Cutaneous vascular and sweating responses to intradermal administration of prostaglandin E₁ and E₂ in young and older adults: a role for nitric oxide?

Naoto Fujii,¹ Maya Sarah Singh,¹ Lyra Hallili,¹ Pierre Boulay,² Ronald J. Sigal,³ and Glen P. Kenny¹

¹Human and Environmental Physiology Research Unit, University of Ottawa, Ottawa, Canada; ²Faculty of Physical Activity Sciences, University of Sherbrooke, Sherbrooke, Canada; and ³Departments of Medicine, Cardiac Sciences and Community Health Sciences, Faculties of Medicine and Kinesiology, University of Calgary, Calgary, Canada

Submitted 21 December 2015; accepted in final form 6 April 2016


Cyclooxygenase (COX) contributes to cutaneous vasodilation and sweating responses; however, the mechanisms underlying these responses remain unknown. We hypothesized that prostaglandin E₁ (PGE₁) and E₂ (PGE₂) (COX-derived vasodilator products) directly mediate cutaneous vasodilation and sweating through nitric oxide synthase (NOS)-dependent mechanisms in young adults. Furthermore, we hypothesized that this response is diminished in older adults, since aging attenuates COX-dependent cutaneous vasodilation and sweating. In 9 young (22 ± 5 yr) and 10 older (61 ± 6 yr) adults, cutaneous vascular conductance (CVC) and sweat rate were evaluated at four intradermal forearm skin sites receiving incremental doses (0.05, 0.5, 5, 50, 500 μM each for 25 min) of PGE₁ or PGE₂ with and without coadministration of 10 mM N⁶-nitro-L-arginine, a nonspecific NOS inhibitor. N⁶-nitro-L-arginine attenuated PGE₁-mediated increases in CVC at all concentrations in young adults, whereas it reduced PGE₂-mediated increases in CVC at lower concentrations (0.05–0.5 μM) in older adults (all P < 0.05). However, the magnitude of the PGE₁- and PGE₂-mediated increases in CVC did not differ between groups (all P > 0.05). Neither PGE₁ nor PGE₂ increased sweat rate at any of the administered concentrations for either the young or older adults (all P > 0.05). We show that although cutaneous vascular responsiveness to PGE₁ and PGE₂ is similar between young and older adults, the cutaneous vasodilator response is partially mediated through NOS albeit via low-to-high concentrations of PGE₁ in young adults and low concentrations of PGE₂ in older adults, respectively. We also show that in both young and older adults, PGE₁ and PGE₂ do not increase sweat rate under normothermic conditions.

Sweating, aging; thermoregulation; prostanoids; EP receptor; IP receptor; cAMP; microcirculation

Previous studies have shown that cyclooxygenase (COX) contributes to cutaneous vasodilation (20, 33, 36) and sweating (10), the two main avenues for heat dissipation in humans in vivo. However, the precise mechanisms mediating these responses are still unresolved. The products of COX, prostaglandin E₁ (PGE₁) and E₂ (PGE₂), can directly induce forearm vasodilation in humans in vivo (1, 47) and sweat secretion in vitro (50, 51). Moreover, PGE₁ and PGE₂ can augment nitric oxide (NO) production as demonstrated under in vitro situations in aortic rings from mice (21) and human polymorphonuclear neutrophils (46). Increases in NO have been shown to contribute to cutaneous vasodilation and sweating responses mediated by cholinergic agents under both normothermic resting conditions (12, 29) and those elicited by exercise-induced whole body heat stress (10, 34, 35, 57) in humans. Therefore, it is plausible that PGE₁ and PGE₂ may directly induce cutaneous vasodilation and sweating through NO synthase (NOS)-dependent mechanisms.

Aging diminishes the contribution of COX to cutaneous vasodilation (18, 20) and sweating (11); however, the mechanism(s) underpinning this response remains unresolved. It is plausible that aging may reduce the ability for COX to produce vasodilator prostanoids (i.e., PGE₁ and PGE₂). Alternatively, aging may impair the peripheral sensitivity of the end organs (i.e., skin blood vessels and sweat glands) to prostanoids. The reduced peripheral sensitivity to prostanoids is postulated to be age related as evidenced by lower forearm vasodilation responses to prostacyclin (a COX-derived prostanoid) in human adults aged 61–73 yr relative to those aged 19–45 yr in vivo (44). Similarly, PGE₁- and PGE₂-mediated cutaneous vasodilation and sweating occurring through NOS-dependent mechanisms, if any, may be diminished in older compared with young adults.

The primary objectives of this study were 1) to assess the mechanism(s) underpinning the cutaneous vasodilator and sweating responses to the administration of PGE₁ and PGE₂, and 2) to determine whether aging may influence the pattern of these responses. We evaluated the hypothesis that in young adults, local administration of PGE₁ and PGE₂ directly induces increases in cutaneous blood flow and sweating through NOS-dependent mechanisms, and that these responses are attenuated in older adults. By advancing our understanding of the role of PGE₁ and PGE₂ in the regulation of cutaneous vasodilation and sweating, we can address important knowledge gaps in the mechanism(s) underpinning the regulation of heat loss especially in the context of aging; a factor that has been shown to induce marked impairments in the body’s capacity to dissipate heat (13, 26).

Materials and Methods

Ethical Approval

This study was approved by the University of Ottawa Health Sciences and Science Research Ethics Board, which conform to the guidelines set forth by the Declaration of Helsinki. We obtained verbal and written informed consent from all volunteers before their participation in this study.

Participants

Nine young (19–34 yr, 4 males and 5 females) and 10 older (53–71 yr, 3 males and 7 females) habitually active (2–5 days per wk, ≥30 min of exercise per day) adults participated in one screening and one
experimental session conducted on separate days. Individuals were excluded from the study if they had a history of specific medical conditions (cystic fibrosis, transmembrane conductance regulator mutations, skin disorders, hypertension, heart disease, diabetes, or autoimmune disorders), were taking prescription medication, were smokers, or had smoked within the last 10 years. To control for female menstrual cycles (28), the experimental session took place during the early follicular phase (within 6 days from the beginning of menstruation) in the young adults. None of the young females were taking oral contraceptives, and all older females were postmenopausal.

During the preliminary session, body height was measured using an eye-level physician stadiometer (model 2391, Deteco Scale, Webb City, MO), and body mass was measured using a digital weight scale (model IND560, Mettler Toledo). The participant characteristics are presented in Table 1, including eye-level physician stadiometer (model 2391, Deteco Scale, Webb City, MO), and body mass was measured using a digital weight scale (model IND560, Mettler Toledo). All participants were instructed to abstain from taking medications (including nonsteroidal anti-inflammatory drugs, vitamins, and minerals, and other over-the-counter medications) for at least 48 h before the experimental session. Furthermore, at least 12 h before the experimental session, participants were asked to refrain from consuming alcohol, caffeine, or performing strenuous physical activity. Participants were also restricted from consuming food 2 h before and during the experimental session. Upon arrival to the laboratory, the participants provided a urine sample to evaluate hydration status as assessed by the measurement of urine specific gravity using a handheld total solids refractometer (model TS400, Reichter, Depew, NY). Urine specific gravity evaluated before the experiment was 1.015 ± 0.005 and 1.015 ± 0.004 in the young and older adults, respectively, indicating that participants were euhydric (5). Participants were then required to rest in a semirecumbent upright position on a bed in a thermoneutral environment (ambient air temperature of 27°C). During this time, at four skin sites on the anterior forearm (each separated by 4 cm), a 25-gauge needle was inserted into the unanesthetized skin 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) was then threaded through the lumen of the needle. The needle was subsequently withdrawn leaving the fiber in the dermal layer of the dorsal forearm skin. Each fiber was connected to the outlet port of a liquid switcher (model 110, CMA Microdialysis, Kista, Sweden). At ~10 min after the placement of the microdialysis fibers, two of four fibers were perfused with lactated Ringer (Baxter, Deerfield, IL), whereas the other two fibers were perfused with 10 mM Nω-nitro-L-arginine (L-NNA, Sigma-Aldrich, St. Louis, MO) dissolved in lactated Ringer to nonselectively inhibit NOS. Perfusion at each of the skin sites was maintained at a rate of 4.0 μl/min using a microinfusion pump (model 4004, CMA Microdialysis, Solna, Sweden). L-NNA was continuously perfused for at least 60 min to ensure NOS blockade. The concentration of L-NNA was determined based on previous studies employing intradermal microdialysis in the human skin (8, 36).

After the insertion of the microdialysis probes, a rest period (87 ± 6 min, range: 67–119 min) was employed to ensure that the localized trauma associated with the insertion of the probe had subsided. Thereafter, baseline measurements were acquired for at least 10 min (Baseline). The infusions of PGE1 and PGE2 (both obtained from Cayman Chemical, Ann Arbor, MI) were subsequently administered at a rate of 4.0 μl/min in an incremental manner with five different concentrations (0.05, 0.5, 5, 50, and 500 μM). PGE1 was administered to one of the lactated Ringer (PGE1) and L-NNA sites (PGE1 + L-NNA). Similarly, PGE2 was administered to the other lactated Ringer site (PGE2) as well as to the other L-NNA site (PGE2 + L-NNA). All doses of PGE1 and PGE2 infusions continued for 25 min. The five incremental concentrations of PGE1 and PGE2 were determined in a pilot study wherein two young adults were tested. The administered PGE1 and PGE2 solutions were dissolved in lactated Ringer with pH of 6.5. All solutions containing PGE1, PGE2, and/or L-NNA used in the present study had similar pH levels (~6–7) as assessed by pH test paper (Hydrion pH paper, Sigma-Aldrich). A basic solution (pH > 7.4) was not used since it can degrade both PGE1 and PGE2 to prostaglandin A and B (61). This degradation prevents us from assessing the effects of PGE1 and PGE2 on cutaneous vasodilation and possibly sweating responses, as both prostaglandin A and B can cause vasodilation in humans in vivo (47).

After the infusion of the final concentration of PGE1 and PGE2 (i.e., 500 μM, 50 mM of sodium nitroprusside (SNP, Sigma-Aldrich)) was administered at each microdialysis site at a rate of 6.0 μl/min. The administration of SNP lasted for 20–30 min, until a stable plateau of cutaneous vascular conductance (CVC) was observed over a minimum 2-min period. At this point, blood pressure was measured to quantify maximal CVC. Immediately after the maximal cutaneous vasodilation response was achieved, a higher concentration of PGE1 and PGE2 (10 mM), each dissolved in 20% of dimethyl sulfoxide (Sigma-Aldrich), was infused to the PGE1 and PGE2 sites, respectively, in two young participants. This was done to evaluate if this higher concentration of PGE1 and PGE2 would modulate sweat rate.

**Measurements**

Cutaneous red blood cell flux expressed in perfusion units was collected continuously at a sampling rate of 32 Hz with laser-Doppler flowmetry (PeriFlux System 5000, Perimed, Stockholm, Sweden). Integrated laser-Doppler flowmetry probes with a 7-laser array (model 413, Perimed) were placed in the center of each sweat capsule (see below for details) over each microdialysis fiber for simultaneous measurement of both local forearm sweat rate and cutaneous red blood cell flux. Manual auscultation was performed using a mercury column sphygmomanometer (Baumanometer Standby model, WA Baum, Copiague, NY).
water content of the effluent air from the sweat capsule was measured with high-precision dew point mirrors (model 473, RH systems, Albuquerque, NM). Long vinyl tubes were employed for connections between the gas tank and the sweat capsule, and between the sweat capsule and the dew point mirror. Local forearm sweat rate was calculated every 5 s based on the difference in water content between influent and effluent air multiplied by the flow rate and normalized for the skin surface area under the capsule (mg·min⁻¹·cm⁻²).

Data Analysis

Because we did not observe sex-related differences in the pattern of responses in cutaneous vasodilation and sweating, males and females were combined for data analysis in both groups. Furthermore, in the present study, all data from our older adults between the ages of 53 to 71 yr were combined for analysis since there were no observed age-related differences in sweating and cutaneous vasodilation responses within this group. All CVC and sweat rate values used for data analysis were obtained by averaging measurements made over the last 5 min of each stage (Baseline, 0.05, 0.5, 5, 50, and 500 µM of PGE₁ or PGE₂). PGE₁- or PGE₂-induced changes in CVC from Baseline were also evaluated as ΔCVC at each skin site.

Statistical Analysis

Statistical analyses were performed using the software package SPSS 23 for Windows (IBM, Armonk, NY). Based on sweat rate (54) and CVC results (9) obtained in our previous works with 80% power and a significance level of 0.05, a minimum sample size was calculated. We found that the minimal sample size was n = 8 for sweat rate and n = 9 for CVC. CVC and sweat rate before and during PGE₁ administration were analyzed using a three-way, mixed-model, analysis of variance (ANOVA) with the factors of treatment site (two levels: PGE₁, PGE₁ + l-NNA), age (two levels: young, older), and stage (six levels: Baseline, 0.05, 0.5, 5, 50, or 500 µM of PGE₁). A two-way, mixed-model, ANOVA with the factors of treatment site (two levels: PGE₁, PGE₁ + l-NNA) and age (two levels: young, older) was employed for ΔCVC obtained during PGE₁ administration (separately performed at each stage of 0.05, 0.5, 5, 50, or 500 µM of PGE₁), as well as maximal absolute CVC (perfusion units mmHg⁻¹).

The data analyses detailed above were also performed for PGE₂ data. When a significant interaction or main effect was detected, post hoc comparisons were carried out using Student’s paired (for between-treatment site comparison) or unpaired (for between-group comparison) two-tailed t-tests; Student’s paired one-tailed t-tests corrected for multiple comparisons using a Holm Bonferroni procedure were employed to test whether sweat rates obtained during administration of each dose of PGE₁ or PGE₂ were higher than that obtained at Baseline. The Kolmogorov-Smirnov test assessed the normal distribution of the data. In the instance that a normal distribution was not detected, the Wilcoxon signed-rank test was used for all pairwise comparisons and the Mann-Whitney U test was used for all between-group comparisons. The level of significance for all analyses was set at P ≤ 0.05. All values are reported with a mean ± 95% confidence interval (1.96 × standard error of the mean).

RESULTS

Cutaneous Vascular Conductance

Figure 1 shows the time-course changes in an index of cutaneous blood flow in response to PGE₁ (Fig. 1A) and PGE₂ (Fig. 1B) obtained in a representative young subject. Cutaneous vascular responses (expressed both in CVC and ΔCVC from Baseline) are presented in Table 2 (during Baseline) and Figs. 2 and 3 (during Baseline and PGE₁ and PGE₂ administration). There were no age-related differences in CVC and ΔCVC measured at the Control site (all P > 0.11) throughout the protocol from Baseline to the last concentration of administered PGE₁ or PGE₂. However, CVC at the l-NNA + PGE₁ site was lower in young relative to older adults (all P < 0.05). The lower CVC response is associated with an l-NNA-mediated reduction in CVC, which occurred before and/or during the infusion of PGE₁ in the young adults only (all P < 0.05).

Similarly, ΔCVC during PGE₁ administration was lower at the l-NNA site compared with the Control site in young adults (all P < 0.05). By contrast, l-NNA coadministration resulted in a lower CVC response (including ΔCVC) during PGE₂ administration in the older adults (all P < 0.05). However, this reduction was not observed in the young adults (all P > 0.08). There were no differences in maximal absolute CVC between treatment sites and groups (P > 0.18 for main effects of age levels: PGE₁, PGE₁ + l-NNA), age (two levels: young, older), and stage (six levels: Baseline, 0.05, 0.5, 5, 50, or 500 µM of PGE₁). A two-way, mixed-model, ANOVA with the factors of treatment site (two levels: PGE₁, PGE₁ + l-NNA) and age (two levels: young, older) was employed for ΔCVC obtained during PGE₁ administration (separately performed at each stage of 0.05, 0.5, 5, 50, or 500 µM of PGE₁), as well as maximal absolute CVC (perfusion units mmHg⁻¹).

The data analyses detailed above were also performed for PGE₂ data. When a significant interaction or main effect was detected, post hoc comparisons were carried out using Student’s paired (for between-treatment site comparison) or unpaired (for between-group comparison) two-tailed t-tests; Student’s paired one-tailed t-tests corrected for multiple comparisons using a Holm Bonferroni procedure were employed to test whether sweat rates obtained during administration of each dose of PGE₁ or PGE₂ were higher than that obtained at Baseline. The Kolmogorov-Smirnov test assessed the normal distribution of the data. In the instance that a normal distribution was not detected, the Wilcoxon signed-rank test was used for all pairwise comparisons and the Mann-Whitney U test was used for all between-group comparisons. The level of significance for all analyses was set at P ≤ 0.05. All values are reported with a mean ± 95% confidence interval (1.96 × standard error of the mean).

Table 2. Cutaneous vascular conductance measured during Baseline (in %max)

<table>
<thead>
<tr>
<th></th>
<th>Young</th>
<th>Older</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGE₁</td>
<td>21 ± 6</td>
<td>17 ± 9</td>
</tr>
<tr>
<td>PGE₁ + l-NNA</td>
<td>8 ± 3*</td>
<td>21 ± 10*</td>
</tr>
<tr>
<td>PGE₂</td>
<td>17 ± 5</td>
<td>19 ± 4</td>
</tr>
<tr>
<td>PGE₂ + l-NNA</td>
<td>12 ± 5*</td>
<td>18 ± 8</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± 95% confidence interval. Data from 9 young and 10 older participants are presented. PGE₁ and PGE₂; prostaglandin E₁ and E₂, respectively; l-NNA, N⁷-nitro-l-arginine, a nonspecific nitric oxide synthase inhibitor. *PGE₁ vs. PGE₁ + l-NNA site or PGE₂ vs. PGE₂ + l-NNA site in young groups (P < 0.05); †Young vs. older at PGE₁ + l-NNA site (P < 0.05).
and treatment site and an interaction between age and treatment site) (Table 3).

**Sweating**

Sweating responses are presented in Fig. 4. No increases in sweat rate were measured relative to Baseline at any of the administered doses of both PGE1 and PGE2 irrespective of treatment sites and groups (all \( P > 0.91 \)). Sweat rate did not differ between treatment sites and groups throughout the protocol from Baseline to the last concentration of administered PGE1 and PGE2 (all \( P > 0.11 \)). Furthermore, sweat rate was unaffected by the infusion of a high dose of PGE1 and PGE2 (10 mM) at the end of the experimental trial (as verified in two young adults).

**DISCUSSION**

We show for the first time that in response to a low-to-high concentration of PGE1, NOS contributes to cutaneous vasodilation in young adults. This is in marked contrast to the pattern of response measured in older adults wherein NOS contributes to cutaneous vasodilator responses only during administration of low concentrations of PGE2. However, the magnitude of increase in the PGE1- and PGE2-mediated modulation of cutaneous vasodilation did not differ between young and older adults. We also show that neither administration of PGE1 nor PGE2 influenced the sweating response in both young and older adults under nonheat stressed conditions.

**Cutaneous Vascular Response**

In accordance with previous studies (3, 15, 23), we found that in young adults, NOS contributes to resting cutaneous blood flow responses under normothermic conditions (Fig. 2, A and B, Table 2). However, in older adults, the NOS-mediated contributions are absent indicating that this contribution becomes diminished with aging. The abolition of a NOS-mediated influence in older adults may be associated with age-
induced cutaneous vasodilation may occur via Ca\(^{2+}\)-dependent mechanisms as an elevation in intracellular Ca\(^{2+}\) has been shown to activate NOS in the endothelial cells of porcine aorta in vitro (4).

In contrast to the influence of NOS on PGE\(_1\), we showed that NOS does not contribute to the PGE\(_2\)-induced increase in cutaneous vasodilation in young adults (Figs. 2B and 3B) demonstrating that the mechanisms underpinning the PGE\(_1\)-mediated modulation of cutaneous vasodilation differs as a function of the type of prostaglandin (i.e., PGE\(_1\) and PGE\(_2\)). In line with the evidence from cultured hamster ovary cells in vitro (42), it is possible that the disparate responses may be related to the fact that 1) PGE\(_1\), but not PGE\(_2\), activates IP receptors; 2) PGE\(_2\), but not PGE\(_1\), activates prostaglandin F (FP) receptors; and/or 3) PGE\(_2\) has a higher affinity for the EP1 receptor (one of the prostaglandin E receptor) compared with PGE\(_1\).

In older adults, the contribution of NOS to PGE\(_1\)-induced cutaneous vasodilation is diminished (Figs. 2A and 3A) relative to the response measured in young adults. Given IP receptor may underlie the PGE\(_1\)-induced NOS-dependent cutaneous vasodilation as discussed above, the diminished NOS contribution could be due to age-related reduction in IP receptors sensitivity, as was observed in human forearm circulation in vivo (44). Noteworthy, we show that NOS contributes to PGE\(_2\)-induced cutaneous vasodilation only in older adults (Figs. 2B and 3B). At the moment, we are not aware of any plausible explanation for this response and future studies are required to elucidate the underlying mechanisms mediating this response.

Previous in vivo human studies reported that aging attenuates COX-dependent cutaneous vasodilation during administration of acetylcholine (20) and application of whole body heat stress (18). In relation to these responses, we found that similar cutaneous blood flow responses to PGE\(_1\) and PGE\(_2\) occur between young and older adults (Figs. 2 and 3), suggesting that aging does not impair cutaneous vascular responsiveness to prostanooids. Therefore, the previously observed age-related attenuation of COX-mediated cutaneous vasodilation

### Table 3. Maximal absolute cutaneous vascular conductance

<table>
<thead>
<tr>
<th></th>
<th>Young</th>
<th>Older</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGE(_1)</td>
<td>2.30 ± 0.46</td>
<td>2.00 ± 0.63</td>
</tr>
<tr>
<td>PGE(_1) + l-NNA</td>
<td>2.53 ± 0.10</td>
<td>2.32 ± 0.47</td>
</tr>
<tr>
<td>PGE(_2)</td>
<td>2.37 ± 0.44</td>
<td>2.22 ± 0.65</td>
</tr>
<tr>
<td>PGE(_2) + l-NNA</td>
<td>1.82 ± 0.18</td>
<td>2.06 ± 0.49</td>
</tr>
</tbody>
</table>

All values are expressed as means ± 95% confidence interval. Data from 9 young and 10 older participants are presented (in perfusion units mmHg \(-1\) m\(^2\)).
(18, 20) may denote a reduction in the biouavailability of COX-derived vasodilator prostaglandins in human skin.

We show that in both young and older adults, the PGE$_1$- and PGE$_2$-induced increases in cutaneous blood flow were still observed even in the presence of NOS inhibition. This suggests the possible involvement of non-NOS mechanisms, which may be associated with the cAMP signaling cascade. In this regard, using chondrocytes isolated from chicks in vitro, O’Keefe and colleagues (45) showed that both PGE$_1$ and PGE$_2$ can increase cAMP. If this increase occurs in the vascular smooth muscle cells, it would result in vasorelaxation. Alternatively, the activation of potassium channels may also contribute to this response. Both calcium-activated potassium (K$_{Ca}$) channels (2, 3, 31) and ATP-sensitive potassium (K$_{ATP}$) channels (17) can influence the cutaneous vasodilator response by hyperpolarizing vascular smooth muscle cells in the human skin under in vivo situations. Importantly, PGE$_1$ and PGE$_2$ can activate large-conductance calcium-activated potassium (BK$_{Ca}$) channels in human corposal or coronary artery smooth muscle cells in vitro (30, 60) and K$_{ATP}$ channels in rat thoracic aortic rings (7). Finally, PGE$_2$ can cause axon-reflex-mediated cutaneous vasodilation in humans in vivo (48), which may also be involved in non-NOS-dependent cutaneous vasodilation during PGE$_2$ administration. Indeed, an in vivo human study shows that most of the axon-reflex-mediated cutaneous vasodilation can occur independently of NOS (40). These potential mechanisms warrant further investigation.

McCord et al. (33) demonstrated that COX contributes to cutaneous vasodilation during passive heating at rest in young adults in vivo. There are some possible mechanisms mediating this COX-dependent cutaneous vasodilation. A rise in core and/or skin temperatures associated with whole body heat stress would increase the release of neurotransmitters from cholinergic nerves, mediating cutaneous vasodilation in humans in vivo (22, 24). Acetylcholine, a cholinergic neurotransmitter, appears to mediate the aforementioned COX-dependent cutaneous vasodilation during passive heat stress at rest. This is supported by previous studies that have consistently shown that intradermal administration of acetylcholine achieved via microdialysis induces cutaneous vasodilation that is greatly due to COX activation (12, 20, 25, 36). Moreover, the COX-dependent cutaneous vasodilation during heat stress occurs independently of NOS (33). Given that PGE$_2$, but not PGE$_1$, induces NOS-independent cutaneous vasodilation in young adults in the current study (Figs. 2B and 3B), PGE$_2$ may be involved in the COX-dependent cutaneous vasodilation during passive heating at rest (33). Additionally, COX-induced increases in CVC during passive heating at rest was reported to be 16%max (33), which is equivalent to the increase in CVC induced by 0.5 μM PGE$_2$ (Fig. 3B). Therefore, cutaneous vasodilation mediated by 0.5 μM PGE$_2$ may represent COX-dependent cutaneous vasodilation occurring during passive heat stress at rest in young adults.

**Sweating**

We show that the infusion of PGE$_1$ and PGE$_2$ had no effect on sweating (Fig. 4), which is in stark contrast to in vitro studies, which reported that both PGE$_1$ and PGE$_2$ can directly increase sweat secretion (50, 51). The underlying reasons for the disparity between in vivo and in vitro observations remain unclear. However, sympathetic nerves, sensory nerves, vessels, adipose tissues, and keratinocytes surround sweat glands under in vivo conditions, which differ from in vitro conditions wherein adjacent tissues are not present. Therefore, the surrounding tissues may directly or indirectly participate in the sweat secretion process in vivo. In line with this, all four prostaglandin E (EP) receptors (EP$_1$, EP$_2$, EP$_3$, EP$_4$) are expressed in keratinocytes within the human skin (49). Future studies elucidating if other surrounding tissues (sympathetic nerves, sensory nerves, vessels, and adipose tissues) that possess EP receptors would provide more insight into whether and how PGE$_1$ and PGE$_2$ modulate sweating in vivo. Also, differential mechanisms between in vivo and in vitro are not unique to PGE-mediated responses. In fact, in our previous work we showed comparable results for ATP whereby no effect of ATP was measured during infusion in human skin in vivo (8), although increases in sweating were recorded during in vitro work (52).

We previously reported that COX contributes to the sweating response in young adults exercising in the heat (10). Although we are not aware of any evidence showing the presence of prostanoid receptors in the human sweat gland, a study shows that COX is present (41); the latter of which is consistent with the observed COX-dependent sweating (10). Given that PGE$_1$ and PGE$_2$ did not elicit sweating in human skin under normothermic conditions (Fig. 4), the COX-dependent increase in sweating observed during exercise in the heat (10) is likely the result of an augmentation in the signaling cascade mediated via muscarinic receptor activation, which is a key mechanism that was previously identified in the regulation of sweating in vivo (24, 32, 38, 53). However, we also showed that under normothermic resting conditions in vivo, COX inhibition does not modulate muscarinic receptor activation-induced sweating (9). Thus it is plausible that increases in body temperature (i.e., skin and/or core temperature) are necessary to observe COX-dependent modulation of sweating responses. This is supported by our previous observation that a COX-dependent increase in sweating occurred with marked increases in body temperature induced by exercise in the heat (10). Mechanistically, it has been shown that local heating of skin can activate the heat shock protein 90 in the human skin in vivo (58), which can in turn activate COX, leading to an increase in the production of prostanoids, as evidenced by rat fibroblastic cells in vitro (56).

**Considerations**

It is important to consider that there are several types of prostaglandin receptors, which include, but are not limited to, EP (EP$_1$, EP$_2$, EP$_3$, EP$_4$), IP, and FP receptors. PGE$_1$ and PGE$_2$ can activate multiple prostaglandin receptors. For example, PGE$_1$ can activate IP receptors, PGE$_2$ can activate FP receptors, and both PGE$_1$ and PGE$_2$ can activate all four EP receptor subtypes as assessed in Chinese hamster ovary cells in vitro (42). Therefore, it is uncertain as to which prostaglandin receptor(s) is(are) responsible for the observed cutaneous vasodilation responses associated with PGE$_1$ and PGE$_2$. Future studies investigating which prostanoid receptors exist in human skin vessels (endothelial and/or vascular smooth muscle cells) would provide some insight into addressing this knowledge gap. However, elucidating information about prostanoid recep-
tor is particularly challenging in humans in vivo given the significant safety concerns associated with the infusion of pharmacological agents (both agonist and/or antagonist) that would be required to assess the specific prostanoid receptors, which to date have yet to be tested in humans in vivo. However, prostacyclin or its analogue, which activates IP receptors, has been widely used in humans in vivo for clinical (i.e., pulmonary arterial hypertension) and experimental (14, 44) purposes. If the observed responses with PGE1 in the present study are primarily due to activation of IP receptors as discussed above, administration of prostacyclin or its analogue, therefore, should replicate cutaneous vasodilation responses induced by PGE1. Thus at a minimum, advancing our understanding of the role of IP receptors in PGE1-induced cutaneous vasodilation is an important direction to take in future studies.

It is also important to consider that the effect of NOS on cutaneous vasodilation in response to PGE1 and PGE2 differs slightly depending on whether absolute CVC or ΔCVC is employed. Using ΔCVC from Baseline may be a more appropriate approach as CVC during Baseline differs between sites. When ΔCVC is used, the effect of NOS on cutaneous vasodilation is limited to lower concentrations of PGE1 and PGE2 (Fig. 3) relative to absolute CVC (Fig. 2). Nevertheless, the between-group differences in NOS contribution to cutaneous vasodilation during PGE1 and PGE2 administrations are clearly depicted using both approaches.

Prostaglandins can cause inflammatory responses in the skin (16). Therefore, the increase in cutaneous blood flow in response to the administration of PGE1 and PGE2 observed in the present study may reflect an inflammatory response, rather than PGE1- and PGE2-induced direct activation of cutaneous vessels.

We did not precisely measure pH in the pharmacological solutions used for the present study. Therefore, we do not know how much pH differed between solutions and the extent to which the difference in pH influenced our results.

**Perspectives and Significance**

Our results show that both PGE1 and PGE2 can induce substantial cutaneous vasodilation in older adults to a similar degree as observed in young adults. Heat dissipation in older adults is normally attenuated (13, 26). Thus interventions that increase vasodilator prostanoids (e.g., PGE1 and PGE2) may augment heat loss response of cutaneous vasodilation and thus heat transfer from the body core to the surface of the skin, mitigating or reversing the age-related impairments in the physiological capacity of the body to dissipate heat. Direct evaluation of this possibility during challenges to human heat balance associated with elevations in environmental heat load, metabolic rate during physical activity in the heat, and the combination of the two is required in future studies.

In conclusion, we show that NOS partially contributes to a prostaglandin-mediated increase in cutaneous vasodilation in young and older adults. However, the response is dependent on the type of prostaglandin (i.e., PGE1 and PGE2) such that NOS partly contributes to cutaneous vasodilation in response to low-to-high concentrations of PGE1 (0.05–50 μM) in young adults, whereas this contribution is only observed in response to lower concentrations of PGE2 (0.05–0.5 μM) in older adults. Furthermore, cutaneous vasodilation responsiveness to PGE1 and PGE2 is similar between young and older adults at all concentrations tested. Finally, we show that for all concentrations of PGE1 and PGE2 employed in the present study, there is no influence on the sweating response in both young and older adults under normothermic conditions. This study advances our physiological knowledge associated with COX-dependent modulation of cutaneous vasodilation and sweating, and provides valuable insights into how aging modulates these mechanisms.

**ACKNOWLEDGMENTS**

We greatly appreciate all of the volunteers for taking their time to participate in this study.

**GRANTS**

This study was supported by the Natural Sciences and Engineering Research Council of Canada (Discovery Grant, RGPIN-06313-2014 and Discovery Grants Program-Accelerator Supplement, RGPAS-462252-2014; funds held by G. P. Kenny). G. P. Kenny was supported by a University of Ottawa Research Chair Award. N. Fujii, M. S. Singh, and L. Halili were supported by the Human and Environmental Physiology Research Unit.

**DISCLOSURES**

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

**AUTHOR CONTRIBUTIONS**

N.F. and G.P.K. conception and design of research; N.F., M.S.S., and L.H. performed experiments; N.F. and M.S.S. analyzed data; N.F., M.S.S., L.H., P.B., R.J.S., and G.P.K. interpreted results of experiments; N.F. prepared figures; N.F. drafted manuscript; N.F., M.S.S., L.H., P.B., R.J.S., and G.P.K. edited and revised manuscript; N.F., M.S.S., L.H., P.B., R.J.S., and G.P.K. approved final version of manuscript.

**REFERENCES**

heat loss responses in older males exercising in the heat? J Physiol 593:
12. Fuji
i N, Reinke MC, Brun
t VE, Minson CT. Impaired acetylcholine-
induced cutaneous vasodilation in young smokers: roles of nitric oxide and 
13. Greaney JL, Stanhe
dewicz AE, Proctor DN, Alexander LM, Kenney WL. Impairments in central cardiovascular function contribute to attenu-
ated reflex vasodilation in aged skin. J Appl Physiol 119: 1411–1420, 
2015.
14. Hellmann M, Roust
t M, Gaillard-Bigot F, Cracowski JL. Cutaneous i
ontophoresis of treprostinil, a prostacyclin analog, increases microvascu-
lar blood flux in diabetic malleolar area. Eur J Pharmacol 758: 123–128, 
2015.
16. Hohlo
17. Ho
js N, Struel M, Cankar K. The effect of glibenclamide on acetylchol
18. Holowat
19. Holowat
t LA, Thompson CS, Kenney WL. Acute ascorbate supplementation alone or combined with arginine inhibition augments reflex cuta-
20. Holowat
21. Hristovsk
22. John
son JM, Minson CT, Kellogg DL Jr. Cutaneous vasodilator and vasoconstric
23. Kellog
26. Kenne
27. Kut
28. K
29. Medow MS, Glo
ver JL, Stewart JM. Nitric oxide and prostaglandin inhibition during acetylcholine-mediated cutaneous vasodilation in hu-
31. Metzler-We
34. Muller-Dee
35. Narumi
36. Nasral
37. Nichol
38. O’Keke
e RJ, Crabb ID, Puzas JE, Rosier RN. Influence of prostaglan
39. Pallapi
es D, Peskar BA. Effect of prostaglandin (PG) E1 and its initial metabolites on neutrophil-induced inhibition of human platelet aggrega
40. Robi
nson BF, Collier JK, Karim SM, Somers K. Effect of prostaglan
41. Robinson BF, Collier JK, Karim SM, Somers K. Role of prostaglan
42. Rusk
43. Rundhau
46. Sato K, Ohtsu
48. Staple
ton JM, Fiji
49. Takahash
i HK, Iwagaki H, Tamura R, Xue D, Sano M, Mori S, Yoshino T, Tanaka N, Nishibori M. Unique regulation profile of pro-
50. Tanioka T, Naka

