Diabetes: Sweat Response and Heart Rate Variability During Electrical Stimulation in Controls and People With Diabetes

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ABSTRACT
Twelve subjects with type II diabetes and 13 control subjects participated in a study to examine whether electrical stimulation might be used as a predictive tool for assessing autonomic nervous system dysfunction in people with diabetes. Subjects ranged in age from 32 to 67 years. Inclusion criteria were by diagnosis of type II diabetes and male. Exclusion criteria were uncontrolled blood pressure (140/90 mmHg), beta blocking medication, and alpha antagonists. The percent change in the local sweat response to electrical stimulation was measured on the skin over the distal quadriceps. Electrodes were placed 15 cm apart and a sweat capsule was placed between the electrodes. Subjects were tested in a thermally neutral environment and a globally heated environment. Heart rate variability (HRV) was measured with continuous electrocardiogram. Mean control HRV was 3.2 bpm and people with diabetes had a mean HR of 1.9 bpm. The results of the experiment showed that electrical stimulation did cause an increase in sweat response between the 2 electrodes. Control subjects showed a 20.2% increase in sweat rate and people with diabetes showed an 18.2% change in sweat rate. Electrical stimulation was most effective in increasing the sweat response in a thermally neutral environment. Electrical stimulation does predict autonomic dysfunction at the endothelial cell in people with diabetes. The mechanism for facilitating the sweat response appears to be shear stress that activates nitric oxide production in the endothelial cell and causes vasodilation.

INTRODUCTION
Diabetes causes microvascular damage. The microvascular damage occurs as a result of poor glycemic control causing precapillary damage. Precapillary damage inhibits normal function of the endothelial cells and blocks the normal nitric oxide (NO) pathways that cause vasodilatation. Endothelial cell damage impacts vascular tone by causing a loss of distensibility in the vessels that affects the ability of the vessels to vasodilate. Vessels remain in a vasoconstricted
state\textsuperscript{3,4} and heart rate variability (HRV) is diminished. People with diabetes have lower HRV than control subjects that is expressed with a near flat appearance.\textsuperscript{1}

The initial injury in the diabetic patient is from chronic hyperglycemia, and the first damage occurs in the blood vessels at the endothelial cell\textsuperscript{5} interfering with the myelinated nerve fibers and causing damage and interfering with nerve blood flow.\textsuperscript{1} The endothelial cell is pivotal in managing vascular tone.\textsuperscript{5}

Microvascular damage causes a decrease in the local reflexes, NO production, and nerve blood flow.\textsuperscript{2,5}

Electrical stimulation is a stressor that can affect the vascular system and has been found to increase blood flow, which is mediated in part by the release of NO. The sweat response is also a vascular response increasing with exposure to heat\textsuperscript{2} and is mediated by the release of NO.

It is possible that since electrical stimulation increases blood flow, it may also increase the sweat response using a similar mechanism as both responses are mediated by the release of NO.\textsuperscript{2} In vascular endothelial cells, shear stress may activate calcium channels resulting in the release of NO.\textsuperscript{2,6} Shear stress activates the cells mechanically to facilitate NO synthase to produce NO, rather than chemically such as with acetylcholine (ACh).\textsuperscript{3}

Electrical stimulation studies set the level of stimulation below the pain threshold, so the effect of the stimulation was studied rather than the individual’s response to pain.\textsuperscript{7,8}

Lowered sweat rates in diabetic patients place them more at risk for heat disorders when in a heat stressed environment,\textsuperscript{9} and it is possible that with moderate fiber damage the sweat response may be lost.\textsuperscript{10}

Nitric oxide is a potent dilator synthesized and released by vascular endothelial cells, certain autonomic nerves, and other tissues, such as sweat glands.\textsuperscript{8} The sympathetic cholinergic nerves release ACh that evokes the release of NO at the endothelial cell causing vasodilatation that mediates sweating.\textsuperscript{6} Shear stress is a mechanical method for activating the phosphorylation cascade utilizing protein kinase to facilitate endothelial NO synthase activation and production of NO.\textsuperscript{3}

Schlereth and colleagues conducted a study using electrical stimulation to obtain a sweat response that was found to be arranged somatotopically in the lower extremity.\textsuperscript{10} A focal loss of the sweat response may be asymmetric and while the people with diabetes experience hypohydrosis in the lower extremities, hyperhydrosis is experienced at the head and chest to compensate for the lower extremities.\textsuperscript{10}

**PATIENTS AND METHODS**

**Subjects**

Two groups of male subjects, one group with diabetes and one of non-diabetic controls, participated. Female subjects were excluded because of possible hormonal changes during the menstrual cycle or menopause that might alter the sweat rate. Controls were recruited from the community at large, and a physician managed people with diabetes recruited

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (years)</th>
<th>Height (cm)</th>
<th>Weight (kg)</th>
<th>BMI (kg/m\textsuperscript{2})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (n = 13)</td>
<td>47.2 ± 11.1</td>
<td>173.9 ± 6.8</td>
<td>94.3 ± 20.4</td>
<td>31.8 ± 7.2</td>
</tr>
<tr>
<td>People with diabetes (n = 12)</td>
<td>52.4 ± 6.1</td>
<td>177.4 ± 7.4</td>
<td>107.8 ± 20.4</td>
<td>34.9 ± 6.1</td>
</tr>
<tr>
<td>\textit{t}-test</td>
<td>\textit{P} = 0.05</td>
<td>\textit{P} = 0.12</td>
<td>\textit{P} = 0.06</td>
<td>\textit{P} = 0.21</td>
</tr>
</tbody>
</table>
from the Loma Linda University Diabetes Treatment Center. Subjects included in the study were non-smokers and had a resting blood pressure not exceeding 140/90 mmHg. Subjects taking beta-blocking medication or alpha antagonists were excluded. Subjects were assigned to their group based on diagnosis. The subjects ranged in age from 32 to 67 years. The non-diabetic control group had 13 subjects and the diabetic group had 12 subjects for a total of 25 subjects. Table 1 shows the mean age, height, weight, and body mass index (BMI). Glycosolated hemoglobin was generally 6%-7% in the diabetic patients. All protocols and procedures were explained to each subject, who signed an informed consent document.

**Electrical Stimulation**

A Challenge 8000 Power Muscle Stimulator was used in this study (Maximum Performance Technologies, Tustin, California). Two disposable UniPatch RE-PLY carbon electrodes #658 (Wabasha, Minnesota), 2 × 4 cm were placed 15 cm apart at the distal third of the upper leg over the skin of the quadriceps. The current was controlled at 15 milliamps of monophasic current.

**Sweat Hygrometry**

Sweat was measured using the Q-Sweat hygrometry system produced by W.R. Medical Electronics Corporation (Stillwater, Minnesota). In this system, a constant source of air pressure was applied through the sweat capsules placed on the skin. Each capsule was 0.781 cm² and the air source to each capsule was dried in Dri-rite. The application of air at a constant flow rate allowed assessment of the humidity of the outgoing air from the capsules. Sweat rate was then calculated by the computerized measuring system utilizing the air temperature, the flow rate, and the humidity in the capsule. A sweat capsule was placed between the 2 carbon electrodes over the skin of the quadriceps at the upper leg. Sweat recordings ran continuously.

**Table 2. Mean Percent Change in Sweat for Controls During Stimulation After Exposure to a Thermally Neutral Environmental Temperature.**

<table>
<thead>
<tr>
<th>Stimulation Period 1</th>
<th>Stimulation Period 1</th>
<th>Stimulation Period 2</th>
<th>Stimulation Period 2</th>
<th>Post Stimulation 2</th>
<th>Post Stimulation 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Start</td>
<td>End</td>
<td>Start</td>
<td>End</td>
<td>Minutes</td>
<td>Minutes</td>
</tr>
<tr>
<td>Upper leg</td>
<td>0.72 ± 1.0</td>
<td>1.72 ± 28.0</td>
<td>2.73 ± 36.3</td>
<td>10.8 ± 31.5</td>
<td>17.7 ± 28.3</td>
</tr>
</tbody>
</table>

**Statistical Analysis**

SPSS Version 12 was used to calculate means and standard deviations. Comparisons were made using a mixed factorial analysis of variance and independent t-tests. The Bonferroni adjustment for multiple comparisons was used in the factorial analysis. The level of significance was $P < 0.05$. All data are expressed with ± standard deviations. Percent change in sweat rate during stimulation was expressed as the change between the starting sweat rate and each data point through 3 minutes post stimulation divided by the starting sweat rate.

**Procedures**

The experiments were conducted in individual sessions on 2 separate days. On the first day, the subject rested comfortably in a 23°C room for 15 minutes. During this 15-minute time interval, the electrodes and the sweat capsules were set in place. A sweat capsule was placed between 2 carbon electrodes at the upper leg over the skin at the distal quadriceps. The upper leg was stimulat-
ed with 15 milliamps of monophasic electrical current for 2 minutes, switched off for 1 minute, on for 2 minutes, then off. Sweat rate was measured continuously throughout the experiment.

On the second day, the subject rested in a 34°C room and the same electrical stimulation procedure as Day 1 was repeated with sweat capsule measurements taken at the same time points.

RESULTS

Controls
Percent change in sweat rates from rest were recorded and are summarized in Table 2. Sweat on the skin at the upper leg increased significantly (20.2%; \( P = 0.005 \)) during stimulation between the start of the first stimulation period (1) to 3 minutes post stimulation in the control subjects. Mean HRV was 3.2 bpm.

People With Diabetes
Table 3 shows the mean change in sweat rates for people with diabetes. The percent change in sweat at the upper leg increased (18.2%; \( P = 0.001 \)) from the start of the first period of stimulation (1) to 3 minutes post stimulation. Mean HRV was 1.9 bpm and had a near flat appearance.

Comparison of 2 Groups
Figure 1 shows the percent change in sweat rate between the controls and people with diabetes in a thermally neutral environment. The controls had greater sweat rate percent changes. The sweat rate increased for both groups when the first electrical stimulation was started. The percent change in sweat rate increased again when the second stimulation was started in the controls; however, the percent change in the people with diabetes did not increase at the second trial. Figure 2 shows the percent change in sweat rate between the controls and people with diabetes in a globally heated environment. People with diabetes had a slower, more sluggish response than the control group. Heart rate variability in controls was significantly higher than in people with diabetes (\( P < 0.05 \)).

DISCUSSION

People with diabetes have poor circulation due to vascular endothelial cell impairment.\(^3,13\) As a consequence of either reduced production or decreased sensitivity to NO, blood vessels remain constricted and show diminished ability to vasodilate.\(^5\) The HRV observed here supports previous literature\(^1\) that states HRV is lower in people with diabetes showing a very small variation in heart rate that is nearly flat in appearance.

Compounding the problem, there is sudomotor damage from diabetes due to sympathetic nerve damage and local damage to sweat glands.\(^12\) The release of the neurotransmitters facilitating vasodilatation at the blood vessel or the sweat glands is also impaired,\(^14-16\) and if the neurotransmitters are not released effectively, NO is diminished, vasodilatation is impacted, and sweat responses are diminished. Thus, patients with diabetes have lowered sweat rates,\(^9\) and with moderate fiber damage the sweat response may be lost.\(^10\)

Electrical stimulation is a stressor

<table>
<thead>
<tr>
<th>Stimulation Period 1 Start</th>
<th>Stimulation Period 1 End</th>
<th>Stimulation Period 2 Start</th>
<th>Stimulation Period 2 End</th>
<th>Post Stimulation 2 Minutes</th>
<th>Post Stimulation 3 Minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper leg</td>
<td>0.28 ± 6.1</td>
<td>3.1 ± 16.3</td>
<td>8.4 ± 17.3</td>
<td>7.5 ± 26.1</td>
<td>14.2 ± 26.7</td>
</tr>
</tbody>
</table>
that can be used to induce a reflex change in the autonomic nervous system. Electrical stimulation is a simple test to perform and is done with equipment readily available to the physical therapist that produces a local vascular response as opposed to a global response. In the present study, sweat between the electrodes in the controls was greater than for the people with diabetes. The sweat response is similar to the blood flow response and increases by NO release and vasodilatation. Nitric oxide production is activated both chemically (eg, in response to ACh) and mechanically via shear stress. Acetylcholine is a chemical activator of calcium-dependent channels. Shear stress is the mechanical activator of NO production through calcium-independent channels.

Sweat increased for both groups during electrical stimulation, but the sweat increase was greater in the controls. Utilizing electrical stimulation to look at the sympathetic response is new. Electrical stimulation causes mechanical shear stress at the endothelial cell. The mechanical shear stress causes a phosphorylation cascade that removes phosphate groups from proteins and kinases activating endothelial NO synthase to synthesize NO. Nitric oxide is produced facilitating the release of cyclic guanosine monophosphate and a change in potassium permeability. The relaxation of the smooth muscle and vasodilatation of the vessels allows an exchange between vessels and sweat gland facilitates the production of sweat.

The release of ACh is regulated by the hypothalamus and, in the case of the sweat response, acts as a response to an increase in blood and/or skin temperature. The mechanical response initiated by shear stress is not dependent on a temperature increase and in the current experiment it appears that the electrical

Figure 1. Comparison of sweat rates between controls (■) and people with diabetes (○) at the upper leg after exposure to a thermally neutral environment during electrical stimulation (mean ± SD). S1S = stimulation period (1) start; S1E = stimulation period (1) end; SOI = stimulation off initial; SOE = stimulation off end; S2S = stimulation period (2) start; S2E = stimulation period (2) end; PS = immediate post stimulation; PS1 = post stimulation 1 minute; PS2 = post stimulation 2 minutes, PS3 = post stimulation 3 minutes.
stimulation acted as a mechanical shear stress activator.

In the patient with diabetes, hyperglycemia causes a reduction in the activation of the phosphorylation cascade, leaving less endothelial NO synthase available to synthesize NO. The decline in NO synthase activation decreases the bioavailability of NO resulting in less vasodilatation, a decrease in the sweat response, blood flow, and increased vascular stiffness. The endothelial cell is key to the maintenance of vascular tone through the production of NO and with a reduction in the bioavailability of NO synthase, vascular tone and vessel remodeling is impaired.\(^3\)

The decreased response during stimulation implies considerable damage to the endothelial cells in people with diabetes. Electrical stimulation assesses damage to the blood vessels at the endothelial cell level independently of the entire sympathetic system and is a viable test that appears to perform better in a thermally neutral environment. The small sample size and recruitment occurring over multiple seasons spanning winter, spring, and summer was a limitation to this study.

Electrical stimulation does act as a predictive tool for early signs of autonomic nervous system dysfunction in people with diabetes by measuring the sweat response. It is a modality that can be used in any physical therapy practice and provides an inexpensive method to assess endothelial impairment. An inexpensive galvanic skin resistance device can be used to measure the change in skin resistance as it relates to sweat rates. As the sweat rate increases, skin resistance increases and a comparison of skin resistance levels from normal subjects to the people with diabetes provides an assessment of microvascular damage at the endothelial cell.

Figure 2. Comparison of sweat rates between controls (■) and people with diabetes (●) at the upper leg after exposure to global heating during electrical stimulation (mean ± SD). S1S = stimulation period (1) start; S1E = stimulation period (1) end; SOI = stimulation off initial; SOE = stimulation off end; S2S = stimulation period (2) start; S2E = stimulation period (2) end; PS = immediate post stimulation; PS1 = post stimulation 1 minute; PS2 = post stimulation 2 minutes, PS3 = post stimulation 3 minutes.
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