Exercise training enhances insulin-stimulated nerve arterial vasodilation in rats with insulin-treated experimental diabetes

T. Dylan Olver,1 Matthew W. McDonald,2 Kenneth N. Grisé,2 Adwitia Dey,2 Matti D. Allen,3 Philip J. Medeiros,4 James C. Lacefield,5 Dwayne N. Jackson,4 Charles L. Rice,3,6 C. W. James Melling,2 Earl G. Noble,2 and J. Kevin Shoemaker1,7

1Neurovascular Research Laboratory, School of Kinesiology, The University of Western Ontario, London, Ontario, Canada; 2Exercise Biochemistry Laboratory, School of Kinesiology, The University of Western Ontario, London, Ontario, Canada; 3Neuromuscular Research Laboratory, School of Kinesiology, The University of Western Ontario, London, Ontario, Canada; 4A. C. Burton Laboratory for Vascular Research, Department of Medical Biophysics, Schulich School of Medicine and Dentistry, The University of Western Ontario, London, Ontario, Canada; 5Department of Electrical and Computer Engineering, Department of Medical Biophysics and Robarts Research Institute, The University of Western Ontario, London, Ontario, Canada; 6Department of Anatomy and Cell Biology, The University of Western Ontario, London, Ontario, Canada; and 7Department of Physiology and Pharmacology, The University of Western Ontario, London, Ontario, Canada

Submitted 14 November 2013; accepted in final form 15 April 2014

Insulin therapy is a common and effective treatment for Type 1 diabetes. In rats with experimental diabetes, concurrent insulin treatment prevents the decline in NO-related vasa nervorum reactivity (37). Furthermore, insulin treatment administered 1 mo following diabetes induction partially restores basal nerve blood flow and motor nerve conduction velocity (MNCV) (10). However, in the aforementioned study, insulin treatment alone did not completely reverse the effects of chronic hyperglycemia. Conceivably, in rats with experimental diabetes, insulin treatment may restore nerve blood flow and vasoreactivity partially through a NO-related mechanism. In addition to the effects of insulin treatment on basal nerve blood flow, acute insulin administration stimulates epineurial arterial vasodilation and increases sciatic nerve blood flow velocity (NBVF) in rats (24, 49). It is uncertain what compounds mediate the insulin-induced dilation (21), but glucose-stimulated insulin-mediated dilation can be prevented by NO synthase (NOS) inhibition (49). The vasodilatory actions of insulin were documented over 70 years ago (1), and since then it has been established that insulin-mediated vasodilation occurs through the activation of the intracellular enzymes phosphatidylinositol-3 kinase (PI3K) and protein kinase B (Akt), which phosphorylate and activate endothelial NOS (eNOS) (27, 45). How the relationship between insulin sensitivity and eNOS

Exercise training enhances insulin-stimulated nerve arterial vasodilation in rats with insulin-treated experimental diabetes

Olver TD, McDonald MW, Grisé KN, Dey A, Allen MD, Medeiros PJ, Lacefield JC, Jackson DN, Rice CL, Melling CW, Noble EG, Shoemaker JK. Exercise training enhances insulin-stimulated nerve arterial vasodilation in rats with insulin-treated experimental diabetes. Am J Physiol Regul Integr Comp Physiol 306: R941–R950, 2014. First published April 16, 2014; doi:10.1152/ajpregu.00508.2013.—Insulin stimulates nerve arterial vasodilation through a nitric oxide (NO) synthase (NOS) mechanism. Experimental diabetes reduces vasa nervorum NO reactivity. Studies investigating hyperglycemia and nerve arterial vasodilation typically omit insulin treatment and use sedentary rats resulting in severe hyperglycemia. We tested the hypotheses that 1) insulin-treated experimental diabetes and inactivity (DS rats) will attenuate insulin-stimulated nerve arterial vasodilation, and 2) deficits in vasodilation in DS rats will be overcome by concurrent exercise training (DX rats; 75–85% V˙O2 max, 1 h/day, 5 days/wk, for 10 wk). The baseline index of vascular conductance values (VCi = nerve blood flow velocity/mean arterial blood pressure) were similar (P > 0.05). Motor nerve conduction velocity (MNCV) was lower in DS rats versus control sedentary (CS) rats and DX rats (P < 0.01). Motor nerve conduction velocity (MNCV) was lower in DS rats versus CS rats and DX rats (P < 0.01). When compared with CS rats, DX rats expressed greater nerve endothelial NOS (eNOS) protein content (P = 0.04). In a separate analysis, we examined the impact of diabetes in exercise-trained rats alone. When compared with exercise-trained control rats (CX), DX rats had a lower AUC during the EHC, lower MNCV values, and lower sciatic nerve eNOS protein content (P < 0.03). Therefore, vasa nervorum and motor nerve function are impaired in DS rats. Such deficits in rats with diabetes can be overcome by concurrent exercise training. However, in exercise-trained rats (CX and DX groups), moderate hyperglycemia lowers vasa nervorum and nerve function.

Exercise; diabetes; nerve blood flow; Doppler ultrasound

Diabetes leads to several clinical comorbidities including vascular disease and peripheral neuropathy (12, 61). The pathology of diabetes peripheral neuropathy is multifactorial and often studied in the context of a metabolic or vascular etiology (29). From a metabolic perspective, experimental diabetes in rats (model for Type 1 diabetes that causes chronic, severe hyperglycemia ∼20–40 mmol/l) results in increased nerve glucose, fructose, polyols, aldose reductase activity, protein kinase C activity, and monoenzymatic protein glycosylation as well as a reduction in nerve myoinositol and sodium potassium ATPase activity (13, 15, 29, 32, 51, 63). From a vascular perspective, experimental diabetes is linked with reduced endothelial function (37, 50) and nerve blood flow (10, 16, 36, 40, 43, 50, 64). Together, these factors contribute to nerve dysfunction (10, 16, 40, 64). Although, reduced nerve blood flow does not always precede diabetes-induced deficits in nerve function (35, 63, 71), impaired vasa nervorum reactivity is implicated in peripheral nerve dysfunction and may be related to altered nitric oxide (NO) signaling or bioavailability (17, 19, 22, 23, 37, 38, 43, 50) as well as enhanced adrenergic-mediated vasa nervorum constriction (23, 36, 63).

Insulin therapy is a common and effective treatment for Type 1 diabetes. In rats with experimental diabetes, concurrent insulin treatment prevents the decline in NO-related vasa nervorum reactivity (37). Furthermore, insulin treatment administered 1 mo following diabetes induction partially restores basal nerve blood flow and motor nerve conduction velocity (MNCV) (10). However, in the aforementioned study, insulin treatment alone did not completely reverse the effects of chronic hyperglycemia. Conceivably, in rats with experimental diabetes, insulin treatment may restore nerve blood flow and vasoreactivity partially through a NO-related mechanism. In addition to the effects of insulin treatment on basal nerve blood flow, acute insulin administration stimulates epineurial arterial vasodilation and increases sciatic nerve blood flow velocity (NBVF) in rats (24, 49). It is uncertain what compounds mediate the insulin-induced dilation (21), but glucose-stimulated insulin-mediated dilation can be prevented by NO synthase (NOS) inhibition (49). The vasodilatory actions of insulin were documented over 70 years ago (1), and since then it has been established that insulin-mediated vasodilation occurs through the activation of the intracellular enzymes phosphatidylinositol-3 kinase (PI3K) and protein kinase B (Akt), which phosphorylate and activate endothelial NOS (eNOS) (27, 45). How the relationship between insulin sensitivity and eNOS
expression influences peripheral nerve blood flow control remains to be elucidated fully.

Aside from insulin therapy, other therapeutic agents directly or indirectly targeted toward NO have been used to prevent experimental diabetes-induced reductions in nerve blood flow or vasoreactivity (17–19, 23, 37, 38, 43, 50). Nonpharmacological interventions, such as concurrent exercise training, have also been used to prevent diabetes-related peripheral neuropathy (6, 58). However, the effects of exercise training on the preservation of nerve blood flow and vasorum reactivity have yet to be established. In skeletal muscle, insulin-stimulated vasodilation is impaired by insulin resistance (68), improved by exercise training (which is known to increase insulin sensitivity) (53), and appears to be mediated in part by eNOS (45, 59, 66) as well as upstream targets pE3K and Akt (33). Thus it is plausible that chronic hyperglycemia-induced impairments in insulin signaling and NO-mediated vasodilation (10, 37, 42, 43, 50) may be avoided by concurrent exercise training (31, 67).

Previous observations suggest impaired vasa nervorum reactivity is related to altered NO signaling and peripheral nerve dysfunction (17, 19, 22, 23, 37, 38, 43, 50). Therefore, we tested the hypothesis that insulin-treated experimental diabetes reduces vasa nervorum reactivity to insulin, as well as decreases sciatic nerve eNOS expression, and that these alterations would be prevented by concurrent exercise training. To address this hypothesis we compared control sedentary rats (CS) to rats with insulin-treated experimental diabetes that were either sedentary (DS) or exercise-trained (DX).

The above question relates to the ability of exercise training to ameliorate the effects of diabetes on nerve blood flow control. However, it does not consider the impact of diabetes on exercise-trained rats alone. For example, in a previous study (33) where insulin-stimulated NO-mediated vasodilation in the cutaneous vasculature was studied in sedentary and trained rats with and without experimental diabetes, relative to their sedentary counterparts, insulin administration (10−8 mol/l) induced vasodilation in trained rats with diabetes but not trained control rats. These data suggest insulin-mediated vasodilation may be different in trained rats with and without diabetes. However, in the aforementioned study the analyses focused on the effects of trained versus untrained and not the effects of diabetes on exercise training. Therefore, to assess the effects of insulin-treated experimental diabetes on exercise-trained rats alone, we conducted a secondary study whereby the responses of DX rats were contrasted with a control exercise group (CX). This study tested the hypothesis that insulin-mediated vasa nervorum dilation would be attenuated in DX rats versus CX rats.

METHODS

Study 1

Procedures. All procedures complied with the Animal Care guidelines and ethics approval board from The University of Western Ontario. Eight-week-old Sprague-Dawley rats were obtained from Charles River Laboratories (Saint-Constant, Quebec, Canada) and were housed in pairs at a constant temperature of 20 ± 1°C with a 12-h light/dark cycle. Rats had ad libitum access to commercial chow (protein = 26%, carbohydrate = 60%, fat = 14%; enriched with vitamins and minerals; PROLAB RMH 3000, Brentwood, MO) and water. Upon arrival, rats were divided into three groups: control sedentary (CS; n = 7), insulin-treated experimental diabetes sedentary (DS; n = 9), and insulin-treated experimental diabetes exercised (DX; n = 9). Each week all rats were weighed and their fed-state blood glucose was measured (saphenous vein sample). At 10 wk (rats ~21.5 wk old) following control conditions, insulin therapy or insulin therapy and exercise training, rats were anesthetized and underwent a euglycemic hyperinsulinemic clamp, and NBFV was measured at baseline and every 10 min for 80 min. After the insulin clamp; final blood samples were collected; MNCV was measured; and the right sciatic nerves were harvested, flash frozen in liquid nitrogen, and (along with the blood samples) stored at −70°C for later analyses.

Animals and diabetes induction. To achieve insulin-treated experimental diabetes (i.e., moderately hyperglycemic state), 8-wk-old rats received daily intraperitoneal injections of 20 mg/kg streptozotocin (STZ; Sigma-Aldrich, St. Louis, MO; citrate buffer pH 4.5) for 5 consecutive days. After the confirmation of two consecutive blood glucose measures of ≥18 mmol/l, rats received a surgically implanted subcutaneous insulin pellet (release rate 1 IU/12 h, Linplant, Linshin, Toronto, Ontaria, Canada). Each week, pellet size was adjusted (increased or decreased) to maintain moderate hyperglycemia (fed-state blood glucose obtained during dark cycle = 14.5 mmol/l) (44).

Exercise training. Before the exercise-training protocol was started, rats were familiarized with treadmill running. Familiarization consisted of two 15-min incremental treadmill running exercise sessions. The exercise-training protocol consisted of running on a motorized treadmill at 27 m/min on a 6° incline, for 60 min, 5 days/wk for 10 wk. In healthy rats, this represented an exercise intensity of ~75–85% maximal oxygen consumption (VO2 max) (8).

Surgical procedures. Before surgery, rats were fasted for 12 h. They were anesthetized with inhaled isoflurane gas (4%) and an intraperitoneal injection of urethane (25 mg/kg) and α-chloralose (4 mg/kg). At doses 50 times greater, urethane cause an increase of blood glucose of ~2–3 mmol/l; however, in the present study, the dose of urethane was small and exogenous insulin during the euglycemic hyperinsulinemic clamp was used to stabilize blood glucose concentrations (4). After ~20 min, the isoflurane gas was removed and the urethane α-chloralose mixture alone maintained surgical depth. To maintain body temperature at 37°C (assessed via rectal probe) a warming blanket was placed beneath the animal. To facilitate infusions of anesthetic, insulin (10 mU·kg−1·min−1), 0.4 μU/ml; Eli Lilly, Toronto, Ontario, Canada), and glucose (20 mg·kg−1·min−1; 0.2 g/ml; EMD Millipore, Darmstadt, HE, Germany), a catheter was inserted into the right jugular vein. To measure continuous blood pressure, another catheter was inserted into the right carotid artery and connected to a pressure transducer (PX272, Edwards Lifesciences, Irvine, CA). To measure NBFV, the left sciatic nerve was exposed. This required sciatic nerve separation from surrounding muscle beds (gluteus maximus and biceps femoris) via blunt dissection (65). To identify and maintain the same nerve segment throughout the imaging protocol, a thin slice of paraffin (serving as a landmark) was woven beneath the sciatic nerve.

Euglycemic hyperinsulinemic clamp. For the original description of the euglycemic hyperinsulinemic clamp see DeFronzo et al. (25). After surgery, all rats were stabilized for ~60 min before the start of the experiment, and mean arterial pressure (MAP) as well as NBFV were stable for ~15 min before rats underwent the euglycemic hyperinsulinemic clamp protocol. Briefly, insulin infusion at 10 mU·kg−1·min−1 (0.4 IU/ml) was maintained using an infusion pump. Blood glucose concentrations were sustained using a separate pump. To maintain euglycemia, blood glucose was infused at a variable rate that was determined by glucose measures performed every 5 min for the first 20 min and every 10 min thereafter (FreeStyle Lite, Abbott Diabetes Care, Alameda, CA). Sciatic nerve blood velocity and MAP values were recorded at baseline and every 10 min for 80 min. These values were used to calculate the index of vascular conductance (VC = NBFV/MAP) and area under the curve with respect to increase above baseline (30) (AUCi; surrogate for total dilation) throughout the insulin clamp. After the euglycemic hyperinsulinemic clamp experi-
ment, a final blood sample was collected, and plasma samples were stored at −70°C for later analysis. Plasma insulin (ALPCO Diagnostics, Salem, NH) and norepinephrine (Biotang, Lexington, MA) concentrations were determined using enzyme-linked immunosorbsent assay kits.

Data acquisition and Doppler parameters. The analog blood pressure signal was sampled at 1,000 Hz (Powerlab; ADInstruments, Colorado Springs, CO). Pulsatile arterial pressure was averaged over 10 beats to calculate MAP. A 40-MHz high-frequency linear array probe (MS550D, VisualSonics, Toronto, Canada) and the Vevo 2100 ultrasound system (VisualSonics) were used to measure NBFV. The probe was positioned over the sciatic nerve and an artery was located using power Doppler. In duplex imaging mode (frequency of 32 MHz with 100% power, PRF between 4 and 5 kHz and wall filter of 40–50 Hz) the pulsed-wave Doppler gate was positioned over the power Doppler signal (insolation angle of 60°), and this arterial segment was imaged throughout the insulin clamp. At 40 MHz, this ultrasound system did not have sufficient resolution to measure changes in the sciatic nerve supply artery diameter in B-mode images. Thus peak blood flow velocity (outer envelope of the mean blood flow velocity waveform) represents a surrogate for blood flow rate.

Sciatic MNCV. Sciatic-fibular (peroneal) MNCV was measured after the insulin clamp protocol. Single, 100-μs square wave pulses were applied directly to the exposed nerve at the sciatic notch and separately in the popliteal fossa via a clinical electrical stimulator (NeuroscanComperio, Compumedics Medical Systems, El Paso, TX). The elicited compound muscle action potentials (CMAP) were recorded with tungsten electrodes inserted into the tibialis anterior. Subsequently, CMAP were displayed, stored and analyzed on a clinical electromyography (EMG) system (NeuroscanComperio, Compumedics Medical Systems, El Paso, TX). All EMG signals were sampled at 10 kHz and band-pass filtered at 10 Hz to 10 kHz. The MNCV (m/s) was calculated as: MNCV = distance between stimulation sites/time difference in CMAP latencies.

Sciatic nerve eNOS expression. After euthanasia, sciatic nerves were harvested and flash frozen in liquid nitrogen and stored at −70°C for later analysis.

Nerves were homogenized under liquid nitrogen with mortar and pestle, immersed in ice-cold lysis buffer (50 mM Tris, pH 7.5, 150 mM sodium chloride, 5 mM ethylene glycol tetraacetic acid, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate, and 1% Triton-X 100) containing protease inhibitors [104 mM 4-(2-aminoethyl)benzene-sulfonyl fluoride hydrochloride (AEBSF), 80 μM aprotinin, 2.1 mM leupeptin, 3.6 mM bestatin, 1.5 mM pepstatin A, 1.4 mM E-64, Pierce Rockford, IL], and then sonicated. Tissue lysates were centrifuged for 15 min at 14,000 rpm at 4°C. Supernatant was collected and stored at −70°C until protein concentration was determined.

Total protein concentration of samples was determined by Bradford assay. Thirty-five micrograms of protein from each sample were transferred to polyvinylidene fluoride membranes and the total protein concentration was determined.

The elicited compound muscle action potentials (CMAP) were recorded with tungsten electrodes inserted into the tibialis anterior. Subsequently, CMAP were displayed, stored and analyzed on a clinical electromyography (EMG) system (NeuroscanComperio, Compumedics Medical Systems, El Paso, TX). All EMG signals were sampled at 10 kHz and band-pass filtered at 10 Hz to 10 kHz. The MNCV (m/s) was calculated as: MNCV = distance between stimulation sites/time difference in CMAP latencies.

Statistics

Body mass (at surgery), fed-state blood glucose, insulin pellet dose, and glucose infusion rate during the insulin clamp as well as norepinephrine and insulin concentrations at the end of the insulin clamp were compared using a one-way ANOVA or independent two-tailed t-tests (Sigma Stat for Windows, version 8.0). These comparisons were divided into two separate analyses; study 1 (CS rats, DS rats, and DX rats; Table 1) and study 2 (CX rats and DX rats). The effect of group and time on baseline and peak VCi, as well as NBFV and MAP (at baseline and the peak VCi) during the insulin clamp, were studied using a mixed model ANOVA. The effects of group on AUC for VCi during the insulin clamp, MNCV, and the eNOS-to-β-actin ratio were compared using a one-way ANOVA (CS rats, DS rats, and DX rats) or independent two-tailed t-tests (CX rats and DX rats). Where necessary a post hoc Tukeys tests was used to determine the location of significance. A regression analyses for eNOS-to-β-actin ratio versus AUC for VCi was constructed and an adjusted $r^2$ was calculated. All data are presented as means ± SD and the significance level was set at $P \leq 0.05$.

RESULTS

Study 1 (CS Rats, DS Rats, and DX Rats)

Intervention data. Body mass (at surgery), fed-state blood glucose, insulin pellet dose, and glucose infusion rate during the insulin clamp as well as norepinephrine and insulin concentrations at the end of the insulin clamp are presented in Table 1. The insulin intervention and experimental protocols were the same in the two groups.

Table 1. General characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>CS</th>
<th>DS</th>
<th>DX</th>
<th>CX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass (at surgery), g</td>
<td>533 ± 43*</td>
<td>461 ± 38</td>
<td>423 ± 58</td>
<td>522 ± 43†</td>
</tr>
<tr>
<td>Fed-state blood glucose, mmol/l</td>
<td>4.4 ± 0.2</td>
<td>15.2 ± 1.1‖</td>
<td>15.0 ± 1.6‖</td>
<td>4.1 ± 0.2</td>
</tr>
<tr>
<td>Insulin pellet, IU/kg</td>
<td>NA</td>
<td>6.6 ± 2.7‖</td>
<td>3.8 ± 1.5</td>
<td>NA</td>
</tr>
<tr>
<td>Average glucose infusion rate, mg·kg⁻¹·min⁻¹</td>
<td>22 ± 2§</td>
<td>14 ± 5</td>
<td>19 ± 6</td>
<td>36 ± 6†</td>
</tr>
<tr>
<td>Norepinephrine at 80 min, pg/ml</td>
<td>129 ± 16</td>
<td>143 ± 6</td>
<td>123 ± 10§</td>
<td>125 ± 9</td>
</tr>
<tr>
<td>Insulin at 80 min, pmol/l</td>
<td>2,905 ± 727</td>
<td>3,038 ± 717</td>
<td>2,971 ± 739</td>
<td>3,104 ± 745</td>
</tr>
</tbody>
</table>

Values are means ± SD, NA, not applicable. *Significantly greater than insulin-treated experimental diabetes sedentary rats (DS) and exercise rats (DX) (P ≤ 0.02); †Significantly greater than control sedentary (CS) or exercise rats (CX) (P < 0.001); ‖Significantly greater than insulin-treated experimental diabetes exercise rats (P ≤ 0.02); §Significantly different from DS rats (P = 0.01).
Average glucose infusion rate was greater in CS rats compared with DS rats ($P = 0.01$; Table 1). There were no statistical differences for the average glucose infusion rate between DX rats and CS rats or DS rats ($P \geq 0.09$; Table 1). At the end of the insulin clamp, plasma norepinephrine concentrations were elevated in the DS rats compared with DX rats ($P = 0.01$; Table 1), but the difference in norepinephrine concentration between DS rats and CS rats did not reach significance ($P = 0.09$; Table 1). Norepinephrine concentrations were not statistically different between CS rats and DX rats ($P = 0.36$; Table 1). Circulating insulin concentrations at 80 min of the euglycemic hyperinsulinemic clamp were the same between CS rats, DS rats, and DX rats ($P = 0.94$).

**Euglycemic hyperinsulinemic clamp data.** Baseline NBFV was similar between CS rats, DS rats, and DX rats ($P > 0.99$; Fig. 1A). In DS rats, peak nerve blood velocity was greater during the EHC than baseline ($P = 0.01$; Fig. 1A). However, peak NBFV was attenuated in DS rats compared with CS rats and DX rats ($P < 0.01$; Fig. 1A). There were no main or interaction effects for MAP when comparing CS rats, DS rats, and DX rats ($P \geq 0.15$; Fig. 1B). Baseline VC$_s$ was similar between CS rats, DS rats, and DX rats ($P = 0.68$; Fig. 1C). In DS rats the peak VC$_s$ was greater than baseline ($P < 0.01$; Fig. 1C). Nonetheless, the peak VC$_s$ was attenuated in DS rats compared with CS rats and DX rats ($P \leq 0.01$; Fig. 1C). The AUC$_s$ for the VC$_s$ throughout the insulin clamp was lower in DS rats compared with CS rats and DX rats ($P < 0.01$; Fig. 1D).

**Data collected following the insulin clamp.** Motor nerve conduction velocity was lower in DS rats compared with CS rats and DX rats ($P \leq 0.01$; Fig. 2B). Expression of eNOS was greater in DX rats compared with DS rats ($P = 0.04$; Fig. 2A). There were no differences in eNOS expression between CS rats and DS rats or CS rats and DX rats ($P \geq 0.31$; Fig. 2A).

**Study 2 (DX Rats and CX Rats)**

**Intervention data.** Body mass was greater in CX rats versus DX rats ($P < 0.01$; Table 1). The 10-wk average fed-state blood glucose was greater in DX rats versus CX rats ($P < 0.001$; Table 1). Average glucose infusion rate was greater in

![Fig. 1](http://ajpregu.physiology.org/)

**Fig. 1.** A: nerve blood flow velocity (mm/s) at baseline and peak vascular conductance during the euglycemic hyperinsulinemic clamp in control sedentary (CS; solid bar) rats, diabetes sedentary (DS; shaded bar) rats, and diabetes exercise (DX; open bar) rats. B: mean arterial pressure (mmHg) at baseline and peak vascular conductance during the euglycemic hyperinsulinemic clamp. C: baseline and peak sciatic nerve arterial index of vascular conductance (VC$_s$; mm·s$^{-1}$·mmHg$^{-1}$) measured during the euglycemic hyperinsulinemic clamp. D: area under the curve (AUC$_s$) for the VC$_s$ (mm·s$^{-1}$·mmHg$^{-1}$·80 min $^{-1}$) measured throughout the euglycemic hyperinsulinemic clamp. †Significantly greater than baseline ($P \leq 0.01$). *Significantly less than CS and DX rats ($P \leq 0.01$).
CX rats versus DX rats (P < 0.01; Table 1). There were no differences in plasma norepinephrine between CX rats and DX rats (P = 0.71; Table 1).

**Euglycemic hyperinsulinemic clamp data.** There was a main effect of time for MNCV (peak > baseline; P < 0.01; Fig. 3A) and MAP (baseline > peak; P < 0.01; Fig. 3B) in CX rats and DX rats. There was a main effect of time for the VC1 in CX rats and DX rats (peak > baseline; P < 0.01; Fig. 3B). However, AUC1 for VC1 was reduced in DX rats versus CX rats (P = 0.03; Fig. 3D).

**Data collected following the insulin clamp.** Sciatic nerve MNCV and eNOS expression were lower in DX rats versus CX rats (P ≤ 0.02; Fig. 4, A and B).

**Group data for eNOS expression and AUC1 for the VC1 during the insulin clamp.** The correlation coefficient between eNOS expression and AUC1 for the VC1 during the insulin clamp was 0.54 (adjusted r2; N = 21; P < 0.01; Fig. 5).

**DISCUSSION**

In study 1, insulin-treated experimental diabetes had little impact on basal VC1 but severely diminished the VC1 response to systemic insulin infusion and decreased MNCV. In rats with insulin-treated experimental diabetes, exercise training pre-served vasa nervorum NBFV responses, as well as MNCV, at levels observed in the CS group. Also, in rats with insulin-treated experimental diabetes that exercise trained, eNOS expression was elevated compared with their sedentary counterparts (DS rats). In study 2 (CX rats and DX rats), diabetes was associated with reduced vasa nervorum responsiveness to insulin and nerve function. Therefore, in this model, insulin treatment combined with exercise training attenuated the effects of chronic, moderate hyperglycemia on vasa nervorum reactivity to insulin and MNCV. However, study 2 demonstrated that in exercise-trained rats alone (CX vs. DX), chronic, moderate hyperglycemia lowers the level of insulin-stimulated vasa nervorum dilation, and reduces MNCV.

It is uncertain whether hyperglycemia or hypoinsulinemia causes the reduced nerve blood flow commonly observed in experimental diabetes. Chronically severe hyperglycemia often reduces basal nerve blood flow (10, 16, 36, 40, 43, 50, 64), but conflicting evidence exists (20, 35, 51, 60, 71). In the aforementioned studies linking experimental diabetes with reductions in nerve blood flow, chronic, severe hyperglycemia values in rats (~20–40 mmol/l) exceeded representative ranges observed in humans with insulin-treated Type 1 diabetes mellitus. The standard glycemic status for patients with poorly controlled Type 1 diabetes mellitus is ~9% glycylated hemoglobin (54), which corresponds to ~13.5 mmol/l blood glucose concentration (56). Thus experimental diabetes in rats that emphasizes the pronounced effects of chronic, severe hyperglycemia may not represent the insulin-treated, Type 1 diabetes mellitus observed clinically.

Based on VC1 (or NBFV) in the current study, baseline blood flow values were similar between insulin-treated experimental diabetes and control groups. This may have been the result of the chronic insulin treatment used in the present study or, alternatively, chronic hyperglycemia does not reduce basal nerve blood flow (20, 35, 51, 60, 71). Acute insulin administration stimulates nerve arterial vasodilation (20, 41), and prolonged insulin treatment in rats with experimental diabetes can prevent declines in vasoactivity (30) and partially restore decrements in basal nerve blood flow (8). In study 1, the DX rats received a lower insulin (pellet) dose but achieved the same level of hyperglycemia as the DS rats. The effect of hyperglycemia on baseline VC1 or NBFV may have been reduced in both DS rats and DX rats, as the blood glucose concentrations observed in the current study were substantially less than those recorded during previous experimental diabetes/nerve blood flow studies (10, 16, 36, 40, 43, 50, 64). Considering blood glucose and basal VC1 were similar between the two groups with diabetes, the smaller insulin dose in DX versus DS rats suggests that insulin sensitivity and not total insulin contributes to the maintenance of the VC1 under basal conditions.

In study 1, insulin-stimulated VC1 responses were attenuated in DS rats. In addition, compared with CS rats, DS rats required a lower glucose infusion rate during the clamp to maintain euglycemia. Together, these data are suggestive of depressed insulin sensitivity. Insulin-mediated vasodilation operates through the activation of intracellular enzymes PI3K and Akt, and the subsequent phosphorylation and activation of eNOS (27, 45). Therefore, reduced insulin-stimulated nerve arterial dilation in DS rats may be explained by an impaired NO signaling mechanism. For example, glucose-stimulated...
insulin-mediated increases in the VCₐ operate through a NO mechanism (41). Also, rats with experimental diabetes express impaired NO-mediated vasa nervorum dilation (37, 43), and reductions in basal nerve blood flow can be reversed with NO-donor treatment (17). Likewise, experimental diabetes impairs insulin-mediated vasodilation in the cutaneous microvasculature, likely through the PI3K pathway (33). In study 1, sciatic nerve eNOS expression was not statistically different between CS and DS rats, but the overall impact of hyperglycemia on upstream eNOS signaling, NO bioavailability, and reactivity were not examined.

Sympathetic overactivity may provide another explanation for the attenuated VCₐ response in DS rats. Insulin stimulates the sympathetic nervous system and elicits a robust norepinephrine response (41, 57) and, in turn, norepinephrine can cause nerve arterial vasoconstriction (43, 72). Furthermore, perivascular adrenergic innervation in the sciatic nerve (26) and vasa nervorum sensitivity to norepinephrine increases in rats with experimental diabetes (43). Whether exercise training reduces perivascular adrenergic innervation in the sciatic nerve or decreases vasa nervorum norepinephrine sensitivity is unknown. Evidence from humans suggests insulin resistance is associated with reduced norepinephrine clearance (7), consistent with the present observations. Thus, in study 1, increased norepinephrine (potentially the result of the diabetes, the insulin infusion, or an interaction of these variables) alongside increased responsiveness to adrenergic activity in the vasa nervorum may have restrained insulin-mediated dilation.

The lower insulin-stimulated VCₐ responsiveness in DS rats was abolished by concurrent exercise training. The ability of insulin to stimulate a greater vasa nervorum response in DX rats compared with DS rats may have been influenced by their enhanced insulin sensitivity (indicated by a lower insulin dose during the study in DX rats vs. DS rats). To achieve the same level of hyperglycemia in DS rats and DX rats, DX rats required less exogenous insulin. This suggests DX rats were more responsive to insulin and supports the findings that when exposed to the same level of hyperinsulinemia during the clamp, the vasodilatory response to insulin was greater in DX rats versus DS rats. Alternatively, the elevated sciatic nerve eNOS content or reduced circulating norepinephrine may help explain the preservation of the insulin-stimulated VCₐ response observed in the DX rats in study 1. In rats with experimental diabetes, exercise training enhances insulin-mediated microvascular vasodilation in cutaneous tissues, likely through a PI3K/eNOS mechanism (27, 33). Although NO bioavailability

![Figure 3](http://ajpregu.physiology.org/)

**Fig. 3.** A: nerve blood flow velocity (mm/s) at baseline and peak vascular conductance during the euglycemic hyperinsulinemic clamp in CX (hatched bar) rats and DX (open bar) rats. B: mean arterial pressure (mmHg) at baseline and peak vascular conductance during the euglycemic hyperinsulinemic clamp. C: baseline and peak sciatic nerve arterial VCₐ (mm·s⁻¹·mmHg⁻¹) measured during the euglycemic hyperinsulinemic clamp. D: AUCₐ for the VCₐ (mm·s⁻¹·mmHg⁻¹·80 min⁻¹) measured throughout the euglycemic hyperinsulinemic clamp. *Significantly less than CX (P = 0.03).
was not assessed in the present study, it is important to note both DX rats and DS rats were exposed to similar levels of hyperglycemia throughout the study and were normoglycemic during the insulin clamp. Therefore, any effects of hyperglycemia on NO bioavailability should have been similar between the two groups.

**Perspectives and Significance**

By intention, blood glucose values in the current study were maintained at a moderate hyperglycemic concentration and similar to clinical values observed in people with poorly controlled Type 1 diabetes mellitus (49). In study 1, compared with control and DX groups, sedentary rats with insulin-treated experimental diabetes had lower insulin sensitivity and attenuated vasa nervorum responsiveness during the insulin clamp. In addition, MNCV was lower in DS rats indicating a possible loss of large myelinated motor units and alterations in nerve physiology (5, 52, 58). Therefore, despite having similar baseline VCi or NBFV values, similar blood glucose concentrations and greater circulating insulin concentrations compared with the DX group, DS rats displayed impaired nerve function. These data suggest that basal blood and hemodynamic variables may be inadequate clinical markers of vasa nervorum health and that functional tests are necessary to detect the onset of decrements to vasa nervorum and nerve function. Furthermore, it appears that impaired insulin responsiveness and peripheral neuropathy were not caused by lower basal nerve blood flow, hyperglycemia, or hypoinsulinemia and presumably were the result of a combination of hyperglycemia and inactivity. Insulin treatment alone was insufficient to preserve vasa nervorum and nerve function in a rat model of chronic, moderate hyperglycemia.

Exercise training appears to counteract the effects of insulin-treated experimental diabetes on nerve function and vasa nervorum dilation, possibly by increasing insulin responsiveness, augmenting eNOS expression and altering sympathetic function. With similar blood glucose, but lower basal insulin concentrations in DX rats versus DS rats, it appears the effects of insulin to dilate the vasa nervorum were amplified by exercise training. Whereas exercise training has been observed to maintain nerve morphology (including large myelinated motor units) in rats with experimental diabetes (58), interventions to prevent deficits in vasa nervorum function have focused largely on pharmacological means (14, 23, 34, 40, 46–48, 55). The current data suggest exercise training is a clinically effective treatment to avoid vasa nervorum and nerve dysfunction. Furthermore, unlike medication, exercise training offers additional benefits that prevent other chronic diseases and diabetes-related comorbidities as well as promote health and longevity in populations with and without Type 1 diabetes mellitus (11, 21). A future study that investigates the relationship between diabetes and exercise training at various time points throughout an intervention (i.e., pre-, peri- and postintervention) would help clarify the progression, prevention, and treatment of vasa nervorum dysfunction.

![Fig. 4.](image)

**Fig. 4.** A: MNCV (m/s) measured following the euglycemic hyperinsulinemic clamp in CX (hatched bar) rats and DX (open bar) rats. B: eNOS expression (normalized to β-actin) in the sciatic nerve. *Significantly less than CX (P ≤ 0.02).

![Fig. 5.](image)

**Fig. 5.** Linear regression between eNOS expression and AUCi for the VCi (mm·s⁻¹·mmHg⁻¹·80 min⁻¹) measured throughout the euglycemic hyperinsulinemic clamp in CS rats, CX rats, DS rats, and DX rats. Adjusted $r^2 = 0.54; P < 0.01$. 

---

**R947 INSULIN-STIMULATED NERVE ARTERIAL DILATION**

*AJP-Regul Integr Comp Physiol* • doi:10.1152/ajpregu.00508.2013 • www.ajpregu.org
Study 2. In exercise-trained rats, insulin-treated experimental diabetes attenuates vasa nervorum reactivity and nerve health. For example, compared with CX rats, DX rats displayed lower vasa nervorum responsiveness to insulin (indicated by a lower AUC for VC_i during the insulin clamp), had lower MNCV, and lower sciatic nerve eNOS expression. Therefore, while exercise training is beneficial, it does not abolish the effects of chronic moderate hyperglycemia.

Considerations. A limitation in the current study may be that the artery insonated was too small to resolve accurately with the ultrasound B-mode imaging device. Therefore, it was assumed that the diameter of the artery did not change between baseline and the insulin clamp conditions. This may have resulted in an underestimation of the dilatory response.

Also, because Doppler ultrasound measures NBFV from a single epineurial vessel, it may not be representative of total flow. Epineurial arteries may travel along the nerve or branch into the nerve (61) and become peri- or endoneurial arteries, but these vascular networks may possess different control features and it was not possible to quantify endoneurial-specific flow. In humans with diabetes and poor glycemic control, epineurial blood vessels are often tortuous and subject to arteriovenous shunting (62, 69). This may result in a redistribution of blood flow that favors greater epineurial versus endoneurial blood flow. Thus, because Doppler ultrasound is limited to a single epineurial vessel, it may not capture reductions in endoneurial-specific flow. However, the sciatic nerve contains feed arteries originating from multiple locations that do not necessarily branch into a capillaryplexus and has been described as an anastomotic vascular network that may contain bidirectional flow (3, 9, 70). Such a pattern of tissue perfusion limits the interpretation of gross flow measurements and highlights the benefit of quantifying exclusive arterial inflow responses.

Another possible limitation is that nerve temperature may have fluctuated and influenced the dilatory response to insulin, as well as MNCV (2). Kihara et al. (39) documented a positive relationship between limb temperature and nerve blood flow, whereas Dines et al. (28) reported an inverse relationship between limb temperature and nerve blood flow, with the effect of temperature being attenuated in rats with diabetes. In the present experiment room and body temperature were kept constant, but nerve temperature was not recorded. The exposure of the sciatic nerve may have led to a decrease in limb temperature. However, any effects of temperature likely occurred during the stabilization period and would have been consistent between groups. Also, baseline VC_i values were similar between groups and, despite nerve exposure and possible limb cooling, all rats displayed vasodilation.

Also, during the insulin clamp, the total volume load each rat received would have been different. Insulin infusion rate was based on mass and glucose infusion rate was variable and based on insulin sensitivity. Notably, CX rats were more insulin sensitive and required a greater glucose infusion rate to maintain euglycemia. Potentially, the increased volume associated with the elevated glucose infusion rate may have contributed to their augmented VC_i. However, despite a higher glucose infusion rate in CX rats, peak NBFV and VC_i were similar between CX rats and DX rats. Also, volume-induced increases in flow velocity would likely occur on account of increased perfusion pressure, but during the insulin clamp there was a main effect of time (baseline > peak) for MAP in CX rats and DX rats, suggesting blood pressure decreased. The vasa nervorum does not autoregulate but instead responds passively to changes in MAP. Therefore, the increase in the VC_i (increases in NBFV that are not caused by increases in MAP) during the insulin clamp was likely not the result of the volume load but instead the vasodilatory properties of insulin.

Although glucose-stimulated insulin-mediated dilation appears to operate through a NO mechanism (41), the exact NO isoform mediating the insulin dilation is unknown. The vasa nervorum dilatory response to acetylcholine and insulin may differ (20), suggesting insulin may function through an eNOS independent pathway. In the present study, eNOS expression alone was quantified, but upstream targets such as PI3K were not. Furthermore, posttranscriptional or translational modifications to eNOS as well as NO bioavailability were not measured. To our knowledge, no studies to date have elucidated the contribution of each NO isoform to insulin-mediated dilation.

In conclusion, insulin-treated experimental diabetes lowered whole body insulin sensitivity, insulin-stimulated VC_i responses, as well as MNCV. These effects were offset by concurrent exercise training in a separate group of rats with insulin-treated experimental diabetes (DX rats). However, when compared with CX rats, DX rats exhibited lower whole body insulin sensitivity, vasa nervorum responsiveness, MNCV, and sciatic nerve eNOS expression. From this perspective, exercise training is an effective treatment for insulin-treated diabetes by lowering the need for exogenous insulin and improving neural outcomes.

GRANTS

This study was funded by the Canadian Institutes of Health Research (CIHR) Team Grant in Physical Activity, Mobility and Neural Health (no. 217532) (to K. Shoemaker, Nominated Principal Investigator). K. Shoemaker is a Canada Research Chair in Integrative Physiology of Exercise and Health.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES

Insulin-Stimulated Nerve Arterial Dilatation


sucrose-fed Otsuka Long-Evans Tokushima fatty rats: effect of an aldose
DA. Evaluation of alpha(1)-adrenoceptor antagonist on diabetes-induced
changes in peripheral nerve function, metabolism, and antioxidative de-
49. Oliver TD, Mattar L, Grisé KN, Twynstra J, Noble EG, Lacefield JC,
Shoemaker JK. Glucose-stimulated insulin secretion causes an insulin-
dependent nitric oxide-mediated vasodilation in the blood supply of the rat
sciatic nerve. Am J Physiol Regul Integr Comp Physiol 305: R157–R163,
2013.
50. Omawari N, Dewhurst M, Vo P, Mahmood S, Stevens E, Tomlinson
DR. Deficient nitric oxide responsible for reduced nerve blood flow in
diabetic rats: effects of l-NAME, l-arginine, sodium nitroprusside and
51. Pugliese G, Tilton RG, Speedy A, Chang K, Santarelli E, MAP, Eades
Ramji N, Toth C, Kennedy J, Zochodne DW. Exercise training im-
proves insulin-mediated capillary recruitment in association with glucose
52. Ramji N, Toth C, Kennedy J, Zochodne DW. Does diabetes mellitus
53. Rattigan S, Wallis MG, Youm JD, Clark MG. Exercise training im-
proves insulin-mediated capillary recruitment in association with glucose
54. Riveline JP, Schapelynck P, Chaillous L, Renard E, Sola-Gazagnes A,
Penfornis A, Tubiana N, Sulmont V, Catargi B, Lukas C, Rademecker
RP, Thivolet C, Moreau F, Benhamou PY, Guerci B, Leguerrier AM,
Penfornis A, Tubiana N, Sulmont V, Catargi B, Lukas C, Rademecker
RP, Thivolet C, Moreau F, Benhamou PY, Guerci B, Leguerrier AM,
Millot L, Sachon C, Charpentier G, Hanaire H. Assessment of patient-
led or physician-driven continuous glucose monitoring in patients with
poorly controlled type 1 diabetes using basal-bolus insulin regimens. Diabe-
55. Robertson S, Cameron NE, Cotter MA. The effect of the calcium
antagonist nifedipine on peripheral nerve function in streptorotoxin-dia-
56. Rohling CL, Weidmeyer HM, Little RR, England JD, Tennill A,
Goldstein DE. Defining the relationship between plasma glucose and
57. Rowe JW, Young JB, Minaker KL, Stevens AL, Pallotta J, Landsberg
L. Effect of insulin and glucose infusions on sympathetic nervous system
58. Severo Do Nascimento P, Lovatel GA, ilha J, Schaan BD, Achaval M
Diabetes increases mechanical sensitivity and causes morphological ab-
normalities in the sural nerve that are prevented by treadmill training.
59. Steinberg H, Brechtel G, Johnson A, Fineberg N, Baron AD. Insulin-
mediated skeletal muscle vasodilation is nitric oxide dependent. A novel
action of insulin to increase nitric oxide release. J Clin Invest 94:
60. Suter SP, Chang K, Marvel J, Williamson JR. Concurrent increases in
regional hematocrit and blood flow in diabetic rats: prevention by sorbinil.
61. Tesfaye S, Chaturvedi N, Eaton SE, Ward JD, Manes C, Lonescu-
Tirgoviste C, Witte DR, Fuller JH. Vascular risk factors and diabetic
62. Tilton RC, Chang K, Nyengaard JR, Van den Enden M, Ido Y,
Williamson JR. Inhibition of sorbitol dehydrogenase. Effects on vascular
and neural dysfunction in streptozocin-induced diabetic rats. Diabetes 44:
63. Tuck RR, Schmelzer JD, Low PA. Endoneurial blood flow and oxygen
tension in the sciatic nerves of rats with experimental diabetic neuropathy.
64. Uysal CA, Mizuno H, Hyakusoku H. Sciatic nerve anatomy in rat
revisited: a more proximal intervention. J Plast Reconstr Aesthet Surg 62:
65. Vincent MA, Barrett EJ, Lindner JR, Clark MG, Rattigan S. Inhib-
itng NOS blocks microvascular recruitment and blunts muscle glucose
uptake in response to insulin. Am J Physiol Endocrinol Metab 285:
66. Walberg-Henriksson H, Gunnarsson R, Henriksson J, Defronzo R,
Felg P, Ostman J, Wahren J. Increased peripheral insulin sensitivity and
muscle mitochondrial enzymes but unchanged blood glucose control in
67. Wallis MG, Wheatley CM, Rattigan S, Barrett EJ, Clark ADH, Clark
MG. Insulin-mediated hemodynamic changes are impaired in muscle of
68. Ward JD. Abnormal microvasculature in diabetic neuropathy. J Clin
EL, Holdsworth DW, Shoemaker JK. Intrinsc microvasculature of the
70. Zochodne DW, Ho IT. Normal blood flow but lower oxygen tension in
diabetes of young rats: microenvironment and the influence of sympathe-
71. Zochodne DW, Low PA. Adrenergic control of nerve blood flow. Exp