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Dissociation of Thermal Nociception and Epidermal Innervation in Streptozotocin-Diabetic Mice

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Abstract

The quantification of epidermal innervation, which consists primarily of heat-sensitive C-fibers, is emerging as a tool for diagnosing and staging diabetic neuropathy. However, the relationship between changes in heat sensitivity and changes in epidermal innervation has not yet been adequately explored. Therefore, we assessed epidermal nerve fiber density and thermal withdrawal latency in the hind paw of Swiss Webster mice after two and four weeks of streptozotocin-induced diabetes. Thermal hypoalgesia developed after only two weeks of diabetes, but a measurable reduction in PGP9.5-immunoreactive epidermal nerve fiber density did not appear until four weeks. These data suggest that impaired epidermal nociceptor function contributes to early diabetes-induced thermal hypoalgesia prior to the loss of peripheral terminals.

Keywords

diabetic neuropathy; epidermal nerve fiber density; GAP-43; PGP9.5; substance P; thermal hypoalgesia

Nearly half of all diabetic patients will develop some form of peripheral neuropathy, with distal symmetric polyneuropathy the most common form [24,33]. Depletion of intra-epidermal nerve fibers (IENFs), representing the peripheral terminals of nociceptive unmyelinated C-fibers, appears to be an early index of diabetic neuropathy [30]. Resulting sensory disorders in diabetic patients range from hyperalgesia and allodynia to progressive hypoalgesia that, in conjunction with microvascular disease and impaired wound healing, leaves limbs vulnerable to infections and amputation.

Diabetes-induced reductions in epidermal innervation have been observed in multiple clinical studies, as well as in diabetic primates and rodents [for review see 1,17 and references therein]. IENF loss has been correlated with measures of nerve function to assess its predictive value for other aspects of diabetic neuropathy. In humans, reductions in IENF density have been correlated with changes in pressure and vibration perception, total neurological disability score and neuropathy status [25,31]. In rats, IENF loss correlates with changes in caudal sensory nerve conduction velocity [16].

One would logically predict that loss of thermal sensation is a consequence of diminished epidermal innervation. Indeed, reductions in IENF density induced by topical application of

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capsaicin to the skin of normal volunteer subjects have been associated with a loss of heat and heat-pain sensitivity [18,20,29]. However, few studies of diabetic humans have reported any correlation between changes in heat sensitivity and loss of epidermal innervation [25,26,28], while this association remains largely unexplored in animal models of diabetes.

The primary aim of this study was to explore the relationship between changes in heat-pain sensitivity and epidermal innervation in diabetic mice. Experiments were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. We evaluated thermal withdrawal latency and PGP9.5-immunoreactive nerve profile density in the hind paw of mice after both 2 and 4 weeks of streptozotocin (STZ)-induced diabetes. Male Swiss Webster mice were given a single injection of STZ (180 mg/kg i.p.) to induce insulin-deficient diabetes. Hyperglycemia was confirmed 3 days later, and only mice with blood glucose levels exceeding 15 mmol/l were used. Thermal withdrawal latency was measured using a modification of a method described in detail elsewhere [11]. Briefly, mice were placed in enclosures with a warmed glass floor and a mobile radiant heat source was directed at the plantar surface of one hind paw. The heat was increased 0.9°C per second to ensure the paw withdrawal response involved activation of C-fibers [41]. The time from initiation of heating to paw withdrawal was recorded in 4 separate trials 5 minutes apart, with the median value of the last 3 trials used for each animal. Mice were then deeply anesthetized, weighed and decapitated before collection of a final blood sample, plantar skin from the ipsilateral foot and both sciatic nerves. Final body weight of control mice was significantly greater than that of diabetic mice after either 2 or 4 weeks of diabetes (2 weeks: 31.5 ± 0.6 g versus 21.4 ± 0.7 g; 4 weeks: 31.7 ± 0.6 g versus 23.2 ± 1.1 g; mean $\pm$ SEM, both $p < 0.01$ by unpaired t test). Final blood glucose levels of diabetic mice after either 2 or 4 weeks of diabetes were all greater than 33.3 mmol/l, while median values of control mice were 13.0 (range 9.9–17.6) and 10.6 (range 6.3–12.3) mmol/l, respectively.

Foot skin, obtained from the region where thermal withdrawal latency measurements were made, was fixed for 24 hours in 4% paraformaldehyde in 0.1 M phosphate buffer. Samples were embedded in paraffin and cut at a thickness of 6 μ m. Sections were collected onto glass slides and incubated with an antibody against the pan-neuronal marker PGP9.5 (1:1000, Biogenesis Ltd., UK) to visualize innervation of the skin (Fig. 1A–C). To visualize substance P-immunoreactive nerve profiles in mice after 2 weeks of diabetes, another set of slides was incubated with an antibody against this neuropeptide (1:50, Abcam Inc., Cambridge, MA). Other slides were incubated with an antibody against growth associated protein 43 (GAP-43: 1:1000, Abcam Inc., Cambridge, MA) to visualize profiles of sprouting nerves (Fig. 1D). Using a light microscope (x400 magnification), the total numbers of IENF and sub-epidermal immunoreactive profiles immediately subjacent to the epidermis were counted using slides coded to obscure group identity. The length and area of each section were determined using point-counting methods and a grid reticle containing 100 squares, each 25 μ m². The number of intersections between the stratum granulosum and the grid lines were counted and length calculated using a previously derived formula [13]. The epidermal area of each section was determined by counting the number of grid intersections between the dermal-epidermal interface and upper edge of the stratum granulosum and then multiplying by the grid-square area. An estimation of epidermal thickness was determined by dividing section area by length.

At sacrifice, left and right sciatic nerves were removed and immediately frozen in liquid nitrogen. Substance P was extracted by boiling nerves in an extraction buffer containing 2M glacial acetic acid, 10mM hydrochloric acid, 1mM ethylene diamine tetraacetic acid and 1mM DL-dithiothreitol, followed by homogenization, centrifugation and freeze drying of the supernatant. Substance P content of the reconstituted supernatant was measured using a competitive enzyme immunoassay (Substance P EIA, Cayman Chemical Co., Ann Arbor, MI).

After 2 weeks of diabetes, there was a significant ($p<0.001$) increase in paw thermal withdrawal latency in the diabetic group compared to the control group (Fig. 2A). The linear densities of both PGP9.5-immunoreactive IENF and sub-epidermal nerve profiles of control and diabetic mice were not significantly different (Figs. 2B, F). However, when PGP9.5-immunoreactive IENF counts were normalized to epidermal area, the diabetic group had a significantly ($p<0.05$) increased profile density compared to the control group (Fig. 2C), reflecting the significant ($p<0.001$) thinning of the epidermis in skin from diabetic mice (Fig. 2D). There was no correlation between thermal withdrawal latency and PGP9.5-immunoreactive IENF profile density after 2 weeks of diabetes (Fig. 2E). Both linear and area densities of substance P-immunoreactive IENF profiles were significantly (both $p<0.01$) increased in diabetic mice compared to controls (Table 1). With respect to GAP-43, there was a non-significant increase in linear density and a significant ($p<0.05$) increase in area density of immunoreactive IENF profiles (Table 1). Densities of either substance P- or GAP-43-immunoreactive IENF profiles were markedly lower than corresponding values obtained using PGP 9.5 immunostaining (Fig. 2B, C). No significant differences in either sub-epidermal substance P- or GAP-43-immunoreactive nerve profile densities were evident between the diabetic and control groups (Table 1). There was a significant ($p=0.05$) decrease in substance P levels in the sciatic nerves of mice after 2 weeks of diabetes compared to controls (Table 1).

After 4 weeks of diabetes, we again observed a significant ($p<0.01$) increase in thermal withdrawal latency in the diabetic group compared to the control group (Fig. 3A). However, there was also a significant ($p<0.01$) reduction in linear PGP9.5-immunoreactive IENF profile density in diabetic mice compared to control mice (Fig. 3B). Despite significant ($p<0.01$) thinning of the epidermis in the diabetic mice (Fig. 3D), there was a significant ($p<0.01$) decrease in the area density of PGP9.5-immunoreactive IENF profiles (Fig. 3C), reflecting the robust loss of epidermal innervation after 4 weeks of diabetes. Regression of thermal withdrawal latency against linear PGP9.5-immunoreactive IENF profile density demonstrated a significant ($p<0.01$; $r^2=0.5597$) inverse relationship (Fig. 3E). Despite the significant decrease in IENF profile density after 4 weeks of diabetes, we did not detect a change in sub-epidermal nerve profile density (Fig. 3F).

The present time-course study of sensory fiber function and structure has identified a disassociation between thermal sensitivity and IENF density in the paw of diabetic mice. It is plausible that immunostaining with PGP9.5 does not identify early structural damage to IENF's, as PGP9.5 is a cytoplasmic enzyme [39]. However, we compared footpad linear IENF density measured using PGP9.5 immunostaining with that measured using immunostaining for the structural protein, tau, and found that similar values were obtained with antibodies directed against either antigen [1]. The early loss of thermal sensation could also reflect loss of a small sub-population of IENFs subsumed within the total PGP9.5-immunoreactive population. Therefore, we measured epidermal and dermal fibers expressing substance P, a neuropeptide associated with thermal nociception [3,15,19,36], but unexpectedly found a statistically significant increase in IENF linear and area density. This increase was also seen in epidermal fibers immunoreactive to an antibody against GAP-43, a protein expressed by developing and regenerating nerve fibers [8], suggesting that IENFs sprout in response to the onset of diabetes and prior to subsequent degeneration. GAP-43 immunoreactive profiles were also detected in the skin of control mice, likely reflecting epidermal neural remodeling resulting from continuous migration of basal keratinocytes to more superficial epidermal layers [35].

Our time-course data implies that heat-sensitive IENFs are subject to a diabetes-induced disruption of function that precedes the loss of PGP9.5-immunoreactive profiles. The coefficient of determination (r^2) for the regression of thermal withdrawal latency against IENF linear density after 4 weeks of diabetes also indicates that factors other than fiber loss contribute to thermal hypoalgesia. Functional hypoalgesia could arise from impairment of heat detection

at peripheral terminals. TRPV1 is a heat-sensitive receptor found on C-fiber terminals [4] and a reduction in TRPV1-immunoreactive fiber density has recently been reported in skin biopsies from patients with diabetic neuropathy [10]. TRPV1 expression is also decreased in the cell bodies of C-fibers of diabetic rats [12] and in the spinal cord, dorsal root ganglia and paw skin of diabetic mice [22]. A similar loss of TRPV1 could plausibly contribute to the thermal hypoalgesia in our diabetic mice. Changes in expression of substance P may also affect C-fiber nociceptive function. In STZ-diabetic rats, reduced substance P levels have been reported in dorsal root ganglia [14,40] and both the peripheral [27,40] and central [2] projections of primary afferents. Reductions in substance P reflect reduced synthesis of this neuropeptide and consequent decreases in the amount undergoing axonal transport [34] and released in the spinal cord after peripheral noxious stimulation [2]. Our finding of a similar decline in sciatic nerve substance P content in mice after 2 weeks of diabetes suggests that diminished synthesis, transport and spinal release of this neuropeptide may contribute to early thermal hypoalgesia.

Two other anatomic features of our study are worth noting. First, loss of IENF profiles after 4 weeks of diabetes was associated with a normal sub-epidermal nerve profile linear density, suggesting that the loss of epidermal innervation represents a distal retraction of peripheral C-fiber terminals rather than somatofugal neuronal loss. Second, our observation of epidermal thinning in diabetic mice presents issues concerning IENF quantification [1], as well as issues relating to the physical and functional contribution of epidermal cells to heat transduction and sensation during diabetes. Epidermal thinning in our insulin-deficient and hyperglycemic mice is consistent with reports that insulin and IGF-1 are involved in keratinocyte proliferation and differentiation [37,38], and that high glucose levels decrease proliferation of cultured keratinocytes [32]. Further, it has been suggested that epidermal nerve fibers and keratinocytes make synaptic connections [5]. Given that keratinocytes express a variety of heat-sensitive receptors [7,9,23], these cells may be involved in the detection of heat, with epidermal thinning contributing to thermal hypoalgesia, even when there is no loss of epidermal innervation.

In summary, diabetes-induced thermal hypoalgesia develops before a detectable reduction in linear PGP9.5-immunoreactive IENF profile density. While the appearance of thermal hypoalgesia before a reduction in IENF density was surprising, it is not entirely unprecedented as the onset of thermal hypoalgesia preceded a reduction in innervation of leg skin from diabetic mice [6] and was absent from mice fed a high-fat diet that displayed thermal hypoalgesia [21]. Neurochemical and functional disorders of epidermal nociceptors appear to contribute to early stages of diabetes-induced thermal hypoalgesia before the quantifiable loss of peripheral terminals.

Acknowledgements

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References

1. Beiswenger KK, Calcutt NA, Mizisin AP. Epidermal nerve fiber quantification in the assessment of diabetic neuropathy. *Acta Histochem.*
2. Calcutt NA, Stiller C, Gustafsson H, Malmberg AB. Elevated substance-P-like immunoreactivity levels in spinal dialysates during the formalin test in normal and diabetic rats. *Brain Res* 2000;856(1–2):20–27. [PubMed: 10677607]
3. Cao YQ, Mantyh PW, Carlson EJ, Gillespie AM, Epstein CJ, Basbaum AI. Primary afferent tachykinins are required to experience moderate to intense pain. *Nature* 1998;392(6674):390–394. [PubMed: 9537322]
4. Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D. The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature* 1997;389:816–824. [PubMed: 9349813]

5. Chateau Y, Misery L. Connections between nerve endings and epidermal cells: are they synapses? *Exp. Dermatol* 2004;13(1):2–4. [PubMed: 15009109]
6. Chen YS, Chung SSM, Chung SK. Noninvasive monitoring of diabetes-induced cutaneous nerve fiber loss and hypoalgesia in *thy1-YFP* transgenic mice. *Diabetes* 2005;54:3112–3118. [PubMed: 16249433]
7. Chung MK, Lee H, Caterina MJ. Warm temperatures activate TRPV4 in mouse 308 keratinocytes. *J. Biol. Chem* 2003;278(34):32037–32046. [PubMed: 12783886]
8. De Graan PNE, Van Hoff COM, Tilly BD, Oestreicher AB, Schotman P, Gispen WH. Phosphoprotein B-50 in nerve growth cones from fetal rat brain. *Neurosci. Lett* 1985;61(3):235–241. [PubMed: 2934647]
9. Denda M, Fuziwara S, Inoue K, Denda S, Akamatsu H, Tomitaka A, Matsunaga K. Immunoreactivity of VR1 on epidermal keratinocyte of human skin. *Biophys. Res. Commun* 2001;285(5):1250–1252.
10. Facer P, Casula MA, Smith GD, Benham CD, Chessell IP, Bountra C, Sinisi M, Birch R, Anand P. Differential expression of the capsaicin receptor TRPV1 and related novel receptors TRPV3, TRPV4 and TRPM8 in normal human tissues and changes in traumatic and diabetic neuropathy. *B.M.C. Neurol* 2007;7:11.
11. Hargreaves K, Dubner R, Brown F, Flores C, Joris J. A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. *Pain* 1998;32:77–88. [PubMed: 3340425]
12. Hong S, Wiley JW. Early painful diabetic neuropathy is associated with differential changes in the expression and function of vanilloid receptor 1. *J. Biol. Chem* 2005;280(1):618–627. [PubMed: 15513920]
13. Kalichman MW, Powell HC, Calcutt NA, Mizisin AP. Mast cell degranulation and blood-nerve barrier permeability in rat sciatic nerve after 7 days of hyperglycemia. *Am. J. Physiol* 1995;268(2 pt2):H740–H748. [PubMed: 7864200]
14. Kanbayashi H, Itoh H, Kashiwaya T, Atoh K, Makino I. Spatial distribution of nociceptive neuropeptide and nerve growth factor depletion in experimental diabetic peripheral nervous system. *J. Int. Med. Res* 2002;30(5):512–519. [PubMed: 12449521]
15. Laneville O, Dorais J, Couture R. Characterization of the effects produced by neurokinins and three agonists selective for neurokinin receptor subtypes in a spinal nociceptive reflex of the rat. *Life Sci* 1988;42(13):1295–1305. [PubMed: 2451105]
16. Lauria G, Lombardi R, Borgna M, Penza P, Bianchi R, Savino C, Canta A, Nicolini G, Marmiroli P, Cavaletti G. Intraepidermal nerve fiber density in rat foot pad: neuropathologic-neurophysiologic correlation. *J. Periph. Nerv. Syst* 2005;10:202–208.
17. Lauria G, Devigili G. Skin biopsy as a diagnostic tool in peripheral neuropathy. *Nat. Clin. Prac. Neurol* 2007;10(3):546–557.
18. Malmberg AB, Mizisin AP, Calcutt NA, von Stein T, Robbins WR, Bley KR. Reduced heat sensitivity and epidermal nerve fiber immunostaining following single applications of a high-concentration capsaicin patch. *Pain* 2004;111(3):360–367. [PubMed: 15363880]
19. Nichols ML, Allen BJ, Rogers SD, Ghilardi JR, Honore P, Luger NM, Finke MP, Li J, Lappi DA, Simone DA, Mantyh PW. Transmission of chronic nociception by spinal neurons expressing the substance P receptor. *Science* 1999;286(5444):1558–1561. [PubMed: 10567262]
20. Nolano M, Simone DA, Wendelschafer-Crabb G, Johnson T, Hazen E, Kennedy WR. Topical capsaicin in humans: parallel loss of epidermal nerve fibers and pain sensation. *Pain* 1999;81(1–2):135–145. [PubMed: 10353501]
21. Obrosova IG, Ilnytska O, Lyzogubov VV, Pavlov IA, Mashtalir N, Nadler JL, Drel VR. High-fat diet induced neuropathy of pre-diabetes and obesity: effects of “Healthy” diet and aldose reductase inhibition. *Diabetes* 2007;56(10):2598–2608. [PubMed: 17626889]
22. Pabidi RM, Yu SQ, Peng S, Khardori R, Pauza ME, Premkumar LS. Influence of TRPV1 on diabetes-induced alterations in thermal pain sensitivity. *Mol. Pain* 2008;4:9. [PubMed: 18312687]
23. Peier AM, Reeve AJ, Andersson DA, Moqrich A, Earley TJ, Hergarden AC, Story GM, Colley S, Hogenesch JB, McIntyre P, Bevan S, Patapoutian A. A heat-sensitive TRP channel expressed in keratinocytes. *Science* 2002;296(5575):2046–2049. [PubMed: 12016205]
24. Pirart J, Lauvaux JP, Rey W. Blood sugar and diabetic complications. *N. Engl. J. Med* 1978;298:1149. [PubMed: 643042]

25. Pittenger GL, Ray M, Burcus NI, McNulty P, Basta B, Vinik AI. Intraepidermal nerve fibers are indicators of small-fiber neuropathy in both diabetic and nondiabetic patients. *Diabetes Care* 2004;27:1974–1979. [PubMed: 15277426]
26. Quattrini C, Tavakoli M, Jeziorska M, Kallinikos P, Tesfaye S, Finnigan J, Marshall A, Boulton AJ, Efron N, Malik RA. Surrogate markers of small fiber damage in human diabetic neuropathy. *Diabetes* 2007;56(8):2148–2154. [PubMed: 17513704]
27. Robinson JP, Willars GB, Tomlinson DR, Keen P. Axonal transport and tissue contents of substance P in rats with long-term streptozotocin-diabetes. Effects of the aldose reductase inhibitor 'statil'. *Brain Res* 1987;426(2):339–348. [PubMed: 2446712]
28. Shun CT, Chang YC, Wu HP, Hsieh SC, Lin WM, Lin YH, Tai TY, Hsieh ST. Skin denervation in type 2 diabetes: correlations with diabetic duration and functional impairments. *Brain* 2004;127:1593–1605. [PubMed: 15128619]
29. Simone DA, Nolano M, Johnson T, Wendelschafer-Crabb G, Kennedy WR. Intradermal injection of capsaicin in humans produces degeneration and subsequent reinnervation of epidermal nerve fibers: correlation with sensory function. *J. Neurosci* 1998;18(21):8947–8959. [PubMed: 9787000]
30. Smith AG, Russell J, Feldman EL, Goldstein J, Peltier A, Smith S, Hamwi J, Pollari D, Bixby B, Howard J, Singleton JR. Lifestyle intervention for pre-diabetic neuropathy. *Diabetes Care* 2006;29(6):1294–1299. [PubMed: 16732011]
31. Sorensen L, Molyneaux L, Yue DK. The relationship among pain, sensory loss, and small nerve fibers in diabetes. *Diabetes Care* 2006;29:883–887. [PubMed: 16567832]
32. Spravchikov N, Sizyakov G, Gartsbein M, Accili D, Tennenbaum T, Wertheimer E. Glucose effects on skin keratinocytes: implications for diabetes skin complications. *Diabetes* 2001;50:1627–1635. [PubMed: 11423485]
33. Thomas PK. Classification, differential diagnosis, and staging of diabetic peripheral neuropathy - current state and perspectives of diabetes research: chronic complications. *Diabetes* 1997;46:S54–S57. [PubMed: 9285500]
34. Tomlinson DR, Robinson JP, Willars GB, Keen P. Deficient axonal transport of substance P in streptozotocin-induced diabetic rats. Effects of sorbinil and insulin. *Diabetes* 1998;37(4):488–493. [PubMed: 2454210]
35. Verze L, Parainfo A, Ramieri G, Viglietti C, Panzica GC. Immunocytochemical evidence of plasticity in the nervous structures of the rat lower lip. *Cell Tissue Res* 1999;297:203–211. [PubMed: 10470490]
36. Vierck CJ, Kline RH, Wiley RG. Intrathecal substance p-saporin attenuates operant escape from nociceptive thermal stimuli. *Neuroscience* 2003;119(1):223–232. [PubMed: 12763083]
37. Wertheimer E, Trebicz M, Eldar T, Gartsbein M, Nofeh-Moses S, Tennenbaum T. Differential roles of insulin receptor and insulin-like growth factor receptor in differentiation of murine skin keratinocytes. *J. Invest. Dermatol* 2000;115(1):24–29. [PubMed: 10886503]
38. Wertheimer E, Spravchikov M, Trebicz M, Gartsbein M, Accili D, Avinoah I, Nofeh-Moses S, Sizyakov G, Tennenbaum T. The regulation of skin proliferation and differentiation in the IR null mouse: implications for skin complications of diabetes. *Endocrinology* 2001;142:1234–1241. [PubMed: 11181540]
39. Wilkinson KD, Lee KM, Deshpande S, Duerksen-Hughes P, Boss JM, Pohl J. The neuron-specific protein PGP 9.5 is a ubiquitin carboxyl-terminal hydrolase. *Science* 1989;246(4930):670–673. [PubMed: 2530630]
40. Willars GB, Calcutt NA, Compton AM, Tomlinson DR, Keen P. Substance P levels in peripheral nerve, skin, atrial myocardium and gastrointestinal tract of rats with long-term diabetes mellitus. Effects of aldose reductase inhibition. *J. Neurol. Sci* 1989;91(1–2):153–164. [PubMed: 2473171]
41. Yeomans DC, Pirec V, Proudfit HK. Nociceptive responses to high and low rates of noxious cutaneous heating are mediated by different nociceptors in the rat: behavioral evidence. *Pain* 1996;68(1):133–140. [PubMed: 9252008]

Figure 1. PGP9.5-immunostained epidermal (circles) and sub-epidermal (arrows) nerve fiber profiles in glabrous skin from the hind paw of control (A) and diabetic mice after 2 (B) or 4 (C) weeks of diabetes. GAP-43-immunostained epidermal and sub-epidermal nerve profiles in a diabetic mouse after 2 weeks of diabetes (D). Bar = 40 microns.

Figure 2. The Impact of Two Weeks of Diabetes

Paw thermal withdrawal latency (A), PGP9.5-immunoreactive IENF profile counts normalized to epidermal length (B) or epidermal area (C), epidermal thickness (D), a plot of paw thermal withdrawal latency against immunoreactive profile linear density (E) and sub-epidermal immunoreactive profile linear densities (F) for control and diabetic mice after 2 weeks of diabetes. Bar graphs show mean + SEM and were analyzed by unpaired t-test, ***p<0.001, *p<0.05. In the linear regression analysis, closed circles represent diabetic mice and open circles control mice.

Figure 3. The Impact of Four Weeks of Diabetes

Paw thermal withdrawal latency (A), PGP9.5-immunoreactive IENF profile counts normalized to epidermal length (B) or epidermal area (C), epidermal thickness (D), a plot of paw thermal withdrawal latency against immunoreactive profile linear density (E) and sub-epidermal immunoreactive profile linear densities (F) for control and diabetic mice after 4 weeks of diabetes. Bar graphs show mean + SEM and were analyzed by unpaired t-test, ** $p < 0.01$. In the linear regression analysis, closed circles represent diabetic mice and open circles represent control mice, $p < 0.01$.

Table 1

Sciatic nerve levels of substance P as well as substance P and GAP-43 immunoreactive epidermal and sub-epidermal profile densities of foot pad skin from control and diabetic mice after two weeks of diabetes.

Substance P		GAP-43					
Sciatic nerve (pg/mm)		IENF profiles (#/mm)	IENF profiles (#/mm ²)	SNP profiles (#/mm)	IENF profiles (#/mm)	IENF profiles (#/mm ²)	SNP profiles (#/mm)
Control	14.9±1.3	0.5±0.2	21.7±8.0	5.6±0.7	6.8±1.2	206.9±33.5	25.2±1.2
Diabetic	11.3±1.5	1.1±0.3	96.1±21.2	4.6±0.7	9.4±1.8	507.2±101.5	28.1±1.3
	p=0.05	p<0.01	p<0.01	NS	NS	p<0.5	NS

Data are presented as mean ± SEM (N=7-8 per group) and were analyzed by unpaired t-test. NS = not significant. IENF = intra-epidermal nerve fiber. SNP = sub-epidermal nerve plexus.