SINGLE-FIBER CONDUCTION VELOCITY TEST ALLOWS EARLIER DETECTION OF ABNORMALITIES IN DIABETES

LUCA PADUA, MD, PhD,[1,2] ERIK STÅLBERG, MD, PhD,[3] PIETRO CALIANDRO, MD, PhD,[1,2] GIOVANNA MUSCOGIURI, MD,[4] COSTANZA PAZZAGLIA, MD,[1,2] GIAN PIO SORICE, MD,[4] GIUSEPPE GRANATA, MD,[1,2] ENRICA SALOMONE, MD,[4] ALFREDO PONTECORVI, MD,[4] and ANDREA GIACCARI, MD, PhD[4]

[1] Institute of Neurology, Università Cattolica del Sacro Cuore, Largo F. Vito 1, Rome 00168, Italy
[2] Don Carlo Gnocchi Onlus Foundation, IRCCS, Milan, Italy
[3] Department of Clinical Neurophysiology, Uppsala University Hospital, Uppsala, Sweden
[4] Division of Endocrinology, Universiì Cattolica del Sacro Cuore, Rome, Italy

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ABSTRACT: *Introduction:* The purpose of this study was to determine whether single-fiber conduction velocity (SF-CV) of a small number of axons increases sensitivity for identification of motor nerve conduction alterations in patients with diabetes. *Methods:* Twenty-one consecutive diabetic patients in good metabolic control were studied. For each patient, conventional (C-CV) and SF-CV results were correlated with the presence of neuropathic symptoms. *Results:* Nine of 21 patients reported symptoms suggestive of mild nerve impairment. Three patients had abnormal sural nerve CV, 1 of whom also had abnormal motor nerve conduction. Eighteen patients had normal findings on conventional tests, 3 of whom had slowing of SF-CV. *Conclusions:* SF-CV is able to detect mild myelin damage with higher sensitivity than conventional tests. The use of SF-CV may be a helpful tool in the early identification of diabetic polyneuropathy, and it may be useful for tailoring an approach to diabetic polyneuropathy.

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Diabetic polyneuropathy (DP) is the most common neurological complication of diabetes.[1] DP is a progressive process characterized by a long asymptomatic stage that is often difficult to identify and manage. Many of the DP consequences (pain, imbalance, foot deformity, risk for infection, ulcerations, and amputations) can be prevented by appropriate clinical management, including early diagnosis, intensification of glycemic control, and checking for foot complications.[2] Therefore, identification of DP at its earliest stages is a major challenge in the clinical care of diabetes.

Nerve conduction velocity (NCV) is the most commonly used and reliable method to assess myelin nerve function. Usually, NCV is measured using a surface electrode (conventional conduction velocity, C-CV). C-CV does not optimally assess the overall conduction properties of a nerve, because it reflects mainly the fastest conducting subset of the alpha motor axon population.[3] However, in its early stages, polyneuropathy does not affect nerves uniformly.[4] This reduces the sensitivity of C-CV in the evaluation of polyneuropathies that involve mainly slow-conducting fibers or polyneuropathy with only partial involvement of the nerve fibers.

Single-fiber CV (SF-CV) evaluation is a technique based on the use of a single-fiber electromyography (SFEMG) electrode. The procedure, which entails recordings from different sites in the muscle, allows study of the CV of a small sample of axons. SF-CV may be useful in detecting early, mild, or partial myelin damage, because it can detect abnormalities in a few axons and may show nerve conduction slowing when conventional tests are normal.[5]

The aim of this study was to determine whether SF-CV could improve detection of mild motor nerve function abnormalities in the early stages of DP.

METHODS

Patient Sample. Twenty-one consecutive Caucasian patients affected by type 2 diabetes, who had been in good metabolic control for at least 1 year and did not have glutamic acid decarboxylase autoantibody, were enrolled in the endocrinology unit of the Hospital "A. Gemelli," Catholic University of Rome. Informed consent was obtained from all participants. The study was approved by local ethics committee. Glycated hemoglobin (HbA_{1c}; 2 days before the neurophysiological tests) and fasting plasma glucose (FPG; the same morning) were measured in all patients. Only participants with HbA_{1c} <8% and fasting glucose <140.0 mg/dl (7.8 mmol/L) were included. Other exclusion criteria were: (1) neuropathy with etiology different from diabetes; (2) peripheral arterial disease (documented by lower limb echo color Doppler) with arterial stenosis >30%; and (3) other concurrent neurological diseases. Individuals who had previous foot ulcer, toe amputation, or pre-ulcers (pre-ulcer is defined as intact skin but a high-risk foot because of dense callus with or without preulcerative macerating changes)[6] were excluded. Anthropometric parameters, lipids, creatinine, and urinary albumin-to-creatinine ratio were also measured in all subjects. Before neurological examination and neurophysiological tests, all patients completed a

Abbreviations: C-CV, conventional conduction velocity; CMAP, conventional compound muscle action potential; CV, conduction velocity; DP, diabetic polyneuropathy; FPG, fasting plasma glucose; HbA_{1c}, glycated hemoglobin; NCV, nerve conduction velocity; SF-CV, single-fiber conduction velocity; SFEMG, single-fiber electromyography; SW-DOM, Semmes–Weinstein dominant; SW-NDOM, Semmes–Weinstein non-dominant

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Correspondence to: L. Padua; e-mail: lpadua@rm.unicatt.it

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standardized questionnaire based on the experience of foot pain, numbness, and tingling.

The patients then underwent a foot examination for deformities, calluses, pre-ulcers, ulcers, toenail integrity, and amputation. Afterwards, all patients underwent examination of distal strength and muscle bulk, deep tendon reflexes, and lower extremity sensory perception. Clinical polyneuropathy was defined as the loss of protective sensation, assessed through application of a 10-g Semmes–Weinstein monofilament wire. It was applied to the dorsum of the great toe of both the dominant (SW-DOM) and non-dominant (SW-NDOM) side midway between the nail fold and the interphalangeal joint. The filament was applied perpendicularly and briefly (for <1 second) with even pressure. The patient was tested with eyes closed and was asked to respond "yes" if the pressure was felt. This procedure was repeated 10 times. Eight affirmative responses out of 10 were considered normal, with 0–7 responses considered abnormal. Peripheral pulses (dorsalis pedis and posterior tibial) were assessed.

Conventional Neurophysiological Tests. The non-dominant fibular nerve (in 1 case tibial nerve) was studied through motor nerve conduction studies. The fibular nerve was studied with recording from the extensor digitorum brevis and stimulation at the ankle and fibular head (the tibial nerve was studied with recording from the abductor hallucis and stimulation at the medial malleolus and popliteal fossa). Conventional nerve conduction studies were performed using surface recording electrodes.

Sensory nerve conduction studies of the radial, median, and ulnar nerves were performed on the non-dominant side and on the sural nerves bilaterally. The following segments of upper extremity nerves were studied: from digit I to wrist for the radial nerve; from digits I and III to wrist for the median nerve; and from digit V to wrist for the ulnar nerve. The sural nerves were studied antidromically from the calf to the ankle. To minimize the effects of temperature on nerve conduction velocity the limb was warmed with an infrared lamp to maintain a skin temperature over 33°C.

Determination of abnormality was made by comparing the results obtained from individual patients with the established reference values of our laboratory: for the radial nerve from digit I to wrist, amplitude was considered pathological when it was <8 μV and velocity was <41 m/s; for the median nerve from digit I to wrist, amplitude was pathological when it was <9 μV and velocity was <42 m/s, and from digit III to wrist the amplitude was pathological when it was <11 μV and velocity was <44 m/s; for the ulnar nerve, amplitude was abnormal when it was <8 μV and velocity was <42 m/s; and for the sural nerve, amplitude was abnormal when it was <7 μV and velocity was <41 m/s.

Single-Fiber Conduction Velocity. The single-fiber conduction velocity (SF-CV) test was described previously.[5] SF-CV differs from C-CV evaluation in the use of a recording SFEMG electrode instead of surface electrodes and in the procedures used to assess the reliability of the recording. It entails insertion of a SFEMG needle electrode into the muscle and application of a supramaximal stimulus of the nerve at two different sites (distal and proximal), as in C-CV evaluation. The supramaximal stimulation was first determined for both sites with surface-recorded conventional compound muscle action potentials (CMAPs). During execution of the SF-CV, we used a stimulus of 15% greater than the one that produced a maximal CMAP. Filters were set at 500 Hz (high-pass) and 10,000 Hz (low-pass). The following procedure was used to verify that the SFEMG responses were actually generated by activation of the same axon at distal and proximal stimulation. Using an SFEMG needle electrode, we recorded the potential obtained in response to the supramaximal stimulation of the nerve at the distal site. The criteria used for an acceptable recording were: sharp, spiky, and fast rise time. We verified that the needle's position had not changed after the first stimulus by applying a second supramaximal stimulus, which should give a potential of the same shape, amplitude, and delay (Fig. 1). SF-CV was calculated at the onset, or sometimes at a well-identifiable peak of the response. For each nerve, we acquired 10 SF-CVs, moving the SFEMG electrode randomly each time.

In our previous study we theoretically[5,7–12] assessed 36 m/s as the low limit of normal SF-CV. In a subsequent series of 23 healthy subjects (12 females, mean age 43 years, range 18–76 years), this value was confirmed (mean value less 2 SD: 49 $\pm$ 6.5 m/s), and in no instance was a value of <36 m/s observed. The fibular nerve was studied in the leg with recording from the extensor digitorum brevis and stimulation at the ankle and fibular head.[5,12]

RESULTS

Patient Sample. We studied 21 consecutive diabetic patients (see clinical features in Fig. 2). The mean FPG was 120.0 $\pm$ 20.3 mg/dl (range 74–139 mg/dl). Three patients did not take any hypoglycemic drugs, 7 received oral hypoglycemic drugs, 9 received insulin therapy and hypoglycemic drugs, and 2 received insulin therapy only.

Figure 2 summarizes clinical and neurophysiological results. No patient had a clinical picture unequivocally suggestive of polyneuropathy, but

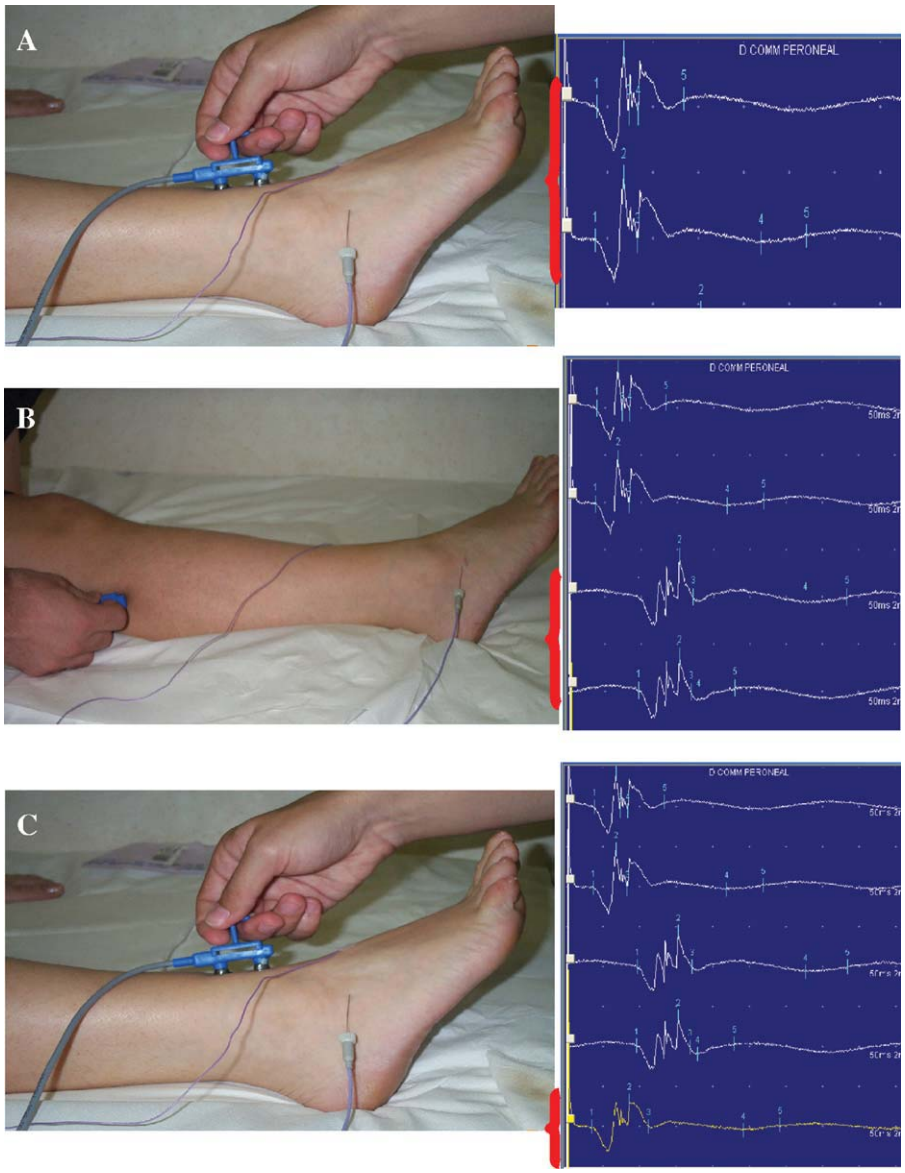

FIGURE 1. Procedure used to verify that the SFEMG responses were generated by activation of the same axons at distal and proximal stimulation sites. **(A)** Distal stimulation (ankle). We recorded two potentials obtained in response to the supramaximal stimulation of the nerve at the distal site. **(B)** Proximal stimulation (fibular head). We recorded two potentials obtained in response to the supramaximal stimulation of the nerve at the proximal site. **(C)** Another distal stimulation (ankle). To verify that the needle's position had not changed, we applied a supramaximal stimulus at the distal site, which gave a potential of the same shape, amplitude, and delay as the first one. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

from the standardized form 9 patients reported transient, asymmetric, neuropathic symptoms (paresthesias, dysethesias, burning feet) in the legs, suggestive of nerve impairment.

C-CV Studies. In 3 patients, C-CV studies showed abnormal findings: all had abnormal sural nerve CV, and 1 also showed slowing on fibular motor nerve conduction studies. None had abnormal neurophysiological findings in the arms or complained of neuropathic symptoms.

SF-CV Studies. Among the 18 patients who had normal C-CV tests, SF-CV showed slowing of nerve CV in 3 patients. Moreover, SF-CV was also abnormal in all 3 patients with abnormal findings on C-CV tests. Globally, 6 patients had abnormal SF-CV. Interestingly, symptoms reported by the patients were associated with abnormal SF-CV and not with conventional conduction tests (see Fig. 2).

DISCUSSION

DP is characterized by a long subclinical and "subneurophysiological" stage, whose identification may be very difficult. In a previous study we used a simulation model to evaluate the distribution of motor fiber CV in motor nerves with patchy and segmental demyelination, and we observed that

patient	age	gender	BMI	HB 1Ac	neuropathic symptoms	abnormal MNCV	abnormal SFCV	number of abnormal SFEMG sites out of 10 sites	lowest SFCV	Nerve evaluated with SFCV	abnormal sural SNCV	abnormal sural SAP	year of known diagnosis
1	61	F	28	7,8	1	0	0	0	37	tibial nerve	0	0	8
2	37	F	26,8	6,9	1	0	0	0	39	peroneal nerve	0	0	8
3	62	F	21	7,9	0	0	0	0	39	peroneal nerve	0	0	12
4	66	F	27	6,9	0	0	1	1	34	peroneal nerve	0	1	3
5	73	M	23,8	6,1	0	0	0	0	43	peroneal nerve	0	0	2
6	71	F	27	7,2	0	0	0	0	43	peroneal nerve	0	0	23
7	56	F	31	6,0	1	0	0	0	37	peroneal nerve	0	0	5
8	33	M	32	7,6	1	0	1	3	29	peroneal nerve	0	0	5
9	71	M	23	6, 1	1	0	0	0	41	peroneal nerve	0	0	3
10	48	F	30	6,4	0	0	0	0	38	peroneal nerve	0	0	4
11	63	F	34,7	7,9	1	0	1	3	27	peroneal nerve	0	0	16
12	64	M	31,5	7,0	0	0	0	0	39	peroneal nerve	0	0	5
13	52	F	29,8	7,9	0	1	1	4	26	peroneal nerve	1	1	12
14	59	M	26,6	6,5	0	0	0	0	40	peroneal nerve	0	0	4
15	51	F	28,7	5,9	1	0	0	0	41	peroneal nerve	0	0	2
16	62	F	32,4	6,8	0	0	0	0	41	peroneal nerve	0	0	2
17	71	F	39,7	7,7	0	1	1	1	32	peroneal nerve	0	1	12
18	62	F	30,5	5,5	0	0	0	0	39	peroneal nerve	0	0	1
19	55	F	28	6,0	0	0	0	0	40	peroneal nerve	0	0	2
20	42	F	29	7,7	1	0	0	0	42	peroneal nerve	0	0	2
21	68	M	25	6,5	1	0	1	2	34	peroneal nerve	0	0	22

FIGURE 2. Clinical picture and neurophysiological (standard conduction studies and SFCV) results from 21 diabetic patients. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

C-CV was pathological only when severe myelin damage involved a large number of axons.[13] This is because conventional nerve conduction tests are heavily dependent on the fastest conducting axons. In that study, the simulation demonstrated that the CV of individual axons may have greater sensitivity in detecting myelin damage. Recently, SFEMG electrodes have been employed to measure the SF-CV in different groups of individual axons in patients with neuropathy. Comparison between SF-CV and C-CV showed that the SFEMG electrode increases neurophysiological sensitivity in cases of demyelinating nerve impairment and may therefore be useful in detecting early, mild, or partial myelin damage.[5]

The sample of diabetic patients in this study was characterized by a lower risk of DP because they had good metabolic control (although it is known that polyneuropathy may also occur at the time of diagnosis). We chose a diabetic sample with these features in order to evaluate the neurophysiological sensitivity in early/mild nerve impairment. The clinical and conventional pattern showed overall mild impairment. Only a few cases had neuropathic symptoms, which were mild, and in no case was there an unequivocal pattern of polyneuropathy. Similarly, conventional conduction tests showed abnormal results in very few cases. Conversely, we confirmed the ability of SF-CV to detect slowing of nerve conduction velocity, which occurred in only a few axons when partial and patchy damage of the motor nerve was seen. In this pattern, the conventional motor conduction test, being based on an overall detection—through surface electrodes—of the maximum CV, is not able to show abnormal results.

Interestingly, in this study, SF-CV was related to the mild neuropathic symptoms reported by the patients more than conventional conduction studies. It is common in clinical practice to observe diabetic patients with neuropathic symptoms with normal results at conventional neurophysiological studies and vice versa. Some of these could be electrophysiologically diagnosed through SF-CV.

DP, at least in the early stages, is usually considered a predominantly sensory axonal polyneuropathy. But this has been partially refuted by some studies.[14–17] We can hypothesize that DP is considered a predominant sensory axonal polyneuropathy because of the low sensitivity of conventional neurophysiological tests in assessing motor fiber involvement. In this debate, SF-CV could provide more information.

Hermann et al.[18] provided strong evidence in support of a significant component of amplitude-independent motor conduction slowing in intermediate nerve segments in DP. Their results support the view that DP cannot be explained by a distal-length-dependent, dying-back axonopathy alone. Hermann and colleagues underscored the importance of multifocal axonal degeneration and secondary segmental demyelination in proximal nerve trunks in diabetes. The hypothesis was that motor CV slowing would not produce any symptoms or deficits, per se. CV assessment of a broad range of sensory axons, not just the fastest represented on C-CV, may be the more sensitive

technique. Unfortunately, the approach of selective recording is not presently applicable to sensory fibers.

Another study utilized SFEMG electrodes in diabetic patients. Shields and colleagues[19] observed that SFEMG is a sensitive indicator of axonal degeneration in diabetes, because it allows detection of increased jitter (a sign of neuromuscular transmission involvement) and fiber density (a sensitive sign of remodeling of motor unit potentials). They performed SFEMG on diabetic subjects both with and without clinical or electromyographic evidence of polyneuropathy. They found abnormalities in both groups. Shields and colleagues unequivocally demonstrated motor fiber involvement in diabetic patients.

The main problem regarding the role of SF-CV in diabetic patients is its use. Some diabetologists believe that early recognition is crucial for reducing the risk of foot injury and morbidity. For this reason, an electrophysiological evaluation that can identify early stages of DP may be recommended in diabetic patients when HbA_{1c} is or has been over the target values even in absence of clinical evidence of nerve injury.[20] On the other hand, some diabetologists believe that even conventional neurophysiological evaluation has a negative advantage/disadvantage ratio (being a partially invasive and uncomfortable test and providing results without evidence of usefulness in treatment), and the clinical picture suggestive of polyneuropathy is enough to make diagnosis. In this setting, a more invasive neurophysiological test has to find its role; that is: (1) it could be of interest only in clinical trials as a more sensitive diagnostic and outcome measure; or (2) being more sensitive, it could show that an early diagnosis could be useful for decisions about treatment.

Note that increased diagnostic sensitivity (identification of additional diabetic patients with peripheral neuropathy beyond standard electrodiagnosis) was small, probably due to sampling of patients at low risk of having DP and in which we do not expect DP. Further studies on a sample of patients with higher risk for DP could be useful.

Moreover, only further studies that include follow-up may provide answers to this question that is so far only speculative. At the moment we can say that earlier evidence of nerve impairment is available in diabetic patients.

REFERENCES

1. Sosenko JM. The prevalence of diabetic neuropathy according to ethnicity. Curr Diab Rep 2009;9:435–439.
2. Writing Team for the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group. Effect of intensive therapy on the microvascular complications of type 1 diabetes mellitus. JAMA 2002;287:2563–2569.
3. Arasaki K, Iijima M, Nakanishi T. Normal maximal and minimal motor nerve conduction velocities in adults determined by a collision method. Muscle Nerve 1991;14:647–653.
4. McLeod JG, Prineas JW, Walsh JC. The relationship of conduction velocity to pathology in peripheral nerves. In: Desmedt JE, editor. New developments in electromyography and clinical neurophysiology. Vol. 2. Basel: Karger; 1973. p 249–258.
5. Padua L, Caliandro P, Stålberg E. A novel approach to the measurement of motor conduction velocity using a single fibre EMG electrode. Clin Neurophysiol 2007;118:1985–1990.
6. de Sonnaville JJ, Colly LP, Wijkel D, Heine RJ. The prevalence and determinants of foot ulceration in type II diabetic patients in a primary health care setting. Diabetes Res Clin Pract 1997;35:149–156.
7. Thomas PK, Sears TA, Gilliatt RW. The range of conduction velocity in normal motor nerve fibers to the small muscles of the hand and foot. J Neurol Neurosurg Psychiatry 1959;22:175–181.
8. Cummins KL, Dorfman LJ, Perkel DH. Nerve fiber conduction-velocity distributions. II. Estimation based on two compound action potentials. Electroencephalogr Clin Neurophysiol 1979;46:647–658.
9. Dorfman LJ. Computer analysis of compound action potentials recorded with surface electrodes. In: Desmedt JE, editor. Computer-aided electromyography and expert systems. Amsterdam: Elsevier; 1989. p 125–135.
10. Dorfman LJ, Cummins KL, Abraham GS. Conduction velocity distributions of the human median nerve: comparison of methods. Muscle Nerve 1982;5:148–153.
11. Kakuda N, Nagaoka M, Tanaka R. Conduction velocities of alpha-motor fibers innervating human thenar muscles and order of recruitment upon voluntary contraction. Muscle Nerve 1992;15:332–343.
12. Van Asseldonk JT, Van den Berg LH, Kalmijn S, Wokke JH, Franssen H. Criteria for demyelination based on the maximum slowing due to axonal degeneration, determined after warming in water at 37 degrees C: diagnostic yield in chronic inflammatory demyelinating polyneuropathy. Brain 2005;128:880–891.
13. Caliandro P, Stålberg E, La Torre G, Padua L. Sensitivity of conventional motor nerve conduction examination in detecting patchy demyelination: a simulated model. Clin Neurophysiol 2007;118:1577–1585.
14. Sharma KR, Cross J, Farronay O, Ayyar DR, Shebert RT, Bradley WG. Demyelinating neuropathy in diabetes mellitus. Arch Neurol 2002;59:758–765.
15. Gorson KC, Ropper AH, Adelman LS, Weinberg DH. Influence of diabetes mellitus on chronic inflammatory demyelinating polyneuropathy. Muscle Nerve 2000;23:37–43.
16. Krendel DA, Costigan DA, Hopkin LC. Successful treatment of severe neuropathies in patients with diabetes mellitus. Arch Neurol 1995;52:1053–1061.
17. Lozeron P, Nahum L, Lacroix C, Ropert A, Said G. Symptomatic diabetic and non-diabetics neuropathies in a series of 100 diabetic patients. J Neurol 2002;249:565–575.
18. Hermann DN, Ferguson ML, Logigian EL, Conduction velocity slowing in diabetic distal polyneuropathy, Muscle Nerve 2002;26:232–237.
19. Shields RW Jr. Single-fiber electromyography is a sensitive indicator of axonal degeneration in diabetes. Neurology 1987;37:1394–1397.
20. Pugliese G. Do advanced glycation end products contribute to the development of long-term diabetic complications? Nutr Metab Cardiovasc Dis 2008;18:457–460.