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Early-stage Type 2 Diabetes Mellitus Impairs Erectile Function and Neurite Outgrowth From the Major Pelvic Ganglion and Downregulates the Gene Expression of Neurotrophic Factors

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Abstract

Objective—To assess neurite sprouting and gene expression of neurotrophic factors, nerve markers, and apoptosis in the major pelvic ganglia (MPGs) of rats with type 2 diabetes mellitus (T2DM) as it relates to erectile function.

Materials and Methods—Male rats were fed high-fat diet for 2 weeks followed by 2 low-dose injections of streptozotocin (20 mg/kg). In 3 groups (controls, 3-week, or 5-week T2DM), erectile function was measured by ratios of intracavernosal pressure to mean arterial pressure after cavernous nerve stimulation. MPGs were harvested, and gene expressions of neurotrophic factor 3, nerve growth factor, glial cell line-derived neurotrophic factor, brain-derived neurotrophic factor, caspase-1, -3, -9, beta tubulin type III, and neuronal nitric oxide synthase were quantified by quantitative polymerase chain reaction. Additional MPGs were harvested and cultured in Matrigel. Neurite outgrowth from the MPG was evaluated at 48 hours after culture.

Results—Erectile function was significantly decreased in all rats with T2DM. Gene expressions of neurotrophic factor 3, nerve growth factor, glial cell line-derived neurotrophic factor, and brain-derived neurotrophic factor were slightly lower at 3 weeks and significantly lower at 5 weeks after T2DM induction. Gene expression of apoptotic markers caspase-1, -3, -9, and neuronal markers beta tubulin type III and neuronal nitric oxide synthase remained unchanged. Rats with T2DM had shorter neurite length and less neurite sprouting than did the control MPG.

Conclusion—Early-stage T2DM downregulates neurotrophic factors, induces erectile dysfunction, and impairs MPG neurite outgrowth, suggesting that erectile dysfunction may be prevented by supplementing neurotrophic factors at early-stage T2DM.

Diabetes mellitus (DM) is a common cause of erectile dysfunction (ED).¹ The prevalence of ED among men with DM is more than 50%.² Multiple factors contribute to the pathogenesis of diabetic ED, including smooth muscle or endothelial dysfunction, and neuropathy.¹ Although the molecular mechanisms that govern smooth muscle and endothelial dysfunction

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have been widely studied,^{3,4} the molecular basis of neurogenic ED in DM remains to be elucidated. Our current understanding is that cavernous neuropathy in insulin-dependent (type 1) DM occurs in 2 phases.⁵ In the first phase, nitrenergic nerves lose some of the neuronal nitric oxide synthase (nNOS) content and function. In the second phase, the nitrenergic neurons undergo apoptosis. Insulin replacement can reverse the first phase, whereas the second phase is irreversible,⁵ suggesting that early intervention is necessary to prevent neurogenic ED in DM. An alternate mechanism proposed for DM neuropathy of the penis is through the reduction of neurotrophic factors in rats with type 1 DM (T1DM). In support of this, supplementation of neurotrophic factors has improved erectile function.^{6,7}

Nonetheless, these results were obtained from rats with streptozotocin (STZ)-induced T1DM, whereas 90% of the clinical DM cases are type 2 DM (T2DM).⁸ Albersen et al established a model of T2DM by feeding rats a high-fat diet (HFD) with a low-dose STZ injection. They characterized the metabolic, hormonal, and morphological changes of the penis at the late stages of T2DM.⁹ This animal model of T2DM can be used to study the molecular changes that occur in the major pelvic ganglion (MPG) and penis to further understand the pathogenesis of T2DM neuropathic ED.

We hypothesize that rats with early-stage T2DM will also display neurogenic ED, and that the potential mechanisms responsible for the decline in erectile function may be due to changes in the expression of neurotrophic factors in the MPG and subsequent impaired neuritogenesis. To test this hypothesis, we assessed erectile function and transcriptional changes of the endogenous neurotrophic factors, neuronal markers, and markers of apoptosis in the MPGs of rats with early-stage T2DM in vivo, and evaluated the neurite outgrowth from the MPG ex vivo.

Materials and Methods

Experimental Animals

Male Sprague-Dawley rats (n = 30, Charles River, Wilmington, MA), weighing 250-300 g, were used and divided into 3 groups: rat group with T2DM at 3 weeks after DM induction (DM-3 wks), rat group with T2DM at 5 weeks after DM induction (DM-5 wks), and the nondiabetic control group. To induce T2DM, the DM-3 wks and DM-5 wks groups were fed HFD containing 60% kcal from fat (D12492, Research Diets, New Brunswick, NJ) and received 2 intraperitoneal injections of STZ (20 mg/kg, Sigma-Aldrich, St. Louis, MO) at 2 weeks after the initiation of HFD.⁹ Injections of STZ took place 3 days apart. DM was confirmed with blood glucose greater than 200 mg/dL at 3 days after the second injection of STZ. The control rats were fed a normal chow diet and received injections of citrate buffer. The DM-3 wks and DM-5 wks groups continued to have HFD until they were sacrificed. All animals were weighed, and non-fasting blood glucose was taken before erectile function testing (n = 6 per group). Following erectile function testing, retroperitoneal, epididymal, and mesenteric fat pads were also weighed. For the ex vivo study, we used additional rats from the control, DM-3 wks, and DM-5 wks (n = 3 per group) groups to harvest and culture the MPGs.

All of the experiments were conducted in accordance with the Johns Hopkins School of Medicine Guidelines for Animal Care and Use, and the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Measurement of Erectile Responses In Vivo

Under ketamine-xylazine (100:10 mg/kg) anesthesia (intraperitoneal), the prostate was exposed by midline laparotomy, and the right cavernous nerve (CN) was identified posterolateral to the prostate.¹⁰ To measure intracavernous pressure (ICP), the right crus was cannulated with a 25G needle connected to a pressure transducer and the right carotid artery was cannulated to measure mean arterial pressure (MAP). The CN was stimulated with a square pulse stimulator (Grass Instruments, Quincy, MA) at a frequency of 20 Hz, duration of 0.5 ms, with no delay at 6 volts for 1 minute with 3- to 5-minute intervals between stimulations.¹¹ The ratio of ICP to MAP was calculated to normalize for variations in systemic blood pressure. Total ICP was measured as the area under the curve (AUC) during the time of stimulation. The time for the ICP to reach 80% of maximal ICP (T80) was also evaluated.¹²

Quantitative Polymerase Chain Reaction (qPCR)

Real-time qPCR was used to evaluate relative gene expressions of neurotrophic factors and neuronal and apoptotic markers in the MPGs. T2DM MPGs were harvested at 3 and 5 weeks after diabetes induction. Total RNA was purified from homogenized MPGs using RNeasy System (Qiagen, Hilden, Germany), according to the manufacturer's instruction. RNA was then reverse transcribed with Ready-To-Go You-Prime First-Strand Beads (GE Healthcare, Pittsburgh, PA). We used TaqMan Gene Expression Assays (Applied Biosystems, Foster City, CA) for neurotrophic factor 3 (NT3: Rn00579280), nerve growth factor (NGF: Rn01511601), glial cell line-derived neurotrophic factor (GDNF: Rn00569510), brain-derived neurotrophic factor (BDNF: Rn02531967), caspase (CASP)-1 (Rn00562724), CASP3 (Rn00563902), CASP9 (Rn00581212), beta tubulin type III (TUBB3: Rn01431594), nNOS (Rn00583793), and hypoxanthine guanine phosphoribosyl transferase (Rn01527840). Hypoxanthine guanine phosphoribosyl transferase was used as an endogenous control. All experiments were performed on 7 different whole paired MPGs from each group, with triplicate technical replicate PCR reactions per sample.¹³

MPG Culture and Neurite Outgrowth Assessment

Whole MPGs were carefully harvested from rats in the control, DM-3 wks, and DM-5 wks groups. MPGs were kept on ice in serum-free media (RPMI 1640 with 1% penicillin-streptomycin, GIBCO, Grand Island, NY) until they were embedded and cultured in reduced growth factor Matrigel (Corning, Tewksbury, MA) with vascular endothelial growth factor (VEGF; 25 µg/mL, R&D Systems, Minneapolis, MN) (n = 3 per group).¹³

As previously described, MPGs were embedded in ~600 µL reduced growth factor Matrigel (diluted 1:3 with media) and covered with 1 mL of media and VEGF (25 µg/mL, R&D Systems).¹³ Media and VEGF were changed every 24 hours and maintained at 37°C in a humidified atmosphere with 5% carbon dioxide. With Nikon TE200 inverted microscope attached to a CCD camera, photographs of neurite outgrowth from the MPGs were taken 48

hours after culture. The images were analyzed with Elements software (Nikon Instruments, Melville, NY). The 5 longest neurites in each area were measured (20-25 neurites per MPG). The average lengths of longest neurites were compared between the groups.¹³ To compare neurite sprouting from the MPG, the lengths of MPG edge from which neurites grow were measured (1) and the total circumference of each MPG (2). The ratio of the 2 lengths (1):(2) was compared between the groups (Fig. 2E).

Statistical Analysis

Data are expressed as mean $\pm$ standard error of the mean. We compared the differences between the 3 groups by a 1-way analysis of variance followed by Tukey multiple comparisons test (GraphPad Prism 5). *P* values of less than .05 were used as criteria for statistical significance.

Results

Blood Glucose Levels and Body Weights

Blood glucose levels were significantly elevated in rats in the DM-3 wks and DM-5 wks groups compared with controls (Table 1). HFD and low-dose STZ injection successfully induced DM in DM-3 wks and DM-5 wks groups. The final body weights of rats in the DM-3 wks and DM-5 wks groups were significantly lower than the final body weights of rats in the control group (*P* = .04, Table 1). However, there was no difference in adiposity of the rats as the ratio of retroperitoneal, epididymal, and mesenteric fat pad and body weight was similar (*P* > .05, Table 1).

Erectile Responses

Erectile function was evaluated by measuring ICP-MAP and AUC. DM-3 wks and DM-5 wks had significantly decreased ICP-MAP than the control (Fig. 1A; *P* = .04). Rats in the DM-3 wks and DM-5 wks groups also demonstrated significantly decreased AUCs (Fig. 1B; *P* = .008). Neurogenic ED is suggested by longer T80 of the DM-3 wks and DM-5 wks groups (Fig. 1C). There was no difference in erectile responses between rats in the DM-3 wks group and rats in the DM-5 wks group. The representative tracings of ICP and MAP further demonstrate these results (Fig. 1D).

Neurite Outgrowth from the MPG

The effect of the early-stage T2DM on neuritogenesis in the MPG in each group was evaluated at 48 hours after culture (Fig. 2). The DM-3 wks and DM-5 wks groups had significantly shorter average neurite length than control MPGs (Fig. 2d; *P* < .01). Furthermore, DM-5 wks MPGs demonstrated significantly shorter average neurite length than DM-3 wks MPGs (*P* = .01), suggesting that neurite outgrowth is impaired in a time-dependent fashion. Interestingly, less neurite sprouting was apparent from the MPGs of DM-3 wks and DM-5 wks groups than in controls. To demonstrate this difference quantitatively, the ratios of (1) the lengths of MPG edges from which neurites grow and (2) the total circumferences of the MPGs were measured (Fig. 2E). Therefore, a bigger ratio (1):(2) translates into greater MPG neurite sprouting. The ratio (1):(2) was significantly smaller in the DM-3 wks and DM-5 wks MPGs (Fig. 2F).

Gene Expressions of Neurotrophic and Apoptotic Factors

The gene expressions of NT3, NGF, GDNF, and BDNF in the MPGs were quantified by qPCR (Fig. 3A). The DM-3 wks group's MPGs had decreased gene expression of GDNF, NGF, BDNF, and NT3 compared with control MPGs (Fig. 3A). However, the decreased expression was statistically significant only for NT3 ($P<.05$). The DM-5 wks group showed significantly decreased ($P<.05$) gene expression of GDNF, NGF, BDNF, and NT3 compared with the control group (Fig. 3A). Overall, there was no significant difference in the gene expressions of CASP1, CASP3, and CASP9 (Fig. 3B; $P>.05$). There was no significant difference in the neuronal marker TUBB3 or nNOS in control and diabetic MPGs (Fig. 3C).

Discussion

The present study showed that rats with early-stage T2DM displayed ED and had an increased time to reach maximal ICP, which is indicative of neurogenic ED. Furthermore, MPGs from rats with T2DM demonstrated reduced neurite sprouting and elongation when grown in culture. In addition, early-stage T2DM downregulated the gene expression of neurotrophic factors in the MPG, whereas the gene expression of neuronal and apoptosis markers remained unchanged.

Although 90% of the diabetic cases are T2DM in clinical settings,⁸ rats with T1DM have been used in the majority of preclinical studies on diabetic ED.^{3,6,7} Furthermore, these rats with T1DM experience severe weight loss,^{3,6,7} reflecting malnutrition due to impaired insulin-dependent glucose uptake. On the other hand, obesity is a general problem with genetic rat models of T2DM.⁹ Albersen et al reported that the combination of HFD with low-dose STZ induced a lean model of T2DM, resembling the natural history and the metabolic, hormonal, and morphological changes of the penis at the late stages of T2DM.⁹ In the present study, although the rats with early-stage T2DM had lower body weights than the controls, both groups had similar visceral adiposity (Table 1).

Albersen et al confirmed ED in this rat model of T2DM 6 weeks after DM induction, but it was still unknown whether rats demonstrate ED at early stages of T2DM.⁹ In the present study, ED was evident as early as 3 and 5 weeks following T2DM induction. Celtek et al demonstrated that rats with T1DM start to lose penile nNOS content 4 weeks after DM induction.⁵ Therefore, a reduction in penile nNOS could explain the pathogenesis of ED in early-stage T2DM. In fact, the representative ICP tracings of the rats in the present study showed delayed T80, which is indicative of neurogenic ED.^{12,14} Interestingly, we did not see a decrease in the gene expression of nNOS in the MPGs of rats with T2DM; however, the protein expression or activity was not assessed.

Neurite outgrowth from the MPG was impaired in a time-dependent fashion in rats with T2DM. The MPGs from rats with T2DM had fewer areas of neurite sprouting than controls. Previous studies have examined neurite outgrowth by measuring average neurite lengths.^{13,15,16} This method evaluates only neurite elongation and does not reflect the amount of neurite sprouting from the MPG. By comparing the ratios of length of growth with total MPG circumference, rats with T2DM demonstrated diminished neurite sprouting from the MPG in addition to decreased neurite elongation. These data represent a global

impairment in axonal health, which may be caused by a number of metabolic changes of neuronal factors, including neurotrophic growth factors.

The gene expression of GDNF, NGF, BDNF, and NT3 was downregulated in the MPG of rats with early-stage T2DM in a time-dependent fashion. Neurotrophic factors are known to promote neurite sprouting.¹⁷ Conversely, reduced neurotrophic factors can downregulate neurite sprouting from the MPG, which is a possible explanation for the reduced sprouting from the MPGs of DM-3 wks and DM-5 wks groups. Neurotrophic factors also protect neurons and promote peripheral nerve regeneration.^{16,18-20} Chen et al demonstrated that protein expressions of NGF, NT3, and neurotrophic factor 4 were increased in the penises of rats with T1DM.⁶ These increases could represent a compensatory mechanism by the penis in an attempt to provide a signal for the CN to regenerate and reconnect. Because these rats with T1DM remain dysfunctional, retrograde axonal transport in the CN could be impaired or nerve damage is too great for regeneration toward the neurotrophic signal. Future studies are required to examine if similar changes to neurotrophic factor proteins are evident in the penis of rats with T2DM. Additionally, the potential changes decreased neurotrophic factor receptor expression, or binding affinity in T2DM should be addressed to confirm that the impaired growth is not due to the neuron's ability to respond to neurotrophic factors.

In the late stages of T1DM model, Schmeichel et al demonstrated that oxidative stress leads to neuronal injury and induces apoptosis in the dorsal root ganglion.²¹ Conversely, in this study, the gene expressions of CASP1, CASP3, and CASP9 remained unchanged, suggesting that apoptosis was not induced in the MPG at the early stage of T2DM. Furthermore, we demonstrated that there was no change in the gene expression of the neuronal marker, TUBB3, or nNOS between groups. These data suggest that the reduction of neurotrophic factors, and not the induction of apoptosis, or less overall neurons could contribute to impaired neurite outgrowth from the MPG at the early stage of T2DM. Further studies that directly measure apoptosis and count the number of neurons in the MPG in this model of T2DM are required.

Peripheral nerves are constantly damaged in DM because neurons are susceptible to hyperglycemia.²⁰ In addition, advanced glycation endproducts, which are increased in the penises and MPGs of rats with DM, induce degeneration of nitrergic neurons.^{22,23} Therefore, prevention of neuronal death or regeneration of nitrergic neurons is required to prevent or recover ED in DM. Generally, functional recovery of peripheral nerves requires more than 1 process following peripheral nerve insult. Initially, neurite sprouting needs to occur for neurites to regenerate following nerve injury.¹⁷ Following sprouting, neurites must elongate toward the target organ or tissue of innervation. Once these neurites reach the target organ (ie penis), unnecessary innervations have to be trimmed to achieve functional recovery.¹⁷ Findings from this study suggest that in this preclinical animal model, T2DM is a neuronal insult that impairs both (1) sprouting and (2) elongation of neurites from the MPG, contributing to the failed function of CN and the pathogenesis of neurogenic ED. These data are similar to the impaired neurite elongation evident in a severe model of T1DM in which diabetic MPG neurite grew 30% less than controls in the presence of BDNF.²⁴ The current study has several limitations. The transcriptional changes of the neurotrophic factors were measured, but the translational and post-translational effects of T2DM were not

evaluated. Decreased nNOS, advanced glycation endproducts, or oxidative stress may have induced neurogenic ED; however, we did not evaluate their effect on nitrergic neurons in this study.

Conclusion

The present study demonstrated that early-stage T2DM induced neurogenic ED. The data from the ex vivo study suggest that T2DM impaired neurite outgrowth (both sprouting and elongation) from the MPG in a time-dependent fashion. The gene expressions of GDNF, NGF, BDNF, and NT3 were downregulated in the MPG of rats with T2DM, whereas the gene expressions of CASP1, CASP3, CASP9, TUBB3, and nNOS were unchanged. These results suggest that reduced gene expressions of neurotrophic factors, not the induction of apoptosis, contribute to the pathogenesis of neurogenic ED of the early-stage T2DM. Furthermore, our data suggest that it is possible that ED may be prevented by supplementing neurotrophic factors at the early stage of T2DM.

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References

1. Malavige L, Levy J. Erectile dysfunction in diabetes mellitus. *J Sex Med.* 2009; 6:1232–1247. [PubMed: 19210706]
2. Selvin E, Burnett A, Platz E. Prevalence and risk factors for erectile dysfunction in the US. *Am J Med.* 2007; 120:151–157. [PubMed: 17275456]
3. Bivalacqua T, Kendirici M, Champion H, et al. Dysregulation of cGMP-dependent protein kinase 1 (PKG-1) impairs erectile function in diabetic rats: influence of in vivo gene therapy of PKG1alpha. *BJU Int.* 2007; 99:1488–1494. [PubMed: 17355372]
4. Musicki B, Burnett A. Endothelial dysfunction in diabetic erectile dysfunction. *Int J Impot Res.* 2007; 19:129–138. [PubMed: 16775612]
5. Cellek S, Foxwell N, Moncada S. Two phases of nitrergic neuropathy in streptozotocin-induced diabetic rats. *Diabetes.* 2003; 52:2353–2362. [PubMed: 12941776]
6. Chen Y, Yang R, Yao L, et al. Differential expression of neurotrophins in penises of streptozotocin-induced diabetic rats. *J Androl.* 2007; 28:306–312. [PubMed: 17079742]
7. Sun C, Lin H, Yu W, et al. Neurotrophic effect of bone marrow mesenchymal stem cells for erectile dysfunction in diabetic rats. *Int J Androl.* 2012; 35:601–607. [PubMed: 22428616]
8. Stumvoll M, Goldstein B, van Haeften T. Type 2 diabetes: principles of pathogenesis and therapy. *Lancet.* 2005; 365:1333–1346. [PubMed: 15823385]
9. Albersen M, Lin G, Fandel T, et al. Functional, metabolic, and morphologic characteristics of a novel rat model of type 2 diabetes-associated erectile dysfunction. *Urology.* 2011; 78:476, e1–476.e8.
10. Hannan J, Albersen M, Kutlu O, et al. Inhibition of Rho-kinase improves erectile function, increases nitric oxide signaling and decreases penile apoptosis in a rat model of cavernous nerve injury. *J Urol.* 2013; 189:1155–1161. [PubMed: 23021998]
11. Hannan J, Kutlu O, Stopak B, et al. Valproic acid prevents penile fibrosis and erectile dysfunction in cavernous nerve-injured rats. *J Sex Med.* 2014; 11:1442–1451. [PubMed: 24636283]
12. Kutlu O, Ross A, Schaeffer E, et al. Increased expression of nestin in the major pelvic ganglion following cavernous nerve injury. *Int J Impot Res.* 2012; 24:84–90. [PubMed: 21993267]

13. Hannan J, Albersen M, Stopak B, et al. Temporal changes in neurotrophic factors and neurite outgrowth in the major pelvic ganglion following cavernous nerve injury. *J Neurosci Res*. 2015; 93:954–963. [PubMed: 25644064]
14. Gratzke C, Strong T, Gebska M, et al. Activated RhoA/Rho kinase impairs erectile function after cavernous nerve injury in rats. *J Urol*. 2010; 184:2197–2204. [PubMed: 20851436]
15. Lin G, Chen K, Hsieh P, et al. Neurotrophic effects of vascular endothelial growth factor and neurotrophins on cultured major pelvic ganglia. *BJU Int*. 2003; 92:631–635. [PubMed: 14511050]
16. Lin G, Shindel A, Fandel T, et al. Neurotrophic effects of brain-derived neurotrophic factor and vascular endothelial growth factor in major pelvic ganglia of young and aged rats. *BJU Int*. 2010; 105:114–120. [PubMed: 19493269]
17. Klimaschewski L, Hausott B, Angelov D. The pros and cons of growth factors and cytokines in peripheral axon regeneration. *Int Rev Neurobiol*. 2013; 108:137–171. [PubMed: 24083434]
18. Bennett N, Kim J, Wolfe D, et al. Improvement in erectile dysfunction after neurotrophic factor gene therapy in diabetic rats. *J Urol*. 2005; 173:1820–1824. [PubMed: 15821595]
19. Burgers J, Nelson R, Quinlan D, et al. Nerve growth factor, nerve grafts and amniotic membrane grafts restore erectile function in rats. *J Urol*. 1991; 146:463–468. [PubMed: 1856953]
20. Peeraer E, Van Lutsenborg A, Verheyen A, et al. Pharmacological evaluation of rat dorsal root ganglion neurons as an in vitro model for diabetic neuropathy. *J Pain Res*. 2011; 4:55–65. [PubMed: 21559351]
21. Schmeichel A, Schmelzer J, Low P. Oxidative injury and apoptosis of dorsal root ganglion neurons in chronic experimental diabetic neuropathy. *Diabetes*. 2003; 52:165–171. [PubMed: 12502508]
22. Celtek S, Cameron N, Cotter M, et al. Pathophysiology of diabetic erectile dysfunction: potential contribution of vasa nervorum and advanced glycation endproducts. *Int J Impot Res*. 2013; 25:1–6. [PubMed: 22914567]
23. Usta M, Kendirci M, Gur S, et al. The breakdown of preformed advanced glycation end products reverses erectile dysfunction in streptozotocin-induced diabetic rats: preventive versus curative treatment. *J Sex Med*. 2006; 3:242–250. [PubMed: 16490017]
24. Bai G, Zhou F, Hui Y, et al. Effects of Icariside II on corpus cavernosum and major pelvic ganglion neuropathy in streptozotocin-induced diabetic rats. *Int J Mol Sci*. 2014; 15:23294–23306. [PubMed: 25517034]

Figure 1.

Erectile function of rats with type 2 diabetes mellitus. Intracavernous pressure-mean arterial pressure (ICP-MAP) (A) and area under the curve (B) were significantly lower in the DM-3 wks and DM-5 wks groups than in the control group. The representative tracings of ICP and MAP further demonstrate the results (C). * $P < .05$ vs control.

Figure 2.

Neurite outgrowth from the major pelvic ganglion (MPG). Representative images of the neurite outgrowth from the MPG at 48 hours after culture (**A**, control; **B**, rats with type 2 diabetes mellitus [T2DM] at 3 weeks after DM induction [DM-3 wks]; **C**, rats with T2DM at 5 weeks after DM induction [DM-5 wks]). Arrows indicate the longest neurites in each area. The scale bar is 100 μm. The average lengths of the longest neurites of DM-3 wks and DM-5 wks were significantly shorter than the control (**D**). The ratio of the length of MPG growth (1) to total MPG circumference (2) was used to compare sprouting (**E**). The DM-3 wks and DM-5 wks groups had significantly smaller ratio than the control group (**F**), indicating less overall neurite sprouting. * $P < .05$ vs control. # $P < .05$ vs DM-3 wks.

Figure 3.

Quantitative polymerase chain reaction results of the neurotrophic factors and apoptosis-related peptides. The gene expressions of glial cell line-derived neurotrophic factor (GDNF), nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and neurotrophic factor 3 (NT3) were reduced in the rat group with type 2 diabetes mellitus (T2DM) at 3 weeks after DM induction (DM-3 wks) compared with the control group without significant difference, except NT3 (A). The rat group with T2DM at 5 weeks after DM induction (DM-5 wks) had significantly reduced gene expressions of GDNF, NGF, BDNF, and NT3 (A). Gene expressions of caspase-1, -3, and -9 were not changed among the groups (B). Gene expressions of beta tubulin type III (TUBB3) and neuronal nitric oxide synthase (nNOS) were also not changed among the groups (C). * $P < .05$ vs control.

Table 1
Animal blood glucose and body composition measurements

Measurement	Control	DM-3 wks	DM-5 wks
Blood glucose (mg/dL)	76 ± 1.8	215 ± 13 *	223 ± 12 *
Final weight (g)	604 ± 12	497 ± 41 *	507 ± 23 *
Retroperitoneal fat-final weight (%)	2.7 ± 0.30	3.3 ± 0.28	2.9 ± 0.46
Epididymal fat-final weight (%)	2.1 ± 0.17	2.4 ± 0.14	2.3 ± 0.26
Mesenteric fat-final weight (%)	1.3 ± 0.12	1.1 ± 0.09	0.9 ± 0.15

DM, diabetes mellitus.

* $P < .05$ vs control.