Type 2 diabetes specifically attenuates purinergic skin vasodilatation without affecting muscarinic and nicotinic skin vasodilatation and sweating

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New Findings (85/100 words):

What is the central question of this study?

It remains to be determined whether type 2 diabetes attenuates muscarinic and nicotinic cutaneous vasodilatation and sweating as well as purinergic cutaneous vasodilatation.

What is the main finding and its importance?

We show that type 2 diabetes specifically attenuates purinergic cutaneous vasodilatation without influencing muscarinic and nicotinic cutaneous vasodilatation and sweating. Our results provide a new valuable information regarding the receptor-specific influence of type 2 diabetes on microvascular function.

ABSTRACT

The present study evaluated whether type 2 diabetes (T2D) attenuates muscarinic and/or nicotinic cutaneous vasodilatation and sweating as well as purinergic cutaneous vasodilatation. Cutaneous vascular conductance and sweat rate were evaluated in 12 healthy nondiabetic older adults (Control, 60±8 years) and 13 older adults with T2D (62±10 years) at three intradermal forearm skin sites perfused with the following: 1) methacholine (muscarinic receptor agonist, 5 doses: 0.0125, 0.25, 5, 100, 2000 mM), 2) nicotine (nicotinic receptor agonist, 5 doses: 1.2, 3.6, 11, 33, 100 mM), or 3) ATP (purinergic receptor agonist, 5 doses: 0.03, 0.3, 3, 30, 300 mM). Each agonist was administered for 25 min per dose. At the end of the protocol, 50 mM sodium nitroprusside was administered to all skin sites to elicit maximum cutaneous vasodilatation. Cutaneous vascular conductance during methacholine and nicotine administration did not differ between groups (all $P>0.05$). By contrast, cutaneous vascular conductance during administration of 30 mM (42±28 vs. 63±26 %max, $P \leq 0.05$) and 300 mM ATP (56±24 vs. 71±20 %max, $P \leq 0.05$) was attenuated in individuals with T2D in comparison to the Control participants. Further, cutaneous vascular conductance during administration of 50 mM sodium nitroprusside was lower in individuals with T2D relative to Control ($P = 0.04$). Methacholine- and nicotine-induced sweating was similar between groups (all $P > 0.05$). Thus, T2D attenuates purinergic mediated cutaneous vasodilatation without affecting muscarinic and nicotinic cutaneous vascular and sweating responses.

(235/250 words)

INTRODUCTION

Type 2 diabetes (T2D) is a metabolic disorder characterized by insulin resistance and hyperglycemia. Further, T2D is a well-established risk factor for cardiovascular disease (Gu *et al.*, 1999) and is associated with endothelial dysfunction, as reflected by impaired acetylcholine-induced forearm vasodilatation (Makimattila *et al.*, 1999; Woodman *et al.*, 2005). In addition, T2D is associated with altered cutaneous vascular (microvascular) responses (Caballero *et al.*, 1999; Colberg *et al.*, 2002; Sokolnicki *et al.*, 2007), though this is not always observed especially when evaluated in relatively healthy individuals with well-controlled T2D and free of comorbidities such as peripheral neuropathy (Sokolnicki *et al.*, 2007; Fujii *et al.*, 2017b). To date, there remains a paucity of information on how T2D may modulate, if at all, the mechanisms regulating cutaneous blood flow.

Studies have shown that T2D attenuates acetylcholine-induced cutaneous vasodilatation (Veves *et al.*, 1998; Caballero *et al.*, 1999; Pek *et al.*, 2017), a well-established index of microvascular endothelial function, though this is not a universal finding (Brooks *et al.*, 2008a; Brooks *et al.*, 2008b). Given that acetylcholine activates both muscarinic and nicotinic receptors (Pappano, 2011), whether the impaired vasodilatory response to acetylcholine (Veves *et al.*, 1998; Caballero *et al.*, 1999; Pek *et al.*, 2017) is due to either altered muscarinic or nicotinic receptor function, or a combination of both, cannot be discerned. Hence it remains to be determined if T2D impairs muscarinic and/or nicotinic receptor function in mediating cutaneous vasodilatation.

T2D is associated with a reduced ability to produce (Stump *et al.*, 2003; Petersen *et al.*, 2005) and release (Richards *et al.*, 2014) ATP, potentially reducing ATP-sensitive purinergic receptor activation. It is therefore plausible that T2D leads to altered purinergic receptor function, which are known to contribute to cutaneous vascular regulation (Wingo *et al.*, 2010; Fujii *et al.*, 2015a; Fujii *et al.*, 2015b; Kalsi *et al.*, 2017). However, no study to date evaluated whether T2D modulates the cutaneous vascular response to purinergic receptor activation elicited by ATP. In human skin low concentrations of ATP may cause vasoconstriction (Lang *et al.*, 2017) whereas high concentrations have been shown to induce a dose-dependent vasodilatation (Wingo *et al.*, 2010; Fujii *et al.*, 2015a; Fujii *et al.*, 2015b; Kalsi *et al.*, 2017) without affecting sweat rate (Fujii *et al.*, 2015a; Fujii *et al.*, 2015b). Further, previous work has demonstrated that ATP induced vasodilatation in

conduit arteries (forearm) is impaired in individuals with T2D (Thaning *et al.*, 2010). It is possible therefore that T2D may attenuate ATP-mediated cutaneous vasodilatation.

Human skin possesses eccrine sweat glands that are important in dissipating heat therefore the regulation of body core temperature. Most but not all previous studies showed that T2D impairs the local sweating response during whole-body heat stress or acetylcholine administration (Fealey *et al.*, 1989; Levy *et al.*, 1991; Caselli *et al.*, 2003; Petrofsky *et al.*, 2005). Blunted acetylcholine-induced sweating in individuals with T2D suggests that attenuated peripheral sweat gland function. However, given that acetylcholine activates both muscarinic and nicotinic receptors as mentioned above, it remains to be determined whether T2D impairs muscarinic and/or nicotinic sweating. In the present study, we examined muscarinic and nicotinic cutaneous vasodilatation and sweating and purinergic cutaneous vasodilatation in older adults with T2D. We hypothesized that T2D attenuates cutaneous vasodilatation and sweating in response to muscarinic and nicotinic agonists as well as ATP induced cutaneous vasodilatation.

MATERIALS AND METHODS

Ethical approval

This study was approved by the University of Ottawa Health Sciences and Science Research Ethics Board and conformed to the *Declaration of Helsinki*. Written informed consent was received from all volunteers before their participation.

Participants

Thirteen older adults diagnosed with T2D for >5 years and 12 healthy nondiabetic older adults (Control) were tested in this study. None of the participants reported a history of specific medical conditions (cystic fibrosis transmembrane conductance regulator mutations, skin disorders, uncontrolled hypertension, heart disease, and/or peripheral neuropathy), were smoking or had smoked in the past 10 years. All participants with T2D were taking following medications (number of subjects are indicated in parenthesis): metformin (13), statin (5), dipeptidyl peptidase-4 inhibitor (4), sulfonylurea (3), angiotensin converting enzyme inhibitor (2), insulin (1), angiotensin receptor blocker (3), sodium/glucose cotransporter 2 inhibitor (2), and diuretic (2). Participants in the Control

group were not taking prescription medication. Further, older females were not undergoing hormone replacement therapy. During the preliminary session, height was assessed using a stadiometer (Model 2391, Detecto Scale Company, Webb City, MO, USA), and body mass was determined using a digital weight scale platform (Model CBU150X, Mettler Toledo Inc., Schwerzenbach, Switzerland) with a weighing terminal (Model IND560, Mettler Toledo Inc). Resting arterial blood pressure was obtained by manual auscultation using a mercury column sphygmomanometer (Baumanometer Standby Model).

Experimental session

Prior to the experimental session, all participants refrained from consuming over-the-counter medications for >48 h (e.g., nonsteroidal anti-inflammatory drugs, vitamins, and minerals), alcohol and caffeine for >24 h, and performing any strenuous physical activity for >12 h. Further they were restricted from consuming food >2 h prior to the start of the trial. Upon arrival to the laboratory, participants rested in a semi-recumbent position on a bed in a thermoneutral environment (24ºC). During this time, a 25-gauge needle was inserted into the unanesthetized dermal layer of the skin of the left dorsal forearm using an aseptic technique with the entry and exit points separated by ~2.5 cm. A microdialysis fiber (30 kDa cutoff, 10 mm membrane; MD2000, Bioanalytical Systems, West Lafayette, IN, USA) was then passed through the lumen of the needle, which was then withdrawn. Three microdialysis fibers were placed in the skin, each separated by ~4 cm between sites to avoid between-site interference of drug administration. Each fiber was connected to a liquid switcher (Model 110, CMA Microdialysis AB, Kista, Sweden) to ensure continuous infusion when switching between doses.

Approximately 10 min after the placement of the three microdialysis fibers, all skin sites were perfused with lactated Ringer's solution (Baxter, Deerfield, IL, USA) for a period of >80 min to allow trauma associated with fiber/needle insertion to subside. Perfusion at each of the skin sites was maintained at a rate of 4.0 μl min^{-1} using a micro-infusion pump (Model 4004, CMA Microdialysis, Solna, Sweden). Thereafter, a 10 min baseline measurement was initiated after which pharmacological agents were administered at the three intradermal forearm skin sites as follows: 1) methacholine (Sigma-Aldrich, St. Louis, MO, USA) (muscarinic receptor agonist, 5 doses: 0.0125, 0.25, 5, 100, 2000 mM),

2) nicotine (MP Biomedicals, Santa Ana, CA) (nicotinic receptor agonist, 5 doses: 1.2, 3.6, 11, 33, 100 mM), or 3) ATP (Cayman Chemical, Ann Arbor, MI, USA) (purinergic receptor agonist, 5 doses: 0.03, 0.3, 3, 30, 300 mM). All doses for each of the agents were determined based on our previous work for methacholine (Fujii *et al.*, 2015a; Fujii *et al.*, 2016), nicotine (Fujii *et al.*, 2017a), and ATP (Fujii *et al.*, 2015b) administration. Methacholine, nicotine, and ATP were all infused in a dose dependent manner, with each dose administered for 25 min at a rate of 4.0 µl min^{-1}. After the completion of drug administration, 50 mM of sodium nitroprusside (Sigma-Aldrich) was administered for 20-30 min at each microdialysis site at a rate of 6.0 µl min^{-1} to elicit maximal cutaneous blood flow. This response can also represent endothelium-independent vasodilatation. Our pilot work (unpublished data) confirmed that 50 mM sodium nitroprusside induces maximal cutaneous vasodilatation to levels similar to those observed with local skin heating to 44°C. The criterion for maximal blood flow was >2 min of stable plateau in cutaneous blood flow.

Measurements

Sweat capsules were attached with the aid of topical skin glue (Collodion HV, Mavidon Medical products, Lake Worth, FL, USA) on the intradermal microdialysis skin site, each covering 1.1 cm^2 area of the skin. This capsule was specifically designed for use with intradermal microdialysis (Meade *et al.*, 2016). An integrated laser-Doppler flowmetry probe (model 413; Perimed, Stockholm, Sweden) was inserted within a form-fitting hole located at the center of the sweat capsule, which permitted the simultaneous measurement of cutaneous blood flow and sweating. Each laser Doppler probe was connected to a recording system (PeriFlux System 5000, Perimed, Stockholm, Sweden). To obtain sweat rate, dry compressed air equilibrated to room temperature (~24˚C) was supplied to each capsule at a rate of 0.2 l min^{-1}. A high-precision dew point mirror (Model 473, RH systems, Albuquerque, NM, USA) was utilized to measure the water content of the effluent air from the sweat capsule. This water content was used to evaluate local forearm sweat rate every 5 s. The measured sweat rate was normalized to capsule area (mg min^{-1} cm^{-2}). Cutaneous red blood cell flux, an index of cutaneous blood flow expressed in perfusion units, was obtained at a rate of 32 Hz. Cutaneous vascular conductance (CVC) was evaluated by

cutaneous red blood cell flux divided by mean arterial pressure (diastolic arterial pressure plus one-third the difference between systolic and diastolic pressures). Manual auscultation was performed using a mercury column sphygmomanometer (Baumanometer Standby Model) to obtain blood pressures every 10-15 min. CVC data were presented as %max to minimize the effect of site-to-site heterogeneity on cutaneous blood flow (Minson, 2010).

Data analysis

Baseline CVC and sweat rate were obtained by averaging values over the final 5 min of the 10 min baseline measurement at each of the three skin sites before commencing any receptor agonist administration. The maximum absolute CVC elicited by sodium nitroprusside administration was obtained over a minimum 2 min period for each skin site. In order to minimize between-site variations in baseline CVC and sweat rate as well as maximum absolute CVC, responses were averaged over three skin sites for each participant to compare values between groups. All CVC and sweat rate values during each dose of methacholine and ATP administration were obtained by averaging measurements made over the last 5 min of the 25 min infusion for each dose. Given that nicotinic sweating has been shown to fluctuate transiently (Fujii *et al.*, 2017a), peak sweat rate (5-min average) achieved during each dose of nicotine administration was analyzed. CVC during nicotine administration was evaluated by averaging values over the last 5 min of the 25 min infusion period for each dose. CVC and sweat rate data during administration of each agonist were presented as relative changes from Baseline.

Statistical analysis

Statistical analyses were conducted using SPSS 24 (IBM, Armonk, NY, USA). Based on CVC (mean difference: 17 %max, standard deviation: 16 %max) (Fujii *et al.*, 2014) and sweat rate (mean difference: 0.1 mg min^{-1} cm^{-2}, standard deviation: 0.08 mg min^{-1} cm^{-2}) (Stapleton *et al.*, 2014) responses obtained in our previous works, with a 80% power and a significance level of 0.05, a minimal sample size of n = 9 for CVC and n = 8 for sweat rate was determined. CVC and sweat rate were analyzed with a two-way, mixed-model analysis of variance with the non-repeated factor of group (T2D and Control) and the repeated factor of stage (baseline and each dose of pharmacological agent). When a significant interaction

or main effect was detected, *post hoc* multiple comparisons were carried out using modified version of the Bonferroni correction (Holm-Bonferroni's method). Student's *t*-tests were employed where applicable. The level of significance for all analyses was set at $P \leq 0.05$. All values are reported with a mean ± 95 % confidence interval (1.96 × standard error of the mean) unless otherwise indicated.

RESULTS

Participant characteristics

Age, height, body mass, and resting mean arterial pressure were all matched between groups (all $P > 0.05$, Table 1).

Cutaneous vascular conductance

No between-group differences in baseline CVC were observed (all $P > 0.05$, Figure 1A). Relative to Baseline, CVC increased in response to the administration of each agonist at all concentrations in both groups (all $P \leq 0.05$, Figure 2) with the exception of no significant increase in CVC with 0.03 and 0.3 mM doses of ATP in the T2D group (all $P > 0.10$, Figure 2C). Throughout methacholine and nicotine administration, CVC was similar between groups (Figure 2A and B, all $P > 0.05$). However, CVC was blunted in the T2D versus the Control group during administration of 30 and 300 mM ATP (both $P \leq 0.05$, Figure 2C). Similarly, maximum absolute CVC was lower in T2D individuals ($P = 0.04$, Figure 3).

Sweating

Baseline sweat rate was higher in the T2D relative to Control group ($P = 0.03$, Figure 1B). Sweat rate was elevated above Baseline when methacholine and nicotine were administered at all doses in both groups (all $P \leq 0.05$, Figure 4A and B) except no significant increase in CVC was observed with 11 and 33 mM nicotine in the Control group and 33 mM nicotine in the T2D group ($P = 0.08$-0.14, Figure 4B). Administration of ATP did not influence sweat rate in either group (all $P > 0.05$, Figure 4C). There were no between-group differences in sweat rate at any treatment site (Figure 4).

DISCUSSION

We showed that older adults with well-controlled T2D demonstrated similar methacholine- and nicotine-mediated cutaneous vasodilatation and sweating responses relative to their healthy age-matched counterparts. However, ATP-induced cutaneous vasodilatation was attenuated in T2D individuals. Further, we showed that cutaneous vasodilatation induced by sodium nitroprusside, a nitric oxide donor, was attenuated in older individuals with T2D. Taken together, we showed that T2D is associated with the attenuation of purinergic-mediated cutaneous vasodilatation with no effect on muscarinic and nicotinic cutaneous vascular and sweating responses.

Cutaneous vasodilatation

Both healthy older adults and those with T2D displayed dose-dependent augmentations in cutaneous vasodilatation in response to exogenous ATP (Figure 2C), findings consistent with observations in young adults (Fujii *et al.*, 2015b). These responses appear to be mediated via the activation of purinergic receptors (i.e., P2Y) located on the endothelial cells, as was demonstrated in resistance artery of human skin in vitro (Martin *et al.*, 1991). However, the magnitude of ATP-induced cutaneous vasodilatation was attenuated in the individuals with T2D relative to their healthy age-matched counterparts (Figure 2C), suggesting that T2D attenuates purinergic cutaneous vasodilatation. Our findings build upon a previous report showing that T2D reduces ATP-induced vasodilatation in human conduit arteries (Thaning *et al.*, 2010). Altogether, these findings indicate that T2D may impair both micro- and macro-vascular response to ATP in humans *in vivo*.

ATP induced cutaneous vasodilatation has been shown to be partly induced via nitric oxide-dependent mechanisms with no influence of cyclooxygenase (Fujii *et al.*, 2015b). Previous work has shown that T2D does not alter the magnitude of contribution of nitric oxide in mediating cutaneous vasodilatation during a passive- (Sokolnicki *et al.*, 2009) or exercise-induced heat stress (Fujii *et al.*, 2017b). This suggests that the reduced ATP-mediated cutaneous vasodilatation in individuals with T2D may not stem from diminished nitric oxide bioavailability. In addition to nitric oxide synthase and cyclooxygenase, endothelium-dependent hyperpolarization has also been shown to play an important role in the regulation of vasodilatation (Edwards *et al.*, 2010). Given nitric oxide synthase and cyclooxygenase do not appear to be involved in the attenuated ATP induced

cutaneous vasodilatation in individuals with T2D, cutaneous vasodilatation associated with endothelium-dependent hyperpolarization may be impaired in this population. Further research is required to evaluate this possibility.

We found that cutaneous vasodilatation elicited by methacholine infusion was similar between groups (Figure 2A). This is consistent with previous work using acetylcholine (Brooks *et al.*, 2008a; Brooks *et al.*, 2008b), though other studies reported that acetylcholine-mediated cutaneous vasodilatation is impaired by T2D (Arora *et al.*, 1998; Veves *et al.*, 1998; Caballero *et al.*, 1999). While the underlying reasons for the disparate findings are unclear, it is important to note that, to the best of our knowledge we are the first to evaluate methacholine induced cutaneous vasodilatation in individuals with T2D, whereas all other studies employed acetylcholine (Veves *et al.*, 1998; Caballero *et al.*, 1999; Fuchs *et al.*, 2017; Pek *et al.*, 2017). Methacholine specifically activates muscarinic receptors only whereas acetylcholine has been shown to stimulate both muscarinic and nicotinic receptors (Pappano, 2011). Hence our study was able to separate muscarinic and nicotinic receptor distinct activation in mediating cutaneous vasodilatation, and suggests that muscarinic cutaneous vasodilatation is not influenced by T2D.

Nicotine-induced cutaneous vasodilatation did not differ between groups (Figure 2B), suggesting that T2D has no effect on nicotinic cutaneous vasodilatation. Nicotinic receptor activation can facilitate acetylcholine release via a cholinergic axon reflex (Schlereth *et al.*, 2005). In keeping with this prior observation, we recently reported that muscarinic receptor blockade abolished nicotinic cutaneous vasodilatation at 1.2-11 mM nicotine (Fujii *et al.*, 2017a). Thus, the lack of cutaneous vasodilatory response with the administration of nicotine at concentrations of 1.2-11 mM in individuals with T2D in the current study suggests that T2D does not modulate the cholinergic axon reflex. It should be noted that cutaneous vasodilatation elicited by high doses of nicotine (33-100 mM) occurs independently of muscarinic receptors (Fujii *et al.*, 2017a). Nicotinic receptor activation on cutaneous vascular endothelial cells (Hagforsen *et al.*, 2002) may underpin this vasodilatory response and, based on our results, T2D may not alter endothelial nicotinic receptor function.

In addition to our observed attenuation in the ATP induced cutaneous vasodilatation, we also found an attenuated endothelium-independent cutaneous vasodilatation in the T2D

individual as assessed by the level of cutaneous perfusion elicited by a high dose of sodium nitroprusside (Figure 3). Indeed, previous studies demonstrate endothelium-independent cutaneous vasodilatation in individuals with T2D (Williams *et al.*, 1996; Caballero *et al.*, 1999; Sokolnicki *et al.*, 2007). It is plausible that the lower cutaneous perfusion during sodium nitroprusside in individuals with T2D reflects a lower vascular smooth muscle responsiveness to vasodilators (e.g., nitric oxide). This potential mechanism is supported by the observation that T2D can increase proliferation, adhesion and/or migration of vascular smooth muscle cells (Faries *et al.*, 2001). Further, a previous study reported that T2D does not alter skin capillary density (Jaap *et al.*, 1996). Thus, our observation of a lower cutaneous perfusion during sodium nitroprusside administration does not appear to be due to cutaneous capillary rarefaction.

Sweating

Consistent with the healthy young individuals of our previous work (Fujii *et al.*, 2014; Fujii *et al.*, 2017a), methacholine and nicotine but not ATP activated sweating in the current study (Figure 4). Regarding the between-group comparison, methacholine-induced sweating was similar between groups (Figure 4A) and are consistent with recent work showing that local sweating in individuals with T2D was similar to their age-matched counterparts as assessed during a passive- (Kenny *et al.*, 2016) and exercise-induced (Fujii *et al.*, 2017b) heat stress. Moreover, the present study showed that nicotinic sweating did not differ between groups (Figure 4b). We recently demonstrated that nicotinic sweating is exclusively mediated via the activation of muscarinic receptors in young healthy individuals at doses of 1.2-100 mM (Fujii *et al.*, 2017a); a response possibly associated with axon-reflex induced acetylcholine release. Thus, consistent with our observations of nicotinic cutaneous vasodilatation, we also show that T2D does not appear to alter cholinergic axon reflex in human skin.

Baseline sweat rate was higher in individuals with T2D relative to their healthy counterparts (Figure 1B). This may be associated with regional changes in sweat rate with T2D characterized by a relative hyperhidrosis on the upper body (including forearm) and lower body anhidrosis (Fealey *et al.*, 1989). However, we did not assess sweating responses

on the lower body. Thus, future studies are necessary to delineate the physiological mechanisms underpinning the higher resting sweat rate in individuals with T2D.

Considerations

We evaluated cutaneous vascular and sweating responses in relatively healthy individuals with well controlled T2D and no peripheral neuropathy. This may explain why we did not observe an attenuation in muscarinic and nicotinic cutaneous vasodilatation and sweating in our T2D participants. It has been shown that individuals with T2D who have peripheral neuropathy exhibit greater attenuation in cutaneous vascular and sweating responses in comparison to those without peripheral neuropathy (Ishibashi *et al.*, 2014; Pek *et al.*, 2017). It is therefore possible that muscarinic and nicotinic differences in cutaneous vasodilatation and sweating would be observed in older diabetic adults with poor glucose control and/or peripheral neuropathy. Regardless, in the current study we observed marked attenuations in cutaneous vasodilatation in response to ATP and sodium nitroprusside, suggesting deterioration of the cutaneous vascular function even in relatively healthy adults with T2D.

All participants with T2D were on prescription medications in the present study. We do not know whether the chronic intake of prescription medications modulates our results. However, we did not observe between-group differences in CVC and sweat rate during methacholine and nicotine administration. Moreover, previous work showed a similar pattern of whole-body (Kenny *et al.*, 2013) and local (Kenny *et al.*, 2013; Fujii *et al.*, 2017b) heat loss responses in individuals with T2D regardless of differences in prescribed medication. While this may suggest that medication use and or type might not greatly modulate local control of sweating and cutaneous perfusion in this group, future studies must be conducted to assess this response.

Perspective and significance

The present study demonstrates that ATP-induced cutaneous vasodilatation is attenuated by T2D albeit in the absence of changes in muscarinic and nicotinic cutaneous vasodilatation and sweating. Given that microvascular dysfunction may precede cardiovascular disease (Ijzerman *et al.*, 2003; Kraemer-Aguiar *et al.*, 2008), we speculate that restoring purinergic receptor function in individuals with T2D might serve as a

management therapy to maintain normal or near normal microvascular function thereby possibly reducing the risk of cardiovascular disease. Moreover, it may help preserve or re-establish near normal levels of skin perfusion, which represents an important avenue of heat dissipation during heat stress. Type 2 diabetes is associated with higher rates of heat illness and death during heat stress when compared to the general population (Kenny *et al.*, 2010; Kenny *et al.*, 2016).

CONCLUSION

We showed that T2D impairs purinergic cutaneous vasodilatation while no effect on muscarinic and nicotinic cutaneous vasodilatation and sweating was observed.

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COMPETING INTERESTS

None.

AUTHOR CONTRIBUTIONS

N.F. and G.P.K. conceived and designed experiments. N.F. and B.D.M.. contributed to data collection. N.F. performed data analysis. N.F., R.D.M., B.D.M., T.N., R.J.S., and G.P.K. interpreted the experimental results. N.F. drafted the manuscript. N.F., R.D.M., B.D.M., T.N., R.J.S., and G.P.K. edited and revised the manuscript. All authors approved the final version of the manuscript. All experiments took place at the Human and Environmental Physiology Research Unit located at the University of Ottawa.

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622 **Table 1**. Participant characteristics

	Control	T2D
Number of subjects (male/female)	12 (3/9)	13 (3/10)
Age (years)	60 ± 8	62 ± 10
Height (m)	1.68 ± 0.10	1.64 ± 0.06
Body mass (kg)	80 ± 13	84 ± 13
Mean arterial pressure (mmHg)	95 ± 8	89 ± 7
HbA_{1C} (%)		7.5 ± 1.0
Duration of diabetes (years)	n/a	12.8 ± 9.9

623 All values are expressed as means ± standard deviation.

624 Values did not differ between groups (all $P > 0.05$).

FIGURE LEGENDS

Figure 1: **Baseline cutaneous blood flow and sweat rate.**
Cutaneous vascular conductance (panel A) and sweat rate (panel A) measured at Baseline in older healthy adults (Control, open bar, n = 12) and those with type 2 diabetes (T2D, filled bar, n = 13). Data are presented as mean ± 95% confidence interval.

Figure 2: **Cutaneous vascular response to each agonist.**
Cutaneous vascular conductance during administration of methacholine (panel A), nicotine (panel B), and ATP (panel C) in older healthy adults (Control, open bar, n = 12) and those with type 2 diabetes (T2D, filled bar, n = 13). All values are expressed as means ± 95% confidence interval. * control vs. type 2 diabetes ($P \leq 0.05$).

Figure 3: **Maximum cutaneous blood flow.**
Maximum absolute cutaneous vascular conductance achieved by sodium nitroprusside administration in older healthy adults (Control, open bar, n = 12) and those with type 2 diabetes (T2D, filled bar, n = 13). Data are presented as mean ± 95% confidence interval.

Figure 4: **Sweating response to each agonist.**
Sweat rate during administration of methacholine (panel A), nicotine (panel B), and ATP (panel C) in older healthy adults (Control, open circle, n = 12) and those with type 2 diabetes (T2D, filled circle, n = 13). All values are expressed as means ± 95% confidence interval. Sweat rate during methacholine, nicotine, and ATP administration was not different between groups ($P > 0.29$ for a main effect of group or an interaction between group and stage).

Figure 1

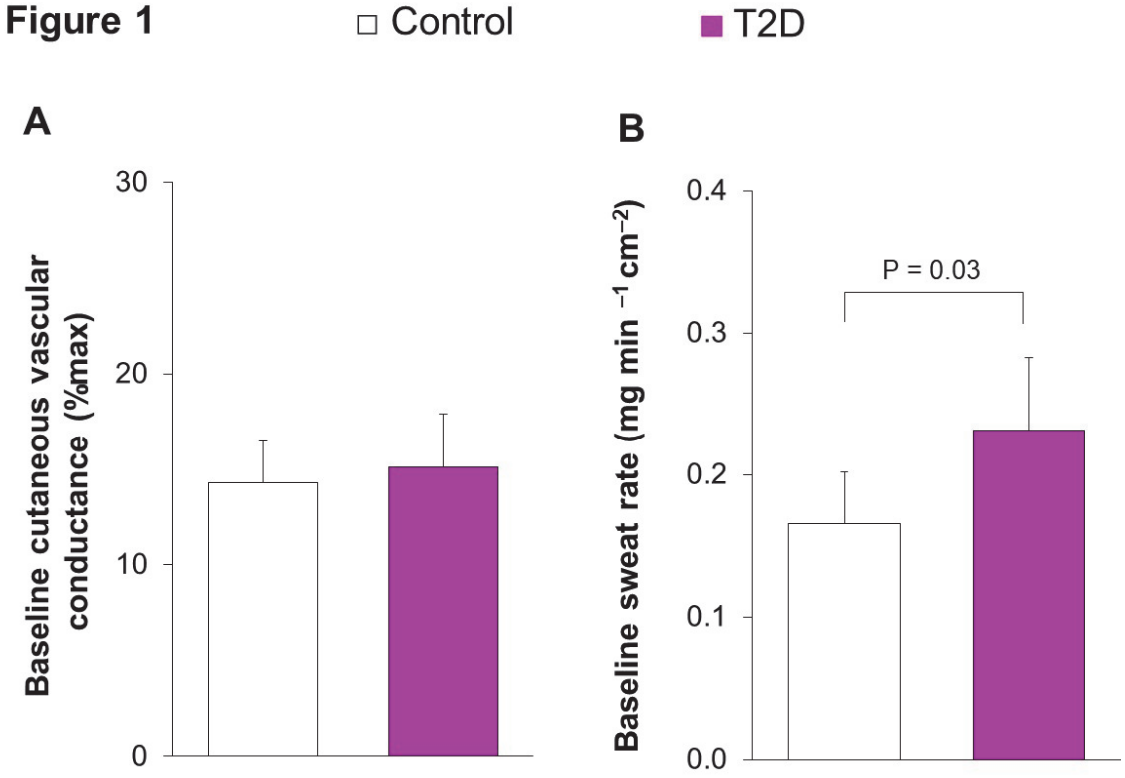

□ Control
■ T2D
A
Baseline cutaneous vascular conductance (%max)
30
20
10
0
B
Baseline sweat rate (mg min −1 cm−2)
0.4
0.3
0.2
0.1
0.0
P = 0.03

Figure 2

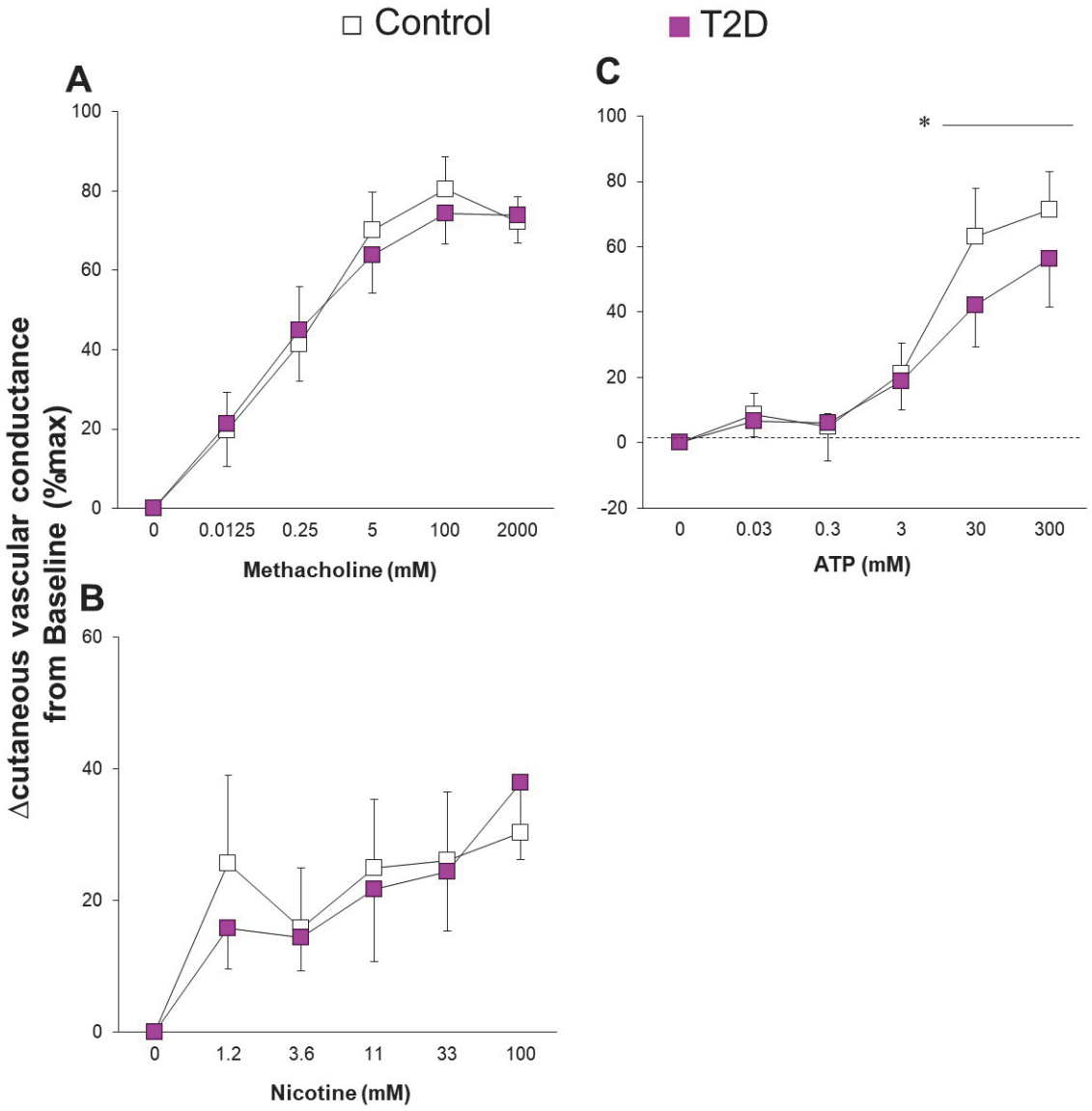

□ Control
■ T2D
A
100
80
60
40
20
0
0
0.0125
0.25
5
100
2000
Methacholine (mM)
C
100
80
60
40
20
0
-20
*
0
0.03
0.3
3
30
300
ATP (mM)
B
60
40
20
0
0
1.2
3.6
11
33
100
Nicotine (mM)
Δcutaneous vascular conductance from Baseline (%max)

Figure 3

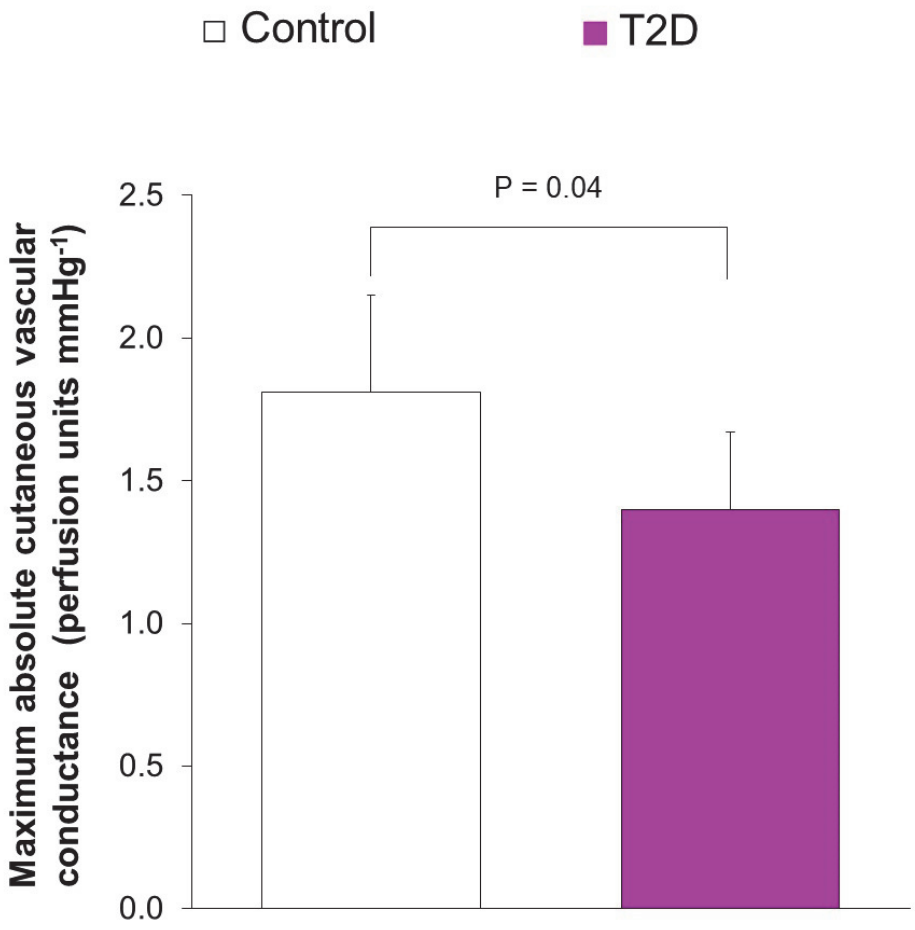
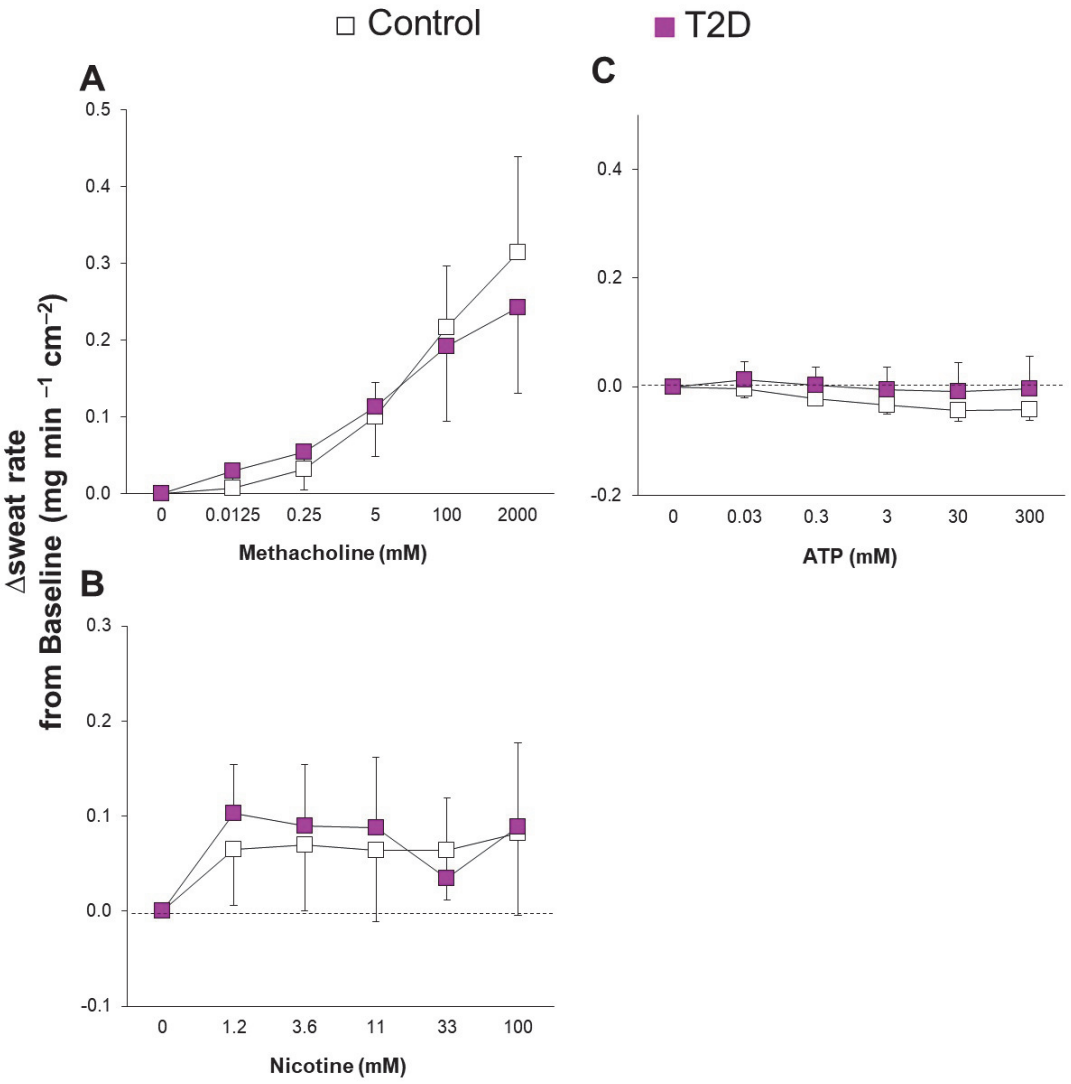

□ Control
■ T2D
P = 0.04
Maximum absolute cutaneous vascular conductance (perfusion units mmHg-1)
2.5
2.0
1.5
1.0
0.5
0.0

Figure 4


□ Control
■ T2D
A
B
C
Δsweat rate from Baseline (mg min −1 cm−2)
Methacholine (mM)
Nicotine (mM)
ATP (mM)
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
0
0.0125
0.25
5
100
2000
1.2
3.6
11
33
0.03
0.3
3
30
300