

Treadmill training increases the size of A cells from the L5 dorsal root ganglia in diabetic rats

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Summary. The aim of this study was to evaluate the effects of physical training on the L5 dorsal root ganglion (DRG) cells in streptozotocin diabetic rats. Male adult rats were divided into 3 groups: (control, diabetic and trained diabetic). Treadmill training was performed for 10 weeks (5 days/week, twice a day). Blood glucose concentrations and body weight were evaluated 48h after diabetes induction and every 30 days thereafter. Then, animals were killed and the right L5 DRG removed. Histological and morphometric analysis consisted of evaluating nuclear and cellular volumes and areas in A and B cells at light and ultrastructural levels. Blood glucose concentrations were higher in both diabetic groups vs controls at all periods. Body weights were lower in all diabetics vs controls at all periods after diabetes induction, with a significant time vs group interaction. In A cells, the cellular and nuclear volumes were lower than control animals only in the diabetic group; control and trained diabetic animals did not differ; in B cells the cellular and nuclear volumes were lower in diabetic and trained diabetic rats. The cellular areas of A cells were smaller in diabetic rats than in control and trained diabetic rats, while the cellular areas of B cells were smaller in the diabetic and trained groups. In conclusion, treadmill training was able to increase the size of A cells from the DRG in diabetic rats and improved the morphological features of these cells.

Key words: Dorsal root ganglion neurons, Diabetic neuropathies, Treadmill training, Morphometry, Electron microscopy

Introduction

Neuropathy is one of the most debilitating complications in diabetes, often associated with considerable morbidity and mortality (Ewing et al., 1976). The pathogenesis of diabetic neuropathy involves hyperglycemia-induced molecular changes (Kamiya et al., 2005), microvascular insufficiency (Ram et al., 1991; Brownlee, 2001), oxidative stress (Zherebitskaya et al., 2009), nitrosative stress (Obrosova et al., 2007) and defective neurotrophic support (Hellweg and Hartung, 1990; Huang et al., 2005).

Streptozotocin (STZ)-induced diabetes in rats causes degenerative changes in autonomic (Schmidt and Plurad, 1986; Schaan et al., 1997; Rosengård-Bärlund et al., 2009) and sensory neurons (Sidenius and Jakobsen, 1980; Zherebitskaya et al., 2009), which are positively correlated with their hyperglycemic state (Schaan et al., 2004). Also, the dorsal root ganglion (DRG) neurons were reported to have smaller cell volumes (Sidenius and Jakobsen, 1980) and abnormalities in growth factor support, with increased expression of vascular endothelial growth factor (VEGF; Samii et al., 1999), decreased activity of insulin-like growth factors I and II (IGF-I and IGF II; Ishii and Lupien, 1995; Zhuang et al., 1996) and decreased axonal transport of nerve growth factor (NGF), as well as expression of its low affinity p75 receptor (Delcroix et al., 1997). While DRG cells have higher metabolic requirements (Zochodne and Ho, 1991), unlike brain cells they do not have a selective blood barrier (Allen and Kiernan, 1994), which facilitates cell body damage.

Although no definitive treatment for diabetic neuropathy has yet been established, several studies have shown that it is possible to reduce its effects through

intensive therapy/optimal glycemic control (The Diabetes Control and Complications Trial Research Group, 1993) and nerve growth factor therapies (Huang et al., 2005; Calcutt et al., 2006). Although the beneficial effects of regular physical exercise are well-known and used as part of the treatment of diabetic patients (American Diabetes Association, 2008), very little data on its effectiveness in human diabetic neuropathy has been reported (Balducci et al., 2006) and there is no data available on the effectiveness of physical training on diabetic neuropathy in animals. Moreover, some studies in rats have shown the benefits of exercise training in diabetes-induced cardiovascular and autonomic dysfunction (De Angelis et al., 2000; Harthmann et al., 2007). Also, there is no data currently available on the effects of exercise training on the morphological aspects of DRG cells. Thus, the aim of this study was to evaluate the effects of an exercise training protocol on the morphological and morphometric features of the nucleus and soma in the DRG cells of STZ-diabetic rats.

Materials and methods

Animal model

Fifteen male Wistar rats (12 weeks old) from a local breeding colony (ICBS, UFRGS) were housed under standard laboratory conditions with food and water available *ad libitum* and maintained under a 12:12 light-dark cycle (lights on at 8:00h). All efforts were made to minimise the number of animals studied and their suffering. In addition, all the animals were cared for in accordance with Brazilian law and the recommendations of the Brazilian Society for Neurosciences, Review Committee of the School of Veterinary Surgery, University of Buenos Aires, and the International Brain Research Organization, and are in compliance with the National Institute of Health's Guidelines for Care and Use of Laboratory Animals (publication no. 85-23, revised 1985).

Experimental design

The rats were divided into 3 groups, as follows: non-diabetic rats, control (C; n=5); diabetic rats (D; n=5) and diabetic rats submitted to treadmill training (trained diabetic rats; TD; n=5).

Diabetes induction

After an overnight fasting period (6 h), the rats received a single intravenous injection of streptozotocin (Sigma Chemicals Co., USA) diluted in 10mM citrate buffer, pH 4.5 (50 mg/kg of body weight). Non-diabetic animals received only citrate buffer (Junod et al., 1969).

The body weight and blood glucose concentrations were measured 48h after induction of diabetes and every 30 days thereafter. Blood glucose concentrations were evaluated in blood collected from the rat-tail using test

strips (Advantage, Roche, Indianapolis, USA) after an overnight fasting period (6 h). Diabetes was defined as a fasting glucose >300mg/dL in tail vein blood 48 h after STZ injection (Junod et al., 1969).

At the 7th week after diabetes induction, all animals underwent adaptation to a treadmill originally designed for human use (Runner, Brazil) and modified for use by rats during 10 minutes at 5 m/min for 4 days. On the 5th day they were submitted to a Maximal Exercise Test (MET), consisting of graded exercise on the treadmill, with speed increments of 5 m/min every 3 minutes, starting at 5 m/min and continuing up to the maximal intensity attained by each rat, and was stopped when each animal remained more than 50% of the time without giving signs of intention to advance (Melo et al., 2003; Rodrigues et al., 2007; Ilha et al., 2008). The values obtained in the MET were used to plan the treadmill training program, which started in the eighth week after diabetes induction. In order to correct the exercise intensity, a second MET was performed in the fifth training week.

Treadmill training

Exercise was performed on a treadmill twice a day with an interval of 4 h between each session, 5 days per week (Tancrède et al., 1982), and the training intensity increased gradually, according to the MET. During the first week the running sessions lasted 10 min, and duration increased each week, reaching 60 min in the 8th week, which was maintained until the tenth week. Moreover, each training session had a warm-up period, a main period and a cooling-off period. During the warm-up the rats ran 15% of the session time, with 20 to 30% of the maximum velocity determined by the MET; in the main period the rats ran 70% of the session time, with 40 to 50% of the maximum velocity; and in the cooling-off period the rats ran 15% of the session time, with 20 to 30% of the maximum MET values.

Histological and morphometric analysis

The rats were anaesthetised with sodium thiopental (i.p; 50 mg/kg; Cristalia, Brazil). Heparin (1000 IU; Cristalia, Brazil) was injected into the left cardiac ventricle, then the animals were transcardially perfused through the left ventricle using a peristaltic pump (Control Company, Brazil, 20 mL/min) with 400 mL of 0.9% saline solution, followed by 400 mL of a fixative solution containing 0.5% glutaraldehyde (Sigma Chemicals Co., USA) and 4% paraformaldehyde (Reagen, Brazil) in a 0.1M phosphate buffer (PB), pH 7.4 at room temperature.

The right L5 dorsal root ganglia (L5 DRG) were removed and postfixed for 1 h at room temperature in the same fixative solution, then washed in PB 0.1 M and postfixed in 1% OsO₄ (Sigma Chemicals Co., USA) in PB for 1 h at room temperature. L5 DRG were then washed in PB 0.1M and dehydrated in a graded series of

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alcohol and propylene oxide (Electron Microscopy Sciences, USA), embedded in resin (Durcupan, ACM-Fluka, Switzerland), kept in vacuum for 24 h and afterwards put in resin blocks and polymerised for 48 h at 60°C.

For morphometric studies, the ganglia were sectioned using an ultramicrotome (MT 6000-XL, RMC, Tucson, USA), and the serial semithin sections (1 µm) were stained with toluidine blue (Merck, Germany) diluted in 1% sodium tetraborate (Ecibra, Brazil). For ultrastructural analyses, ultrathin sections (80- 95 nm) containing the DRG neurons were obtained with the same ultramicrotome and mounted on copper grids (200 mesh). These sections were stained with 1% uranyl acetate (1 h; Merck, Darmstadt, Germany) followed by 1% lead citrate (30 minutes; Merck, Darmstadt, Germany; Reynolds, 1963).

Images of the DRG neurons of the semithin sections were captured and digitalized using a Nikon Eclipse E-600 microscope (Tokyo, Japan; initially 200X and further amplified 200% for analysis) coupled to a Pro-Series High Performance CCD camera and Image Pro Plus Software 6.0 (Media Cybernetics, USA). For the ultrastructural study, ultrathin sections were examined at the Electron Microscopy Centre (UFRGS) using a transmission electron microscope (JEOL, JEM 1200 EXII, Japan), coupled to a Bioscan camera (Digital Micrograph, Gatan, München, Germany, model 792).

The A cells were identified based on the presence of a large pale cytoplasm and a large nucleus containing one central nucleolus, while the B cells were identified based on the presence of a dark cytoplasm, containing a large pale nucleus which had several eccentrically located nucleoli (Sidenius and Jakobsen, 1980; Tandrup, 1993).

For morphometric quantification of the cellular and nuclear volumes, fifteen B cells and ten A cells were selected per animal from each experimental group. Both the cellular bodies and nucleus were analysed separately, and only entire cell bodies and their nuclei found within the series of sections were used for quantitative evaluations (Rocha et al., 2007). The mean values of cellular and nuclear volumes were obtained per animal and later used for further comparisons. B cells were only analysed when they appeared in a minimum of twelve

sections and A cells when they appeared in a minimum of nineteen sections.

Morphometric quantification of the cellular and nuclear volumes of the L5 DRG A and B cells was determined using a point counting technique in association with the Cavalieri method (Cruz-Orive and Weibel, 1990; Howard and Reed, 1998; Hermel et al., 2006; Rocha et al., 2007). The mean values of cellular and nucleus volumes were calculated using the equation: $V = \sum P.a/p.T$

Where V corresponds to volume; $\sum P$ corresponds to the total counted points; a/p corresponds to area-point and T corresponds to section thickness. All measurements were done by two blinded examiners.

For morphometric quantification of the cellular areas, 60 A and 60 B cells were selected in the region of the cell where the nucleolus appeared. The area was determined using a point counting technique (Ilha et al., 2008). The mean values of the cellular areas were calculated using the equation:

$$\hat{A} = \sum P.a/p$$

Where \hat{A} corresponds to area; $\sum P$ corresponds to the sum of the points and a/p corresponds to area-point.

Statistical analysis

Blood glucose concentrations and body weight data were analysed using repeated measures analysis of variance (ANOVA), and differences between the groups were assessed using the Duncan *post-hoc* test. The mean values of the cellular and the nuclear areas and volumes were analysed using one-way ANOVA, and the differences between the groups were assessed using the Bonferroni *post-hoc* test. The statistically significant level was set as $P < 0.05$. Data were run on Statistica 6.0 software package (StatSoft, Inc., USA). All data are represented by the mean \pm standard error of mean (SEM).

Results

Blood glucose concentrations and body weight data

As expected, in all the evaluations performed, blood glucose concentrations were higher in diabetic rats (D

Table 1. Time course of blood glucose concentrations and body weight data in the studied rats.

Group	48 h		30 days		60 days		90 days		120 days	
	BGC (mg/dl)	Weight (g)								
C	121±8	276±8	95±1	309±8	101±2	325±11	90±3	345±13	90±3	368±13
D	421±27*	269±6	427±54*	260±5	432±19*	263±6*	447±10*	278±4*	423±13*	288±4*
TD	396±30*	269±8	448±13*	257±7	480±32*	256±8*	458±27*	251±10*	456±15*	276±7*

C: control group; D: diabetic group; TD: trained diabetic group. *: corresponds to $P < 0.05$ when compared to the C group. BGC: Repeated measures ANOVA group effect ($F(2,12) = 200.980$; $P < 0.000001$), time effect ($F(4,48) = 0.736$; $P = 0.572$), time vs group interaction ($F(8,48) = 1.277$; $P = 0.277$). Body weight: Repeated measures ANOVA group effect ($F(2,12) = 22.526$; $P = 0.000087$), time effect ($F(4,48) = 36.718$; $P < 0.000001$), time vs group interaction ($F(8,48) = 17.177$; $P < 0.000001$).

and TD groups) when compared to the C group ($P < 0.001$). However, blood glucose concentrations in both diabetic groups were similar over the follow-up period ($P > 0.05$; Table 1), i.e., treadmill training had no influence on the control of the blood glucose concentrations of these animals.

There were no differences in the body weight between groups from 48 h until the 60th day post-

diabetes induction ($P > 0.05$; Table 1). However, on the 90th and 120th days, the diabetic rats (D and TD groups) had lower mean body weights than the C group ($P < 0.05$; Table 1). Furthermore, there were no differences between the diabetic groups over the whole experimental period. Additionally, the body weight of the C group increased gradually and significantly over the experimental period ($P < 0.05$; Table 1).

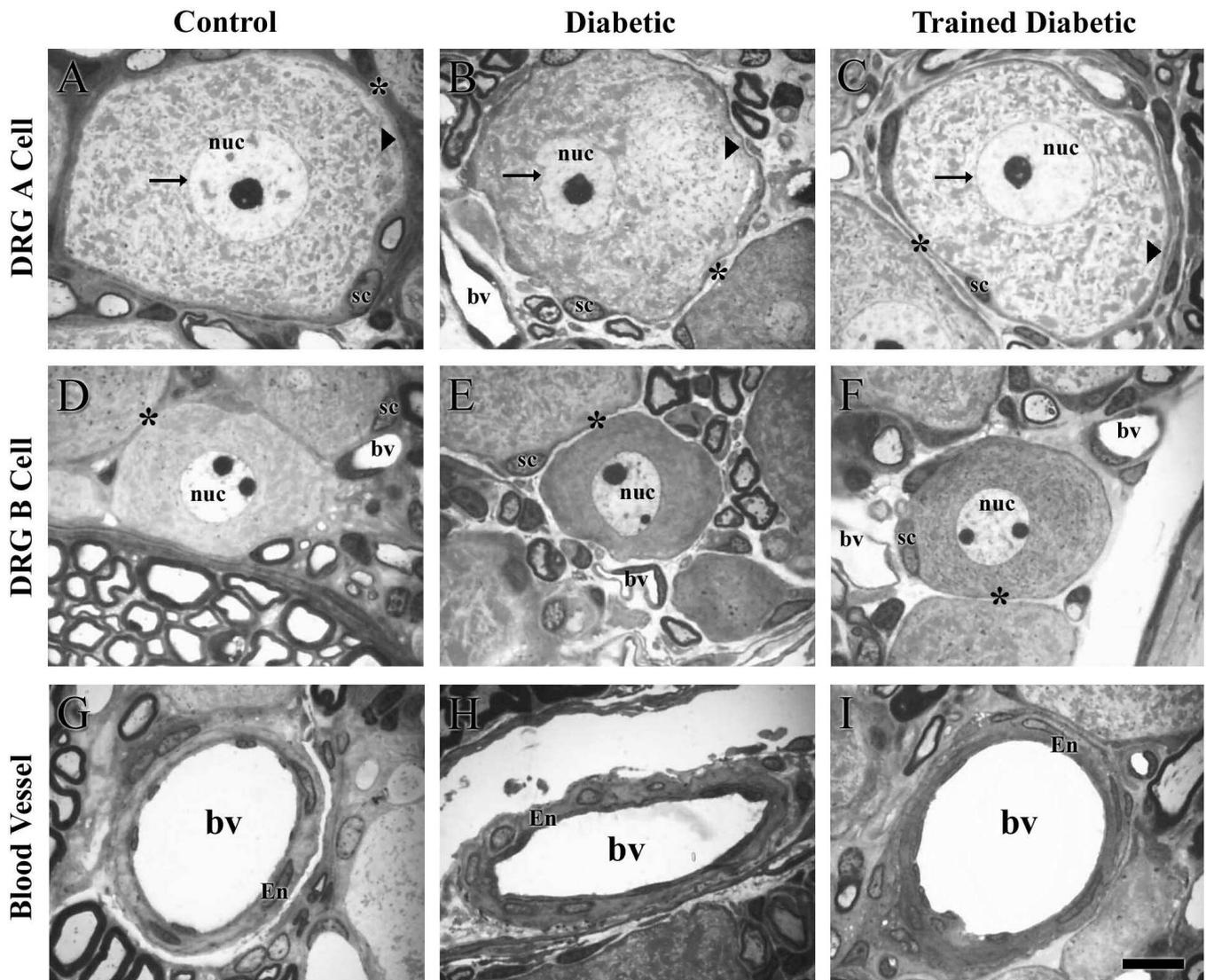


Fig. 1. Digitalized images of transverse semithin sections ($1 \mu\text{m}$) obtained from L5 DRG. **A, B and C** images show examples of an A cell from Control, Diabetic and Trained Diabetic groups, respectively. Note the irregularities in the nuclear (arrow) and plasma (arrowhead) membranes and the altered aspect of both the nucleus and cytoplasm in Diabetic (**B**) compared with Control (**A**) and Trained Diabetic (**C**). Note the increase in the interstitial space (asterisk) in the Diabetic group (**B**). Images **D, E and F** show examples of a B cell from Control, Diabetic and Trained Diabetic groups, respectively. Note that B cells showed an altered aspect of both nucleus and cytoplasm in Diabetic (**E**) compared with Control (**D**) and Trained Diabetic (**F**). The Diabetic group (**E**) showed an increased interstitial space (asterisk). Images **G, H and I** show blood vessels from the Control, Diabetic and Trained Diabetic groups, respectively. Note that the Diabetic group (**H**) had a shrunken aspect of the blood vessels, when compared to Control (**G**) and Trained Diabetic (**I**). Nuclear membrane (arrow); plasma membrane (arrowhead); nucleus (nuc); interstitial space (*); satellite cell (sc); nerve fibre (nf); blood vessel (bv); vascular endothelium (En). Scale bar: $10 \mu\text{m}$.

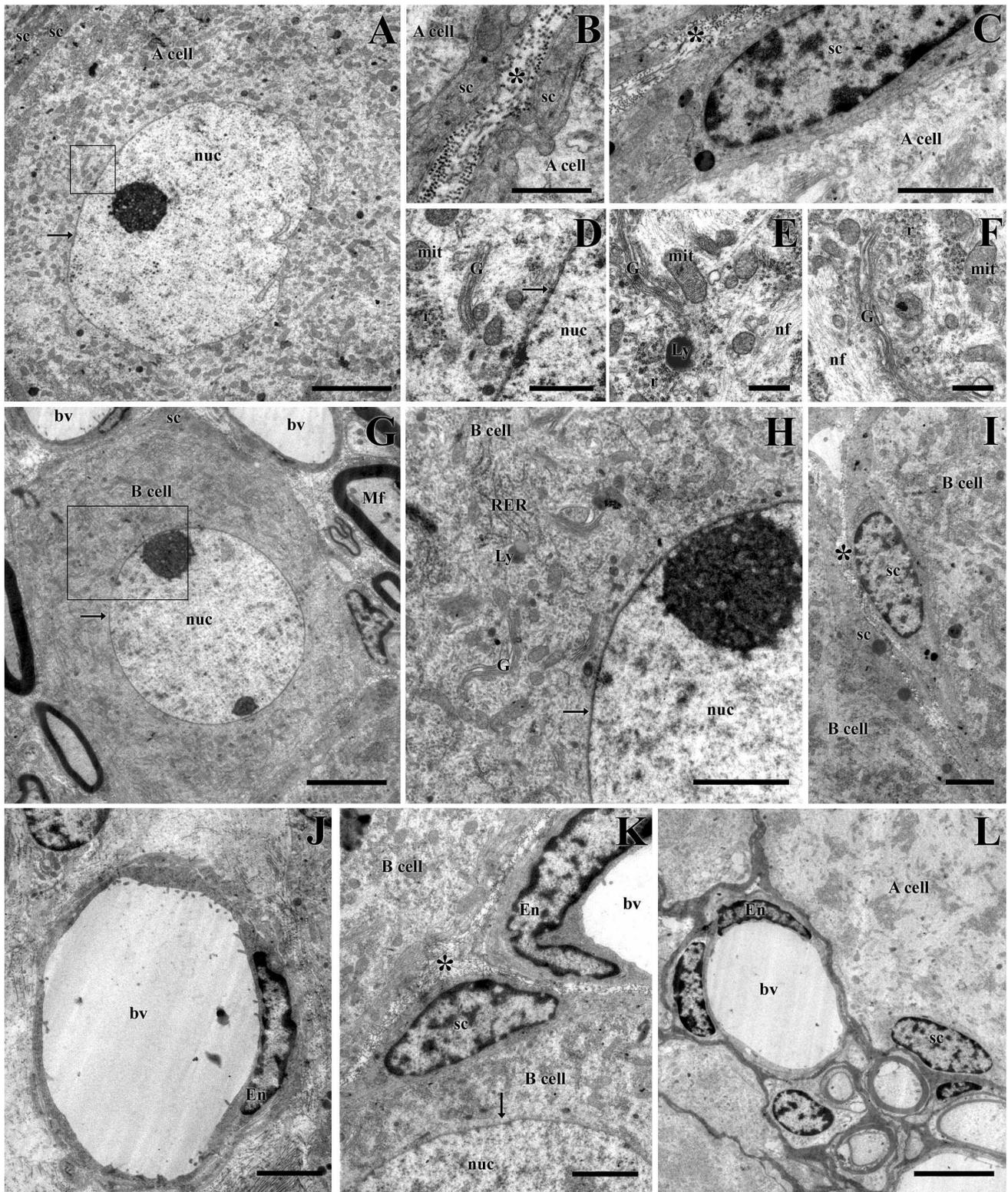


Fig. 2. Electron micrographs of L5 DRG cells from Control group. **A-F** images show cytoplasm from A cell. In **A** note the regular well-defined nuclear (arrow) and plasma membranes. In **B** note the relationship between A cells and their satellite cells (sc) and the extracellular space among them (*). In **C** note the A cell closely surrounded by satellite cell (sc). In **D** (a higher magnification of the boxed area in **A**), **E** and **F** note the cytoplasm containing well-defined Nissl bodies separated by spaces containing many cytoskeleton filaments, giving the appearance of a pale cytoplasm, free ribosomes (r); the Golgi apparatus with long parallel cisternae with many vesicles associated; mitochondria with their cristae oriented transversely. **G, H and I** show the cytoplasm from B cell. In **G** note the well-defined nuclear membrane (arrow); the relationship between B and satellite cell (sc). In **H**, a higher magnification of the boxed area in **G**, note the rough endoplasmic reticulum (RER); scanty lysosomes (Ly); and the Golgi apparatus with long and parallel cisternae (G). In **I** note the relationship between B cells and their satellite cells (sc) and the extracellular space among them (*). **J, K and L** display blood vessels with well-defined walls and regular lumen. Nuclear membrane (arrow); nucleus (nuc); extracellular space (*); satellite cell (sc); neurofilaments (nf); rough endoplasmic reticulum (RER); ribosomes (r); Golgi apparatus (G); lysosome (Ly); mitochondria (mit); blood vessel (bv); vascular endothelium (En); myelinated fibers (Mf). Scale bars: A, G, L, 5 μ m; B, D, 1 μ m; C, H-K, 2 μ m; E, F, 0.5 μ m.

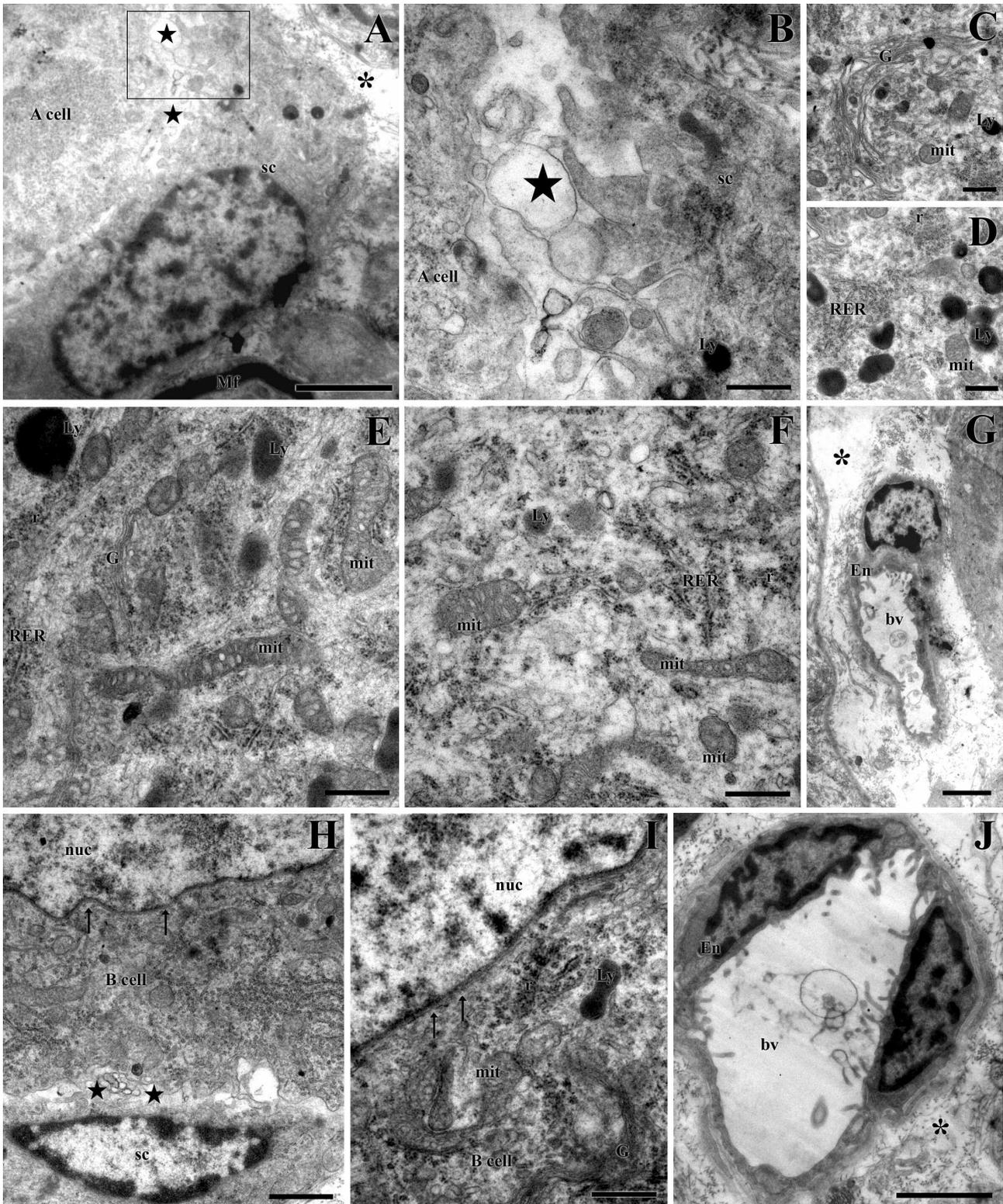


Fig. 3. Electron micrographs of L5 DRG cells from Diabetic group. **A, B, C, D, E and F** images show cytoplasm from A cell. In **A** note the relationship between A cell and satellite cell (sc), showing the enlarged interstitial space between them (star). In **B**, a higher magnification of the boxed area in **A**, show the enlarged and irregular aspect of the interstitial space between A and satellite cell. In **C** note the dilated Golgi apparatus (G). In **D** note the numerous lysosomes with dense content (Ly). **E and F** show the many vacuolated and degenerating mitochondria (mit), and the intracellular oedema evidenced by the dilated cisternae of the rough endoplasmic reticulum (RER); free ribosomes (r) separated by spaces containing fewer cytoskeleton filaments. **H and I** show the cytoplasm of B cell. In **H** note the irregular nuclear membrane (arrow) and the relationship between B cell and satellite cell (sc) with an enlarged interstitial space between them (stars). In **I** note the double-layered envelope surrounding the nucleus (arrows), lysosome (Ly), and vacuolated and degenerating mitochondria (mit). In **G and J** note blood vessels with irregularities in the lumen, which was coarse wrinkled protuberant like fingers or villi. Nuclear membrane (arrow); nucleus (nuc); extracellular space (*); satellite cell (sc); neurofilaments (nf); rough endoplasmic reticulum (RER); ribosomes (r); Golgi apparatus (G); lysosome (Ly); mitochondria (mit); blood vessel (bv); vascular endothelium (En); myelinated fibers (Mf); interstitial space between satellite cells and DRG neurons (star). Scale bars: A, G, J, 2 μ m; B-F, I, 0.5 μ m; H, 1 μ m.

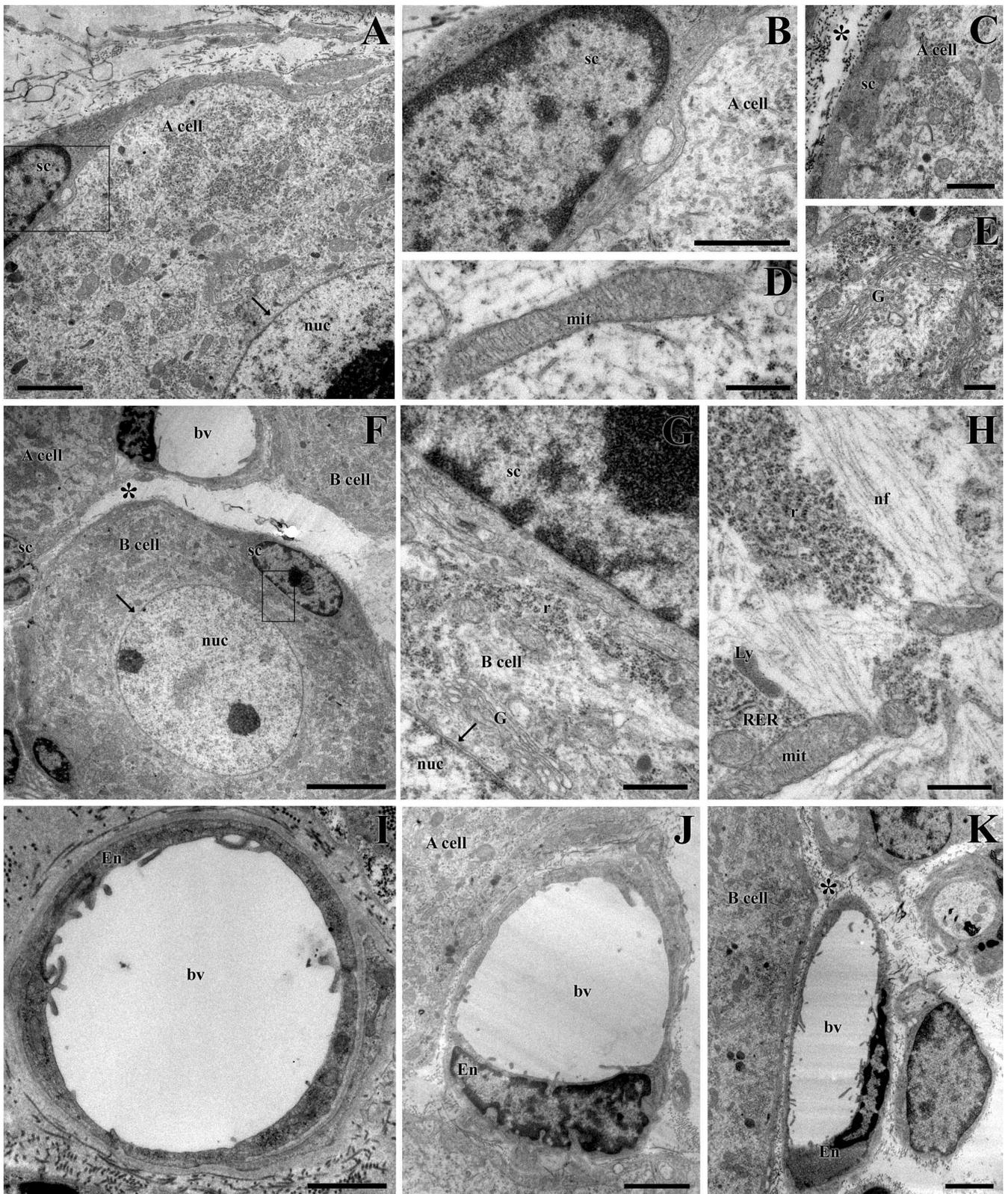


Fig. 4. Electron micrographs of L5 DRG cells from Trained Diabetic group. **A, B, C, D, E and H** images show cytoplasm of A cell. In **A** note the well-defined nuclear membrane (arrow). In **B**, a higher magnification of the boxed area in **A**, note the A cell closely surrounded by a satellite cell (sc). In **C** note the relationship between A and satellite cell. In **D** note the mitochondria with cristae oriented transversely. In **E** note the large Golgi apparatus with an increased amount of vesicles. **F and G** show the cytoplasm of B cell. In **G**, a higher magnification of the boxed area in **F**, note the B cell closely surrounded by the satellite cell (sc), the well-defined nuclear membrane (arrow), ribosomes (r) and the large Golgi apparatus (G). In **H** note lysosomes (Ly), rough endoplasmic reticulum (RER) numerous cytoskeleton filaments distributed throughout the cytoplasm (nf), mitochondria (mit) and lysosomes (Ly). In **I, J, K** note blood vessels with rounded lumen and well-defined walls. Nuclear membrane (arrow); nucleus (nuc); extracellular space (*); satellite cell (sc); neurofilaments (nf); rough endoplasmic reticulum (RER); ribosomes (r); Golgi apparatus (G); lysosome (Ly); mitochondria (mit); blood vessel (bv); vascular endothelium (En); myelinated fibers (Mf). Scale bars: A, J, K, 2 μ m; B, C, I, 1 μ m; D, E, G, 0.5 μ m; F, 5 μ m.

Morphological outcomes

Under light microscopy, A cells from the C group (Fig. 1A) had granular and large pale cytoplasm and one nucleolus in their nuclei. B cells (Fig. 1D) had dark homogeneous cytoplasm, large pale nuclei, with well-defined nuclear membranes and multiple nucleoli. Satellite glial cells surrounded both B and A cells. In the interstitial space there were fibroblasts (data not shown), nerve fibres and blood vessels with regular well-defined walls (Fig. 1G).

Morphological alterations of B and A cells in the D group (Fig. 1E,B; respectively) included irregular nuclei in both B and A cells and an irregular aspect in both nuclei and cytoplasm. Additionally, the interstitial space was increased with numerous fibroblasts (data not shown), blood vessels and nerve fibres. In the D group, the blood vessels (Fig. 1H) had a shrunken aspect (Fig. 1G).

B and A cells from the TD group (Fig. 1F,C; respectively) also displayed morphological changes, although they were not as common as in the D group (Fig. 1E,B). Cell-shrinkage was less frequently seen.

Blood vessels (Fig. 1I) had a rounded lumen with regular well-defined walls, similar to the control group (Fig. 1G).

At the ultrastructural level, A and B cells from the C group displayed well-defined nuclear and plasma membranes, which were closely surrounded by satellite cells (Fig. 2A,G). The cytoplasm of A cells contained well-defined Nissl bodies separated by spaces containing many cytoskeleton filaments, giving the appearance of a pale cytoplasm (Fig. 2A,D-F). Some of the ribosomes were attached to the outer surfaces of the cisternae of the rough endoplasmic reticulum (RER; Fig 2E,F), and others lay free in the cytoplasmic matrix (Fig. 2F). Also, these cells displayed a Golgi apparatus with long parallel cisternae to which many vesicles were attached to or lay free (Fig. 2D-F). Their cytoplasm contained many mitochondria (Fig. 2D-F), in which the cristae were mainly oriented transversely. The scanty lysosomes had a dense content (Fig. 2E,F). B cells displayed all of these features, although in their cytoplasm there were numerous Nissl bodies which gave a dark and homogeneous aspect to these cells, masking the cytoskeleton (Fig. 2G-I). As evidenced by light

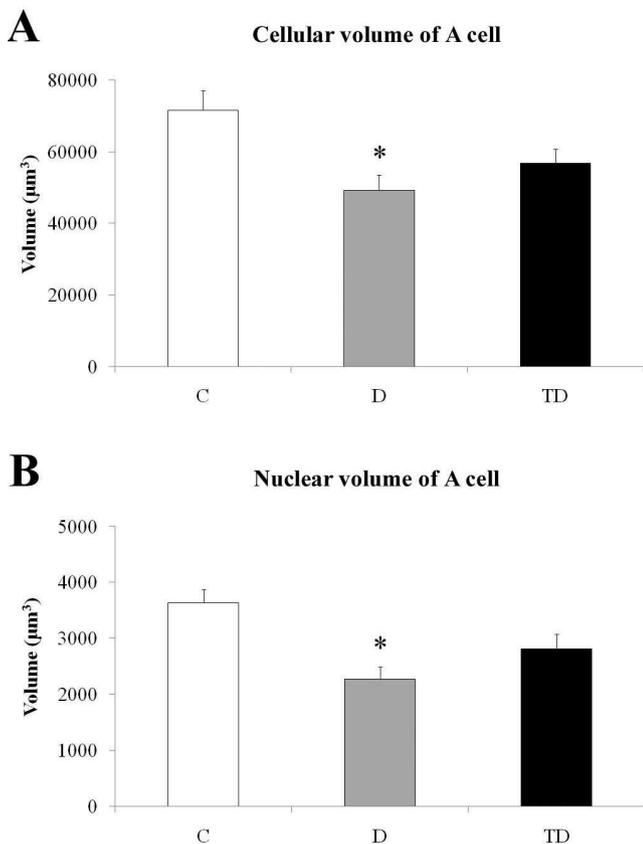


Fig. 5. A. Cellular volume of A cell (mean \pm S.E.M). *: corresponds to $P < 0.05$ when compared to C group. **B.** Nuclear volume of A cell (mean \pm S.E.M). *: corresponds to $P < 0.05$ when compared to C group.

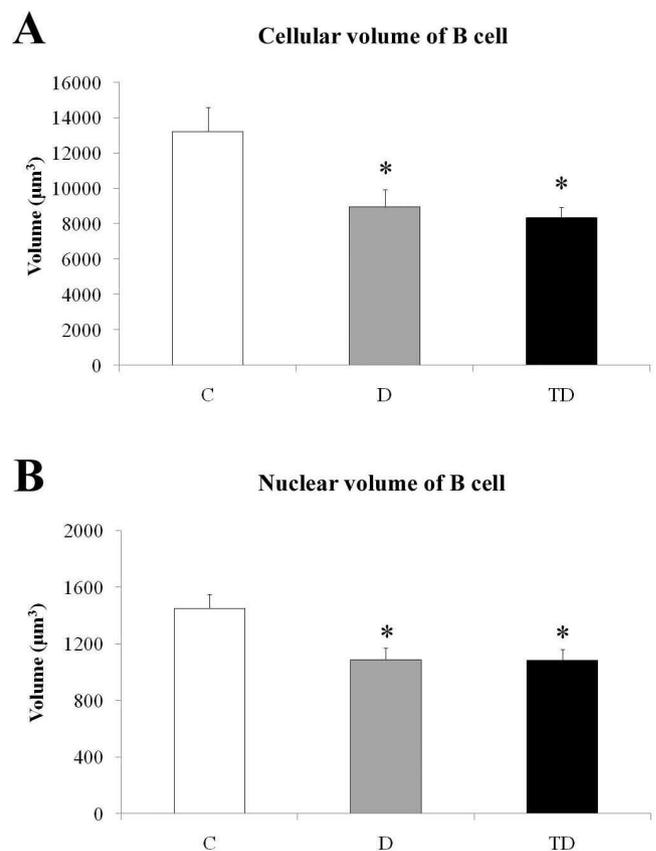


Fig. 6. A. Cellular volume of B cell (mean \pm S.E.M). *: corresponds to $P < 0.05$ when compared to C group. **B.** Nuclear volume of B cell (mean \pm S.E.M). *: corresponds to $P < 0.05$ when compared to C group.

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microscopy, in the interstitial space there were blood vessels with regular well-defined walls (Fig. 2J-L).

In the D group, A cells showed an intracellular oedema evidenced by large numbers of ribosome attached to the dilated cisternae of the RER, while others lay free in the cytoplasmic matrix and were separated by spaces containing apparently fewer cytoskeleton filaments (Fig. 3E,F). However, in the TD group, ribosomes were attached to the outer surfaces of the cisternae of the RER with normal aspect, others lay free in the cytoplasmic matrix and there were numerous

cytoskeleton filaments distributed throughout the cytoplasm (Fig. 4H). In diabetic animals, these cells displayed a dilated Golgi apparatus with which vesicles were associated (Fig. 3C,E). A cells from the TD group displayed a large Golgi apparatus with which a greater amount of vesicles were associated, including dense vesicles, which were probably packaging peptides (Fig. 4E). In addition, in the D group, A cell cytoplasm contained many vacuolated and degenerating mitochondria (Fig. 3E,F), while in the TD group there were numerous mitochondria, some of which were

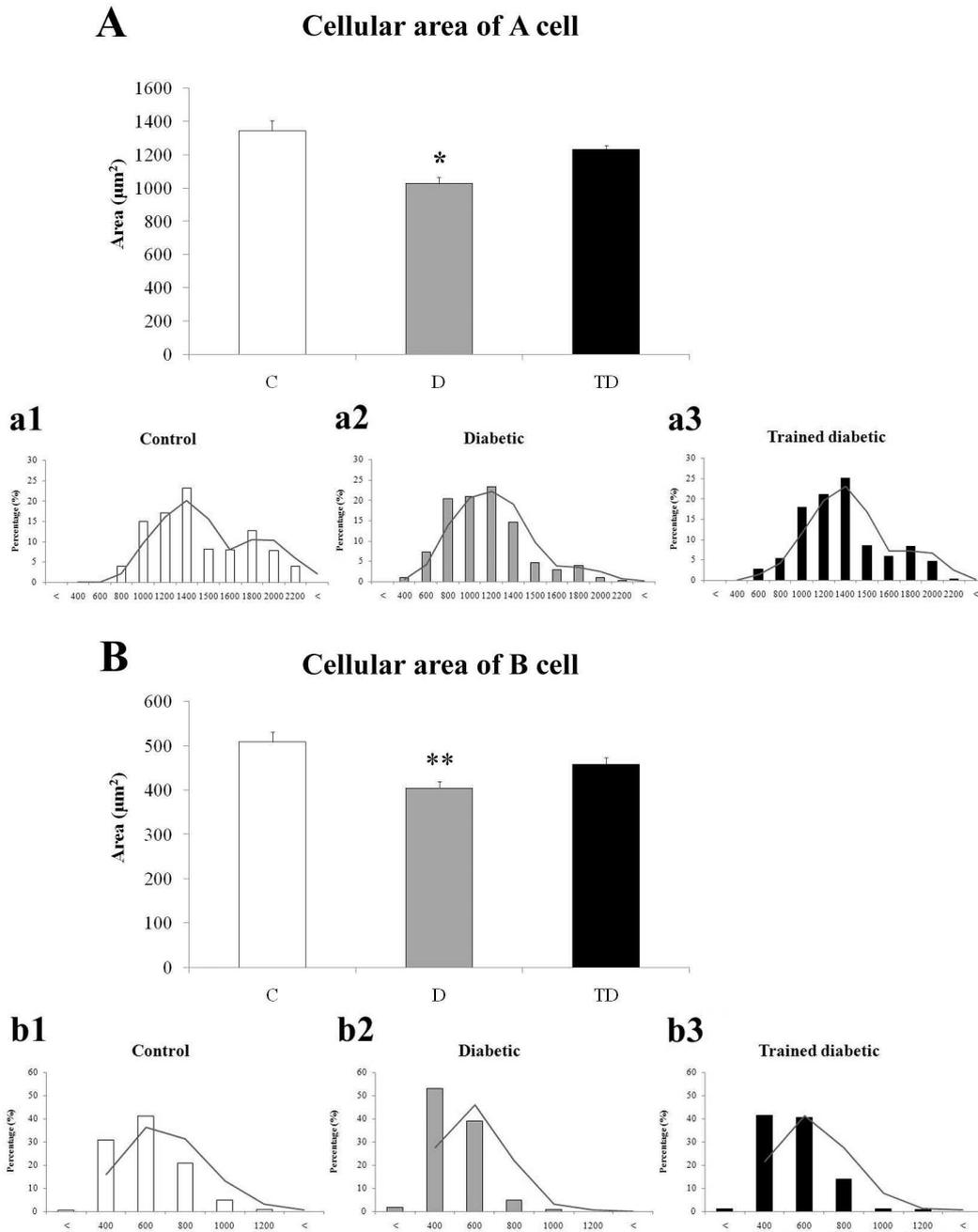


Fig. 7. A. Cellular area of A cell (mean ± S.E.M). **a1.** Frequency histogram of cellular area of A cell in control group. **a2.** Frequency histogram of cellular area of A cell in diabetic group. **a3.** Frequency histogram of cellular area of A cell in trained diabetic group. **B.** Cellular area of B cell (mean ± S.E.M). **b1.** Frequency histogram of cellular area of B cell in control group. **b2.** Frequency histogram of cellular area of B cell in diabetic group. **b3.** Frequency histogram of cellular area of B cell in trained diabetic group. *: corresponds to P<0.05 when compared to C and TD groups. **: corresponds to P<0.05 when compared to C group.

vacuolated and in the process of degenerating, while others had their cristae mainly oriented transversely, as seen in the C group (Fig. 4D,H). There were numerous lysosomes in the D group, which had dense content (Fig. 3B-F), while fewer lysosomes were seen in the TD group than in the D group (Fig. 4C,E). In the cytoplasm of B cells from the D (Fig. 3H,I) and TD groups (Fig. 4F,G) there were numerous Nissl bodies, dilated Golgi apparatus with which vesicles were associated, vacuolated and degenerating mitochondria, and numerous lysosomes, which had dense content. B cells from the D group showed an irregular double-layered envelope surrounding the nucleus (Fig. 3H,I), while B cells from the TD group showed a regular double-layered envelope surrounding the nucleus (Fig. 4F,G). In the D group, the cellular space between the A and B cells and their corresponding satellite cells was enlarged (Fig. 3A,B). The satellite glial cells showed an irregular aspect in the D group, while in the TD group there was a relationship between the satellite cells and the DRG neurons similar to that seen in non-diabetic animals (Fig. 2C).

In the D group, the blood vessels showed irregularities in the lumen, which had coarse, protuberant finger or villi-like wrinkles (Fig 3 G,J), and it was noteworthy that the blood vessel profiles of the TD group (Fig. 4I-K) were similar those seen in the C group (Fig. 2J-L).

Morphometrical outcomes

In the analysis of volumes, the mean cellular (Fig. 5A) and nuclear (Fig. 5B) volumes of A cells were smaller in the D group than in the C group ($P<0.05$). There were no differences in cellular and nuclear volumes of A cells between the C and TD groups ($P=0.1$). With respect to the cellular (Fig. 6A) and the nuclear (Fig. 6B) volumes of B cells, the D group had smaller cellular and nuclear volumes ($P=0.03$) than the C group. The same was true with the TD group, which had smaller cellular and nuclear ($P<0.05$) volumes than the C group.

Moreover, the analysis of cellular areas showed that the mean cellular area of A cells (Fig 7A) was lower in the D group than in the C group ($P<0.05$). The TD group and C group had similar mean areas ($P=0.2$), while the D group had a lower mean area than the TD group ($P<0.02$). Summaries of the area histograms of the control, diabetic and trained diabetic rats are shown in figures 7a1, a2 and a3, respectively. The shape of the histograms shows that while in the C and TD groups the peaks of cell areas were between 1400 and 1800 μm^2 , in the D group the peak was between 800 and 1200 μm^2 , demonstrating a clear shift to the left. Also, the mean B cell cellular area (Fig. 7B) was lower in the D group than the C group ($P<0.05$), but similar to the TD group ($P=0.1$). Summaries of the area histograms of control, diabetic and trained diabetic rats are shown in figures

7b1, b2 and b3, respectively. Although the TD group was not different from the D group, the histograms show that the peaks in the three groups were between 400 and 600 μm^2 , in the D group there were fewer cells with more than 800 μm^2 .

Discussion

This is the first study to evaluate the effects of physical training on the morphological and morphometric features of DRG cells in diabetic rats. As expected, diabetic rats displayed higher blood glucose levels when compared to control animals, but physical training did not improve the control of the blood glucose concentration, which is in accordance with previous data (Midaoui et al., 2006). As expected, rats rendered chronically insulin deficient by the injection of STZ would not have benefits on their blood glucose concentration by physical training. This is similar to that observed in human type 1 diabetes mellitus (Berger et al., 1977; Zinman et al., 1984).

Diabetic rats exhibited morphological alterations in both B and A cells from the DRG, including irregular nuclei and a shrunken aspect in both the nuclei and cytoplasm, with modified matrix and cell components in the interstitial space. The blood vessels in the untrained diabetic group had numerous irregular profiles, and showed irregularities in the lumen, which had coarse, protuberant finger or villi-like wrinkles. Previous studies showed neuronal cytoplasmic vacuolations (Kishi et al., 2002), and reduced somatic volumes (Sidenius and Jakobsen, 1980); thickening and reduplication of capillary basal lamina, hyperplasia and proliferation of endothelial cells in the nerve (Sasaki et al., 1997; Cameron et al., 2001). A previous study associated the folds in the plasma membrane with loss of elasticity of the blood vessels (Lu et al., 2004).

These irregularities in the lumen of blood vessels in diabetic animals were less frequently identified in trained diabetic animals, so that blood vessels of trained diabetic rats appeared to be similar to those of controls. Studies showed that low-intensity training can increase both capillary density and capillary/fiber ratio in the gracilis and myocardium (Amaral et al., 2000) and also the medial wall thickness of systemic arteries (Segal et al., 1993) in non-diabetic rats.

The idea that diabetes causes alterations in DRG cell volume is already widely accepted (Sidenius and Jakobsen, 1980; Noorafshan et al., 2001; Kishi et al., 2002). In our study, diabetic rats showed decreased cellular and nuclear volumes of B and A cells, as well as cellular areas. The exercise training employed was able to increase cellular and nuclear volumes and cellular areas of A cells, so that the cellular areas of A cells from trained diabetic rats became similar to those of non-diabetic rats. This is corroborated by the findings from the neuronal area distributions, which show that A cells from trained diabetic rats had a distribution pattern

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similar to that of the control animals. However, in the trained diabetic rats, there was no change in the morphometric values of cellular and nuclear volumes of B cells, and the findings for the cellular areas showed that the trained diabetic rats had means similar to both the control and diabetic rats. In addition, the distribution of the neuronal areas of B cells shows that in the D and TD groups there was a predominance of small cells, with areas between 400 and 600 μm^2 , with few cells larger than 800 μm^2 .

Since B cells send nerve fibres that form A δ and C nociceptors (Harper and Lawson, 1985), we could speculate that exercise does not produce direct effects on the volumes and areas of these cells. Moreover, nerve growth factor (NGF) is responsible for the maintenance of small sensory neurons and promotes cell survival, maintenance of calcitonin gene-related peptide content (CGRP), substance P content (Donnerer et al., 1992) and neurofilament gene expression (Verge et al., 1990). Diabetes causes a loss of NGF and CGRP content in the sciatic nerve (Ferynhough et al., 1994), deficits in NGF retrograde transport (Delcroix et al., 1997), deficits in axonal transport structural proteins (Jakobsen and Sidenius, 1980) and aberrant neurofilament phosphorylation in DRG cells (Ferynhough et al., 1999). All of these features could explain the persistence of the B cell alterations even after the training program.

Loss of vibratory sensation and reduction in nerve conduction velocity in large diameter A β / γ fibres, which send axons from A cells is a hallmark of diabetic neuropathy (Harper and Lawson, 1985). Since diabetes causes a reduction in nerve conduction velocity (Cameron et al., 1986) and this is proportional to the size of the neuronal soma (Harper and Lawson, 1985), one may suggest that exercise training may improve nerve conduction velocity by changing, among other factors, the cellular size.

The morphometric results on DRG cellular and nuclear volumes in diabetic rats were in accordance with previous data (Sidenius and Jakobsen, 1980; Noorafshan et al., 2001). The molecular and cellular basis for decreased cell volumes in the DRG of diabetic rats is still unknown, but multiple factors could be implicated, such as high susceptibility to blood glucose variations due to blood-nerve barrier properties and greater metabolic demands in pseudounipolar cells (Zochodne and Ho, 1991). Furthermore, impaired expression of cytoskeleton proteins, like neurofilaments and α -tubulin, can contribute to alterations in neuronal volume (Scott et al., 1999).

Also, in the D group, some alterations shown at the ultrastructural level, such as the more numerous lysosomes, could indicate that these cells were in the autophagic process, as occurs in myogenic satellite cells from diabetic rats (Brannon et al., 1989). It is well established that oxidative stress (Vincent et al., 2005; Zhrebetskaya et al., 2009) and mitochondrial damage are a hallmark in diabetic neuropathy, causing a direct

insult to neurons (Srinivasan et al., 2000). Cells from the D group showed a visibly dilated Golgi apparatus, an irregular double-layered envelope surrounding the nucleus and fewer cytoskeleton filaments. All these factors could indicate that these cells are damaged, and explain the loss of their volume. Additionally, in contrast to the control animals, in which the DRG neurons were closely surrounded by satellite cells (Pannese et al., 1994), in diabetic animals the space between the A or B cells and their respective satellite cells was larger, perhaps suggesting that the former had shrunk.

Exercise is an important therapeutic tool in the treatment of diabetes. Doing exercise was shown to produce improvements to cardiovascular and autonomic dysfunctions, which were maintained even after three weeks of detraining in rats (Mostarda et al., 2009).

Moreover, the practice of exercise leads to increased protein synthesis (Tipton and Wolfe, 1998), increased expression and translocation of GLUT4 to the plasma membrane (Dela et al., 1993), increased capillary supply in skeletal muscle (Gute et al., 1996) and it enhanced skeletal muscle blood flow capacity (Laughlin and Roseguini, 2008). In addition, exercise leads to the production of more heat shock proteins (Atalay et al., 2004; Lappalainen et al., 2010) contributing to the defence system against oxidative stress in trained STZ-diabetic rats.

Furthermore, the mammalian target of rapamycin (mTOR) controls cell size (Schmelzle and Hall, 2000) in a wide variety of cells and is activated by growth factors, such as brain-derived neurotrophic factor (BDNF), insulin and insulin-like growth factor (IGF), which signal upstream phosphoinositide-3 kinase / protein-kinase B/Akt (Oldham and Hafen, 2003; Johnson-Farley et al., 2007). Brain-derived neurotrophic factor induces the phosphorylation of mTOR in cortical neurons *in vitro* (Takei et al., 2001). Also, physical activity is able to increase the BDNF production in muscles (Gómez-Pinilla et al., 2001) and the levels of BDNF mRNA in DRG cells in culture (Molteni et al., 2004). Therefore, activation of mTOR by neurotrophins signalling may be involved in exercise-induced effects in DRG neurons.

In summary, our results show that diabetes causes morphological alterations in DRG cells and blood vessels, and that some of these can be prevented/reverted by physical training, as evidenced by improvement in morphological features of DRG cells and blood vessels. Furthermore, exercise training was able to increase the cellular and nuclear volumes, as well as cellular areas of A cells from the DRG in diabetic rats.

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