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Effects of the menstrual cycle on muscle recruitment and oxidative fuel selection during cold exposure

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Blondin DP, Maneshi A, Imbeault M-A, Haman F. Effects of the menstrual cycle on muscle recruitment and oxidative fuel selection during cold exposure. *J Appl Physiol* 111: 1014–1020, 2011. First published July 7, 2011; doi:10.1152/jappphysiol.00293.2011.—Differences in core temperature and body heat content, generally observed between the luteal and follicular phase of the menstrual cycle, have been reported to modulate the thermogenic activity of cold-exposed women. However, it is unclear how this change in whole body shivering activity will influence fuel selection. The goal of this study was to quantify the effects of the menstrual cycle on muscle recruitment and oxidative fuel selection during low-intensity shivering. Electromyographic activity of eight large muscles was monitored while carbohydrate, lipid, and protein utilization was simultaneously quantified in the follicular and luteal phases of the menstrual cycle in nonacclimatized women shivering at a low intensity. The onset (~25 min), intensity (~15% of maximal voluntary contraction), and pattern (~6 shivering bursts/min) of the shivering response did not differ between menstrual cycle phases, regardless of differences in core temperature and hormone levels. This resulted in lipids remaining the predominant substrate, contributing 75% of total heat production, independent of menstrual phase. We conclude that hormone fluctuations inherent in the menstrual cycle do not affect mechanisms of substrate utilization in the cold. Whether the large contribution of lipids to total heat production in fuel selection confers a survival advantage remains to be established.

energy metabolism; electromyography; shivering thermogenesis; shivering pattern

THE MUSCLE RECRUITMENT and energy requirements of shivering thermogenesis have been well characterized in cold-exposed men (see Refs. 8 and 35 for review). However, studies of thermogenic responses and fuel selection pattern of cold-exposed women have been substantially less comprehensive (6, 16, 19, 24, 28). Some of the work conducted with women demonstrated that shivering activity can be influenced by the menstrual cycle phase. For example, during low-intensity shivering [~2 times resting metabolic rate (RMR)], Hessemer and Bruck (16) reported reduced muscle shivering activity and delayed onset of shivering in two large muscles (latissimus dorsi and rectus femoris) during the luteal phase (LP) compared with the follicular phase (FP) of the menstrual cycle. Such effects have been attributed to a higher baseline mean core body temperature (~0.5°C) in LP than FP (1, 6, 16). Accordingly, the modulation of thermogenic activity is also likely to affect fuel selection. It is unclear how these differences in thermogenic responses between FP and LP might affect fuel selection during cold exposure.

Fuel selection in shivering muscles can be modulated by recruiting 1) different metabolic pathways within the same fibers, 2) specific subpopulations of fibers within the same muscle, and 3) different muscles varying in fiber composition (8, 35). By combining measurements of fuel oxidation (indirect calorimetry) and muscle fiber recruitment [electromyography (EMG)], cold exposure studies in men have shown that the first two of these mechanisms are used for shivering (35). In glycogen-depleted and -loaded men, the same thermogenic rate could be sustained using widely different fuels [from carbohydrate (CHO)- to lipid-dominant] without modifying EMG activity of eight large muscles, indicating that this change in fuel selection was occurring within the same muscle fibers. In contrast, during high-intensity shivering, the second mechanism of fuel selection, whereby increases in CHO oxidation were achieved by recruiting fuel-specific fibers within the same muscle, was observed (10). These combined electrophysiological and metabolic approaches have not been used in cold-exposed women and may yield important information about the effects of the menstrual cycle on muscle recruitment and energy requirement of shivering.

The estimated CHO and lipid utilization data in women during low- to moderate-intensity shivering appear contradictory (5, 28, 33). Pettit et al. (28) showed a greater reliance on lipids in women [~36% of heat production (\dot{H}) for CHO and ~64% of \dot{H} for lipids] than in men (~47% of \dot{H} for CHO and ~53% of \dot{H} for lipids) during low-intensity shivering. However, no such sex differences were reported in the other two studies eliciting low-intensity (5) and moderate-intensity (33) shivering. Only one of these studies examined the effects of the menstrual cycle (5), and neither accounted for the potentially significant contribution of protein oxidation to total \dot{H} [which accounts for as much as 20% of \dot{H} during low-intensity shivering in men (12)]. They showed that hormone fluctuations during the menstrual cycle had no effect on thermogenic rate and substrate utilization between LP and FP. These variations in fuel selection in the few available studies may be related to 1) differences in cold exposure, 2) differences in controlling for the effects of the menstrual cycle, and 3) failure to account for the contribution of proteins in the total energy balance. Furthermore, while the total thermogenic rate may not be different between the menstrual cycle phases, the muscle recruitment pattern may be different and, ultimately, could affect fuel selection.

Therefore, the overall purpose of this study was to quantify the effects of the menstrual cycle on whole body shivering activity and oxidative fuel selection. Using EMG and indirect calorimetry, we monitored the recruitment levels of eight large muscles and quantified CHO, lipid, and protein utilization in LP and FP during low-intensity shivering. Based on previous

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findings, we hypothesize that shivering onset will be delayed and contraction intensity reduced in LP vs. FP because of the warmer initial core temperature. We also hypothesize that lipid oxidation will be greater and CHO oxidation will be lower in LP than FP. To ascertain the fuel selection mechanism responsible for this modulation (i.e., recruitment of specific metabolic pathways or muscle fibers), burst and continuous shivering activity will be quantified in LP and FP. These two patterns are linked with the recruitment of specific muscle fibers (22, 23, 27). Continuous, low-intensity shivering is associated with low-threshold, type I (slow-oxidative, fatigue-resistant) fibers, whereas high-intensity shivering bursts are linked to high-threshold, type II (fast-glycolytic, more fatigable) fibers. As in men (8–10, 14), this analysis may help us better understand the mechanisms that dictate fuel utilization in women.

MATERIALS AND METHODS

Participants. Six healthy, non-cold-acclimatized women volunteered for the study, which was approved by the Health Sciences Ethics Committee of the University of Ottawa. Written informed consent was obtained from all participants. The women were eumenorrheic, with normal and regular menstrual cycles (confirmed by daily logs; see below), and had not taken hormone contraceptives for ≥ 6 mo. Age, body mass, height, and body composition (dual-energy X-ray absorptiometry; see Table 2) of the participants were as follows: 22.7 ± 1.8 yr, 63.4 ± 0.9 kg, 166 ± 3.2 cm, and $27.8 \pm 2.4\%$, respectively. Peak O_2 consumption ($\dot{V}O_2$), estimated 5–7 days before the experiments by incremental treadmill exercise to volitional fatigue, averaged 48.6 ± 2.1 ml·kg⁻¹·min⁻¹.

Determination of menstrual cycle phase. At 3 mo prior to testing, using an electronic thermometer, the women charted daily basal sublingual temperature. For each participant, the monthly temperatures were averaged, and ovulation was estimated to occur when sublingual temperatures were consistently over the average temperature of that month. Participants also indicated illness, alcohol consumption, and poor sleep, which can affect body temperature and were considered when the log books were analyzed for ovulation. With the use of the log books, FP (4–7 days postmenses) and LP (4–7 days postovulation) were estimated. On the day of the experiments, menstrual cycle phase was verified by measurement of oral temperature upon awakening and by quantification of plasma estrogen and progesterone levels (human estradiol and human progesterone ELISA kits, Alpha Diagnostic, San Antonio, TX).

Experimental protocol. Each woman was exposed to the cold on two separate occasions: during FP and LP. The order of trials was randomly assigned following a crossover design. Each trial consisted of a 30-min baseline period followed by 120 min of shivering at an intensity equivalent to ~ 2.5 times the RMR. Experiments were conducted between 0800 and 1200, following 36 h without heavy physical activity. The last evening meal was standardized (3,220 kJ or 770 kcal, with 42% of energy from CHO), and participants were asked to report to the laboratory at 0730 on the next morning after a 12- to 14-h fast. On the day of the experiment, care was taken to minimize exercise or thermal stresses between awakening and the start of the experiment. Upon their arrival to the laboratory, participants, wearing only shorts and a sports bra, were weighed and instrumented with thermal probes (aural canal and skin thermocouples) and EMG surface electrodes (Delsys, Boston, MA). Once participants were fitted with a three-piece liquid-conditioned suit (Allen-Vanguard, Ottawa, ON, Canada), they performed a series of exercises to estimate their maximal voluntary contraction (MVC). After this series of exercises, an indwelling catheter (18 gauge, 32 mm; Arlington Medical Supply, Arlington, TX) was inserted into the antecubital vein for blood sampling. Participants were then asked to empty their bladder and remain seated for the next 30 min at $24.5 \pm 0.5^\circ\text{C}$ (750 ± 2 mmHg,

$40 \pm 3\%$ relative humidity). After this baseline period, the liquid-conditioned suit was perfused with 4°C water (*time 0*) using a temperature- and flow-controlled circulation bath (Endocal, NESLAB; and model 200-00, Micropump, Vancouver, WA). Thermal responses, muscle activity, metabolic rate, and substrate utilization were measured at baseline prior to cooling and every 30 min during the subsequent 120 min of cold exposure.

Thermal response. Changes in \dot{H} were calculated by indirect respiratory calorimetry corrected for protein oxidation, as described elsewhere (13). Aural canal (T_{au}) and mean skin (T_{sk}) temperatures were continuously monitored prior to and during cold exposure using aural canal (Mon-a-therm Tympanic, Mallinckrodt Medical, St. Louis, MO) and skin [Concept Engineering, Old Saybrook, CT; area-weighted equation from 6 sites: left side of face (14%), left upper chest (19%), left forearm (11%), lower back (19%), right medial quadriceps (32%), and posterior surface of the hand (5%) (25)] thermocouples.

Muscle recruitment. Shivering EMG signals were recorded from eight muscles: trapezius, latissimus dorsi, pectoralis major, rectus abdominis, vastus lateralis, rectus femoris, vastus medialis, and adductor magnus. Surface electrodes (Delsys) were placed bilaterally over the belly of each muscle, and their exact positions were identified with an indelible skin marker to allow consistent placement between experimental sessions. Raw EMG signals were collected at 1,000 Hz, filtered to remove spectral components < 20 Hz and > 500 Hz as well as 60-Hz contamination and related harmonics, and analyzed using custom-designed MATLAB algorithms (MathWorks, Natick, MA). Shivering activity of the eight individual muscles was monitored 15 min before and 10 times during cold exposure at 5–15, 20–30, 35–45, 50–60, 65–75, 80–90, 95–105, 105–110, 110–115, and 115–120 min, but only the last 25-min period of recording was used for the analyses. Participants were asked to avoid voluntary movements during recording periods to minimize voluntary muscle activity as much as possible throughout cold exposure.

Shivering intensity of individual muscles was determined from root-mean-square (RMS) values calculated from raw EMG signals using a 50-ms overlapping window (50%). Briefly, baseline RMS values ($\text{RMS}_{\text{baseline}}$: 15-min RMS average measured prior to cold exposure) were subtracted from shivering RMS (RMS_{shiv}) values and RMS values obtained from the MVCs of individual muscles (RMS_{MVC}). Shivering intensity was normalized to RMS_{MVC} by using the following equation

$$\text{shivering intensity (\%MVC)} = \frac{\text{RMS}_{\text{shiv}} - \text{RMS}_{\text{baseline}}}{\text{RMS}_{\text{MVC}} - \text{RMS}_{\text{baseline}}} \times 100 \quad (1)$$

Whole body shivering intensity was determined by averaging the shivering intensity from each muscle.

Shivering pattern was determined as previously described (10), where an example of EMG signal showing the two shivering patterns is illustrated. Briefly, a shivering burst was defined as an EMG interval with a duration > 0.2 s, an interburst interval > 0.75 s, and an amplitude higher than the intensity threshold at each recording period. Intensity threshold was determined by 1) averaging shivering intensity (A_{EMG}) over the entire recording period, 2) averaging the remaining values above A_{EMG} (B_{EMG}), and 3) setting the intensity threshold at B_{EMG} . Whole body burst shivering was calculated as the average of the burst rate of individual muscles.

Onset of shivering was visually determined and defined on the following criteria: four of the eight muscles evaluated on the right side of the body achieved an RMS_{shiv} that was at least double that of $\text{RMS}_{\text{baseline}}$ for ≥ 10 s. A separate analysis of shivering onset was also done using only the latissimus dorsi and rectus femoris muscles, to allow for a comparison with the studies by Hessemmer and Bruck (16, 17). In that instance, the onset of shivering in latissimus dorsi and rectus femoris was defined using the approach described above, but

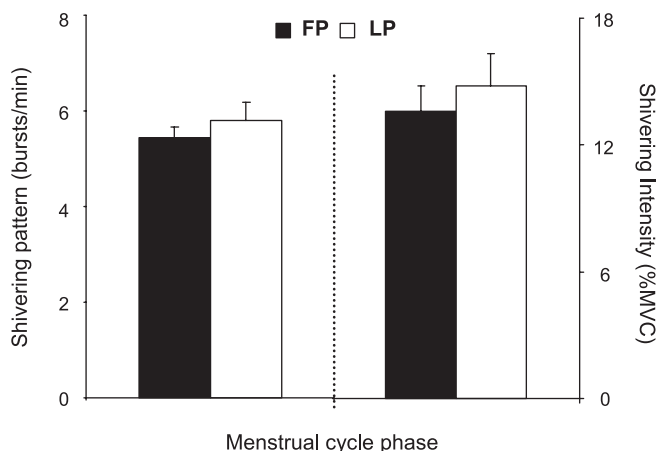


Fig. 1. Shivering pattern and intensity of women in the follicular (FP) and luteal (LP) phases of the menstrual cycle during the final 30 min of cold exposure. MVC, maximal voluntary contraction.

both muscles had to meet these criteria. The onset of shivering for each participant and each trial was determined twice by the same researcher. If there was a discrepancy of >30 s between the first two assessments, then the onset of shivering was evaluated a third time.

Measurement of heat production and fuel utilization. $\dot{V}O_2$ and CO_2 dioxide production ($\dot{V}CO_2$) were measured using a calibrated metabolic system (MOXUS, Applied Electrochemistry, Pittsburgh, PA) and expressed in STPD. Total protein (RP_{ox}), carbohydrate (RG_{ox}), and lipid (RF_{ox}) oxidation rates (in g/min) were calculated as described previously (12, 13)

$$RP_{ox}(\text{g/min}) = 2.9 \times \text{urea}_{\text{urine}}(\text{g/min}) \quad (2)$$

$$RG_{ox}(\text{g/min}) = 4.59 \dot{V}CO_2(\text{l/min}) - 3.23 \dot{V}O_2(\text{l/min}) \quad (3)$$

$$RF_{ox}(\text{g/min}) = -1.70 \dot{V}CO_2(\text{l/min}) + 1.70 \dot{V}O_2(\text{l/min}) \quad (4)$$

where $\dot{V}O_2$ (l/min) and $\dot{V}CO_2$ (l/min) are corrected for the volumes of O_2 and CO_2 corresponding to protein oxidation (1.010 and 0.843 l/g, respectively). RP_{ox} was estimated from urinary urea excretion ($\text{urea}_{\text{urine}}$) in urine samples collected for 120 min in the cold. Urinary urea concentration was determined using a commercial urine assay kit (BioAssay Systems). Energy potentials of 16.3 kJ/g (CHO), 40.8 kJ/g (lipids), and 19.7 kJ/g (proteins) were used to calculate the relative contributions of each fuel to total \dot{H} (21, 26).

Statistical analyses. Values are means \pm SE. Statistical significance was set at $P \leq 0.05$. Two-way ANOVA with menstrual cycle phase (FP and LP), time [0, 30, 60, 90, and 120 min (Figs. 2–4) and baseline and cold (see Table 3)], and interaction as independent variables was used to analyze differences in \dot{H} , T_{au} , \bar{T}_{sk} , CHO and lipid oxidation, and the relative contributions of CHO, lipid, and protein to \dot{H} throughout the cold exposure. Significant interactions were followed up with a 2 (phase) \times 2 (time) repeated-measures ANOVA. Significant effects of time or condition were followed up with a one-way repeated-measures ANOVA. A paired-sample t -test was used to compare menstrual cycle differences in sex hormones, as well as burst rate and shivering intensity, during cold exposure. The statistical power of significant effects for key parameters (oxidation rates and relative contributions of plasma glucose, muscle glycogen, total CHO, and lipids to \dot{H}) ranged from 0.889 to 1.000.

RESULTS

Menstrual phase. Average morning basal sublingual temperatures were taken upon awakening (~ 0600), and sex hormone concentrations are presented in Table 2. Thermal and hormonal parameters measured in the morning prior to the experiment

indicated that the women were in the appropriate menstrual phase.

Shivering EMG activity. Whole body shivering intensity was not significantly different between the two menstrual cycle phases: $13.6 \pm 1.2\%$ MVC and $14.8 \pm 1.5\%$ MVC in FP and LP, respectively (Fig. 1). Similarly, whole body burst shivering rate did not differ between menstrual phases: 5.4 ± 0.2 and 5.7 ± 0.4 bursts/min in FP and LP, respectively. Whole body onset of shivering occurred at the same time in FP and LP: 23.7 ± 3.2 and 25.6 ± 3.3 min, respectively. In addition, this onset occurred at the same \bar{T}_{sk} ($29.5 \pm 0.5^\circ\text{C}$ and $29.5 \pm 0.9^\circ\text{C}$ in FP and LP, respectively) and at same T_{au} ($36.9 \pm 0.2^\circ\text{C}$ and $37.0 \pm 0.2^\circ\text{C}$ in FP and LP, respectively).

Thermal response. Changes in \dot{H} , T_{au} , and \bar{T}_{sk} in FP and LP are presented in Fig. 2. Total \dot{H} progressively increased throughout cold exposure in both menstrual cycle phases, stabilizing at 2.4 times RMR by the final 30 min of exposure in FP (from 4.8 ± 0.4 to 11.3 ± 0.6 kJ/min) and LP (from 4.7 ± 0.1 to 11.9 ± 0.7 kJ/min; Fig. 1A). T_{au} gradually decreased throughout the cold exposure period, falling by an average of 0.5°C by the end of cold exposure in both phases (from $36.7 \pm 0.1^\circ\text{C}$ to $36.3 \pm 0.3^\circ\text{C}$ in FP and from $36.9 \pm 0.3^\circ\text{C}$ to $36.3 \pm 0.3^\circ\text{C}$ in LP), but no differences were seen between FP and LP. Similarly, \bar{T}_{sk} decreased by 16% in FP (from $32.7 \pm 0.4^\circ\text{C}$ to $27.5 \pm 0.6^\circ\text{C}$) and 18% in LP (from $33.0 \pm 0.9^\circ\text{C}$ to $27.0 \pm$

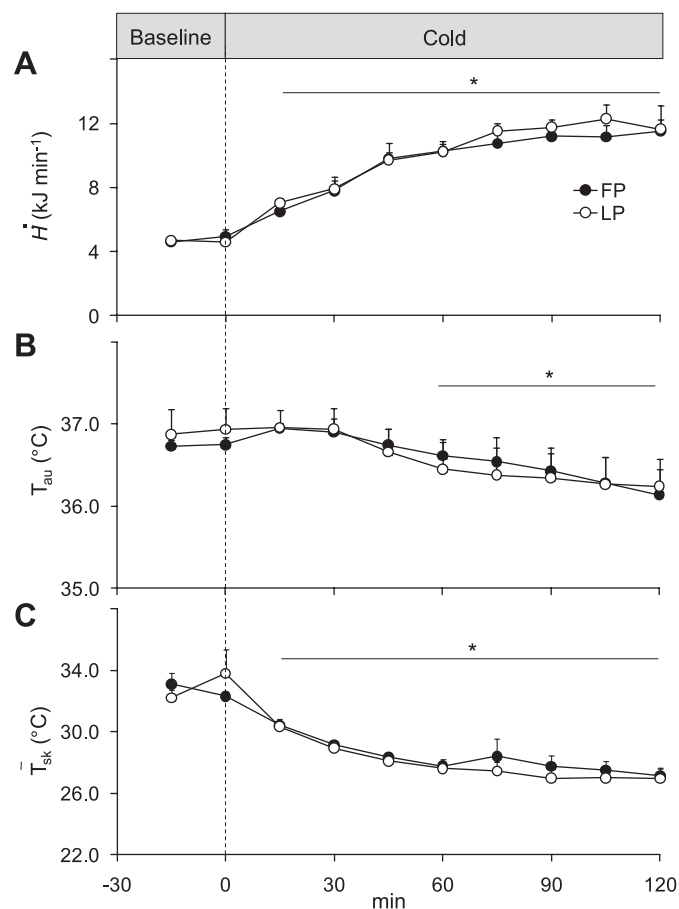


Fig. 2. Rate of heat production (\dot{H}) and aural canal (T_{au}) and mean skin (\bar{T}_{sk}) temperatures before (baseline) and during cold exposure in FP and LP. *Significantly different from baseline before cold exposure, $P < 0.05$.

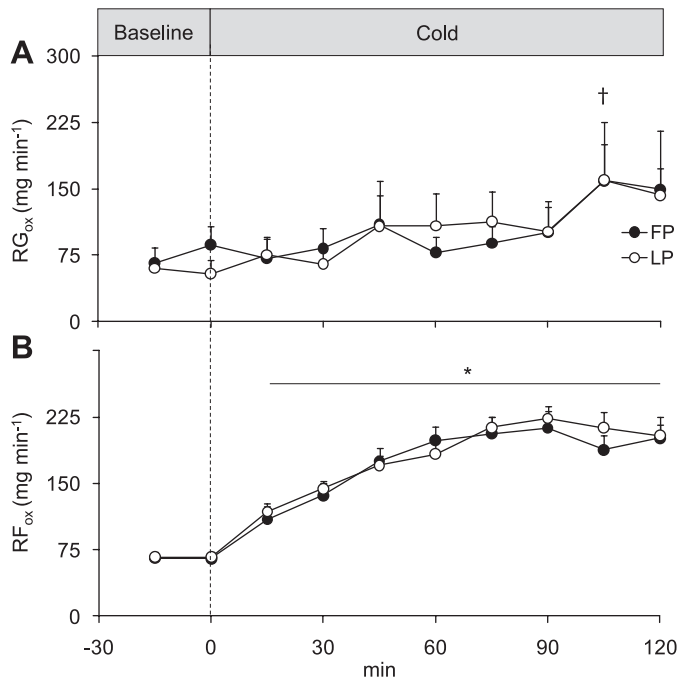


Fig. 3. Changes in absolute carbohydrate (RG_{ox} ; A) and lipid (RF_{ox} ; B) oxidation rates before (baseline) and during cold exposure in FP and LP. Significantly different from baseline before cold exposure: * $P < 0.05$; † $P = 0.06$.

0.6°C) as a result of cold exposure, but no differences were observed between menstrual cycle phases.

Measurement of heat production and fuel utilization. Absolute rates of CHO and lipid oxidation are presented in Fig. 3. CHO and lipid oxidation rates increased significantly with cold exposure but did not differ between menstrual cycle phases. In response to cold exposure, RG_{ox} only increased significantly by the final 30 min in FP (from 76 ± 18 to 136 ± 30 mg/min) and also in LP (from 57 ± 13 to 135 ± 51 mg/min; Fig. 3A, see Table 3), with the latter failing to reach statistical significance ($P = 0.08$). Similarly, RF_{ox} increased significantly from 65 ± 5 mg/min during baseline to 200 ± 17 mg/min during cold exposure in FP and from 67 ± 6 to 213 ± 18 mg/min in LP (Fig. 3B, see Table 3). No differences were present in RP_{ox} during cold exposure between menstrual cycle phases: 45 ± 5 and 50 ± 6 mg/min in FP and LP, respectively (see Table 3).

Changes in the relative contribution of CHO, lipid, and protein oxidation to total \dot{H} (% \dot{H}) in FP and LP are presented in Fig. 4. During cold exposure, % \dot{H} from CHO oxidation transiently decreased, returning to baseline during the final 30 min of exposure ($19.6 \pm 3.9\%$ of \dot{H} in FP and $16.7 \pm 5.7\%$ of \dot{H} in LP; Fig. 4A, see Table 3). A gradual increase and eventual plateau in % \dot{H} from lipid oxidation was observed in LP and FP from the onset of cold exposure (from $56.2 \pm 3.4\%$ to $72.4 \pm 3.7\%$ of \dot{H} in FP and from $58.8 \pm 5.1\%$ to $74.5 \pm 5.8\%$ of \dot{H} in LP; Fig. 4B, see Table 3). A twofold decrease in % \dot{H} from protein oxidation was found in FP (from $19.2 \pm 2.8\%$ to $8.0 \pm 1.0\%$ of \dot{H}) and LP (from $21.3 \pm 2.5\%$ to $8.8 \pm 1.4\%$ of \dot{H}) due to cold exposure (see Table 3).

DISCUSSION

The overall purpose of this study was to examine the effects of menstrual cycle-associated fluctuations in core temperature

and sex hormone levels on muscle activity and substrate utilization during low-intensity shivering. The methodology employed to identify FP and LP was adequate to observe significant differences in resting body temperature, as well as circulating estradiol and progesterone levels, prior to cold exposure (see Table 2). However, contrary to our expectation, these physiological differences between menstrual cycle phases did not alter muscle recruitment and oxidative fuel selection in the cold. Whole body muscle activity (intensity and EMG pattern) and substrate (CHO, lipids, and proteins) utilization remained the same in both menstrual phases (see Table 3, Figs. 1 and 3). These combined results indicate that fuel selection mechanisms are not altered during the menstrual cycle. When we compared values obtained in women (present study) with values previously obtained in men (8, 35), we found that women displayed a similar burst shivering rate ($\sim 5\text{--}6$ bursts/min) but oxidized substantially more lipids at the same shivering intensity ($\sim 75\%$ vs. 50% \dot{H} for men).

Previous work in men indicated that metabolic fuel selection can be altered through the selective recruitment of specific fibers within the same muscles and/or metabolic pathways within the same muscle fibers (8, 10, 11, 34). For example, during moderate-intensity shivering, variations in whole body CHO and muscle glycogen utilization were closely related to changes in burst shivering rate (or recruitment of more type II

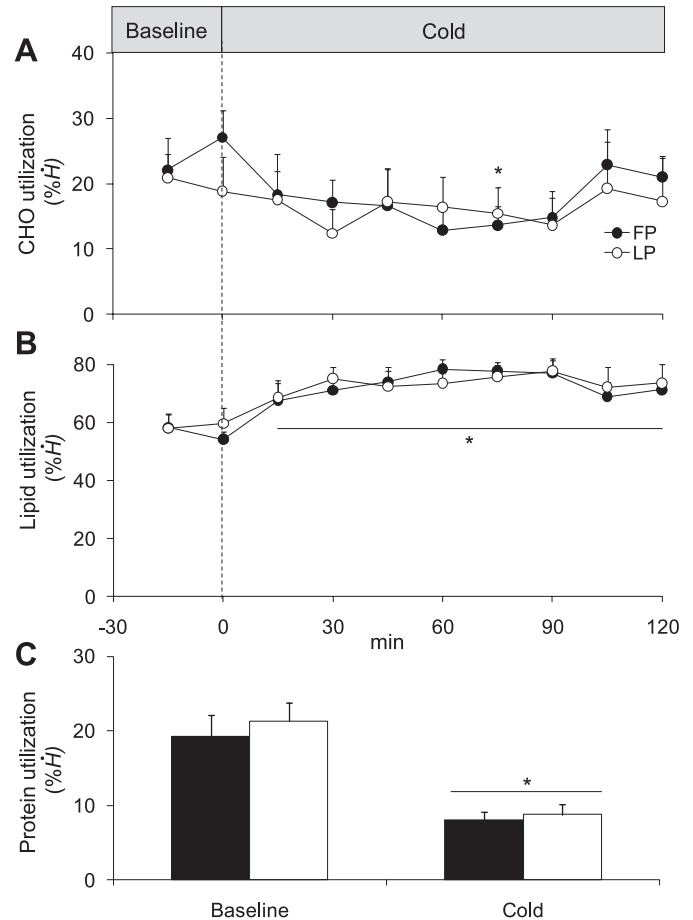


Fig. 4. Relative contributions of carbohydrates (CHO), lipids, and proteins to total \dot{H} before (baseline) and during cold exposure in FP and LP. *Significantly different from baseline before cold exposure, $P < 0.05$.

Table 1. *Physical characteristics of participants*

Subj No.	Age, yr	Height, cm	Body Wt, kg	%Body Fat	Peak O ₂ Uptake, ml·kg ⁻¹ ·min ⁻¹
1	30	171	64.0	17.9	55.8
2	19	171	61.7	25.3	50.1
3	26	161	64.9	29.3	48.6
4	20	152	60.1	35.9	40.8
5	19	168	66.6	28.8	51.2
6	22	172	63.0	29.3	45.3
Mean ± SE	22.7 ± 1.8	166 ± 3.2	63.4 ± 0.9	27.8 ± 2.4	48.6 ± 2.1

fibers) (10, 11). In women, such a combined EMG and fuel selection analysis had never been attempted but was necessary to better understand the effects of thermal and hormonal fluctuations on mechanisms of substrate utilization. To achieve this objective, much effort was given to clearly identify the menstrual cycle.

Menstrual cycle duration and fluctuations in sex hormone levels are affected by a variety of factors, such as age, fatness, fitness, and genetics (15, 20). To reduce interindividual variability in menstrual cycle, particular effort was made in the present study to recruit morphologically and aerobically similar women (Table 1). Using this approach, we found significant differences between LP and FP in oral temperature (~0.3°C taken at 0630) and circulating estradiol and progesterone levels prior to cold exposure (Table 2). Estradiol concentrations were >1.7-fold higher in LP than FP, whereas a >10-fold difference in progesterone level was observed between menstrual phases. Together, these findings confirm that all women were in the anticipated phases of their menstrual cycle prior to cold exposure. Changes in muscle recruitment and fuel selection were then quantified to identify the effect of the respective menstrual cycle phases on these outcome measures.

Effects of menstrual cycle on muscle recruitment in the cold. EMG signals obtained from eight large muscles were analyzed to quantify the effects of the menstrual cycle on shivering onset and intensity. Contrary to our expectation, these parameters were not different between LP and FP, with the onset of shivering beginning at ~25 min and whole body shivering intensity remaining between 13% and 15% MVC (Fig. 1). These findings differ from results of Hessemer and Bruck (16, 17), who reported differences in shivering onset and intensity between FP and LP in latissimus dorsi and rectus femoris. When these same two muscles are considered in the shivering onset and intensity for the present study, there is still no difference between menstrual phases. Hessemer and Bruck associated this delayed shivering onset to a ~0.6°C greater T_{au} in LP than FP, while others similarly suggested that a blunted response may be associated with differences in heat balance (6). However, since shivering thermogenesis is strongly driven by the stimulation of peripheral thermoreceptors (29), method-

Table 2. *Plasma estradiol and progesterone levels and basal morning temperatures in FP and LP*

	FP	LP
Estradiol, pmol/l	310 ± 26	534 ± 33*
Progesterone, nmol/l	2.6 ± 0.3	25.4 ± 1.6*
Morning temperature, °C	36.1 ± 0.09	36.4 ± 0.09*

Values are means ± SE (n = 6). FP, follicular phase; LP, luteal phase. *Significantly different from FP (P < 0.05).

ological differences in cooling procedures are likely contributing more significantly to the discrepant findings between studies. The cooling suit used in the present study elicited identical and consistent skin temperatures during cold exposure in LP and FP without substantially modifying core temperature (Fig. 2). Consequently, the onset of shivering occurred at the same T_{sk} and T_{au} in both menstrual phases, suggesting that differences in core temperature or heat balance between conditions are inconsequential to the thermogenic response in women.

This study also provides the first estimates of the contribution of low-intensity continuous shivering and high-intensity burst shivering in women. In men, such an analysis has greatly improved our understanding of the mechanisms involved in adjusting fuel use during cold exposure (see Ref. 9 for review). Here, results showed that the burst shivering activity was unaffected by the menstrual cycle phase, with the average for the eight muscles monitored remaining at ~6 bursts/min (Fig. 1). This is similar to values previously observed in men (10) suggesting that there are also no sex-related differences. Together, these results indicate that, during mild cold exposure, thermal and hormonal fluctuations associated with the menstrual cycle do not modify muscle recruitment patterns and/or the pattern of fiber recruitment within each muscle. These findings also show that potential differences in fuel selection between sexes are not associated with differences in muscle or fiber recruitment. Instead, different metabolic pathways would need to be recruited in the same fibers.

Menstrual cycle and fuel selection. Studies examining fuel selection in cold-exposed women have been relatively sparse (5, 28, 33), and the cooling protocols varied greatly, limiting

Table 3. *Absolute oxidation rate of substrates and their relative contribution to total heat production (% \dot{H}) in FP and LP before and during cold exposure*

	FP		LP	
	Baseline	Cold	Baseline	Cold
Heat production, kJ/min	4.8 ± 0.4	11.3 ± 0.6*	4.7 ± 0.1	11.9 ± 0.7*
Total CHO				
mg/min	76 ± 18	136 ± 30	57 ± 13	135 ± 51
% \dot{H}	24.6 ± 4.2	19.6 ± 3.9	19.9 ± 4.4	16.7 ± 5.7
Lipids				
mg/min	65 ± 5	200 ± 17*	67 ± 6	213 ± 18*
% \dot{H}	56.2 ± 3.4	72.4 ± 3.7*	58.8 ± 5.1	74.5 ± 5.8*
Proteins				
mg/min		45 ± 5		50 ± 6
% \dot{H}	19.2 ± 2.8	8.0 ± 1.0*	21.3 ± 2.5	8.8 ± 1.4*

Values are means ± SE (n = 6). Baseline, ≤30 min before cold exposure; cold, 90–120 min of cold exposure. *Significantly different from baseline (P < 0.05).

the ability to make comparisons. Only one study investigated the effect of the menstrual cycle on substrate utilization under cold conditions (5). They showed that ~60–70% of total \dot{H} may be attributed to fat oxidation during low-intensity shivering in women, regardless of the menstrual cycle phase. However, fuel selection values have not been corrected to account for protein oxidation in total energy budget, which can have important implications (8). Here we demonstrate that when we account for protein oxidation in the energy budget, lipid oxidation increases threefold, and its contribution to the thermogenic rate increases from 56–59% at rest to 72–75% during low-intensity shivering (Table 3). As a consequence, the reliance on protein oxidation for \dot{H} is diminished from 19–21% at rest to 8–9% during cold exposure (Table 3). These changes occur regardless of the menstrual cycle phase. While differences in fat oxidation between menstrual cycle phases have been reported in resting and exercising women in concurrence with elevated concentrations of estrogen (7, 36), these differences do not appear to be consistent with all exercise studies. Elevated estrogen levels promote lipolysis, increasing fatty acid availability and, in turn, increasing lipid oxidation (2, 30). However, some investigators failed to see changes in fuel selection between menstrual cycle phases, despite observing differences in estradiol concentration (18), while others suggested that these phase-related differences only occur at much greater exercise intensities (90% of lactate threshold) (37). Although large differences in these sex hormones were confirmed between conditions in the present study, substrate utilization did not differ, suggesting that perhaps the cold-induced sympathetic stimulation is masking the effects of these sex hormones.

Sex-related differences: fuel selection and shivering response. The present findings demonstrate that the relative contribution of fat oxidation to total \dot{H} is 25% greater in women than men (~75% vs. ~50%) exposed to the same cold stress (13, 14). Consequently, the reliance on CHO for \dot{H} is significantly diminished in women compared with men (~20% in women compared with 40% in men). These findings are consistent with the pattern previously reported in women and men, matched for body fatness, exposed to 5°C for 2 h (28). In this study (28), men and women were matched for body fatness, and no differences in catecholamine response were observed, suggesting that the sex-related differences could not be attributed to differences in sympathetic stimulation or morphology. Similarly, since the change in \dot{H} and the shivering pattern were the same between men (13) and women (present study), the differences in fuel selection observed here suggest that different metabolic pathways in the same muscle fibers were likely selectively recruited. A similar response was observed in glycogen-loaded and -depleted men exposed to a cold stress similar to that used in the present study (10). In men, glycogen depletion and cold exposure elicited the same \dot{H} and shivering pattern (same fiber recruitment) observed in glycogen-loaded men, but fuel selection patterns were very different, with a significantly greater reliance on lipids in the glycogen-depleted condition. Given the greater basal intramyocellular lipid content in women (3, 31), their high capacity for fatty acid transport at the sarcolemma, cytosol, and mitochondria (4), and greater capacity for fatty acid oxidation, preferential utilization of lipids should be expected. This may explain the fuel selection pattern in women, which resembles that of glycogen-

depleted men. As a consequence, these findings lend further support to the hypothesis, previously suggested by Tarnopolsky and Ruby (32), that women may be better adapted to metabolic stresses where glycogen sparing is advantageous, such as high altitude, prolonged exercise, and now cold exposure. Further research is needed to confirm whether differences in intramuscular substrate utilization explain the sex-related differences in whole body fuel selection observed here.

Conclusions. In summary, we show that menstrual cycle phase does not affect muscle recruitment and/or oxidative fuel selection of cold-exposed women. Since shivering thermogenesis is stimulated by peripheral thermoreceptors, the onset, intensity, and pattern of the shivering response remain the same, regardless of differences in core temperature and hormone levels between menstrual phases. Accordingly, substrate utilization was also the same between menstrual phases, with lipids remaining the predominant thermogenic fuel, contributing 75% of \dot{H} . This is 25% greater than the contribution previously observed in men exposed to the same cold condition, eliciting the same change in \dot{H} and the same shivering pattern. What remains clear is that the increased lipid use in women compared with men may result in differences in shivering endurance and/or nutritional requirements in the cold.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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