CHAPTER IV

ROLE OF L-ASCORBIC ACID IN RENAL HEMODYNAMICS AND FUNCTIONS IN STZ-INDUCED DIABETIC RATS

Introduction

High glucose concentration or diabetic conditions in both *in vitro* and *in vivo* studies have been reported. A high blood glucose concentration induces the abnormality of either glomerular function or structure including decrease in glomerular filtration rate and renal plasma flow as well as glomerular expansion with an increase in matrix materials and thickening of glomerular basement membrane, leading to glomerulosclerosis (Mauer et al., 1984; Osterby et al., 1992). In addition, it has been demonstrated that the pathophysiology occurs not only in glomerular part but also in renal tubular part in diabetes mellitus (Dunlop, 2000; Wang et al., 2001; Morcos et al., 2002; Rosca et al., 2002). L-ascorbic acid (vitamin C) is a powerful antioxidant that has been shown to prevent the oxidative stress in rats treated with toxic agents (Appenroth et al., 1998; Greggi et al., 2000). The decreases in L-ascorbic acid concentration in serum and tissues are always found in diabetes mellitus (Seghieri et al., 1994; Lindsay et al., 1998). Supplementation of L-ascorbic acid to streptozotocin-induced diabetic rats markedly increased the concentration of L-ascorbic acid in both plasma and renal cortex (Lindsay et al., 1998). It can prevent the increase in albumin clearance (Craven et al., 1997). Therefore, these experiments were undertaken to examine the role of L-ascorbic acid supplementation on renal hemodynamics and functions in STZ-induced diabetic rats at the various periods of diabetic condition including week 4, 8, 16 and 24.

Materials and methods

The studies were performed in 96 male Sprague-Dawley rats weighing 180-220 gram. The experiments were divided into 4 groups of 4 observation periods as the experimental designs in CHAPTER III. At the end of each observation period, all

animals were examined the clearances of inulin and para-aminohippuric acid to study the renal hemodynamics such as glomerular filtration rate (GFR), effective renal plasma flow (ERPF). General circulation was assessed during the experiments to access renal vascular resistance (RVR). Urinary and plasma electrolytes (sodium, potassium and chloride) were determined to access fractional excretion of sodium (FE_{Na}), fractional excretion of potassium (FE_K), fractional excretion of chloride (FE_{Cl}), urinary excretion of sodium ($U_{Na}V$), urinary excretion of potassium (U_KV), urinary excretion of chloride ($U_{Cl}V$). Filtration fraction (FF), and urine flow rate were also considered.

Statistics

All values were expressed as means with standard deviations. Statistical comparisons among groups in the same observation periods were analyzed by ANOVA and using Least significant difference (LSD) and Thamhane's as the post hoct tests. The significant difference was indicated at p-value < 0.05.

Results

Effect of L-ascorbic acid supplementation on blood glucose concentration

The means of blood glucose concentration in STZ and STZ-AA had significantly ($p < 0.001$) higher than blood glucose concentration in CON and CON-AA at the specified experimental periods as shown in Table 4-1 and Figure 4-1. At week 4 after diabetic induction, STZ and STZ-AA rats had the blood glucose concentration as 457.87 $\pm$ 65.49 and 494.50 $\pm$ 72.37 mg/dl compared with CON and CON-AA groups (82.44 $\pm$ 11.84 and 74.50 $\pm$ 12.14 mg/dl respectively). At week 8 after diabetic induction, blood glucose concentrations of STZ and STZ-AA rats were 438.45 $\pm$ 57.10 and 436.54 $\pm$ 82.21 mg/dl higher than 80.14 $\pm$ 8.11 and 81.60 $\pm$ 12.17 mg/dl in CON and CON-AA, respectively). At week 16, STZ and STZ-AA had significantly higher blood glucose concentration (413.31 $\pm$ 48.98 and 367.11 $\pm$ 96.52 mg/dl) compared with CON and CON-AA groups (75.30 $\pm$ 5.17 and 77.70 $\pm$ 5.83 mg/dl, respectively). The blood glucose concentration in diabetic rats with L-ascorbic acid supplementation (367.11 $\pm$ 96.52 mg/dl) was significantly decreased compared

with the blood glucose concentration in diabetic rats without L-ascorbic acid supplementation (413.31 $\pm$ 48.98 mg/dl) at $p < 0.05$. However, L-ascorbic acid supplementation for 24 weeks did not significantly decrease the blood glucose concentration in STZ-AA rats. At week 24 after diabetic induction, the significantly higher blood glucose concentration in STZ was 375.70 $\pm$ 23.40 mg/dl and 356.25 $\pm$ 50.31 mg/dl in STZ-AA compared with 80.80 $\pm$ 14.82 and 70.83 $\pm$ 3.43 mg/dl in CON and CON-AA, respectively.

Table 4-1 Comparisons of blood glucose concentrations among groups of streptozotocin-induced diabetic rats and control rats with or without L-ascorbic acid supplementation at week 4, 8, 16 and 24 of the experimental periods.

Groups	Blood glucose concentration (mg/dl)			
	week 4	**week 8**	**week 16**	**week 24**
CON	82.4 ± 11.8 (n = 9)	80.1 ± 8.1 (n = 7)	75.3 ± 5.2 (n = 10)	80.8 ± 14.8 (n = 5)
CON-AA	74.5 ± 12.1 (n = 8)	81.6 ± 12.2 (n = 10)	77.7 ± 5.8 (n = 10)	70.8 ± 3.4 (n = 6)
STZ	457.87 ± 65.49[ab] (n = 15)	438.5 ± 57.1[ab] (n = 11)	413.3 ± 49.0[ab] (n = 13)	375.7 ± 23.4[ab] (n = 10)
STZ-AA	494.5 ± 72.4[ab] (n = 14)	436.5 ± 82.2[ab] (n = 13)	367.1 ± 96.5[abc] (n = 9)	356.3 ± 50.3[ab] (n = 12)

Mean ± SD

[a] compared with CON at the same column, $p \leq 0.001$;

[b] compared with CON-AA at the same column, $p \leq 0.001$;

[c] compared with STZ at the same column, $p \leq 0.05$

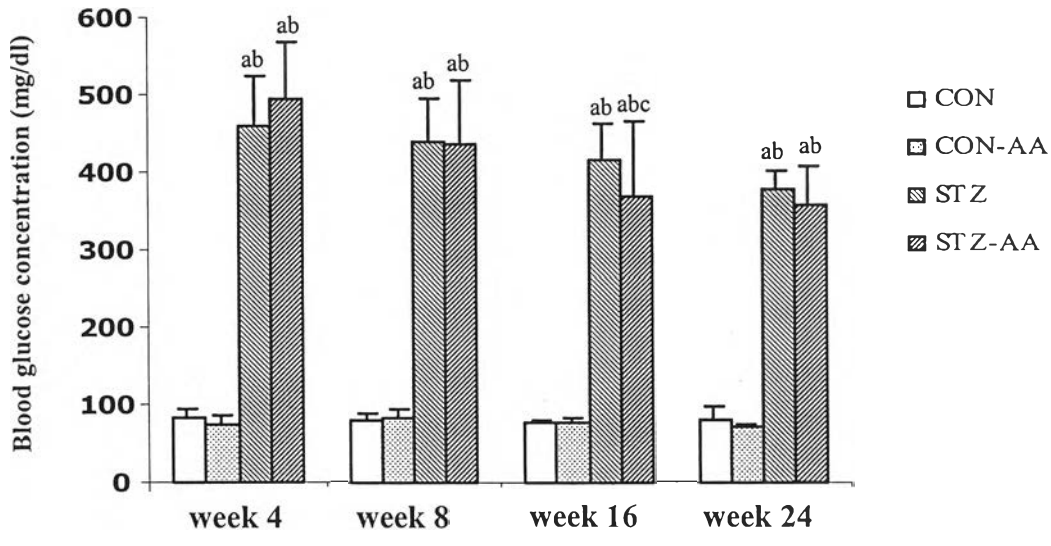

Figure 4-1 Alterations of blood glucose concentrations among groups of streptozotocin-induced diabetic rats and control rats with or without L-ascorbic acid supplementation at week 4, 8, 16 and 24 of the experimental periods. All values are means ± SD. Statistically significant differences are indicated by [a] compared with CON at each period, $p < 0.001$; [b] compared with CON-AA at each period, $p < 0.001$ and [c] compared with STZ at each period, $p < 0.05$.

Effect of L-ascorbic acid supplementation on body weight and kidney weight

The mean values of body weight were not statistically different among groups at the beginning of the experiments as shown in Table 4-2. At the experimental periods, the body weights of rats in diabetic groups with or without L-ascorbic acid supplementation were significantly lower ($p < 0.05$) than those of control groups with or without L-ascorbic acid supplementation as shown in Figure 4-2. At week 4 after diabetic induction, both STZ and STZ-AA rats showed a reduction of the body weight (161.1 $\pm$ 16.3 and 152.8 $\pm$ 22.1 gm) as compared with CON and CON-AA groups (366.7 $\pm$ 16.1 and 374.9 $\pm$ 16.5 gm, respectively). At week 8, STZ and STZ-AA had the lower body weight (140.0 $\pm$ 23.5 and 141.6 $\pm$ 39.8 gm) as compared with CON and CON-AA groups (419.5 $\pm$ 27.6 and 419.1 $\pm$ 33.2, respectively). At week 16, STZ and STZ-AA rats showed a reduction of the body weight (180.8 $\pm$ 51.2 and 216.9 $\pm$ 18.1 gm) as compared with CON and CON-AA groups (482.2 $\pm$ 33.9 and 460.6 $\pm$ 18.0, respectively). At week 24, STZ and STZ-AA rats had the lower body weight (251.7 $\pm$ 53.3 and 254.9 $\pm$ 59.7 gm) as compared with CON and CON-AA groups (504.6 $\pm$ 45.6 and 532.6 $\pm$ 25.5, respectively).

The alterations of kidney weights are shown in Table 4-2 and Figure 4-2. At week 4, the kidney weights of STZ and STZ-AA (2.3 $\pm$ 0.2 and 2.2 $\pm$ 0.3 gm) were less than those of CON and CON-AA (2.85 $\pm$ 0.33 and 3.00 $\pm$ 0.38 gram) at $p < 0.05$. At week 8, the kidney weight of STZ-AA (2.2 $\pm$ 0.2 gm) was less than those in CON, CON-AA (3.0 $\pm$ 0.4 and 2.9 $\pm$ 0.2 gram) at $p < 0.05$. However, kidney weights were not significantly different among groups at the experimental periods of week 16 and 24.

The percentages of kidney weight respected to bodyweight (KW/BW) are shown in Table 4-2 and Figure 4-2. At week 4, the percentage of kidney weight of STZ and STZ-AA (1.4 $\pm$ 0.2 and 1.4 $\pm$ 0.1 %) were higher than those of CON and CON-AA (0. $\pm$ 0.10 and 0.8 $\pm$ 0.1 %) at $p < 0.05$. At week 8, the percentage of kidney weight of STZ and STZ-AA (1.8 $\pm$ 1.0 and 1.7 $\pm$ 0.3 %) were higher than those of CON and CON-AA (0.71 $\pm$ 0.07 and 0.70 $\pm$ 0.05 %) at $p < 0.05$. At week 16, (KW/BW) of STZ-AA (1.30 $\pm$ 0.12 %) were higher than those of CON and CON-AA (0.64 $\pm$ 0.02 and 0.72 $\pm$ 0.08 %) at $p < 0.05$. Interestingly, the diabetic rats, which were supplemented with L-ascorbic acid for 16 weeks, showed the decrease in the percentage of kidney weight when compared with the diabetic rats without the AA

supplementation (1.3 $\pm$ 0.1 *vs* 1.9 $\pm$ 0.9 %). However, these finding were not apparent in the experiment at week 24. The values of KW/BW of STZ and STZ-AA were still higher than those of CON and CON-AA (1.36 $\pm$ 0.15 and 1.23 $\pm$ 0.12 *vs* 0.66 $\pm$ 0.04 and 0.67 $\pm$ 0.04 %, respectively) at $p < 0.05$.

Table 4-2 Comparison of body weight, kidney weight and ratio of kidney weight to body weight among groups of streptozotocin-induced diabetic rats and control rats with or without L-ascorbic acid supplementation at week 4, 8, 16 and 24 of the experimental periods (n=6).

	Groups	week 4	week 8	week 16	week 24
BW (g)	**CON**	366.73 ± 16.13	419.50 ± 27.61	482.20 ± 33.94	504.62 ± 45.64
	CON-AA	374.94 ± 16.48 [a,b]	419.13 ± 33.16 [a,b]	460.58 ± 18.0 [a]	532.63 ± 25.49 [a,b]
	STZ	161.13 ± 16.30 [a,b]	139.98 ± 23.53 [a,b]	180.79 ± 51.23 [a]	251.70 ± 53.25 [a,b]
	STZ-AA	152.78 ± 22.14	141.61 ± 39.83	216.91 ± 18.10	254.92 ± 59.68
KW (g)	**CON**	2.85 ± 0.33	2.96 ± 0.39	3.10 ± 0.21	3.34 ± 0.30
	CON-AA	3.00 ± 0.38 [a,b]	2.91 ± 0.18	3.31 ± 0.45	3.57 ± 0.34
	STZ	2.27 ± 0.17 [a,b]	2.51 ± 1.08 [a,b]	3.15 ± 0.74	3.36 ± 0.42
	STZ-AA	2.17 ± 0.27	2.22 ± 0.23	2.80 ± 0.15	3.13 ± 0.69
KW/BW (%)	**CON**	0.78 ± 0.10	0.71 ± 0.07	0.64 ± 0.02	0.66 ± 0.04
	CON-AA	0.80 ± 0.09 [a,b]	0.70 ± 0.05 [a,b]	0.72 ± 0.08 [a]	0.67 ± 0.04 [a,b]
	STZ	1.42 ± 0.16 [a,b]	1.84 ± 0.95 [a,b]	1.89 ± 0.86 [a,b,c]	1.36 ± 0.15 [a,b]
	STZ-AA	1.43 ± 0.11	1.65 ± 0.33	1.30 ± 0.12	1.23 ± 0.12

Mean ± SD

[a] compared with CON at the same column, $p < 0.05$; [b] compared with CON-AA at the same column, $p < 0.05$; [c] compared with STZ at the same column, $p < 0.05$

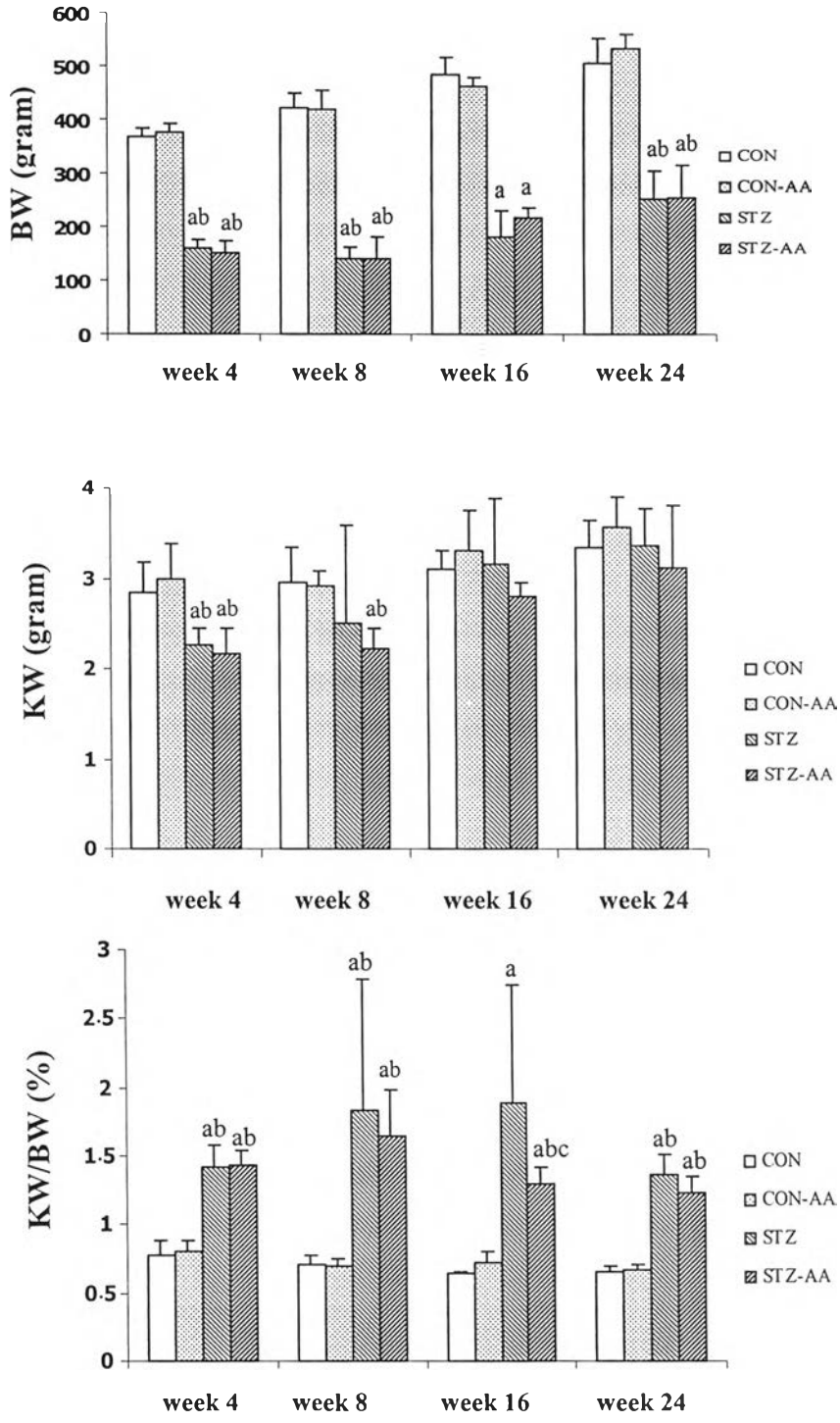

Figure 4-2 Alterations of body weight, kidney weight and percentage of kidney weight respected to body weight in streptozotocin-induced diabetic rats and control rats with or without L-ascorbic acid supplementation at week 4, 8, 16 and 24 of the experimental periods. All values are means $\pm$ SD. Statistically significant differences are indicated by ^{a} compared with CON and ^{b} compared with CON-AA at the same experimental periods, $p < 0.05$.

Effect of L-ascorbic acid supplementation on general circulation

General circulation including mean arterial pressure (MAP), systolic pressure (SP), diastolic pressure (DP), pulse pressure (PP), heart rates (HR) and hematocrit (Hct) of the rats in CON, CON-AA, STZ and STZ-AA at each observation time are presented in Table 4-3, Figure 4-3 and. Figure 4-4

At week 4 after diabetic induction, MAP, SP, DP, PP and HR of STZ and STZ-AA were significantly decreased when compared with CON and CON-AA but no significant difference were shown between STZ and STZ-AA at $p < 0.05$. MAP of STZ and STZ-AA were 86.7 $\pm$ 8.8 and 92.5 $\pm$ 21.1 mmHg while those of CON and CON-AA were 117.8 $\pm$ 10.5 and 117.7 $\pm$ 13.7 mmHg, respectively. SP of of STZ and STZ-AA were 101.2 $\pm$ 8.0 and 106.3 $\pm$ 21.3 mmHg decreasing as compared with 134.9 $\pm$ 11.7 and 135.9 $\pm$ 13.3 in CON and CON-AA, respectively. DP of STZ and STZ-AA were also significantly decreased as compared with CON and CON-AA (77.1 $\pm$ 7.4 and 85.9 $\pm$21.0 mmHg *vs* 109.5 $\pm$ 10.0 and 108.8 $\pm$ 14.0 mmHg, respectively). PP of STZ and STZ-AA were significantly decreased as compared with CON and CON-AA (21.5 $\pm$ 2.8 and 20.5 $\pm$ 2.7 mmHg *vs* 25.4 $\pm$ 3.3 and 27.1 $\pm$ 2.4 mmHg, respectively). HR of STZ and STZ-AA (240.8 $\pm$ 29.3 and 237.6 $\pm$ 59.5 beat/min) significantly declined as compared with 353.8 $\pm$ 32.7 beat/min in CON and 339.1 $\pm$ 24.3 beat/min in CON-AA.

At week 8 after diabetic induction, there was no significant difference of MAP between STZ and STZ-AA. MAP of STZ and STZ-AA were significantly decreased comparing with CON at $p < 0.05$ (92.9 $\pm$ 15.8 mmHg and 94.1 $\pm$ 12.1 *vs* 114.7 $\pm$ 12.4 mmHg, respectively). SP of of STZ and STZ-AA were 106.7 $\pm$ 13.8 mmHg and 108.8 $\pm$ 9.8 mmHg decreased comparing with 132.4 $\pm$ 9.1 and 126.1 $\pm$ 13.7 mmHg in CON and CON-AA, respectively at $p < 0.05$. DP of STZ and STZ-AA were also significantly decreased comparing with CON and CON-AA at $p < 0.05$ (86.3 $\pm$ 16.9 and 87.1 $\pm$ 13.4 mmHg *vs* 106.1 $\pm$ 14.0 mmHg). PP of STZ was significantly decreased as compared with CON and CON-AA (20.38 $\pm$ 3.9 mmHg *vs* 26.4 $\pm$ 5.1 and 27.1 $\pm$ 2.4 mmHg, respectively) at $p < 0.05$. STZ-AA had a significant decrease in PP as compared with CON-AA (21.7 $\pm$ 5.3 *vs* 26.4 $\pm$ 5.1 mmHg). The change of HR at this observation time was similar to the change of HR at week 4. Namely, HR of STZ and STZ-AA (198.4 $\pm$ 46.7 and 213.5 $\pm$ 34.1 beat/min) significantly declined

as compared with 296.3 ± 26.2 beat/min in CON and 318.2 ± 53.7 beat/min in CON-AA at $p < 0.05$.

At week 16 after diabetic induction, there was a significant difference of MAP between STZ-AA and STZ at $p < 0.05$ (107.3 ± 8.8 mmHg *vs* 102.2 ± 3.5 mmHg). SP was decreased in STZ as compared with CON and CON-AA at $p < 0.05$ (116.3 ± 3.9 mmHg *vs* 128.1 ± 11.1 and 121.4 ± 9.2 mmHg). STZ-AA had a significant increase in DP (99.4 ± 8.8mmHg) as compared with 95.34 ± 3.7 mmHg in STZ at $p < 0.05$. PP of STZ and STZ-AA were significantly decreased comparing with CON-AA (20.9 ± 2.6 and 24.1 ± 4.6 mmHg *vs* 28.6 ± 2.4 mmHg, respectively. Simultaneously, STZ had a significant decrease in PP as compared with PP of CON at $p < 0.05$ (20.9 ± 2.6 mmHg *vs* 27.3 ± 3.3 mmHg). HR of STZ was statistically decreased comparing with CON and CON-AA at $p < 0.05$ (265. 7 ± 17.7 beat/min *vs* 328.9 ± 24.7 and 333.0 ± 7.4 beat/min).

At week 24 after diabetic induction, there was no significant difference of MAP, SP, DP and HR among groups. However, PP in STZ was found a decrease as compared with PP in CON and CON-AA (21.2 ± 2.8 mmHg *vs* 29.4 ± 2.7 and 29.5± 2.2 mmHg) at $p < 0.05$.

No significant difference of hematocrit among groups was seen at week 4 and 8 of the experimental periods. At week 16, there were significantly increased in Hct of STZ and STZ-AA as compared with CON and CON-AA at $p < 0.05$ (.52.1 ± 1.8 and 50.0 ± 2.7 % *vs* 45.5 ± 1.1 and 45.6 ± 2.1 %, respectively). At week 24, only STZ-AA was found the significant increased in Hct as compared with CON and CON-AA at $p < 0.05$ (54.4 ± 3.1 % *vs* 46.3 ± 2.1 and 46.4 ± 0.9 %, respectively).

Table 4-3 General circulation of streptozotocin-induced diabetic rats and control rats with or without L-ascorbic acid supplementation at week 4, 8, 16 and 24 of the experimental periods (n=6).

	Groups	week 4	week 8	week 16	week 24
MAP (mmHg)	**CON**	117.8 ± 10.5	114.7 ± 12.4	109.7 ± 10.7	116.5 ± 14.5
	CON-AA	117.7 ± 13.7 [ab]	107.4 ± 12.2 [a]	102.2 ± 8.5	119.2 ± 18.0
	STZ	86.7 ± 8.8 [ab]	92.9 ± 15.8 [a]	102.2 ± 3.5 [c]	105.9 ± 5.3
	STZ-AA	92.5 ± 21.1	94.1 ± 12.1	107.3 ± 8.8	116.5 ± 4.2
SP (mmHg)	**CON**	134.9 ± 11.7	132.4 ± 9.1	128.1 ± 11.1	136.3 ± 15.3
	CON-AA	135.9 ± 13.3 [ab]	126.1 ± 13.7 [ab]	121.4 ± 9.2 [ab]	139.0 ± 17.7
	STZ	101.2 ± 8.0 [ab]	106.7 ± 13.8 [ab]	116.3 ± 3.9	120.2 ± 5.4
	STZ-AA	106.3 ± 21.3	108.8 ± 9.8	123.5 ± 9.6	131.8 ± 8.0
DP (mmHg))	**CON**	109.5 ± 10.0	106.1 ± 14.0	100.8 ± 10.6	106.8 ± 14.2
	CON-AA	108.8 ± 14.0 [ab]	98.3 ± 11.5 [a]	92.8 ± 8.2	109.5 ± 18.2
	STZ	77.1 ± 7.4 [ab]	86.3 ± 16.9 [a]	95.3 ± 3.7 [c]	99.0 ± 5.5
	STZ-AA	85.9 ± 21.0	87.1 ± 13.4	99.4 ± 8.8	109.0 ± 2.9

Mean ± SD

[a] compared with CON at the same column, $p < 0.05$; [b] compared with CON-AA at the same column, $p < 0.05$; [c] compared with STZ at the same column, $p < 0.05$

Table 4-3 General circulation of streptozotocin-induced diabetic rats and control rats with or without L-ascorbic acid supplementation at week 4, 8, 16 and 24 of the experimental periods (n=6). (continued)

	Groups	week 4	week 8	week 16	week 24
PP (mmHg)	**CON**	25.4 ± 3.3	26.4 ± 5.1	27.3 ± 3.3	29.4 ± 2.7
	CON-AA	27.1 ± 2.4 [ab]	27.8 ± 3.1 [ab]	28.6 ± 2.4 [ab]	29.5± 2.2 [ab]
	STZ	21.5 ± 2.8 [ab]	20.4 ± 3.9 [b]	20.9 ± 2.6 [b]	21.2 ± 2.8
	STZ-AA	20.5 ± 2.7	21.7 ± 5.3	24.1 ± 4.6	22.8 ± 6.4
HR (beat/min)	**CON**	353.8 ± 32.7	296.3 ± 26.2	328.9 ± 24.8	335.5 ± 60.9
	CON-AA	339.1 ± 24.3 [ab]	318.2 ± 53.7 [ab]	333.0 ± 7.39 [ab]	343.7 ± 29.4
	STZ	240.8 ± 29.3 [ab]	198.4 ± 46.7 [ab]	265.7 ± 17.7	301.0 ± 31.6
	STZ-AA	237.6 ± 59.5	213.5 ± 34.1	254.1 ± 75.9	236.3 ± 86.9
Hct (%)	**CON**	47.0 ± 2.28	45.3 ± 1.0	45.5 ± 1.1	46.3 ± 2.1
	CON-AA	44.8 ± 2.7	44.6 ± 1.5	45.6 ± 2.1 [ab]	46.4 ± 0.9
	STZ	45.5 ± 1.8	46.1 ± 4.2	52.1 ± 1.8 [ab]	49.7 ± 3.8 [ab]
	STZ-AA	46.8 ± 1.8	47.6 ± 3.1	50.0 ± 2.7	54.4 ± 3.1

Mean ± SD

[a] compared with CON at the same column, $p < 0.05$; [b] compared with CON-AA at the same column, $p < 0.05$; [c] compared with STZ at the same column, $p < 0.05$

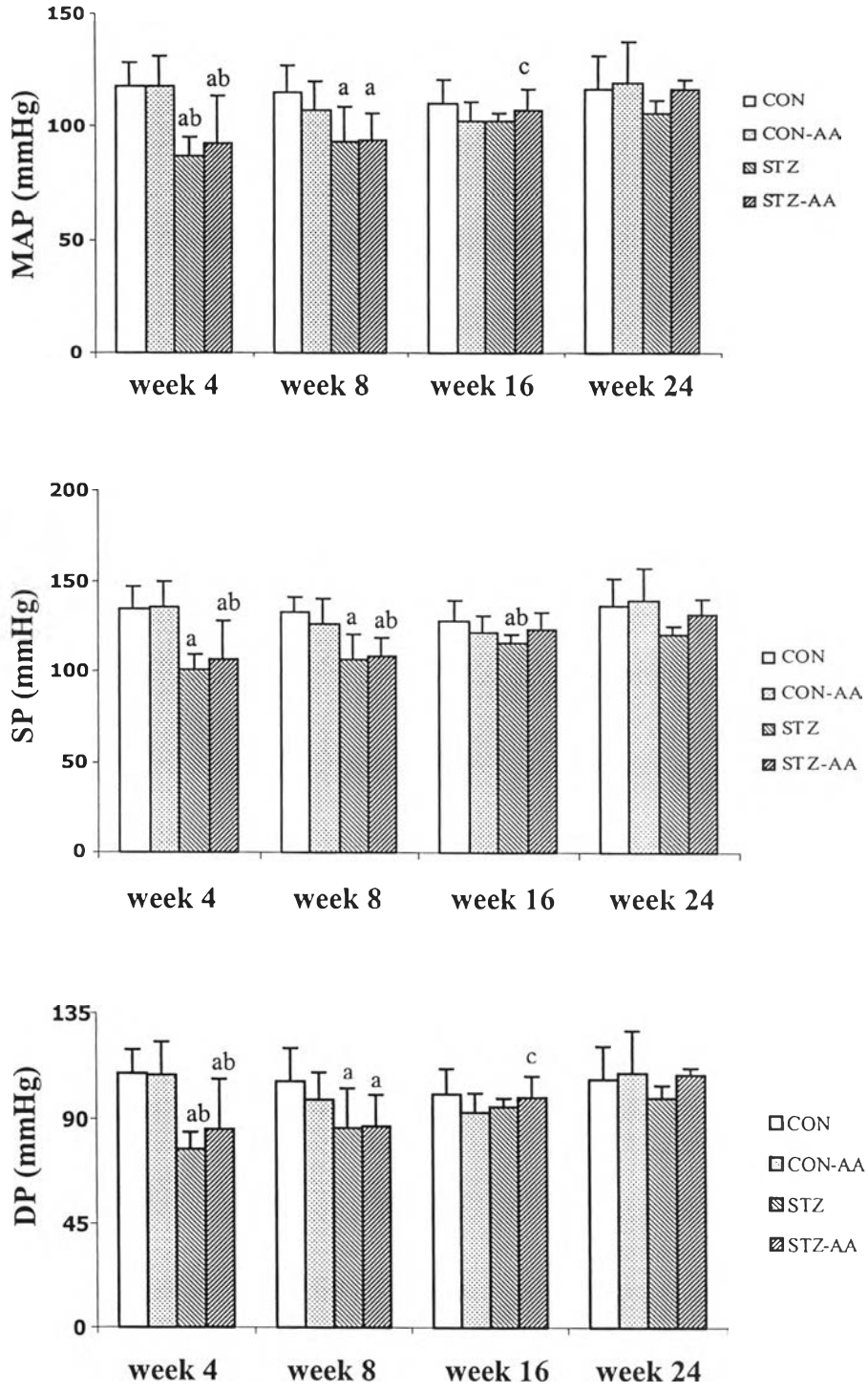

Figure 4-3 Alterations of mean arterial pressure (MAP), systolic pressure (SP) and diastolic pressure (DP) in streptozotocin-induced diabetic rats and control rats with or without L-ascorbic acid supplementation at week 4, 8, 16 and 24 of the experimental periods. All values are means ± SD. Statistically significant differences are indicated by [a] compared with CON, [b] compared with CON-AA and [c] compared with STZ at the same observation period, $p < 0.05$.

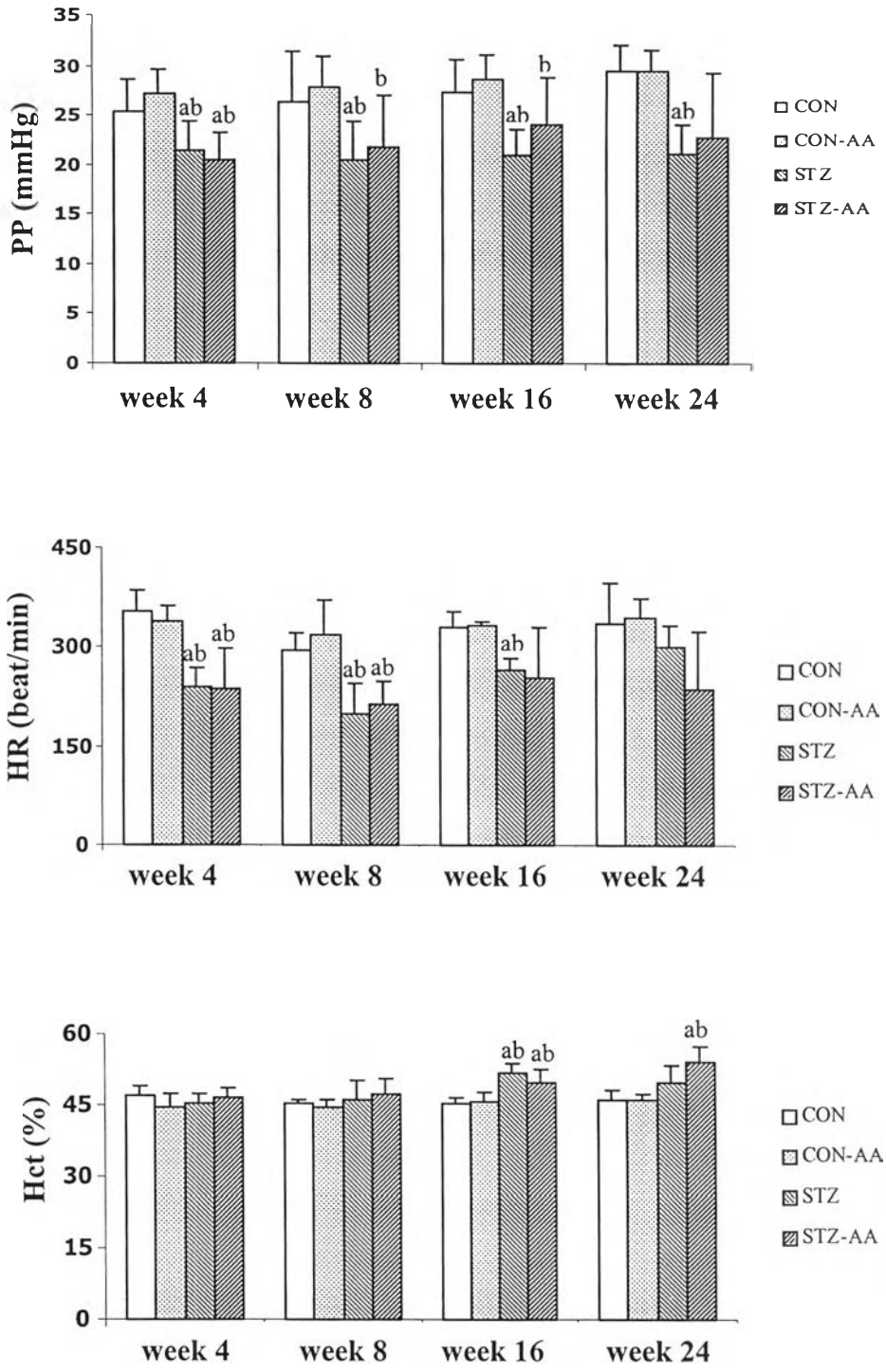

Figure 4-4 Alterations of pulse pressure (PP), heart rate (HR) and hematocrit (Hct) in streptozotocin-induced diabetic rats and control rats with or without L-ascorbic acid supplementation at week 4, 8, 16 and 24 of the experimental periods. All values are means ± SD. Statistically significant differences are indicated by [a] compared with CON and [b] compared with CON-AA at the same observation period, $p < 0.05$.

Effect of L-ascorbic acid supplementation on renal hemodynamics and glomerular functions

Renal hemodynamics and functions including glomerular filtration rate (GFR), effective renal plasma flow (ERPF), effective renal blood flow (ERBF), renal vascular resistance (RVR), filtration fraction (FF) of the rats in CON, CON-AA, STZ and STZ-AA at each observation time are presented in Table 4-4, Figure 4-5 and Figure 4-6.

At week 4 after diabetic induction, GFR of STZ was significantly decreased as compared with CON-AA at $p < 0.05$ (0.5 ± 0.2 *vs* 0.8 ± 0.2 ml/min/g KW). STZ-AA had significant decrease in GFR as compared with CON and CON-AA at $p < 0.05$ (0.4 ± 0.1 *vs* 0.6 ± 0.2 and 0.8 ± 0.2 ml/min/g KW). GFR of CON-AA was significantly increased in comparison with CON at $p < 0.05$ (0.8 ± 0.2 *vs* 0.6 ± 0.2 ml/min/g KW). ERPF of STZ and STZ-AA were 0.7 ± 0.3 and 0.6 ± 0.2 ml/min/g KW decreasing as compared with CON and CON-AA at $p < 0.05$ (1.9 ± 0.7 and 2.6 ± 0.7 ml/min/g KW, respectively). CON-AA had markedly higher GFR than CON at $p < 0.05$ (2.6 ± 0.7 *vs* 1.9 ± 0.7 ml/min/g KW). ERBF had similar changes to ERPF as shown in Table 4. FF of STZ and STZ-AA was markedly increased as compared with CON and CON-AA at $p < 0.05$ (92.8 ± 64.7 and 76.5 ± 23.8 % *vs* 36.0 ± 6.3 and 31.7 ± 3.5 %, respectively). The RVR of both STZ and STZ-AA were significantly increased as compared with CON and CON-AA (92.0 ± 47.1 and 101.4 ± 41.1 mmHg/ml/min/g KW *vs* 38.3 ± 12.5 and 28.6 ± 13.9 mmHg/ml/min/g KW, respectively).

At week 8 after diabetic induction, GFR in STZ and STZ-AA were markedly decreased as compared with CON and CON-AA at $p < 0.05$ (0.29 ± 0.16 and 0.39 ± 0.14 ml/min/g KW *vs* 0.98 ± 0.16 and 0.95 ± 0.110 ml/min/g KW). ERPF of STZ and STZ-AA were 0.47 ± 0.19 and 0.56 ± 0.15 ml/min/g KW decreasing as compared with 3.14± 0.24 and 3.55 ± 0.37 ml/min/g KW at $p < 0.05$. CON-AA had a significant higher ERPF than CON at $p < 0.05$ (3.55 ± 0.37 *vs* 3.14± 0.24 ml/min/g KW). ERBF had similar changes to ERPF as shown in Table 4. Changes of FF at this time were similar to those at week 4. FF of STZ and STZ-AA was markedly increased as compared with CON and CON-AA at $p < 0.05$ (90.17 ± 69.13 and 75.48 ± 28.21 % *vs* 31.28 ± 3.50 and 26.86± 2.59 %, respectively). RVR in both STZ and STZ-AA were significantly increased as compared with CON and CON-AA (145.26

± 61.28 and 97.13 ± 33.60 mmHg/ml/min/g KW *vs* 20.51 ± 2.0 and 16.29±1.97 mmHg/ml/min/g KW, respectively). Interestingly, L-ascorbic acid supplementation could reduce the increase in RVR significantly in diabetic rats (97.13 ± 33.60 in STZ-AA as compared with 145.26 ± 61.28 mmHg/ml/min/g KW in STZ).

At week 16 after diabetic induction, GFR in STZ and STZ-AA were significantly decreased as compared with CON and CON-AA at $p < 0.05$ (0.36 ± 0.12 and 0.59 ± 0.16 ml/min/g KW *vs* 0.82 ± 0.13 and 0.95 ± 0.18 ml/min/g KW). Fascinatingly, L-ascorbic acid supplementation was able to ameliorate the decrease in GFR of diabetic rats. Namely, GFR was significant increased from 0.36 ± 0.12 ml/min/g KW in STZ to 0.59 ± 0.16 ml/min/g KW in STZ-AA. ERPF of STZ and STZ-AA (0.49 ± 0.20 and 0.70 ± 0.12 ± 0.19 ml/min/g KW) were markedly decreased as compared with CON and CON-AA (2.82 ± 0.54 and 3.05 ± 0.50 ml/min/g KW) at $p < 0.05$. An increase in ERPF was consistent with the increase in GFR of STZ-AA. ERPF of STZ-AA was significantly increased as compared with ERPF in STZ at $p < 0.05$ (0.70 ± 0.12 *vs* 0.49 ± 0.20 ml/min/g KW). Changes of FF at this time were similar as those at week 4 and 8. FF of STZ and STZ-AA was markedly increased as compared with CON and CON-AA at $p < 0.05$ (79.26 ± 18.51 and 85.18 ± 18.49 % *vs* 30.00 ± 6.70 and 31.26 ± 1.48 %, respectively). RVR in both STZ and STZ-AA were significantly increased as compared with CON and CON-AA (130.47 ± 73.17 and 79.54 ± 9.91 mmHg/ml/min/g KW *vs* 21.70 ± 5.36 and 18.72 ± 3.84 mmHg/ml/min/g KW, respectively). Interestingly, L-ascorbic acid supplementation showed the amelioration of the increase in RVR as similar as shown at week 8 of the observation time (79.54 ± 9.91 mmHg/ml/min in STZ-AA as compared with 130.47 ± 73.17 mmHg/ml/min in STZ).

At week 24 after diabetic induction, GFR in STZ was significantly decreased as compared with CON at $p < 0.05$ (0.58 ± 0.08 *vs* 0.97 ± 0.18 ml/min/g KW). STZ-AA had the significant decrease in GFR as compared with CON and CON-AA at $p < 0.05$ (0.32 ± 0.07 *vs* 0.97 ± 0.18 and 0.94 ± 0.25 ml/min/g KW, respectively). At this time, L-ascorbic acid supplementation did not ameliorate the decrease in GFR of diabetic rats. Conversely, GFR of STZ-AA was significantly decreased as compared with GFR in STZ at $p < 0.05$ (0.32 ± 0.07 *vs* 0.58 ± 0.08 ml/min/g KW). ERPF had similar changes as ERBF. ERPF of STZ-AA was significantly decreased as compared with STZ at $p < 0.05$ (0.23 ± 0.17 *vs* 0.67 ± 0.09 ml/min/g KW). ERBF of

STZ-AA was 0.43 ± 0.28 ml/min/g KW markedly decreased comparing with 1.26 ± 0.16 ml/min/g KW of STZ at $p < 0.05$. FF of STZ and STZ-AA was markedly increased as compared with CON and CON-AA at $p < 0.05$ (84.30 ± 10.16 and 154.29 ± 57.86 % *vs* 26.63 ± 2.44 and 28.58 ± 4.20 %, espectively). FF of STZ-AA was significantly increased comparing with STZ at $p < 0.05$ (154.29 ± 57.86 *vs* 84.30 ± 10.16 %). RVR in both STZ and STZ-AA were significantly increased as compared with CON and CON-AA (83.09 ± 10.29 and 339.55 ± 131.09 mmHg/ml/min/g KW *vs* 19.17 ± 5.17 and 21.26 ± 8.51 mmHg/ml/min/g KW, respectively). L-ascorbic acid supplementation to the diabetic for 24 weeks did not decrease RVR in STZ-AA (339.55 ± 131.09 in STZ-AA as compared with 83.09 ± 10.29 mmHg/ml/min/g KW).

Table 4-4 Renal hemodynamics of streptozotocin-induced diabetic rats and control rats with or without L-ascorbic acid supplementation at week 4, 8, 16 and 24 of the experimental periods (n=6).

	Groups	week 4	week 8	week 16	week 24
GFR (ml/min/g KW)	**CON**	0.63 ± 0.18	0.98 ± 0.16	0.82 ± 0.13	0.97 ± 0.18
	CON-AA	0.82 ± 0.23 [a]	0.95 ± 0.11 [ab]	0.95 ± 0.18 [ab]	0.94 ± 0.25 [a]
	STZ	0.49 ± 0.16 [ab]	0.29 ± 0.16 [ab]	0.36 ± 0.12 [abc]	0.58 ± 0.08 [abc]
	STZ-AA	0.39 ± 0.06	0.39 ± 0.14	0.59 ± 0.16	0.32 ± 0.07
ERPF (ml/min/g KW)	**CON**	1.9± 0.7 [a]	3.14± 0.24 [a]	2.82 ± 0.54	3.59 ± 0.83
	CON-AA	2.60 ± 0.7 [ab]	3.55 ± 0.37 [ab]	3.05 ± 0.50 [ab]	3.37 ± 1.01 [ab]
	STZ	0.7 ± 0.3 [ab]	0.5 ± 0.2 [ab]	0.5 ± 0.2 [abc]	0.7 ± 0.1 [abc]
	STZ-AA	0.57 ± 0.21	0.56 ± 0.15	0.70 ± 0.12	0.23 ± 0.17
ERBF (ml/min/g KW)	**CON**	3.6± 1.4 [a]	5.8± 0.5 [a]	5.4 ± 1.2	6.7 ± 1.5
	CON-AA	4.8 ± 1.5 [ab]	6.4 ±0.5 [ab]	5.6 ± 1.0 [ab]	6.2 ± 1.8 [ab]
	STZ	1.2 ± 0.6 [ab]	0.8 ± 0.3 [ab]	1.0 ± 0.4 [ab]	1.3 ± 0.2 [abc]
	STZ-AA	1.1 ± 0.4	1.1 ± 0.3	1.4 ± 0.3	0.4 ± 0.3

Mean ± SD

[a] compared with CON at the same column, [b] compared with CON-AA at the same column, [c] compared with STZ at the same column; $p < 0.05$

Table 4-4 Renal hemodynamics of streptozotocin-induced diabetic rats and control rats with or without L-ascorbic acid supplementation at week 4, 8, 16 and 24 of the experimental periods (n=6). (continued)

	Groups	week 4	week 8	week 16	week 24
FF (%)	**CON**	36.0 ± 6.3	31.3 ± 3.5	30.0 ± 6.7	26.6 ± 2.4
	CON-AA	31.7 ± 3.5 [ab]	26.9 ± 2.6 [ab]	31.3 ± 1.5 [ab]	28.6 ± 4.2 [ab]
	STZ	92.8 ± 64.7 [ab]	90.2 ± 69.1 [ab]	79.3 ± 18.5 [ab]	84.3 ± 10.2 [abc]
	STZ-AA	76.5 ± 23.8	75.5 ± 28.2	85.2 ± 18.5	154.3 ± 57.9
RVR (mmHg/ml/min/g KW)	**CON**	38.3 ± 12.5	20.5 ± 2.0	21.7 ± 5.4	19.2 ± 5.2
	CON-AA	28.6 ± 13.9 [ab]	16.29 ± 2.0 [ab]	18.7 ± 3.8 [ab]	21.3 ± 8.5 [ab]
	STZ	92.0 ± 47.1 [ab]	145.3 ± 61.3 [abc]	130.5 ± 73.2 [abc]	83.1 ± 10.3 [abc]
	STZ-AA	101.4 ± 41.1	97.1 ± 33.6	79.5 ± 9.9	339.6 ± 131.1

Mean ± SD

[a] compared with CON at the same column, [b] compared with CON-AA at the same column, [c] compared with STZ at the same column; $p < 0.05$.

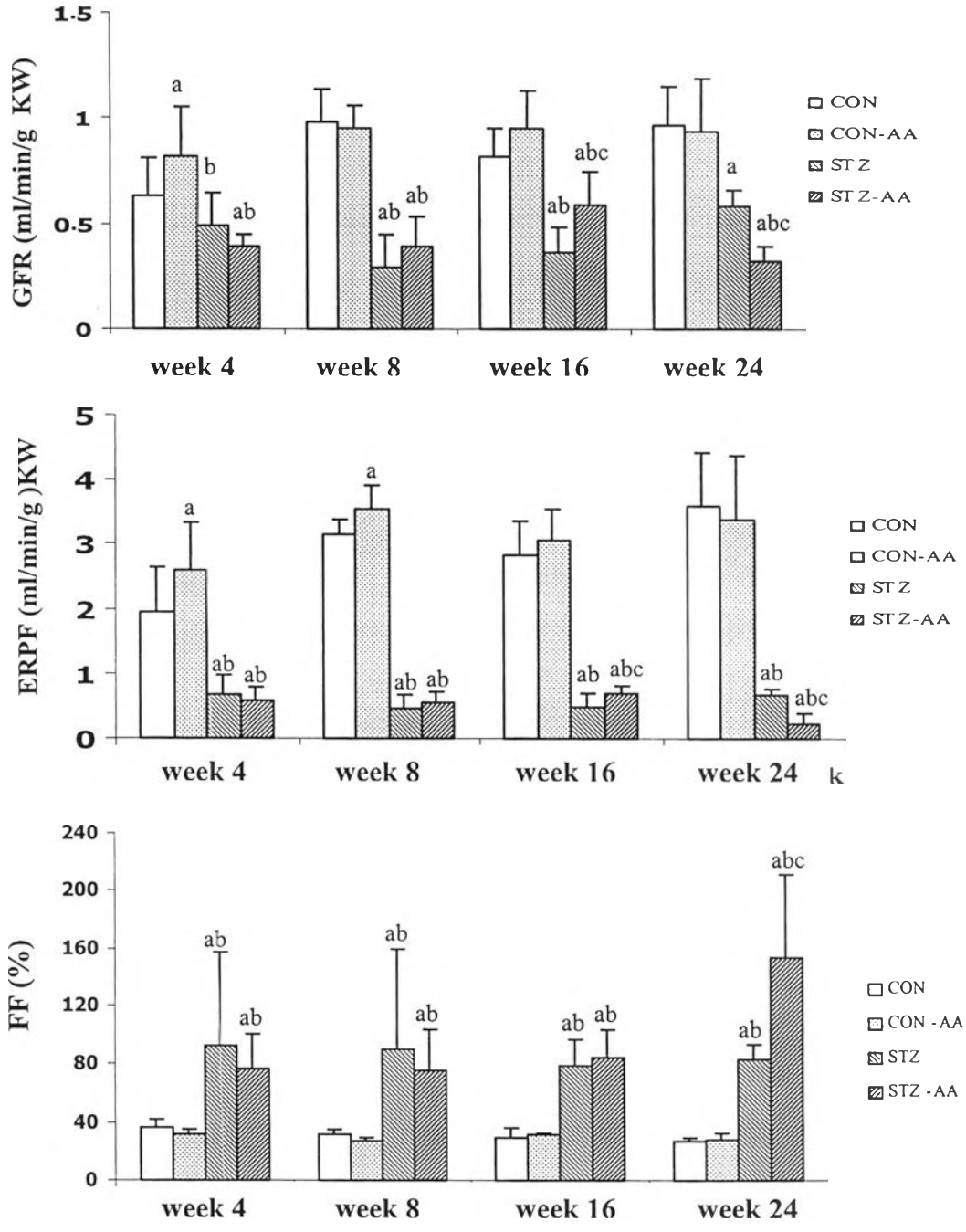

Figure 4-5 Alterations of glomerular filtration rate (GFR), effective renal plasma flow (ERPF) and filtration fraction (FF) in streptozotocin-induced diabetic rats and control rats with or without L-ascorbic acid supplementation at week 4, 8, 16 and 24 of the experimental periods. All values are means ± SD. Statistically significant differences are indicated by [a] compared with CON, [b] compared with CON-AA and CON and [c] compared with CON-AA at the same observation period, $p < 0.05$.

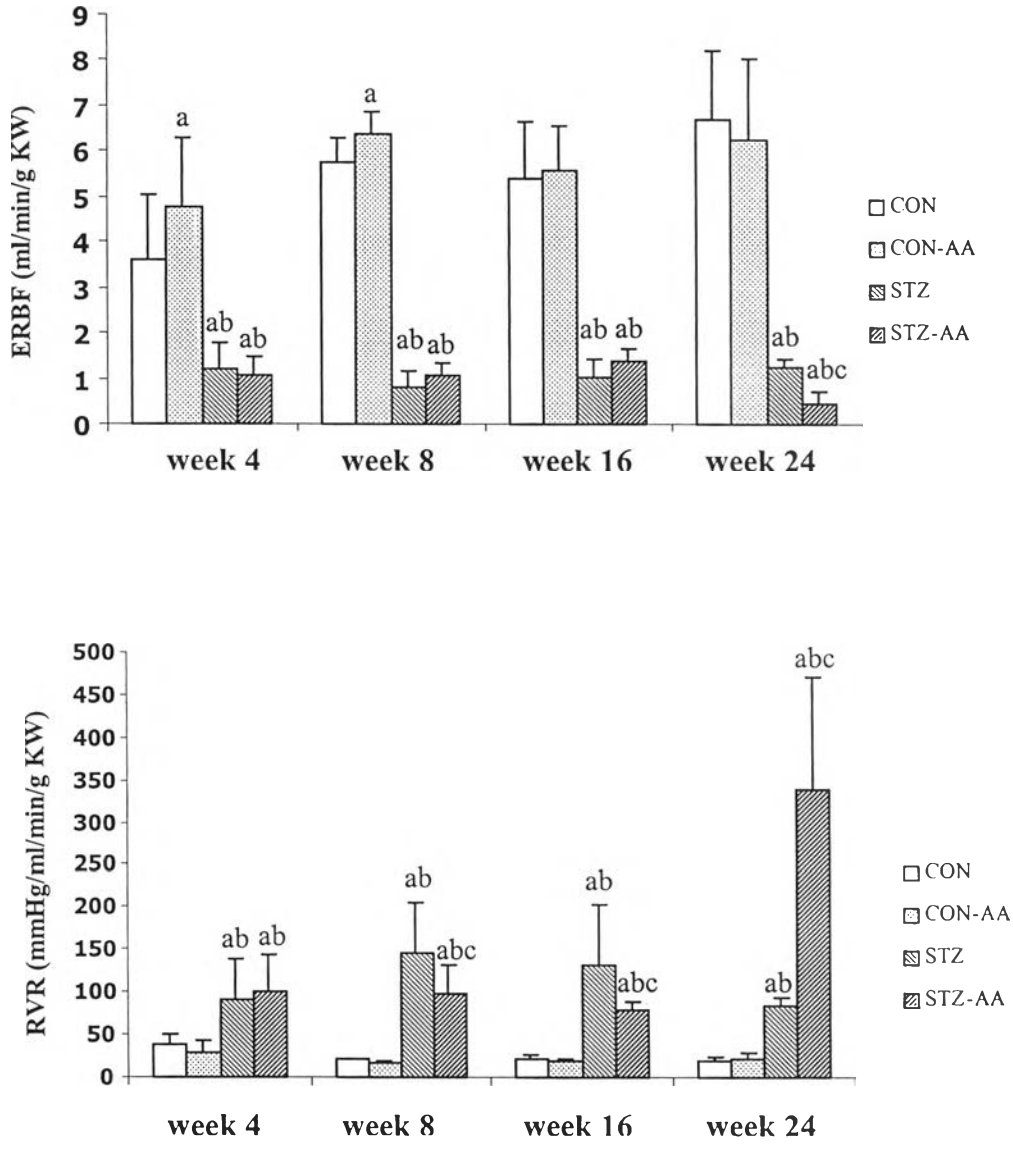

Figure 4-6 Alterations of effective renal blood flow (ERBF), renal vascular resistance (RVR) in streptozotocin-induced diabetic rats and control rats with or without L-ascorbic acid supplementation at week 4, 8, 16 and 24 of the experimental periods. All values are means ± SD. Statistically significant differences are indicated by [a] compared with CON, [b] compared with CON-AA and CON and [c] compared with CON-AA at each experimental period, $p < 0.05$.

Effect of L-ascorbic acid supplementation on renal tubular functions

Renal tubular functions including urine flow rate (V), fractional excretion of urine (V/GFR), urinary excretion of electrolytes (UV_{Na}, UV_K UV_{Cl}) and fractional excretion of electrolytes (FE_{Na}, FE_K, FE_{Cl}) of the rats in CON, CON-AA, STZ and STZ-AA at each observation time were reported in Table 4-5, 4-6 and 4-7, Figure 4-7, 4-8 and 4-9. In addition, plasma electrolytes were shown in Table 4-8 and Figure 4-10.

At week 4 after diabetic induction, urine flow rate of STZ-AA was significantly decreased compared with CON and CON-AA at $p < 0.05$ (5.8 $\pm$ 2.0 *vs* 10.5 $\pm$ 4.0 and 11.1 $\pm$ 6.2 µl/min/g KW, respectively). But no significant difference of V/GFR was shown among groups. $U_{Na}V$ of STZ and STZ-AA were significantly decreased compared with CON and CON-AA at $p < 0.05$ (1.4 $\pm$ 0.8 and 1.0 $\pm$ 0.5 mEq/min/g KW *vs* 3.4 $\pm$ 1.0 and 3.6 $\pm$ 1.3 mEq/min/g KW, respectively). U_KV and $U_{Cl}V$ had similar changes as $U_{Na}V$. Namely, they were significantly decreased in STZ and STZ-AA compared with CON and CON-AA. U_KV of STZ and STZ-AA were 0.4 $\pm$ 0.2 and 3.0 $\pm$ 0.2 mEq/min/g KW compared with 0.8 $\pm$ 0.1 and 0.9 $\pm$ 0.3 mEq/min/g KW. in CON and CON-AA, respectively. UV_{Cl} of STZ and STZ-AA (0.4 $\pm$ 0.2 and 0.7 $\pm$ 0.4 mEq/min/g KW) were significantly decreased ($p < 0.05$) as compared with those of CON and CON-AA (1.9 $\pm$ 1.0 and 2.5 $\pm$ 1.2 mEq/min/g KW).

FE_{Na} of STZ and STZ-AA at week 4 after diabetic induction were significantly decreased compared with CON and CON-AA at $p < 0.05$ (2.1 $\pm$ 0.6 and 1.4 $\pm$ 0.5 % *vs* 4.2 $\pm$ 1.1 and 3.6 $\pm$ 1.0 %, respectively). FE_K of STZ and STZ-AA were significantly decreased compared with CON at $p < 0.05$ (26.27 $\pm$ 5.73 and 20.5 $\pm$ 7.0 % *vs* 46.2 $\pm$ 12.4 %). L-ascorbic acid had an effect on FE_K in CON-AA. FE_K of CON-AA was decreased compared with CON at $p < 0.05$ (28.7 $\pm$ 4.6 *vs* 46.2 $\pm$ 12.4 %). The manifest decreases in FE_{Cl} were found in STZ and STZ-AA comparing with CON and CON-AA at $p < 0.05$ (0.9 $\pm$ 0.2 and 0.8 $\pm$ 0.5 % *vs* 2.4 $\pm$ 1.1 and 3.3 $\pm$ 0.7 %)

At week 8 after diabetic induction, V/GFR of STZ and STZ-AA was significantly increased comparing with CON and CON-AA at $p < 0.05$ (2.1 $\pm$ 0.6 and 1.9 $\pm$ 0.2 % *vs* 1.2 $\pm$ 0.3 and 1.2 $\pm$ 0.6 %, respectively). Urinary excretion of the electrolytes of STZ and STZ-AA were markedly decreased compared with CON and CON-AA. $U_{Na}V$ of STZ and STZ-AA were 1.0 $\pm$ 0.5 and 1.1 $\pm$ 0.5 mEq/min/g KW

while $U_{Na}V$ of CON and CON-AA were higher (4.1 ± 1.1 and 3.6 ± 1.5 mEq/min/g KW, respectively). U_KV of STZ and STZ-AA (0.2 ± 0.1 and 0.3 ± 0.1 mEq/min/g KW) were decreased comparing with 0.9 ± 0.15 mEq/min/g KW in CON and 0.83 ± 0.2 mEq/min/g KW in CON-AA. $U_{Cl}V$ of STZ and STZ-AA (0.6 ± 0.5 and 0.4 ± 0.3 mEq/min/g KW) were decreased comparing with 2.8 ± 1.2 and 2.5 ± 1.4 mEq/min/g KW. in CON and CON-AA, respectively ($p < 0.05$).

AA could decrease FE_{Na} and FE_K in diabetic rats at week 8 after the supplementation with AA. FE_{Na} of STZ-AA was significantly decreased comparing with CON, CON-AA and STZ at $p < 0.05$ (1.9 ± 0.3 % *vs* 3.0 ± 05, 3.0 ± 0.8 and 2.7 ± 0.4 %, respectively). FE_K of STZ-AA was similarly changed as FE_{Na}. Namely, it was significantly decreased comparing with CON, CON-AA and STZ at $p < 0.05$ (18.3 ± 2.6 % *vs* 27.3 ± 3.3, 27.1 ± 4.1 and 26.1 ± 7.0 %, respectively). FE_{Cl} of STZ-AA was significantly decreased comparing with CON and CON-AA at $p < 0.05$ (0.9 ± 0.5 % *vs* 2.4 ± 0.7, 2.5 ± 1.1 and 1.8 ± 0.8 %).

At week 16 after diabetic induction, V/GFR of STZ was significantly increased comparing with CON at $p < 0.05$ (1.8 ± 0.8 *vs* 1.1 ± 0.3 %, respectively). At this observation time, V/GFR of STZ-AA was not different from CON (1.4 ± 0.3 vs 1.3 ± 0.6 %). $U_{Na}V$ of STZ and STZ-AA (0.6 ± 0.4 and 1.3 ± 1.0 mEq/min/g KW) were significantly decreased as comparing with CON and CON-AA (3.8 ± 0.9 and 4.1 ± 1.1 mEq/min/g KW). U_KV of STZ and STZ-AA (0.2 ± 0.1± 0.1 and 0.3 ± 0.1 mEq/min/g KW) were significantly decreased comparing with 0.72 ± 0.08 and 0.71 ± 0.1 mEq/min/g KW in CON and CON-AA, respectively. U_{Cl} Vof STZ and STZ-AA (0.9 ± 0.8 and 0.7 ± 0.3 mEq/min/g KW) were decreased comparing with 2.4 ± 0.9 and 3.0 ± 1.4 mEq/min/g KW. in CON and CON-AA, respectively ($p < 0.05$).

FE_{Na} of STZ (1.3 ± 0.5 %) and STZ-AA (1.7 ± 0.7 %) were significantly decreased as compared with CON (3.8 ± 1.4 %) and CON-AA (3.2 ± 1.1 %). A significant decrease in FE_K of STZ-AA (17.3 ± 1.5) was found as compared with CON (25.6 ± 2.3 %). FE_{Cl} of STZ (1.6 ± 0.5 %) and STZ-AA (1.4 ± 0.5 %) was significantly decreased at $p < 0.05$ comparing with CON (3.2 ± 1.6 %) and CON-AA (3.02 ± 1.8 %).

At week 24 after diabetic induction, V/GFR of STZ and STZ-AA were significantly increased comparing with CON at $p < 0.05$ (2.6 ± 0.9 and 3.1 ± 1.1 % *vs* 0.9 ± 0.3 %, respectively). UV_{Na} STZ (2.5 ± 1.2 mEq/min/g KW) was significantly

decreased (P <0.05) as comparing with CON-AA 3.9 ± 1.6 mEq/min/g KW). L-ascorbic acid could decrease $U_{Na}V$ of STZ-AA (1.0 ± 0.8 mEq/min/g KW) as compared with those of CON (3.3 ± 0.4 mEq/min/g KW), CON-AA and STZ at week 24 after AA supplementation. U_KV had a similar change as $U_{Na}V$. Namely, U_KV of STZ-AA was significantly decreased compared with STZ at $p < 0.05$ (0.4 ± 0.2 vs 0.6 ± 0.2 mEq/min/g KW). It was also markedly decreased when compared with CON and CON-AA (0.7 ± 0.2 and 0.8 ± 0.2 mEq/min/g KW). $U_{Cl}V$ of STZ and STZ-AA (1.0 ± 0.5 and 0.9 ± 0.4 mEq/min/g KW) were decreased ($p < 0.05$) as comparing with 1.9 ± 0.6 and 2.6 ± 1.6 mEq/min/g KW in CON and CON-AA, respectively

FE_{Na}.of STZ-AA (1.4 ± 0.9 %) was significantly decreased (P <0.05) as compared with CON (3.9 ± 1.3 %), CON-AA (3.1 ± 1.0 %) and (3.3 ± 1.2 %). The significant increases ($p < 0.05$) in FE_K of STZ (35.2 ± 9.5) and STZ-AA (36.2 ± 8.8 %) were found compared with CON (21.8 ± 4.8) and CON-AA (23.0 ± 3.4 %).

The alterations of plasma electrolytes are presented in Table 8. No significant change of P_{Na} among groups was seen at week 8 and 16 of the experimental periods. There were the decreases in P_{Na} of STZ-AA compared with CON and CON-AA at week 4 and 24 of the experimental periods. At week 4, P_{Na} of STZ-AA was 134.1 ± 5.1 mEq/L comparing with 138.3 ± 2.2 mEq/L in CON and 139.1 ± 3.4 mEq/L in CON-AA at $p < 0.05$. At week 16s of the experimental periods P_{Na} of STZ-AA was 117.9 ± 13.2 mEq/L comparing with 136.25 ± 1.64 mEq/L in CON and 136.9 ± 1.5 mEq/L in CON-AA at $p < 0.05$. L-ascorbic acid showed the effect on plasma sodium concentration in diabetic rats by significantly reducing P_{Na} in STZ-AA compared with STZ at $p < 0.05$ (117.9 ± 13.2 *vs* 133.8 ± 2.2 mEq/L). At week 4, 8 and 16 of the experimental periods, plasma potassium concentrations were unaltered compared among groups each times. At week 24 of the observation time, P_K of STZ-AA was significantly decreased comparing with CON and CON-AA at $p < 0.05$ (2.8 ± 0.7 *vs* 3.5 ± 0.2 and 3.5 ± 0.2 mEq/L). There was no significant alteration of plasma chloride concentrations compared among groups at week 4 and 8 of the experimental periods. At week 16, P_{Cl} of STZ and STZ-AA were significantly decreased comparing with CON-AA at $p < 0.05$ (98.4 ± 4.1 and 98.4 ± 4.2 mEq/L *vs* 105.4 ± 3.0 mEq/L, respectively). At week 24, P_{Cl} of STZ was significantly decreased comparing with CON and CON-AA at $p < 0.05$ (99.5 ± 3.0 mEq/L *vs* 104.9 ± 2.8 and

105.2 ± 1.4 mEq/L, respectively). STZ-AA had a significant decrease in P_{Cl} (101.0 ± 5.0 mEq/L) compared with 105.2 ± 1.4 mEq/L of CON-AA at $p < 0.05$.

Table 4-5 Urine flow rate and fractional excretion of urine of streptozotocin-induced diabetic rats and control rats with or without L-ascorbic acid supplementation at week 4, 8, 16 and 24 of the experimental periods (n=6).

	Groups	week 4	week 8	week 16	week 24
V (μl/min/gm KW)	**CON**	10.5 ± 4.0	11.9 ± 4.3	13.0 ± 8.7	9.0 ± 2.5
	CON-AA	11.1 ± 6.2	11.7 ± 6.3	11.7 ± 4.9	16.2 ± 9.0
	STZ	7.2 ± 2.9	5.8 ± 3.3 [ab]	8.1 ± 4.6	14.9 ± 6.4
	STZ-AA	5.75 ± 2.04 [ab]	7.22 ± 1.99	8.49 ± 2.45	10.97 ± 2.43
V /GFR (%)	**CON**	2.1 ± 1.0	1.2 ± 0.3	1.1 ± 0.3	0.9 ± 0.3
	CON-AA	1.5 ± 0.6	1.2 ± 0.6 [ab]	1.3 ± 0.6 [a]	2.0 ± 1.1 [a]
	STZ	1.5 ± 0.4	2.1 ± 0.6 [ab]	1.8 ± 0.8	2.6 ± 0.9 [a]
	STZ-AA	1.4 ± 0.4	1.9 ± 0.2	1.4 ± 0.3	3.1 ± 1.1

Mean ± SD

[a] compared with CON at the same column, $p < 0.05$; [b] compared with CON-AA at the same column, $p < 0.05$.

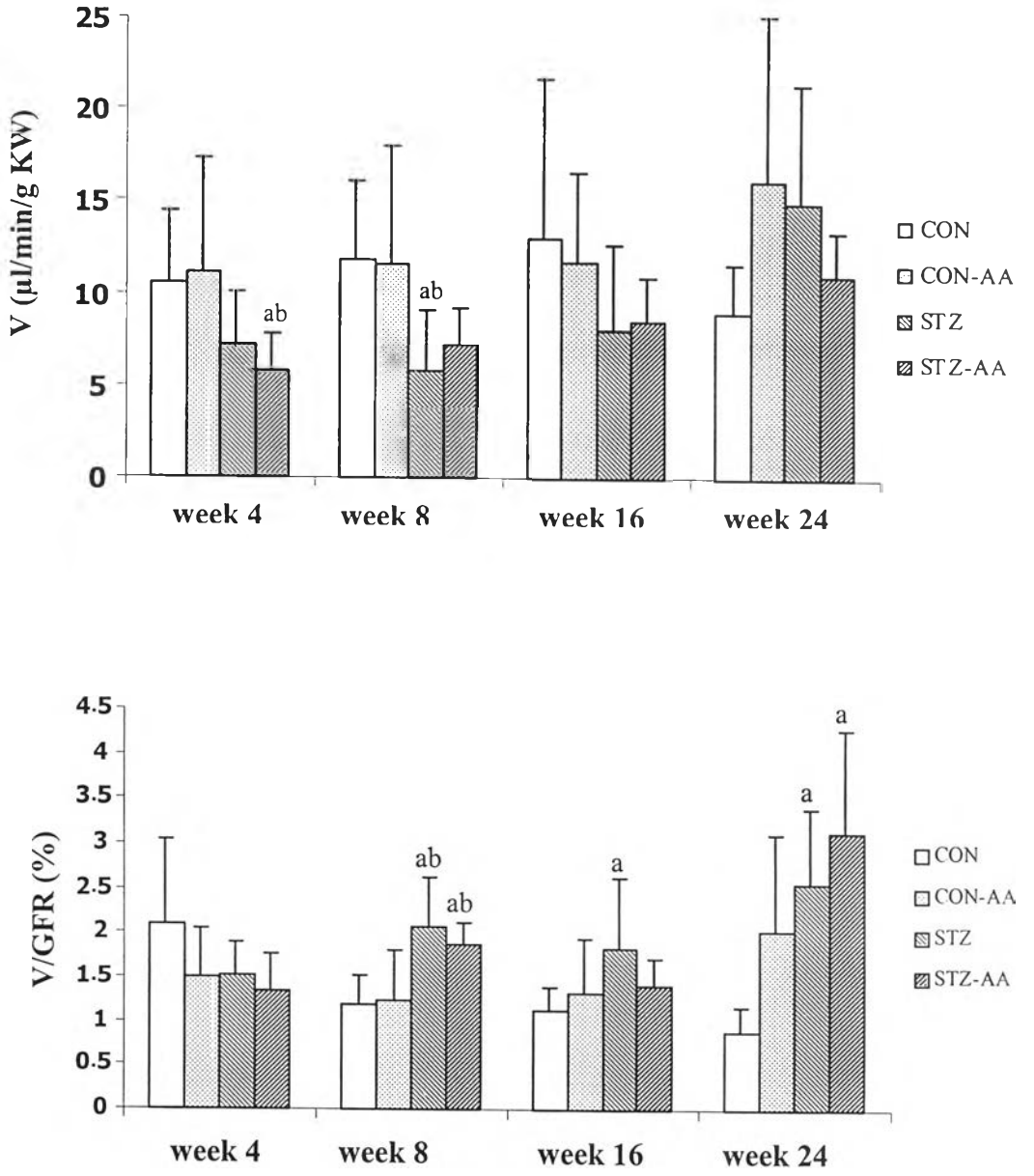

Figure 4-7 Alterations of urine flow rate (V) and percentage of V respected to GFR in streptozotocin-induced diabetic rats and control rats with or without L-ascorbic acid supplementation at week 4, 8, 16 and 24 of the experimental periods. All values are means ± SD. Statistically significant differences are indicated by [a] compared with CON, and [b] compared with CON-AA and CON at each period, $p < 0.05$.

Table 4-6 Urinary excretion of electrolytes of streptozotocin-induced diabetic rats and control rats with or without L-ascorbic acid supplementation at week 4, 8, 16 and 24 of the experimental periods (n=6).

	Groups	week 4	week 8	week 16	week 24
UV_{Na} (μEq/min/gm KW)	CON	3.4 ± 0.9	4.1 ± 1.1	3.8 ± 0.9	3.3 ± 0.4
	CON-AA	3.6 ± 1.3 [ab]	3.62 ± 1.5 [ab]	4.1 ± 1.1 [ab]	3.9 ± 1.6 [b]
	STZ	1.4 ± 0.8 [ab]	1.0 ± 0.5 [ab]	0.6 ± 0.4 [ab]	2.5 ± 1.2 [abc]
	STZ-AA	1.0 ± 0.5	1.1 ± 0.5	1.3 ± 1.0	1.0 ± 0.8
UV_K (μEq/min/gm KW)	CON	0.9 ± 0.3	0.9 ± 0.2	0.7 ± 0.1	0.7 ± 0.2
	CON-AA	0.8 ± 0.1 [ab]	0.8 ± 0.2 [ab]	0.7 ± 0.1 [ab]	0.8 ± 0.2
	STZ	0.4 ± 0.2 [ab]	0.2 ± 0.1 [ab]	0.2 ± 0.1 [ab]	0.6 ± 0.2 [abc]
	STZ-AA	0.3 ± 0.2	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.2
UV_{Cl} (μEq/min/gm KW)	CON	1.9 ± 1.0	2.8 ± 1.2	2.4 ± 0.9	1.9 ± 0.6
	CON-AA	2.5 ± 1.2 [ab]	2.5 ± 1.4 [ab]	3.0 ± 1.4 [ab]	2.6 ± 1.6 [ab]
	STZ	0.7 ± 0.4 [ab]	0.6 ± 0.5 [ab]	0.9 ± 0.8 [ab]	1.0 ± 0.5 [ab]
	STZ-AA	0.4 ± 0.2	0.4 ± 0.3	0.7 ± 0.3	0.9 ± 0.4

Mean ± SD

[a] compared with CON at the same column, $p < 0.05$; [b] compared with CON-AA at the same column, $p < 0.05$; [c] compared with STZ at the same column, $p < 0.05$.

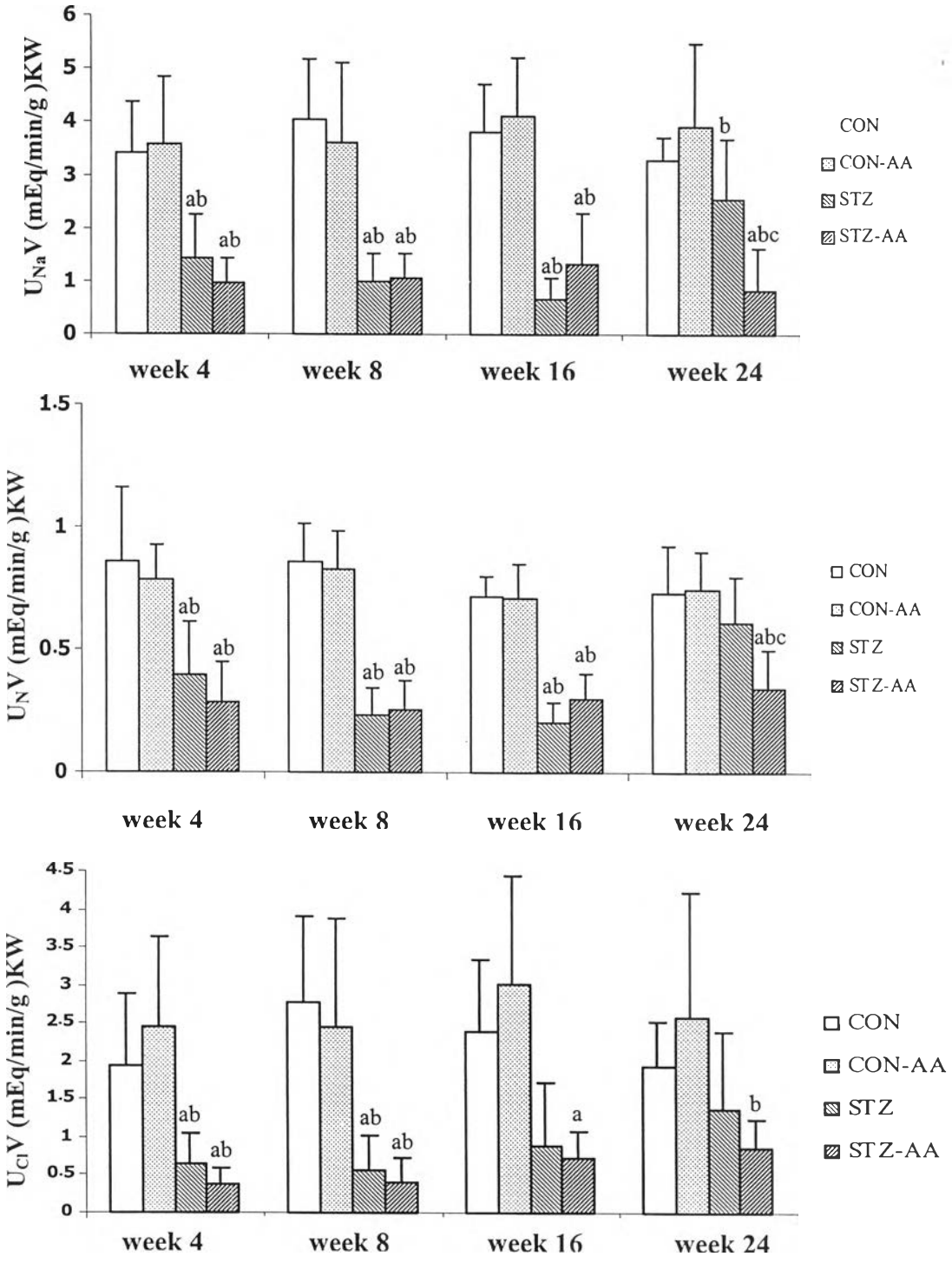

Figure 4-8 Alterations of urinary excretion of chloride in streptozotocin-induced diabetic rats and control rats with or without L-ascorbic acid supplementation at week 4, 8, 16 and 24 of the experimental periods. All values are means ± SD. Statistically significant differences are indicated by [a] compared with CON, [b] compared with CON-AA and [c] compared with STZ at each period, $p < 0.05$.

Table 4-7 Fractional excretion of electrolytes of streptozotocin-induced diabetic rats and control rats with or without L-ascorbic acid supplementation at week 4, 8 16 and 24 of the experimental periods (n=6).

	Groups	week 4	week 8	week 16	week 24
FE_{Na} (%)	CON	4.2 ± 1.1	3.0 ± 0.5	3.8 ± 1.4	3.9 ± 1.3
	CON-AA	3.6 ± 1.0	3.0 ± 0.8	3.2 ± 1.1	3.1 ± 1.0
	STZ	2.1 ± 0.6[a,b]	2.7 ± 0.4[a,b,c]	1.3 ± 0.5[a,b]	3.3 ± 1.2
	STZ-AA	1.4 ± 0.5[a,b]	1.9 ± 0.3	1.7 ± 0.7	1.4 ± 0.9[a,b,c]
FE_K (%)	CON	46.2 ± 12.4[a]	27.3 ± 3.3	25.6 ± 2.3	21.8 ± 4.8
	CON-AA	28.7 ± 4.6[a]	27.1 ± 4.1	22.2 ± 6.7	23.0 ± 3.4[a,b]
	STZ	26.3 ± 5.7[a]	26.1 ± 7.0[a,b,c]	20.3 ± 5.4[a]	35.2 ± 9.5[a,b]
	STZ-AA	20.5 ± 7.0	18.3 ± 2.6	17.3 ± 1.5	36.2 ± 8.8
FE_{Cl} (%)	CON	2.4 ± 1.1	2.4 ± 0.7	3.2 ± 1.6	2.1 ± 1.2
	CON-AA	3.3 ± 0.7[a,b]	2.5 ± 1.1	3.2 ± 1.8[a,b]	2.6 ± 1.3
	STZ	0.9 ± 0.2[a,b]	1.8 ± 0.8[a,b]	1.6 ± 0.5[a,b]	2.2 ± 1.5
	STZ-AA	0.8 ± 0.5	0.9 ± 0.5	1.4 ± 0.5	2.4 ± 1.4

Mean ± SD

[a] compared with CON at the same column, $p < 0.05$; [b] compared with CON-AA at the same column, $p < 0.05$; [c] compared with STZ at the same column, $p < 0.05$

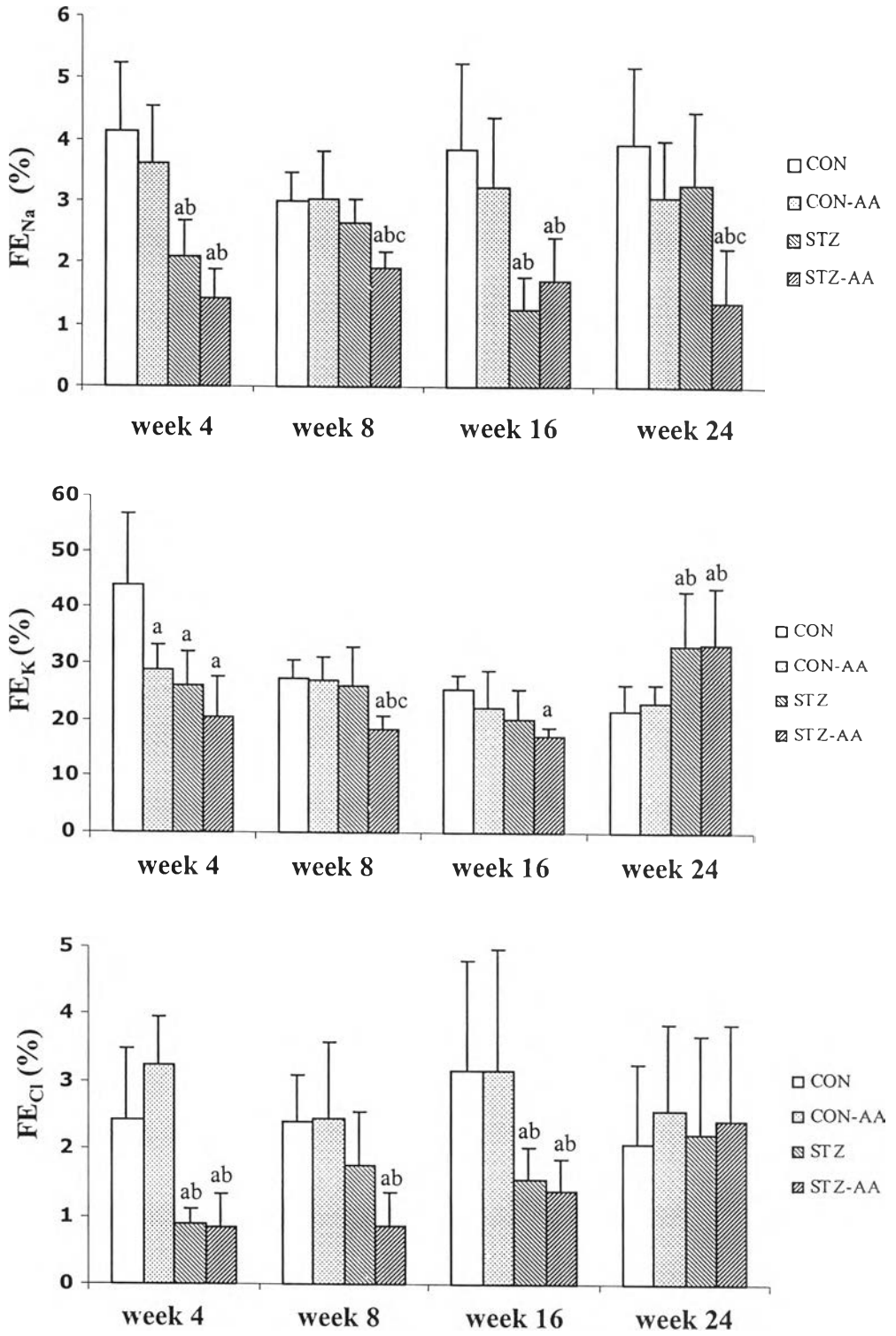

Figure 4-9 Alterations of fractional excretion of sodium in streptozotocin-induced diabetic rats and control rats with or without L-ascorbic acid supplementation at week 4, 8, 16 and 24 of the experimental periods. All values are means ± SD. Statistically significant differences are indicated by [a] compared with CON and [b] compared with CON-AA and CON at each period, $p < 0.05$.

Table 4-8 Plasma electrolytes of streptozotocin-induced diabetic rats and control rats with or without L-ascorbic acid supplementation at week 4, 8, 16 and 24 of the experimental periods (n=6).

	Groups	week 4	week 8	week 16	week 24
P_{Na} (mEq/L)	CON	138.3 ± 2.2	138.6 ± 1.8	136.2 ± 5.3	136.3 ± 1.6
	CON-AA	139.1 ± 3.4	139.0 ± 1.8	140.8 ± 7.8	136.9 ± 1.5
	STZ	135.1 ± 4.2 [ab]	138.2 ± 2.8	134.1 ± 6.5	133.8 ± 2.2 [abc]
	STZ-AA	134.1 ± 5.1	136.8 ± 1.8	135.5 ± 8.1	117.9 ± 13.2
P_K (mEq/L)	CON	3.1 ± 0.5	3.4 ± 0.1	3.4 ± 0.2	3.5 ± 0.2
	CON-AA	3.2 ± 0.3	3.4 ± 0.2	3.3 ± 0.7	3.5 ± 0.2
	STZ	2.9 ± 0.5	3.4 ± 0.3	3.0 ± 0.3	3.2 ± 0.3 [ab]
	STZ-AA	3.2 ± 0.5	3.5 ± 0.2	3.0 ± 0.2	2.8 ± 0.7
P_{Cl} (mEq/L)	CON	100.2 ± 15.8	105.8 ± 3.4	102.0 ± 9.2	104.9 ± 2.8
	CON-AA	103.6 ± 3.5	106.5 ± 1.3	105.4 ± 3.0 [b]	105.2 ± 1.4 [ab]
	STZ	99.1 ± 4.2	101.9 ± 5.9	98.4 ± 4.1 [b]	99.5 ± 3.0 [b]
	STZ-AA	97.9 ± 3.0	102.3 ± 3.4	98.4 ± 4.2	101.0 ± 5.0

Mean ± SD

[a] compared with CON at the same time, $p < 0.05$; [b] compared with CON-AA at the same time, $p < 0.05$; [c] compared with STZ at the same column, $p < 0.05$.

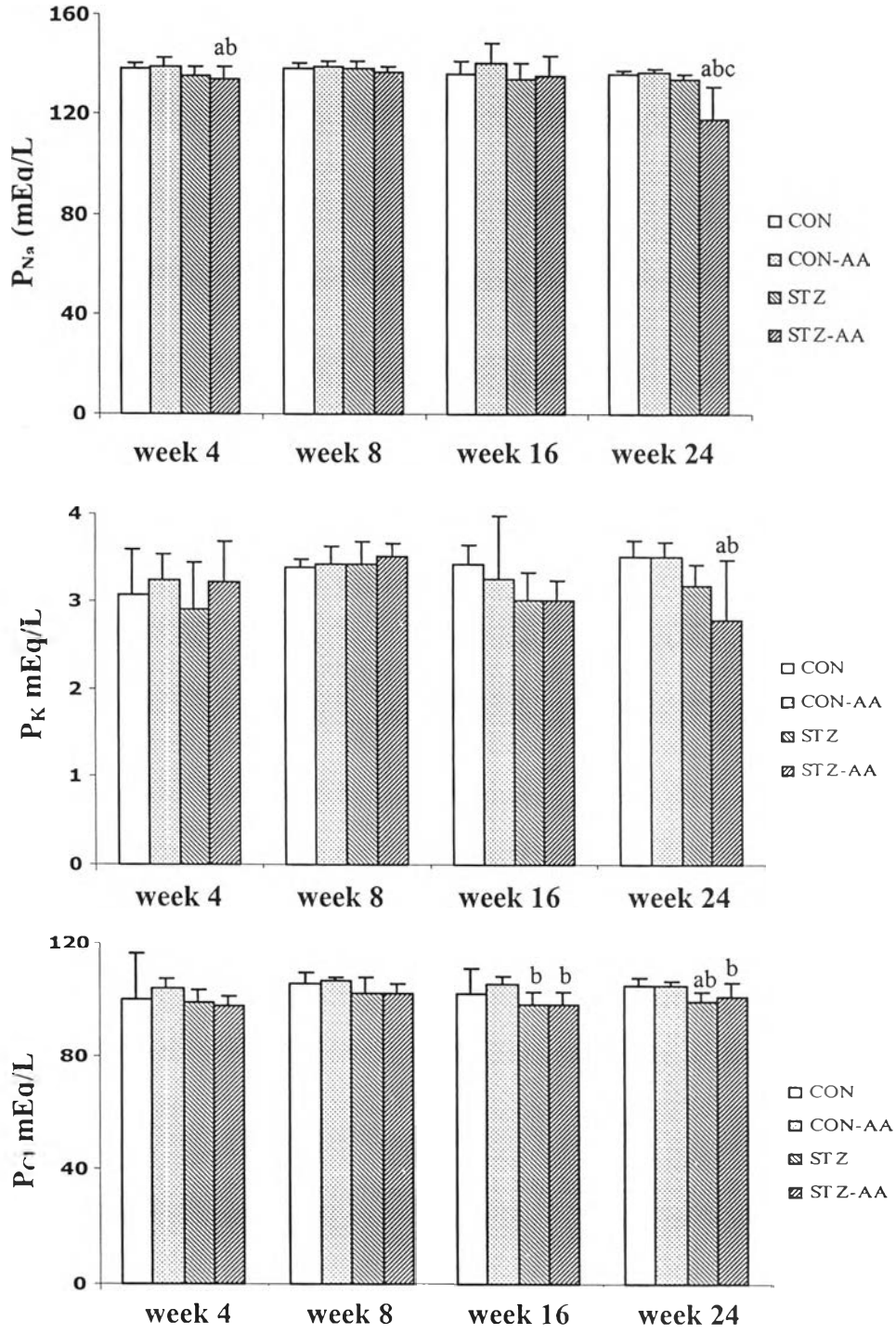

Figure 4-10 Alterations of plasma sodium (P_{Na}), plasma potassium (P_K) and plasma chloride (P_{Cl}) in streptozotocin-induced diabetic rats and control rats with or without L-ascorbic acid supplementation week 4, 8, 16 and 24 of the experimental periods. All values are means $\pm$ SD. Statistically significant differences are indicated by [a] compared with CON, [b] compared with CON-AA and [c] compared with STZ at each period, $p < 0.05$.

Discussion

The present study was performed to clarify whether AA supplementation could ameliorate renal dysfunction in STZ-induced diabetic rats. Normally, kidneys regulate the GFR and renal blood flow nearly the normal level despite considerable change in arterial pressure. This is called renal autoregulation of renal blood flow and glomerular filtration rate. For instance, the renal blood flow and GFR of both kidneys remains very near the normal level of 1200 ml per minute and 125 ml per minute, respectively even when the arterial pressure falls as low as 90 mm Hg or rises to 180 mmHg (Stanton et al., 1993). In the present study the change in the systemic blood pressure would be expected to maintain the autoregulation of the kidneys throughout the experimental periods. Any alterations of renal plasma flow and GFR occurred in diabetic animal should be caused by changes of intrarenal factors. Several investigations of the effect of diabetes mellitus on cell or tissue function indicated endothelial dysfunction (Granstam et al., 2003). The attenuation of NO synthesis in the kidneys occurred at the early onset (ten days) of STZ-diabetes mellitus rats (Pflueger et al., 1999) whereas other works have demonstrated no increase in renal blood flow in spite of significant increases in GFR and blood glucose concentration (Kaizu et al., 1995). The considerable evidences suggested that renal hemodynamics changes were associated with the onset of diabetes mellitus leading to the increases in renal plasma flow, glomerularcapillary hydraulic pressure gradient (Ditzel et al., 1967; Hostetter et al., 1981; Christiansen et al., 1981; Hostetter et al., 1982; Futrakul et al., 2003). Previous studies reported that GFR in diabetics were higher although ERPF was similar in diabetics and controls. The present results supported latter observations regarding a higher value of GFR as the value of body weight. However, in the present study, a marked reduction of the GFR value per gram of kidney weight was apparent. The larger increase in the filtration fractions (GFR/ERPF) of diabetic groups than those of control groups indicated more increase in vasoconstriction of the efferent arterioles than the afferent arterioles in diabetic kidney. An alteration of renal hemodynamics, which was characterized by a markedly elevated efferent arteriolar resistance, an increase in intraglomerular hydrostatic pressure and a markedly decreased renal plasma flow and peritubular capillary flow, was noted in chronically severe glomerulonephritis (Futrakul et al., 2003). In the present study, glomerulosclerosis has been seen diabetes mellitus at week 16 (see Chapter VII).

The RVR of diabetic groups were 6-7 folds more than those of control groups. With advancing diabetic glomerulosclerosis, there is an increase in the severity of arteriolosclerosis (Bohle et al., 1991), the development of renal arteriosclerosis of the small vessels (efferent, afferent arterioles and capillaries) in diabetic groups may be assumed in the present study. The increase in renal vascular resistance led to the decrease in effective renal plasma flow and caused the decrease in GFR in diabetic rats as compared with the normal rats (Table 4-4). After the supplementation of AA for 8 and 16 weeks, STZ-AA rats elucidated the decreased in RVR as compared with diabetic rats without AA treatment. Accordingly, ERPF of STZ-AA was slightly increased at week 8 after AA supplementation and was explicit at week 16 by 40 % of STZ, while GFR was concurrently increased in STZ-AA by 50 % of STZ. AA was effective to repair the renal hemodynamics after the supplementation to the diabetic rats for 16 weeks; however, it could not restore completely the alterations of renal hemodynamics to achieve the normal. The increases in ERPF and GFR are possible due to the ability of AA to increase the levels of nitric oxide in blood vessels. The evidence of NO-dependent vascular reactivity by AA administration in diabetic animals in improvement of vascular elasticity has been noted (Taddei et al., 1998, 2001). Moreover, a study in patients with diabetes mellitus has been shown that intrarenal rennin-angiotensin system (RAS) was activated in type 1 diabetes mellitus (over 80% of patients with type 1 diabetes mellitus). Inhibition of RAS by angiotensin-converting enzyme (ACE) inhibitor or an angiotensin receptor blocker in the patient with diabetes mellitus led to an increase in renal plasma flow (RPF), and a fall in filtration fraction. This factor was generally attributed to predominant efferent arteriolar dilation (Hollenberg et al., 2003). It may be needed a further investigation of the effect of AA on RAS to ameliorate the decrease in renal plasma flow and increase in GFR in STZ-induced diabetic rats

Base on the evidence of endothelial dysfunction in diabetes mellitus, atherosclerosis has been noted that caused the development of hypertension in chronic diabetes (Lim et al., 2004). In this study, blood pressure of both diabetic groups were lower than that of the control groups at the similar period of the study; however, it showed the progression of the increase in systolic, diastolic and mean arterial pressure of diabetic groups in the prolonged period (Table 4-3). The decrease in total body fluid resulting in the lower blood pressure would be expected during diabetes, which

was indicated by the increase in Hct (Table 4-3). In the present study, the ratios of V/GFR in diabetic animals were markedly higher than those of the control animals. It indicated that there was an increase in fractional delivery of filtrate to the diluting sites of ascending limb of Henle's loop in diabetic animals. However, it is not necessary to assume that this increased fractional delivery during diabetes comes from an effect in the anatomic proximal tubule. A marked decrease in GFR in STZ-treated animals would reduce filtered load of sodium ion, which transported to the inner medullary segment of the ascending limb resulting in a reduction of the concentration of sodium ion in the medullary interstitium. In consequence, less water would be absorbed from the descending limb. Therefore, increased filtrate would be delivered to the medullary and cortical diluting segments. In the present study, STZ-AA had slightly decrease in V/GFR when compared with that of STZ and had no significant difference as compared with those of CON and CON-AA. A severe hyperglycemia (over 400 mg/dl) (Table 4-1) in the present study would induce osmotic diuresis resulting in the polyuria, which is commonly found in diabetes mellitus (Spira et al., 1997). Glucose reabsorption was decreased which was consistent with a fall in proximal tubular reabsorption in nephrotic syndrome (Usberti et al., 1979). Loss of body fluid results in an increase in blood tonicity and a decrease in blood volume. The arterial blood pressure is determined directly by two major physical factors, including the arterial blood volume and the arterial compliance. These physical factors are affected in turn by physiologic factors, including the heart rate, stroke volume, cardiac output, and peripheral resistance (Berne and Levy, 1993). In the present study, the diabetic rats showed the decrease in HR, and arterial blood pressures, indicating much of the decrease in stroke volume, even though the peripheral resistance might be present in the diabetic rats. This is possible a reason why hypertension was not apparent. Normally, the decrease in blood volume could stimulate RAS and antidiuretic hormone (ADH). The increase in water reabsorption in distal and collecting tubule would be occurred and consequentially increases in body fluid and blood pressure. These changes are amplified in diabetes mellitus (Lim et al., 2004). It could imply that AA was able to improve the polyuria in diabetic rats. The knowledge of the effect of AA related to RAS on the decrease in polyuria is not available. The role of AA administration on diabetic animals on RAS may be needed more further investigations.

The disturbance of renal tubular function was seen in the present study. A marked decreased in GFR in the diabetic animals would reduce filtered load of the sodium. The decrease in filtered load of sodium ion resulted in the decrease in FE_{Na} as compared with CON or CON-AA, which were seen at week 4 and 8. This result was similar to a previous study in short-term diabetes patients who had no change in GFR and RPF, but lowered fractional excretion of sodium as compared with control subjects (Pelikanova et al., 1997). Since polyuria occurred in the diabetic rats, loss of sodium ion into the urine would be expected and resulted in a reduction of sodium concentration in the medullary interstitium. Therefore, the increased in sodium reabsorption via the passive diffusion was probably occurred in the ascending limb. In addition, the increased luminal glucose could stimulate the proximal sodium reabsorption (Bank, 1990). Other factors that can affect the sodium reabsorption should be considered as well. ADH and aldosterone might be indirectly and directly involved in the sodium reabsorption in the collecting duct. In the present study, acidosis was expected in chronic diabetes mellitus, the renal regulation of acid-base balance was likely to result in the increase in sodium reabsorption in renal tubules and decreased in FE_{Na} . In the present study, at week 24, STZ-AA showed the significant decrease ($p < 0.05$) in P_{Na} without an increase in sodium excretion as compared with the control rats. It might be resulted from polyuria, which caused the loss of sodium into the urine. In addition, P_{Na} of STZ-AA was significantly decreased ($p < 0.05$) as compared with that of STZ. It might be due to the effect of hyperglycemia and hypertriglyceridemia , which is always apparent in diabetes mellitus. The hyperglycemia and hypertriglyceridemia would draw the intracellular water into the plasma space, resulting in the decrease in P_{Na}, called pseudohyponatremia (University of IOWA, Health Care, Holden Comprehensive Cancer Center, 2005). In addition, since ketoacidosis usually occurs in IDDM, H^+ may be eliminated in the form of titrable acid with sodium salt into the urine. The manner of FE_{Na} of STZ-AA seemed to be decreased as compared with STZ at each experimental period and significantly decreased at week 16. It was likely that more sodium ions were used in the co-transport of AA through the proximal cells resulted in the decrease in sodium excretion respected to GFR.

In the present result of week 4-16, the decrease in fractional excretion of potassium might be caused the acutly-induced hyperglycemia (Pelikanova et al.,

1997). Potassium excretion by the kidneys is determined by the rate of potassium secretion by the distal tubule and collecting duct. Potassium secretion by these tubule segments is stimulated by a rise in tubular flow rate (Koeppen and Stanton, 1993). In the present study, U_KV of the diabetic animals was decreased as compared with the control animals resulted from a marked decrease in GFR and/or urine flow rate. The reduction of potassium excretion shown as the decreases in U_KV and FE_K of the diabetic rats during 16 weeks of diabetes mellitus might indicate the appearance of acute metabolic acidosis (Koeppen and Stanton, 1993). Conversely, the rise in FE_K of the diabetic animals were seen at week 24 probably indicated the chronic metabolic acidosis in the diabetes mellitus because the acid accumulation in acidosis is needed more preservation of sodium ion to regulate acid-base balance. The renal tubules are responsible for sodium reabsorption related to an expectable increase in aldosterone. An increase in aldosterone results in the increase in potassium secretion. In addition, with chronic acidosis, the reabsorptions of water and sodium by proximal tubule are inhibited resulting to the increase in the flow of tubular fluid through distal tubule and collecting duct and leading to the increase in potassium secretion. These may also result in the decrease in P_K of STZ-AA at week 24. The more decrease in U_KV of STZ-AA than those of STZ could imply more acidosis in STZ-AA than STZ at week 24. The long-term supplementation of AA might be caused the acid accumulation to the chronic diabetic animals, which always approach the disturbance of renal function. In acidosis, In addition, the decrease in pH reduces the permeability of apical membrane and inhibit Na^+-K^+ATPase, resulting in the decrease in K^+ concentration in renal tubular cells. Therefore, the decrease in K^+ secretion would be expected to occur in the renal tubule of STZ-AA, which results in the decrease in U_KV at week 24.

The present results demonstrate that supplementation of AA for 16 weeks could lower blood glucose concentration in STZ-induced diabetic rats as compared with STZ-induced diabetic rats without AA supplementation. It is possible that AA is able to decrease in cortisol and cathecolamines level in diabetes mellitus. Oxidative stress always occurs in the hyperglycemic condition, which is toxic to the cells (Riedle and Kerjaschki, 1997; Valko, 2004; Karageuzyan, 2005). It has been reported that pancreatic β-cells also were affected by the oxidative stress (O'Brien, 2000). The deficiency of insulin results in the decrease in glucose uptake by cells and the decrease in glycolysis in insulin-dependent diabetes mellitus (IDDM or Type I

diabetes mellitus). In type I diabetes mellitus eventually lose insulin secretion is accompanied by increases in glucagons, catecholamines and cortisol. The abnormalities of those hormones lead to hepatic glucose production and consequent increase in blood glucose level. Namely, glucagon excess exacerbates hyperglycemia by increasing hepatic glucose release and decreasing hepatic glucose uptake (Rizza et al., 1979; Cherrington et al., 1981). Epinephrine and norepinephrine concentrations may be elevated in type I diabetes mellitus (Bolli et al., 1984). Epinephrine impairs insulin action at the hepatic and extrahepatic tissues and stimulates glucagon secretion, leading to further increases in endogenous glucose production (Gerich et al., 1976; Rizza et al., 1980). In addition, cortisol increases endogenous glucose production and lipolysis while decreasing tissue glucose uptake in type I diabetes mellitus (Schade et al., 1978; Dinneen et al., 1995). Previous studies in prolonged exercise runner, subjects with acute psychological stress and in stress soft-shelled turtles indicated that supplemental AA could decrease serum adrenaline level and cortisol level in serum and salivary (Brody et al., 2002; Peters et al., 2001; Zhou et al., 2003). The decrease in catecholamines and cortisol concentration in diabetes mellitus may result in the decrease in endogenous glucose production leading to the attenuation of hyperglycemia. The great amelioration of hyperglycemia has been found in albino rats treated with interferon-alpha (IFN-alpha). IFN-alpha acts as a cytotoxic agent since it increases in free radical and inhibits insulin secretion leading to the development of diabetes mellitus. Administration of vitamin C along with IFN-alpha succeeded in modulating most of the altered parameters affected during IFN-alpha including a decrease in plasma glucose, an increase in pancreatic and serum insulin and decrease in plasma lipids peroxides level. However, the increase in plasma glucagons still persisted (Al-Zuhair et al. 1998). In addition, the decline in hyperglycemia in diabetic rats with supplemental AA was also due to the powerful antioxidant effect of AA to preserve the β-cell function (Bergsten et al., 1994; Kaneto et al., 1999; Steffner et al., 2004).

In conclusion, supplementation of AA to the STZ-induced diabetic rats had the effects on renal hemodynamics to attenuate the increase in renal vascular resistance and decrease in GFR and ERPF. In addition, AA supplementation could reduce hyperglycemia in the STZ-induced diabetic rats. These beneficial effects of AA should be considered as a therapeutic supplemental agent for the diabetic patient to ameliorate the renal dysfunction.