Delayed threshold for active cutaneous vasodilation in patients with Type 2 diabetes mellitus

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Epidemiological evidence suggests decreased heat tolerance in patients with Type 2 diabetes mellitus (T2DM), but it is not known whether the mechanisms involved in thermoregulatory control of skin blood flow are altered in these patients. We tested the hypothesis that individuals with T2DM have a delayed internal temperature threshold for active cutaneous vasodilation during whole body heating compared with healthy control subjects. We measured skin blood flow using laser-Doppler flowmetry (LDF), internal temperature (Tor) via sublingual thermocouple, and mean arterial pressure via Finometer at baseline and during whole body heating in 9 T2DM patients and 10 control subjects of similar age, height, and weight. At one LDF site, sympathetic noradrenergic neurotransmission was blocked by local pretreatment with bretylium tosylate (BT) to isolate the cutaneous active vasodilator system. Whole body heating was conducted using a water-perfused suit. There were no differences in preheating Tor between groups (P > 0.10). Patients with T2DM exhibited an increased internal temperature threshold for the onset of vasodilation at both untreated and BT-treated sites. At BT-treated sites, Tor thresholds were 36.28 ± 0.07°C in controls and 36.55 ± 0.05°C in T2DM patients (P < 0.05), indicating delayed onset of active vasodilation in patients. Sensitivity of vasodilation was variable in both groups, with no consistent difference between groups (P > 0.05). We conclude that altered control of active cutaneous vasodilation may contribute to impaired thermoregulation in patients with T2DM.

IN RECENT YEARS, THE INCIDENCE of Type 2 diabetes mellitus (T2DM) has reached epidemic proportions in the United States and other developed countries (24), leading to increasing interest in mechanisms of metabolic, cardiovascular, and neurological dysfunction in this disease. Importantly, dysfunction in some or all of these areas can lead to significant impairment in mechanisms of thermoregulation, which itself can cause increased morbidity and mortality in patients with T2DM. Epidemiological data indicate that individuals with diabetes are at significantly higher risk for heat illness during heat waves compared with the general population (19, 20). Although physiological/pathophysiological mechanisms are not well understood, some existing data are consistent with the potential for impaired thermoregulation. For example, diabetic patients with length-dependent peripheral neuropathy exhibit impaired sweating in affected areas (7, 14). Furthermore, individuals with diabetes have been shown to have decreased vasodilator responsiveness to pharmacological stimuli in vascular beds, including the skin (3, 11, 21, 22). Such peripheral impairments could lead to a decrease in the ability of the cutaneous vasculature to respond to neural signals involved in reflex cutaneous vasodilation, and thus a decrease in convective heat transfer during body heating, leading to impaired thermoregulation and increased risk of dangerous hyperthermia.

Reflex control of the skin circulation is essential for normal body temperature regulation during exposure to the heat. In humans, skin blood flow is controlled by both sympathetic vasoconstrictor nerves and sympathetic active vasodilator nerves (4, 10). This latter system does not exhibit tonic activity in normothermia, but it is the major mechanism for the control of skin blood flow during heat stress, when large increases in skin blood flow are key to the heat dissipation required for normal thermoregulation (4, 10). During severe hyperthermia, up to 60% of cardiac output is directed to the surface of the skin for purposes of heat dissipation; ~80–90% of this large increase in skin blood flow is controlled by the active vasodilator system (10, 17). Therefore, impairments in control of the active vasodilator system could substantially inhibit the ability of the skin circulation to appropriately respond to whole body hyperthermia.

Despite the key role of neural control of skin blood flow in normal thermoregulation, it is currently unknown whether changes in the mechanisms of this control during whole body heating exist in patients with T2DM, which could contribute to impaired thermoregulation. In the present study, therefore, we set out to test two main hypotheses. First, we hypothesized that individuals with T2DM would exhibit a higher internal temperature (Tor) threshold for the onset of active cutaneous vasodilation compared with control subjects. Second, we hypothesized that the sensitivity of the cutaneous vasodilator response (the responsiveness of cutaneous vasodilation to continuing increases in Tor) would be decreased in the patient group. To address these hypotheses, we measured skin blood flow during whole body heating experiments in 9 patients with T2DM and 10 control subjects of similar age and body size, using local pretreatment with bretylium tosylate (BT) to isolate the active vasodilator system at one measurement site.
METHODS

General methods. The protocol for this experiment was approved by the Institutional Review Board at the Mayo Clinic in Rochester, Minnesota. Subjects for these studies were 9 patients with T2DM and 10 control subjects of similar age and body size. Our goal in these experiments was to study relatively “healthy” individuals with diabetes, to minimize potential confounding influences of several common comorbidities of this disease. Subjects were screened via telephone interview and physical examinations (described below) to rule out comorbidities of this disease. Subjects were reported to the Mayo General Clinical Research Center (GCRC) on 2 separate days. Informed consent was obtained before any procedures began.

Screening visit. On the screening day, blood samples were taken for measurement of hemoglobin, glycosylated hemoglobin, hematocrit, glucose, albumin, cholesterol, and triglycerides. On both screen and study days, subjects with T2DM tested their blood glucose with their personal glucose meter and the meter at the GCRC. Diabetic patients were also seen by a neurologist to screen for neuropathy and by a diabetologist to discuss the regulation of their medication. Patients with T2DM discontinued taking oral antihyperglycemic medications 2 wk before their study date. One patient took synthetic insulin and was switched to regular fast-acting insulin 72 h before the patient’s study date, and this individual did not take insulin the day of the study. In addition to antihyperglycemic medications, two patients and two control subjects were taking a statin, two patients took an angiotensin-converting enzyme inhibitor, and one control and one patient took a β-blocker.

The screening day also consisted of a treadmill exercise test (TMET) using a standard Bruce protocol to rule out occult cardiovascular disease in diabetic and control subjects. Subjects walked for an average of 8 min on the treadmill. Subjects also participated in a thermoregulatory sweat test (TST). The TST is a whole body sweat test used clinically to determine the innervated areas of the skin that produce perspiration (7). For this test, unclothed subjects laid supine in an enclosed area in which air temperature was kept at 45–50°C with relative humidity of 35–40%. Subjects were covered with an indicator powder composed of a mixture of alizarin red, corn starch, and sodium carbonate. When dry, this mixture is light orange in color; as sweat comes into contact with the powder the color changes from orange to purple (7). The heating period lasts 45–65 min. For subject comfort and safety, Tₑₑₑₑ was monitored continuously to prevent excessive hyperthermia.

Study day. On the study day, subjects reported to the GCRC at 7:00 AM after fasting overnight. BT was administered by iontophoresis (Intophor-II, model 6111PM/DX, Life-Tech, Stafford, TX) for 10 min at 240 μA on the left forearm to inhibit all noradrenergic neurotransmission over an area of 0.6 cm². Subjects then waited 1 h to allow the BT to be taken up and have its effect (8). Forty-five minutes into the waiting period the subjects were instrumented with a three-lead ECG and six skin temperature probes. They then put on a two-piece, water-perfused suit. The suit covered the entire body with the exception of hands, feet, and areas of skin blood flow measurement (5, 6). Two laser-Doppler flow (LDF) probes (Periflux System 5000, Perimed, Stockholm, Sweden) were attached by adhesive to the left forearm to measure skin blood flow. The probes were attached to the skin in specialized holders that measure and control local temperature over an area of 12 cm² (Perimed). One probe was placed at the BT-treated site and another at a control site a few centimeters away. Blood pressure was measured continuously using a Finometer (Finapres Medical Systems, Amsterdam, The Netherlands) placed on the left middle finger. Tₑₑₑₑ was monitored via sublingual thermistor (model ON-402-PP, Omega Engineering, Stamford, CT) connected to a bichpoch precision thermistor thermometer with a resolution of 0.01°C (model 5831, Omega Engineering). Subjects were encouraged to keep their mouths closed and prevent movement of this probe for the duration of the study to ensure correct temperature measurement.

Protocol. The subjects rested quietly for a 10-min baseline period. Cool water (~10–12°C) was then perfused through the suit for 2–3 min to test for effective blockade of the vasoconstrictor nerves at the BT-treated site. This body cooling was sufficient to cause cutaneous vasoconstriction but not shivering. Warm water (~45–50°C) was then perfused through the suit until a minimum core temperature increase of 0.6°C was reached. This took ~60–90 min. Because of subjects’ ages and the fact that diabetes may make patients less tolerant to heat, we were careful to ask subjects whether they felt well enough to continue. Excessive discomfort on the part of the subjects was sufficient to terminate the experiment. After whole body heating, cool water was perfused to return subjects to normothermia. Local temperature at both LDF sites was then increased to 42°C at a rate of ~1°C every 7 s. A local temperature of 42°C was maintained for 35 min for assessment of maximum cutaneous vascular conductance (CVC).

LDF and temperature data were collected at 100 Hz; ECG and beat-to-beat blood pressure were collected at 250 Hz. Data were stored on a personal computer using Labview software (National Instruments, Austin, TX). Data were later converted to 1-s averages for post hoc analysis.

Data analysis. Skin blood flow (LDF) was divided by mean arterial pressure to derive an index of CVC. Maximum CVC was assessed as the average of the last 3 min during local warming. CVC data are expressed as a percentage of this maximum.

The sensitivity of cutaneous vasodilation was determined by using linear regression analysis of the CVC-Tₑₑₑₑ relation after a significant increase in CVC had occurred. We defined the sensitivity of the response as the slope of the linear portion of the relationship that constituted the majority of the vasodilation in each subject. For those subjects in whom a plateau was seen in the vasodilator response later in heating, the plateau was not included in the assessment of sensitivity. For assessment of threshold, each individual’s linear regression equation was solved for baseline CVC for that individual to obtain the Tₑₑₑₑ value at which neurally mediated vasodilation was initiated.

Statistics. Results are expressed as means ± SE. Comparisons between control and T2DM patients were made using unpaired Student’s t-test. Statistical significance was set at P < 0.05.

RESULTS

Mean age, height, and weight were 57.1 ± 2.7 yr, 174 ± 2 cm, and 84.8 ± 3.9 kg for T2DM subjects and 56.8 ± 2.4 yr, 174 ± 3 cm, and 79.7 ± 3.7 kg for control subjects, respectively (P > 0.05 for all). All subjects successfully passed the TMET as reviewed by a cardiologist. TST results were reviewed by a neurologist for any abnormalities, and most subjects had normal sweat patterns. A few diabetic individuals had reduced or absent sweating on their toes and small patches of anhidrosis, but none affected the area on the forearm at which skin blood flow was measured. Screening blood test results were as follows: hemoglobin: control 14.5 ± 0.3 g/dl, T2DM 14.1 ± 0.4 g/dl (P > 0.05); hematocrit: control 41.9 ± 0.8%, T2DM 40.7 ± 1.4% (P > 0.05); glycosylated hemoglobin: control 5.2 ± 0.1%, T2DM 6.9 ± 0.5% (P < 0.01); glucose: control 94.5 ± 2.0 mg/dl, T2DM 143 ± 4.9 mg/dl (P < 0.01); albumin: control 4.3 ± 0.0 g/dl, T2DM 4.4 ± 0.1 g/dl (P < 0.05); total cholesterol: control 200.5 ± 13.6 mg/dl, T2DM 190.0 ± 8.4 mg/dl (P > 0.05); triglycerides: control 146.5 ± 38.7 mg/dl, T2DM 157.7 ± 27.1 mg/dl (P > 0.05).

Baseline data. Baseline CVC values are shown in Fig. 1. As shown in this figure, baseline CVC in control subjects was higher at BT-treated sites than at untreated sites, consistent
with inhibition of tonic vasoconstrictor activity at the BT site. In contrast, patients with T2DM exhibited no difference between untreated and BT-treated sites \( (P > 0.05) \); this resulted in CVC values at untreated sites that were significantly higher in T2DM patients than in controls \( (P < 0.05) \).

**Responses to cooling.** The cooling period resulted in a decrease in CVC at untreated sites (control \(-19 \pm 2\% \) of baseline, T2DM: \(-26 \pm 3\% \) of baseline; \( P < 0.05 \) vs. baseline) but not at BT-treated sites (control \(1 \pm 3\% \) of baseline, T2DM \(-3 \pm 3\% \) of baseline; \( P > 0.05 \) vs. baseline), indicating effective blockade of noradrenergic vasoconstriction at BT-treated sites in both groups.

**Responses to heating.** Starting \( T_or \) was not different between groups (control \(36.13 \pm 0.09^\circ C \), T2DM \(36.23 \pm 0.06^\circ C \); \( P > 0.05 \)). Whole body heating resulted in an increase in \( T_or \) of \(0.70 \pm 0.04^\circ C \). The increase in \( T_or \) was similar between groups (control \(0.70 \pm 0.05^\circ C \), T2DM \(0.70 \pm 0.04^\circ C \); \( P > 0.05 \)). The \( T_or \) threshold at which cutaneous vasodilation began was higher in patients with T2DM compared with controls. Figure 2 shows average values for this threshold for both untreated and BT-treated sites for both groups, (control: untreated \(36.35 \pm 0.06^\circ C \), BT: \(36.28 \pm 0.07^\circ C \); T2DM: untreated \(36.50 \pm 0.05^\circ C \), BT \(36.55 \pm 0.05^\circ C \); \( P < 0.05 \) for control vs. T2DM for both untreated and BT sites).

Sensitivity of cutaneous vasodilation exhibited substantial variability between groups, and it was not different between sites or between groups, as follows: control: untreated \(58 \pm 27\% \) maximum/\(^\circ C \); BT \(54 \pm 13\% \) maximum/\(^\circ C \); T2DM: untreated \(90 \pm 24\% \) maximum/\(^\circ C \), BT \(64 \pm 18\% \) maximum/\(^\circ C \) \( (P > 0.05 \) for all comparisons). Figure 3 shows four individual examples of cutaneous vascular conductance during whole body heating, showing the variability in sensitivity of the response. It was likely as a result of this variability that the average total change in CVC during heating was similar between groups: for control, \(+24 \pm 8\% \) maximum; for T2DM, \(+26 \pm 5\% \) maximum \( (P > 0.05) \).

**Local warming.** Absolute values for maximum cutaneous vascular conductance during local heating were lower in patients with T2DM compared with controls (control: \(2.52 \pm 0.18 \) arbitrary units/mmHg; T2DM \(1.99 \pm 0.14 \) arbitrary units/mmHg, \( P < 0.02 \)).

**DISCUSSION**

Our main new findings are consistent with our hypothesis that patients with T2DM exhibit higher \( T_or \) thresholds for the onset of cutaneous vasodilation during whole body heating compared with control subjects of similar age, height, and weight. Furthermore, data from BT-treated sites suggest that this difference in threshold is primarily due to a difference in control of skin blood flow by the active vasodilator system. However, our data did not support the hypothesis that sensitivity of active cutaneous vasodilation would be diminished in patients with T2DM. As has been noted previously in studies of healthy individuals (5, 6), we noted substantial variability of the sensitivity of the response in both controls and T2DM.

**Fig. 1.** Average values for cutaneous vascular conductance [CVC; %maximum (%max)] at baseline for untreated and bretylium tosylate (BT)-treated sites in patients with Type 2 diabetes mellitus (T2DM) and control subjects. Values are means \( \pm \) SE. Note that CVC was significantly lower at untreated sites in control subjects (consistent with tonic activity of sympathetic vasoconstrictor nerves in the skin), but not in T2DM patients, suggesting less tonic vasoconstrictor control of skin blood flow in patients with T2DM. \(*P < 0.05 \).

**Fig. 2.** Group average values for threshold for cutaneous vasodilation for control and T2DM subjects at untreated and BT-treated sites. Values are means \( \pm \) SE. Patients with T2DM exhibited significantly higher thresholds for vasodilation compared with control subjects at both sites. \(*P < 0.05 \).
patients (see Fig. 3) and no consistent difference between groups. Finally, we also noted differences in baseline CVC that might suggest decreased tonic sympathetic vasoconstrictor tone in the T2DM patients.

Epidemiological literature suggests impaired heat tolerance in T2DM (19, 20). Because of the key role of neural control of skin blood flow in normal human thermoregulation, our main goal in the present study was to test whether changes in active vasodilator control of skin blood flow exist that could contribute to this impairment. With regard to mechanism, our finding of a shift in threshold may indicate altered central control of thermoregulation in the patient group (5, 6, 15). Although we are not aware of evidence specific to central neural mechanisms of thermoregulation (such as in the temperature-sensitive neurons of the preoptic/anterior hypothalamic region) in diabetes, there is evidence that the function of hypothalamic neurons is altered in diabetic rats (12, 23). For example, neuronal activity in several regions of the hypothalamus (including the paraventricular and supraoptic nuclei) was altered as evidenced by increased expression of C-fos in these regions in diabetic rats (23). Increased norepinephrine release from the hypothalamus and preoptic area has also been noted in diabetic rats (12). Similar changes in hypothalamic function, if they affect central thermoregulatory control in the preoptic/anterior hypothalamic region, could have contributed to the delayed threshold for active cutaneous vasodilation we observed in the present study.

Alternatively, because patients with T2DM have been shown to demonstrate impaired vasodilator responsiveness (3, 21, 22), it is possible that the skin blood vessels in our patient group exhibited delayed vasodilation in response to a similar neural stimulus. This would also be consistent with reports of decreased axon-reflex mediated cutaneous vasodilator responses in patients with T2DM (2, 9). Because diabetes is a multifactorial disease that can affect central neural, peripheral neural, and vascular mechanisms, it is likely that some combination of the above mechanisms contributed to our present observations.

The shift in threshold in combination with the variable sensitivity within groups did not result in an overall average difference in peak vasodilator response to heating between the two groups, when expressed in terms of percentage of maximum vasodilation (an average peak increase in CVC of ~25% maximum in both groups). Because baseline CVC was higher in T2DM patients at untreated sites, a similar increase in CVC meant that the total CVC (as % maximum) was somewhat higher in patients than in controls. It is functionally important in this context that maximum CVC was, on average, 20% lower in T2DM patients compared with controls. The similar percent increases in CVC across groups, with lower maxima in the T2DM group, suggests less increase in CVC in the T2DM group. However, in the context of higher untreated baseline CVC values in our T2DM patients, absolute levels of skin blood flow during heating (the relevant variable with regard to heat exchange) and overall thermoregulation (in terms of skin blood flow) were probably not significantly impaired in this group. The relevance of the threshold shift in this context is that a larger shift, in combination with more severe local vasodilator impairment, would likely have a greater functional impact than that seen here.

An important consideration with regard to the foregoing discussion is that skin blood flow and vascular conductance values measured using LDF, although excellent for providing continuous measurements of perfusion that are specific to the skin circulation (18), do not provide absolute values of blood flow (e.g., in ml/min). The LDF values in a given area are affected not only by the blood flow in that area but also by the number of microvessels in the area of measurement of the LDF probe. For this reason, data are often expressed as a percentage of maximum to allow for interindividual comparison. A condition such as that reported here, in which the maxima are different between groups, presents a limitation for data analysis. In conjunction with previous reports of decreased local cutaneous vasodilator responsiveness in T2DM (1, 3), it is likely that there was a true decrease in maximum vasodilator capacity in our patient group. Because of the limitations mentioned above, it remained appropriate to consider each individual in the context of his or her own individual maximum CVC, and to recognize that it is likely that this maximum was, overall, lower in T2DM patients. A limitation of the present study is that we did not include measurements of skin blood flow (for example, venous occlusion plethysmography) that would have allowed for assessment of absolute changes in cutaneous vascular conductance with whole body heating.

Because we aimed to study the influences of diabetes while minimizing potential confounds of coexisting conditions, we limited our population to relatively healthy diabetic individuals. Although some had small areas of decreased sweating or minor loss of sensation in their toes, they did not exhibit painful neuropathy, cardiovascular disorders, impaired wound healing, or other cutaneous lesions. With regard to the population as a whole, many individuals with less well-controlled diabetes develop these and other comorbidities that are com-
mon with T2DM. It is possible that if we had studied individuals who were less healthy, we would have found more pronounced impairments in cutaneous vascular control during heating. On the other hand, such a group would have presented significant limitations to data interpretation because of their advanced disease states, and greater safety issues exist when subjecting individuals with heart disease to hyperthermia, which consistently causes tachycardia. In general, a continuing challenge of studying physiological mechanisms in diabetes is how to separate the influence of the disease per se from the myriad potential influences of related cardiovascular, metabolic, and physical conditions (e.g., obesity). We attempted to control for as much of this as was possible by studying relatively healthy diabetic individuals, and comparing them with controls of similar age and body size. In doing so, however, it is likely that we selected those individuals with diabetes who had smaller changes in thermoregulatory control of skin blood flow compared with their less healthy counterparts.

Although not part of the original goals of the study, we were interested to note that baseline CVC was not different between untreated and BT-treated sites during baseline in the T2DM group, although the two sites were different in our control subjects. This resulted in higher CVC values at untreated sites in T2DM patients, suggesting decreased resting sympathetic vasoconstrictor tone in the patient group (13, 16). However, vasoconstrictor responses to body cooling were similar to controls, suggesting that the response of the system to thermal stimuli was not changed.

In summary, we report in the present study that patients with T2DM have significantly higher $T_{soe}$ thresholds for the onset of active cutaneous vasodilation during whole body heating compared with control subjects of similar age and body size. In contrast, sensitivity of active vasodilation was variable within groups, and it was not different between our control and patient groups. Although overall vasodilator responses to body heating were not significantly impaired in our patient group, the shift in threshold suggests altered neural control mechanisms that might be more functionally significant in individuals with less well-controlled diabetes. In those individuals, a greater shift in threshold, in combination with more pronounced local microvascular impairment, could contribute importantly to impaired thermoregulation in this disease.

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