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# INFLUENCE OF PUBERTAL DEVELOPMENT ON POST-EXERCISE FOREARM BLOOD FLOW OF GIRLS EXERCISING IN THE HEAT

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## Abstract

**Introduction:** There is evidence that during exercise in a hot climate, girls respond with a greater shift in blood volume from the central to the peripheral circulation, compared with women. No studies have considered the influence of puberty on the hemodynamic response post-exercise.

**Purpose**: This study measured post-exercise forearm blood flow (FBF) of pre- and early- (PEP) and late- (LP) pubertal girls exercising in the heat.

**Methods**: Eighteen 9- to 16- year-old healthy girls (n=9 per group) cycled in a climate chamber set at 35±1°C and 50±5% relative humidity for two 20-min bouts (10-min rest in between and a 10-min rest at the end). FBF was measured using venous occlusion plethysmography upon chamber entry, 5 min prior to exercise bout 1 (E1), 3 min after the end of E1, at the end of rest 1 (R1), 3 min after the end of exercise bout 2 (E2), and at the end of rest 2 (R2).

**Results**: Initial FBF at rest was similar in PEP (4.9±1.8 ml·100 ml$^{-1}$·min$^{-1}$) and LP (4.7±1.2 ml·100 ml$^{-1}$·min$^{-1}$). The increase of FBF in response to E1 was slightly higher in PEP (9.3±2.5 ml·100 ml$^{-1}$·min$^{-1}$) than in LP (7.9±3.2 ml·100 ml$^{-1}$·min$^{-1}$). The main effect for group ($P$=0.037) and for time ($P$<0.001) were significant but the group × time interaction ($P$=0.79) was not. Forearm skin temperature at rest was not different between PEP (33.2±0.5°C) and LP (33.4±0.3°C) and increased over the first 15 min of exercise to 33.9±0.4°C in PEP and 33.7±0.2°C in LP, with a gradual decrease throughout the session. There was a significant main effect for time ($P$<0.001), but not for group ($P$=0.29) or for a group × time interaction ($P$=0.17).

**Conclusion**: These findings suggest that post-exercise FBF of girls exercising in the heat is influenced by pubertal status.

***Key Words:*** *females, peripheral blood flow, puberty, thermoregulation, climate*

## Introduction

When compared with adults, children have long been considered to demonstrate reduced tolerance to exercise in extreme heat. Specifically, they show a reduced time until the onset of dizziness and fatigue during exercise performed under high heat stress [1,2]. As reviewed by Armstrong & Maresh [3], no studies have observed large exercise-induced differences in rectal temperatures between children and adults. Research has suggested, instead, that children's reduced exercise-heat tolerance is more likely due to the response of their cardiovascular system than to hyperthermia [2]. A recent review article revisited the important issues regarding maturational differences in thermoregulation during exercise in the heat [4].

Evidence for children's circulatory instability in response to high heat stress has been offered. Drinkwater et al. [2] compared the physiological responses of pre-pubertal girls and college women to exercise in the heat. The subjects were matched in fitness (i.e., $\dot{V}O_2$max) and walked on a treadmill at the same relative intensity (~30% $\dot{V}O_2$max) for two 50-min bouts at temperatures of 28°C (45% RH), 35°C (65% RH) and 48°C (10% RH). The pre-pubertal girls had a lower tolerance time than the women during exercise in the two hottest conditions. Although post-exercise forearm blood flow (FBF) was not significantly higher in the girls, the authors suggested that the girls' higher body surface area-to-mass ratio required a larger proportion of their total blood volume to maintain adequate peripheral flow for heat dissipation to the environment. This would decrease venous return and contribute to the girls' lower stroke volume index, which was documented in this study [2]. In contrast, a more recent investigation involving the comparison of girls and women found no age-related differences in cardiovascular adaptation, including FBF response, to exercise in the heat [5]. However, these participants were indigenous to a tropical climate (i.e., heat-acclimatized) and competitive athletes, which the participants of the Drinkwater study [2] were not. Thus, the influence of pubertal development on FBF

of girls exercising in the heat remains to be clarified, although there is some evidence for such an effect in males. A study of the thermoregulatory responses of pre-, mid- and late-pubertal boys considered the FBF response to exercise in the heat while cycling at 50% $\dot{V}O_2$max (three 20-min bouts at 42°C, 20% RH) [6]. It is noteworthy that the boys in this study were tested at a time when they were unlikely to be heat-acclimatized and under exercise conditions that were low to moderate intensity. Post-exercise FBF was significantly higher in the least mature boys, suggesting that regulation of peripheral blood flow during exercise in the heat may be influenced by pubertal development.

Investigations of the impact of puberty on blood flow regulation in females are absent. Further study on this topic in females is warranted to enhance understanding of how normal growth and development can influence the regulation of blood flow during physiological stress, normally encountered in the lives of children. Further understanding in this area could also be important to provide evidence-informed recommendations for coaches and sport organizations responsible for young female athletes competing in warm climates.

The purpose of this study was, therefore, to measure the post-exercise FBF of pre- and early-pubertal and late-pubertal girls exercising in the heat. Based on earlier findings in boys, we hypothesized that pre- and early- pubertal girls would have a higher post-exercise FBF than late-pubertal girls.

## Methods

*Participants*: Healthy, pre- and early- pubertal (PEP, n = 9) and late-pubertal (LP, n = 9) girls volunteered for this study. Based on previous FBF data from a study involving boys [6], we performed a sample size calculation and determined that 9 subjects would be required in each group to maintain statistical power at ~80%. Each girl provided a pubertal rating by self-assessment using breast development criteria proposed by Tanner [7]. PEP was defined as stages 1 and 2 and LP as stages 4 and 5. None of the girls in the PEP group had reached menarche. However, all of the participants in the LP group were post-menarcheal and were tested during the follicular phase (prior to Day 10) of their menstrual cycle. Participants were recruited from the local community; they participated in recreational physical activity, but none were competitive athletes. Participant characteristics are summarized in Table 1. All procedures for this study were approved by the research ethics board of the Faculty of Health Sciences, McMaster University. A parent signed an informed consent after the participant gave verbal assent.

*Study design and protocol*: There were two visits to the Children's Exercise and Nutrition Centre. During the first visit, anthropometric measurements were taken, followed by resting and post-exercise FBF to familiarize the subjects with the technique. Finally, a $\dot{V}O_2$max test was performed on a cycle ergometer using the McMaster All-out Progressive Cycle test. The second visit was completed in a climate chamber set at 35°C and 45-50% RH. Because pre-existing hypohydration at the start of the session could have an impact on FBF results, we instructed the girls to eat and drink regularly and to arrive for the experimental session 2 h after a light meal, having refrained from exercise for at least 8 h. They were also asked to drink a cup of water about half an hour prior to coming to the laboratory. At the laboratory, each participant performed two 20-min cycling tasks. After each bout of exercise, a 10-min rest period was provided. Participants exercised at a power output corresponding to 50% $\dot{V}O_2$max. Forearm skin temperatures ($T_{sk}F$) and heart rate (HR) were recorded throughout the session. FBF was measured upon chamber entry, 5 min prior to exercise bout 1, 3 min after exercise bout 1, at the end of rest 1, 3 min after exercise bout 2 and at the end of rest 2. Body mass was measured at the beginning, middle and end of each session, after toweling off sweat, if applicable. Participants were encouraged to drink chilled (~10°C) water to avoid dehydration.

*Measurements and Calculations*: Height was measured using a Harpenden wall-mounted Stadiometer

Table 1. *Subject characteristics*

| | PEP | LP |
|---|---|---|
| n | 9 | 9 |
| Age, yr | 10.5 ± 1.3 | 14.5 ± 1.2* |
| Height, cm | 142.1 ± 8.5 | 164.8 ± 5.9* |
| Body mass, kg | 38.4 ± 10.5 | 58.6 ± 7.3* |
| Surface area, $m^2$ | 1.22 ± 0.18 | 1.62 ± 0.12* |
| Surface area/Body mass, $cm^2/kg$ | 324 ± 38 | 279 ± 19* |
| Body fat, % | 21.6 ± 7.1 | 25.7 ± 5.0 |
| $\dot{V}O_2$max, $ml \cdot kg^{-1} \cdot min^{-1}$ | 29.9 ± 4.0 | 31.9 ± 5.7 |

Values are mean ± SD. $\dot{V}O_2$max, maximal oxygen uptake. * Significantly different ($P<0.05$) than PEP.

2109, accurate to 0.1 cm. An Ancaster electro-scale model UMC-600, accurate to 20g (Brantford, ON, Canada) was used for body mass, and a bioelectrical impedance analyzer (BIA-101A, RJL Systems, Clinton Twp, MN) was used to estimate adiposity. Body surface area (BSA) was calculated from height and mass, according to DuBois and DuBois [8]. $T_{sk}F$ were determined by a Micron portable digital infrared thermometer, model M80G-OCH, 0.1°C accuracy. HR was monitored using the Polar Vantage XL HR monitor, Polar CIC (Port Washington, New York).

FBF was estimated by venous occlusion plethysmography, using a mercury-in-rubber strain gauge (Hokanson EC6 strain gauge plethysmograph, D.E. Hokanson Inc., Bellevue, WA). The strain gauge was placed around the largest circumference of the forearm. By allowing arterial inflow, but inhibiting venous outflow, the rate of swelling of the forearm in ml·100 $ml^{-1}$ of tissue·$min^{-1}$ (% per min) was calculated by changes in forearm circumference. The Noninvasive Vascular Program (NIVP3) software package was used to capture the waveforms sent by the EC6 plethysmograph, to edit the arterial inflow slopes, and to control the rapid cuff inflator. Throughout the measurement, the left forearm rested on an adjustable support at about the level of the right atrium to facilitate venous drainage. A rapid version vascular cuff was placed around the upper left arm and another cuff was placed around the wrist so that hand flow could be excluded during the measurement. Each measurement took 15 sec and consisted of the following sequence: the wrist cuff was manually inflated to ~200 mmHg (and remained inflated for 1 min), the arm cuff was then inflated automatically by the NIVP3 system (0.3 sec inflation time) for 6 sec. This was followed by an automatic deflation of the arm cuff (0.2 sec deflation time), allowing 9 sec for venous drainage. Four values were therefore recorded in 1 min. When all four FBF measurements were obtained in 1 min, the highest and lowest values were excluded and FBF was then calculated as the mean of the remaining two measures. When three values were available, the median was recorded. With two values, the mean was taken, but only if there was ≤15% difference between them. Participants had to remain completely still during the 6 sec sampling period to avoid any artifact caused by slight body movements. To prevent any small movements of the fingers, they were tucked underneath an elastic bandage that was attached to the support. The participant also held her breath during the 6 sec sampling period.

*Statistical Analysis*: Independent t-tests were used to determine inter-group differences in physical characteristics. A two factor (group × time) ANOVA with repeated measures for time was used to analyze $T_{sk}F$, HR and % change in initial body mass (i.e., dehydration). A General Linear Model (GLM) option was used to analyze the FBF data. This approach allows unequal sample sizes at different time points, so that the maximum number of observations can be used in the analysis. GLM was appropriate for FBF analysis due to missing data that occurred when subjects did not keep still during the measurement. Intra- and inter-tester reliability for the editing of FBF measurements was 0.89 for both. When appropriate, *post-hoc* analyses were performed using a Tukey test. Statistical significance was set at $P \leq 0.05$ for all comparisons. Data are reported as the mean ± SEM, unless otherwise specified.

**Results**

Initial FBF (Figure 1) at rest was similar in PEP (4.91.8 ml·100 $ml^{-1}$·$min^{-1}$) and LP (4.7±1.2 ml·100 $ml^{-1}$·$min^{-1}$). The increase of FBF in response to exercise bout 1 was slightly higher in PEP (9.3±2.5 ml·100 $ml^{-1}$·$min^{-1}$) than in LP (7.9±3.2 ml·100 $ml^{-1}$·$min^{-1}$). GLM analyses of FBF revealed a significant main effect for group ($P$=0.037) and for time ($P$<0.001). However the group × time interaction was not significant ($P$=0.79).

$T_{sk}F$ at rest was not different between PEP (33.2±0.5°C) and LP (33.4±0.3°C). During the first 15 min of the session it increased to 33.9±0.4°C in PEP and 33.7±0.2°C in LP, with a gradual decrease throughout the session (Figure 2). GLM analyses of $T_{sk}F$ revealed a significant main effect for time ($P$<0.001) but not for group ($P$=0.29). The group × time interaction was not significant ($P$=0.168).

HR prior to exercise bout 1 was similar in PEP (98±4.6 bpm) and in LP (96±4.9 bpm). The main effect for time was significant ($P$<0.001), with HR increasing markedly during the initial period of exercise to 150±3.7 bpm in PEP and to 143±2.9 bpm in LP. At the end of exercise bout 2, HR was 165±4.2 bpm in PEP and 157±3.2 bpm in LP. There was no main effect for group ($P$=0.329) or a group × time interaction ($P$=0.865).

The changes in initial body mass throughout the session (i.e., dehydration) were minimal and not significant, and similar in PEP (–0.30±0.14%) and LP (–0.15±0.10%) confirming that all subjects remained euhydrated.

**Discussion**

To our knowledge, this is the first study to investigate the impact of pubertal development in females on post-exercise FBF in response to exercise in the heat. The main finding is that late-pubertal girls demonstrated a lower FBF, compared with pre- and early-pubertal girls. The impact of puberty appeared most visible for post-exercise FBF, as evidenced by a drop in FBF from the end of exercise to the end of each rest

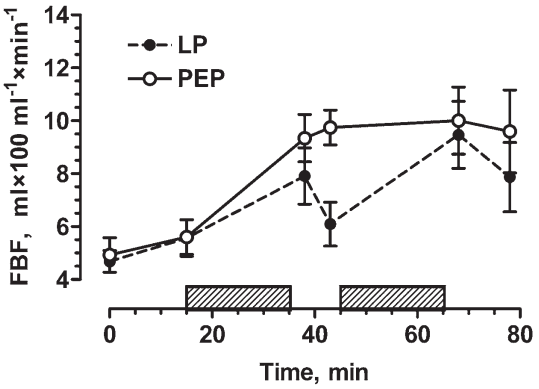


**Fig. 1.** *Forearm blood flow (FBF) throughout the session in pre- and early--pubertal (PEP), and late-pubertal (LP) girls. Values are mean ± SEM. Horizontal hatched bars denote exercise bouts 1 and 2. Main effect for group, P=0.037; main effect for time, P<0.001.*

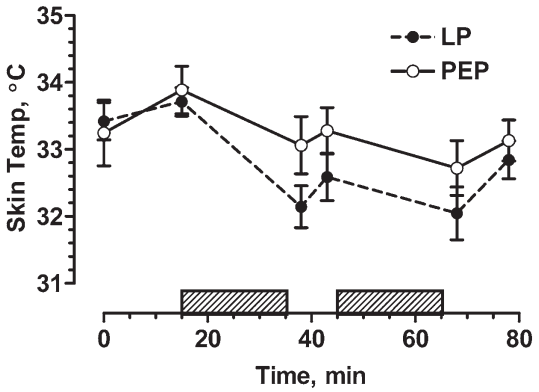


**Fig. 2.** *Forearm skin temperature throughout the session in pre- and early--pubertal (PEP), and late-pubertal (LP) girls. Values are mean ± SEM. Horizontal hatched bars denote exercise bouts 1 and 2. Main effect for time, P<0.001.*

period (Figure 1). In contrast, pre- and early-pubertal girls maintained an elevated and relatively constant post-exercise FBF throughout these same time periods.

In only a few studies that have compared the FBF response to exercise in the heat between girls and women, the results have been inconsistent, most likely due to very different groups of participants. Drinkwater et al. [2] reported a tendency for a higher FBF in pre-pubertal girls, compared with women, during treadmill walking (at 30% $\dot{V}O_2max$) in the heat. According to Drinkwater et al. [2], when pre-pubertal girls exercise in the heat, a greater proportion of their total blood volume may shift from the central to the peripheral circulation. This reflects the larger BSA-to-mass ratio in smaller individuals. The girls in the study by Drinkwater et al. had a lower blood volume per skin surface area, compared to the women (2,269 ml·m$^{-2}$ vs. 3,095 ml·m$^{-2}$). In contrast to these findings, Rivera-Brown et al [5] reported no age-related differences of FBF responses in pre-pubertal girls and women cycling (at 60% $\dot{V}O_2max$) in the heat (33.4°C, 20% RH). However, it should be pointed out that the participants in the latter study were heat-acclimatized (by living in a tropical climate) and competitive athletes. Therefore differences in the subjects' fitness level and degree of heat-acclimatization can likely explain the discrepancy of FBF between these two studies. However, methodological differences such as environmental conditions, exercise mode, intensity, and duration could also help explain differences in FBF responses in these studies.

As in the females studied by Drinkwater et al. [2], we also observed a higher FBF in pre- and early-pubertal compared to late-pubertal girls who were not athletes. This may suggest that the effect of puberty on FBF responses might be more apparent in females who are either less physically fit or non-acclimatized to the heat or both. The post-exercise FBF response of our girls is also consistent with previous work in non-athletic and non-acclimatized males [6] and support the idea that regulation of peripheral blood flow following exercise in the heat may be influenced by growth and maturation. Falk et al. [6] reported a higher post-exercise FBF in pre-pubertal boys (i.e., Tanner 1), compared with more mature boys (i.e., Tanner 2 to 5) following exercise at 42°C, 20% RH. Despite these puberty-related FBF differences, the boys maintained rectal (i.e., core) temperature at almost identical levels. We did not measure rectal temperature in the current study, although together our results support the idea that before puberty children may utilize different strategies to regulate body temperature [9]. In our pre- and early-pubertal girls, higher post-exercise FBF indicate that peripheral blood flow was enhanced to dissipate heat generated during exercise; this mechanism of convective heat exchange would counteract the lower sweating rates (i.e., evaporative heat loss) observed before puberty [10].

FBF can also be influenced by local skin temperature. While the FBF response to heat stress arises primarily through neurogenic vasoconstrictor and vasodilator systems, local warming of the skin can also vasodilate the underlying resistance vessels. Consistent with the FBF data, girls in the PEP group generally had higher $T_{sk}F$ values than did girls in the LP group (Figure 2), although there were no statistical differences. However, $T_{sk}F$ did not exceed 35.5°C in any of the subjects. FBF shows a fairly small increase per °C increase in the local surface temperature between 5°C and 35°C [11]. It is not until skin temperature exceeds ~38°C that FBF rises more steeply. It is therefore possible that even small group differences in local skin temperature may have contributed to small group differences in post-exercise FBF in the present study.

There are also the possible effects of the female reproductive steroid hormones on FBF responses. Women's resting core temperature fluctuates during the menstrual cycle [12], such that there is an upward shift of ~0.5°C following ovulation that persists until the onset of menstruation (luteal phase). While there is some controversy over the effect of menstrual cycle on sweating and FBF during rest or exercise [13-16], we can only speculate that female hormones indeed

have a role in blood flow regulation during exercise in the heat. Charkoudian and Johnson [17] reported that vasodilation is initiated at higher internal temperatures during the high-hormone phase of oral contraceptive use (similar to the luteal phase, when both estrogen and progesterone are elevated). Even though the LP girls were tested during the follicular phase (days 1-14), it is possible that changes in hormones within this time frame could have increased the threshold for cutaneous vasodilation, which could explain the lower FBF response in the LP group, compared with the PEP group.

In this study, we faced limitations that should be acknowledged. Despite our intention to record core temperature data in this study, we could not obtain permission from the girls and their parents for measuring it rectally and alternate methods of assessing it were not available to us. This markedly reduced our ability to fully interpret thermoregulatory responses and the thermal impact on FBF in our girls. We put a lot of effort to obtain FBF values for all subjects at each time point, however we had to accept missing data points due to technical problems (when subjects did not keep still during FBF measurement). This could reduce the sensitivity of our statistical analysis. Our findings therefore, would likely be statistically stronger, if that problem had been avoided. It is also possible that a higher ambient temperature or a more intense and prolonged exercise task would have placed greater stress on the cardiovascular system, creating the possibility that group differences might have been accentuated. We can not discount this possibility; however, we designed this study so that we could compare our results with those of Falk et al. [6], who used a similar protocol, and to recreate typical conditions that many children are exposed to everyday. Given the results of this study, we plan to pursue some of these questions in future research.

In conclusion, under the conditions of the current study, pre- and early-pubertal girls demonstrated higher FBF, compared with late-pubertal girls, with group differences accentuated post-exercise. These data add to the understanding that as children mature they rely on different strategies for effective thermoregulation in response to exercise.

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A – Study Design
B – Data Collection
C – Statistical Analysis
D – Data Interpretation
E – Manuscript Preparation
F – Literature Search
G – Funds Collection