Temperature regulation during rest and exercise in the cold in premenarcheal and menarcheal girls

Panagiota Klentrou, 1 Melora Cunliffe, 1 Jill Slack, 1 Boguslaw Wilk, 2 Oded Bar-Or, 2 Mary Jane De Souza, 3 and Michael Plyley 1
1 Faculty of Applied Health Sciences, Brock University, St. Catharines, Ontario L2S 3A1; 2Children’s Exercise and Nutrition Centre, Department of Pediatrics, McMaster University, Hamilton, Ontario L8N 3Z5; and 3Faculty of Physical Education and Health, University of Toronto, Toronto, Ontario, Canada M5J IP3

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温度调节在寒冷中的作用与年龄、体脂率和性别的关系可能存在差异。尽管以前的研究已经表明，雌激素和孕激素水平的波动会影响温度调节，但这些影响与早熟的热调节是否有差异还不清楚。极少数研究对年轻女性的温度调节做了研究，因为这些研究可能受卵巢激素水平的影响。

女性的生殖健康状况，如月经周期，可能会影响温度调节。早期的研究表明，月经周期不同阶段的温度调节存在差异，但这些差异是否主要由几何差异或与年龄相关的外周血管收缩有关还不清楚。此外，研究还表明，没有明显的生殖相关的温度调节差异。

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plateau in oxygen consumption (\(\dot{V}_\text{O}_2\)).

\section*{METHODS}

\textbf{Subjects.} Thirteen female adolescents, 13–18 yr of age, volunteered to participate in the research. Each was involved in sports such as artistic and rhythmic gymnastics, dance, wrestling, and rowing. The average training volume was 8 h/wk. The Ethics Boards of both Brock University and McMaster University approved the study. Informed written consent was obtained from both the subjects and their parents or guardians before their involvement with the study. During a debriefing meeting, the researcher provided oral and written information regarding procedures and possible risks involved in the experiments to all subjects and their parents or guardians, and subjects completed the menstrual status questionnaire for assignment to one of the following experimental groups: 1) eumenorrheic menarcheal (EM; \(n = 6\)): menarcheal girls with regular menses \(\geq 9\) cycles/yr, and 2) premenarcheal (PM; \(n = 7\)): nonmenstruating girls (20).

\textbf{Experimental protocol.} After being assigned to one of the groups, each subject was contacted to schedule the experimental visits at the Children’s Exercise and Nutrition Centre, Chedoke Hospital. During their first visit to the center, subjects were familiarized with the study procedures. After a medical examination, anthropometric variables (Table 1), including height, weight, and percent body fat, were measured for normalization of data. Skinfold thickness (sum of subcutaneous adipose tissue, in mm) was assessed at two sites (subscapular and triceps) by using Harpenden calipers. Relative body fat (%) was measured by using bioelectrical impedance analysis (10/ARJL System, Clinton Township). Height was assessed by using a Harpenden Stadiometer (model 2109), and body mass was measured by using a Mott Electro-scale (model UMC-600). Body surface area and body surface area-to-mass ratio were calculated (9). After completion of the anthropometric measurements, maximal oxygen consumption (\(V_{\text{O}_2\text{max}}\)) was directly measured on a Monark cycle ergometer at a cycling rate of 60 rpm via a SensorMedics Vmax 29 series metabolic cart. Power output (W) was increased every 2 min by 1.1 and a \(0.3 \text{ m/s air velocity}.\) This test consisted of a 10-min rest period, followed by 20 min of exercise on a cycle ergometer at 30% of \(V_{\text{O}_2\text{max}}\) and then by an additional 30 min of rest in thermoneutrality. 2) The second was a cold-stress test at \(5^\circ\text{C}\) air temperature, 40% air humidity, and \(<0.3\) m/s air velocity. This test started with a 20-min rest period in a seated position, followed by 40 min of cycling at 30% of \(V_{\text{O}_2\text{max}}\). Total exposure time to cold was 60 min.

\textbf{Experimental protocol.} Power output (W) was increased every 2 min by using a Mott Electro-scale (model UMC-600). Body surface area and body surface area-to-mass ratio were calculated (9). After completion of the anthropometric measurements, maximal oxygen consumption (\(V_{\text{O}_2\text{max}}\)) was directly measured on a Monark cycle ergometer at a cycling rate of 60 rpm via a SensorMedics Vmax 29 series metabolic cart. Power output (W) was increased every 2 min until \(V_{\text{O}_2\text{max}}\) was achieved. The criteria used to verify the achievement of \(V_{\text{O}_2\text{max}}\) were a respiratory gas exchange ratio \(\geq 1.1\) and a plateau in oxygen consumption (\(V_{\text{O}_2}\)) with increasing power output.

During subsequent visits to the center, subjects performed two tests in the climate chamber. 1) The first was a test for the determination of metabolic rate in thermoneutrality (22.5°C). This test consisted of a 10-min rest period, followed by 20 min of exercise on a cycle ergometer at 30% of \(V_{\text{O}_2\text{max}}\) and then by an additional 30 min of rest in thermoneutrality. 2) The second was a cold-stress test at \(5^\circ\text{C}\) air temperature, 40% air humidity, and \(<0.3\) m/s air velocity. This test started with a 20-min rest period in a seated position, followed by 40 min of cycling at 30% of \(V_{\text{O}_2\text{max}}\). Total exposure time to cold was 60 min.

\begin{table}[h]
\centering
\caption{Physical characteristics for eumenorrheic menarcheal and premenarcheal female adolescents}
\begin{tabular}{lcc}
\hline
 & EM (n = 6) & PM (n = 7) \\
\hline
Age, yr & 15.6±0.9 & 13.1±0.5 \\
Height, cm & 166.4±2.9 & 154.1±2.3* \\
Body mass, kg & 55.7±1.3 & 43.7±2.5† \\
Fat-free body mass, kg & 46.9±1.4 & 36.9±2.0* \\
Body surface area, m² & 1.61±0.03 & 1.39±0.05* \\
Surface area-to-mass ratio, cm²/kg & 289.2±2.4 & 323.3±9.5* \\
Body fat, % & 15.7±0.7 & 15.5±0.9 \\
\(V_{\text{O}_2\text{max}}, \text{ml/min}^\cdot1^{-1}\text{kg LBM}^\cdot1^{-1}\) & 46.3±1.2 & 47.9±2.1 \\
HR at \(V_{\text{O}_2\text{max}}, \text{beats/min}\) & 193.5±4.4 & 199.0±3.0 \\
\hline
\end{tabular}
\end{table}

Values are means ± SE; n, no. of subjects. EM, eumenorrheic menarcheal; PM, premenarcheal; \(V_{\text{O}_2\text{max}},\) maximal oxygen consumption; LBM, lean body mass; HR, heart rate. *P < 0.05 between groups. †P < 0.01 between groups.

All tests were completed in the afternoon. A light meal was allowed \(\sim2\) h before testing. During all tests, the subjects wore shorts, T-shirt, socks, and running shoes. Blood samples were collected through a single venipuncture before the neutral sessions and after the cold-stress sessions. All subjects were visually screened during cold exposure for adverse reactions to the cold, including Raynaud’s phenomenon. Subjects consumed \(\sim50\) ml of room temperature water during the 30-min rest period in the neutral environment. Subjects in the PM group were required to complete the cold stress test once. Subjects in the EM group were tested in the climate chamber twice: 1) during early-follicular phase of their cycle (days 1–5) and 2) during mid-luteal phase of the same cycle (days 19–22).

Rectal (°C) temperature was monitored continuously throughout the testing protocol and recorded every 5 min by a thermistor (400 series, Yellow Springs Instrument) inserted \(\sim10\) cm beyond the anal sphincter. Skin temperature (°C) was recorded every 5 min by using thermisters fixed at five sites (upper back, upper arm, forearm, finger, and thigh). Heart rate (beats/min) was monitored continuously throughout the testing protocol by a Polar heart rate monitor (3000 system).

Mean skin temperature \((T_s)\) was calculated by using the formula of Bonner et al. (3)

\[
T_s = 0.03 (T_{\text{fr}} + T_{\text{up}}) + 0.2 (T_{\text{th}} + T_{\text{p}})
\]

where \(T_{\text{fr}}\) is subcapsula temperature, \(T_{\text{up}}\) is upper arm temperature, \(T_{\text{th}}\) is thigh temperature, and \(T_p\) is finger temperature.

Heat production (W/kg and W/m²) was determined from \(V_{\text{O}_2}\) and respiratory exchange ratios. The increase in metabolic heat production due to cold was estimated from \(V_{\text{O}_2}\) which was measured every 10 min, and was defined as the excess \(V_{\text{O}_2}\) measured in the 5°C environment compared with the values obtained at the same work rate in the thermoneutral environment. Heat conductance (W/m²°C) was calculated by using heat production, and core and skin temperature gradient.

A rating of perceived effort (RPE) was determined using the Borg 6–20 category scale (4). A thermal sensation rating of the body was determined by using a 9-point scale, with –4 being very cold, neutral being 0, and +4 being very hot (10). Thermal comfort was assessed for the body by using a 5-point scale, with 0 being comfortable and 4 being very uncomfortable (. 10).

\textbf{Determination of menstrual status and phase.} Menarcheal subjects reported their age at menarche onset and \textit{day 1} (i.e., the first day of flow) for their last three menstrual cycles. They were then instructed to report on \textit{day 1} of their next cycle, after the initial study visit. Testing sessions were scheduled to correspond to each of the desired phases on the basis of this information. Progesterone and 17\(\beta\)-estradiol were measured to verify menstrual status and phase. For EM subjects, blood draws were performed during \textit{days 1–5, 13–15, and 19–22} of the menstrual cycle. For the PM subjects, blood was drawn before their testing sessions.

Intravenous blood samples were taken from the antecubital vein into a vacutainer. All blood samples were immediately centrifuged for 15 min at 1,500 g. The separated plasma was removed from the sample tube, placed in 2-ml microtubes, and stored in a 20°C freezer until analysis. 17\(\beta\)-Estradiol and progesterone levels were analyzed by using a chemiluminescence-based immunoassay system (Immulite, Diagnostic Products, Los Angeles, CA). The analytic sensitivity of the estradiol assay was 55 pmol/l. The intra-assay and interassay coefficients of variation for the estradiol assay were 7.8 and 6.2%, respectively. The analytic sensitivity of the progesterone assay was 0.6 nmol/l. The intra-assay and interassay coefficients of variation for the progesterone assay were 7.5 and 7.9%, respectively. All samples were run in duplicate at the Women’s Exercise and Bone Health Laboratory at the University of Toronto.

\textbf{Statistical analysis.} One-way ANOVA was performed to assess group and phase differences for all variables. An ANOVA with repeated measures was performed to assess changes in time for both

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Table 2. Resting serum estrogen and progesterone levels in eumenorrheic menarcheal and premenarcheal adolescents

<table>
<thead>
<tr>
<th></th>
<th>PM (n = 7)</th>
<th>Early follicular (n = 6)</th>
<th>Midluteal (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>17β-Estradiol, pmol/l</td>
<td>75.3 ± 1.8</td>
<td>75.2 ± 1.2</td>
<td>206.9 ± 18.9*</td>
</tr>
<tr>
<td>Progesterone, nmol/l</td>
<td>0.8 ± 0.01</td>
<td>0.9 ± 0.1</td>
<td>11.6 ± 2.3*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of subjects. *P < 0.01, PM vs. midluteal, and early follicular vs. midluteal.

Groups and during both phases. Post hoc analysis of significant differences was completed via a Bonferroni test where appropriate. Statistical significance was accepted at P < 0.05. Pearson product-moment correlation and regression analyses were used to explain differences between and within key variables. All data are expressed as means ± SE.

RESULTS

Physical characteristics and hormonal levels. No significant differences were observed in age, $V_{O2\ max}$, heart rate at $V_{O2\ max}$, or percent body fat (Table 1). However, height, weight, and surface-to-mass ratio were significantly ($P < 0.01$) different between the PM and EM groups (Table 1). The mean plasma estradiol and progesterone are presented in Table 2. The mean plasma estradiol and progesterone were similar in the PM group and the early follicular phase of EM group. For the EM group, both mean estradiol and progesterone levels were significantly elevated during the luteal phase compared with the follicular phase and PM levels (Table 2).

Rest to exercise in thermoneutral environment. No differences were observed in resting or exercise core temperature in thermoneutrality. Neither menstrual phase nor menstrual status altered core temperature responses in the neutral environment (Table 3). Similarly, neither menstrual status nor menstrual phase affected mean skin temperature (Table 3). Heat production increased from rest to exercise in the neutral environment. Heat production per unit body mass was significantly higher in the PM, compared with the EM, girls only at rest ($P < 0.05$). There was no influence of menstrual phase.

Rest to exercise in the cold. There were no significant differences in core temperature at the beginning of cold exposure (Fig. 1). During exercise in the cold after 20 min of cold exposure, the EM subjects displayed a slight, but not significant, increase in rectal temperature; at the same period, the core temperature of the PM subjects significantly ($P < 0.05$) declined. For the total cohort, the core temperature changes from 0 to 60 min in the cold were significantly correlated with body surface area-to-mass ratio and heat production at 60 min. With the use of stepwise regression analysis, only body surface area-to-mass ratio was entered in the model, explaining 42% [$R^2 = 0.42$; SE of estimate (SEE) = 0.78; $P < 0.01$] of the variance in the core temperature changes. However, for the PM group, body fat alone explained 79% ($R^2 = 0.79$; SEE = 0.57; $P < 0.01$) of the variance in core temperature changes. There was a significant difference between the EM-follicular and the PM results for rectal temperature at the conclusion of the cold exposure ($P < 0.05$). Skin temperature in the cold rapidly decreased during the initial 10 min of cold exposure (Fig. 1).

Between the 10th and 20th min, all groups demonstrated a
leveling off of skin temperature. There was no menstrual phase or menstrual status differences in skin temperature throughout the cold-exposure period (Fig. 1).

Both groups displayed a significant \( P < 0.01 \) increase in heat production during rest and exercise in the cold compared with the thermoneutral environment. The largest heat production change was found in the PM subjects. There was a significantly \( P < 0.05 \) increased heat production per unit body mass in the PM, compared with the EM girls, during rest and exercise (Fig. 2). Heat conductance during rest and exercise was significantly increased in the PM, compared with the EM, group \( P < 0.05 \). Exercise did not significantly alter perceived comfort or sensitivity for any of the groups. There were no significant differences for thermal comfort and thermal sensitivity between the groups; however, a phase-related trend showed that the EM group felt more comfortable and less sensitive to the cold during the luteal phase. There were no menstrual phase-related differences in RPE during the cold session.

**DISCUSSION**

Reproductive status and temperature regulation. Several findings of significance from the present study illustrate the altered thermoregulatory responses of girls of different reproductive status. The main finding was that, despite their higher heat production in the cold, the PM girls could not maintain their rectal temperature as effectively as the EM girls. The sharp decrease in core temperature of the PM girls began after the light exercise began and was strongly associated with surface area-to-mass ratio. This, in combination with the non-significant decrease of the skin temperature of these girls, suggests that the enhanced metabolic response to cold could not compensate for the greater heat loss, liking a result of a limited peripheral vasoconstriction.

Smolander et al. (27) reported that, despite their larger surface area-to-mass ratio, young boys maintained their core temperature during cold exposure as effectively as men, by increasing their metabolic heat production. Because Smolander et al. used the same cold-exposure protocol as was used in the present study, comparisons can be drawn. Although the PM girls had the same surface area-to-mass ratio and displayed similar increases in heat production as the boys (27), the drop in core temperature for the girls was significant, whereas that for the boys was not. Presumably, the mechanism of increasing heat storage was not sufficient for the girls to overcome their geometric disadvantage, as their core temperature continued to decline even after starting the exercise. Moreover, the drop in core temperature after 60 min in the cold for the PM girls was more evident than that observed in previous studies (18) in amenorrheic athletes after 60 min of rest in the cold. The lower core temperatures in the cold observed in the amenorrheic, compared with eumenorrheic, athletic young women were linked to a slower metabolic response. In contrast, in the present study, an increased metabolic response was observed in the PM girls at the beginning of the cold session, suggesting that, unlike young adult women, a limited metabolic response is not the reason for the inability of the PM girls to maintain rectal temperature.

Heat conductance was significantly higher for PM subjects in the cold during exercise. Despite the larger heat production, the PM girls displayed a larger skin-core temperature gradient. They produced more heat per unit body mass, with more heat being conducted toward the skin. This suggests an altered vasoconstrictive response in the PM subjects and illustrates the greater thermoregulatory distress that the nonmenstruating girls experienced during the cold session. Skin temperature did not differ with menstrual status in either the neutral or cold environment. Although a rapid decline was observed during the initial rest period, cold-exposure skin temperature was stabilized after 10–20 min of exercise, suggesting that the exercise stimulus was sufficient to maintain skin temperature for both groups. These results are in agreement with those of Falk et al. (9), who, in their examination of young boys and adults, found no significant difference in mean skin temperature among the groups. Graham et al. (18) found lower skin temperatures in amenorrheic women compared with eumenorrheic controls. Graham (17) also suggested that, compared with men, women maintained a lower skin temperature. However, the premenarcheal girls in the present study did not demonstrate the same response as either eumenorrheic adolescents or amenorrheic women, suggesting that the premenarcheal adolescents have an altered thermoregulatory response and that both maturation status and age influence temperature regulation in the cold.

No significant differences were found between the groups during rest or exercise in the cold session in terms of thermal comfort and thermal sensitivity. Even though the PM group had a lower core temperature in the cold, their perceived thermal comfort and thermal sensation did not display this distress. Similarly, Smolander et al. (27) found no differences in thermal comfort and thermal sensitivity between boys and men. The RPE was not altered by reproductive status during either the neutral or cold sessions. Although the relative work-
loads were the same for both groups, the RPE of the EM girls decreased during the session in the cold, whereas that of the premenarcheal girls exhibited an increase, suggesting that the RPE of the premenarcheal girls is influenced by the cold.

**Menstrual phase and temperature regulation.** In the present study, no menstrual phase differences were detected in core temperature in the neutral environment, suggesting that the young eumenorrheic girls do not exhibit the same phase-related temperature fluctuations as seen in older women. Both Frascarolo et al. (11) and Hessemer and Bruck (21) have suggested that the higher temperature in the luteal phase resets all thermoregulatory responses at higher thresholds compared with the follicular phase. More specifically, Hessemer and Bruck found that skin and core temperatures, as well as heart rate, were higher during the luteal phase. Frascarolo et al. (11) established that women have higher internal body temperatures (rectal temperature) during the luteal phase of the menstrual cycle, even though neither heat production nor heat loss was altered between the cycle phases (11). It has been suggested that, during the luteal phase, there is a resetting of the basal body temperature, causing an increase in the thermoregulatory set point after ovulation. The lack of thermoregulatory differences between phases in the eumenorrheic adolescents can be explained on the basis of differences in gynecological age. A shortened luteal phases and lower progesterone levels have been reported in adolescents compared with women (1, 2). The present estradiol levels reported in our study did not reach adult levels reported in the literature for both follicular and luteal phase, but they are similar to those previously reported for adolescents (1). However, the plasma progesterone levels reported in the presented study are in the same range as that reported for adults (5), a level not associated with thermoregulatory differences (6).

During cold exposure, the menstruating girls demonstrated a similar core temperature at rest and during exercise during both the luteal and follicular phases of the same menstrual cycle. This is in agreement with previous research (7, 14, 16) in which it was reported that there was no significant core temperature difference related to menstrual phase in adult women. In contrast, Gonzalez and Blanchard (15) found an elevated core temperature, lower heat debts, and longer tolerance times during the luteal phase, under resting conditions in which ambient air temperature was gradually decreased, which may account for the differing results. Recently, Sunderland and Nevill (30) found no significant differences between phases in the thermoregulatory responses during exercise in the heat of seven 20-yr-old eumenorrheic women.

No phase-related differences were found in skin temperature during rest or exercise in the EM girls. This is in agreement with previous research during exercise in either neutral or hot environment (11). In the cold, Glickman-Weiss et al. (14) and Graham (16) found that skin temperature was not changed during the menstrual cycle. In the present study, a gradual decrease in skin temperature was observed during rest in the cold, and a leveling off, similar to that seen during the neutral session, was observed during exercise.

As expected, heat production was higher in the cold than in the neutral temperature environment during both phases. This provides evidence that the cold stress during exercise places a strain on the thermoregulatory system. According to Horvath and Drinkwater (22), $\dot{V}_{O_2}$ increases threefold in a cold environment. On the other hand, Kruk et al. (23) observed that $\dot{V}_{O_2}$ increased proportionally with intensity and that it was higher at 5°C than at 24°C. Although the young menarcheal girls responded with a similar increase, heat production in the cold was not altered by phase for the EM subjects. This is in agreement with Glickman-Weiss et al. (14) and Frascarolo et al. (11), who reported similar results for women aged 18–30 yr during cold and heat exposure, respectively. Furthermore Stephenson and Kolkka (28) and Stephenson et al. (29) found that heat production in women was consistent between phases during the day. Because our experiments were consistently conducted at the same time during the day, heat production in the younger girls appears to follow the same pattern during the menstrual cycle as seen in older women.

The EM girls were more comfortable in the neutral session during the follicular phase, and they were slightly more comfortable in the cold during the luteal phase. Thermosensitivity was also influenced by menstrual phase, with the girls feeling less cold during the luteal, compared with the follicular, phase.

**Body size vs. hormonal status.** The PM girls were significantly different in body surface area-to-mass ratio from the EM girls, which would have influenced their core temperature response during cold exposure. A larger exposed surface area relative to the body mass is a hurdle for temperature regulation, and that may well have compromised thermoregulatory control in the premenarcheal girls. Again, Smolander et al. (27) found that boys with a larger surface area-to-mass ratio maintained core temperature as effectively as men through a greater reduction in skin temperature and an increased metabolic rate. Whereas a reduction in skin temperature was not observed in the PM girls, an increased metabolic rate was seen. It is important to notice that, although the two groups were matched for body fat, body fat was found to explain 79% ($P < 0.01$) of the variance in the decrease of core temperature within the PM group. Thermoregulation in the cold has been linked previously to adiposity (26). However, Gallow et al. (13) suggested that temperature regulation in women was more influenced by metabolic responses than morphological characteristics. Graham et al. (18) also concluded that the differences in thermoregulatory responses observed in the cold between eumenorrheic and amenorrheic women were physiological in nature because there was no difference in body surface area or body surface per unit of mass. In addition, the PM girls had a significantly lower core temperature during cold exposure, particularly when compared with the EM menarcheal girls at a time when their estradiol and progesterone levels were significantly elevated in the luteal phase.

On the basis of the above, it appears that young PM female individuals demonstrate an altered thermoregulatory response during cold exposure compared with EM female individuals, likely as a result of differences in their hormonal milieu. There is evidence that the absence of reproductive hormones may have an effect on the thermoregulatory responses of female individuals in the cold (18). In addition, the effects of exogenous hormones on temperature control in both younger and older women highlight the differences that an altered menstrual status may have on thermoregulatory responses (6, 12). However, limited data are available concerning thermoregulation in amenorrheic women, and no information has been reported previously for PM young girls.
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