Effects of magnesium and vitamin E on the development of nephropathy in diabetic rats

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Zusammenfassung

Männliche Streptozotocin-diabetische und nicht-diabetische Wistar-Ratten erhielten 5½ Monate lang Mg-arme (80 ppm Mg), normale (620 ppm Mg) und Mg-supplementierte (5800 ppm Mg) Diät mit niedrigem (48 ppm) oder hohem (6800 ppm) Vitamin E-Gehalt (D, L-α-Tocopherolacetat). Zusätzlich wurden diabetische und nicht-diabetische Ratten, die eine Diät mit 620 ppm Mg und niedrigem Vitamin E-Gehalt erhielten, mit Captopril (50 mg/kg Körpergewicht × Tag) behandelt.
Während des Versuchs hypertrophierten die Nieren der diabetischen Ratten unabhängig von der Mg- und Vitamin E-Zufuhr.
Elektronenmikroskopisch fanden sich Zeichen einer diabetischen Nephropathie, nämlich eine vermehrte mesangiale Matrix mit einer Vermehrung von Kollagen Typ I sowie eine Verdickung der Basalmembranen der glomerulären Kapillaren. Diese Veränderungen waren bei den Mg-arm ernährten diabetischen Ratten am stärksten ausgeprägt und bei den Mg-supplementierten und Captopril-behandelten diabetischen Ratten nahezu normalisiert. Bei nicht-diabetischen hypertensiven Mg-Mangelratten war die mesangiale Matrix vermehrt. Vitamin E hatte keinen signifikanten Effekt. Die Ergebnisse wurden hinsichtlich der Wirkungen von Mg auf verschiedene pathobiochemische Mechanismen des Diabetes mellitus diskutiert.

Summary

Streptozotocin-diabetic male Wistar rats and age-matched control rats were fed for 5½ months with Mg-deficient (80 ppm), normal Mg (620 ppm) and high Mg (5800 ppm) diet containing either 48 ppm or 6800 ppm D, L-α-tocopherol acetate.
Additionally, diabetic and non-diabetic rats fed with 620 ppm Mg and low vitamin E diet were treated with captopril (50 mg/kg body weight × day).
During this time the kidneys of all diabetic rats hypertrophied independent of Mg and vitamin E nutrition.
Electron microscopy yielded signs of diabetic nephropathy such as increased mesangial matrix with an increase of collagen type I and thickening of the basement membranes of glomerular capillaries.
The alterations were most expressed in Mg-deficient diabetic rats and almost normalized in Mg-supplemented and captopril-treated diabetic rats. In non-diabetic, hypertensive Mg-deficient rats mesangial matrix was increased. Vitamin E had no significant effect.
The results were discussed with respect to the effects of Mg on the various pathobiochemical mechanisms of diabetes mellitus.

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Introduction

Diabetic nephropathy is now the most common cause of renal failure requiring kidney transplantation or continuous dialysis [1].
The glomerulus is one of the insulin-independent tissues [2, 3]. Thus, all biochemical mechanisms related to hyperglycemia may operate in the kidney of diabetics. These mechanisms may be (see also Ref. [4]):

- Increased sorbitol pathway [2],
- increase of diacylglycerol and activation of protein kinase (PK) C-β [3, 5, 6],
- reduced myoinositol uptake [7],
- increased formation of reactive oxygen species (ROS) [8],
- increased non-enzymatic glycosylation [9],
- increased secretion of cytokines, particularly TGFβ [10].

The mechanisms underlying high-glucose-induced upregulation of TGFβ may be via PKCβ activation due to an increase of diacylglycerol and via advanced glycation end products (AGE), since mesangial cells have specific AGE receptors which may result in enhanced matrix and cytokine production.
Moreover, mechanic (shear) stress by osmotic diuresis may stimulate release of TGFβ [10] and endothelin [11, 12]. Also vasoactive agents such as endothelin and angiotensin II (AII) are implicated in the pathogenesis of diabetic nephropathy [10]. The most important inducer of TGFβ in renal cells may be AII, implying that part of the renal protective effect of ACE inhibitors is related to blockade of TGFβ production. Therefore, in the present experiment, part of the rats were treated with the ACE inhibitor captopril.
Some of the above-mentioned mechanisms depend on the extracellular Mg^{2+} concentration, e.g. myoinositol transport [7], or are improved by vitamin E such as ROS- [13] or PKC related mechanisms [3, 5, 6]. Furthermore, albuminuria was higher in hypomagnesemic diabetic patients [14] and Mg deficiency activated the juxtaglomerular apparatus [15]. In patients with diabetes mellitus, serum Mg concentration is often reduced due to osmotic diuresis [16]. Therefore, we investigated the effects of Mg and vitamin E on the kidneys of diabetic rats.

Materials and methods

Material and methods were described in detail in an accompanying paper [4]. Briefly, in male Wistar rats weighing

Tab. 1: Kidney weight (mean of 2 kindneys) of control and diabetic rats fed diets with various Mg and vitamin E content. Mean ± SEM of 5–6 rats per dietary group. Significant difference between diabetic and controls by unpaired Student's t-test; a, $p < 0.05$; b, $p < 0.01$; c, $p < 0.001$.

Dietary Group	Diabetics		Controls	
	weight g	rel. weight mg/g b.w.	weight g	rel. weight mg/g b.w.
Mg def. – Vit E def.	1.94 ± 0.41	7.02 ± 1.43	1.48 ± 0.19	4.57 ± 0.68
Mg def. – Vit E rich	2.30 ± 0.41[a]	7.30 ± 0.67[c]	1.15 ± 0.06	3.32 ± 0.12
Mg norm. – Vit E def.	2.03 ± 0.12[c]	6.50 ± 0.58[c]	1.36 ± 0.02	2.63 ± 0.07
Mg norm. – Vit E def. + Capt.	2.32 ± 0.04[c]	7.18 ± 0.38[c]	1.49 ± 0.09	2.68 ± 0.06
Mg norm. – Vit E rich	2.27 ± 0.27[b]	6.96 ± 0.99[b]	1.36 ± 0.06	2.77 ± 0.15
Mg rich – Vit E def.	2.23 ± 0.11[c]	5.78 ± 0.19[c]	1.44 ± 0.09	2.58 ± 0.14
Mg rich – Vit E rich	2.23 ± 0.08[c]	5.87 ± 0.49[c]	1.39 ± 0.03	2.77 ± 0.06

308 g, diabetes mellitus was induced by i.p. injection of 65 mg/kg streptozotocin. Thereafter, the diabetic and control rats received a semi-synthetic diet with about 80, 620 and 5800 ppm Mg and either 48 mg/kg or 6800 mg/kg D, L-α-tocopherol acetate and distilled water ad libitum for 5½ months. One diabetic and control group fed with 620 ppm Mg and vitamin E-deficient diet received 50 mg/kg body weight × day captopril in the drinking water throughout the experiment. At the end of the experiment, blood was taken from the anaesthetized rats by heart puncture. The kidneys were removed, weighed and corresponding pieces of the kidneys were fixed in cold (4° C) Karnovsky's solution for electron microscopy. Electron microscopy was performed as described in detail elsewhere [4].

Results

General observations

The nutritional state with respect to Mg and vitamin E, the degree of lipid peroxidation measured by malondialdehyde content of plasma and the diabetic state measured by plasma glucose concentration were reported in an accompanying paper [4].

All diabetic rats consumed more food and water (180 ml/ rat × day vs. 40 ml/rat × day of controls) and excreted more urine than the control rats.

Due to the increased diuresis, kidneys of all diabetic rats were hypertrophied as can be seen from the increased kidney weight of the diabetic rats (Tab. 1). There were no significant differences in the absolute kidney weights within the dietary groups of diabetics or controls. Also, in another experiment with streptozotocin-diabetic rats, supplementation with vitamin E had no significant effect on kidney weight [17].

When related to body weight, the relative kidney weight was higher in Mg-deficient diabetic and Mg-deficient control rats than in the Mg-supplemented groups and relative kidney weight was higher in all dietary groups of diabetic rats than in non-diabetic control rats due to the lower body weight of the diabetic and Mg-deficient rats.

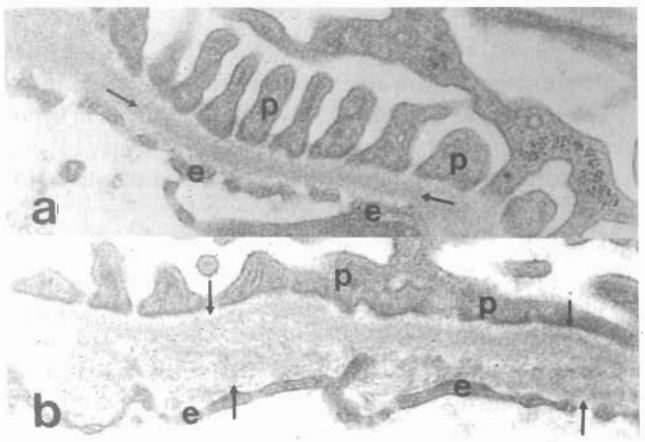

Fig. 1a: Kidney of a non-diabetic rat fed a Mg-rich and vitamin E-rich diet. Normal thickness of a glomerular basement membrane (arrows) p, processes of podocytes; e, endothelial cell with pores; X 32,000.
1b: Kidney of a diabetic rat fed a Mg-deficient and vitamin E-deficient diet. Thickening of glomerular basement membrane (arrows); p, confluent processes of podocytes; e, endothelial cell; X 32,000.

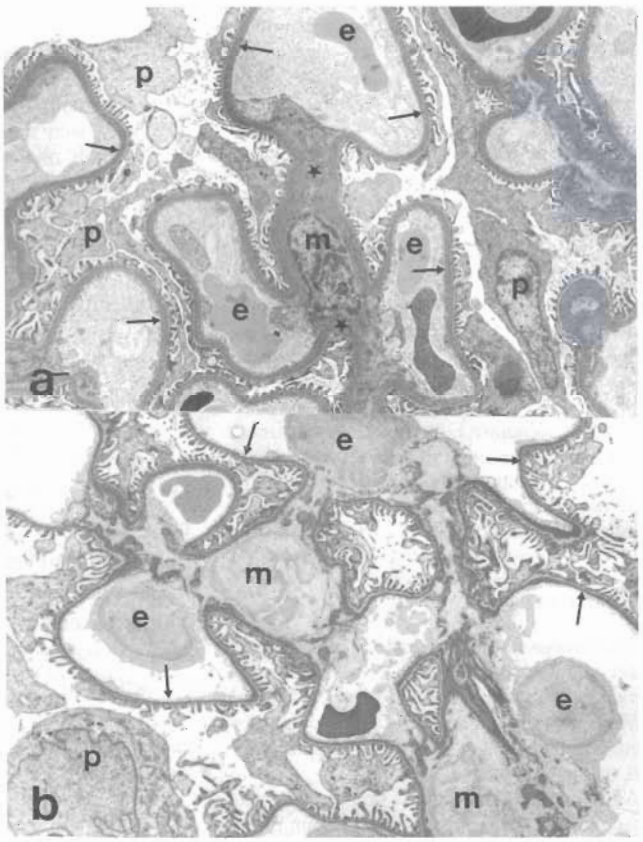

Fig. 2a: Kidney of a diabetic rat fed a Mg-deficient and vitamin E-deficient diet. Thickening of glomerular basement membranes (arrows); increase of mesangial matrix (*); m, extended mesangial cell; p, podocyte; e, erythrocytes in glomerular capillaries; X 2,700.
2b: Kidney of a diabetic rat fed a Mg-rich and vitamin E-rich diet. Normal thickness of basement membranes (arrows); m, plump mesangial cells with little matrix in their neighborhood; e, endothelial cells; p, podocytes; X 2,700.

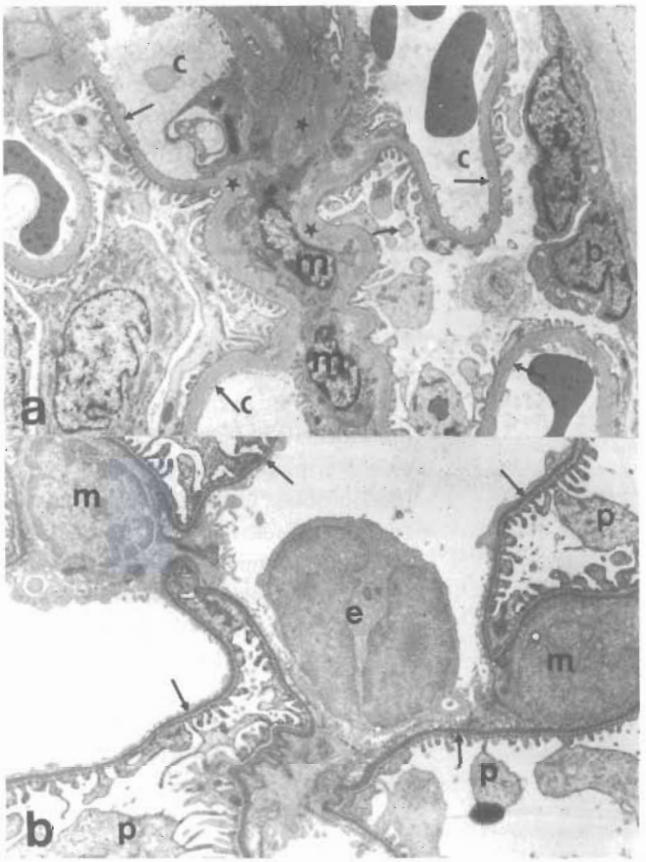

Fig. 3a: Kidney of a diabetic rat fed a Mg-deficient and vitamin E-deficient diet. **m**, mesangial cells with elongated processes and increased matrix in their neighborhood (⋆); thickened glomerular basement membranes (arrow); **p**, podocytes; **c**, glomerular capillaries, **b**, Bowman's epithelial cell; X 2,800.
3b: Kidney of a diabetic rat fed a Mg-rich and vitamin E-deficient diet. **m**, plump mesangial cells with little matrix; normal basement membranes (arrows); **p**, podocytes; **e**, endothelial cells; X 3,200.

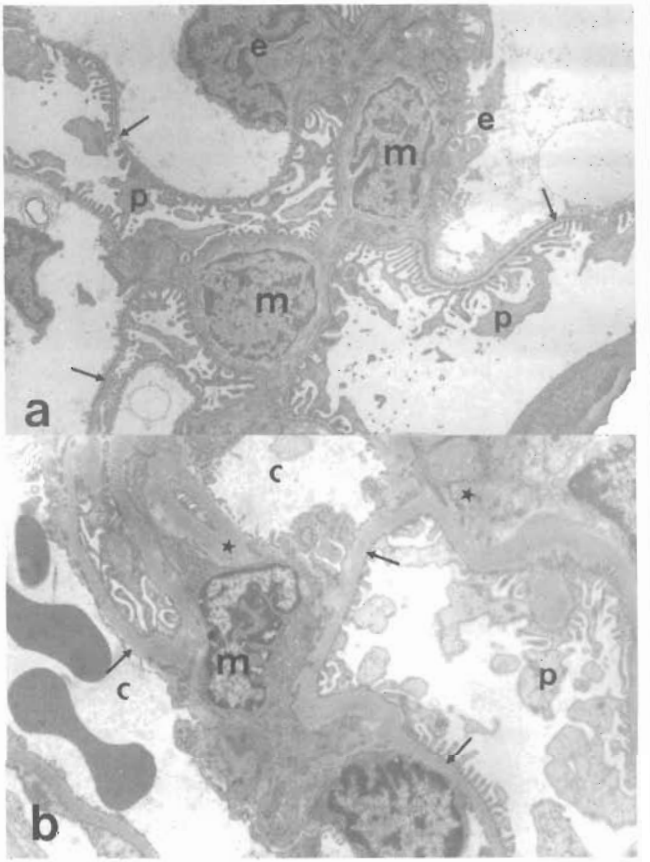

Fig. 4a: Kidney of a diabetic rat fed a Mg-rich and vitamin E-deficient diet. Normal glomerular basement membranes (arrows), **m**, mesangial cells; **p**, podocytes; **e**, capillary with endothelial cell; X 3,700.
4b: Kidney of a diabetic rat fed a Mg-deficient and vitamin E-deficient diet. Thickened glomerular basement membranes (arrows); elongated, irregularly shaped mesangial cells (**m**); increased mesangial matrix (⋆); **p**, podocyte; **c**, capillaries; X 6,500.

Electron microscopy

Thickening of glomerular basement membranes, enlargement of the mesangium and accumulation of extracellular matrix are the most consistent morphological findings in diabetic nephropathy [18].

In the present experiment, electron microscopy yielded the same alterations: The glomerular basement membranes of diabetic rats were thickened (Fig. 1b).

The mesangial matrix was increased (Fig. 2a) and fibrillar collagen was occasionally found within the mesangial matrix, indicating the formation of collagen type I.

There were more contacts between endothelial and mesangial cells. The number of processes of the mesangial cells was increased and the processes were elongated. Some mesangial cells expressed extended processes (Fig. 3a) through neighboring endothelial cells into the lumen of capillaries.

The number of openings and pores of glomerular endothelial cells were decreased (Fig. 1b). These alterations were heterogeneously expressed among various parts of the kidneys. There were areas with strongly expressed and less expressed glomerulosclerosis besides almost normal glomeruli.

These alterations were most expressed in Mg-deficient diabetic rats and less expressed in Mg supplemented (compare Figs. 2a and 2b, 3a and 3b, 4a and 4b) and captopril-treated rats (not shown). Diabetes-induced thickening of glomerular basement membranes was normalized by feeding a Mg-rich diet (compare Figs. 2a and 2b, 3a and 3b, 4a and 4b). Diabetes-induced increase of mesangial matrix was reduced by Mg supplementation (compare Figs. 2a and 2b, 3a and 3b, 4a and 4b). Kidneys of Mg-supplemented diabetic rats expressed almost normal morphology (Figs. 2b, 4a). In the kidneys of Mg-deficient, non-diabetic rats, mesangial matrix was increased (compare Figs. 5a with 5b), whereas basement membranes seemed to be not significantly thickened by Mg deficiency.

Vitamin E supplementation appeared to have no significant effect on the morphological alterations.

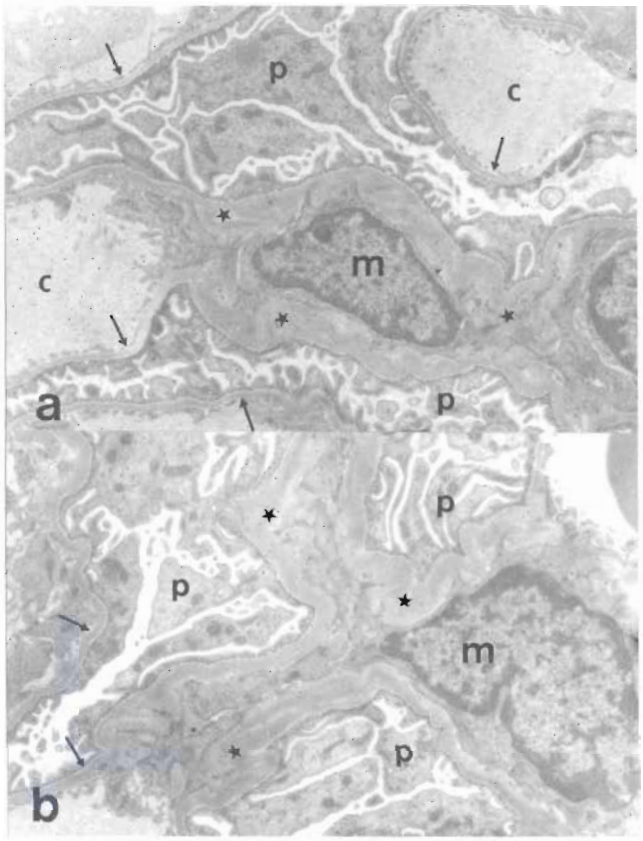

Fig. 5a: Kidney of a non-diabetic rat fed a Mg-deficient and vitamin E-deficient diet. Normal glomerular basement membranes (arrows); m, slightly extended elongated mesangial cell, increased mesangial matrix (*); p, podocytes; c, glomerular capillaries; X 7,000.
5b: Kidney of a diabetic rat fed a Mg-deficient and vitamin E-deficient diet. m, mesangial cell with long, irregularly shaped processes and increased mesangial matrix (*); thickened glomerular basement membranes (arrows); p, podocytes; X 7,000.

Discussion

One significant effect of the present experiment was renal hypertrophy (Tab. 1). The mechanisms of renal hypertrophy are not well understood. According to one hypothesis the increased glomerular filtration rate may be responsible for renal hypertrophy. Another hypothesis suggests that a hyperglycemia-induced increase in intracellular Na^+ and/or Ca^{2+} may play a role [1]. $[Ca^{2+}]_i$ may be increased by reduction of Ca-ATPase [1, 19]. For controversial results on Ca-ATPase activity in diabetes mellitus see Ref. [20]. The increased $[Ca^{2+}]_i$ in cooperation with increased PKC isoforms may induce kidney hypertrophy [1]. Mg deficiency should favor the increase of $[Ca^{2+}]_i$ via the changed extracellular Mg^{2+}/Ca^{2+} ratio and Ca^{2+} antagonism of Mg^{2+} [21] and vitamin E supplementation should reduce PKC activity [21]. However, the different Mg and vitamin E contents of the diets had no significant effect on kidney weight and hypertrophy.

The other significant alteration concerned glomerular basement membranes and the mesangium and may thus reflect morphological signs of diabetic nephropathy [18].

Glomerular mesangial cells have various functions including the production and metabolism of various macromolecules such as collagen type IV, laminin or fibronectin.

Remarkably, according to our electron microscopical results, mesangial matrix was increased and within the matrix, also collagen type I and/or III were accumulated. Moreover, mesangial cells regulate glomerular filtration rates through their ability of contraction induced by angiotensin II, vasopressin or endothelin [22] and by relaxation induced by atrial natriuretic peptide [23]. Thus, angiotensin II may play a significant role in diabetic nephropathy, also explaining the beneficial effect of captopril.

In addition to its action on glomerular capillaries, angiotensin II can induce the formation of endothelin [24], TGFβ-1, platelet-derived growth factor, fibroblast growth factor, collagen, fibronectin, etc. [24]. The beneficial effect of captopril treatment supports this mechanism. Additionally, other cytokines such as IL1 and TNFα are involved in the development of diabetic nephropathy [25]. The formation of IL1 and TNFα can be increased by Mg deficiency [26]. Therefore, diabetic nephropathy may be favored by Mg deficiency. The relevant mechanisms for the induction of angiotensin II may be mechanical stretch and activation of mitogen-activated protein (MAP) kinase and extracellular signal-regulated kinase (ERK) [27] and co-stimulation by hyperglycemia [24]. The accumulation of mesangial matrix in diabetes is supported by decreased degradation due to hyperglycemia [28]. The accumulation of extracellular matrix can lead to glomerulosclerosis via apoptosis [29]. The vasoactive agents angiotensin II and endothelin operate via PKC, IP_3 and the increase of $[Ca^{2+}]_i$. In the increase of $[Ca^{2+}]_i$ influx of extracellular Ca^{2+} is involved. A significantly altered extracellular Mg^{2+} concentration by Mg deficiency or Mg supplementation can modify Ca^{2+} influx by an alteration of extracellular Ca^{2+}/Mg^{2+} antagonism [21]. Thus, an altered Ca^{2+}/Mg^{2+} antagonism can explain the aggravating effect of Mg deficiency and the beneficial effect of Mg supplementation on diabetic nephropathy.

The mechanisms of diabetic nephropathy are not exactly defined. Probably, TGFβ may play a major role in

kidney hypertrophy and nephropathy [10]. TGFβ is mainly operating by autocrine production and activation [10]. We have not measured TGFβ in kidneys and other tissues. Therefore, we have no results on the effects of Mg and vitamin E supplementation on TGFβ production and action. In another experiment, TGFβ was increased in glomerular epithelial and endothelial cells of diabetic rats and reduced to control values by vitamin E, whereas TGFβ in plasma was not significantly changed [17].

However, vitamin E supplementation did not prevent glomerular hypertrophy and had no effect on increased albumin excretion in diabetic rats [17], indicating that other factors than TGFβ contribute to permeability changes in diabetes mellitus.

As shown in the present experiment, also Mg deficiency alone (in non-diabetic rats) induced an increase in mesangial matrix. This effect of Mg deficiency may be caused by IL1 and TNFα, as just discussed.

However, the increase in mesangial matrix may also be produced by hypertension which developed in chronic Mg-deficient, non-diabetic rats [30].

In agreement with this assumption, in spontaneously hypertensive rats glomerular basement membranes were thickened, mesangial matrix was increased and morphology of mesangial cells was changed [31] very similar to the alterations in streptozotocin-diabetic rats. On the other hand, Mg supplementation prevented thickening of glomerular basement membranes and reduced the increase of mesangial matrix in diabetic rats. Therefore, it can be concluded that hyperglycemia, hypertension and Mg deficiency are operating in concert via above discussed mechanisms.

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