was observed in 70 % of diabetic animals.

The histological picture of kidneys in animals given the sucrose diet with 9 % protein showed slight thickening of basement membranes of glomerular capillaries, more accentuated thickening of the mesangium, hyaline thickening and arteriolar dilatation. Typical Armanni-Ebstein cells were found in tubuli of 40 % of animals (Fig. 2). Glomerular and tubular lesions affected 70 % of animals. Histological changes of glomeruli and tubuli were more marked in animals given the sucrose diet containing 18 % of protein; 90 % of animals in this group were affected. More severe intercapillary nodular lesions with destruction of mesangial cells were found (Fig. 3).

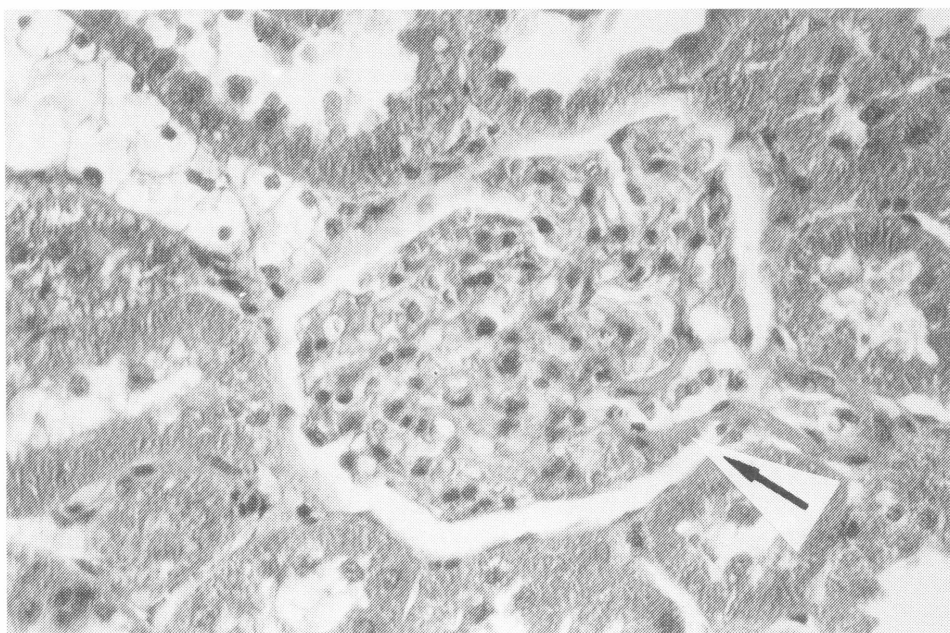


Fig. 2

Hyaline thickening of arteriolar wall and of a section of its branching in a glomerulus (arrow). In the left upper corner light Armani-Ebstein cells (chronic diabetes, 9 % protein with sucrose) (haematoxylin-eosin, x400)



Fig. 3

Intercapillary nodular thickening of mesangium with hyperplasia and necrosis of mesangial cells (arrow). Dilatation of capillaries surrounding the mesangial "nodular lesion" (chronic diabetes, 18 % protein with sucrose) (haematoxylin-eosin, x400)

In comparison to the other group, diffuse thickening of the mesangium and glomerulosclerotic lesions with lobulation and atrophy of glomeruli were found (Fig. 4). Armani-Ebstein cells were observed in 60 % of animals. Dystrophic changes were

accompanied in 30 % of cases by deposition of calcium salts in the distal tubuli.

As the effect of diabetes and the diet, there was a major increase in the level of triglycerides, especially in animals given the diet with 70 % of

sucrose and 9 % of protein. Cholesterolaemia increased in both groups regardless of the sucrose and/or protein content of the diet (Table 2). When considering the lipoprotein spectrum, the increase was most evident in the VLDL fraction, but it was also present to a lesser extent in their metabolites (IDL, LDL) (Tables 3 and 4). Due to the large variance of values, the differences between both dietary regimes

were either not significant or only borderline significant. Accumulation of triglycerides in the liver was the same in both groups, elevated cholesterol levels were found in the group receiving the diet with a higher protein content (Table 2). Body weights of diabetic animals were the same at the end of the three-months observation period (Table 1).



Fig. 4

Glomerulosclerosis with glomerular lobulation and atrophy (right lower corner) and hyaline thickening of arterioli, Armani-Ebstein cells (left) (chronic diabetes, 18 % protein and sucrose) (PAS, x400)

Discussion

In the present study, both diets elevated triglyceridaemia and cholesterolaemia, regardless of the different protein content of either diet. Several factors could be responsible for this hyperlipoproteinaemia: 1. hyperglycaemia and subsequent glycosylation of lipoprotein lipase lead to a slower catabolism of primary lipoproteins (Niskanen *et al.* 1990), 2. sucrose stimulates the synthesis and secretion of primary lipoproteins (Kazumi *et al.* 1989), and 3. loss of smaller molecules caused by impaired kidney function (albumin, HDL, apoproteins) could contribute to the described changes (Jensen 1988). Accumulation of primary lipids and their metabolites, and apparently the decreased participation of HDL in cholesterol transport, represent important risk factors in the pathogenesis of glomerular injury, potentiating the development of focal glomerulosclerosis (Keane *et al.* 1988).

Kidneys of experimental animals were heavier and their protein content was higher, regardless of the different protein content. This is in accordance with the

findings of other authors (Wen *et al.* 1985). Dilatation of glomerular capillaries and tubuli with signs of hypertrophy and hyperplasia were the concomitant morphological correlate of these changes. Changes characteristic for experimental diabetes can be attributed to glomerular hyperfiltration with a subsequent elevation of glomerular volume and capillary filtration surface area (Ellis *et al.* 1986). Besides metabolic factors and vasoactive systems (Hostetter 1992), the development and persistence of glomerular hyperfiltration in diabetic animals is also influenced by increased protein intake (O'Donnell *et al.* 1988). Glomerular hyperfiltration is associated with increased permeability of glomerular capillaries, which enables deposition of macromolecules into the mesangium and subsequent development of mesangial lesions and glomerular sclerosis (Wen *et al.* 1985). In our experiment, the sucrose diet with 18 % of protein stimulated the development of changes from mesangial lesions (Fig. 3) to glomerulosclerosis (Fig. 4). The low-protein diet was shown to reduce glomerular filtration in experimental animals (Meyer *et al.* 1983). However, in our experiment with 9 % protein diet glomerular

and tubular lesions were not eliminated, but their progression was slowed. Both diets, but especially that containing 18 % of protein, accentuated arteriolar lesions and lesions in the vascular pole of glomeruli indicating arteriolar hyalinosis, which is usually found in human diabetic nephropathy (Mogensen *et al.* 1981).

The dietary regimen could contribute to metabolic disorders similar to human diabetes, as indicated by changes observed in the lipid spectrum (Keane *et al.* 1988). We do not think that we succeeded to induce identical changes because only focal lesions were found in experimental model and nodular sclerosis was not observed. Nevertheless, the study indicates that the selected dietary regimen, as well as the strain of experimental animals can serve as a model

for the induction of a broad scale of metabolic and morphological changes in experimental diabetes.

Low protein intake did not eliminate diabetic nephropathy but it slowed its development and degree. It can be assumed that the effects of low-protein diet could even be more pronounced if other carbohydrate components were selected, e.g. starch, which supports hypertrophy and development of glomerulosclerosis in the kidney, stimulates lipogenesis and influences the lipoprotein spectrum, as shown by several studies (Kazumi *et al.* 1989) as well as by our results.

Finally, we can conclude that the major factor, which may considerably influence the progress of diabetic nephropathy is dietary protein, and not hyperlipoproteinaemia.

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