

CHAPTER IV

RESULTS

1. Severity of diabetes

1.1 Body weight

The body weight was significantly decreased in the DV and DI groups when compared to the C group from the 2nd week to the end of the study (Figure8). There were no differences between the DV and DI groups throughout the experiment.

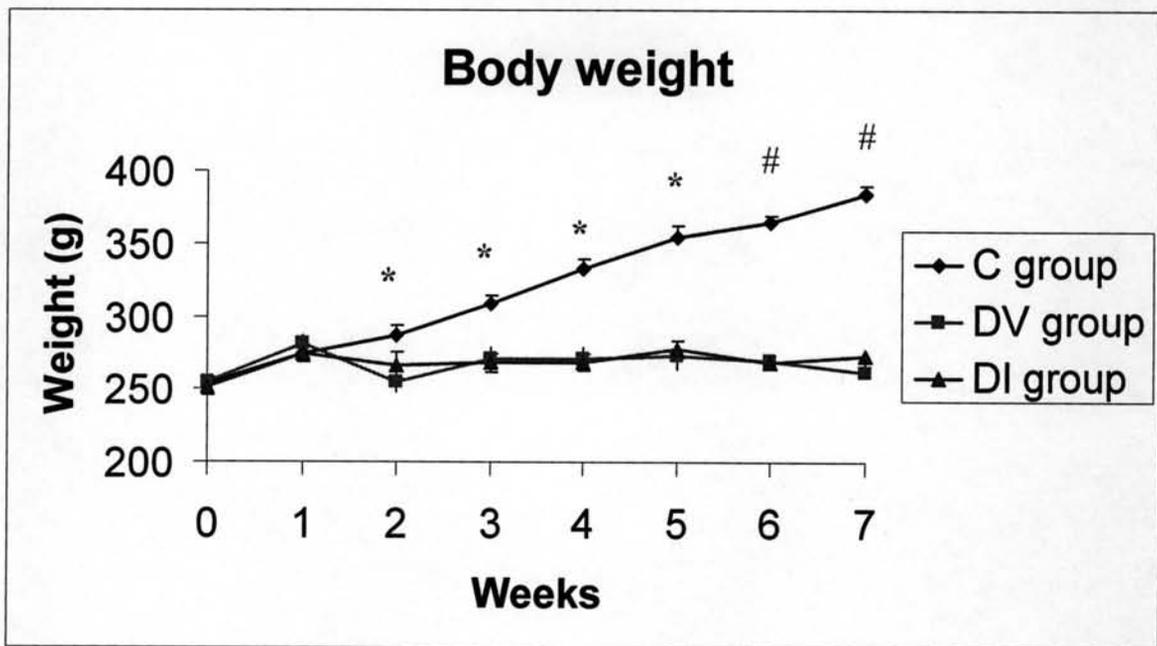


Figure 8 Changes in the body weight of C, DV and DI rats. Data are mean \pm SD. (* $p < 0.05$, *# $p < 0.01$)

1.2 Plasma glucose

The plasma glucose in the DV and DI groups was markedly higher compared with the C group at the end of the study (week 7). There were no significant differences between the DV and DI groups (Figure 9).

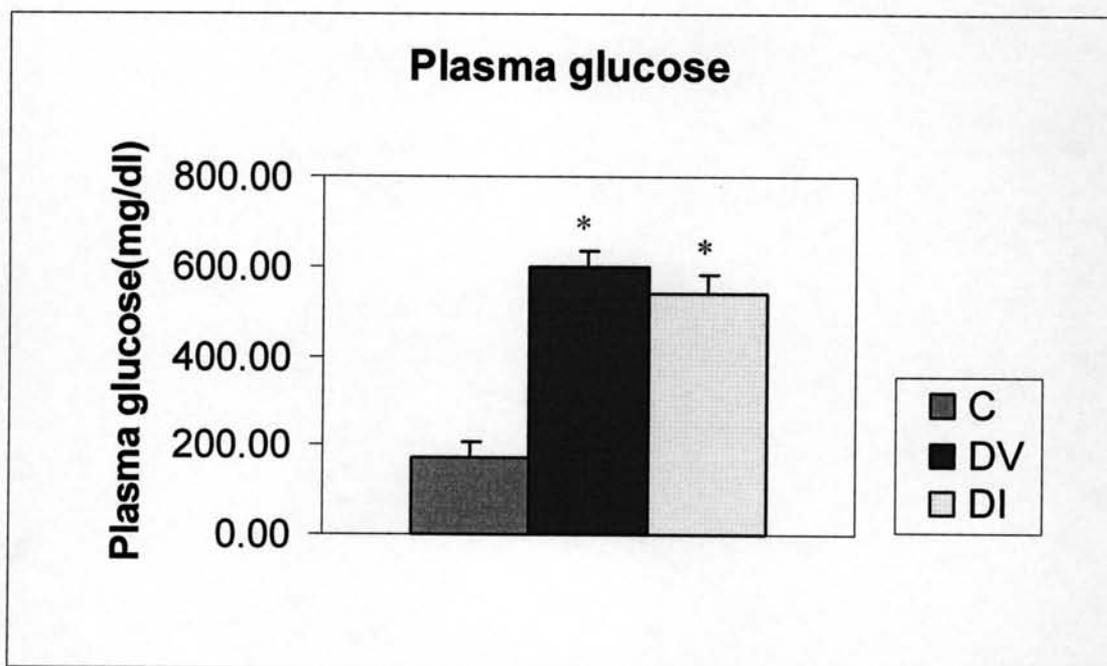


Figure 9 Plasma glucose at week 7 after the onset of diabetes. Data are mean \pm SD. (* $p < 0.001$)

2. Electrophysiological measurement

No difference between groups was observed at baseline determination (data not shown). Significant decrease in motor nerve conduction velocity was found in the sciatic nerve of the DV and DI rats compared to the controls in the fourth week ($p < 0.01$) (Figure 10). However, the abnormality was not detected at week 7. There were no significant differences between the DV and DI groups throughout the study.

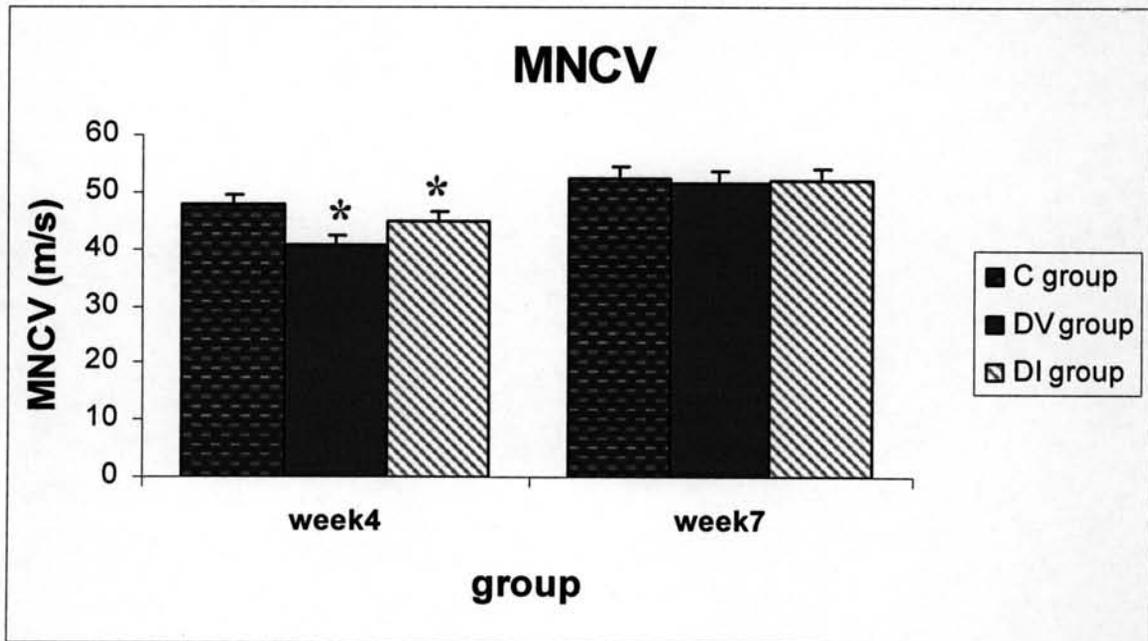


Figure 10 Motor nerve conduction velocity (MNCV) in the C, DV and DI groups at week 4 and the end of experiment (week 7). Data are mean \pm SD. (* $p < 0.01$)

3. Western blot analysis of ERK phosphorylation

Western blot analysis showed that the level of ERK-P was significantly higher in the DV when compare to the C group ($p < 0.05$) (Figure 11). As expected, in the DI group, the level of ERK-P was lower than the DV and similar to the C group. Therefore, the level of ERK phosphorylation in DRG was elevated in the DV group and the ERK inhibitor (u0126) can inhibit the enhanced of ERK phosphorylation in the DI group. In addition, no differences in the level of ERK-T were observed among groups.

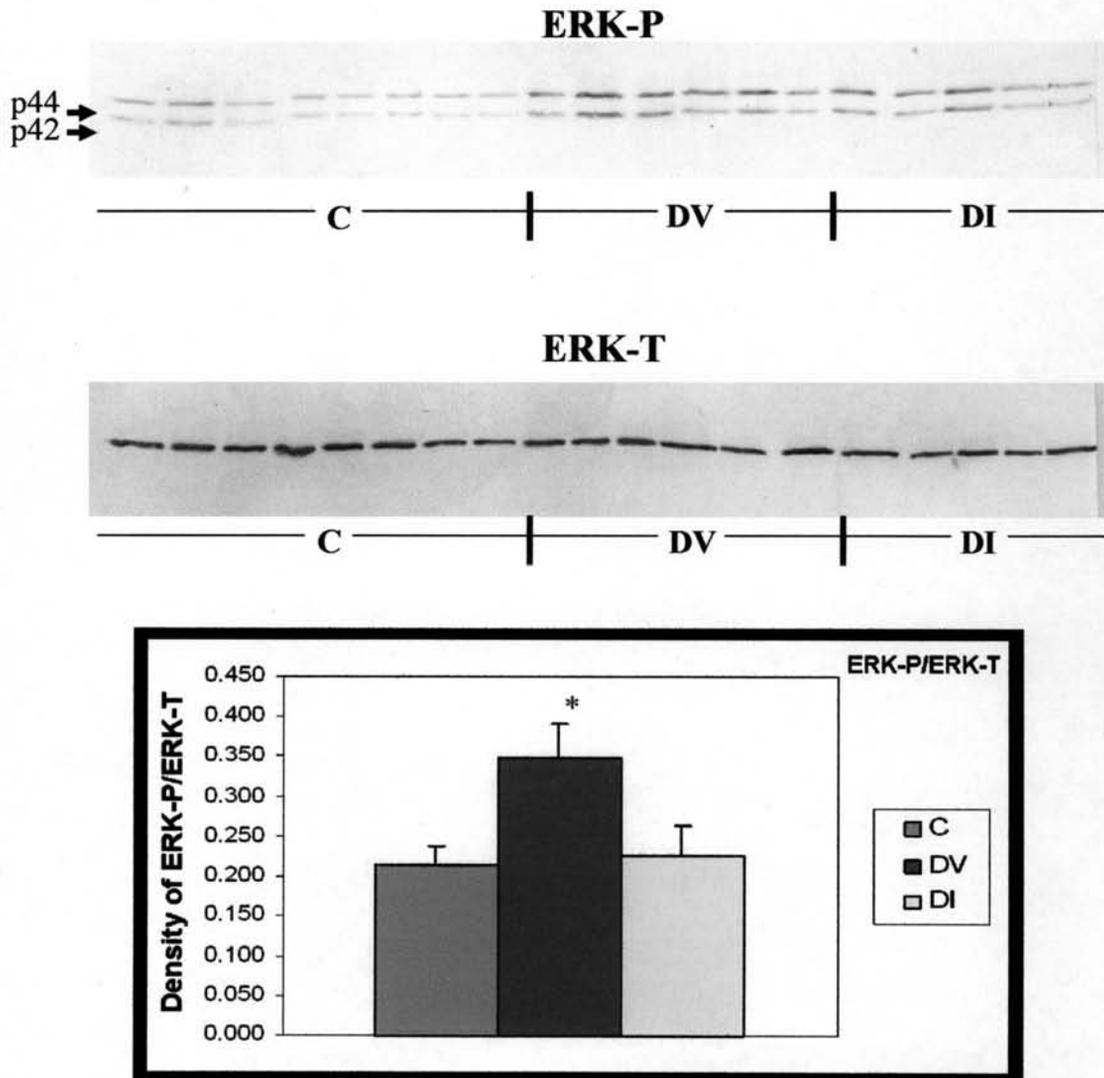


Figure 11 Western blot results of the ERK phosphorylation in DRG from the C, DV and DI groups. Above, two blots were probed for ERK-P and ERK-T, respectively. Two isoform of ERK at 44 and 42 kDa are demonstrated. Below the graph shows the ratio of ERK-P (from isoform p44 and p42) to ERK-T, * $p < 0.05$

4. Microscopic examinations

4.1 Qualitative examination

- Sciatic nerve

In Epon semithin transverse sections of the sciatic nerve, no dramatic changes in the axons or myelin sheath were observed in the DV and DI groups compared to the C group (Figure 12). Endoneurial and perineurial vessels appeared normal.

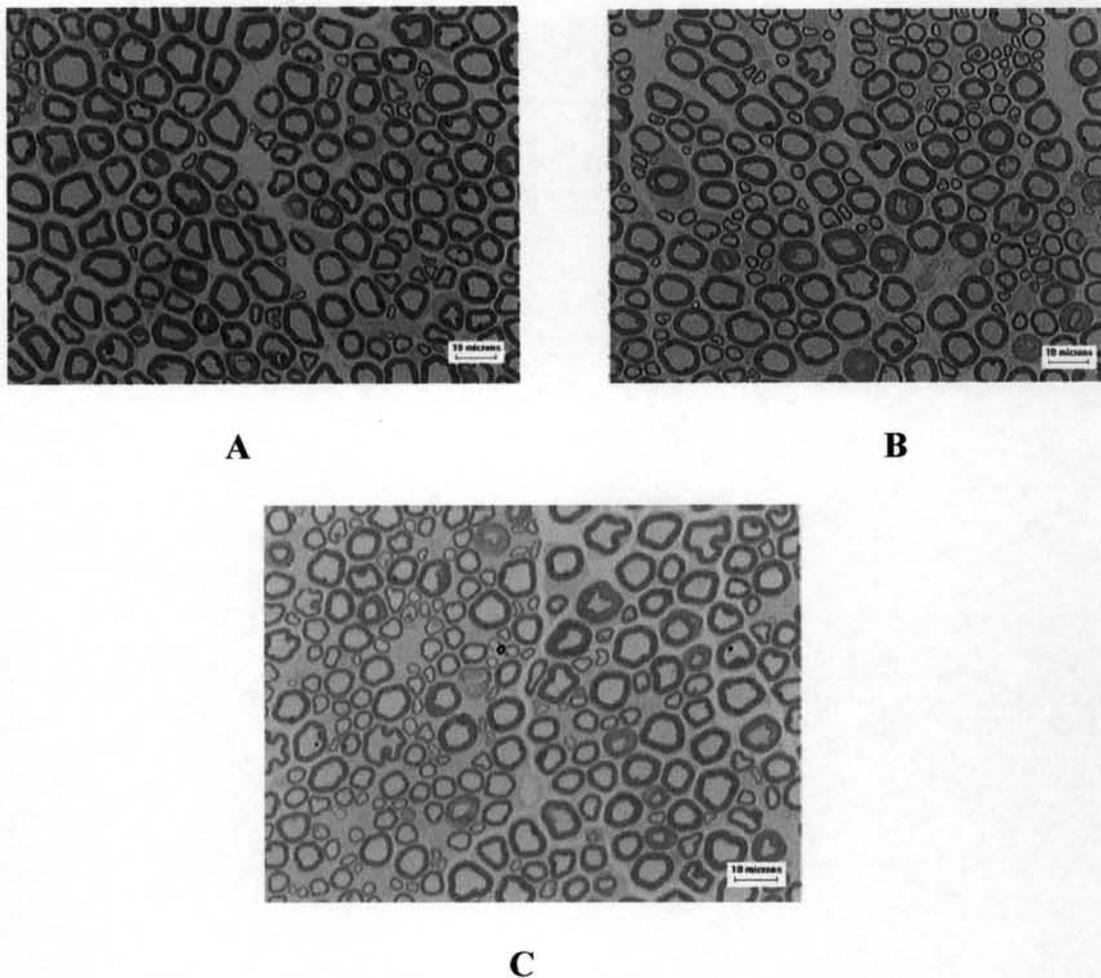
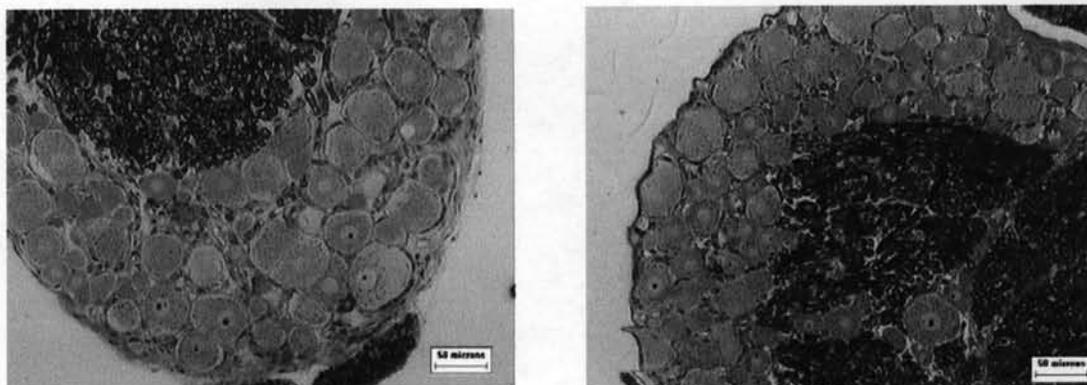


Figure 12 Representative micrographs of sciatic nerves from the C (A), DV (B) and DI (C) groups stained with paraphenylenediamine. Scale bar represent 10 μ m.

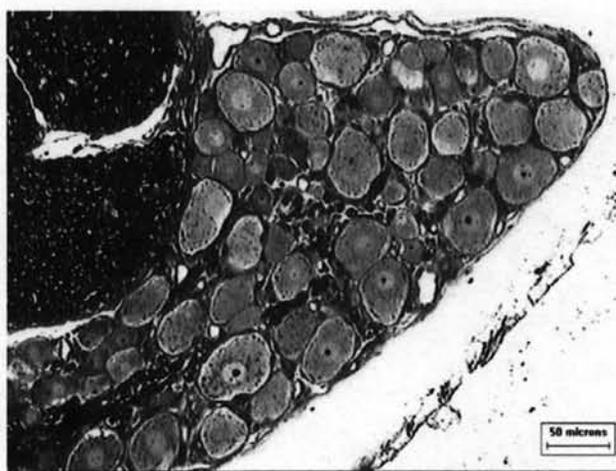
- DRG

In the dorsal root ganglia, no dramatic pathological changes were observed in any groups (Figure 13).



A

B



C

Figure 13 Representative micrographs of L4 dorsal root ganglia from the C (A), DV (B) and DI (C) groups. Scale bar represent 50 μ m.

4.2 Quantitative evaluation

4.2.1 Nerve morphometry

The morphometric data are summarized in Table 1 and Figure 14. There was a significant decrease in myelin thickness and diameter of myelinated fiber (8-9 μ m) in the DV and DI groups when compared to the C group. Although we found significant differences only in these two parameters, there was a trend toward a decrease in other parameters such as fiber and axon diameters, percentage of myelinated fiber larger than 10 μ m in the DV and DI groups when compared to the control group.

Table 1 Summary of the nerve morphometric results.

Morphometry DATA	C Group		DV group		P-value	DI group		P-value
	Mean	SD	Mean	SD		Mean	SD	
Lt. sciatic n	15597.22	\pm 2153.27	15183.34	\pm 1738.34	0.73	15366.67	\pm 1422.10	0.78
Total fiber								
Fiber Diameter	6.54	\pm 0.30	6.22	\pm 0.17	0.6	6.42	\pm 0.31	0.97
Axon Diameter	3.61	\pm 0.19	3.51	\pm 0.14	0.22	3.53	\pm 0.14	0.8
Myelin Thickness	1.52	\pm 0.07	1.3	\pm 0.09	0.006**	1.45	\pm 0.08	0.042*
g-ratio	0.53	\pm 0.01	0.57	\pm 0.02	0.68	0.54	\pm 0.01	0.4
MF (%)								
(\varnothing > 1-2 μ m)	0.91	\pm 0.45	1.33	\pm 0.76	0.428	0.43	\pm 0.41	0.435
(\varnothing > 2-3 μ m)	8.48	\pm 1.46	11.57	\pm 3.21	0.076	10.5	\pm 2.96	0.155
(\varnothing > 3-4 μ m)	12.87	\pm 4.19	13.37	\pm 1.92	0.075	12.39	\pm 0.88	0.121
(\varnothing > 4-5 μ m)	9.85	\pm 1.16	11.03	\pm 0.86	0.746	11.36	\pm 3.32	0.997
(\varnothing > 5-6 μ m)	9.21	\pm 1.71	8.97	\pm 3.47	0.876	9.87	\pm 1.45	0.946
(\varnothing > 6-7 μ m)	13.12	\pm 2.78	12.93	\pm 5.19	0.858	13.96	\pm 1.83	0.876
(\varnothing > 7-8 μ m)	14.46	\pm 1.61	11.96	\pm 2.17	0.532	14.48	\pm 1.10	0.428
(\varnothing > 8-9 μ m)	11.82	\pm 1.50	10.98	\pm 1.49	0.048*	10.01	\pm 3.04	0.035*
(\varnothing > 9-10 μ m)	10.09	\pm 1.33	12.33	\pm 3.47	0.929	10.77	\pm 2.35	0.663
(\varnothing > 10 μ m)	9.19	\pm 3.02	5.52	\pm 1.83	0.921	5.84	\pm 3.57	0.998

@ MF= myelinated fiber , \varnothing = diameter of myelinated fiber

(* , ** DV & DI vs. C)

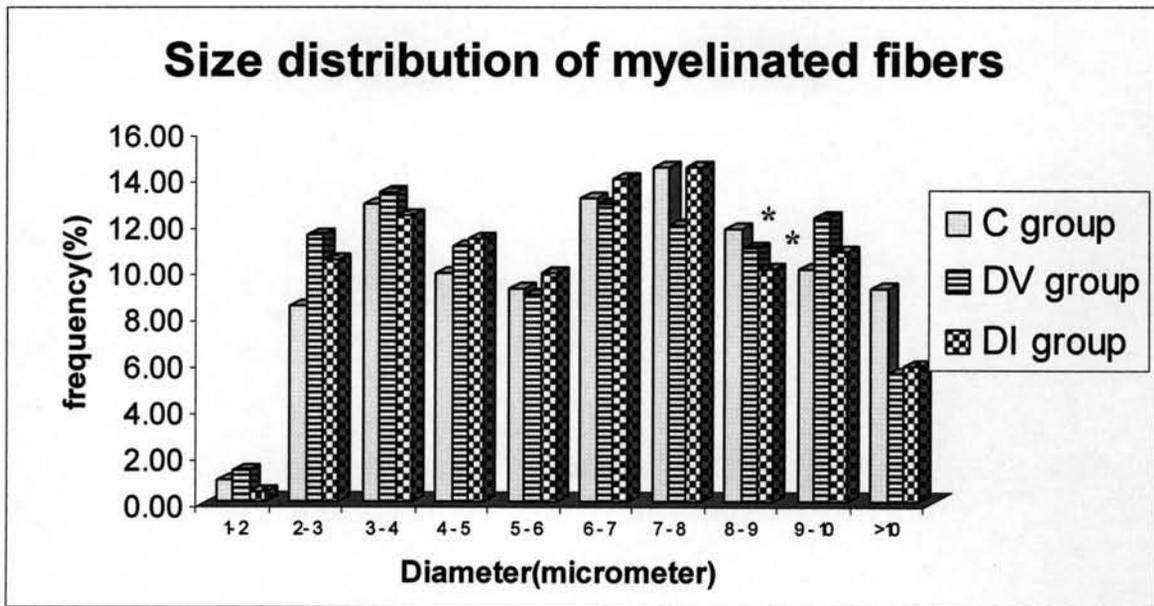


Figure14 Distribution of myelinated fibers according to the diameter in the sciatic nerves from the C, DV and DI groups (* $p < 0.05$ vs. C)

DRG morphometry

The mean number of L4 DRG neurons \pm SD was 15385 ± 1065 , 11956 ± 836 and 10863 ± 935 for the C, DV and DI groups, respectively (Figure 15). Although there was a trend toward a decrease in the total number of neurons in the diabetic compared with the control groups, the differences were not statistically significant.

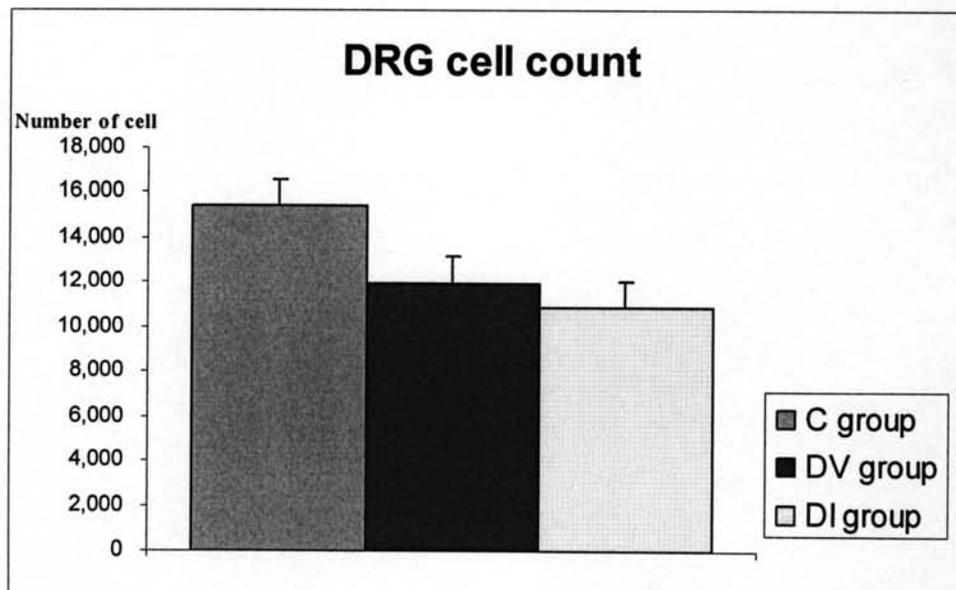


Figure15 Total number of neurons in L4 DRG from the C, DV and DI groups

In addition, there were no significant differences between the groups in the average areas of nucleus and nucleolus of L4 DRG neuron (Figure 16).

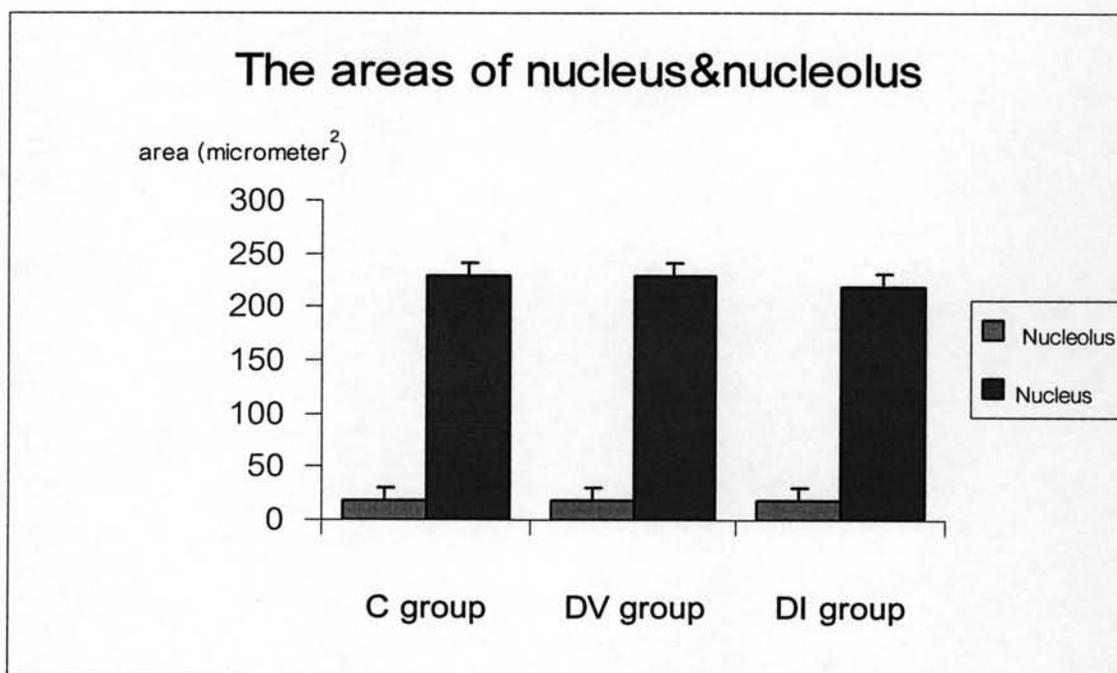


Figure16 The areas of nucleus and nucleolus in L4 DRG neurons of the C, DV and DI groups.

The means \pm SD areas of the nucleolus were 18.67 ± 1.60 in control, 17.94 ± 1.55 in DV, and 17.87 ± 1.81 in DI groups. And, the mean \pm SD areas of the nucleus were 228.21 ± 37.63 in control, 228.31 ± 14.99 in DV, and 219.38 ± 29.25 in DI groups.