Glucose-Stimulated Insulin Secretion Causes an Insulin-Dependent Nitric Oxide-Mediated Vasodilation in the Blood Supply of the Rat Sciatic Nerve

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Abstract
This study tested the hypothesis that acute hyperglycemia reduces sciatic nerve blood flow in Sprague-Dawley rats. Anaesthetized rats underwent cannulation of their right jugular vein (for anaesthetic/nutrient/drug infusion) and right carotid artery (for continuous blood pressure measurement via pressure transducer). The left sciatic nerve was exposed and nerve blood velocity (NBV) was assessed from an arterial segment lying superficially along the sciatic nerve (Doppler ultrasound, 40 MHz, VisualSonics Vevo 2100). NBV and mean arterial pressure (MAP) values were collected, and an index of nerve vascular conductance (NVC) was made (NBV/MAP) at baseline and at 5, 10, 20 and 30 min (and 80 min for insulin) following i) Low Glucose infusion 1 g/kg (50% solution), ii) High Glucose infusion 3 g/kg, iii) High Glucose infusion in the absence of a functioning pancreas, iv) euglycemic hyperinsulinemic clamp - insulin infusion (10 mU/kg/min; 0.4IU/mL), v) High Glucose infusion + L-NAME infusion (30 mg/kg) and vi) L-NAME alone followed 20 min later by High Glucose infusion. High Glucose infusion increased NVC ~120% relative to baseline (P<0.001) and this dilation was attenuated in rats without a functioning pancreas (i.e. without insulin secretion) (P=0.004) and following L-NAME infusion (P=0.011). Therefore, the vasodilation in rat sciatic nerve during glucose infusion was dependent upon the insulin response and acted through a nitric oxide synthase pathway.

Keywords: Nerve Blood Flow; Glucose; Insulin; NO; Doppler ultrasound
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Introduction:

Nerve metabolism and function relies heavily on glucose as a substrate and, therefore, proper glucose and insulin regulation (6, 8, 9, 11, 14, 20, 25). Experimental diabetes (ie. chronic overt hyperglycemia) appears to decrease nerve blood flow in rats (6, 16, 25) and insulin treatment may partially restore such decrements (6). Unlike skeletal muscle blood flow, the acute hemodynamic actions of glucose and/or insulin on nerve blood flow control remain unclear (6, 8, 20). In skeletal muscle, acute insulin infusion (1, 19) or glucose-stimulated insulin secretion (GSIS; 3, 29) stimulates a nitric oxide (NO)-mediated (21, 24) vasodilation. This vasodilation enhances insulin binding and glucose uptake in skeletal muscle (4). Whether a similar hemodynamic pathway exists in the vasa nervorum requires further study.

Using video-microscopy, Davidson and colleagues documented insulin-stimulated epineurial vasodilation (8). Applying the laser Doppler method, Biessels and co-workers likewise observed an increase in nerve red blood cell flux following insulin infusion in streptozotocin induced diabetic rats (6). These data suggest that glucose may lead (via insulin stimulation) to neural artery vasodilation.

In contrast, Saini and colleagues (20) observed reductions in nerve blood flow following glucose infusion (3 g/kg). In the aforementioned experiments, insulin increased (8) or restored (6) nerve blood flow, but GSIS did not (20). Potentially, hyperglycemia attenuates nerve blood flow and insulin-stimulated vasodilation (20). This vasoconstrictor effect of glucose on the nerve vasculature (19) may relate to adverse effects of hyperglycemia on NO production and availability (7, 12, 16) or potentially to a glucose inhibitory effect on adenosine-mediated dilation (20). The discrepancies between these studies and outcomes also may result from variations in methods used to measure nerve blood flow. Nonetheless, a detailed examination of
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the relationship between acute glucose concentration, insulin secretion and nerve arterial vascular conductance warrants further study.

Therefore, the purpose of this study was to assess the independent vasoactive effects of acute hyperglycemia and hyperinsulinemia on an index of vascular conductance in the sciatic nerve vasculature of rats. Based on data by Saini and colleagues (20), this study tested the hypothesis that glucose infusion will reduce nerve blood flow and vascular conductance through an NO-independent vasoconstrictor mechanism. In contrast to the hypothesis, the results indicated that glucose stimulated an insulin-mediated, NO-dependent dilation in sciatic nerve blood supply.

Methods:

Procedures and Animals

All procedures complied with the Animal Care guidelines and ethics approval board from The University of Western Ontario. We studied a total of 37 male Sprague Dawley rats (238-400 g) within four separate and sequential experiments whose objectives were as follows: 1) determine the effect of glucose on whole leg blood flow (femoral artery) which includes and emphasizes the skeletal muscle vasculature; 2) determine the effect of glucose infusion at two doses (Low; 1 and High; 3 g/kg) on sciatic nerve vasomotor control; 3) determine if the glucose-stimulated change in nerve vascular control in #2 above was stimulated by concurrent insulin release; and 4) determine if the glucose-stimulated and insulin-mediated nerve vasomotor response in objectives #2 and #3 operated through an NO-dependent mechanism.

To assess the effect of hyperglycemia on nerve vasomotor control independent from the accompanying insulin response, we implemented a non-insulin secreting rat model (NIS). In this
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model rats received a 60 mg/kg streptozotocin (STZ; Sigma-Aldrich, St. Louis, MO, USA; citrate buffer pH 4.5) injection to impair pancreatic-beta cell function and attenuate GSIS. Forty eight hours after STZ injection (blood glucose ≥16 mmol/L), these rats also received a surgically implanted subcutaneous insulin pellet (Linplant, Linshin, Toronto, ON,CAN; release rate 1 IU/12 h) to reverse hyperglycemia and maintain normoglycemia. Experimental testing took place ~72 h post implantation of the insulin pellet.

Surgical Procedures

Surgeries were performed on the animals after a 12-h overnight fast. To achieve an appropriate surgical depth, rats inhaled isoflurane gas (5%). Thereafter, rats underwent an intraperitoneal injection of urethane (50 mg/kg) and α-chloralose (8.0 mg/kg) at the onset of surgery. After ~ 30 min, the urethane α-chloralose mixture alone maintained surgical depth for the remainder of the experiment. A warming blanket placed beneath the animal maintained rat body temperature at 37°C (rectal probe). Catheter insertion into the right jugular vein facilitated all anaesthetic, glucose (EMD Millipore, Darmstadt, HE, Germany), insulin (Eli Lilly, Toronto, ON, CAN) and L-NG-nitroarginine methyl ester (L-NAME; Sigma-Aldrich, St. Louis, MO, USA) infusions. A second catheter was inserted into the right carotid artery and connected to a pressure transducer (PX272, Edwards Lifesciences, Irvine, CA, USA) to enable continuous blood pressure measurement. Subsequently, the left femoral artery or sciatic nerve blood supply was exposed. Sciatic nerve arterial exposure required sciatic nerve separation from surrounding muscle beds (gluteus maximus and biceps femoris) via blunt dissection (26). Following separation, parafilm weaved beneath the sciatic nerve and the underlying tissue provided a landmark to ensure visual consistency throughout the imaging protocol.

Experimental Protocol
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Animals were allowed to stabilize for one hour after surgery. Thereafter, anaesthetized rats underwent one of seven experimental intravenous infusion protocols over the four experiments outlined above. To establish the effect of GSIS on skeletal muscle blood flow (i: n=6; 297±37 g) we measured femoral blood velocity (FBV) following the Low Glucose (50% solution) infusion via the jugular cannula (experiment 1). To examine the effect of GSIS on nerve vasomotor control, we measured nerve blood velocity (NBV) and mean arterial pressure (MAP) during infusion of Low Glucose (ii: n=5; 284±30 g), or High Glucose (iii: n=6; 291±57 g) (experiment 2). To establish whether the endogenous glucose-induced increase in insulin caused the observed effect (see Results), we assessed NBV and MAP during infusion of High Glucose alone in NIS rats (iii: n=5; 350±41 g) or during administration of a euglycemic hyperinsulinemic clamp (EHC; v: insulin: 10 mU/kg/min; 0.4UI/mL; n=6; 260±9 g) (experiment 3). To determine if the GSIS affected nerve vasomotor control through an NO pathway, we co-infused High Glucose with L-NAME (non-specific NOS blocker, 15 mg/kg) (iv: n=5; 357±48 g) (experiment 4). In a separate group (v: n=5; 277±8 g), to assess whether the effects of L-NAME were exclusively on baseline values or if they inhibited the response to glucose infusion, L-NAME (15 mg/kg) infusion occurred alone at baseline and preceded High Glucose infusion by 20 min (experiment 4). In each of the above protocols (excluding the EHC), isovolumetric saline infusions were also made. In each protocol, measures of MAP, FBV or sciatic NBV were made at baseline, 5, 10, 20 and 30 min (and 80 min for EHC) following the infusion. The index of vascular conductance (VC) was calculated as VC= peak BV/MAP.

Details of the Euglycemic Hyperinsulinemic Clamp

For the original description see Deforonzo et al. (10). Briefly, an infusion pump maintained insulin infusion at 10 mU/kg/min (0.4 IU/mL) and induced a hyperinsulinemic state.
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Using a separate pump, glucose infusion commenced at 20 mg/kg/min (20% solution) to defend euglycemia. Maintenance of euglycemia was made through adjustments to the glucose infusion rate that, in turn, were based on blood glucose measurements made every five minutes during the first 20 min, and every 10 min thereafter.

Data Acquisition and Analysis

The analog blood pressure signal was sampled at 1000 Hz and stored online (Powerlab; ADInstruments, Colorado Springs, CO, USA). Pulsatile arterial pressure over ~ 10 heart beats was averaged to calculate MAP.

Blood flow velocity was measured using Doppler ultrasound (Vevo 2100 ultrasound system VisualSonics, Toronto, CA) and a 40-MHz linear array probe (MS550D) transducer placed superficially along the femoral artery or an artery lying superficially along the sciatic nerve. The arteries were located using power or colour Doppler. Thereafter, this segment was studied using duplex imaging mode (frequency of 32MHz with 100% power, PRF between 4-5 kHz and wall filter of 40-50 Hz) with the pulsed wave Doppler gate (~0.12 mm width) positioned over the site of high power Doppler signal (insonation angle of 60°). The smallest vessels that could be detected in B-mode images acquired using the 40-MHz linear array probe were ~130 um diameter (17). Based on the width of the power Doppler image, it has been approximated that the size of a sciatic supply artery vessel is ~70 - 80 um. Moreover, the B-mode caliper resolution was ~15 um/pixel. Therefore, insufficient resolution existed for confident measurements of either femoral or nerve arterial diameter changes that might have occurred in this study. Nonetheless, the greatest vasomotor changes occur downstream in the microvascular bed and these changes will form the dominant contribution to total changes in
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flow velocity through the insonated vessel segment. Therefore, blood flow velocity represents our surrogate for blood flow volume.

Blood samples obtained from the arterial catheter line enabled measurements of glucose (FreeStyle Lite, Abbot Diabetes Care, Alameda, CA, USA) and insulin (enzyme linked immunosorbent assay kit for human and rat insulin; ALPCO Diagnostics, Salem, NH, USA) concentrations.

Statistics

Statistical analyses were performed using Sigma Stat for Windows (version 8.0). Paired two-tailed t-tests were used to test the effect of infused agent on NBV and MAP (baseline versus peak response) for within-group comparisons. The interaction effect of group and time on the glucose, insulin and NVC changes were assessed using a mixed model (one-factor repetition) ANOVA. Where necessary, post-hoc Tukey tests determined the location of significance. The significance level was set at $P<0.05$. Data are presented as mean ± SD.

Results:

General observations

Blood glucose and insulin concentrations: Glucose infusion produced a dose-dependent rise in blood glucose concentration (3 >1 g/kg; $P<0.001$; Table 1). Glucose concentration in the NIS group following High Glucose surpassed the measuring range of the glucometer (>32 mmol/L). Insulin concentration in the High Glucose (3 g/kg) and hyperinsulinemic clamp groups exceeded those observed in the Low Glucose (1 g/kg) and NIS groups ($P<0.001$; Table 1).
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Nerve blood velocity and mean arterial pressure: Compared to their respective baseline levels, sciatic nerve NBV was greater in i) High Glucose, ii) High Glucose + L-NAME, and iii) insulin, (P≤0.05; Table 2). In all cases L-NAME significantly elevated MAP (P≤0.05; Table 2).

Experiment 1: Impact of glucose on femoral blood velocity, mean arterial pressure and femoral vascular conductance

In the femoral artery, infusion of Low Glucose increased FBV from 223±60 mm/s at baseline to 432±94 mm/s at peak (P=0.001) with no change in MAP (baseline=87±17 vs peak=94±19 mmHg; P=0.153). Consequently, FVC increased from 2.7±0.8 mm/s/mmHg at baseline to 4.8±1.5 mm/s/mmHg at peak (P=0.007; Fig 1).

Experiment 2: Effect of glucose infusion at Low and High doses on sciatic nerve vasomotor control

Compared to baseline, Low Glucose did not alter NVC. However, High Glucose infusion increased NVC above baseline (P<0.001; Figure 2).

Experiment 3: Determine whether glucose stimulates an insulin-dependent or independent dilation

First, the nerve arterial vascular response to hyperglycemia was measured in the NIS group. In this model, glucose does not stimulate insulin secretion and insulin concentrations remain stable (pellet release rat1 IU/12). Compared with baseline, we observed no change in NVC following High Glucose infusion (P=0.66; Figure 3). Second, the nerve arterial vascular response was assessed during the euglycemic hyperinsulinemia clamp (EHC). A group x time interaction (P=0.003) was observed where the EHC caused an increase in NVC compared to both baseline EHC (P<0.001) and the peak NIS value (P=0.004; Figure 3).
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Experiment 4: Determine if the glucose-mediated and insulin-dependent nerve vasomotor response was dependent upon NO production

This experiment examined whether NO mediated the glucose-stimulated insulin-dependent dilation. The addition of L-NAME 20 min pre-, or co-infused with, High Glucose abolished any increase in NVC induced by High Glucose alone (P>0.2; Figure 4). A group x time interaction (P<0.001) was observed where, compared with other conditions, High glucose alone stimulated increases in NVC (all comparisons P≤0.011; Figure 4).

Discussion:

The major new findings of the current study are: 1) glucose infusion did not cause a decrease in NVC, 2) High, but not Low, Glucose resulted in increases in NVC (~120% from baseline), 3) GSIS mediated the nerve arterial dilation in response to glucose infusion, and 4) GSIS-mediated dilation was caused by an NO mechanism. These findings do not support the hypothesis that acute hyperglycemia produces vasoconstriction in the rat sciatic nerve arteries, as per earlier results (20). Rather, the findings indicate that the sciatic nerve vasculature reacts similarly to skeletal muscle by vasodilating with glucose infusion through an NO mechanism in response to the acute insulinemic response.

Experiment 2: Glucose infusion increased NVC

The current data contrast with those of Saini and co-workers (19) who observed a reduction in nerve blood flow following the same intravenous glucose infusion protocol (3 g/kg). Both studies used Sprague-Dawley rats and MAP responses appear to be similar. Importantly, we performed measurements at 0, 5, 10, 20 and 30 min following glucose infusion, whereas Saini and colleagues (20) took measurements at 0, 15, 30, 45, 60, 90 and 120 min following
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glucose infusion. The peak glucose values obtained following High Glucose (3 g/kg) infusion were ~three fold greater in our experiment (~ 30 mmol/L) than in the earlier study (19; ~10 mmol/L). The reason for this difference remains unclear. Conceivably, insulin secretion would be greater in the current study due to the greater glucogenic stress. Insulin values are not known from this earlier study and independent manipulation of blood glucose and insulin concentrations were not performed. In addition, in the current study, the use of fixed probe Doppler ultrasound system limited nerve blood flow velocity measures to a single segment of a superficial nerve artery. Alternatively, Saini et al. (20) used laser Doppler to detect net sciatic nerve vascular flux. This measure of flux represents the net signal from all red blood cells flowing in any direction. The sciatic nerve contains feed arteries arising from multiple positions (for example via gluteal and popliteal arteries) located primarily at the nerve junctions (2, 5, 30). Such an arrangement led to the proposition of bidirectional flow in the nerve, as well as the presence of arterial-venous anastomoses (2, 5, 30). If this scenario exists, measures of blood flow flux in a large region of the nerve may not capture arterial inflow responses. The combined methodological differences make it difficult to compare results between the current and previous studies.

Experiment 3: EHC not hyperglycemia stimulated the rise in NVC

The novel findings from this experiment on the blood supply of the sciatic nerve compliment previous data (6, 8) that suggest acute insulin administration increases nerve blood flow. Biessels and colleagues (6) speculated that the effect of insulin to increase nerve blood flow may relate to either a reduced glucose-stimulated vasoconstriction or perhaps insulin itself exhibits vasodilatory properties. Here we demonstrated that GSIS produced a dominant dilatory response in the sciatic nerve vasculature.
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Using hydrogen clearance, reductions in basal nerve blood flow have been observed following chronic uncontrolled hyperglycemia (25). Employing a similar model of hyperglycemia, Biessels and colleagues (6) partially reversed decrements in basal nerve blood flow with insulin treatment. Specifically, acute insulin treatment restored basal nerve blood flow in chronically hyperglycemic rats to ~85% of healthy control values. In the present experiment the insulin infusion rate was lower than that used by Biessels and colleagues (6) but, nonetheless, a ~50% greater vasodilation was observed. Importantly, Sprague-Dawley rats were used in the current study, rather than Wistar rats, as used previously. Finally, the rats used here were not subjected to prior chronic hyperglycemia, a procedure that may impair endothelial function and NO production (12, 15, 16).

**Experiment 4: L-NAME infusion attenuates GSIS-mediated rises in NVC**

Previous studies report a role for insulin, acetylcholine (8), eNOS (16) and nNOS (12) in sciatic nerve arterial vascular control. Therefore, this study included an examination of the potential contributions of NO to the GSIS dilatory effect. The evidence that L-NAME, given before or with glucose, abolished the glucose-stimulated, insulin-mediated dilation points to an important role for NO in this response. Taken together, it appears NO influences nerve blood flow control and the vasodilatory response to combined glycemic and insulinemic stress. Whether insulin and glucose act synergistically to stimulate a NO-mediated dilation remains unclear. A study conducted by Oomen et al. (18) documented a trend towards enhanced skin blood flow following hyperglycemia + hyperinsulinemia compared with euglycemia + hyperinsulinemia in Type I Diabetic participants suggesting an interaction may occur. Nonetheless, despite differences in circulating concentrations of glucose between the High Glucose infusion (~30 mmol/L) and the EHC (~5 mmol/L) condition in the present study, GSIS
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and insulin infusion resulted in comparable sciatic nerve arterial vasodilation. Potentially the combination of elevated glucose and insulin enhances any vasodilatory actions; but this question requires further study.

Perspectives and Significance

The current study highlighted the independent contributions of insulin and glucose to nerve blood flow control under healthy conditions and clarifies earlier observations (6, 8) regarding the potential vasodilatory actions of insulin in the rat sciatic nerve vasculature. In particular, insulin appears to vasodilate the blood supply of the sciatic nerve through an NO-mediated mechanism.

These results may have particular relevance to altered nerve function in diabetes. In the context on type I and II diabetes, the absence of chronic circulating insulin (6, 16, 25) or alternatively, vascular insulin resistance in the vasa nervorum (8) may contribute to impaired NO signalling and help explain previously observed decrements in sciatic nerve blood flow and nerve function. Experimental diabetes or uncontrolled hyperglycemia appears to reduce nerve blood flow and impair nerve function in rats (6, 16, 25). Whether this occurs as a result of hyperglycemia or hypoinsulinemia remains uncertain. Insulin treatment may partially restore nerve blood flow and nerve function (6), but these adaptations occur alongside reductions in blood glucose concentration rendering it difficult to isolate the exclusive effects of insulin. Whether stable basal circulating insulin concentrations (similar to the NIS group), or the fluctuating insulin concentrations resulting from pulsatile insulin secretion, cause differential effects on nerve health requires further study.

In the present study, NO inhibition alone did not alter baseline vascular conductance. Instead, NO inhibition impaired GSIS-mediated vasodilation. If basal insulin concentrations
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contribute to the maintenance of basal nerve blood flow, the mechanism may be NO independent. Alternatively, numerous mechanisms may operate in conjunction with some degree of redundancy to maintain basal nerve blood flow during NO inhibition. Attenuated vasodilation during NO inhibition suggests that the sciatic nerve vasculature may operate similarly to skeletal muscle vasculature (4), in that the hemodynamic actions of insulin may serve to enhance insulin binding and perpetuate any downstream effects.

Likely, both chronic insulin concentrations and insulin-mediated vasodilation serve metabolic and vascular functions to preserve neural health. Elucidating their independent contributions to nerve health is of particular importance when evaluating the efficacy of various insulin delivery therapies (for insulin-dependent diabetes). For example, use of a continuous insulin delivery system, such as the insulin pump, may preserve vasa nervorum health to a greater extent than conventional insulin injections. Considering the current data and reports that indicate a role for insulin in the maintenance of basal (6) and vasomotor control of nerve blood flow (8), the hemodynamic actions of insulin must be considered when studying the vascular etiology of peripheral neuropathy in diabetes.

Considerations

Although core temperature was maintained at 37°C, sciatic nerve/limb temperature was not recorded. Previously, Kihara and co-workers (13) demonstrated a positive relationship between limb temperature and sciatic nerve blood flow. Presumably, in the current experiment exposure of the sciatic nerve may have cooled the surrounding tissue and reduced nerve blood flow velocity. However, any effects of cooling likely took place during the stabilization period as baseline conductance values were similar between groups and blood flow velocity did not decrease during the baseline period. Further, during all protocols, the index of conductance either
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remained unchanged or increased. While the possibility remains that limb cooling may have attenuated the dilatory responses, this occurrence would not affect the overall conclusions of the current study.

The exact isoform of nitric oxide synthase was not determined in the current study as L-NAME acts as a non-selective NOS inhibitor. Although insulin stimulates the endothelial NOS pathway in skeletal muscle arterioles (21, 24), evidence suggests both endothelial (8, 9, 16) and neuronal (12) NO may serve a role in nerve blood flow control. The current approach may affect both of these sources of NO but separate roles for endothelial, neuronal or inducible forms of NO in the insulin-dependent dilation remain uncertain.

Assuming no change in arterial diameter, the Doppler ultrasound method reflects an effective technique to detect sciatic nerve arterial flow waveforms and changes in blood flow (26). The advantage of this method includes quantifiable changes in flow and waveform patterns through arterial segments, excluding venous contributions to overall flow flux or hydrogen clearance. However, we can only measure the Doppler signal on a single artery at a time. Moreover, the inability to obtain arterial diameters forced us to rely on blood velocity measurements alone as the analog of changes in total flow. This approach, often used in studies of cerebral blood flow velocity patterns (22, 23, 28), assumes the diameter of the interrogated vessel does not change such that any changes in velocity reflect events happening to the downstream vascular bed. Certainly, the validity of this assumption requires confirmation in the sciatic nerve arterial network. Nonetheless, failure to capture any local dilation (ie. increases in arterial diameter) in interrogated supply vessel would underestimate the overall dilator responses under investigation in the current study, a limitation that does not alter the overall conclusions.

Conclusion
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This experiment demonstrated that glucose infusion stimulates an insulin-dependent NO-mediated dilation along the superficial artery of the sciatic nerve. In addition, increasing the glucose infusion from 1 to 3 g/kg enhances the vasodilatory response. Lastly, glucose-stimulated insulin secretion and insulin infusion elicit a similar relative vasodilation.
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References


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Figure Captions

Fig. 1. Femoral artery index of vascular conductance (mm/s/mmHg) pre- and post- infusion of Low Glucose (1 g/kg). *, Significantly greater than baseline (P=0.007).

Fig. 2. Sciatic nerve arterial index of vascular conductance (mm/s/mmHg) pre- and post- Low (1g/kg) and High (3 g/kg) Glucose infusions. *, Significantly greater than baseline (P<0.001).

Fig. 3. Sciatic nerve arterial index of vascular conductance (mm/s/mmHg) pre- and post- hyperglycemia+euinsulinemia (NIS) and euglycemia+hyperinsulinemia (EHC). *, Significantly greater than corresponding baseline and peak NIS (P=0.004).

Fig. 4. Sciatic nerve arterial index of vascular conductance (mm/s/mmHg) pre- and post- glucose and L-NAME infusions. *, Significantly greater than corresponding baseline, High Glucose (3g/kg) co-infused with L-NAME (15 mg/kg), L-NAME alone and High Glucose infused 20 min following L-NAME (P≤0.011).
### Tables

#### Table 1: Glucose and Insulin Concentrations

<table>
<thead>
<tr>
<th>Group</th>
<th>Glucose (mmol/L)</th>
<th>Insulin (ulU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>10 min</td>
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<tr>
<td>Low Glucose (1 g/kg; n=5)</td>
<td>5.0±0.9</td>
<td>11.9±2.2^§</td>
</tr>
<tr>
<td>High Glucose (3 g/kg; n=6)</td>
<td>5.0±0.9</td>
<td>29.1±2.5^§*</td>
</tr>
<tr>
<td>High Glucose NIS (n=5)</td>
<td>4.6±1.5</td>
<td>&gt;32^§*♫a</td>
</tr>
<tr>
<td>Euglycemic hyperinsulinemic clamp (EHC; n=6)</td>
<td>5.3±0.8</td>
<td>5.6±1.4</td>
</tr>
</tbody>
</table>

Values are Mean ± SD.

Non-insulin secreting (NIS)

^Significantly greater than baseline (within group comparison; P≤0.01)

§Significantly greater than the EHC group (P<0.001) at the same time point

*Significantly greater than the Low Glucose dose (P<0.001) at the same time point

†Significantly greater than the NIS group (P<0.001) at the same time point

♫Significantly greater than the High Glucose dose (P<0.001) at the same time point

a Too high for the glucometer to measure (>32 mmol/L; input 32 mmol/L for stats)

b Insulin sample acquired at 80 min
### Table 2: Nerve Blood flow Velocity and Mean Arterial Pressure Values

<table>
<thead>
<tr>
<th>Group</th>
<th>Nerve Blood Flow Velocity (mm/s)</th>
<th>Mean Arterial Pressure (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>At Peak VC</td>
</tr>
<tr>
<td>Low Glucose (1 g/kg; n=5)</td>
<td>83±30</td>
<td>103±24*</td>
</tr>
<tr>
<td>High Glucose (3 g/kg; n=6)</td>
<td>82±48</td>
<td>138±64*</td>
</tr>
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<td>High Glucose NIS (n=5)</td>
<td>62±19</td>
<td>73±21</td>
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<td>Euglycemic hyperinsulinemic clamp (EHC; n=6)</td>
<td>75±30</td>
<td>167±62*</td>
</tr>
<tr>
<td>High Glucose + L-NAME (15 mg/kg; n=5)</td>
<td>81±23</td>
<td>130±36*</td>
</tr>
<tr>
<td>L-NAME alone (n=5)</td>
<td>59±5</td>
<td>69±20</td>
</tr>
<tr>
<td>High Glucose added to L-NAME (n=5)</td>
<td>83±42</td>
<td>110±45</td>
</tr>
<tr>
<td>Isovolumetric saline infusion (n=6)</td>
<td>86±36</td>
<td>86±38</td>
</tr>
</tbody>
</table>

Values are Mean ± SD. *, Significantly greater than baseline of the same protocol (P≤0.05). Non-insulin secreting (NIS)