

Original article

Long-term effects of vitamin C supplementation on glomerular changes in streptozotocin-induced diabetic rats

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Background: Deficiency of vitamin C (L-ascorbic acid; AA) may induce renal glomular dysfunction in diabetes. Few data are available for the role of continuous supplementation of AA on glomerular dysfunction and pathology induced during diabetes.

Objective: To investigate long-term effects of AA supplementation on glomerular changes in streptozotocin (STZ)-induced diabetic rats.

Methods: Diabetes was induced in Sprague-Dawley rats (180-220 g) by injection of STZ (55 mg/kg bw, iv). The rats were divided into controls (CON), AA-supplemented controls (CON-AA), diabetic (STZ) and AA-supplemented diabetic rats (STZ-AA). AA (1 g/L) was continuously supplemented to the rats for 4, 8, 16 and 24 weeks. The glomerular filtration rate (GFR), effective renal plasma flow (ERPF), renal vascular resistance (RVR) malondialdehyde (MDA) and transforming growth factor- β 1 (TGF- β 1) levels were measured in the renal cortex. Glomerular morphology was examined histologically. Renal hypertrophic index was calculated using kidney-to-body weight ratio (KW/BW).

Results: Decreases in GFR and ERPF were ameliorated at week 16 and deteriorated at week 24 after AA supplementation in STZ-AA rats. High blood glucose concentration was attenuated only at week 16. MAD and TGF- β 1 levels in renal cortex decreased significantly in STZ-AA rats at week 16 but not at week 24. The number of abnormal glomeruli and KW/BW decreased significantly at week 16 in STZ-AA rats.

Conclusion: Long-term supplementation of AA may ameliorate the glomerular changes induced by diabetes.

Key words: Glomerular changes, long-term effect, diabetic rats, vitamin C (L-ascorbic acid).

Diabetic nephropathy is a major cause of end-stage renal disease. Features include glomerular and tubular sclerosis [1]. Glomerulosclerosis is a conspicuous morphological change in diabetes mellitus [2]. Decreases in the glomerular filtration rate (GFR) and the effective renal plasma flow (ERPF), glomerular expansion, and thickening of glomerular basement membrane occur in chronic diabetes mellitus [2, 3]. Hyperglycemia is a known cause of renal pathophysiology [4, 5]. It enhances oxidative stress and directly affects mesangial cells to develop glomerulopathy [6, 7].

In diabetes mellitus, malondialdehyde (MDA) has been detected [8]. A link of oxidative stress has been noted together with overproduction of transforming growth factor- β 1 (TGF- β 1) [9, 10]. High concentrated

glucose ambient can induce mesangial cells to increase TGF- β 1 production, resulting in synthesis of extracellular matrix protein [11, 12].

It has been known that vitamin C (L-ascorbic acid; AA) is deficient in diabetes mellitus [13, 14]. Recent studies using diabetic rats [3, 15, 16] have shown that renal hemodynamics and function can be improved by supplementation of AA. Furthermore, immunohistochemistry has shown that AA supplementation can prevent glomerular TGF- β 1 production in streptozotocin (STZ)-induced diabetic rats [17].

A number of studies have indicated that AA is an antioxidant and thus of benefit for diabetes mellitus [18, 19, 20, 21]. In particular, functional defects of vital organs relating to renal function, have been reported [3, 16, 17, 22]. However, few data are available for the role of continuous supplementation of AA on glomular pathophysiological changes

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induced during diabetes. The objective of this study is to elucidate long-term effects of vitamin C supplementation on glomerular dysfunction and pathological changes using STZ-induced diabetic rats. By examining changes in MDA and TGF- β 1 levels, we evaluated the damage of renal cortical glomeruli.

Materials and methods

Animal preparation

This study was conducted according to the guideline for the use of experimental animals of the National Research Council of Thailand (1999). The research protocol has been approved by the ethical committee, Faculty of Pharmacy, Chulalongkorn University (Bangkok, Thailand).

Ninety-six Sprague-Dawley rats (male, 2 month old, 180-220 g body weight) were used for this study. Rats were divided into control and diabetic groups. The diabetes was induced by intravenous injection of STZ (Sigma Chemical Co., USA) (55 mg/kg body wt). Control rats were injected with an equivalent volume of citrate buffer as a placebo. Two days after the injection, fasting blood samples were collected and the hyperglycemic state (blood glucose concentration >200 mg/dl) was measured using a glucometer (Advance Glucometer, Boehringer Mannheim, Germany).

Total of ninety-six rats were divided into four subgroups as follows. The control (CON) or diabetic rats (STZ) were given tap water. Another control (CON-AA) or diabetic rats (STZ-AA) was supplemented with AA solution. AA (L-ascorbic acid 99%, Sigma, USA) was supplemented with drinking water at a concentration of 1g/L [14], which was prepared daily. The supplementation was started after examining the blood glucose concentration. The fresh AA solution was prepared by dissolving 1 g/l AA in tap water every day [19] and supplemented to CON-AA and STZ-AA. All animals were fed with a standard rat chow diet and given either tap water or AA solution *ad libitum* throughout the experimental periods of 4, 8, 16 and 24 weeks.

Experimental procedure

We measured the fasting blood glucose concentrations at week 4, 8, 16 and 24 of the experimental periods, and renal clearances of inulin (C_{in}) and para-aminohippuric acid (C_{PAH}) at the end of the experimental periods.

On the day of the experiment, the animal was anesthetized with pentobarbitone sodium (60 mg/kg bw, i.p.). After tracheostomy, the carotid artery was catheterized for blood pressure measurement and for blood collection. The femoral vein was catheterized for infusion of normal saline or the solution of inulin and para-aminohippuric acid. Both ureters were cannulated for urine collection approaching via midline incision at *linea alba*. Normal saline solution was infused at the rate of 10 ml/kg/hr. to replace the body fluid loss during surgery. The solution of inulin (1 g/dl) and para-aminohippuric acid (0.2 g/dl) in normal saline was infused throughout the experiments. At the mid-point of each urine collection, a blood sample from the carotid artery was obtained for renal clearance measurement. After each blood collection, the total 0.8 ml of the replacing blood was transferred immediately to the rat. The replacing blood was prepared by using red blood cells from another rat which were washed 3 times with normal saline solution before mixing with 6 % bovine serum albumin in normal saline to access the same total volume [23]. The mean arterial blood pressure (MAP) was recorded continuously throughout the experiments. The blood hematocrit (Hct) was measured. The concentrations of inulin and PAH in the plasma and urine samples were determined for renal clearances.

Renal hemodynamics

The GFR, ERPF and RVR were calculated using the equations:

$$GFR = C_{in} = U_{in} V/P_{in}, \quad (1)$$

$$ERPF = C_{PAH} = U_{PAH} V/P_{PAH}, \quad (2)$$

$$ERBF = ERPF/\{1-(Hct/100)\}, \quad (3)$$

$$RVR = MAP/ERBF. \quad (4)$$

In the above, GFR = C_{in} = glomerular filtration rate (ml/min/g kidney wt), clearance of inulin (ml/min), U_{in} = urinary inulin concentration (mg/ml), V = urine flow rate (ml/min), P_{in} = plasma inulin concentration (mg/ml), ERPF = effective renal plasma flow (ml/min/g kidney wt), C_{PAH} = clearance of PAH (ml/min), U_{PAH} = urinary PAH concentration (mg/ml), P_{PAH} = plasma PAH concentration (mg/ml), ERBF = effective renal blood flow (ml/min/g kidney wt), Hct = hematocrit (%), RVR = renal vascular resistance (mmHg/ml/min/g kidney wt), MAP = mean arterial pressure (mmHg).

The urine and plasma samples were analyzed using the anthrone and Smith modified methods [24]. The C_{in} and C_{PAH} levels were calculated for determination of GFR and ERPF, respectively. At the end of the renal clearance study, both kidneys were immediately excised. The adhering fat was removed and the kidney was weighed. The renal cortex was isolated using a stereomicroscope (20x), and homogenized in phosphate buffer (0.1 M, pH 7.4). The renal cortex homogenate was determined for MDA concentration by reaction with 2-thiobarbituric acid (TBA) [25] and TGF- β 1 protein by enzyme-linked immunosorbent assay (ELISA) kit (DRG Instruments GmbH, Germany). The concentration of total protein in the renal cortex homogenate was determined by Lowry's method [26] and the concentrations of MDA and TGF- β 1 was corrected

Glomerular pathology

Kidney-to-body weight ratio (KW/BW) was used as an index of renal hypertrophy. A cross-section of the kidney was isolated, and 2 mm-thickness pieces were preserved in 10 % formalin. The sections were embedded in paraffin and stained using the periodic acid-Schiff staining method (PAS). The sections were observed under light microscope to examine the glomerulosclerosis.

Total of one hundred glomeruli per rat were examined for number of normal and abnormal glomeruli. Abnormal glomeruli were examined in terms of vascular injury (loop collapse), regional adhesion of glomerular tuft to Bowman's capsule, and expansion of mesangial matrix. These were classified into 5 grades [27, 28] as follows:

Grade 0: normal;

Grade 1: 1-25 % of the capillary damage, proliferation of mesangial cells and/or mesangial expansion;

Grade 2: 26-50 % of the capillary damage with mesangial expansion and/or proliferation of mesangial cells;

Grade 3: 51-75 % of the damage and mesangial expansion;

Grade 4: more than 75 % of the sclerosed capillaries.

Statistics

Results were expressed as means \pm standard deviation (SD). Statistical analysis was made by analysis of variance (ANOVA) using the least

significance test (LSD). A probability (P) less than 0.05 was considered to be significantly different.

Results

Changes in renal hemodynamics

Table 1 shows levels of renal hemodynamics measured on different weeks in control and diabetic rats with or without AA supplementation. Apparently, the GFR and ERPF level decreased significantly in STZ and STZ-AA rats, compared to CON or CON-AA. No significant change of GFR and ERPF was seen between STZ and STZ-AA at week 4 and 8. GFR and ERPF in STZ-AA were around 50 % and 40 %, which were significantly higher compared to STZ at week 16. At week 24, GFR and ERPF in STZ-AA decreased significantly down to approximately 50 % and 70 % of those in STZ rats. RVR in STZ and STZ-AA increased, compared to CON and CON-AA. At week 8 and 16, RVR of STZ-AA decreased significantly about 50 % compared to STZ.

Changes in KW/BW, MDA and TGF- β 1

Table 2 shows kidney to body weight ratio (KW/BW), MDA and TGF- β 1 levels measured on different weeks in diabetic and control rats with or without AA supplementation. The KW/BW in STZ and STZ-AA increased significantly, compared to those in CON and CON-AA. Significant decrease appeared in KW/BW in STZ-AA, compared to STZ at week 16 and 24.

The MDA level in the renal cortex did not increase significantly in STZ and STZ-AA, compared to CON and CON-AA at week 4. At week 8, the MDA level in STZ was approximately 39 % which was higher than that of CON. The MDA level in STZ-AA increased slightly up to 28 %, which was higher than that in CON. AA supplementation decreased significantly the MDA at weeks 16, but this effect disappeared 24 weeks after AA supplementation.

The TGF- β 1 levels in renal cortex did not show a significant difference among groups at week 4 of the experimental periods. At week 8, the TGF- β 1 concentration of STZ was about 46 %, and higher than that in CON and CON-AA. The TGF- β 1 levels in STZ-AA did not increase significantly. At week 16, the TGF- β 1 level in STZ was about 30 %. After AA supplementation for 16 weeks, the TGF- β 1 level in STZ-AA decreased significantly by 27 % from the value of STZ. This effect was not seen 24 weeks after AA supplementation.

Table 1. Renal hemodynamic parameters measured at weeks 4, 8, 16 and 24 in diabetic and control rats with or without AA supplementation (n=6).

	Groups	CON	CON-AA	STZ	STZ-AA
	Periods				
GFR (ml/min/g kidney wt)	week 4	0.70 ± 0.14 ^a	0.82 ± 0.23 ^a	0.49 ± 0.16 ^b	0.39 ± 0.06 ^b
	week 8	0.98 ± 0.16 ^a	0.95 ± 0.11 ^a	0.29 ± 0.16 ^b	0.39 ± 0.14 ^b
	week 16	0.82 ± 0.13 ^a	0.95 ± 0.18 ^a	0.36 ± 0.12 ^b	0.59 ± 0.16 ^c
	week 24	0.97 ± 0.18 ^a	0.95 ± 0.24 ^{ab}	0.58 ± 0.08 ^b	0.32 ± 0.07 ^c
ERPF (ml/min/g kidney wt)	week 4	2.02 ± 0.76 ^a	2.60 ± 0.73 ^a	0.66 ± 0.31 ^b	0.57 ± 0.21 ^b
	week 8	3.14 ± 0.24 ^a	3.55 ± 0.37 ^b	0.47 ± 0.19 ^c	0.56 ± 0.14 ^c
	week 16	2.82 ± 0.54 ^a	3.05 ± 0.50 ^a	0.49 ± 0.20 ^b	0.70 ± 0.12 ^c
	week 24	3.59 ± 0.83 ^a	3.37 ± 1.01 ^a	0.67 ± 0.09 ^b	0.23 ± 0.17 ^c
MAP (mmHg)	week 4	117.8 ± 10.5 ^a	117.7 ± 13.7 ^a	86.7 ± 8.8 ^b	92.5 ± 21.1 ^b
	week 8	114.7 ± 12.4 ^a	107.4 ± 12.2 ^{ab}	92.9 ± 15.8 ^{bc}	94.1 ± 12.1 ^{bc}
	week 16	109.7 ± 10.7 ^a	102.2 ± 8.5 ^a	102.2 ± 3.5 ^{ab}	107.3 ± 8.8 ^{ac}
	week 24	116.5 ± 14.5 ^a	119.2 ± 18.0 ^a	105.9 ± 5.3 ^a	116.5 ± 4.2 ^a
RVR (mmHg ml/g kidney wt)	week 4	38.3 ± 12.5 ^a	28.6 ± 13.9 ^a	92.0 ± 47.1 ^b	101.4 ± 41.1 ^b
	week 8	20.5 ± 2.0 ^a	16.3 ± 2.0 ^a	145.3 ± 61.3 ^b	97.1 ± 33.6 ^c
	week 16	21.7 ± 5.4 ^a	18.7 ± 3.8 ^a	130.5 ± 73.2 ^b	79.5 ± 9.9 ^c
	week 24	19.2 ± 5.2 ^a	21.3 ± 8.5 ^a	83.1 ± 10.3 ^b	339.6 ± 131.1 ^c

Values are expressed in terms of means ± SD. Different or same letters in the rows indicate significant difference ($p < 0.05$) or non-significant difference, respectively.

Glomerular pathological changes

Figure 1 shows glomerular morphological changes in STZ-induced diabetic rats. Normal glomeruli seen in CON and CON-AA presented clear images of capillary lumens, normal mesangial cells without hyperproliferation and no mesangial material matrix expansion (**A**). Glomerulosclerosis was frequently observed in diabetic rats, showing glomerular mesangial expansion, proliferation of mesangial cells (**B**) and obliteration of the capillary lumens with either diffuse or nodular lesions (**C**).

Table 3 shows changes of abnormal glomeruli at different stages in which grade 0 corresponds to normal

glomeruli seen in CON and CON-AA. There was no significant difference in the percentages of abnormal glomeruli among groups at the specified period of week 4 and 8. At week 16 and 24, normal glomeruli in STZ and STZ-AA decreased significantly in percentage, but the percentage increased in grade 2, grade 3 or grade 4-abnormal glomeruli, compared to CON or CON-AA. After AA supplementation for 16 and 24 weeks, STZ-AA decreased significantly in grade 2-abnormal glomeruli, compared to STZ. The percentages of grade 3 and grade 4-abnormal glomeruli of STZ-AA appeared to decrease, compared to STZ at these periods.

Table 2. Kidney-to-body weight ratio (KW/BW), concentrations of MDA and TGF-β1 among groups of diabetic and control rats with or without AA supplementation at weeks 4, 8, 16 and 24 (n=6).

	Groups	CON	CON-AA	STZ	STZ-AA
	Periods				
KW/BW (%)	week 4	0.8 ± 0.1 ^a	0.8 ± 0.1 ^a	1.4 ± 0.2 ^b	1.4 ± 0.1 ^b
	week 8	0.7 ± 0.1 ^a	0.7 ± 0.1 ^a	1.8 ± 0.9 ^b	1.7 ± 0.3 ^b
	week 16	0.6 ± 0.0 ^a	0.7 ± 0.1 ^a	1.6 ± 0.2 ^b	1.3 ± 0.1 ^c
	week 24	0.7 ± 0.0 ^a	0.7 ± 0.0 ^a	1.4 ± 0.2 ^b	1.2 ± 0.1 ^c
MDA (nmol/mg protein)	week 4	1.2 ± 0.6 ^a	1.6 ± 0.8 ^a	2.1 ± 1.2 ^a	2.2 ± 1.1 ^a
	week 8	1.8 ± 0.5 ^a	2.3 ± 0.2 ^b	2.5 ± 0.4 ^b	2.3 ± 0.2 ^b
	week 16	2.3 ± 0.5 ^a	2.4 ± 0.3 ^a	2.7 ± 0.5 ^a	1.9 ± 0.4 ^b
	week 24	1.6 ± 0.2 ^a	1.6 ± 0.1 ^a	1.9 ± 0.1 ^b	1.8 ± 0.3 ^b
TGF-β1 (pg/mg protein)	week 4	351.3 ± 106.7 ^a	379.2 ± 113.6 ^a	319.7 ± 88.1 ^a	340.0 ± 111.83 ^a
	week 8	411.4 ± 71.2 ^a	482.7 ± 60.1 ^{ab}	601.5 ± 140.7 ^c	521.1 ± 70.9 ^{bc}
	week 16	497.4 ± 85.8 ^a	489.6 ± 39.2 ^a	645.0 ± 128.7 ^b	468.3 ± 85.4 ^a
	week 24	395.3 ± 60.4 ^{ab}	373.8 ± 50.6 ^a	473.4 ± 48.6 ^c	455.1 ± 83.3 ^{bc}

Values are expressed in terms of means ± SD. Different or same letters in the rows indicate significant difference ($p < 0.05$) or non-significant difference, respectively.

Discussion

Our results show that GFR, ERPF and RVR changed significantly in STZ-diabetic rats with and without supplementation of AA, compared to control rats with and without supplementation of AA (Table 1). In general, any alterations of ERPF and GFR might be caused by unknown intrarenal changes occurring in diabetic animals. In the present experiment, the mean arterial blood pressure (MAP) remained within the normal range (90-180 mmHg) as shown in Table 1. This indicates that kidney autoregulation was maintained throughout the period of our clearance study.

In the present experiment, GFR level decreased significantly in diabetic rats, compared to control rats. Hosteller et al. [29] reported a significant increase in the GFR level per body weight in diabetes mellitus. Our result indicates that the GFR level was reduced markedly per gram of kidney weight. RVR also increased significantly in diabetic rats, compared to

control rats (Table 1). This increase coincided with an appearance of glomerulosclerosis in diabetes (Table 3). The increase in RVR may be due to matrix materials accumulated in glomeruli, mesangia and endothelium. GFR and ERPF increased significantly at week 16 after supplementation of AA, compared to the control levels of STZ-diabetic rats. These increases correlated well with the decrease in RVR. A number of reports have indicated that AA administration can improve the vascular elasticity through nitric oxide (NO)-dependent reactivity in diabetic animals [30]. The present ERPF and GFR increases might come from nitric oxide increasing in renal blood vessels by supplementation of AA.

Our experimental results have shown that the renal cortical MDA level decreased significantly 16 weeks after supplementation of AA. This decrease corresponds to the decrease in TGF-β1, a nephropathic biomarker. This finding suggests that AA supplementation might reduce damage in the

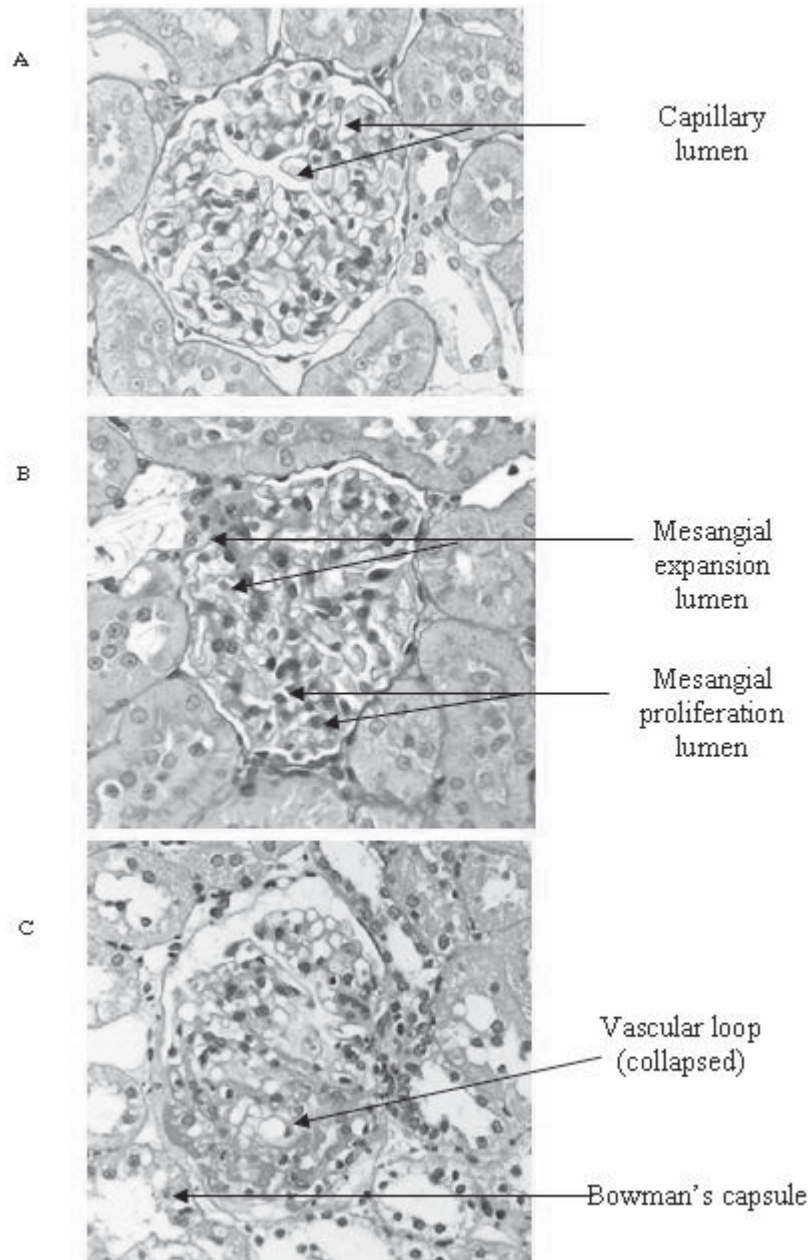


Fig. 1 Histological images showing glomerular morphological changes in STZ-induced diabetic rats. **A:** a normal glomerulus, characterized by the well-visualized capillary lumen, no mesangial proliferation and expansion. **B, C:** abnormal glomeruli (glomerulosclerosis), characterized by mesangial proliferation, expansion of matrix material and glomerular tuft adhering to Bowman's capsule.

renal cortex, relating with the occurrence of the glomerulopathy as shown in **Table 3**. Interestingly, our obtained TGF- β 1 level in the renal cortex was not significantly different among groups at week 4. In fact, the TGF- β 1 level increased markedly in both diabetic groups of STZ and STZ-AA since week 8 to 24. Its level was significantly different at week 16 and 24 (**Fig. 2**). According to Makino et al. [31], TGF- β 1

mRNA expression appeared in the first month of diabetes mellitus. Thus, it is reasonable to suppose that TGF- β 1 might contribute to diabetic nephropathy. It took about 8 weeks to develop the nephropathy.

In our experiment, the level of TGF- β 1 increased markedly in diabetic rats at week 8, 16 and 24 of diabetes. These results are in agreement with the appearance of glomerular expansion and

Table 3. Percentages of normal and abnormal glomeruli of STZ-induced diabetic and control rats with or without AA supplementation for 4, 8, 16 and 24 weeks (n=5).

Periods	Groups	Percentages of glomeruli							
		Normal glomeruli		Abnormal glomeruli					
				grade 1		grade 2		grade 3	grade 4
week 4	CON	68.6	7.6 ^a	28.2	6.8 ^a	3.2	2.2 ^a	0±0 ^a	0±0 ^a
	CON-AA	67.4	9.5 ^a	29.2	7.3 ^a	3.4	2.7 ^a	0±0 ^a	0±0 ^a
	STZ	64.2	3.8 ^a	28.2	2.3 ^a	7.6	2.7 ^a	0±0 ^a	0±0 ^a
	STZ-AA	66.2	7.1 ^a	26.6	2.7 ^a	7.2	4.9 ^a	0±0 ^a	0±0 ^a
week 8	CON	61.3	15.7 ^a	29.8	5.7 ^a	1.0	1.2 ^a	0±0 ^a	0±0 ^a
	CON-AA	68.0	7.7 ^a	26.4	6.2 ^a	5.8	3.1 ^a	0±0 ^a	0±0 ^a
	STZ	65.6	6.3 ^a	29.4	8.4 ^a	2.8	3.0 ^a	0±0 ^a	0±0 ^a
	STZ-AA	64.3	14.0 ^a	29.0	8.2 ^a	6.7	6.4 ^a	0±0 ^a	0±0 ^a
week 16	CON	68.6	12.0 ^a	28.4	10.1 ^a	3.0	3.0 ^a	0±0 ^a	0±0 ^a
	CON-AA	66.0	13.7 ^a	27.8	10.2 ^a	6.5	4.5 ^b	0±0 ^a	0±0 ^a
	STZ	33.0	15.6 ^b	40.3	10.0 ^a	21.3	7.2 ^c	0.5	1.0 ^a
	STZ-AA	53.3	3.9 ^a	35.8	2.9 ^a	10.8	2.6 ^{bd}	0.3±0.5 ^a	0±0 ^a
week 24	CON	69.8	8.1 ^a	20.5	2.6 ^a	7.8	6.8 ^a	1.8	2.1 ^a
	CON-AA	63.0	3.8 ^a	26.8	2.4 ^b	9.8	3.4 ^a	0.8	1.5 ^a
	STZ	45.8	7.1 ^b	33.0	4.5 ^c	18.3	8.6 ^b	2.5	2.6 ^a
	STZ-AA	63.2	6.4 ^a	27.8	5.1 ^{bc}	7.8	3.9 ^a	1.0	0.9 ^a

Values are expressed in terms of means ± SD. Different or same letters in the rows indicate significant difference ($p < 0.05$) or non-significant difference, respectively.

thickening of glomerular basement membrane. These morphological changes occurred at week 8 after diabetic induction [2]. The TGF-β1 level in STZ rats significantly increased from week 8, compared to CON and CON-AA (Table 2). These results are in accordance with the significant increase in KW/BW (Table 2). We note that though the KW/BW significantly increased in STZ and STZ-AA, the TGF-β1 level was not significantly different among groups at week 4. The increases in the TGF-β1 levels at week 8, 16 and 24 correspond to the increases in the KW/BW. In STZ-AA groups, the TGF-β1 level

declined significantly at week 16 after AA supplementation, compared to STZ group. This may be a reflection of decrease in the KW/BW in STZ-AA group. It indicates that AA supplementation might decrease TGF-β1, is associated with decreases in the renal cortical MDA level and renal hypertrophy.

There was a decrease in total numbers of abnormal glomeruli (Grade: 1-4) at week 16 after AA supplementation, compared to STZ group. This indicates a beneficial effect of AA on pathological changes of glomeruli. In addition, in the STZ-AA group, normal glomeruli increased in number, but

abnormal glomeruli (grade 2) decreased. These coincided with decreases in the MDA, TGF- β 1 level and renal hypertrophy index (KW/BW) at week 16.

In conclusion, long-term supplementation of AA to STZ-induced diabetic rats for 16 weeks can ameliorate glomerular pathophysiological changes, including increase in the GFR and ERPF, decrease in the RVR, renal hypertrophy and severity of glomerulosclerosis.

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References

1. Brownlee M, Spiro RG. Glomerular basement membrane metabolism in the diabetic rat. *Diabetes*. 1979;28:121-5.
2. Mauer SM. Structural-functional correlations of diabetic nephropathy. *Kidney Int*. 1994;45:612-22.
3. Yusuksawad M, Chaiyabutr N. Effect of continuous supplementation of L-ascorbic acid on renal functions in streptozotocin-induced diabetic rats. *Thai J Physiol Sci*. 2007;20:19-30.
4. Osterby R. Glomerular structural changes in type 1 (insulin-dependent) diabetes mellitus: causes, consequences, and prevention. *Diabetologia*. 1992;35:803-12.
5. Mauer SM., Steffes MW, Ellis EN, Sutherland DER, Brown DM. Structure-function relationships in diabetic nephropathy. *J Clin Invest*. 1984;74:1143-55.
6. Rasch R. Prevention of diabetic glomerulopathy in streptozotocin diabetic rats by insulin treatment: The mesangial regions. *Diabetologia*. 1979;17:243-8.
7. Heidland A, Sebekova K, Schinzel R. Advanced glycation end products and the progressive course of renal disease. *Am J Kidney Dis*. 2001;38:S100.
8. Cam M, Yavuz O, Guven A, Ercan F, Bukan N, Ustundag N. Protective effects of chronic melatonin treatment against renal injury in streptozotocin-induced diabetic rats. *J Pineal Res*. 2003;35:212-20.
9. Sharma K, Jin Y, Guo J, Ziyadeh FN. Neutralization of TGF- β by anti-TGF- β antibody attenuates kidney hypertrophy and the enhanced extracellular matrix gene expression in STZ-induced diabetic mice. *Diabetes*. 1996;45:522-30.
10. Sharma K, Ziyadeh FN, Alzahabi B, McGowan TA, Kapoor S, Kurnik BR, Kurnik PB, Weisberg LS. Increased renal production of transforming growth factor- β 1 in patient with type II diabetes. *Diabetes*. 1997;46:854-9.
11. Ziyadeh, F. N. Role of transforming growth factor beta in diabetic nephropathy. *Exp. Nephrol*. 1994;2:137.
12. Ziyadeh FN, Hoffman BD, Han DC, Iglesias-de la Cruz M, Hong SW, Isono M, et al. Long-term prevention of renal insufficiency, excess matrix gene expression, and glomerular mesangial matrix expansion by treatment with monoclonal antitransforming growth factor- β antibody in *db/db* diabetic mice. *PNAS*. 2000;97:8015-20.
13. Seghieri G, Martinoli L, Miceli M, Ciuti M, D'Alessandri G, Gironi A, Palmieri L, Anichini R, Bartolomei G, Franconi F. Renal excretion of ascorbic acid in insulin dependent diabetes mellitus. *Int J Vitam Nutr Res*. 1994;64:119-24.
14. Lindsay RM, Jamieson NSD, Walker SA, McGuigan CC, Smith W, Baird J. Tissue ascorbic acid and polyol pathway metabolism in experimental diabetes. *Diabetologia*. 1998;41:516-23.
15. Shamsi M, Amin A, Adeghate E. Effect of vitamin c on liver and kidney functions in normal and diabetic rats. *Ann NY Acad Sci*. 2006;1084:371-90.
16. Yusuksawad MS, Chaiyabutr N. Changes in renal hemodynamics in streptozotocin-induced diabetic rats with L-ascorbic acid supplementation. *Clin Hemorheol Microcirc*. 2006;34:391-9.
17. Craven PA, DeRubertis FR, Kagan VE, Melhem M, Studer RK. Effects of supplementation with vitamin C or E on albuminuria, glomerular TGF- β , and glomerular size in diabetes. *J Am Soc Nephrol*. 1997;8:1405-1414.
18. Paolisso G, D'Amore A, Balbi V, Volpe C, Galzerano D, Giugliano D, et al. Plasma vitamin C affects glucose homeostasis in healthy subjects and in non-insulin-dependent diabetics. *Am J Physiol Endocrinol Metab*. 1994;266:E261-E268.
19. Dai S, McNeill JH. Ascorbic acid supplementation prevents hyperlipidemia and improves myocardial performance in streptozotocin-diabetic rats. *Diabetes Res Clin Practice*. 1995;27:11-18.
20. Laight DW, Carrier MJ, Anggard EE. Antioxidants, diabetes and endothelial dysfunction. *Cardiovasc Res*. 2000;47:457-64.
21. Howarth FC, Jacobson M, Shafiullah M, Adeghate E. Long-term effects of streptozotocin-induced diabetes

- on the electrocardiogram, physical activity and body temperature in rats. *Exp Physiol.* 2005;90:827-35.
22. Kedziora-Kornatowska K, Szram S, Kornatowski T, Szadujkis-Szadurski L, Kedziora J, Bartosz G. Effect of vitamin E and vitamin C supplementation on antioxidative state and renal glomerular basement membrane thickness in diabetic kidney. *Nephron Exp Nephrol.* 2003;95:e134-e143.
 23. Suanarunsawat T, Klongpanichapak S, Chaiyabutr N. Role of nitric oxide in renal function in rats with short and prolonged periods of streptozotocin-induced diabetes. *Diabetes Obes Metab.* 1999;1:339-46.
 24. Smith HW. *Principle of Renal Physiology.* London: Oxford University Press;1962.
 25. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analy Biochem.* 1979;95:351-8.
 26. Lowry OH, Rosebrough JN, Farr AL, Randall RJ. Protein measurement with the Folin reagent. *J Biol Chem.* 1951;193:265-75.
 27. Reyes AA, Pukerson ML, Karl I, Klahr S. Dietary supplementation with L-arginine ameliorates the progression of renal disease in rats with subtotal nephrectomy. *Am J Kidney Dis.* 1992;20:168-72.
 28. Fornoni A, Lenz O, Striker L J, Striker GE. Glucose induces clonal selection and reversible dinucleotide repeat expansion in mesangial cells isolated from glomerulosclerosis-prone mice. *Diabetes.* 2003;52:2594-602.
 29. Hostetter TH, Rennke HG, Brenner BM. The case for intrarenal hypertension in the initiation and progression of diabetic and other glomerulopathies. *Am J Med.* 1982;72:375-80.
 30. Jariyapongskul A, Yamaguchi S, Patumraj S. Long-term oral vitamin C supplementation improves cerebral vasodilatory impairment in diabetes: in vivo evidence using diabetic rats. *Asian Biomed.* 2007;1:159-66.
 31. Makino H, Mukoyama M, Sugawara A, Mori K, Suganami T, Yahata K, et al. Roles of connective tissue growth factor and prostanoids in early streptozotocin-induced diabetic rat kidney: the effect of aspirin treatment. *Clin Exp Nephrol.* 2003;7:33-40.