Original Article

Effects of green tea catechin on prostaglandin synthesis of renal glomerular and renal dysfunction in streptozotocin-induced diabetic rats

Soon-Jae Rhee[1] PhD, Mi-Ji Kim[1] PhD and Oh-Gye Kwag[2] PhD

[1]*Department of Food Science and Nutrition, Catholic University of Daegu, Gyungsan-si, Gyungbuk, Korea*
[2]*Department of Nursing Science, Taegu Science College, Taegu, Korea*

The purpose of the present study was to investigate the effects of green tea catechin on prostaglandin synthesis of renal glomerular and renal dysfunction in rats with streptozotocin–induced diabetes. Sprague-Dawley rats weighing 100 ± 10 g were randomly assigned to one normal group and three groups with streptozotocin-induced diabetes. The diabetic groups were classified to a catechin-free diet (DM group), a 0.25% catechin diet (DM-0.25C group) and a 0.5% catechin diet (DM-0.5C group) according to the levels of catechin supplement in their diet. The animals were maintained on an experimental diet for 4 weeks. At this point, they were injected with streptozotocin to induce diabetes. They were killed on the sixth day. The catechin supplementation groups (DM-0.25C, DM-0.5C groups) showed a decrease in thromboxane A_2 synthesis but an increase in prostacyclin synthesis, compared to the DM group. The ratio of prostacyclin/thromboxane A_2 was 53.3% and 38.1% lower in the DM and DM-0.25C groups, respectively, than in the normal group. The ratio in the DM-0.5C group did not differ from that in the normal group. The glomerular filtration rate in catechin feeding groups (DM-0.25C and DM-0.5C groups) was maintained at the normal level. The urinary β_2-microglobulin content in the DM-0.5C group was significantly lower than that in the normal group. On the sixth day after induction of diabetes, the urinary microalbumin content in the DM, DM-0.25C and DM-0.5C groups had increased 5.40, 4.02, 3.87 times, respectively, compared with the normal group. In conclusion, kidney function appears to be improved by green tea catechin supplementation due to its antithrombotic action, which in turn controls the arachidonic acid cascade system.

Key words: antithrombotic activity, diabetes, green tea catechin, renal dysfunction, renal glomerulus.

Introduction

Diabetes is a chronic metabolic disease characterized by hyperglycemia. However, diabetics with a control problem due to hyperglycemia also exhibit changes in the function and shape of their kidneys.[1,2] The renal disorder caused by diabetes infects the renal system, hardens the renal artery, and damages the glomerulus. As a result, patients experience a decline in the excretion function of their kidneys, albuminuria and edema appear, and blood pressure rises. If these symptoms persist, this further weakens the renal functions, leading to uremia, and finally death.[3]

A recent study raised the issue of the relationship between functional damage to the capillary vessels, such as the glomerulus or renal tubule, and the presence of eicosanoid, which is the metabolic product of arachidonic acid generated by the renal cells.[4] Linos *et al.* previously reported that, since the function of the kidney is to excrete non-volatile materials or metabolic wastes, controlling the blood flow is important in renal functions, such as the filtration of the glomeruli.[5] This is closely related to prostaglandin.[5] In another study, chronic diabetics caused an increase in blood pressure and albuminuria and a decline in the glomerular filtration function. A previous study of diabetic rat kidneys reported hardened glomeruli, an increase in thromboxane A_2 (TXA_2), and a decrease in prostacyclin (PGI_2), resulting in an imbalanced PGI_2/TXA_2 ratio.[6] When the PGI_2/TXA_2 balance is broken, renal microvascular ageing and artery hardening are both accelerated. Accordingly, maintaining a balanced PGI_2/TXA_2 ratio can improve renal function by alleviating the hardening of the renal arteriole and controlling the renal blood flow and glomerular filtration rate, thereby contributing to the prevention of diabetic renal failure. Studies of the physiological mechanism of the renal microvascular system are important in order to identify ways to enhance it. There have already been several case studies on the relationship between prostaglandin synthesis and renal

Correspondence address: Dr Soon-Jae Rhee, Department of Food Science and Nutrition, Catholic University of Daegu, Gyungsan-si, Gyungbuk, 712-702, Korea.
Tel: +82 53 850 3523; Fax: +82 53 850 3504
Email: sjrhee@cataegu.ac.kr
Accepted 14 January 2002

dysfunction in the renal glomerulus, which in diabetics is the most vulnerable organ and has the worst prognosis.

The polyphenol compound chemical catechin, found in green tea, has been reported to exhibit pharmacological functions.[7–12] The antioxidant activity of catechin and its effects on the cohesion of thrombocytes has already been reported in several studies.[13–16] In the current study, rats were fed different green tea catechin diets and then diabetes was induced. The glomeruli in the renal organs were separated to ascertain the effects of catechin on prostaglandin synthesis and identify any improvement in renal function.

Materials and methods

Experimental animals and diets

Male Sprague-Dawley rats weighing between 70 and 80 g were purchased from KRITC (Taejon, Korea). The rats were individually housed in stainless steel cages in a room with controlled temperature (20–23°C) and lighting (alternating 12 h period of light and dark). They were fed a pelleted commercial non-purified diet (Samyang, Seoul, Korea) for 6 days after arrival. They were randomly divided into one normal group and three diabetic groups of 10 experimental rats each. The four groups were fed experimental diets for 4 weeks and the diabetic groups were classified to a catechin-free diet (DM group), a 0.25% catechin diet (DM-0.25C group) or a 0.5% catechin diet (DM-0.5C group; Table 1). The crude catechin powder, which is about 87.5% pure, was prepared according to the method of Matzuzaki and Hata (Table 2).[13] Crude catechin was made up of 51.86% epigallocatechin gallate (EGCG), 12.48% epicatechin gallate (ECG), 27.65% epigallocatechin (EGC) and 8.01% epicatechin (EC). The experimental design was approved by the committee for the care and use of laboratory animals at the Catholic University of Daegu.

Experimental diabetes

Diabetes was induced by an intravenous injection of streptozotocin (STZ; 55 mg/kg body weight) in a citrate buffer (pH 4.3) via the tail vein. Rats with a blood glucose concentration of 16.7 nmol/L after 6 days were used for the experiment.

Table 1. Classification of experimental groups

	Catechin (% in diet)	Diabetes (streptozotocin-induced)†
Normal	0	–
DM	0	+
DM-0.25C	0.25	+
DM-0.5C	0.5	+

†Intravenous injection of streptozotocin (55 mg/kg body weight) in 0.1 mol/L sodium citrate buffer (pH 4.3) via tail vein. DM, catechin-free diet group; DM-0.25C, 0.25% catechin diet group; DM-0.5C, 0.5% catechin diet group.

Preparation of glomeruli

The glomerulus was prepared according to the method of Spiro.[17]

Measurement of thromboxane A_2 and 6-keto prostaglandin F_{1a}

The isolated glomerulus was incubated in a 280 nmol Tris KCl/L buffer, pH 7.4 at 37°C under continuous agitation and then centrifuged at 10 000 *g* for 5 min. The supernatant was adjusted with a buffer to a final volume of 1.0 mL and used as a source of the TXA_2 and 6-keto prostaglandin F_{1a} (PGF_{1a}). The 6-keto PGF_{1a} and TXA_2 contents were measured using a commercially available radioimmunoassay kit (RPA 515 and RPA 516, Amersham Life Science, Cleveland, OH, USA). The radioactivity of the iodinated final products was measured using a Packard liquid scintillation counter (Diagnostic Product Corporation, Miami, FL, USA).

Microalbumin determination

Urinary microalbumin was determined by Albumin-RIA kit (Fullerton, CA, USA).

β_2-Microglobulin determination

Urinary β_2-microglobulin was determined by SPAC-SV-B_2 micro kit (Diachi Radioisotope Labs, Tokyo, Japan).

Glomerular filtration rate determination

Glomerular filtration rate (GFR) was determined by sicdia creatinine reagent kit.

Statistical analysis

Results were assessed by ANOVA and Tukey's Honestly Significant Difference test. Differences were considered significant at $P < 0.05$.

Result

Production of thromboxane A_2 and prostacyclin in kidney glomeruli

The formation of TXA_2 in the kidney microsomes was 45.8% greater in the DM group than in the normal group. However, there was no difference between the DM-0.25C, DM-0.5C, and normal groups (Fig. 1a). The formation of PGI_2 in the kidney glomeruli was 31.9% and 24.8% lower in the DM and DM-0.25C groups, respectively, than in the normal group, which did not differ from the DM-0.5C group (Fig. 1b). The ratio of PGI_2/TXA_2 was 53.3% and 38.1% lower in the DM and DM-0.25C groups, respectively, than in the normal group. The ratio of PGI_2/TXA_2 in the DM-0.5C group did not differ from that in the normal group (Fig. 1c).

Table 2. Composition of crude catechin powder from green tea

Catechin powder	EGC	EC	EGCG	ECG	Total
100 µg	27.65 ± 0.24	8.00 ± 0.01	51.86 ± 0.06	12.48 ± 0.06	100

EC, epicatechin; ECG, epicatechin gallate; EGC, epigallocatechin; EGCG, epigallocatechin gallate.

Glomerular filtration rate

Changes in GFR can provide information about the metabolic function of the kidney. Figure 2 shows the results of the changes in the GFR. When compared to the normal group, the DM group showed a 291.7% increase in GFR by the sixth day after the induction of diabetes. The catechin-fed groups (DM-0.25C and DM-0.5C groups) maintained a normal level during the experimental period (Fig. 2).

β_2-Microglobulin contents in urine

The β_2-microglobulin contents in urine, which show the index of renal tubular damage, are shown in Fig. 3. By the sixth day after the induction of diabetes, the levels in the DM and DM-0.25C groups increased 3.47 and 1.15 times, respectively, compared to the normal group. However, the β_2-microglobulin levels in the DM-0.5C group were significantly reduced compared to the DM group (Fig. 3).

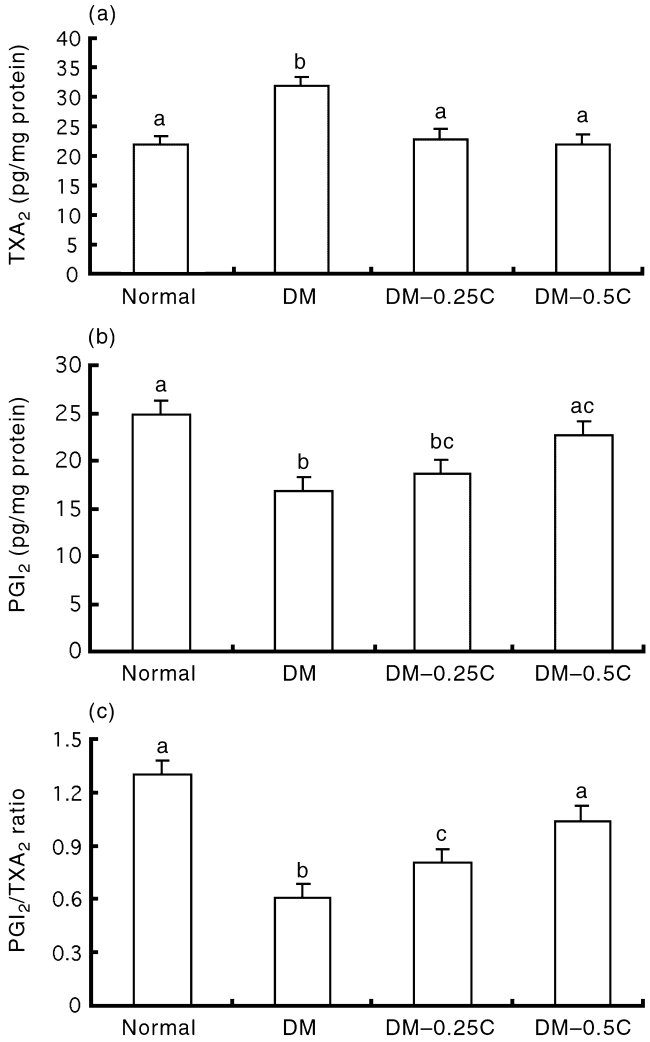

Figure 1. Effects of green tea catechin on (a) glomerular thromboxane A_2 (TXA_2), (b) prostacyclin (PGI_2) synthesis and (c) PGI_2/TXA_2 ratio in streptozotocin-induced diabetic rats. All values are mean ± SE (n = 10). Values with different superscripts are significantly different at $P < 0.05$.

Microalbumin contents in urine

The urinary microalbumin contents indicate glomerular damage (Fig. 4). By the sixth day after the induction of diabetes, the levels in the DM, DM-0.25C, and DM-0.5C groups increased 5.4, 4.02, and 3.87 times, respectively, compared to the normal group (Fig. 4). As seen in Fig. 4, the

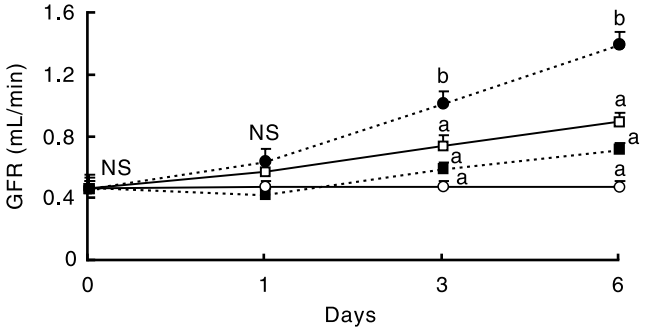

Figure 2. Effects of green tea catechin on glomerular filtration rate (GMR) in rats with streptozotocin-induced diabetes. All values are mean ± SE (n = 10). Values with different superscripts are significantly different at $P < 0.05$. (–○–), Normal; (–□–), DM-0.25C; (· · ● · ·), DM; (· · ■ · ·), DM-0.5C. NS, not significant.

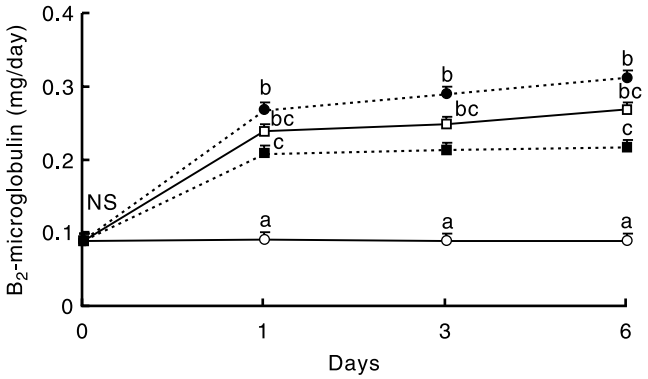

Figure 3. Effects of green tea catechin on urinary β_2-microglobulin contents in rats with streptozotocin-induced diabetes. All values are mean ± SE (n = 10). Values with different superscripts are significantly different at $P < 0.05$. (–○–), Normal; (–□–), DM-0.25C; (· · ● · ·), DM; (· · ■ · ·), DM-0.5C. NS, not significant.

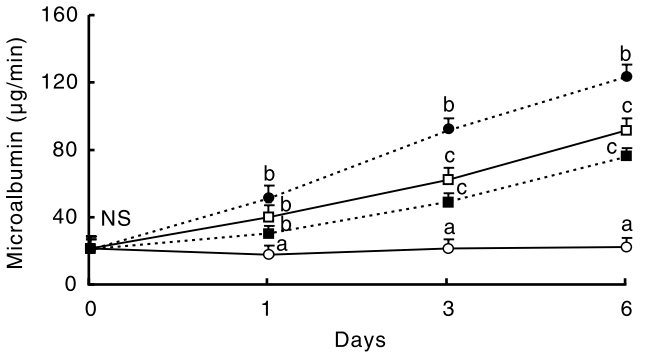

Figure 4. Effects of green tea catechin on urinary microalbumin contents in rats with streptozotocin-induced diabetes. All values are mean ± SE (n = 10). Values with different superscripts are significantly different at $P < 0.05$. (–○–), Normal; (–□–), DM-0.25C; (· · ● · ·), DM; (· · ■ · ·), DM-0.5C. NS, not significant.

urinary microalbumin contents were reduced by increasing catechin supplementation.

Discussion

The current study was conducted to identify the effects of catechin on renal dysfunction and prostaglandin synthesis of the renal glomeruli in rats with streptozotocin-induced diabetes. Among the metabolic products of eicosanoid, TXA_2, PGI_2, and PGE_2 are all known to be related to renal function or renal disease. Klahr *et al.* reported that renal synthesis of thromboxane and prostacyclin alleviates blood vessel contraction and controls the blood flow and glomerular filtration rate.[18] In contrast, TXA_2 accelerates the contraction of blood vessels, generates thrombus, shrinks the mesangial cells, and decreases the glomerular filtration rate, thereby aggravating renal disease. The results from the current research confirmed those previously reported by Kwag *et al.*[19] In their study of diabetic rats, they found that the strong antioxidant, vitamin E, decreased TXA_2 synthesis in the renal glomeruli and increased PGI_2 synthesis. The current research also showed that the PGI_2/TXA_2 ratio increased in ascending order according to the amount of catechin included in the diet. Kwag *et al.* explained their results based on the fact that the PGI_2 synthesis enzyme, prostacyclin synthetase, was affected by a lower level of lipid peroxide than the TXA_2 synthesis enzyme, thromboxane synthetase.[20] An analysis of the current results showed that the antioxidant catechin was also able to prevent lipid peroxidation, similar to vitamin E.

Diabetic renal disease is one of the main causes of terminal renal failure. For insulin-dependent diabetics, urine microalbumin is recognized as an early sign of renal disease.[21] For non-insulin-dependent diabetes mellitus, urine microalbumin is not only a sign of diabetic renal disease but is also a warning of cardiovascular disease, thereby increasing the risk of death.[22] Decker *et al.* have argued that, in diabetics, the pathological and biochemical mechanism of urine microalbumin has not been accurately identified, although they admit that urine microalbumin clearly indicates a functional disorder, such as overfiltration of the blood vessels including the renal glomeruli.[23]

In the current study, catechin was found to alleviate damage to the glomeruli. The renal tubule albumin, β_2-microglobulin, has been reported to appear early in renal tubule disorders as well as in glomerular disorders in diabetic renal disease.[24] Since 99.9% of the urine that passes through the glomeruli is re-absorbed by the nearby renal tubule cells, measuring the level of β_2-microglobulin in the urine can be an accurate method for early diagnosis of disease in the nearby renal tubule.[25] In the current study, it was found that the level of β_2-microglobulin decreased according to the level of catechin. The oxidation stress due to diabetes damages the nearby renal tubule and inhibits the re-absorption of microalbumin and β_2-microglobulin, thereby increasing their levels in the urine. However, in the current study, the strong antioxidant catechin alleviated the oxidation stress and normalized renal function.

In the early stage of diabetes, the glomerular filtration rate generally increases. This was originally reported in the study of Steffes *et al.*[26] According to the current study, rats with streptozotocin-induced diabetes exhibited an increased renal glomerular filtration rate seemingly caused by an increase in the filtration surface size due to an increase in the renal glomeruli size. There have also been previous reports of an increase in the renal glomeruli size in human diabetics and animal experiments.[27–29] In the current research, renal dysfunction, such as structural damage and an increase in the glomerular filtration rate, was identified in the diabetic kidneys. Yet the dietary supplementation of catechin from green tea was found to alleviate such structural and functional damage.

In conclusion, catechin from green tea can alleviate diabetic renal blood flow disorders by increasing the PGI_2/TXA_2 ratio and controlling the glomerular filtration rate. The current study also showed that the PGI_2/TXA_2 ratio increased according to the level of catechin in the diet. This conclusion was based on the effect of catechin decreasing the TXA_2 level in the renal glomeruli of the diabetic rats and increasing the PGI_2 synthesis. Finally, catechin was found to contribute to the prevention of renal dysfunction by lowering the microalbumin and β_2-microglobulin levels in the urine.

Acknowledgement. This work was supported by Health Technology and Evaluation Board Grant (HMP-00-B-22000–00151).

References

1. Brenner BM, Rector FC. Diabetic nephropathy. The kidney, 3rd edn. Philadelphia: WB Saunders, 1986: 1377–1402.
2. Cotran RS, Kumar V, Robbins SL. The kidney, pathologic basis of disease, 4th edn. Philadelphia: WB Saunders, 1989: 1044–1047.
3. Pedro C, Francis D, Jossse G, Nathan WL. Relationship between renal function and metabolic alteration in early streptozotocin-induced diabetes in rats. Diabetes 1987; 76: 80–87.
4. Schlondorff D, Arvaillou R. Prostaglandin and other arachidonic acid metabolites in kidney. Kidney Int 1986; 29: 108–119.
5. Linos EA, Andres GA, Dunn MJ. Glomerular prostaglandin and thromboxane synthesis in rat nephrotoxic serum nephritis. J Clin Invest 1983; 72: 1439–1448.
6. Purkerson ML, Hoffstein PE, Klahr S. Pathogenesis of the glomerulopathy associated with renal infection in rats. Kidney Int 1976; 9: 407–417.
7. Muramatsu K, Fukuyo M, Hara Y. Effect of green tea catechins on plasma cholesterol-fed rats. J Nutr Sci Vitaminol 1986; 32: 613–622.
8. Suzuki KT, Yaguchi K, Ohnuki R, Kishikawa M, Yamada YK. Extent of cadmium accumulation and its effect on essential metal in liver, kidney and body fluid. J Toxicol Environ Health 1983; 11: 713–726.
9. Sakanaka S, Kim M, Taniguchi M, Yamamoto T. Antibacterial substances in Japanese green tea extract against *Streptococcus mutans* a carcinogenic bacterium. Agric Biol Chem 1989; 53: 2307–2311.
10. Cheng SJ. The preliminary study of inhibitory effects of green tea antioxidant on mutation. Acta Exp Biol 1986; 9: 328–334.
11. Yang JA, Choi JH, Rhee SJ. Effects of green tea catechin on phospholipase A_2 activity and antithrombus in streptozocin diabetic rats. J Nutri Sci Vitaminol 1999; 45: 337–346.
12. Kada T, Kaneko K, Matsuzaki S, Matsuzaki T, Hara Y. Detection and chemical identification of natural bioantimutagens. Mutat Res 1985; 50: 127–132.

13. Matsuzaki T, Hata Y. Antioxidative activity of tea leaf catechins. Nippon Nogeikagaku Kaishi 1985; 59: 129–134.
14. Yoon YH, Rhee SJ. Effect of Korean green tea, oolong tea and black tea beverage on the antioxidative detoxification mechanism in cadmium. Kor J Nutr 1994; 27 (10): 1007–1017.
15. Takahashi R, Morita I, Murata S, Ito H. Regulation of platelet aggregation and arachidonate metabolism in streptozotocin-diabetic rats. Prostaglandins Leukot Essent Fatty Acids 1986; 25: 123–130.
16. Kazuko N, Midori Y, Chikusa T, Michiko I, Mitsuo N. Platelet aggregation inhibitory activity of tea extracts. Nippon Shokuhin Kogyo Gakkaishi 1991; 38: 189–195.
17. Spiro RG. Studies on the renal glomerular basement membrane preparation and chemical composition. J Biol Chem 1967; 242 (8): 1915–1922.
18. Klahr S, Schreiner G, Ichikawa I. The progression of renal disease. N Engl J Med 1988; 318: 1657–1666.
19. Kwag OG, Kim SO, Choi JH, Rhee IK, Choi MS, Rhee SJ. Vitamin E improves microsomal phospholipase A_2 activity and the arachidonic acid cascade in kidney of diabetic rats. J Nutr 2001; 131: 1297–1301.
20. Particia FEM, Nievelstein PF, Sixma JJ, Wynne ORM, Groot HTD, Banga JD. Platelet adhesion and aggregate formation in type I diabetes under flow conditions. Diabetes 1991; 40: 1410–1417.
21. Parving HH, Oxenboll B, Svendsen PA, Christiansen JS, Anderson AR. Early detection of patients at risk of developing diabetic nephropathy. Acta Endocrinol 1982; 100: 550–555.
22. Nelsen RG, Knower WC, Pattit DJ, Saad MF, Charles MA, Bennett PH. Assessment of risk of overt nephropathy in diabetic patients from albumin excretion in untimed urine specimens. Arch Intern Med 1991; 151: 1761–1763.
23. Decker T, Feidt-Rasmussen B, Borch-Johnsen K, Jesan I, Koford Enevoldson A. Albuminuria reflects widespread vascular damage: the steno hypothesis. Diabetologia 1989; 32: 219–226.
24. Bernard AM, Moreau D, Lauways R. Comparison of retinol-binding protein and β_2-microglobulin determination in urine for the early detection of tubular proteinuria. Clin Chem Acta 1982; 126: 1–8.
25. Schardijn GH, Statius van Eps LW. β_2-Microglobulin, its significance in the evaluation of the renal function. Kidney Int 1987; 32: 635–641.
26. Steffes MW, Osterby R, Chavers B, Mayer SM. Mesangial expansion as a central mechanism for loss of kidney function in diabetic patients. Diabetes 1989; 38: 1077–1081.
27. Mogensen CE, Andersen MJF. Increased kidney size and glomerular filtration rate in early juvenile diabetes. Diabetes 1973; 22 (9): 706–712.
28. Butcher D, Kirkkawa R, Klein L, Miller M. Size and weight of glomeruli isolated from human diabetic and nondiabetic kidney. J Lab Clin Med 1977; 89: 544–553.
29. Rasch R. Prevention of diabetic glomerulopathy in streptozotocin diabetic rats by insulin treatment, kidney size and glomerular volume. Diabetologia 1979; 16: 125–128.