Protective and therapeutic effects of swimming exercise training on diabetic peripheral neuropathy of streptozotocin-induced diabetic rats

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ABSTRACT. ***Background:*** **Diabetic peripheral neuropathy (DPN) is a typical complication of diabetes. No definitive treatment and prevention of DPN has been established, and very few data on the role of exercise training on DPN have been reported.** ***Aim of the study:*** **The protective and therapeutic effects of aerobic physical activity on the development of DPN in Type 1 were investigated.** ***Methods:*** **Rats were assigned to 5 groups: C (control), E (exercise), D (diabetic), DEx (exercise after diabetic), ExD (diabetic after exercise); C containing 10 animals and E, D, DEx, ExD containing 15 animals. Diabetes was induced with streptozotocin (STZ) (45 mg/kg, ip). Development of diabetes was confirmed by measuring blood glucose levels 2 days after STZ treatment. Body weights of all the animals were evaluated weekly throughout the experiment. Motor dysfunction defined by a significant increase in compound muscle action potential (CMAP) latency was recorded. The amplitude of CMAP which mainly reflects axonal dysfunction was also measured using standard techniques. Sciatic nerve morphometry and blood glucose levels were analyzed in all the groups.** ***Results:*** **Blood glucose level significantly increased 2 days after STZ injection. All diabetic rats showed decreased body weight compared to control rats. An increase in motor latency of CMAP and a decrease in amplitude of CMAP, indicative of neuropathy, were seen in STZ rats. On the completion of the study (the 56th day post-STZ), histological examination revealed significant myelin loss (thinner myelin) in sciatic nerves of STZ rats. Treatment with swimming exercise had no effect on glycemic control but restored body weight, CMAP amplitude, CMAP latency or motor dysfunction in the diabetic animals.** ***Conclusions:*** **This study suggests that swimming exercise training has protective and therapeutic effects on DPN of STZ-induced diabetic rats.**

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INTRODUCTION

It is well known that neuropathy occurs in spontaneous and experimental diabetes, the streptozotocin (STZ) model being widely used to investigate the experimental diabetic peripheral neuropathies (1). Diabetic neuropathy is the most common form of peripheral neuropathy with functional, morphological, and metabolic changes in peripheral nerves (2). Neuropathy contributes to the greatest morbidity and mortality and severely impairs the quality of life because of paresthesia, pain, and neuropathic injury, the leading cause of non-traumatic amputation (3, 4).

Diabetic neuropathy is a multifactorial problem with a unique etiology. It has been described by some investigators to be a disease of the vasculature leading to nerve ischemia and altered nerve function (5, 6). Other investigators have proposed that diabetic neuropathy causes a defect in Na^+-K^+-ATPase activity and an alteration of signal transduction pathways in the nerve (7). Hyperglycemia is critical for the development and progression of diabetic neuropathy (8), with the two main pathogenic hypotheses focusing on a metabolic *vs* vascular etiology. Hyperglycemia increases oxidative stress *via* an overproduction of reactive oxygen species (ROS). A considerable amount of clinical and experimental evidence now exists, suggesting that many biochemical pathways strictly associated with diabetes increase the production of ROS (9). Increasingly, data indicate that oxidative stress plays an important role in the chronic complications of insulin-dependent diabetes mellitus (10, 11). Although no definitive treatment for diabetic neuropathy has been established yet, several studies have shown that intensive therapy and optimal glycemic control can significantly reduce diabetic neuropathy (12).

Daily moderate exercise can also be beneficial to diabetes due to reducing blood glucose and free radical production, and to increasing peripheral blood flow (9). Nevertheless, very few data on the effectiveness of exercise treatment on diabetic neuropathy have been reported (13, 14).

Abnormal nerve conduction is an early feature of diabetic nerve damage (15). The changes in excitability involve a reduced conduction velocity (due to myelination) together with diminished amplitude (loss of axons). Tesfaye et al. (16) showed that conduction velocity increased significantly after exercise in normal subjects. This study aimed to investigate the efficacy of physical exercise in treating diabetic peripheral neuropathy (DPN). The protective effects of physical exercise on neuropathy in diabetic rats were also evaluated. To accomplish this, rats with STZ-induced diabetes were used. Therefore, the amplitude and the latency (thus, conduction velocity) of compound muscle action potentials (CMAP) in diabetic rats trained by swimming exercise were measured. The thickness of the myelinated sheath of the sciatic nerve and the blood glucose levels were also evaluated.

Key-words: Action potential, diabetic peripheral neuropathy, swimming exercise training.

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MATERIALS AND METHODS

Animals and treatment

All procedures were approved by the Medical Faculty Experimentation Ethics Committee of Mersin University and followed the guidelines of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes. Seventy healthy male Wistar albino rats with a mean initial weight of 241±29.99 g and an average of 12 weeks old were housed at 23±2 C under a reversed dark-light-cycle (dark: 9:00 h to 21:00 h) and fed on standard rat pellets (Tavas animal food product Co., Turkey) and tap water *ad libitum*. The rats were randomly allotted into one of the 5 experimental groups: C (control), E (exercise), D (diabetic), DEx (exercise after diabetic), ExD (diabetic after exercise), C containing 10 animals and E, D, DEx, ExD containing 15 animals.

Induction of diabetes

Diabetes was induced in rats with STZ (Sigma-Aldrich) using a previously described method (8). STZ was administered ip at a dose of 45 mg/kg body weight dissolved in citrate buffer (0.1 M, pH 4.5). Group D received STZ at the beginning of the study. Group ExD was subjected to swimming training for 4 weeks, 5 days a week, 1 h a day, after which the group received STZ, and the swimming training continued for another 4 weeks. Group DEx received STZ at the beginning of study and was subjected to the same swimming exercise protocol from the beginning to the end of the study (8 weeks). Group E only underwent the same swimming training for 8 weeks. Group C was injected with the same volume of isotonic NaCl as the diabetic groups received. Blood glucose was measured 48 h after induction of diabetes. The diabetic state was confirmed when the glucose concentration exceeded 200 mmol/dl.

Blood glucose assays

Blood glucose levels were measured from tail vein blood samples in all 5 groups immediately before the induction of diabetes and served as the baseline data (0^{th} day). Other blood glucose levels were measured from tail vein blood samples 2 days after STZ injection (the 2^{nd} day) and at the end of the experiment (the 56^{th} day). Development of diabetes was confirmed by measuring blood glucose levels two days after STZ treatment. Rats with blood glucose levels of 200 mmol/dl or higher were considered to be diabetic. Plasma glucose levels in control and exercise group animals remained normal during the study. The diabetes mellitus was confirmed by Acu-Check Go One Touch Glucometer (Roche Diagnostics).

Body weight assay and exercise program

Body weight of all animals was measured weekly throughout the experiment. However, data were analyzed in all the groups only on the 0^{th}, 28^{th} (the 4^{th} week) and the 56^{th} day (the 8^{th} week). All the rats were adapted to the water before the beginning of the experiment. The adaptation consisted of keeping the animals in shallow water at 31±1 C (17), 5 days/week, from 09:00 to 21:30 h. The adaptation to the water proceeded along the entire experimental period. The purpose of the adaptation was to reduce stress without, however, promoting physical training adaptations (18).

As rats are natural swimmers, exercise protocols without overload based on swimming are widely used (19). The exercise-trained groups swam 1 h a day, 5 days a week for 8 weeks of housing (between 13:00-16:00 h on each training day). This exercise intensity without overload provides a mild to moderate aerobic stress for animals. This exercise protocol was selected because Gobatto et al. and Matsuo et al. (18, 20) demonstrated that it corresponds to moderate aerobic exercise training for rats.

The control rats were housed under the same conditions as the swimming rats and were handled as often as the exercise group. Exercise was performed by swimming in one glass tank (length 100 cm, width 90 cm, depth 60 cm) containing tap water maintained at 31±1 C. The duration of the first swimming exercise was limited to 15 min and increased by 15 min daily until it reached 1 h. Thus, continuous exercise (1 h) was performed from the 4^{th} day until the end of the training period. The non-exercise-trained rats (the control and the diabetic) were placed in shallow water at 31±1 C, 1 h, 5 days/week for 8 weeks of housing.

Electrophysiological techniques

Prior to electrophysiological recordings the rats were anesthetized with 50 mg/kg ketamine hydrochloride (Ketalar, Eczacibasi Ilac Sanayi ve Ticaret A.S., Istanbul, Turkey) administered intramuscularly. The hind limbs of all the rats were placed in a standard position during the electrophysiological recordings and then shaved. Electrophysiological recordings (CMAP) across nerve segment were made by using BIOPAC MP 100 acquisition system (Santa Barbara, USA). CMAP were recorded on the 0^{th}, 28^{th}, and 56^{th} days in all 5 groups. Standardized electromyography technique was used to record CMAP of the gastrocnemius muscle (21). Briefly, bipolar stimulating electrodes (Medelec small bipolar nerve electrodes, 6894T, Oxford, UK) were placed around the sciatic nerve at sciatic notch. The supramaximal stimulus consisted of single square pulse (intensity 10 V, duration 0.5 msec). The ground electrode was placed on the other thigh, where the stimulation was not applied. CMAP were recorded from the gastrocnemius muscle by the surface disc electrodes (Medelec, number 017K006, Oxford, UK) which were always positioned on the distal 1/3 of the leg. During the study, body temperature of rats was maintained at 37 C using a heating pad and continuously monitored by rectal probe digital thermometer. BIOPAC Acknowledge Analysis Software (ACK 100 W) was used to measure CMAP amplitude and CMAP latency. The amplitude of the CMAP is the height in millivolts from the baseline to the peak of the negative phase. The CMAP latency is the time in msec from the application of a stimulus to the initial deflection from the baseline, either positive or negative.

Morphological analysis of nerve

Sciatic nerves were harvested from the sacrificed animals at the end (56^{th} day) of the study. Histology was performed blindly. The fragments from nerve tissues were immediately fixed with 10% neutral formaline solution and embedded in paraffin. Cross sections (4 μm) were then cut by microtome and stained with hematoxylin and eosin to evaluate the degeneration of myelin. One hundred myelin sheath thicknesses in each slide were measured by an ocular micrometer attached to an Olympus BX50 light microscope with 1000 magnification.

Statistical analyses

All the results were expressed as the mean±SD. Data were analyzed using the STATISTICA statistical program (version 6.0). To determine the effect of swimming exercise on myelin sheath

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thickness, data were analyzed by using one way analysis of variance (ANOVA) and Tukey's test was used for *post hoc* test. To determine the effect of swimming exercise on the other parameters (the blood glucose, the body weight, the CMAP amplitude, and the distal latency) of diabetic rats, data were analyzed by using repeated measures ANOVA. Tukey's multiple range test was then used to analyze pair-wise comparisons by taking into account the significant interactions between time and groups. In all cases, the statistical significance was set at $p<0.05$.

RESULTS

The entire diabetic and training diabetic animals showed persistent polydipsia and polyuria. Two animals from group D, 3 animals from group DEx, and 4 animals from group ExD died before the end of the experiment.

Effects of diabetes and swimming training on blood glucose levels

Measured on the 0th, 2nd, and 56th days of the experiment, respectively, the blood glucose level of the control group was 115.40±7.34, 131.00±8.78, and 123.20±7.35 mmol/dl; that of the group E was 105.08±22.78, 132.67±12.52, and 118.88±10.45 mmol/dl; that of group D was 120.50±19.38, 416.13±49.73, and 546.14±90.77 mmol/dl; that of group ExD was 127.63±21.23, 383.13±59.85, and 598.00±196.48 mmol/dl; and that of group DEx was 122.75±5.95, 466.63±110.89, and 577.00±32.14 mmol/dl. There was no significant difference in the blood glucose values on the 0th day in all of the groups. Blood glucose level significantly increased 2 days after STZ injection. Swimming exercise had no effect on the blood glucose level of the hyperglycemic rats (Fig. 1).

Effects of diabetes and swimming training on body weight

Rats were weighed on the 0th, 28th, and 56th days of the experiment. Respectively, the body weight of group C was 251.30±36.33, 306.70±44.16, and 332.20±56.15 g; that of group E was 243.00±22.22, 294.67±20.91, and 336.75±29.37 g; that of group D was 257.25±34.55, 208.00±52.11, and 191.29±49.33 g; that of ExD group was 244.00±18.63, 282.78±22.91, and 242.00±46.22 g; and that of group DEx was 209.25±15.04, 166.25±15.79, and 140.83±33.10 g. There was no significant difference in the body weight values on the 0th day in all 5 groups. Mean body weight increased rapidly in the control group and exercise group. Compared to the control group, the diabetic group and the exercise after diabetic group showed significant weight loss. Also, compared to the exercise group, the diabetic group and the exercise after diabetic group showed significant weight loss. However, group ExD rats gained weight significantly more than the other two diabetic groups. Swimming exercise therefore showed a preventive effect on the weight loss of group ExD (Fig. 2).

Effects of diabetes and swimming training on electrophysiological parameters

The CMAP in the gastrocnemius muscle were also evaluated. CMAP were recorded on the 0th, 28th and 56th days in all 5 groups. Records of CMAP at control, exercise and diabetic rats at the beginning (0th day) and at the end (56th day) of experiment are shown in Figure 3. Respectively, the CMAP amplitude of group C was 6.82±0.37, 6.43±0.43, and 6.49±0.80 mV; that of group E was 6.47±0.94, 5.79±1.16, and 6.29±0.74 mV; that

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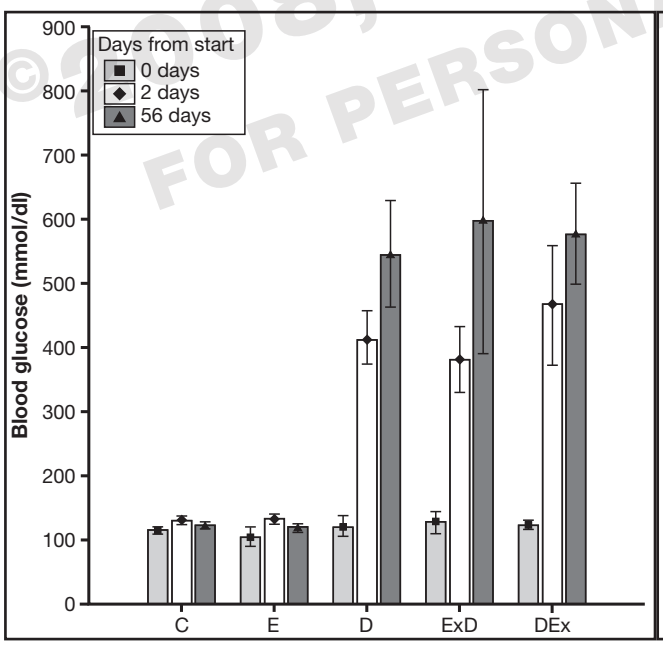


List of significant differences
Between groups for each time

2nd day		56th day	
Pairwise comparisons	***p***	**Pairwise comparisons**	***p***
D *vs* C	0.0001	D *vs* C	0.0001
D *vs* E	0.0001	D *vs* E	0.0001
ExD *vs* C	0.0001	ExD *vs* C	0.0001
ExD *vs* E	0.0001	ExD *vs* E	0.0001
DEx *vs* C	0.0001	DEx *vs* C	0.0001
DEx *vs* E	0.0001	DEx *vs* E	0.0001

Between times for each group

D

Pairwise comparisons	*p*
0th *vs* 2nd	0.0001
0th *vs* 56th	0.0001
2nd *vs* 56th	0.0004

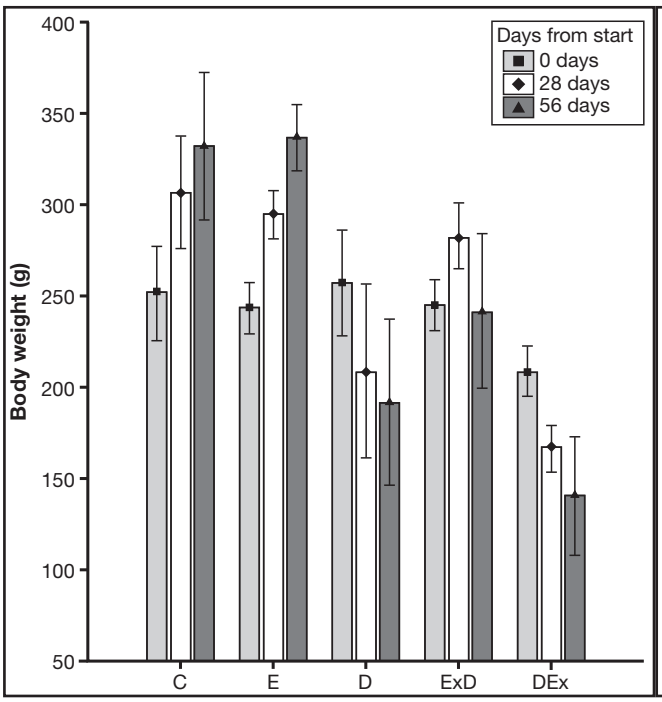
ExD

Pairwise comparisons	*p*
0th *vs* 2nd	0.0001
0th *vs* 56th	0.0001
2nd *vs* 56th	0.0001

DEx

Pairwise comparisons	*p*
0th *vs* 2nd	0.0001
0th *vs* 56th	0.0001

Fig. 1 - *Blood glucose level on the day of injection [streptozotocin (STZ)] and, 2 days and 56 days later. C: control group; E: exercise group; D: diabetic group; ExD: diabetic after exercise group; DEx: exercise after diabetic group. Statistical significances are shown to the right of the figure.*



List of significant differences

Between groups for each time

28nd day		56th day	
Pairwise comparisons	***p***	**Pairwise comparisons**	***p***
C *vs* D	0.0001	C *vs* D	0.0001
C *vs* DEx	0.0001	C *vs* DEx	0.0001
E *vs* D	0.0001	C *vs* ExD	0.0001
E *vs* DEx	0.0001	E *vs* D	0.0001
D *vs* ExD	0.0001	E *vs* DEx	0.0001
D *vs* DEx	0.0008	E *vs* ExD	0.0001
DEx *vs* ExD	0.0001	D *vs* ExD	0.0001
		D *vs* DEx	0.0021
		DEx *vs* ExD	0.0001

Between times for each group

C

Pairwise comparisons	***p***
0th *vs* 28th	0.0001
0th *vs* 56th	0.0001

E

Pairwise comparisons	***p***
0th *vs* 28th	0.0001
0th *vs* 56th	0.0001
28th *vs* 56th	0.0001

D

Pairwise comparisons	***p***
0th *vs* 28th	0.0002
0th *vs* 56th	0.0001

ExD

Pairwise comparisons	***p***
0th *vs* 28th	0.0008
28th *vs* 56th	0.0004

DEx

Pairwise comparisons	***p***
0th *vs* 28th	0.026
0th *vs* 56th	0.0006

Fig. 2 - *Body weight on the day of injection [streptozotocin (STZ)], 28 days and 56 days later. C: control group; E: exercise group; D: diabetic group; ExD: diabetic after exercise group; DEx: exercise after diabetic group. Statistical significances are shown to the right of the figure.*

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of group D was 5.96±1.58, 3.73±0.53, and 2.75±0.47 mV; that of group ExD was 6.58±0.57, 5.86±1.16, and 5.67±0.56 mV; and that of group DEx was 6.34±0.59, 2.77±0.53, and 3.59±0.79 mV. There was no significant difference in the amplitude values on the 0th day in all of the groups. As shown in Figure 3 and 4, compared to the control group, the amplitude of CMAP, which mainly reflects axonal dysfunction, decreased by 37% in group D and 56% in group DEx on the 28th day. Also, compared to the control group, the amplitude of CMAP decreased by 54% in group D and 43% in group DEx on the 56th day. However, exercise, which was applied before induction of diabetes, showed a preventive effect on the axonal dysfunction of group ExD (Fig. 3 and 4). Motor dysfunction defined by a significant increase in CMAP latency was also recorded. Respectively, the CMAP latency of group C was 0.98±0.06, 0.97±0.05, and 0.99±0.08 msec; that of group E was 0.98±0.06, 0.92±0.09, and 0.93±0.03 msec; that of group D was 0.89±0.08, 1.12±0.13, and 1.12±0.13 msec; that of group ExD was 0.98±0.07, 0.92±0.08, and 0.95±0.02 msec; and that of group DEx was 0.99±0.04, 0.93±0.05, and 0.94±0.09 msec. There was no significant difference in the CMAP latency values (conduction velocity values) on the 0th day in 5 groups. The results on CMAP latency measured 28 days after STZ injection showed that the observed increment in CMAP latency in the diabetic group (+25%) was prevented by 50% by exercise. When exercises were applied according to the training schedule, the CMAP latency increment in diabetic group on the 56th day was 13% when compared to non-diabetic controls, and exercise partially counteracted this increment (Fig. 5).

Effects of diabetes and swimming training on myelinated fibers morphometry

Measured from the sacrificed animal's sciatic nerve at the end (the 56th day) of the study, the myelin sheath thickness of group C was 3.16±0.09 μm, that of the group E was 3.50±0.09 μm, that of group D was 2.56±0.08 μm; that of group ExD was 3.17±0.09 μm, and that of group DEx was 2.94±0.18 μm. Average myelin sheath thicknesses of group D were significantly smaller when compared to the control group (Fig. 6). A significant increase in average myelin sheath thicknesses was found in groups ExD and DEx when compared to the group D values. We can therefore say that the exercise, which was applied before and after induction of diabetes, prevents myelin damage. Also, there was a significant difference in the average myelin sheath thicknesses between the control and exercise groups (3.16±0.09 μm and 3.50±0.09 μm, respectively) (Fig. 6).

DISCUSSION

The results of this study demonstrate the protective and the therapeutic effects of swimming exercise on peripheral diabetic neuropathy of STZ-induced diabetic rats. STZ as an antibiotic produced by Streptomyces achromogenes and anticancer agent has been widely used to induce diabetes in a variety of animals by affecting degeneration and necrosis of pancreatic β-cells (22). STZ

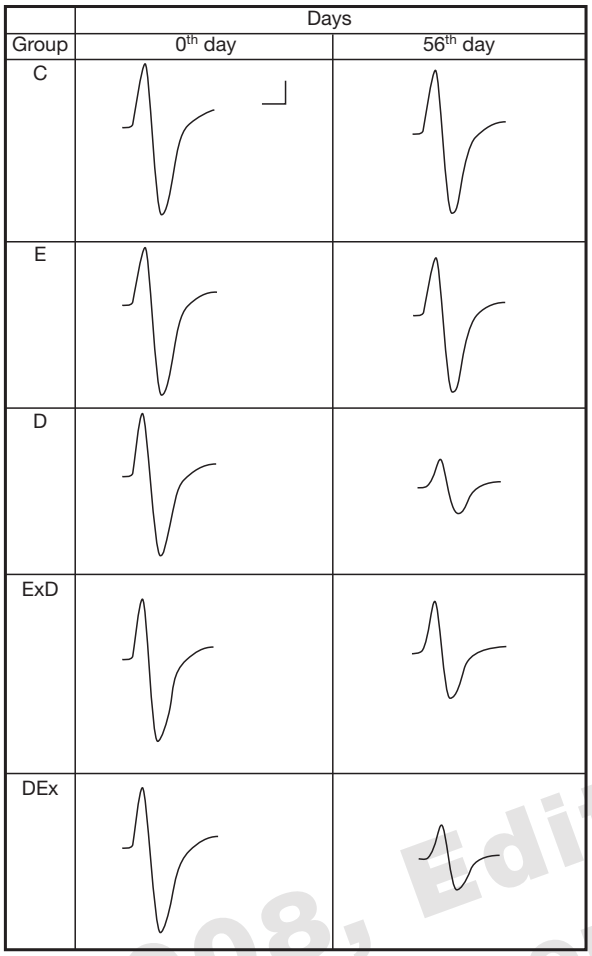

Fig. 3 - *Records of the compound muscle action potential (CMAP) in control, exercise, and diabetic rats at the beginning (0th day) and at the end (56th days) of experiment. Calibrations for all traces are shown in upper left; vertical bar =2.33 mV; horizontal bar =1.50 msec.*

causes Type 1 (insulin-dependent) diabetes mellitus (23). Different strategies have been proposed to inhibit diabetes-induced abnormalities. Neuropathy is the most severe and the least understood complication of diabetes (24). The treatment of diabetes peripheral neuropathy has traditionally focused on the control of hyperglycemia. The impact of an intensive glycemic control on diabetes peripheral neuropathy has been widely evaluated (25), but with controversial results.

Increased blood glucose and reduced nerve perfusion are important factors in the etiology of DPN. Also, the development of therapies to prevent the action of generation of free radicals may influence the progression of DPN. Daily moderate exercise can be beneficial to diabetes due to reducing free radical production and blood glucose, and to increasing peripheral blood flow. The treatment of exercise may affect CMAP amplitude, CMAP latency, and nerve conduction velocity (NCV) values are used as an index of neuropathy, thereby inducing adaptive changes in the neuromuscular system in response to exercise training. Exercise training has been known to be effective in Type 2 diabetes by increasing insulin sensitivity, but there is not enough knowledge as to how exercise acts in Type 1 experimental diabetes (26).

In the present study, it was found that the treatment of exercise inhibited the progression of diabetic neuropathy (Fig. 2-6). However, exercise treatment did not affect glucose levels in either ExD and DEx groups (Fig. 1), although the exact reason is unclear. In our study, the severity of diabetes, estimated as plasma glucose concentration, was not affected by the exercise training. In our study, the elevated blood glucose concentration at the beginning and the end of the experimental period clearly indicates the persistent hyperglycemia in the STZ-induced diabetic rats. Likewise, Wallberg-Henriksson et al. (27) demonstrated that physical training in insulin-dependent diabetics results in unchanged blood glucose control. Also, Franz indicated (28) that exercise may cause a further rise in blood glucose levels in persons with marked hyperglycemia and physical training improves glucose tolerance in individuals with non-insulin-dependent diabetes mellitus. However, our result is not consistent with another study result (26), which indicated a significant decrease by exercise training in the elevated serum glucose in STZ-induced diabetic rats with the elapse of the experiment.

Carrington et al. (29) reported that all diabetic rats showed decreased body weight compared to control rats. In our study, exercise affected body weight, which increased in non-diabetic rats (groups C and E), while it decreased in group D (Fig. 2). Exercise treatment protected group ExD from the weight loss caused by STZ-induced diabetes along with protecting against the diabetes. However, in group DEx decreases were not protected in body weights of D group. Lee et al. (30) reported that, compared to the control group, the hyperglycemia group showed significant weight loss but treadmill exercise had no effect on the weight of the hyperglycemic rats. It is therefore clear that our results, obtained by using swimming exercise treatment, are not in line with their data obtained by using treadmill exercise treatment.

In many studies, CMAP amplitude, CMAP latency, and nerve conduction velocity (NCV) values are used as an index of neuropathy in diabetic rats (8, 24, 31). Slowed nerve conduction velocity (therefore increased CMAP latency) develops within 2 weeks just after the onset of hyperglycemia in diabetic rats (32). Saini et al. conclude (33) that acute hyperglycemia attenuates motor NCV and nerve blood flow.

There have been reports on the effect of diabetes on NCV, CMAP amplitude and CMAP latency. While many researchers (8, 24, 29, 31, 34) have reported decreased CMAP amplitude and NCV, and increased CMAP latency after diabetes, compared to the control group, some investigations on animal models have shown increased CMAP amplitude and NCV, and decreased CMAP latency by drug therapy in diabetics (8, 24, 31, 34). Tesfaye et al. (16) have reported impaired peripheral nerve blood flow and NCV in diabetic neuropathy and they have also shown that exercise has induced conduction velocity increment. In our study, CMAP amplitude and CMAP la-

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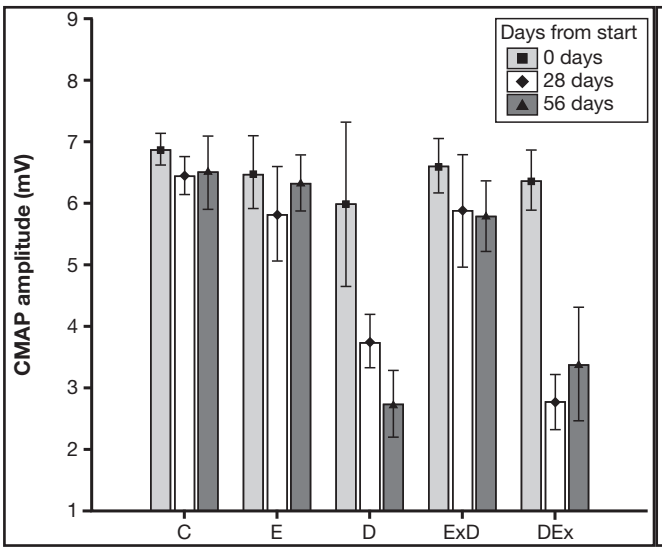

List of significant differences
Between groups for each time

28nd day		56th day	
Pairwise comparisons	***p***	**Pairwise comparisons**	***p***
C *vs* D	0.0001	C *vs* D	0.0001
C *vs* DEx	0.0001	C *vs* DEx	0.0001
E *vs* D	0.0004	E *vs* D	0.0001
E *vs* DEx	0.0002	E *vs* DEx	0.0002
D *vs* ExD	0.0007	D *vs* ExD	0.0001
DEx *vs* ExD	0.0002	DEx *vs* ExD	0.0052

Between times for each group

D

Pairwise comparisons	***p***
0th *vs* 28th	0.0131
0th *vs* 56th	0.0001

DEx

Pairwise comparisons	***p***
0th *vs* 28th	0.0026
0th *vs* 56th	0.0251

Fig. 4 - *Exercise applied before induction of diabetes prevents the decrease in amplitude of compound muscle action potential (CMAP), which mainly reflects axonal dysfunction, in diabetic rats. C: control group; E: exercise group; D: diabetic group; ExD: diabetic after exercise group; DEx: exercise after diabetic group. Statistical significances are shown to the right of the figure.*

tency values were used as an index of neuropathy in diabetic rats. The measurement of CMAP amplitude is important because it informs us about the summated activity of the synchronously activated muscle fibers innervated by the axons and motor units represented in that muscle. Thus, amplitude provides an estimate of the amount of functioning nerve and muscle. CMAP latency is the time required for the action potentials in the fastest conducting fibers to reach the nerve terminals and activate the muscle fibers.

Our findings show that CMAP amplitude values in group D were less than in the C and E groups (Fig. 3 and 4), suggesting that sciatic nerves with diabetic neuropathy have axonal degeneration. In this study, it was demonstrated that moderate exercise application markedly increased the amplitude of CMAP in the ExD group only (Fig. 3 and 4). Also, our findings show that CMAP latency values in the D group were higher than in the C and E groups (Fig. 5), suggesting that rats with diabetic neuropathy have a motor dysfunction. The CMAP latency increment in dia-

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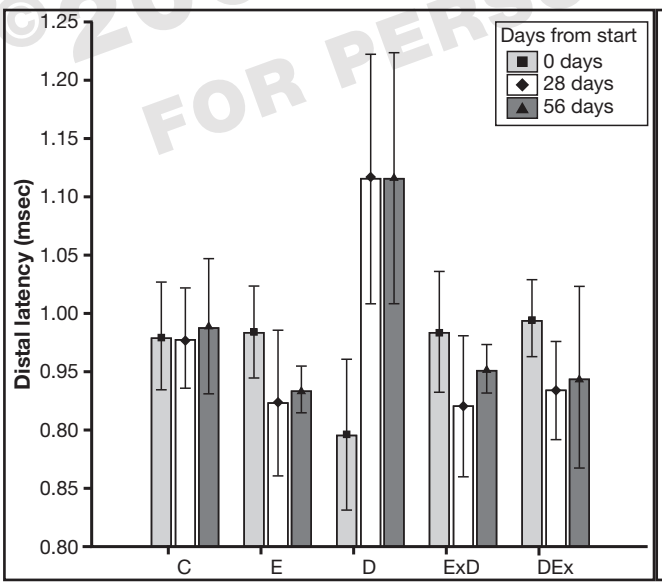

List of significant differences
Between groups for each time

28nd day		56th day	
Pairwise comparisons	***p***	**Pairwise comparisons**	***p***
C *vs* D	0.007	C *vs* D	0.044
D *vs* E	0.0001	D *vs* E	0.0001
D *vs* ExD	0.0002	D *vs* ExD	0.0001
D *vs* DEx	0.0002	D *vs* DEx	0.0004

Between times for each group

D

Pairwise comparisons	***p***
0th *vs* 28th	0.0001
0th *vs* 56th	0.0001

Fig. 5 - *Exercise restores the increment in compound muscle action potential (CMAP) latency in diabetic rats. C: control group; E: exercise group; D: diabetic group; ExD: diabetic after exercise group; DEx: exercise after diabetic group. Statistical significances are shown to the right of the figure.*

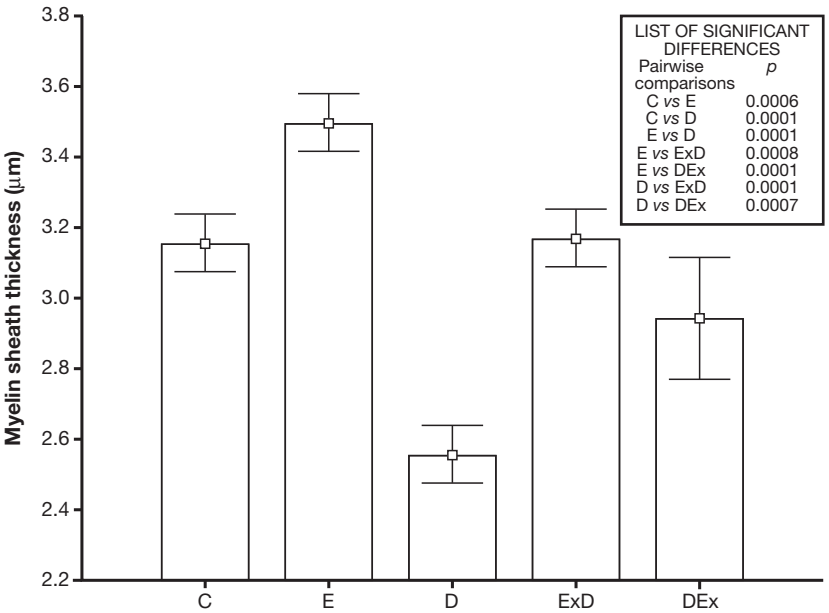

Fig. 6 - *Exercise applied before and after induction of diabetes prevents myelin damage. C: control group; E: exercise group; D: diabetic group; ExD: diabetic after exercise group; DEx: exercise after diabetic group. Statistical significances are shown to the right of the figure.*

betic rats was significantly prevented by exercise treatment in both ExD and DEx groups (Fig. 5). The CMAP latency increment, in other words slowing of nerve conduction, has been documented early in the course of diabetes in experimental rats (hyperglycemic neuropathy) (34). A decreased sodium concentration gradient across the axonal membrane has been linked to slowing nerve conduction (35). Several exercise-induced vascular and metabolic changes could be invoked to explain the effects of training on DPN development. Human and experimental studies suggest that short-term exercise stimulates endothelium-dependent vasodilatation (14) and therefore, increases endoneurial blood flow. It is known that exercise training exposes the vessels to repeated episodes of hyperemia (14). Some investigators have proposed that diabetic neuropathy causes a defect in Na^+-K^+-ATPase activity and an alteration of signal transduction pathways in the nerve (7). Therefore, in our study, exercise-induced nerve function changes could also be related to an improvement of Na^+-K^+-ATPase activity. In fact, training has been reported to increase the concentration of Na^+-K^+-ATPase in rat muscle cells (36).

The beneficial effect of exercise on diabetic neuropathy was also confirmed by morphological analyses at the end of experiment. Morphometrical analysis of the swimming exercise trained nerves verified the electrophysiological results in our study. Eight weeks after the STZ injection average myelin sheaths of group D were significantly thinner compared to control values (Fig. 5), as previously reported (24, 37, 38). On the other hand, morphometric analysis of exercise-trained diabetic groups for 8 weeks shows significantly thicker myelin sheaths when compared to untrained diabetic rats (Fig. 5). Average myelin sheath thicknesses did not differ significantly when the two exercise treatment groups (ExD and DEx) were compared. In this study, the group ExD was used to demonstrate the protective effect and the group DEx was used to demonstrate the therapeutic effect of swimming exercise on DPN of STZ-induced diabetic rats. Indeed, it was seen that only the exercise before the induction of diabetes (ExD) had positive effects on the prevention of the decrease in body weight and the therapy of the axonal damage induced by diabetes (Fig. 2, 3, and 4). But both the exercise before induction of diabetes (ExD) and the exercise after the induction of diabetes (DxE) had positive effects on the therapy of myelin damage as shown electrophysiologically (Fig. 5) and morphologically (Fig. 6).

The results of this study demonstrate an amelioration effect of swimming exercise in a rat model of DPN. In this model, exercise partially reversed diabetes-induced loss in nerve functions (CMAP amplitude and CMAP latency) and body weight, and impairment in myelin sheath thickness. Exercise was effective as a protective and therapeutic method where it was applied for 4 weeks before the induction of diabetes (ExD), and also in the other method where it was applied immediately, after the induction of diabetes (DEx).

Since DPN develops motor and sensory neuropathy, DPN is a damaging factor in the life quality of diabetic patients (4) and a leading cause of non-traumatic foot amputation (3). Our findings show that sub-maximal aerobic exercise training, such as swimming, can positively affect and modify motor neuromuscular parameters in diabetic patients; hence it can increase the life quality of diabetic patients. In conclusion, these results indicate that swimming exercise has a beneficial effect on peripheral neuropathy in STZ-induced diabetic rats, irrespective of blood glucose levels.

REFERENCES

1. Sugimura K, Windebank AJ, Natarajan V, Lambert EH, Schmid HH, Dyck PJ. Interstitial hyperosmolarity may cause axis cylinder shrinkage in streptozotocin-diabetic nerve. J Neuropathol Exp Neurol 1980, 39: 710-21.

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2. Yasuda H, Terada M, Maeda K, et al. Diabetic neuropathy and nerve regeneration. Prog Neurobiol 2003, 69: 229-85.
3. Boulton AJ. Lowering the risk of neuropathy, foot ulcers and amputations. Diabet Med 1998, 15 (Suppl 4): S57-9.
4. Poncelet AN. Diabetic polyneuropathy. Risk factors, patterns of presentation, diagnosis, and treatment. Geriatrics 2003, 58: 16-8.
5. Cameron NE, Cotter MA. The relationship of vascular changes to metabolic factors in diabetes mellitus and their role in the development of peripheral nerve complications. Diabetes Metab Rev 1994, 10: 189-224.
6. Ward JD. Biochemical and vascular factors in the pathogenesis of diabetic neuropathy. Clin Invest Med 1995, 18: 267-74.
7. Kim J, Kyriazi H, Greene DA. Normalization of Na+-K+ ATPase activity in isolated membrane fraction from sciatic nerves of streptozotocin-induced diabetic rats by dietary myoinositol supplementation in vivo or protein kinase C agonists in vitro. Diabetes 1991, 40: 558-67.
8. Bianchi R, Buyukakilli B, Brines M, et al. Erythropoietin both protects from and reverses experimental diabetic neuropathy. Proc Natl Acad Sci USA 2004, 101: 823-8.
9. Naziroglu M, Simsek M, Kutlu M. Moderate exercise with a dietary vitamin C and E combination protects against streptozotocin-induced oxidative damage to the blood and improves fetal outcomes in pregnant rats. Clin Chem Lab Med 2004, 42: 511-7.
10. Lee DM, Hoffman WH, Carl GF, Khichi M, Cornwell PE. Lipid peroxidation and antioxidant vitamins prior to, during, and after correction of diabetic ketoacidosis. J Diabetes Complications 2002, 16: 294-300.
11. Atalay M, Laaksonen DE. Diabetes, oxidative stress and physical exercise. J Sports Sci Med 2002, 1: 1-14.
12. Dahl-Jørgensen K, Brinchmann-Hansen O, Hanssen KF, et al. Effect of near normoglycaemia for two years on progression of early diabetic retinopathy, nephropathy, and neuropathy: The Oslo study. Br Med J (Clin Res Ed) 1986, 293: 1195-9.
13. Richardson JK, Sandman D, Vela S. A focused exercise regimen improves clinical measures of balance in patients with peripheral neuropathy. Arch Phys Med Rehabil 2001, 82: 205-9.
14. Balducci S, Iacobellis G, Parisi L, et al. Exercise training can modify the natural history of diabetic peripheral neuropathy. J Diabetes Complications 2006, 20: 216-23.
15. Thomas PK, Tomlinson DR. Diabetic and hypoglycemic neuropathy. In: Dyck PJ, Thomas PK, Griffin JW, Low PA, Poduslo JF eds. Peripheral Neuropathy (vol 3). Philadelphia: WB Saunders. 1993, 1219-50.
16. Tesfaye S, Harris ND, Wilson RM, Ward JD. Exercise-induced conduction velocity increment: a marker of impaired peripheral nerve blood flow in diabetic neuropathy. Diabetologia 1992, 35: 155-9.
17. Harri M, Kuusela P. Is swimming exercise or cold exposure for rats? Acta Physiol Scand 1986, 126: 189-97.
18. Gobatto CA, de Mello MA, Sibuya CY, de Azevedo JR, dos Santos LA, Kokubun E. Maximal lactate steady state in rats submitted to swimming exercise. Comp Biochem Physiol A Mol Integr Physiol 2001, 130: 21-7.
19. de Oliveira CA, Luciano E, de Mello MA. The role of exercise on long-term effects of alloxan administered in neonatal rats. Exp Physiol 2004, 90: 79-86.
20. Matsuo T, Kang HS, Suzuki H, Suzuki M. Voluntary resistance exercise improves blood hemoglobin concentration in severely iron-deficient rats. J Nutr Sci Vitaminol (Tokyo) 2002, 48: 161-4.
21. Aminoff MJ. Nerve conduction studies: Basic principles and pathologic correlations. In Aminoff MJ ed. Electromyography in Clinical Practice. London: Churchill Livingstone 1998, 113-45.
22. Rakieten N, Rakieten ML, Nadkarni MV. Studies on the diabetogenic action of streptozotocin (NSC-37917). Cancer Chemother Rep 1963, 29: 91-8.
23. Changrani NR, Chonkar A, Adeghate E, Singh J. Effects of streptozotocin-induced type 1 diabetes mellitus on total protein concentrations and cation contents in the isolated pancreas, parotid, submandibular, and lacrimal glands of rats. Ann N Y Acad Sci 2006, 1084: 503-19.
24. Andriambeloson E, Baillet C, Vitte PA, Garotta G, Dreano M, Callizot N. Interleukin-6 attenuates the development of experimental diabetes-related neuropathy. Neuropathology 2006, 26: 32-42.
25. Vinik AI. Diabetic neuropathy: pathogenesis and therapy. Am J Med 1999, 107 (2B): 17S-26S.
26. Coskun O, Ocakcı A, Bayraktaroglu T, Kanter M. Exercise training prevents and protects streptozotocin-induced oxidative stress and beta-cell damage in rat pancreas. Tohoku J Exp Med 2004, 203: 145-54.
27. Wallberg-Henriksson H, Gunnarsson R, Henriksson J, et al. Increased peripheral insulin sensitivity and muscle mitochondrial enzymes but unchanged blood glucose control in type I diabetes after physical training. Diabetes 1982, 31: 1044-50.
28. Franz MJ. Exercise and the management of diabetes mellitus. J Am Diet Assoc 1987, 87: 872-80.
29. Carrington AL, Calcutt NA, Ettlinger CB, Gustafsson T, Tomlinson DR. Effects of treatment with myo-inositol or its 1,2,6-trisphosphate (PP56) on nerve conduction in streptozotocin-diabetes. Eur J Pharmacol 1993: 237: 257-63.
30. Lee HH, Shin MS, Kim YS, et al. Early treadmill exercise decreases intrastriatal hemorrhage-induced neuronal cell death and increases cell proliferation in the dentate gyrus of streptozotocin-induced hyperglycemic rats. J Diabetes Complications 2005, 19: 339-46.
31. Kihara M, Mitsui MK, Mitsui Y, et al. Altered vasoreactivity to angiotensin II in experimental diabetic neuropathy: role of nitric oxide. Muscle Nerve 1999, 22: 920-5.
32. Tomlinson DR, Holmes PR, Mayer JH. Reversal, by treatment with an aldose reductase inhibitor, of impaired axonal transport and motor nerve conduction velocity in experimental diabetes mellitus. Neurosci Lett 1982, 31: 189-93.
33. Saini AK, Arun KH, Kaul CL, Sharma SS. Acute hyperglycemia attenuates nerve conduction velocity and nerve blood flow in male Sprague-Dawley rats: reversal by adenosine. Pharmacol Res 2004, 50: 593-9.
34. Yang O, Kaji R, Takagi T, et al. Abnormal axonal inward rectifier in streptozocin-induced experimental diabetic neuropathy. Brain 2001, 124: 1149-55.
35. Greene DA, Feldman EL, Stevens MJ, Sima AAF, Albers JW, Pfeifer MA. Diabetic neuropathy. In: Porte D, Sherwin R eds. Ellenberg & Rifkins's diabetes mellitus. Stamford (CO): Appleton & Lange. 1997, 1009-76.
36. Kjeldsen K, Richter EA, Galbo H, Lortie G, Clausen T. Training increases the concentration of [3H]ouabain-binding sites in rat skeletal muscle. Biochim Biophys Acta 1986, 860: 708-12.
37. Bestetti G, Zemp C, Probst D, Rossi GL. Neuropathy and myopathy in the diaphragm of rats after 12 months of streptozotocin-induced diabetes mellitus. A light-, electron-microscopic, and morphometric study. Acta Neuropathol 1981, 55: 11-20.
38. Birrell AM, Heffernan SJ, Ansselin AD, et al. Functional and structural abnormalities in the nerves of type I diabetic baboons: aminoguanidine treatment does not improve nerve function. Diabetologia 2000, 43: 110-6.

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