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Cutaneous vasomotor responses in boys and men

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Abstract

Few studies have investigated skin blood flow (SkBF) in children and age-related differences in the underlying mechanisms. We examined mechanisms of SkBF responses to local heating, post-occlusive reactive hyperaemia (PORH), and isometric hand-grip exercise (IHE) in adult and prepubescent males, hypothesizing that SkBF responses would be greater in children compared to adults. We measured SkBF in 12 boys (9 ± 1 y) and 12 men (21 ± 1 y) using laser-Doppler flowmetry at rest, in response to 3-min PORH, 2-min IHE, and local skin heating to 39°C (sub-maximal) and 44°C (maximal). Using wavelet analysis we assessed endothelial, neural, and myogenic activities. At rest and in response to local heating to 39°C , children had higher SkBF and endothelial activity compared to men ($d\geq 1.1$, $p<0.001$) and similar neurogenic and myogenic activities ($d<0.2$, $p>0.05$). Maximal responses to 44°C local skin heating, PORH, and IHE did not differ between boys and men (all $d\leq 0.2$, $p>0.05$). During PORH children demonstrated greater endothelial activity compared to men ($d\geq 0.6$, $p<0.05$); in contrast, men had higher neurogenic activity ($d=1.0$, $p<0.01$). During IHE there were no differences in endothelial, neurogenic, and myogenic activities ($d<0.2$, $p>0.3$), with boys and men demonstrating similar increases in endothelial activity and decreases in myogenic activity ($d\geq 0.8$, $p<0.05$). These data suggest that boys experience greater levels of SkBF at rest and in response to sub-maximal local heating compared to men, while maximal responses appear to be similar. Additionally, endothelial mediators seem to contribute more to vasodilatation in boys than in men.

Keywords: children, laser-Doppler, local heating, exercise, reactive hyperaemia.

Introduction

Skin blood flow plays a major role in human thermoregulation, increasing and decreasing flow in response to changes in both core and skin temperature (Johnson et al. 2014). During systemic heat stress, it has been argued that children thermoregulate as effectively as adults. However, while adults rely more on evaporative heat loss, children rely more on enhanced skin blood flow for heat dissipation (Falk and Dotan 2008, Rowland 2008). Reflex thermoregulatory changes in skin blood flow are controlled by two arms of the sympathetic nervous system: an active vasodilator system and a noradrenergic vasoconstrictor system. In adults, the cutaneous vasodilator response to local skin heating (with no change in core temperature) is primarily endothelial-dependent, with endothelial nitric oxide synthase (eNOS) (Kellogg et al. 2008, 2009) and endothelial-derived hyperpolarizing factor (EDHF) (Brunt and Minson 2012) mediating the response. Skin heating to 39°C has been shown to be primarily eNOS-dependent (Choi et al. 2014), while local heating to 42°C involves a combination of eNOS, EDHF, and neural processes (Johnson et al. 2014). The vasodilator response to local skin heating in children and adolescents has been shown to be larger than in adults (Martin et al. 1995, Hodges et al. 2010). These findings are also supported by the consistent findings that during heat stress, sweating rate is lower in children than adults, suggesting a greater reliance on increasing blood flow to the skin to achieve thermoregulatory balance (Falk and Dotan 2008, Rowland 2008). There have been no studies that have examined the underlying mechanisms of local heating-induced vasodilatation in healthy children and how they compare to adults.

In adults, isometric exercise elicits physiological responses such as increases in heart rate (HR), mean arterial pressure (MAP), cardiac output ($\dot{Q}$), and sympathetic nerve activity directed to skeletal muscle (Johnson 2010). It has also been reported that isometric exercise leads to an increase in skin sympathetic nerve activity (Johnson 2010). However, in normothermia, the data for forearm skin blood flow responses to isometric exercise appear to be equivocal with both vasoconstriction and vasodilatation being reported (Saad et al. 2001, McCord and Minson 2005, Johnson 2010). There is no work examining the skin blood flow responses to isometric exercise in children and no understanding of the underlying mechanisms eliciting the cutaneous vascular responses.

The marked, transient increase in microvascular blood flow following arterial occlusion, known as post-occlusive reactive hyperaemia (PORH), is a common assessment of microvascular function and reactivity. The responsible mechanisms governing the cutaneous PORH are unclear, partly because the mediators of this response are not fully understood. Four factors are currently thought to contribute to the PORH response: endothelial vasodilators, metabolic vasodilators, the myogenic response, and sensory nerves. The involvement of endothelial vasodilators is equivocal. That is, nitric oxide and prostaglandins may (Dakak et al. 1998, Binggeli et al. 2003) or may not (Wong et al. 2003, Zhao et al. 2004, Lorenzo and Minson 2007) be mediators of PORH, while EDHFs probably are involved (Lorenzo et al. 2007). There are no studies that have investigated the mechanisms of PORH in children.

The use of spectral analysis of the laser-Doppler signal has enabled non-invasive examination of the mechanisms underlying cutaneous vascular responses (Stefanovska et al. 1999, Kvandal et al. 2006). Previously, invasive and/or pharmacological approaches (intradermal microdialysis and iontophoresis) were necessary to investigate the endothelial and neural components regulating skin blood flow. Use of spectral analysis of the rhythmic variations in cutaneous blood flow enables the examination of specific oscillatory frequencies within the signal to delineate the underlying mechanisms involved in vascular control from the cardiac rhythms (1 Hz) down to the innate endothelium-related oscillations (0.01 Hz) (Stefanovska et al. 1999). Beyond the ability to compare and contrast the blood flow response, this approach also enables the examination of the mechanisms of action mediating changes in blood flow in children and adults.

There is a dearth of work examining the effects of age and maturation on cutaneous vasodilation in healthy children. Indeed, cutaneous vascular responses to local skin heating, isometric handgrip exercise, and PORH have not been investigated in children relative to adults and neither have the underlying mechanisms. Therefore, the aim of the present study was to examine cutaneous vasomotor responses to PORH, isometric exercise, and local skin heating in the skin of the forearm (non-glabrous) in response to a 3 min reactive hyperaemia, local forearm muscle contraction, and local skin heating respectively, in male youths and young adults. These tests were chosen to potentially provide us with insight regarding mechanistic differences between children and adults: Local heating to 39°C is

99 primarily been shown to be NO-mediated (Choi et al. 2014). PORH is generally considered
100 to be mediated by prostaglandins and EDHF (Wong et al. 2003, Lorenzo and Minson 2007).
101 Isometric response is thought to be neurally-mediated (Crandall et al. 1995, Saad et al.
102 2001). Thus, these 3 assessments were intended to provide us with some insight into NO-
103 dependent, NO-independent, and neural mechanisms and how they might differ between
104 children and adults. We hypothesize that SkBF responses at rest and in response to local
105 heating, PORH, IHE will be greater than those observed in adults. We hypothesize that
106 these larger responses in boys compared to men will be mediated by greater endothelial
107 and neurogenic contributions.

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Materials and methods

Participants

Twelve healthy men and 12 healthy boys participated in the study. They did not have any overt symptoms or history of cardiovascular disease, and were not taking any medications. The study was cleared by Brock University's Bioscience Ethics Review Board (#16-026) and adhered to the Declaration of Helsinki. Prior to participating in this study, adult participants gave written and verbal consent, whereas both children participants and their parents gave written and verbal consent. Participant characteristics are presented in Table 1. No differences were observed between groups in relative maximal oxygen uptake ($\dot{V}O_{2\max}$) and both groups were of normal-weight.

Procedure

Each participant visited the laboratory twice. The first visit involved familiarization with study equipment and procedures, anthropometric measurements (mass and stature) and an incremental cycling test to volitional exhaustion to determine $\dot{V}O_{2\max}$. The second visit was the experimental session in which the cutaneous blood flow response was investigated in response to isometric exercise, PORH and local heating, as outlined in Figure 1.

Measurements

Laser-Doppler flowmetry was used for an index of forearm skin blood flow (PeriFlux 5010 laser-Doppler perfusion monitor; Perimed; Järfälla, Sweden), and was expressed as laser-Doppler flux (LDF). LDF provides an accurate index of skin blood flow and is not influenced by underlying skeletal muscle blood flow (Saumet et al. 1988). An integrated laser-Doppler local heating probe (Probe 413; Perimed) was used. A calibration device (PF 1000, Perimed) standard was used to adjust the laser-Doppler fluxmeter readings to coincide with the readings obtained with Perimed's Motility Standard. Manipulation of local skin temperature (T_{loc}) was achieved with local heating unit and heating probe holders (PF5020 local heating units and PeriFlux 5020 Temperature Unit; Perimed) that controlled and monitored skin surface temperature.

140 *Isometric exercise*

141 Prior to data collection, three maximal voluntary contractions (MVC) were
142 performed. The maximal response was used to determine the MVC. The participant
143 performed a 2-min isometric handgrip exercise at 30% MVC with the LDF probe
144 maintaining T_{loc} at 33°C.

145

146 *Post-occlusive reactive hyperaemia*

147 Post-occlusive reactive hyperaemia (PORH) was performed, as previously described
148 (Tew et al. 2012, Hodges et al. 2017c), by inflating a size-appropriate blood pressure cuff
149 on the upper arm (bicep) to 230 mm Hg and maintaining that pressure for 3 min. The cuff
150 was then rapidly deflated and a 5-min recovery period was recorded. Ten to fifteen
151 minutes of additional time ensured resting levels of skin blood flow were achieved.

152

153 *Local skin heating*

154 Local skin heating was performed in two stages. First, following baseline
155 measurements at a thermoneutral temperature of 33°C. Second, T_{loc} was increased to 39°C
156 at a rate of 3°C·min⁻¹ and held for a minimum 25 min or until a stable plateau had been
157 established. In adults, the cutaneous vasodilatation to this level of local skin heating has
158 been reported to be primarily eNOS-mediated (Choi et al. 2014). Finally, T_{loc} was increased
159 from 39 to 44°C at a rate of 1°C·min⁻¹ and held for a minimum of 25 min. This level of local
160 heating elicits a maximal thermal vasodilatation in adults that is dependent on many
161 factors including endothelial, neural, and other sources (Wong and Fieger 2010, Johnson et
162 al. 2014, Hodges and Sparks 2014, Hodges and Del Pozzi 2014). Each duration of
163 measurement outlined above and in Figure 1 was a minimum, with each level of heating
164 maintained until a stable plateau in LDF was achieved.

165

166 *Determination of $\dot{V}O_{2max}$.*

167 Participants began with a 3–5-minute warm-up and cadence familiarization,
168 followed by the graded exercise to exhaustion. Boys typically started at 25–35 W and
169 increased by 10 W·min⁻¹, while men typically began the graded protocol at 60–70 W and

increased by $10 \text{ W} \cdot \text{min}^{-1}$. The test continued to volitional exhaustion. As has previously demonstrated by Barker *et al.* (2011) we did not rely on the commonly-used fixed criteria for $\dot{V}\text{O}_2\text{max}$ attainment (*e.g.*, 90% predicted max heart rate (HR), or respiratory exchange ratio of 1.05), but rather exceeded them in motivating the participants and verbally encouraging them to reach their respective utmost exhaustion. To verify that the testing protocol indeed elicited highest possible values, supra-maximal testing at 105% of the peak $\dot{V}\text{O}_2$ test's final power, was administered, ~ 10 min after the graded exercise protocol (as suggested by Barker *et al.* (2011)). In no case was an improvement observed relative to the preceding $\dot{V}\text{O}_2\text{max}$ test. $\dot{V}\text{O}_2\text{max}$ was recorded as the average of the highest three consecutive 15-s intervals near the end of the volitional exercise test. Expired gas was collected and analyzed using the Moxus metabolic cart (AEI Technologies, PA, USA), calibrated prior to each test. A cadence of 80 rpm or higher was required throughout each test. The metabolic cart could be switched between standard (adult) and small (pediatric) mixing chambers. The latter was used for children of less than ~ 40 kg body mass.

Other cardiovascular measurements

HR was calculated from R-R intervals that were collected using a standard three-lead electrocardiogram (BioAmp, ADInstruments, Colorado Springs, USA). Blood pressure, was recorded by auscultation from the contralateral arm every 30 min by the same investigator. Additionally, photoplethysmography (Nexfin, Edwards Lifesciences, Irvine, USA) was used to continuously calculate blood pressure and cardiac output ($\dot{Q}$) from finger perfusion. Mean arterial pressure (MAP) was calculated as $0.33 \text{ systolic pressure} + 0.67 \text{ diastolic pressure}$.

Wavelet transform

A Morlet mother wavelet was used to perform wavelet analysis on the LDF signal. Wavelet analysis was chosen as it provides good time and frequency resolution within the uncertainty principle. It uses an adjustable window to provide good frequency resolution for lower and higher frequencies by using a longer and shorter analysis window, respectively. This method characterizes the dynamics of signals over a wide frequency

range, from 0.005 to 1.6 Hz. The six frequency intervals and associated physiological functions were as follows: 0.005-0.01 Hz (endothelial: NO-independent) and 0.01-0.02 Hz (endothelial: NO-dependent) (Kvandal et al. 2006); 0.02-0.05 Hz (neurogenic) (Söderström et al. 2003, Hodges et al. 2017a); 0.05-0.15 Hz (myogenic) (Johnson 1989, Söderström et al. 2003); 0.15-0.40 Hz (respiratory); 0.40-1.6 Hz (cardiac).

Data collection and statistical analysis

Laser-Doppler flowmetry (LDF) and finger photoplethysmography data were collected at 40 Hz, local skin temperature data were collected at 4 Hz, and ECG data were collected at 1 KHz (LabChart, ADInstruments). The data from the entire protocol ~95 min was exported and run through the wavelet transform in a custom written computer script (Iatsenko et al. 2015) (Matlab®, The MathWorks Inc., Natick, MA, USA). Subsequently, median amplitudes were chosen from the appropriate section (Fig. 1) such that data for basal (4 min), hand grip (final 20 s), recovery from hand grip (final 20 s), PORH (10 s), local heating at 39°C and 44°C (4 min) were extracted. The same sections were extracted as means for laser-Doppler data.

Analysis of time segments of 20 min is needed for good low-frequency resolution and detection of oscillations in the low-frequency range (Stefanovska et al. 1999). We ran the wavelet transform on a minimum of 80 min of data. While we have chosen short time windows for certain phases to extract median amplitudes of the vasodilator responses (handgrip, PORH), the fact that the wavelet was run on the entire data laser-Doppler signal ensured that even these smaller extracted portions had appropriate resolution and power to examine the low-frequency bands (Hodges et al. 2017b, 2017a).

Laser-Doppler flowmetry data were normally distributed as assessed by the Shapiro-Wilk test of normality. Data are presented as cutaneous vascular conductance (CVC = $\text{LDF} \cdot \text{MAP}^{-1}$). CVC data are presented as raw ($\text{PU} \cdot \text{mm Hg}^{-1}$) and normalized to maximal thermal vasodilation, local skin heating to 44°C (%max). Isometric handgrip CVC data were analyzed by repeated measures ANOVA with a Dunnett's multiple-comparison test. Two-way repeated measures ANOVA (group x temperature) compared CVC responses in boys and men at 33°C and 39°C, with a Bonferroni *post-hoc* correction for multiple comparisons.

Vasomotion data (AU) were normally distributed as assessed by the same criteria above. A two-way repeated measures ANOVA (group x temperature) was used to examine local heating responses (baseline, 39°C, and 44°C) with a Bonferroni *post-hoc* correction for multiple comparisons. Participant characteristics are provided as mean and SD, while all other data are presented as mean and 95%CI. Analyses were performed using Graphpad Prism (v6.01, GraphPad Software Inc., La Jolla, CA, USA).

Cohen's *d* (Cohen 1988) was used to calculate effect sizes using ESCI-delta (Geoff Cumming, La Trobe University, Australia), and descriptors for magnitudes used is based upon those of Sawilowsky (Sawilowsky 2009): Very small 0.01, Small 0.2, Medium 0.5, Large 0.8, Very large 1.20, Huge 2.00.

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Results

Basal and local heating responses

There were main effects for group and heating level (both $p < 0.05$). Basal CVC was higher in the boys (0.225 ± 0.028 PU·mmHg⁻¹) than in the men (0.102 ± 0.0237 PU·mmHg⁻¹) ($d = 2.7$, $p < 0.001$) (Fig. 2A). This was also the case for CVC normalized to the maximal response (12 ± 2 %max vs. 6 ± 2 %max) ($d = 1.4$, $p = 0.002$) (Fig. 2B). We also observed greater CVC response to local heating of 39°C in the boys compared to the men in both raw CVC (0.442 ± 0.099 vs. 0.268 ± 0.073 PU·mmHg⁻¹) ($d = 1.1$, $p = 0.01$) and normalized to max (23 ± 4 %max vs. 15 ± 4 %max) ($d = 1.0$, $p = 0.019$). Boys had higher basal responses (Table 2). When the responses to 39°C were expressed as percent change from baseline, there was no difference between the boys and men ($125 \pm 81\%$ vs. $183 \pm 80\%$; $d = 0.3$, $p = 0.13$). There were no differences between the groups in raw CVC during local heating at 44°C ($d = 0.2$, $p = 0.6$).

Laser-Doppler flux and wavelet analysis of the local heating responses are presented in Table 2. Laser-Doppler flux comparisons between boys and men mirror those of CVC in that we observed main effects for group and heating level ($p < 0.05$), but no interaction ($p > 0.05$). For the vasomotion analyses, there were main effects for group, heating level, and interaction ($p < 0.05$). Under basal conditions, men have stronger neurogenic and myogenic components than boys (all $d \geq 0.9$, $p < 0.05$); whereas, endothelial NO-independent and NO-dependent influences appear to have a greater role in boys (both $d \geq 0.8$, $p < 0.05$). In response to local heating to 39°C both groups present with increased NO-independent endothelial activity (both $d \geq 1.1$, $p < 0.05$). In response to skin heating to 44°C we observed that cardiac influence increased (both $d \geq 1.2$, $p < 0.05$) in both groups and became the main contributing component to the vasomotor response.

Post-occlusive reactive hyperaemia

There was no difference in the maximal CVC responses to PORH between the boys and men, whether expressed in absolute units or as a percentage of the maximal CVC (both $d \leq 0.1$, $p > 0.3$) (Table 3). However, both time-to-peak ($d = 2.9$, $p < 0.001$) and area-under-the-curve (AUC) ($d = 2.2$, $p < 0.001$) of the PORH were greater in boys compared to men.

There was no difference in the maximal LDF response between the boys and men ($d=0.3$, $p=0.2$) (Table 4). However, both endothelial NO-independent and NO-dependent activities were higher in the children (both $d\geq 0.6$, $p<0.05$); while neurogenic, respiratory, and cardiac frequency bands were higher in the men compared to the boys (all $d\geq 0.8$, $p<0.05$).

Isometric handgrip exercise

During the isometric handgrip exercise trials, cutaneous measurements (LDF, raw CVC, and CVC %max) were significantly greater in the boys compared with men (Fig. 3). In absolute values, MAP and $\dot{Q}$ were lower in boys compared to men (all $d>0.8$, $p<0.05$), but when expressed as changes from baseline, there were no differences between boys and men (all $d<0.2$, $p>0.05$); HR responses did not differ ($d<0.2$, $p>0.05$). Endothelial and neurogenic responses to exercise were greater in the men compared to the boys (both $d\geq 0.8$, $p<0.05$) (Table 5).

Isometric handgrip exercise caused a significant increase in both boys and men in LDF, MAP, and HR (all $d>0.8$, $p<0.05$). When cutaneous vasomotor responses were expressed as CVC, during isometric exercise, there was a marked vasoconstriction only in the boys (all $d>0.8$, $p<0.05$) while there was no change in the men (Fig. 3). $\dot{Q}$ was unchanged during the exercise in both men and boys ($d<0.2$, $p>0.05$). Isometric exercise caused similar increases in NO-independent endothelial activity and decreases in myogenic activity in both men and boys groups (all $d>0.8$, $p<0.05$) (Table 5).

Post-handgrip, there were abrupt, transient increases in CVC in boys ($d>0.8$, $p<0.05$). In the men, $\dot{Q}$ was also temporarily increased relative to the exercise and pre-exercise baseline ($d=0.7$, $p=0.006$). LDF, MAP, and HR all decreased post-exercise in both men and boys (all $d>0.8$, $p<0.05$).

Discussion

We used a combination of laser-Doppler flowmetry and wavelet analysis of the LDF signal to non-invasively examine mechanisms of cutaneous vascular function in both adult and prepubescent males. Cutaneous vascular responses and endothelial activity at rest and in response to local skin heating to 39°C as well as during PORH were greater in boys compared to men; while men had larger neurogenic responses. Maximal LDF responses to 44°C local skin heating, PORH, and isometric handgrip exercise did not differ between boys and men and both boys and men presented with similar increases in endothelial, neurogenic, and myogenic activity. These data suggest that, despite similar maximal responses, children respond with a greater level of vasodilatation at rest and to submaximal stimuli compared to men. Additionally, endothelial-mediators seem to be the predominant mediators of vasodilatation in boys. Finally, we demonstrate that the combination of laser-Doppler flowmetry and wavelet analysis to non-invasively examine mechanisms of cutaneous vascular function can be successfully applied in children.

Local heating

Forearm skin vascular conductance, expressed both as CVC and CVC normalized to maximum (44°C), was higher in boys compared with men at both 33°C and NO-mediated 39°C local heating (Choi et al. 2014), suggesting enhanced microvascular endothelial dilation. This is consistent with previous reports of higher skin blood flow at rest and during local heating (Martin et al. 1995, Hodges et al. 2010) in children and adolescents compared with adults. It is also consistent with the observed greater increase in skin blood flow in younger compared with older boys during systemic heat stress [exercise in the heat (Falk et al. 1992)], and the assumption of greater reliance on dry heat exchange in children compared with adults (Falk and Dotan 2008, Rowland 2008). Note that there were no difference in fitness level ($\dot{V}O_{2\max}$) between the groups and no participants were overweight or obese, both of which can affect endothelial function in both children and adults (Abbott et al. 2002, Roche et al. 2010).

At rest under thermoneutral conditions (33°C) and in response to submaximal local heating, boys had greater NO-dependent and NO-independent activities than the men.

Consistent with previous reports in adults, endothelial activity plays an important role under basal thermoneutral conditions (Del Pozzi et al. 2013, Hodges and Sparks 2014) and in response to local skin heating to 39°C (Choi et al. 2014). In general, the endothelial response, both NO-dependent and -independent, was greater in children, and it would seem that boys are more responsive or are more sensitive to increases in skin temperature than men. This greater sensitivity may be due to higher responsiveness of the mechanisms governing vasodilatation, specifically of the endothelial tissue. Indeed, this postulate is consistent with the greater endothelial-mediated cutaneous vasodilator response to local skin heating in young vs. older men (Hodges et al. 2017b) and may imply that the ‘aging process’ of the endothelium begins at a very young age. This is consistent with the age-related decreases in skin blood flow in response local heating seen in large cross-sectional studies (Martin et al. 1995, Hodges et al. 2010) and with the maturity-related lower skin blood flow observed in late- compared with pre-pubertal boys (Falk et al. 1992). Also, it is important to note that the vasodilator responses to 39°C heating observed in this study were about half the size of those reported in the Choi et al (Choi et al. 2014) study. Whether this is contributing to the reduced endothelial-signal in men compared to boys is unclear and warrants further investigation.

It is of note that the cardiac interval (0.4–1.6 Hz) was largest during local heating at 44°C. We do not consider this observation to mean that cardiac factors were the mediating force behind this vasodilatory response. Rather, the large vasodilatory response will mean the vascular smooth muscle will be almost maximally-relaxed (flux has increased >10 times compared to basal measures), and the larger oscillations in blood flow that occur between systole to diastole are what are contributing more to the overall power of the signal in the cardiac interval.

Post-Occlusive Reactive Hyperaemia (PORH)

There was no difference in the maximal PORH response between boys and men; however, AUC was greater in boys, as was the time-to-peak. As was the case with local heating, endothelial (both NO-dependent and independent) activity was higher in boys. This is an interesting observation as previous work in adults has shown that the

mechanisms responsible for PORH are unclear, partly because the mediators of this response are not fully understood and the data are inconsistent (Johnson et al. 2014). The role of endothelial-derived vasodilators in adults is equivocal. Nitric oxide and prostaglandins may (Dakak et al. 1998, Binggeli et al. 2003) or may not (Wong et al. 2003, Zhao et al. 2004, Lorenzo and Minson 2007) be mediators of PORH, while EDHFs probably are involved (Lorenzo and Minson 2007). Shear stress stimulation, the primary vasodilator action of reactive hyperaemia, has been shown to increase prostaglandin levels, leading to improved vascular function separate from any contribution of nitric oxide (Koller et al. 1994; Walshe et al. 2005). We recently published work that showed that shear stress leads to a larger peak and AUC PORH responses in healthy adults (Hodges et al. 2017c). Thus, the present data indicate that boys have greater endothelial response (both NO and non-NO sources) in the hyperemic response to brief occlusion, which is consistent with the greater endothelial involvement to local skin heating.

A curious observation is that the time-to-peak for the PORH response was longer in the boys than in the men. This was an unexpected finding, as usually the kinetics (neuromuscular and metabolic) are faster in children compared to adults (Falk and Dotan 2008, Black et al. 2008, Falk et al. 2009, Mitchell et al. 2011). This finding might be a consequence of the greater reliance on endothelial-dependent vasodilators and a smaller contribution of neural input, both sensory and sympathetic (Larkin and Williams 1993, Lorenzo and Minson 2007) in boys compared to men (Table 4), such that these metabolic signals that take longer to elicit vascular responses. Furthermore, the AUC was greater in the boys compared to the men. This might be due to a heavier reliance on the endothelial dilators (NO, PG_i , and EDHF) rather than on neural components in boys. Prior work has shown that in adults, endothelial dilators are less important in adults than neural inputs (Wong et al. 2003, Zhao et al. 2004).

Isometric exercise

In both boys and men, the contribution of both endothelial-NO-dependent and NO-independent activities increased with exercise while other factors decreased or did not change. Thus, it would appear that similar mechanisms were employed by both boys and

men to elicit the vasodilatation that occurred during isometric handgrip exercise. Our study supports previous work which reported mild increase in forearm skin vascular conductance during isometric exercise in adults (Saad et al. 2001, McCord and Minson 2005) and extend these findings to boys. Importantly, this is the first study to demonstrate that in boys the increases in forearm skin vascular conductance during isometric exercise is due to a combination of an increase in both NO-dependent and NO-independent endothelial activity and a reduction in basal sympathetic activity (i.e. sympathetic withdrawal).

Limitations

We did not directly inhibit endothelial enzymes, block sensory and sympathetic nerves, or antagonise receptors. However, wavelet analysis is considered an excellent tool to study the mechanisms of vasomotor responses (Stefanovska et al. 1999). It is particularly attractive for the use in children and other vulnerable populations, because it is non-invasive and does not require the use of pharmacological agents. However, its specific accuracy in these populations is not clear. Additionally, wavelet analysis may be unable to delineate local and reflex sympathetic activity. While previous work has clearly demonstrated that the neural frequency band (0.02-0.05 Hz) is mostly sympathetic (Hodges et al. 2017a), there is also likely to be some sensory nerve involvement. Thus, categorical statement of what the neural influences are and whether the observed effects are of local or reflex origin on cutaneous vascular control are not possible at the present time with this approach. The observation of an increase in myogenic activity with local heating is puzzling. Data supporting the vascular oscillation centred around 0.1 Hz and termed myogenic activity is still equivocal. A sympathetic origin of these oscillations has been suggested (Stauss et al. 1998, Cevese et al. 2001); however, studies employing anaesthesia and skin flaps (Söderström et al. 2003) and with localized sympathetic blockade (Hodges et al. 2017a) have been able to uncouple the systems and distinguish separate oscillations. However, it is important to note that there has been no direct confirmation of the role of myogenic activity due to the difficulties in manipulating and measuring microvascular pressure and obtaining a flow measure simultaneously *in vivo* in human skin. That is why we have focused mostly on oscillations associated with

endothelial and neural activities, as these have the most evidence to support their function (Kvandal et al. 2003, 2006, Hodges et al. 2017a). Also, it is important to note that the frequency bins are not completely independent and caution must be exercised in making definitive statements regarding the underlying mechanisms of action. We did not randomize the order of tests. We waited a minimum of 10 min between each assessment, but there could have been an order effect. and importantly, this could be different between the two groups. Finally, we assessed vasomotor function in males only. While previous work has shown no effect of menstrual status on cutaneous vasomotor responses using FFT analysis (Rossi et al. 2009), future work should examine cutaneous vasomotor function in maturing females, as the influence of female sex-hormones may differ than that of androgens.

In summary, we have found that at rest and in response to submaximal local skin heating, boys have higher skin blood flow than men and are more reliant on endothelial-dependent mechanisms. Maximal local heating to 44°C, reactive hyperemic, and isometric hand-grip responses do not differ between boys and men. However, during these stimuli, endothelial-mediators are the predominant mediators of vasodilatation in boys, while in the men, neural and myogenic responses appear to play a larger role. Also, we have demonstrated that the combination of laser-Doppler and wavelet analysis to non-invasively examine mechanisms of cutaneous vascular function can be successfully applied in children.

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Author contributions

GJH conceived and designed the study. GJH and MCM collected and analysed the data. GJH, MCM, SSC, and BF interpreted the data. GJH drafted the manuscript. MCM, SSC, and BF revised the manuscript critically for important intellectual content. All authors

446 approved the final version of the manuscript and agree to be accountable for all aspects of
447 the work.

448

449 **Conflict of interest**

450 The authors have no conflicts of interest to report.

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Figure Legends

Figure 1. Outline of the experimental protocol.

Figure 2. The mean responses in absolute CVC (**A**) and CVC normalized to max (**B**) during passive rest at thermoneutral conditions (33°C) and during local skin heating to 39°C and 44°C. * indicates $p < 0.05$. $n = 12$.

Figure 3. Mean skin blood flow and cardiovascular measures for the isometric handgrip exercise. Note that $n = 12$ for all measure except MAP and cardiac output for children ($n = 8$) due to equipment failure. * indicates $p < 0.05$ compared to adults; \$ indicates $p < 0.05$ compared to baseline; % indicates $p < 0.05$ compared to exercise.

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TablesTable 1. Participant characteristics ($n=12$ per group)

	Boys	Men
Age, y	9 $\pm$ 1	21 $\pm$ 1*
Stature, m	1.42 $\pm$ 0.12	1.76 $\pm$ 0.10*
Mass, kg	35 $\pm$ 9	81 $\pm$ 11*
BMI, kg·m ²	17 $\pm$ 2	25 $\pm$ 2*
$\dot{V}O_2\text{max}$, ml·kg ⁻¹ ·min ⁻¹	51 $\pm$ 5	46 $\pm$ 9
Resting measures		
Systolic blood pressure, mm Hg	89 $\pm$ 4	112 $\pm$ 5*
Diastolic blood pressure, mm Hg	52 $\pm$ 4	70 $\pm$ 3*
Heart rate, bpm	64 $\pm$ 5	66 $\pm$ 5
$\dot{Q}$, L·min ⁻¹	2.98 $\pm$ 0.28	4.38 $\pm$ 0.26*

* indicates $p<0.05$ compared to boys. Note that cardiac output was only obtained in $n=8$ children.

Table 2. Local heating laser-Doppler flux and wavelet data ($n=12$ per group)

	33°C		39°C		44°C	
	Boys	Men	Boys	Men	Boys	Men
LDF, PU	16.57 $\pm$ 1.06	8.41 $\pm$ 0.91*	32.94 $\pm$ 4.39	22.3 $\pm$ 2.9*	150.9 $\pm$ 14.5	162.5 $\pm$ 17.1
Endo NO-Indep, %	20.2 $\pm$ 1.3	15.1 $\pm$ 1.6*	25.8 $\pm$ 2.1†	21.4 $\pm$ 2.5*†	8.0 $\pm$ 0.6†	7.5 $\pm$ 0.5†
Endo NO-Dep, %	20.9 $\pm$ 1.2	12.1 $\pm$ 1.6*	30.4 $\pm$ 1.2†	26.5 $\pm$ 1.5*†	8.3 $\pm$ 0.4†	9.3 $\pm$ 0.9†
Neurogenic, %	13.5 $\pm$ 0.7	19.6 $\pm$ 1.7*	12.1 $\pm$ 1.1	12.6 $\pm$ 1.0†	15.8 $\pm$ 0.9†	14.2 $\pm$ 1.4†
Myogenic, %	10.2 $\pm$ 2.5	18.3 $\pm$ 1.4*	10.6 $\pm$ 0.8	12.7 $\pm$ 1.3†	19.6 $\pm$ 1.3†	20.8 $\pm$ 1.1†
Respiratory, %	11.8 $\pm$ 1.6	17.6 $\pm$ 1.4*	10.8 $\pm$ 1.7	13.9 $\pm$ 1.9†	18.3 $\pm$ 0.9†	17.3 $\pm$ 1.0†
Cardiac, %	23.4 $\pm$ 1.9	17.2 $\pm$ 1.3*	10.3 $\pm$ 1.3†	12.9 $\pm$ 1.6†	30.1 $\pm$ 1.1†	30.9 $\pm$ 1.4†

* indicates $p<0.05$ compared to boys; † indicates $p<0.05$ compared to 33°C. Data are mean $\pm$ 95%CI.

Table 3. Maximal, time-to-peak, and area-under-the-curve post occlusive reactive hyperaemic responses in boys and men ($n=12$ per group)

	Boys	Men
Max CVC (PU·mm Hg ⁻¹)	0.549 $\pm$ 0.035	0.546 $\pm$ 0.054
Max CVC (%max)	27.9 $\pm$ 1.4	28.9 $\pm$ 2.0
Time-to-peak (s)	33.3 $\pm$ 4.7	10.7 $\pm$ 1.9*
AUC, CVC·s ⁻¹	4035 $\pm$ 456	2336 $\pm$ 438*

* indicates $p<0.05$ compared to boys

Table 4. Laser-Doppler flux and wavelet analysis for max post occlusive reactive hyperaemia ($n=12$ per group)

	Boys	Men
LDF, PU	41.7 $\pm$ 2.65	45.2 $\pm$ 3.8
Endo NO-Indep, %	23.7 $\pm$ 1.0	20.8 $\pm$ 1.5*
Endo NO-Dep, %	28.3 $\pm$ 0.9	23.8 $\pm$ 0.8*
Neurogenic, %	16.9 $\pm$ 0.8	23.1 $\pm$ 0.7*
Myogenic, %	15.2 $\pm$ 1.1	14.7 $\pm$ 0.8
Respiratory, %	6.4 $\pm$ 0.3	10.0 $\pm$ 0.9*
Cardiac, %	9.5 $\pm$ 0.8	7.5 $\pm$ 0.5*

* indicates $p<0.05$ compared to boys

Table 5. Wavelet data in response to isometric handgrip exercise ($n=12$)

	Boys		Men	
	Baseline	Exercise	Baseline	Exercise
Endo NO-Indep, %	12.1±1.1	22.3±2.7†	17.2±1.6*	28.4±3.8*†
Endo NO-Dep, %	10.7±1.1	13.2±1.9	17.6±1.7*	17.9±2.4*
Neurogenic, %	13.6±0.9	12.4±0.9	19.6±1.7*	19.8±2.3*
Myogenic, %	21.3±3.1	17.4±2.4	17.8±1.3	14.1±2.1*
Respiratory, %	18.9±1.5	16.7±2.5	12.2±1.4*	8.3±1.0*†
Cardiac, %	23.7±1.8	17.8±1.4†	15.1±1.3*	11.4±2.0*

* indicates $p<0.05$ compared to boys, † indicates $p<0.05$ compared to baseline

Figure 1. Outline of protocol

Phase	Baseline	IHE	Recovery	PORH	Recovery	ΔT_{loc}	Stabilize	ΔT_{loc}	Stabilize
Duration (min)	20	2	5	3	15	2	25	5	25
T_{loc}	33°C					39°C		44°C	

IHE = isometric handgrip exercise
PORH = post occlusive reactive hyperaemia
 T_{loc} = local skin temperature