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The Effects of the Menstrual Cycle on Muscle Activation Patterns and Fuel Utilization in Cold Exposure

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THE EFFECTS OF THE MENSTRUAL CYCLE ON MUSCLE ACTIVATION PATTERNS AND FUEL UTILIZATION IN COLD EXPOSURE

by

Anali Maneshi

B.Sc., University of Waterloo, 2006

THESIS

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Abstract

Cold-induced shivering is characterized by two distinct muscle contraction patterns: continuous low-intensity shivering [at ~1 to 5% of maximal voluntary contraction (MVC)] and bursts of high-intensity shivering (at 10 to 35% MVC) (Meigal, 2002, Haman et al., 2004). In this context, Haman *et al.* (2004) showed that carbohydrate (CHO) oxidation rate is directly related to burst shivering rate which increases the proportional recruitment of glycolitic type II fibers. Because most of these studies have been carried out in men, little is still know on the effects of hormonal fluctuations during the menstrual cycle on the dual shivering pattern and energy metabolism in women.

Therefore, the purpose of this study was to quantify differences in muscle recruitment pattern and fuel selection in two phases of the menstrual cycle. Muscle recruitment in eight muscles and whole body fuel selection were monitored in women exposed to 120 min of cold exposure (5°C liquid-perfusion suit) during FP and LP. Results show that there is no difference in the onset of shivering, shivering intensity, burst duration and burst shivering rate between LP and FP during steady state shivering (in last 15 min). Similarly, during steady state shivering (T=105-120 min), absolute rates and relative contributions of metabolic fuels to total heat production did not differ between FP and LP. We conclude that hormonal fluctuations during the menstrual cycle do not affect muscle recruitment patterns or substrate utilization in the cold. Whether this important difference in fuel selection modifies muscle shivering activity and/or confers a survival advantage remains to be established.

List of Abbreviations

AD – adductor magnus

CHO – carbohydrate

CO_2 – carbon dioxide

EMG – electromyography

FP – follicular phase

Hloss- heat loss rate

$\dot{M}$ – heat production rate

HR – heart rate

LA – latissimus dorsi

LCS – liquid conditioned suit

LH – luteinizing hormone

LP – luteal phase

MVC – maximal voluntary contraction

PE – pectoralis major

RF – rectus femoris

RFox- total lipid oxidation

RGox – total glucose oxidation

RPox- total protein oxidation

RMR – resting metabolic rate

Tau – aural canal temperature

Tco – core temperature

Tes – esophageal temperature

TR - trapezius

Tre- rectal temperature

TU – Trunk region (including trapezius, latissimus dorsi, pectoralis major, rectus abodminus)

Tsk – skin temperature

UL – Upper leg region (vastus lateralis, rectus femoris, vastus medialis and adductor magunus.)

VL – vastus lateralis

VM – vastus medialis

VO_2 - volume of oxygen consumed

$\dot{V}O_{2peak}$ – peak maximal volume of oxygen consumption

%RGox – relative percent of carbohydrate contribution to heat production

% RFox – relative percent of lipid contribution to heat production

% RPox – relative percent of protein contribution to heat production

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Figure 3.10 Changes in the relative contribution of carbohydrates (CHO) to total heat production (M) (%Gox), and whole body overall shivering activity ($\%Shiv_{ov}$) in the follicular (○) and luteal (●) phase of the menstrual cycle in women exposed to 4°C water for 120 min.

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Chapter I - General Introduction

Shivering is an involuntary contraction of muscles to produce heat when exposed to cold (Brooks 2005). Energy required to maintain shivering thermogenesis in men is obtained primarily from the oxidation of CHO and lipids (Vallerand and Jacobs 1989; Vallerand, Zamecnik et al. 1995; Vallerand, Zamecnik et al. 1999; Haman, Péronnet et al. 2002) and to a lesser, yet significant, contribution of protein oxidation to heat production (Vallerand, Jacobs et al. 1989; Haman, Peronnet et al. 2002). The contribution of each substrate to heat production is dependent on: nutritional status, intensity of shivering and muscle recruitment (Haman 2006). Although this notion is clear in men, it is unclear if women respond in the same manner as men when exposed to cold conditions. The hormonal fluctuations of the menstrual cycle present an additional challenge when investigating women and metabolism. It is unknown if the differences in the selection of metabolic pathways in the different menstrual cycle phases results in oxidation of different substrates to produce heat during resting and cold conditions in the two phases of the menstrual cycle: follicular and luteal.

Menstrual Cycle and Thermoregulation

The menstrual cycle affects heat balance by increasing core body temperature in the luteal phase (LP) by up to 0.5° C when compared to the follicular phase (FP), see Appendix A, figure 1.1. The cycle is divided into two primary ovarian phases, the FP and the LP (Silverthorn 2001). Progesterone has been linked to the increase in core body temperature in the LP compared to the FP. Administering progesterone to both males and females has been shown to increase core body temperature (Nakayama, Suzuki et al. 1975). Progesterone has consequently been shown to influence heat production (Nakayama, Suzuki et al. 1975; Horvath and Drinkwater 1982; Hessemer and Bruck

1985). Nakayama & Suzuki discovered that progesterone affects the activity of warm-sensitive neurons in the preoptic area of the hypothalamus (Nakayama, Suzuki et al. 1975). The effect of progesterone on the warm-sensitive neurons leads to an upward 'shift' in thermoregulatory thresholds, causing the increase in core body temperature (Nakayama, Suzuki et al. 1975). It is thought that the fluctuations in both estrogen and progesterone, in the different menstrual cycle phases, may have a "resetting" effect on the shivering threshold thus affecting net heat production (Nakayama, Suzuki et al. 1975; Hessemer and Bruck 1985). Contradictory evidence exists as to whether this increase in core body temperature influences heat production in the different menstrual cycle phases (Horvath and Drinkwater 1982; Hessemer and Bruck 1985; Gonzalez and Blanchard 1998; Pettit, Marchand et al. 1999). However, some evidence does suggest a "resetting" of the shivering threshold and thermoregulatory thresholds (Hessemer and Bruck 1985). This is demonstrated that both shivering and sweating occurred with a higher core temperature in the LP.

Gonzalez and Blanchard (1998) hypothesized that shivering may be blunted during women's LP due to the "resetting" of the shivering threshold. The researchers believed that the increase elevated levels progesterone and estradiol in the LP would heighten the activity of warm-sensitive neurons in preoptic area of the hypothalamus during cold stress. The authors speculated that the heightened activity of warm-sensitive neurons lead to a decrease in the firing of the cold-sensitive neurons resulting in a blunted shivering onset. Gonzalez and Blanchard (1998) verified the hypothesis through an experiment which demonstrated a lower shivering response to a higher given mean core body temperature in the LP compared to the FP (Gonzalez and Blanchard 1998).

Hessemer and Bruck (1998) found similar results when they performed one of the earliest investigations researching the effects of the menstrual cycle on thermoregulation, specifically: shivering, skin blood flow and sweating responses measured at night (Hessemer and Bruck 1985). A decrease in shivering electrical muscle activity, in both amplitude and frequency of the signal, was found in the LP when compared to the FP. The parallel changes in the alteration of thermoregulatory thresholds for shivering, cutaneous vasodilation and sweating thresholds suggest that an ovulation-related "resetting" of the threshold in the LP exist. Shivering, cutaneous vasodilation and sweating all occurred at higher core body temperatures in the LP compared to the FP.

The increase in core body temperature affects the onset of specific physiological responses to not only in cold exposure but exercise and heat exposure (Hessemer and Bruck 1985). These shifts are believed to result from the abovementioned effects of progesterone increasing hypothalamic temperatures therefore affecting hypothalamic thermosensitivity. Previous studies indicate that hypothalamic warming decreases shivering response to cold temperatures (Boulant and Gonzalez 1977). The increase in core body temperature, resulting in hypothalamic warming, is thought to compete with the peripheral afferent information being delivered by the peripheral temperature receptors which are located on the skin surface. A study using animal models and found that warming the hypothalamus decreases or even represses shivering in cold temperature exposure (Nakayama, Suzuki et al. 1975). Li et al (1988) found that blocking the progesterone receptors in women in the LP dropped core body temperature to that observed in the FP (Li, Dockery et al. 1988).

Menstrual Cycle and Metabolism

Little research has been done on the effects of cold exposure on metabolism in women. Studies have been done looking at substrate utilization in women, but few have controlled for the menstrual cycle. Two studies have quantified the metabolic response of women exposed to cold. Glickman-Weiss et al. (2000) found no menstrual cycle or sex difference in substrate utilization when they evaluated acute cold-exposure through water immersion in 10 females and 18 males. Contrarily, Pettit et al. (1999) found that after 2 hours of cold air exposure, women's RER, in the FP, was significantly lower than men's indicating higher fat oxidation. Men oxidized 47% CHO and 53% lipid, and women oxidized 36% CHO and 64% lipids. No sex differences were found when observing plasma catecholamine responses of adrenalin and noradrenalin. It should be noted that RER values were not corrected for protein metabolism as the authors felt that protein contribution was negligible (Pettit, Marchand et al. 1999). Recently, protein oxidation has been shown to contribute up to 20% of heat production during low intensity shivering in men (Weber and Haman 2005). Secondly, the menstrual cycle was controlled for by a gynecological questionnaire determining menstrual phase status. The questionnaire did indicate that the women were in varying menstrual cycle phases, which may have affected metabolic and thermoregulatory results.

Minimal information exists on the effects of the menstrual cycle on cold exposure and metabolism; consequently studies investigating menstrual cycle effects on metabolism and exercise are considered in this section. The menstrual cycle has been shown to affect not only core body temperature but also substrate utilization at rest and during low-intensity exercise (Lariviere, Moussalli et al. 1994; Wenz, Berend et al. 1997;

Hackney 1999; Zderic, Coggan et al. 2001). It is suggested that the differences in selection of metabolic pathways are due to varying estrogen and progesterone levels found in the FP and LP of the menstrual cycle (Wenz, Berend et al. 1997). Dombovy et al. (1987) observed a lower RER in basal and maximal exercise intensities in the LP compared to the FP (Dombovy, Bonekat et al. 1987). Many studies are in agreement demonstrating greater lipid and lower CHO oxidation rates in the LP compared to the FP (Lariviere, Moussalli et al. 1994; Wenz, Berend et al. 1997; Zderic, Coggan et al. 2001). On the other hand, many researchers do not believe that the menstrual cycle affects metabolism (Horton, Pagliassotti et al. 1998; Suh, Casazza et al. 2003; Casazza, Jacobs et al. 2004). Conflicting results regarding the effects of the menstrual cycle on substrate utilization do exist in the literature; however, there is a trend in the literature where menstrual cycle effects are seen at low work intensities but not seen at high work intensities (Stephenson, Kolka et al. 1982; Dombovy, Bonekat et al. 1987; Wenz, Berend et al. 1997; Hackney 1999; Braun, Mawson et al. 2000; Casazza, Jacobs et al. 2004).

Considering that recruiting type I muscle fibers has been linked to lipid oxidation and recruitment of type II muscle fibers is linked to CHO oxidation, a possible cause for the variability in metabolic pathways may be due to differences in muscle fiber recruitment patterns.

Few studies have focused on the neural control of muscle fiber recruitment during shivering (Meigal 2002; Haman, Legault et al. 2004) when compared to exercise. In exercise, muscle fibers are recruited according to size, whereby type I fibers are activated for low force contractions and the larger, faster type II are then recruited to provide greater force contractions as stated by Henneman's size principle (Henneman and Olson

1965; Henneman, Somjen et al. 1965; McPhedran, Wuerker et al. 1965). During shivering, two distinct patterns of muscle activity are seen: continuous low-intensity shivering and high intensity shivering bursts (Meigal 2002; Haman, Legault et al. 2004), see figure 1.2 in appendix A. Low-intensity continuous shivering has been linked to type-I fibers, and high-intensity shivering bursts have been linked to type II fibers through the use of intramuscular electrodes in humans (Meigal, Lupandin Iu et al. 1993).

Quantifying muscle activation patterns allows for an understanding of substrate utilization because of the relationship between muscle fiber type and fuel use. Type I fibers are known as "slow-twitch" fibers that have high fatigable resistance and generally use lipids as their fuel source. Type II fibers, subcategorized into type IIa and b, are known as "fast-twitch" fibers which have low fatigue resistance and which generally use CHO as their main fuel source (Powers 2001). As mentioned above, while low-intensity shivering has been linked to type-I fibers (more lipolitic in nature), high-intensity shivering bursts have been associated to that of type II fibers (glycolitic and fatigable). To date only two studies have investigated the relationship between muscle recruitment and fuel utilization in shivering.

Haman et al. (2004) indicated that greater recruitment of type II fibers exist in shivering compared to exercise at the same metabolic intensity. A greater recruitment of type II muscle fibers correlates with higher CHO oxidation compared to type I muscle fiber recruitment (Haman, Legault et al. 2004). Such differences in substrate utilization between exercise and shivering have lead researchers to investigate the effects of fuel availability on muscle recruitment. Adult men were exposed to 10 °C for 2 hours after a low CHO diet with an exercise protocol and a high CHO diet without an exercise

protocol (Haman, Peronnet et al. 2004). Results indicated that fuel availability did not affect the specific contribution of each muscle to total shivering activity. The same muscle fibers and types were being recruited even when different fuels were being metabolized, suggesting that neural recruitment patterns outweigh the fuel availability. During low-intensity shivering it was found that men were able to sustain the same thermogenic rate by oxidizing different fuels within the same muscle fibers (Haman, 2004); however a study performed by Haman et al. (2004) found that even when different fuels are available, motor unit (MU) recruitment is the same for both the FP and LP. No significant differences in substrate utilization between phases were observed, however a trend of preferential lipid oxidation in the LP was found. According to the findings of Haman et al.'s study (2004) it would not be expected that there would be a difference in MU recruitment, unless there was a hormonal effect of estrogen or progesterone on muscle activation patterns and in turn, motor unit recruitment, which has not been investigated in previous studies.

Understanding the link between substrate utilization and heat production, through muscle recruitment, in the cold is important for optimal fueling to survive the cold. The effects of shivering on substrate utilization and muscle recruitment in cold exposure have previously been investigated but only in men and very little is known about women (Haman, Legault et al. 2004; Haman, Peronnet et al. 2004; Haman, Peronnet et al. 2005). An additional challenge is present when investigating women in thermoregulation and metabolism. As previously mentioned, the hormonal effects of the menstrual cycle have been shown to affect both heat production and substrate utilization (Hessemer and Bruck 1985; Hessemer and Bruck 1985; Romijn, Coyle et al. 2000; Tarnopolsky 2000;

Hamadeh, Devries et al. 2005; Devries, Hamadeh et al. 2006). Consequently, the menstrual cycle should be taken into consideration when investigating heat production and substrate utilization in cold exposure. The few studies investigating women do not properly control for menstrual cycle, or neglect the effects of the menstrual cycle altogether (Pettit, Marchand et al. 1999; Prisby, Glickman-Weiss et al. 1999; Glickman-Weiss, Caine et al. 2000; Tikuisis, Jacobs et al. 2000). By assessing the effects of the menstrual cycle on substrate utilization and muscle actication patterns separately, an inference can be derived on if or how muscle activation patterns and fuel selection interact when affected by the menstrual cycle.

Thermoregulatory responses are elicited by changes in core and skin temperature. It is known that the menstrual cycle affects core body temperature; therefore it is possible that shivering, a thermoregulatory response is affected by the menstrual cycle. If dissimilar activation patterns exist then different fuels may be metabolized. It is possible that the 0.5°C increase in the LP may affect muscle recruitment by blunting the onset of shivering as it will take a longer time from the onset of cold exposure to reach a core body temperature which will initiate shivering. If the onset of shivering is affected then consequently fuel utilization will be affected.

Rationale

Research focusing on substrate utilization in cold exposure has been only performed in on men and very few studies have investigated females. It is unclear if the menstrual cycle phases do affect women in selection of metabolic pathways and thermoregulatory control of the cold responses of shivering. Unknown is the how the dependant relationship of fuel selection and muscle activation patterns in

thermoregulation are affected by menstrual cycle. Many studies measure the metabolic differences between menstrual cycle phases but do not look at difference in muscle activation patterns. Because the two are linked, it is optimal to look at both metabolic and muscle responses to get a clearer picture of what is happening at the whole body level

Purpose and Framework

The main purpose of this thesis is to investigate the effects of the menstrual cycle on thermoregulation and fuel selection. An integration of key links and relationships is presented in appendix B, figure 1.3.

Two components determine thermoregulation: the rate of heat loss and the rate of heat production. By exposing the women to the same conditions of cold exposure in both menstrual cycle phases we can allow for similar conditions resulting in heat loss. Heat production will be affected by thermoregulatory responses to cold exposure. The increase in core temperature due to fluctuating hormone levels in the menstrual cycle may affect these thermoregulatory responses. Shivering is a thermoregulatory response which requires the oxidation of substrate to fuel the contracting muscle.

The hormonal fluctuations of the menstrual cycle may affect substrate utilization through four ways: fuel availability, selection of metabolic pathways, muscle and fiber type recruitment. Fuel availability was controlled for providing a standardized meal to each participant and ensuring that they were fasted for at least 12 hours prior to the trials. Women were also asked to refrain from intense physical activity at least 18 hours prior to their trials. The effects of the menstrual cycle on metabolic pathways have been confounding in the literature however a greater reliance on lipid oxidation has been found in the LP compared to the FP. Lastly muscle activation patterns and fiber type

recruitment may affect substrate utilization. Depending on the type of shivering pattern (low vs. high intensity) different muscle fibers will be recruited leading to differences in fuel oxidation. It is unknown in the literature if shivering patterns or fiber type recruitment patterns are affected by the menstrual cycle however the onset of shivering has been shown to be later in the LP compared to the FP (Hessemer and Bruck 1985). Hessemer and Bruk (1985) also demonstrated that the intensity of muscle activity during shivering was lower in the LP compared to the FP. Therefore it is hypothesized that onset of shivering will be later and at a lower intensity in the LP compared to the FP. Fuel availability has not been shown to affect muscle activation patterns therefore it is thought that continuous vs. burst shivering will not differ nor will fiber type recruitment between the menstrual cycle phases

If dissimilarities in muscle activation patterns are not observed between the menstrual cycle phases then differences in substrate utilization are most likely due to hormonal regulation of metabolic pathways. Conversely, if a divergence in activation patterns is seen in shivering between the LP and FP, then differences in substrate utilization are due to an integration of both hormonal regulation of metabolic pathways and differences in muscle recruitment. Consequently from the framework the following objectives and hypothesis were derived.

Objective of thesis

The main objective of this thesis was to quantify differences in muscle activation patterns and fuel selection in the FP and LP during steady state low-intensity shivering (<2.5 times resting metabolic rate). From the results we can determine if the hormonal fluctuations affect muscle fiber recruitment therefore affecting substrate utilization or if

the hormonal fluctuations affect metabolism directly, or if there is no effect at all. Expected outcomes of this proposed research will improve our understanding of the substrate regulation in women expose to cold.

Hypothesis

It was hypothesized that steady low-intensity shivering in the two menstrual cycle phases will show that:

1) The onset of shivering will be later in the LP than the FP due to the higher core body temperature found in the LP. Shivering intensity will be lower in the LP then the FP. Muscle activation patterns (burst vs. continuous shivering) will be the same between both menstrual cycle phases.

2) In the LP a greater reliance on lipids and less on carbohydrates and protein will exist in than in the follicular phase as generally shown during low-intensity exercise in previous literature.

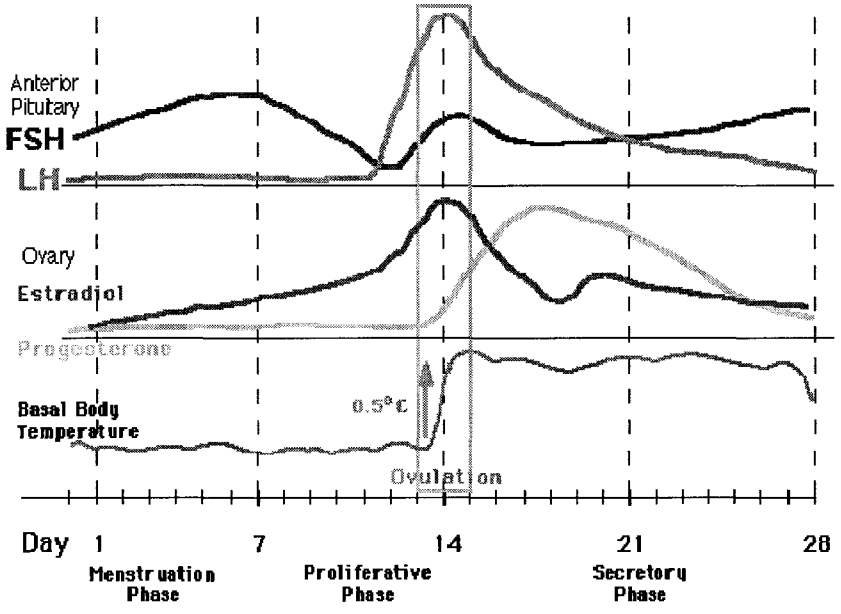

Figure 1.1. Menstrual Cycle (Hill 1997). Early FP is characterized by low estrogen and progesterone. LP is characterized as high estrogen and progesterone. It is important to note the 0.5°C increase in basal body temperature in the LP compared to the FP.

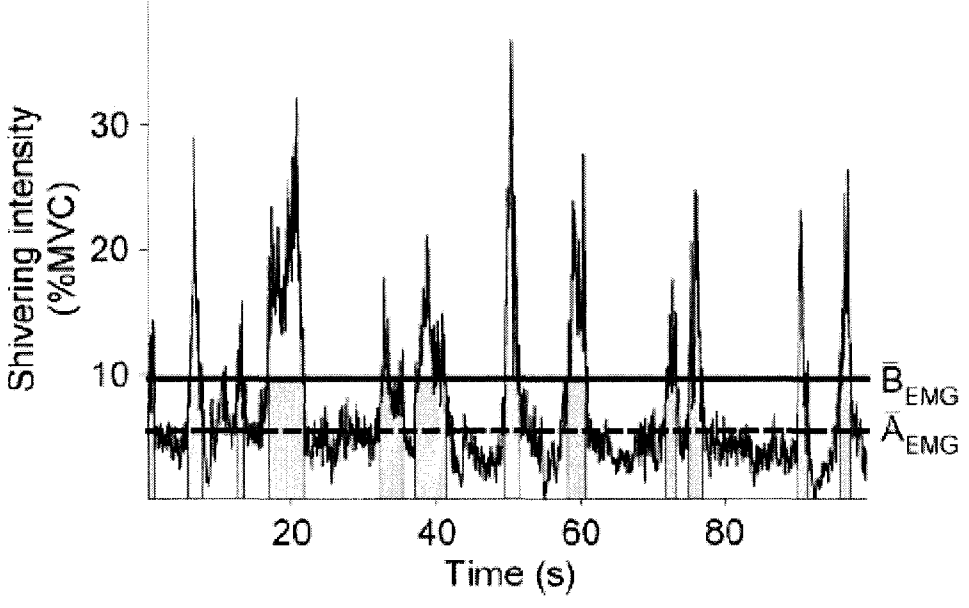

Figure 1.2. Adapted from Haman et al. 2004 (Haman. Legault et al. 2004). Schematization of the procedure for shivering burst identification. A_{EMG} (dashed line), average shivering intensity for the 15 min recording interval. B_{EMG} (solid line), shivering intensity threshold for burst determination. B_{EMG} is the intensity threshold determined by averaging the shivering intensities above the A_{EMG}. A shivering burst was arbitrarily defined in the present study as an EMG interval with duration 0.2 s, an interburst interval 0.75 s, and an amplitude higher than the amplitude threshold at each recording. Gray boxes indicate location and duration of the shivering bursts found in this example.

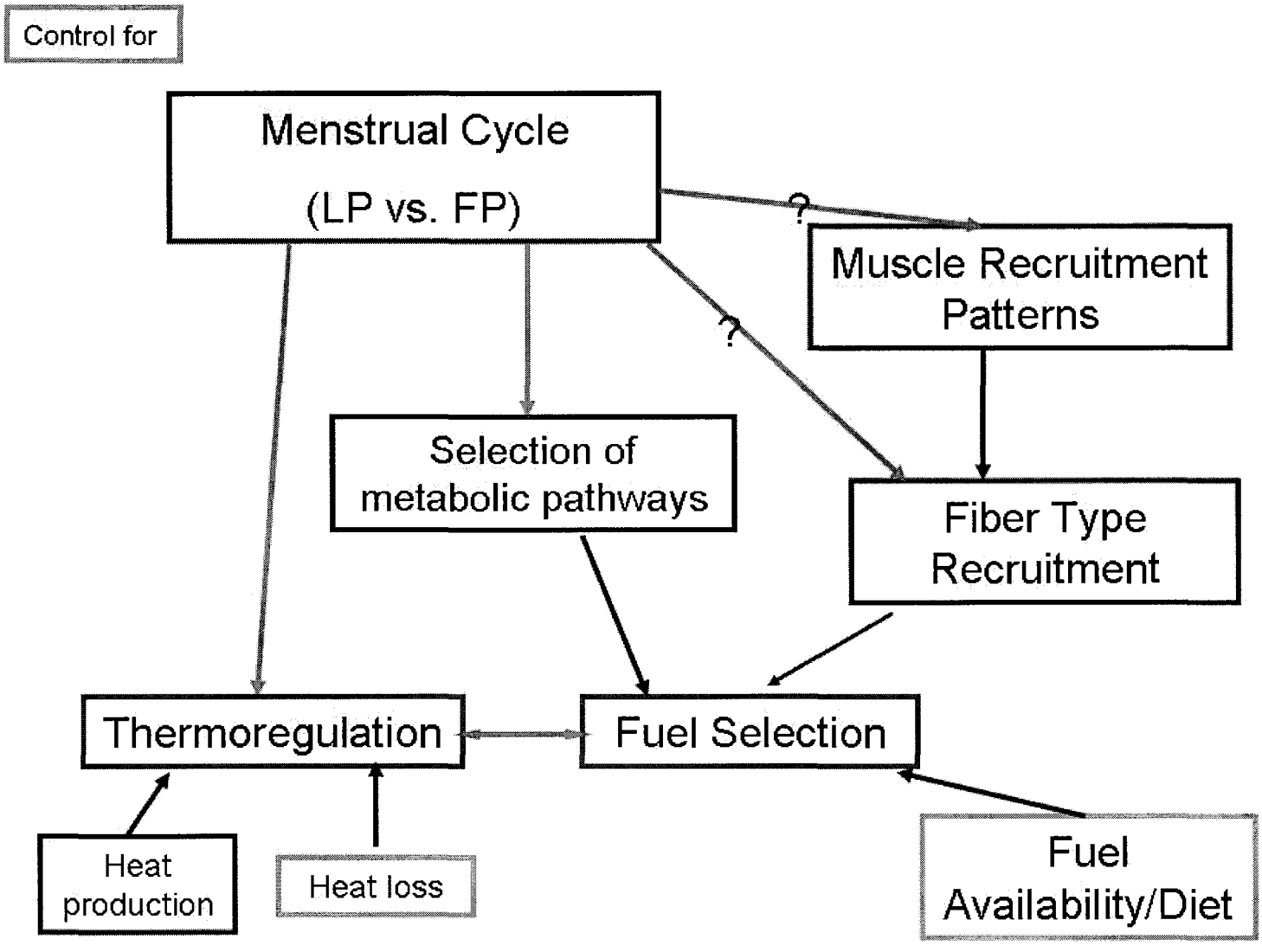
Control for
Menstrual Cycle
(LP vs. FP)
?
Muscle Recruitment Patterns
?
Selection of metabolic pathways
Fiber Type Recruitment
Thermoregulation
Fuel Selection
Heat production
Heat loss
Fuel Availability/Diet

Table 1.1: Summary of cold exposure studies and methods.

Study	Participants	Protocol to control for menstrual cycle	Measurement of temperature	Cold Exposure/Shivering intensity
Haman et al. (2005)	8 men	Not Applicable	Tes, Tskin (12 sites)	90 minutes in liquid-conditioned suit at 10 ° C (Low intensi 40% $Shiv_{peak}$) and 5°C (High intensity – 60% $Shiv_{peak}$)
Haman et al. (2002)	6 men	Not Applicable	Tes, Tskin (12 sites)	120 minutes in liquid-conditioned suit at 10° C (Low intensi 40% $Shiv_{peak}$)
Tikuisis et al. (2000)	11 women, 14 men	None	Tre	90 minute cold water immersion in 18 ° C water up to neck
Glickman-Weiss et al. (2000)	10 women, 16 men	Verification of menstrual cycle phase through serum estradiol and progesterone	Tes	Cold water immersion at 20° C to first thoracic vertebrae wit limbs separated until Tes reached 36.5° C
Gonzalez & Blanchard (1998)	6 women	Oral temperature measurement for one month. Verification of menstrual cycle phase through serum estradiol and progesterone	Tes, Tskin (6 sites)	Cold air exposure from 20° C to -10°C
Prisby et al. (1999)	12 women	Unknown, presumed to be in the FP, but no verification was done.	Tre, Tskin (5 sites)	120 minutes immersed in water at 17°C

Pettit, Marchand & Graham (1999)	9 women, 8 men	Responses to gynecological questionnaires on day of study to assess menstrual phase, but did not control for menstrual cycle	Tre, Tskin (4 sites)	180 min of exposure to cold air at 5°C
Vallerand, Jacobs & Kavanagh (1989)(Vallerand, Jacobs et al. 1989)	9 men	Not applicable	Tre, Tskin (12 sites)	180 minutes of seminude exposure to cold air at 10° C
Vallerand, Zamecnik & Jacobs (1995)(Vallerand, Zamecnik et al. 1995)	6 men	Not applicable	Tre, Tskin (12 sites)	180 minutes of seminude exposure to cold air at 10° C
Martineau & Jacobs (1988)(Martineau and Jacobs 1988)	14 men	Not applicable	Tre, Tskin (12 sites)	90 minutes or until Tre < 35.5° C in 18° C water immersed u shoulders

Ta, Ambient Temperature; Tes, Esophageal Temperature; Tre, Rectal Temperature; Tskin, Skin Temperature; Brackets next to Tskin indicate how many sites heat fl discs were administered.

Chapter II – Methodology

Subjects. Six healthy, physically active, non-acclimatized females between the ages of 18-30 volunteered to participate in this study approved by the Health Sciences Ethical Committee of the University of Ottawa. Participants were not and had not been taking oral contraceptives for a minimum of 6 months prior to testing. Age, body mass, height and percent body fat (estimated by dual energy x-ray absorptiometry) of the subjects were 23.3 ± 1.4 years, 65.3 ± 1.5 kg, 167.6 ± 2.6 cm, and 27.9 ± 2.0 % respectively. Peak oxygen consumption, estimated by incremental treadmill exercise to volitional fatigue (Bruce Protocol), averaged 51.0 ± 2.8 $ml \cdot kg^{-1} \cdot min^{-1}$ and was measured 5-7 days before the experiments.

Verification of menstrual cycle phase. Three months prior to testing, the women charted daily basal sublingual temperature, using an electronic thermometer, to ensure regular ovulation, see Appendix B. Logs were monitored monthly to ensure consistent ovulation through sublingual temperature changes. For each participant the monthly temperatures were averaged. Ovulation was estimated to occur when sublingual temperatures were consistently over the average temperature of that month. Please refer to Appendix B for a sample log and sample of how ovulation was determined. Participants also indicated illness, alcohol consumption and poor sleep which are all factors that can affect body temperature and were all considered when analyzing the logs for ovulation. With the use of the logs, FP and LP were estimated. On the day of the experiments menstrual cycle phase was verified by measurement of oral temperature upon awakening and with the use of plasma concentrations of estrogen and progesterone (Human Estradiol ELISA Kit,

Alpha Diagnostic, San Antanio, TX; Human Progesterone ELISA Kit, Alpha Diagnostic, San Antanio, TX).

Morning basal sublingual temperatures were taken upon awakening (approximately 6:00 am). Morning temperature was significantly lower in the FP compared to the LP, 36.1 ± 0.3°C and 36.4 ± 0.3°C respectively. Average plasma concentrations of estrogen and progesterone and morning temperatures are found in Table 2.1. Plasma concentrations were taken during rest in all cases, except for one, in which plasma was taken 2 days after the study due to difficulties successfully administering the catheter.

Experimental protocol. The subjects were exposed to cold on two separate occasions in the mid follicular phase (4-7 days post menses) and mid-luteal phases (4-7 days post ovulation) of the menstrual cycle. Participants consumed a standardized meal ("President's Choice" meat lasagna) the night prior (before 8pm) the experimental trials and were asked to perform similar activities as much as possible before each trial. Physical activity was discouraged 18 hours prior to the trials. On the day of the experiment care was taken to minimize exercise or thermal stresses between awakening and the start of the experiment. On their arrival in the laboratory (7:30 am; 12 h postabsorptive), subjects were instrumented with EMG electrodes, thermal probes and an indwelling catheter (18G, 32 mm, Medical Arlington, TX) placed in the antecubital vein for blood sampling. The women were then fitted with a liquid-conditioned suit (three piece Delta Temax, Pembroke, ON). After voiding the bladder (time = 0 min) the subject remained seated comfortably for the next 30 minutes at 24.5 ± 0.5° C (750 ± 2 mmHg, 40 ± 3% relative humidity). After this period air conditioning was turned on (18.0 ± 0.3° C,

755 mmHg, 44% relative humidity), and a 4 °C water perfusion started in the LCS by use of a temperature-controlled circulation bath (Endocal, NESLAB and model 200-00, Micropump, Vancouver, WA). Tape was wrapped around the suit limbs and core to ensure skin contact between the suit and participant. Average environmental conditions in the laboratory were the same for both FP and LP conditions. Thermal and metabolic responses were monitored continuously in both baseline and cold exposure conditions.

EMG electrode placement:

Skin sites were prepared by using 3M Red Dot skin prep tape to remove the top layer of skin. Skin was then cleaned with alcohol swabs to remove any excess dead skin and to reduce skin resistance as much as possible. Surface electrodes (Delsys, Boston, MA) were positioned 1 cm apart over the bellies of eight large and centrally located muscles: *trapezius*, *latissimus dorsi*, *pectoralis major*, *rectus abdominis*, *vastus lateralis*, *rectus femoris*, *vastus medialis*, and *adductor magnus*. Surface electrodes were connected to a Myomonitor wireless 3.5 EMG system (Delsys, Boston, MA). Electrode placement was the same between MVC and cold exposure. Electrodes were placed in the area with the most bulk of muscle and in parallel with muscle fiber direction. At the end of the first study the placement of the electrodes was marked with a permanent marker and participants were asked to remark these places daily. This was to keep electrode placement as consistent as possible between trials.

Thermal Measures:

Whole body heat loss rate ($\dot{H}_L$, in kJ/min) was estimated using the following equation:

$$\dot{H}_{L\,s} = (R + C) + (E_{resp} + C_{resp})$$ (Beckman and Reeves 1966)

R and C represent rates of radiative and convective heat loss, respectively and E_{resp} and C_{resp} are rates of evaporative and convective heat loss by ventilation, respectively. R and C are estimated by the use of heat-flux transducers (Concept Engineering, Old Saybrook, CT) placed on the skin surface at 6 sites: left cheek, pectoralis, left side of forearm, lower back, right medial quadriceps, posterior surface of the hand, and calculated with a surface area-weighted equation. Weightings were 0.14, 0.19, 0.11, 0.05, 0.32 and 0.19 respectively for left cheek, pectoralis, left side of forearm, lower back, right medial quadriceps, posterior surface of the hand (Palmes & Parker from Parson 2003). Evaporative heat loss from the skin was assumed to be negligible at baseline and cold exposure conditions. Core temperature was estimated using an aural canal temperature probe (Mon-a-therm Tympanic, Mallinckrodt Medical, St. Louis, MO). The thermal probe was inserted into the participant's aural canal and was covered with a ball of cotton and tape to prevent as much displacement as possible. The aural canal probe can be influenced by environmental temperature and can move once in the auditory canal thus affecting the results and validity of the readings (Lee, Toner et al. 1997) however because there was minimal movement throughout the study, and no wind or cold water.
Heat production ($\dot{M}$) was determined based on metabolic rate calculated by indirect respiratory calorimetry corrected for protein oxidation described by (Haman, Peronnet et al. 2004). Changes in heat production ($\dot{M}$) were calculated by indirect respiratory calorimetry corrected for protein oxidation (as described in Haman et al., 2002).

EMG analysis:

Raw EMG data was collected using EMG Works 3.5 analysis software (Delsys, Boston, MA). Data was collected at 1000Hz and downloaded via fiber optic to a desktop

computer as an .emc file. The signal gain was 1000. EMG signals were filtered band-pass filtered to remove spectral components below 20 Hz and above 500 Hz, as well as 60-Hz contamination (and related harmonics). Resting muscle activity was collected 30 minutes before cold exposure. Shivering activity was recorded for a total of 85 minutes at these time points during cold exposure (0 minutes being the onset of cold exposure): between 5-15min, 20-30 min, 35-45 min, 50-60 min, 65-75 min, 80-90 min, 95-105 min, 105-110 min, 110-115 min and 115-120 min. Participants were asked to avoid voluntary moments during the recording periods. Intervals between recording periods allowed for participants to voluntary move if necessary, have their legs passively stretched and to urinate if necessary.

Normalization of EMG amplitude:

Before each experimental trial, maximal EMG signals of each muscle (RMS_{mvc}) were determined from maximal isometric contractions (MVC). MVC protocols involve maximum force for 2–3 seconds. Participants were verbally encouraged to achieve maximum force during the three trials with 1 min rest between each MVC measurement. The average of the three attempts was taken to be the MVC for that specific muscle. The following procedures were used to determine RMS_{mvc} of individual muscle: 1) For the trapezius muscle, MVC was measured while participants were standing and shrugging their shoulders towards their ears, while keeping their arms at a 90° angle at the elbows. Participants pushed against two researchers who were pushing elbow joint down. 2) For the pectoralis major muscle participants were asked to stand and lean forward at the waist. Participants had a slight bend in the arms and attempted to touch fists while to researchers outwardly pushed on their fists thus preventing this motion. 3) For the

latissimus dorsi muscle, the participants had their arms laterally extended at shoulder height (with elbows bent at 90° - hands facing up to the ceiling). The elbows were being pushed down in the opposite direction by the researchers and the participants were using their latissimus dorsi to prevent their arms from dropping down past their shoulder height. *4*) For the rectus abdominus muscle, while subjects were lying down in a bed, researchers placed the external force on the participant's pectorals. Subjects curled forward, aiming to raise their head and upper back towards the ceiling, using their abdominal muscle maximally. Participant's legs were held down to isolate the abdominals. *5*) For the vastus lateralis, rectus femoris, and vastus medialis muscles, MVC measurements were performed with subjects sitting securely in an upright position and the external force pushing slightly above the ankle. The participant started with their legs at a 90° angle from the vertical position, and subjects performed a maximal knee extension pushing against the external force. *6*) For the adductor magnus muscle, MVCs were measured with participants lying down with feet slightly apart and knees bent to 90°. The external force was placed between both knees on the medial side of the quadriceps, while the participant adducted their legs together against the external force.

Determination of shivering intensity and pattern:

Raw EMG signals were analyzed with the use of custom-designed MATLAB algorithms (Mathworks, Natick, MA). The peak values for each muscle from the MVC exercises were used to normalize each participant's muscle shivering activity to a %MVC scale. Shivering intensity of individual muscles (EMG_{shiv}^{m}, where the index m identifies the muscle) was determined from root-mean-square values (RMS) calculated from EMG signals using a 50-ms overlapping window (50%). Baseline RMS values ($RMS_{baseline}$:

5 min RMS average measured before cold exposure) were subtracted from RMS shivering (RMS_{shiv}) as well as RMS_{mvc} values. $EMG_{shiv}{}^{m}$ was normalized to RMS_{mvc} by using the following equation:

$$EMG_{shiv}{}^{m}\ (\%MVC) = \frac{RMSshiv - RMSbaseline}{RMSmvc - RMSbaseline} \times 100$$

$EMG_{shiv}{}^{m}$ was subsequently quantified with ten outcome variables corresponding to changes in overall shivering, continuous low-intensity shivering, and burst shivering. The ten outcome variables give an overall representation of shivering activity by looking at duration, intensity and mean area under the curve to specifically compare shivering patterns between the two menstrual cycle phases. The outcome variables were determined for the two latter EMG components unless indicated otherwise are *1)* total shivering time (in min), total amount of time for which $EMG_{shiv}{}^{m}$ was over > 0% MVC (i.e. above $RMS_{baseline}$), *2)* summed area, (in %MVC·min) summed area, *3)* mean intensity (in %MVC) over 90 minute recording time; *4)* percent shivering time (in min), summed area of individual EMG components as a percentage of total recording time (%RT); *5)* steady–state intensity (in %MVC), average overall intensity reached in the last 15 min of cold exposure; *6)* summed duration (in min), total duration continuous low-intensity shivering and burst shivering; *7*) number of bursts, total number of bursts during the recording period; *8*) burst rate (in bursts/min), mean number of bursts per minute; *9*) percentage contribution to total whole body shivering ($\%Shiv_{total}$), relative contribution to total whole intensity shivering and burst shivering to total shivering activity, *10*) percentage of burst summed area to total continuous low-intensity shivering area ($\%EMG_{burst}/EMG_{cont}$).

Shivering activity was muscle contraction which was double the baseline EMG signal for at least 10 seconds; this was generally approximately 5% MVC. The maximum level of shivering considered was 50% MVC. Anything that was above and below these thresholds was visually inspected to ensure that muscle activity was in fact involuntary muscle activity. If the movement was voluntary the data was not included in data collection, however if it was in fact an involuntary contraction the data was included in analysis. A shivering burst is defined in the study 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 (5–15, 20–30, 35–45, 50–60, 65–75, 80-90, 95-105, 105-110, 110-115, 115-120 min). Intensity threshold was determined by first calculating the average shivering intensity (A_{EMG}) over the entire recording period. Remaining values above A_{EMG} were averaged again (B_{EMG}), and the intensity threshold was set at B_{EMG} (see Fig. 1.2).

Shivering activity of all muscles were summed up to obtain a whole body index of shivering activity. Muscle mass of each region was taken into account using body composition data for each individual given from the DEXA scans (Meigal, Lupandin Iu et al. 1993):

$$\text{Whole Body Shivering} = \int (f_T \, EMG^T + f_{UL} \, EMG^{UL}) dt \qquad (2)$$

Where EMG^T and EMG^{UL} are trunk (T, average of TR, LA, PE and RA) and upper leg (UL, average of RF, AD, VM, and VL). The coefficients were determined for each participant using their individual DEXA scan to determine relative body mass weightings.

The onset of shivering was defined on the following criteria: 4 of 8 muscles on the right side of the body achieved an amplitude of at least double that of baseline for a minimum duration of 10 seconds. The onset for each participant and each trial was done twice by the same researcher, if there was a discrepancy of more than 30 seconds then the onset was done for a third time.

Metabolic Rate and Fuel Utilization:

Ventilation ($\dot{V}_E$), oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) were measured using a metabolic cart (MOXUS, Applied Electrochemistry Inc., Pittsburgh, PA, USA). Total protein (RP_{ox}), carbohydrate (RG_{ox}) and lipid (RF_{ox}) oxidation rates (in $g \cdot min^{-1}$) were calculated as described previously (Haman, Péronnet et al. 2002; Haman, Péronnet et al. 2004):

$$RP_{ox}(g \cdot min^{-1}) = 2.9 \times Urea_{urine}(g \cdot min^{-1})$$

$$RG_{ox}(g \cdot min^{-1}) = 4.59\ \dot{V}CO_2(l \cdot min^{-1}) - 3.23\ \dot{V}O_2(l \cdot min^{-1})$$

$$RF_{ox}(g \cdot min^{-1}) = -1.70\ \dot{V}CO_2(l \cdot min^{-1}) + 1.70\ \dot{V}O_2(l \cdot min^{-1})$$

Where $\dot{V}O_2$ ($l \cdot min^{-1}$) and $\dot{V}CO_2$ ($l \cdot min^{-1}$) were corrected for the volumes of O_2 and CO_2 corresponding to protein oxidation (1.010 and 0.843 $l \cdot g^{-1}$, respectively). RP_{ox} was estimated from urinary urea excretion (UREA) in urine samples collected for 120 min during the habituation period, and 150 min in the cold. Urinary concentrations were determined using a commercial urine assay kit (BioAssay Systems, CA, USA). Energy potentials of 16.3 $kJ \cdot g^{-1}$ (CHO), 40.8 $kJ \cdot g^{-1}$ (lipids), and 19.7 $kJ \cdot g^{-1}$ (proteins) were used to calculate the relative contributions of each fuel to total heat production (Elia 1991; Péronnet and Massicotte 1991).

Statistical analyses. Statistical differences were considered significant when $P<0.05$. All values presented are means ± SE (n=6), unless indicated otherwise. The main effects of time and conditions as well as their interaction were tested by analysis of variance for repeated measures. Changes in T_{au}, T_{sk} , $\dot{M}$, were assessed by two-way ANOVA for repeated measures. Menstrual cycle difference in burst rate, burst number, mean intensity of continuous and burst shivering, mean duration of bursts, percent of time spent continuous and burst shivering and relative time spent overall, and burst shivering (%RT) were analyzed over the last 30 minutes before and during cold exposure were determined by using a one-way ANOVA to verify the main effect of menstrual cycle phase (FP and LP). Follow-up paired sample t-tests determined differences in conditions.

Chapter III – Results

Thermal Response. Changes in T_{au}, T_{sk} and $\dot{M}$ for FP and LP are presented in Figure 3.1. Tau was initially slightly lower in the FP than the LP (36.8 ± 0.1°C and 37.0 ± 0.2 °C), however it was not significant. Tau decreased as a result of cold exposure in both menstrual cycle phases (36.8 ± 0.1°C to 36.1 ± 0.2°C in FP and 37.0 ± 0.2 °C to 36.1 ± 0.2°C in LP), but no differences were found between FP and LP. Similarly T_{sk} decreased as a result of cold exposure in both menstrual cycles (33.2 ± 0.4°C to 27.7 ± 0.4°C in FP and 33.1 ± 0.6 °C to 27.2 ± 0.5°C in LP), but no differences existed between menstrual cycle phase. $\dot{M}$ did increase significantly with cold exposure in both phases. Resting metabolic rate was not different between FP and LP (7.2 ± 0.1% and 7.1 ± 0.1%). Metabolic rate reached by the end of cold exposure was not different in FP and LP (17.0 ± 0.1 % $\dot{V}O_{2peak}$ and 18.0 ± 0.3 % $\dot{V}O_{2peak}$). $\dot{M}$ averaged at 3.1 ± 0.2 kJ/min in FP and 3.0 ± 0.2 kJ/min in LP at 25.5°C and gradually increased by 2.0-fold in FP to 6.3 ± 0.6 kJ/min and 2.2 – fold in LP to 6.6 ± 0.6 kJ/min. Heat production during cold exposure tended to be higher in the LP compared to the FP however it was not significantly different ($p = 0.088$).

Differences between the FP and LP did not exist in heat loss values. Heat flux values were slightly higher in the LP during rest and during the last 30 minutes of cold exposure, -45.2 ± 5.7 W• m^2 and -72.4 ± 4.6 W• m^2 in the FP and -43.5 ± 2.5 W• m^2 and -71.6 ± 4.9 W• m^2 in the LP during rest and cold respectively.

EMG results. A total of 90 minutes of EMG was collected for eight large muscles corresponding to two regions of the body, trunk (TK) consisting of the *trapezius, latismus dorsi, rectus abdominis* and *pectoralis major* and the *upper leg* (UL) consisting of the

vastus medialis, *vastus lateralis*, *adductor magnus, rectus femoris*, on both sides of the body. An example of a raw EMG signal is shown in Figure 3.2.

Onset of Shivering. Four of the six women showed electrical muscle activity earlier in the FP at 21.2 ± 3.3 minutes compared to 22.4 ± 3.5 minutes in the LP. The women in the FP shivered for 80.3 ± 2.6% of the 120 minutes of recording and the 78.6 ± 2.8% in the LP. Figure 3.3 shows the relationship between mean T_{sk} and onset of shivering. Estimating from the graphs it is predicted that the onset of shivering occurred at 29.5°C in the FP and 30.3°C in the LP. The onset of shivering occurred at T_{au} of 36.90°C in the FP and 36.85°C in the LP.

Overall Body Shivering. Shivering intensity increased continuously during cold exposure but no difference was observed between FP and LP. The relative contributions of individual muscles to total shivering activity were the same between FP and LP.

Shivering pattern. The method used to distinguish the difference between burst shivering and continuous, low-intensity shivering is schematized in Fig.1.2 (taken from Haman, Legault et al. 2004). Separating these two patterns was achieved on the basis of large differences in intensity (2-5 vs. 7-15% MVC) and rate of occurrence (8-10 vs. 0.1-0.2 Hz). Table 3.1 summarizes all parameters calculated for burst shivering and continuous, low-intensity shivering. The effects of the menstrual cycle on burst shivering activity are show in Figure 3.5. Results show that the patterns were the same in the FP and the LP for the trunk region, however there was a significant difference in number of burst and burst rate in the upper leg region between the two menstrual cycle phases. However, burst patterns did not differ between menstrual cycle phases during the last fifteen minutes of cold exposure. Whole body burst shivering intensity, burst shivering rate, and relative

contribution to burst shivering time were not different between FP and LP. Burst shivering intensity increased to 23.5 ± 5.1% of MVC in FP and 26.4 ± 5.4% of MVC in LP during the last fifteen minutes of cold exposure. Burst shivering rate increased to 5.8 ± 0.5 burst/min for FP and 6.0 ± 0.5 burst/min for LP in the last recording interval, and the relative contribution of burst to total recording time increased from 14.6 ± 1.5% of RT to 17.5 ± 0.1 % of RT for FP and 14.8 ± 1.3%RT to 18.1 ± 0.8% RT in the LP. Overall shivering contribution to heat production was similar in both phases of the menstrual cycle as shown by Figure 3.6.

Metabolic Fuel Utilization. Absolute rates of lipid and CHO oxidation are presented in Figure 3.7. Lipid and CHO oxidation rates did not differ between menstrual cycle phases however oxidation rates increased with cold exposure. RF_{ox} increased from baseline at 66 ± 5 mg/min to 204 ± 13 mg/min in the FP and 68 ± 5 mg/min to 205 ± 18 mg/min in the LP. RG_{ox} increased significantly from 75 ± 10 mg/min to 136 ± 19 mg/min in the FP and 56 ± 9 mg/min to 145 ± 70 mg/min in the LP. Lipid utilization and CHO utilization were the same in both LP compared to the FP. Absolute rates of protein oxidation in the two menstrual cycles are shown in Table 3.3. No differences were present in RP_{ox} between menstrual cycle phases, 0.05 ± 0.01 g/min in FP and in the LP.

Changes in the relative contribution of CHO ($\%RG_{ox}$), lipid ($\%RF_{ox}$), and protein ($\%RP_{ox}$) oxidation to total $\dot{M}$ in both FP and LP are presented in Figure 3.8. Before cold exposure $\%RG_{ox}$ was not significantly, higher in the FP (25.1 ± 2.9 % of $\dot{M}$) compared with (18.7 ± 5.2 % of $\dot{M}$) in LP. $\%RG_{ox}$ slightly decreased during the first 30 minutes of cold exposure (12.8 ± 6.6 % $\dot{M}$ in FP and 17.5 ± 5.0 % $\dot{M}$ in LP) and slowly increased again in the last 30 minutes of cold exposure up to 19.3 ± 2.5 % $\dot{M}$ in FP and 17.1 ± 6.6

% $\dot{M}$ in the LP. A continuous increase in $\%RF_{ox}$ was observed for both LP and FP from the onset to 75 minutes of cold exposure (55.9 ± 2.1 % $\dot{M}$ to 78.6 ± 5.1 % $\dot{M}$ in the FP and 60.1 ± 5.5 $\dot{M}$ to 78.0 ± 4.4% $\dot{M}$). As a result of cold exposure there was an increase in $\%RF_{ox}$ of 1.45-fold in FP and 1.30-fold in LP. A 2.05-fold and 2.09-fold decrease in %RPox was found for FP (19.0 ± 2.8% to 6.2 ± 1.4%) and LP (21.3 ± 2.5% to 9.1 ± 1.7%) due to cold exposure.

Figure 3.9 displays absolute CHO, lipid and protein contribution to total heat production before (base) and during the last 30 minutes of cold exposure (t = 105-135 min) in both menstrual cycle phases. No differences were observed between menstrual cycle phases. However there were significant increases in lipid contribution to heat production from baseline measurements to cold exposure.

Table 3.3 summarizes the averages values at baseline conditions 15 minutes before cold exposure (0-15 min) and in the last 30 minutes (105-135 min) of cold exposure at 5°C for fuel utilization variables ($\dot{M}$, RF_{ox}, RG_{ox}, RP_{ox}) estimated in this study in FP and LP. At rest RG_{ox} was slightly higher in FP compared to LP. No significant menstrual cycle phase differences exist in the relative contribution of substrates to total heat production. Absolute values of RF_{ox}, RG_{ox} and $\dot{M}$ increased significantly between baseline and cold exposure conditions in both phases. In both menstrual cycle phases the relative contribution to $\dot{M}$ of lipids increased from rest to cold exposure, and the relative contribution of CHO and protein decreased significantly.

Muscle Activity and Metabolic Fuel Utilization: In the last 30 minutes of cold exposure, CHO, lipids and proteins respectively contributed, 19.3 ± 2.5 %, 78.6 ± 5.1 %, 6.2 ± 1.4 % in FP and 17.1 ± 6.6 %, 78.0 ± 4.4 %, 9.1 ± 1.7 % in the LP. Changes in $\%RG_{ox}$, $\dot{M}$

and $\%Shiv_{ov}$ are presented in Figure 3.10. No significant differences exist between $\%RG_{ox}$, M and $\%Shiv_{ov}$ between the FP and LP. $\dot{M}$ increased by 2.0-fold in FP and 2.2 – fold in LP from baseline to the last 30 mins of cold exposure. $\%Shiv_{ov}$ also increased continuously from the first sampling interval (5-15 mins) 1.6 ± 0.4% of MVC to 10.1 ± 1.7% of MVC in FP and 1.6± 0.4% of MVC to 8.8 ± 0.5% of MVC in LP in the last sampling interval (115-120 min).

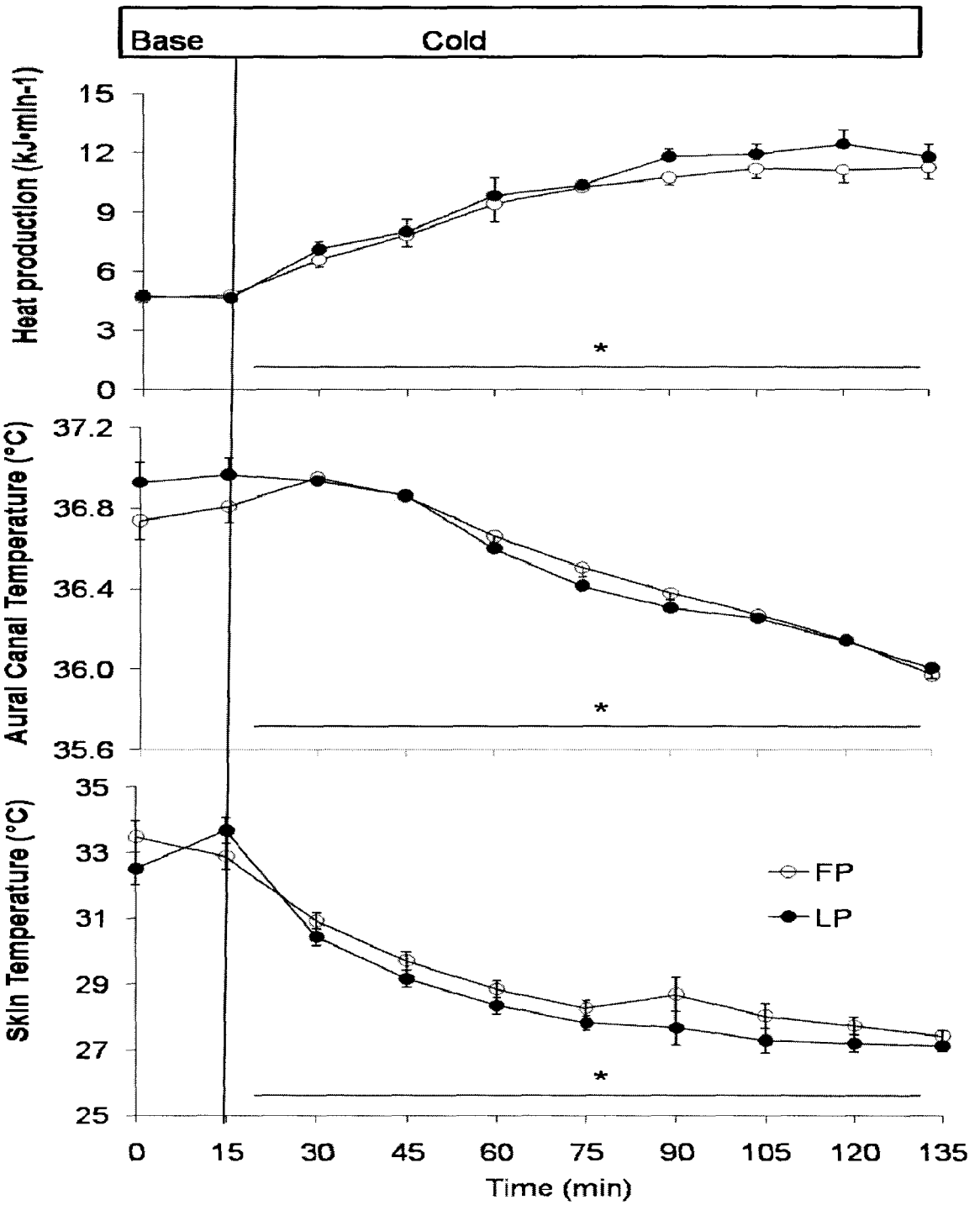

Figure 3.1. Rate of heat production and aural canal temperature and mean skin temperatures before (baseline) and during cold exposure in the follicular (FP) and luteal (LP) phase of the menstrual cycle. *Significantly different from baseline values before cold exposure, $P < 0.05$.

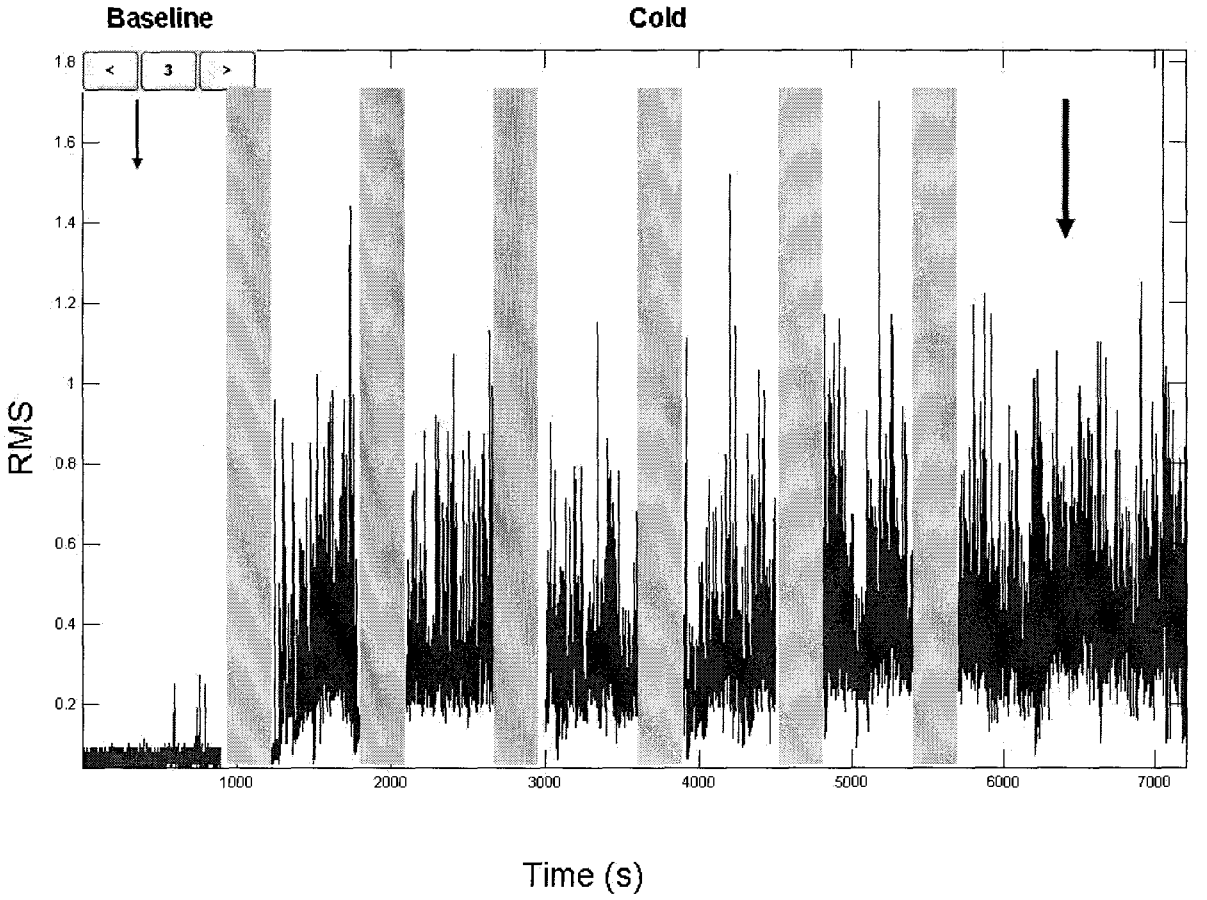

Figure 3.2. Example of RMS recording in pectoralis major before and during cold exposure for 1 subject (A). Gray areas show the time intervals when EMG was not being recorded. Arrow indicates the area used to schematize shivering bursts determination procedure (Fig. 3.1).

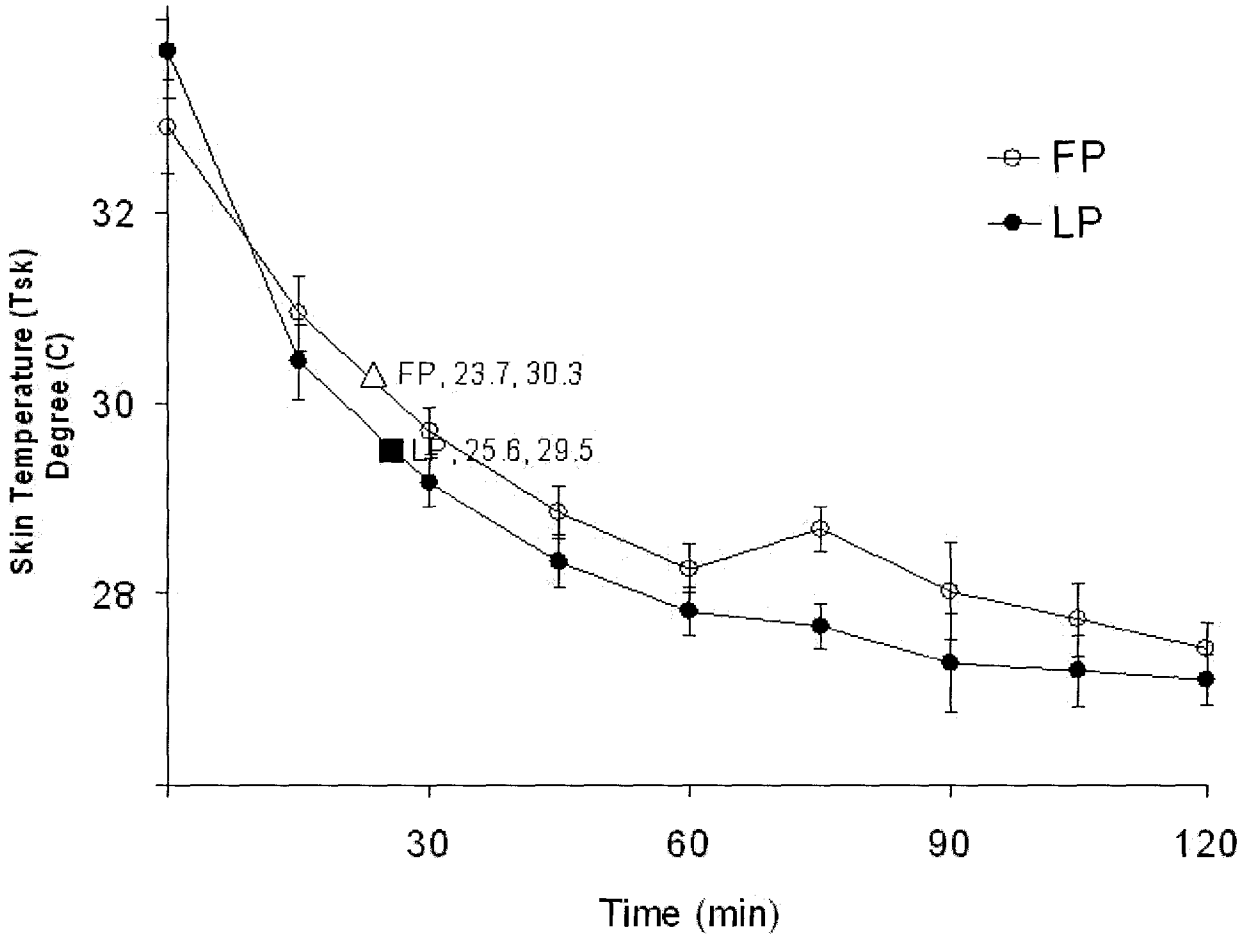

Figure 3.3. Skin temperature during 120 min of cold exposure. Plotted with onset of shivering in the follicular (○) and luteal (●) phase of the menstrual cycle in women exposed to 4°C.

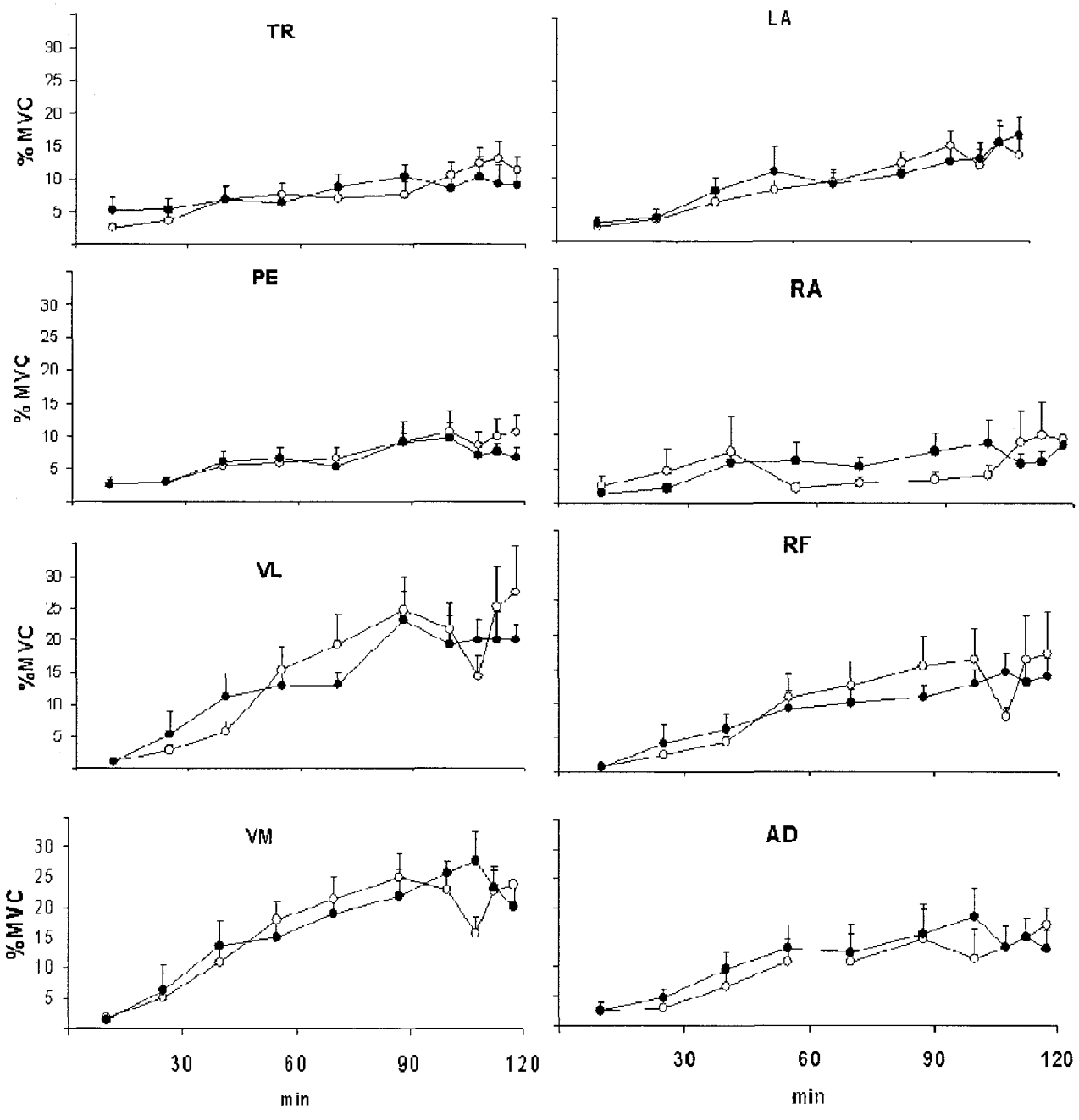

Figure 3.4. Mean changes in individual muscle shivering intensity of women in the FP (°) and LP (•) exposed to 4°C for 120 mins. Values are in means ± SE. LA, latissimus dorsi;RE, pectoralis major; TR, trapizus; RA, rectus abdominus; VL, vastus lateralis; RF, rectus femoris; VM, vastus medialis ; AD, adductor magnus.

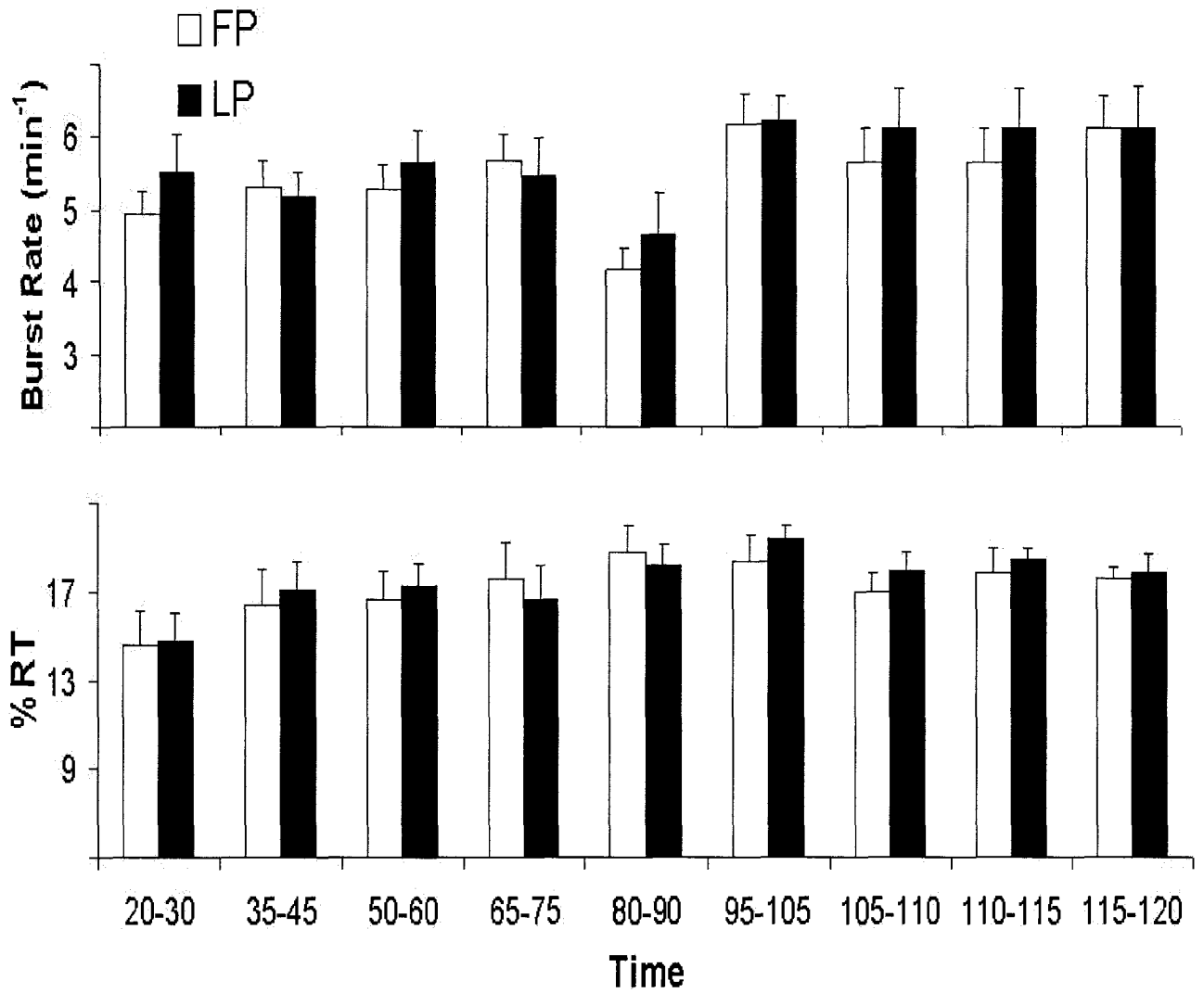

Figure 3.5. Changes in the burst shivering rate and relative contribution to total recording time (RT) averaged at each recording interval of women in the FP (open-bar) and LP (solid-bar) women expose to 4°C liquid perfusion suit for 120 mins. Values are means ± SE.

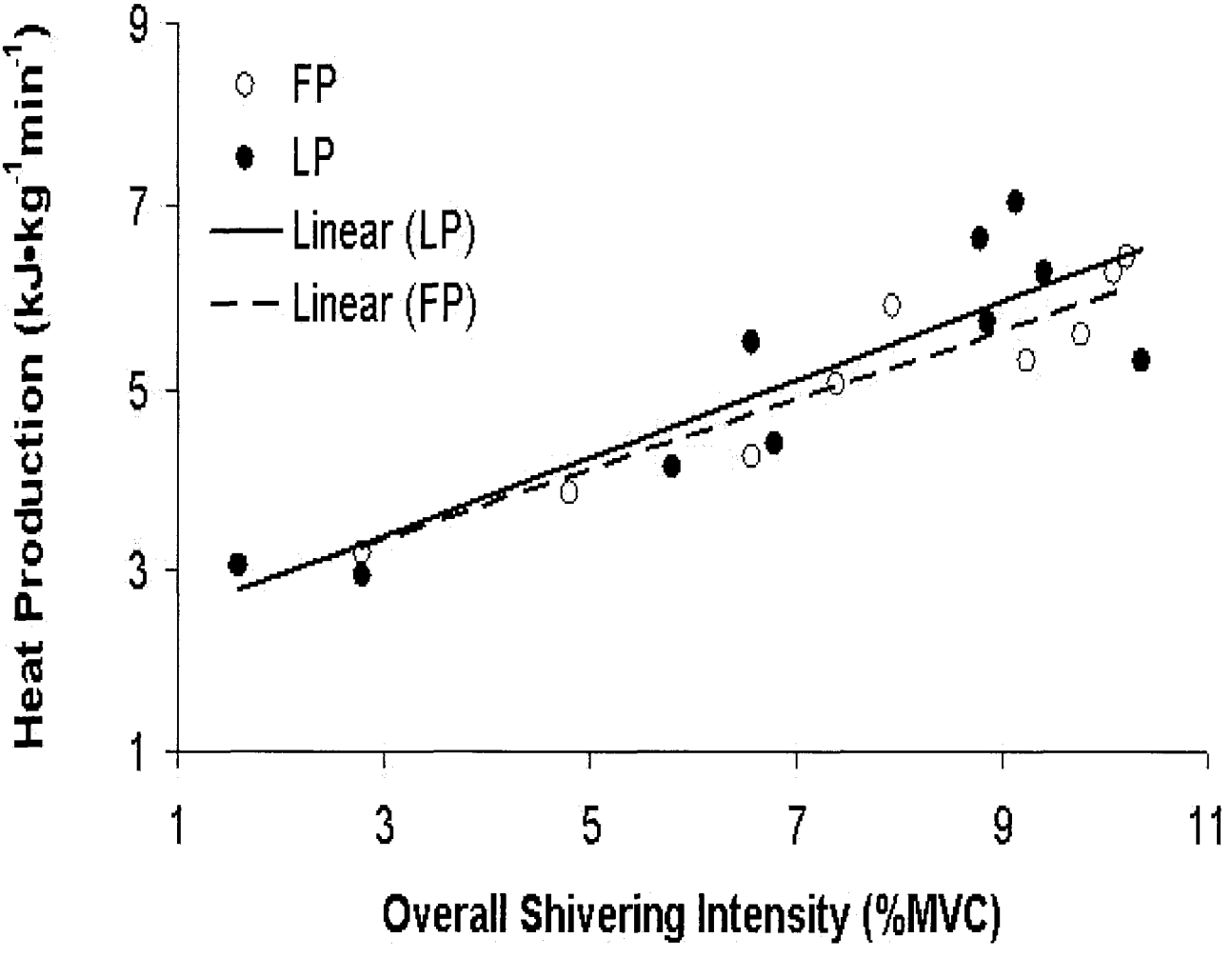

Figure 3.6. Changes in the relative contribution of (CHO) to total heat production (%Gox), and whole body overall shivering activity ($\%Shiv_{ov}$) in the follicular (○) and luteal (●) phase of the menstrual cycle in women exposed to 5°C water for 120 mins.

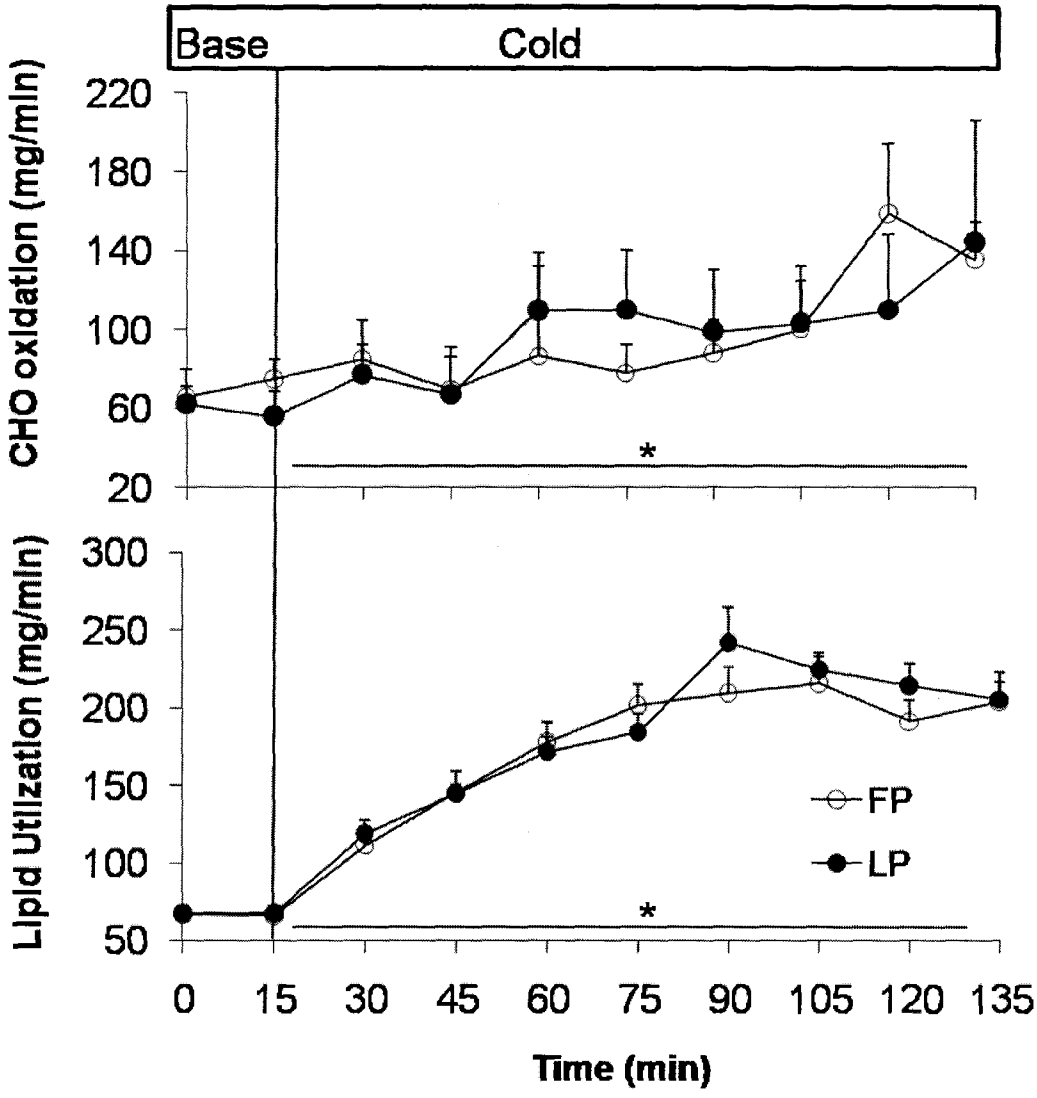

Figure 3.7. Changes in absolute CHO (A) and Lipid (B) rates before (baseline) and during cold exposure for follicular (◦) and luteal (•) phases of the menstrual cycle. *Significantly different from baseline values before cold exposure, $P < 0.05$

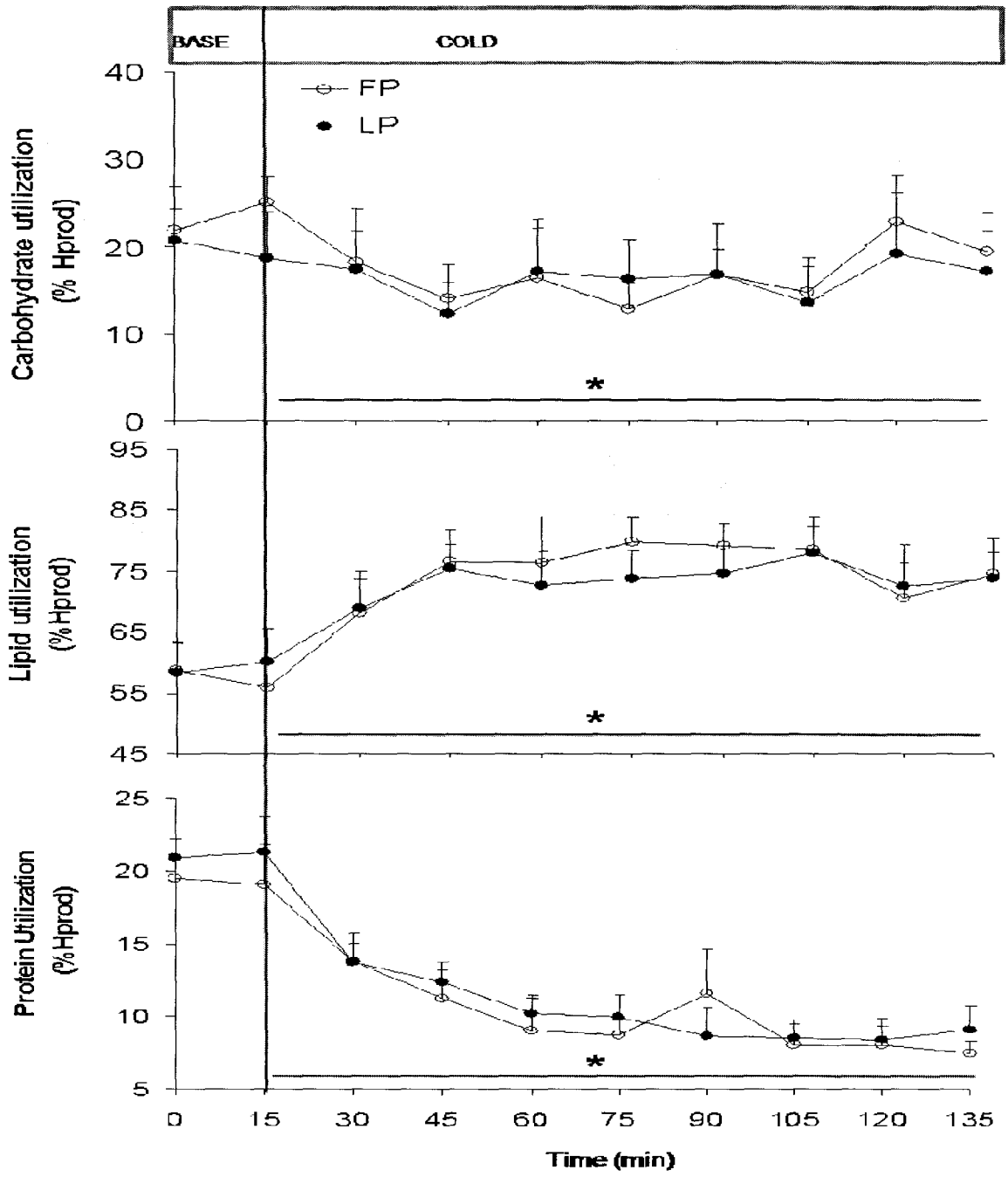

Figure 3.8. Relative contributions of carbohydrates, lipids and proteins to total heat production before (base) and during cold exposure in the FP and LP of the menstrual cycle. *Significantly different from baseline values before cold exposure, $P < 0.05$.

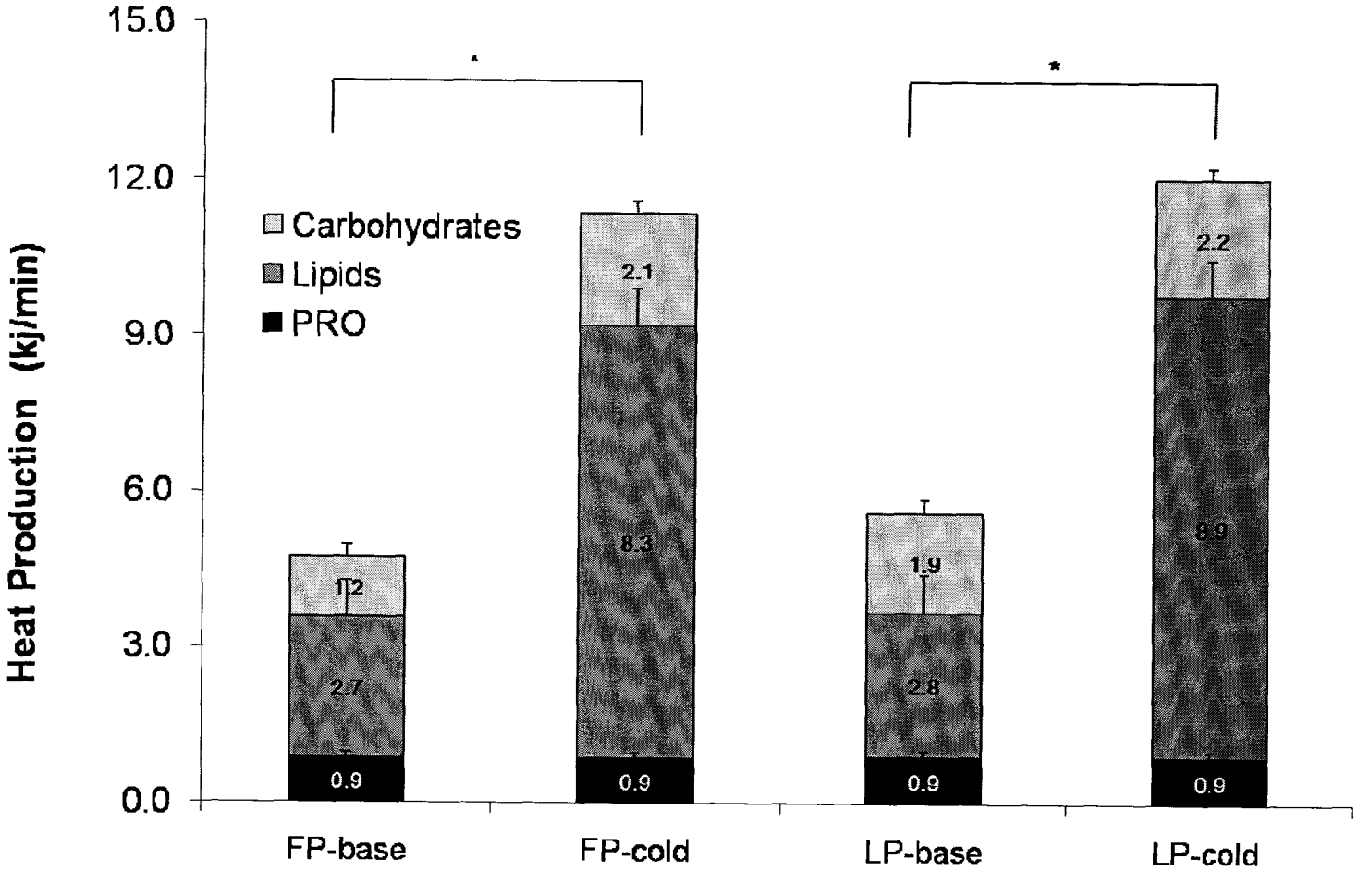

Figure 3.9. Absolute carbohydrate, lipid and protein contribution to heat production before (base) and during the last 30 minutes of cold exposure (t = 90-120 min) in t the follicular and luteal phase. *Significantly different for all substrates from baseline values before cold exposure, $P < 0.05$.

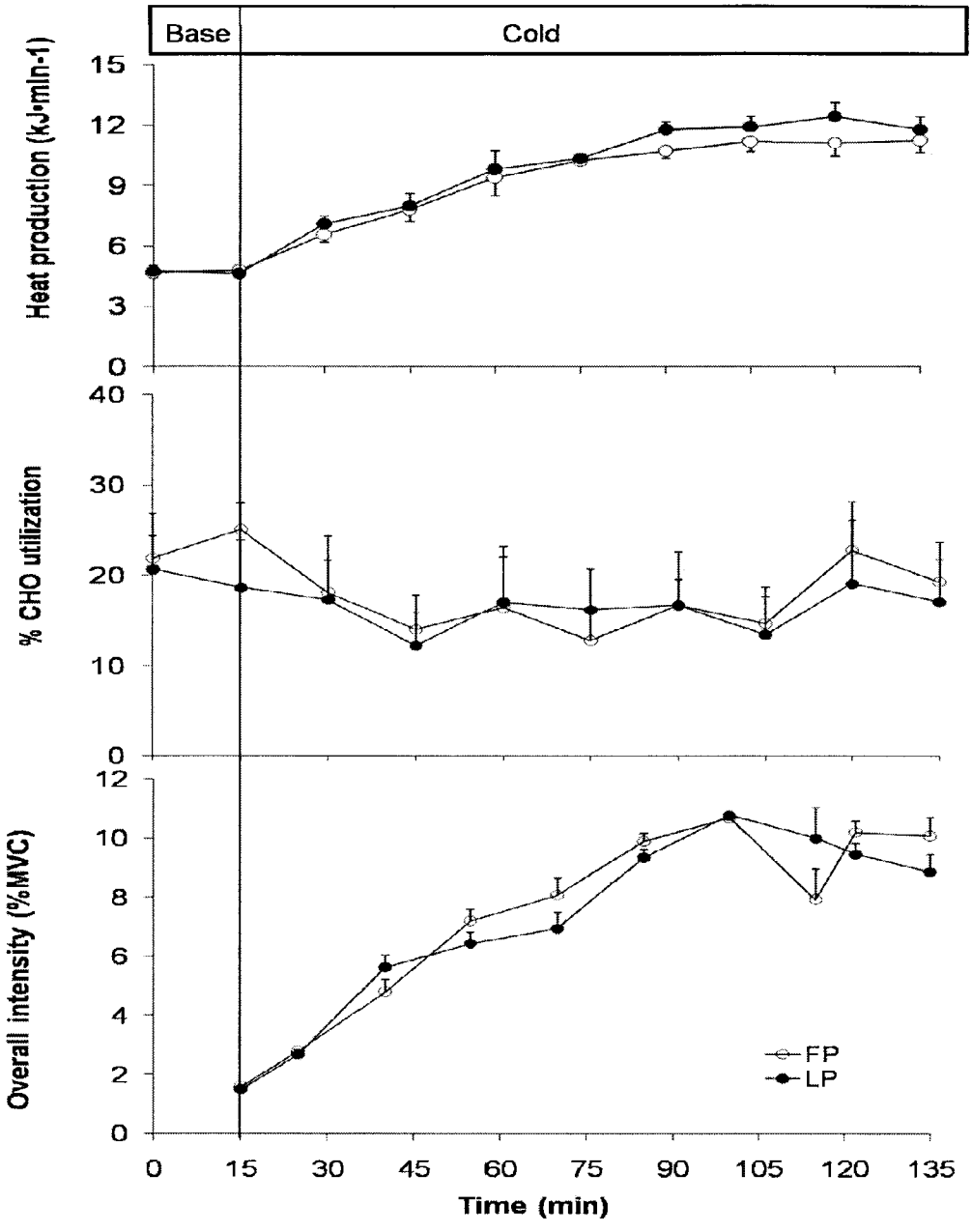

Figure 3.10. Changes in the relative contribution of carbohydrates (CHO) to total heat production (M) (%Gox), and whole body overall shivering activity ($\%Shiv_{ov}$) in the follicular (○) and luteal (●) phase of the menstrual cycle in women exposed to 4°C water for 120 mins. Values are means ± SE. *$P < 0.05$

Table 3.1: Plasma estradiol, progesterone and basal morning temperatures using a digital thermometer in women in the FP and LP of the menstrual cycle.

	FP	LP
Estradiol, pg/ml	92.8 ± 7.1	173.2 ± 21.4*
Progesterone, ng/ml	1.1 ± 0.1	7.9 ± 1.5*
Morning temperature	36.1 ± 0.3	36.4 ± 0.3*

Data are mean ± s.e.m , N = 6; $p \leq 0.05$ for significance. * Significant difference between phases.

Table 3.2. Overall, continuous, and burst EMG activity of trunk and upper leg in women in the FP and LP exposed to a 4°C liquid conditioned suit for 120 min.

	FP				LP			
	Trunk		Upper Leg		Trunk		Upper Leg	
			Overall					
Summed area, %MVC • min	770.8 ± 292.5		1479.9 ± 409.3		824.4 ± 273.9		1410.2 ± 0.8	
Mean intensity, %MVC	8.3 ± 3.2		14.5 ± 3.8		8.9 ± 2.9		14.8 ± 4.4	
Steady State Intensity, %MVC	8.9 ± 3.5		17.0 ± 5.2		10.0 ± 3.3		17.0 ± 5.4	
% RT	80.3 ± 2.09				78.6 ± 2.1			
			Continuous					
Summed area, %MVC• min	768.2 ± 311.0		1480.2 ± 404.7		789.5 ± 270.3		1372.6 ± 429.6	
Summed duration, min	80.6 ± 2.1		83.4 ± 1.6		79.4 ± 2.0		80.6 ± 1.4	
$\%Shiv_{time}$	83.5 ± 2.2		86.6 ± 1.7		83.9 ± 2.2		85.3 ± 1.5	
			Burst					
Number of Burst	499.3 ± 77.0		349.7 ± 46.0*		500.5 ± 74.3		393.6 ± 55.1	
Burst rate, number/min	6.4 ± 0.8		4.8 ± 0.6*		6.3 ± 0.8		5.2 ± 0.7	
Mean duration, s	1.8 ± 0.2		2.2 ± 0.2		1.9 ± 0.2		2.2 ± 0.2	
Summed area, %MVC• min	19.7 ± 7.4		43.1 ± 12.7		18.8 ± 6.0		37.9 ± 11.2	
Mean intensity, %MVC	16.1 ± 3.8		45.3 ± 10.4		15.7 ± 5.4		32.2 ± 9.5	
Summed duration, min	15.7 ± 2.1		12.9 ± 1.6		15.3 ± 1.8		13.7 ± 1.4	
$\%Shiv_{time}$	16.4 ± 2.2		13.4 ± 1.7		16.3 ± 2.0		14.7 ± 5.0	
$\%EMG_{burst}/EMG_{cont}$	2.9 ± 0.7		3.1 ± 0.4		2.8 ± 0.9		3.4 ± 1.0	

Values are means ± SE, n=6 subjects. FP, follicular phase; LP, luteal phase, EMG, electromyography, steady-state intensity, shivering intensity measured in the last 15 minutes of cold exposure under quasi-steady state, %RT, percentage of total recording time (~ 90 min); ($\%Shiv_{time}$, percentage of total shivering time; MVC, maximal voluntary contraction, $\%EMG_{burst}/EMG_{cont}$ ratio of shivering burst summed area to continuous shivering summed area. *P<0.05 significance between phases.

Table 3.3. Absolute oxidation rate and relative contribution of lipids, total CHO and proteins to total heat production in women in the follicular (FP) and luteal phase (LP) before (25.5°C, 0-15min) and during (5°C; 105-135 min) cold exposure.

	FP				LP			
	25.5°C		5°C		25.5°C		5°C	
Rate of Heat Production, kJ/min	4.7 ± 0.3		11.2 ± 0.7*		4.7 ± 0.1		12.1 ± 0.7*	
Lipids								
mg/min	66.2 ± 4.0		181.5 ± 16.7*		67.9 ± 4.6		217.2 ± 12.8*	
% M	57.3 ± 3.4		74.5 ± 4.9*		59.2 ± 5.1*		73.2 ± 5.9*	
Total CHO								
mg/min	70.6 ± 11.7		131.5 ± 24.2*		59.2 ± 5.1		108.9 ± 31.9*	
% M	23.5 ± 3.9		18.9 ± 3.9*		19.7 ± 4.4		16.6 ± 5.9	
Proteins								
mg/min			45.5 ± 4.9				45.0 ± 5.0	
%M	19.3 ± 2.8		6.5 ± 1.6*		21.1 ± 2.5		8.7 ± 1.5*	

Data are mean ± s.e.m , n = 6; $p \leq 0.05$ for significance. * Significant difference between baseline and cold values

Chapter IV – Discussion

The purpose of this study was to determine the effects of the hormonal fluctuations of the menstrual cycle on substrate utilization and muscle activation patterns during low intensity shivering. In accordance to the hypothesis, this study shows that hormonal fluctuations of the menstrual cycle do not affect metabolism or muscle activation patterns when women are exposed to cold using a 4°C LCS for 120 min. Therefore, heat production is obtained by similar muscle activation patterns and similar substrate oxidation in both the FP and LP of the menstrual cycle.

Menstrual cycle status.

The protocols controlling for menstrual cycle phase ensured that women were in the appropriate phase. Each woman showed ovulation in three months of temperature logging. Additionally, serum hormone levels on the experimental days indicated appropriate menstrual cycle phases. Morning sublingual temperature was significantly higher in the LP compared to the FP in all of the participants, indicating appropriate menstrual cycle phases. Aural canal temperature was slightly higher in the LP than the FP at rest but not significantly. The greatest difference in body temperature is seen in the middle of the night (~3:00 am); therefore, upon arrival and preparation at the laboratory (~ 7:30 – 8:00 am), it is possible that the difference was not as distinguishable. We did not find differences in substrate utilization at rest which is similar to some studies (Horton, Miller et al. 2002; Casazza, Jacobs et al. 2004; Horton, Miller et al. 2006) but not to others (Devries, Hamadeh et al. 2006; Horton, Miller et al. 2006). Possible differences may depend on when the measurements are taken; early, mid or late stages of a phase as hormone concentrations may differ therefore affecting metabolic pathways. Similarly our participants had their metabolic data collected after a series of maximal

voluntary contractions therefore an increase in stress hormones such as cortisol and adrenalin may have masked the effects of estrogen and progesterone on metabolism and thermoregulation.

Referring back to the framework of the project, in reverse order, it was demonstrated that there was no effects of the menstrual cycle on thermoregulation, metabolism, and muscle activation patterns or an interaction of the two factors (figure 1.3).

Thermal Data

Additionally, there were no differences in thermal responses to cold exposure between the two menstrual cycle phases which also is in agreement with the muscle activation and metabolic patterns. At the commencement and throughout cold exposure the T_{au} was nearly identical in both phases. T_{au} did decrease significantly with the onset of cold exposure dropping 0.75°C in the FP and 0.81°C in the LP ($p = 0.034$ in the FP and $p = 0.01$ in the LP). Heat flux and heat production measurements did not differ between menstrual cycle phases. This may be because the thermal stress of the cold was masked any potential menstrual cycle differences.

Correspondingly, the menstrual cycle does not affect muscle activation patterns, metabolic pathways or thermoregulatory pathways during cold exposure. This may be due to the overriding affect of the cold stress on the effects of estrogen and progesterone.

Muscle recruitment patterns

EMG signal analysis was performed to analyze two levels to assess potential differences in 1) whole body shivering (monitoring >90% of muscle mass involved in shivering) and 2) detailed EMG activity of individual muscles based on their shivering

patterns (burst vs. continuous shivering). There were no differences in muscle activation patterns at the whole body shivering level or in individual muscles.

Contrary to Hessemer and Bruk (1985) there was no significant difference found on the onset of shivering between the FP and LP of the menstrual cycle. Women also started to shiver at the same T_{au} in both menstrual cycle phases. There was a trend for shivering to start later in the LP, as hypothesized, however the results were not significant. Four of the 6 women did start shivering earlier in the FP at 23.7 ± 3.2 minutes compared to 25.6 ± 3.3 minutes in the LP. Hessemer and Bruk (1985) did find a later onset in muscle activity using EMA by looking at the latissmus dorsi and quadriceps femoris muscle. It was not indicated if the electrodes were put exactly on the same spot for each experiment. The present study looked at 8 muscles, and the onset of shivering was considered when 4 of the 8 muscles were active. This may have been one of the discrepancies between the results of the studies. Hessemer and Bruk (1985) performed their study at 3:30am, when core body temperatures have the greatest difference between phases. The women had been sleeping in the laboratory prior to cold exposure resulting in a lower state of wakefulness, which may have decreased the amount of involuntary movements. In a pilot study that Hessemer and Bruk (1985) performed during the day, they did not find menstrual cycle differences in electrical muscle activity. The authors suggest that this is due to the decreased cycle phase difference in core body temperature throughout the day when compared to larger differences in the early morning; the same could be said for the present study which was done at approximately 8:00 am.

Hessemer and Bruk (1985) attributed the blunted shivering response in the LP to the observation that the rate of basal metabolic rate was higher in the LP compared to the

FP. However the results of this study which are in accordance to those of Gonzalez and Blanchard (1994) do not demonstrate differences between the LP and FP in basal metabolic rate. Gonzalez and Blanchard (1994) did find a blunted shivering response in the LP, but attribute it to differences in thermoregulatory responses between the two menstrual cycle phases. The onset of shivering for their study was observed visually by two researchers. The participants were wearing army ensembles, so it is possible that low-continuous shivering started and was not visually apparent under the thick army clothing, therefore affecting the results of the study. The authors do attribute this blunted shivering response in the LP to the higher core body temperature in the LP resulting in a lower rate of heat loss, which would increase cold tolerance. They believe this heat debt is a linear combination of core temperature as measured by esophageal temperature, mean skin and peripheral temperature as measured by finger temperature. In the current study, the onset of shivering occurred at a mean skin temperature of 0.8°C higher in the LP and an aural temperature that was 0.05°C higher in the LP. Although the present study did not show significant differences in shivering response between menstrual cycle phases, our results do follow a similar trend to those of Gonzalez and Blanchard. Women in both studies had higher core temperatures and skin temperatures in the LP than the FP and consequently elicited a shivering response later in the LP.

Shivering pattern. The EMG signal was analyzed by separating the patterns into "low- intensity continuous" and "high-intensity burst" shivering (Table 3.1). These two patterns were distinguished by differences in intensity (2-5 vs. 7-15% MVC respectively) and rate of occurrence (8-10 vs. 0.1-0.2 Hz respectively) (Fig 4.1). The menstrual cycle does not affect overall shivering patterns, however there was a higher burst rate and total

number of burst in the upper leg in the LP compared to the FP during the 80 min of data collection. It is unclear as to why this difference occurred; however, this difference was not large enough to cause a difference in overall body burst rate and number of bursts.

Women also spent the same amount of time burst shivering and continuous shivering in both menstrual cycle phases. The burst durations were similar between body regions in both phases and consequently, both summed burst and continuous durations were similar. These measurements give an overall and complete look at bursting patterns looking at duration, intensity and type of shivering. These findings indicate that overall whole body and individual muscle shivering patterns were the same between both menstrual cycle phases.

The last 15 minutes of cold exposure, in which the participants had reached a steady-state of shivering, showed no difference in burst rate pattern, burst duration or continuous shivering duration. The lack of difference between shivering patterns in the FP and LP suggests that motor unit recruitment patterns are the same in both the FP and LP during cold exposure. Previous studies have not looked at shivering patterns and the menstrual cycle, therefore it is hard to compare these findings with that of others. It appears that the fluctuations in sex hormones do not affect the neural pathways controlling muscle activation. It would be interesting to look at muscle activation patterns at both higher and lower shivering intensities to determine if differences in sex hormone levels do affect neural pathways at lower or higher shivering intensities. It may be that at high intensities the effects of other catecholamines override the effects of the sex hormones, but not at low intensities. More research is necessary in the area of sex hormones and muscle activation patterns.

The contribution of each muscle to M was the same between in the FP and LP. Figure 3.5 shows us that M and $\%Shiv_{ov}$ are closely correlated over time. Nonshivering thermognesis is generally thought to be negligible in adults, which fits well with our results. The tight correlation between $\%Shiv_{ov}$ and heat production indicate that nonshivering thermogenesis is probably not a factor in heat production. Total shivering activity ($\%Shiv_{ov}$) and the respective contributions of specific muscles to $\%Shiv_{ov}$ were not affected by the menstrual cycle.

It is presumed that because both continuous and burst shivering intensities were the same between both menstrual cycle phases, there were no muscle activation pattern differences. Women started shivering at the same intensity in both phases of the menstrual cycle. This data is compatible with metabolic data which shows no difference in fuel oxidation between menstrual cycle phases.

Selection of metabolic pathways.

During low-intensity shivering women sustain the same thermogenic rate by oxidizing the same fuels in both phases of the menstrual cycle. Differences in relative percent contribution of fuels to heat production were not found between menstrual cycle phases. A significant increase in lipid oxidation and lipid contribution to heat production occurred when women were exposed to cold. Carbohydrate and protein contribution to heat production decreased with exposure to cold. Therefore, it is concluded that the effects of the menstrual cycle have no effects on metabolism during exposure in cold.

Menstrual cycle difference in lipid oxidation

No differences in fat oxidation were present between menstrual cycle phases during baseline conditions and low-intensity shivering. We did observe a trend in which

lipid oxidation was higher in the LP at rest and during cold-exposure. Similarly Glickman-Weiss et. al. did not find menstrual cycle differences, or trends in metabolism after 20°C cold water immersion (Glickman-Weiss, Caine et al. 2000).

Referring to table 2.2 we see that women oxidized less fats in the FP compared to the LP at rest and during the last 30 min of cold exposure (65.8 mg/min to 203.9 mg/min in FP and 68.1 mg/min to 217.21 mg/min in the LP). A 2.8-fold increase in lipid oxidation was present in the in FP and 3.2-fold increase in LP. It is thought that estrogen promotes lipolysis therefore women oxidize more lipids, especially in the LP (Casazza, Jacobs et al. 2004; Tarnopolsky 2008). Although the results are not statistically significant this trend is observed in the present study ($p = 0.058$ in lipid oxidation between menstrual cycle phases from rest to cold exposure). Women rely more heavily on lipid oxidation and lipid contribution to heat production at rest and during cold exposure in the LP when compared to the FP (Tarnopolsky, MacDougall et al. 1990).

Menstrual cycle differences in CHO oxidation

Carbohydrate oxidation was not significantly different between menstrual cycle phases; however absolute RGox values were slightly higher in the FP at baseline and cold exposure values ($p = 0.058$). Petit et al. (1998) exposed men and women (in varying points of their menstrual cycle) to 5.6°C cold air for 2 h and concluded that there were no sex differences in catecholamine responses (Pettit, Marchand et al. 1999). Unfortunately research is not as clear investigating the effects of the menstrual cycle and catecholamines. Literature is confounding regarding catecholamine levels between menstrual cycle phases; some studies indicated that adrenalin and noradrenalin levels are not significant different in the LP and FP of the menstrual cycle (Dean, Perreault et al.

2003), while others indicate that adrenalin levels are higher in the FP compare to LP (Ettinger, Silber et al. 1998; McFetridge and Sherwood 2000). The latter observation does agree with the findings of some previous research indicating that women oxidize more CHO in the FP (Wenz, Berend et al. 1997). Observed increases in CHO oxidation rates may be as a result of higher levels of adrenalin which could cause greater rates of glycolysis. However it is important to note that adrenalin has also been shown to increase lypolysis (Kim, Saidel et al. 2008). It is not clear if adrenalin increases lypolysis or glycolysis more in cold exposure.

Menstrual cycle differences in protein oxidation

The results of the present study indicate that there is no significant difference in protein oxidation between menstrual cycle phases after 2 hours of cold exposure. RP_{ox} was very similar between both menstrual cycle phases; however, there was a trend of higher contribution to heat production in the LP at both baseline and cold exposure compared to the FP. Previous studies investigating the effects of the menstrual cycle on protein oxidation are inconclusive. However, recent data indicates that higher levels of ß-estrogen result in lower levels of leucine oxidation during exercise (Tarnopolsky 2008). Since estrogen levels are almost double as high in the LP compared to the FP, it would be thought that less protein oxidation would occur in the LP; this finding was not present during cold exposure (45 ± 5g/min in the FP and LP). Contrarily, the opposite trend of a slightly higher protein contribution to heat production in the LP at both baseline and cold exposure conditions was found.

Overall Fuel Utilization during cold exposure

Absolute lipid and carbohydrate oxidation were significantly higher during cold exposure, which is expected since the body must produce more heat by metabolizing fuels. The relative contribution of lipids to total heat production was significantly higher during cold exposure compared to baseline. Contrarily the relative contribution of CHO and proteins decreased with exposure to cold. This significant increase in the relative contribution of lipids to $\dot{M}$ in cold exposure is a novel finding in women. It appears that lipids are the preferential fuel during cold exposure in women. Lipid oxidation increased from approximately 58% to 74% from baseline to cold values. Shivering at a moderate intensity resembles low-intensity exercise therefore it is expected that lipids would be the predominant fuel utilized. Future research should focus on lipid oxidation that provides most of the energy during cold exposure in women.

Limitations/Delimitations

The results of this investigation cannot be generalized to the entire population as the participants are relatively young, healthy, physically active females that are not on hormonal contraceptives. Research has shown that 80 percent of all American women born since 1945 have used the birth control pill (Chilcott 2006). Ideally, an additional part would be added to the study which would investigate the effects of oral contraceptives on fuel selection in cold exposure.

This was a very invasive study which required healthy females that had been ovulating regularly for at least one year prior to the study. There was an attempt to normalize for body fat percentage as it could have affected thermoregulatory responses. Sixteen participants were recruited however only 6 participants provided full sets of data

and fit the appropriate criteria for ovulation. Therefore the sample size of this study was very small; however due to the cross-over random assignment the power of this study is increased but it could be strengthened by enhancing the sample size.

A great deal of trust was put onto the participants to keep daily logs of their oral body temperatures and menstrual cycles. This required the participants to record consistent, accurate logs. Follow ups with participants were done twice a week for the first two weeks of the temperature logging via telephone to remind them of the measurements and answer any questions they may have had. Continued follow-up occurred twice per week via the phone and email for the remaining 10 weeks of the daily temperature log. Participants came into the laboratory at least once after one month of recordings to ensure proper recording practices.

Participants were also asked to mark the location of the EMG placement daily; however, it is likely that the placement was not the exact same between trials however because EMG data was normalized relative to the placement for that specific day it is not likely that this had a major effect on the results.

Chapter V- General Conclusions and Perspectives

This is the first study to provide an integrated analysis of fuel metabolism and muscle fiber recruitment during shivering in women. The main purpose of this thesis was to determine whether the hormonal fluctuations of the menstrual cycle affect substrate utilization and muscle activation patterns. By assessing individual menstrual cycle effects in the luteal and follicular phase on substrate utilization, by indirect calorimetry and muscle fiber recruitment patterns, by EMG it was possible to draw the following conclusions:

Menstrual cycle effects on muscle activation patterns

1) The onset of shivering occurred at similar skin and aural canal temperatures in both phases of this study, indicating that thermoregulation is not different between the two phases. The onset of shivering did occur at a slightly later time and a slightly higher tympanic and skin temperature in the luteal phases however differences were not of a high enough magnitude for significance.
2) Shivering pattern of continuous to burst shivering was the same between the two menstrual cycle phases. Burst rates and durations were the same between both phases in the trunk and upper leg region, with the exception of more burst in the upper leg in the LP. Nonetheless overall body burst numbers were the same between both phases.
3) No significant difference was shown in the shivering intensity, bursting and continuous, between menstrual cycle phases. The percent contribution of body regions to overall shivering was the same between menstrual cycle phases.

Menstrual cycle effects on substrate utilization

1) The results of this study do not statistically demonstrate the previously shown trend of higher lipid and lower CHO oxidation in the LP compared to the FP seen during low-intensity exercise. At rest and during cold exposure relative contributions of substrates do not follow this tendency seen by researchers between the menstrual cycle phases. The physiological responses to cold exposure may have masked the possible menstrual phase differences.
2) Women rely predominantly on lipid contribution for heat production when exposed to cold. Protein and CHO contribution decreased from baseline to cold exposure conditions. These are novel findings in women.
3) Heat production did not vary between menstrual cycle phases at rest or during cold exposure. There were no significant heat loss, aural canal or skin temperature differences between menstrual phases therefore these results are as expected.

This experiment is the first to observe both metabolic and muscle activation pattern differences in cold in women. Differences were not significant between menstrual cycle phases in muscle activation patterns and metabolism. No overall muscle activation patterns were found between the two phases however there was non-significant trend in preferential lipid oxidation in the LP compared to the FP. It would be beneficial to perform this study earlier in the morning, similar to Hessemer & Bruk (1985) to see if differences may exist when core body temperature differs the most between the two phases of the menstrual cycle. Additionally, further research done in a larger sample group as well as earlier in the morning may result in significant differences.

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Appendix A: Consent Form

INFORMED CONSENT OF THE SUBJECT

EFFECT OF COLD EXPOSURE ON THE REGULATION OF FAT METABOLISM

University of Ottawa

Investigators:

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The purpose of this study is to investigate the effect of glucose ingestion on the capacity of the body to utilize lipids, proteins and carbohydrates for supplying the energy demand during cold-exposure in humans. This study will be conducted in English (i.e. experiments and documentation). Consequently, participants will need to be fluent in this language.

Only healthy, non-pregnant volunteers can participate in this study. Participants must be between the ages of 18 and 35 years of age and fluent in English. Those on any form of medication, including oral contraceptives, will be excluded, as well as those with any history of cardiovascular or respiratory disease. In addition, I will be asked to abstain from consuming caffeine and/or alcohol at least 24 hrs prior to the experimental sessions.

I have filled out a "Par-Q and You: Physical Activity Readiness Questionnaire. I will be asked to participate in one preliminary session, which will allow me to learn about what the experimental sessions will involve. During the preliminary session, body composition will be determined by a dual Energy X-Ray Absorptiometry device, used to determine tissue density, (a low radioactivity device amounting to 1/20 of the radioactive rays received over an 8-hour period, or 15-20 minutes, in the sunlight) will be performed to estimate my proportion of fat and muscle tissue. It is recommended that I have urinated within 30 min of the test, consumed no alcohol 24 hours prior to the test, and not have exercised within 24 hours of the test. I will also be measured for height, weight as well as waist and hip circumferences. Finally, I will be asked to perform a maximal exercise test on a treadmill while my heart rate will be monitored.

For the experimental sessions, I will be required to sit in a 5°C room wearing a liquid conditioned suit that circulates 5°C water next to the skin for up to 4 hours wearing only shorts and running shoes (and sports bra for women). I will be attached to an intravenous catheter (IV), which will continuously administer glucose into my body. I

am aware that nine blood samples will be taken by a qualified individual (i.e. nurse, medical doctor) at 30-minute intervals using an intravenous catheter (very small and flexible plastic tube). During this test, I will also be connected to a rectal temperature probe, a Polar heart rate monitor, and an automated metabolic cart.

As for the experimental conditions specified above, I will be required to participate in three consecutive five-hour experimental sessions. The only difference between these three experimental sessions is that I will orally consume 0, 50 or 100 g of glucose solution. I will receive $150 that will be paid in increments of $50 at the beginning of each of the experimental sessions. I will receive the $50 even if I do not complete the session in question. However, I will not be compensated for any session for which I do not show up.

The risks and discomforts associated with the study are as follows: The physical practice of exercise of low mild intensity shivering can result in muscular and articular stiffness and/or cramps. In extreme cases, when exposure to cold is prolonged and severe (leading then to hypothermia), heart attacks and even death may occur. These risks depend largely on physical condition and age of an individual. However, all experimental conditions will be performed in a moderately cold environment (5°C) which will not induce hypothermia. Shivering can result in the same effects as low intensity exercise due to the increase in physical demands on the body. There is also the possibility of acute fatigue due to the prolonged shivering. The indwelling catheter for blood samples and administration of 6,6-2H2-glucose pose potential risks ranging in severity from simple redness of the skin to a local inflammation of the vein. On the psychological level, it is possible that certain individuals may feel distressed and anxious with regards to some measurements such as blood sampling, the indwelling catheters or the rectal probe.

I understand that the confidentiality of my data will be maintained at all times. Data will be assessable only by the investigators and will be destroyed within 5 years of publication. Data will be presented in pooled form, and identified by a specific code. In any written reports or publications, I will not be identified. The data collected in this study will be kept at the University of Ottawa in a locked filing cabinet in a room with limited access, meaning that the room can only be accessed by the researchers. The room is restricted, and has a pass code entry system to ensure only authorized personnel such as the researchers have access to it. In addition, the computerized data will be protected by a password. The computer will be kept in the same restricted room. The blood and plasma samples will be kept at the Behavioral and Metabolic Research Unit at Montfort Hospital and will also be destroyed five years after the publication.

There will be no direct benefit to me from these procedures. However, the investigators may be able to better understand the importance of glucose ingestion on fuel selection during shivering in cold temperatures and will share these results at the end of

the study, upon my request. The data collected will also be included in the graduate dissertations of Anali Maneshi (Master student) and Denis Blondin (Master student).

I have talked to the members of the research team about this study and my questions have been answered. If I have any other questions I may call the research assistant, Marie-Andrée Imbeault, at (613) 562-5800 ext. 2458, or Drs François Haman at 562-5800 ext. 4262 or Pascal Imbeault at 562-5800 ext. 4269

I have been given a copy of this consent form for me to keep. Participation in this research is voluntary. I can refuse to participate or if I choose to participate, I can withdraw at any time for any reason.

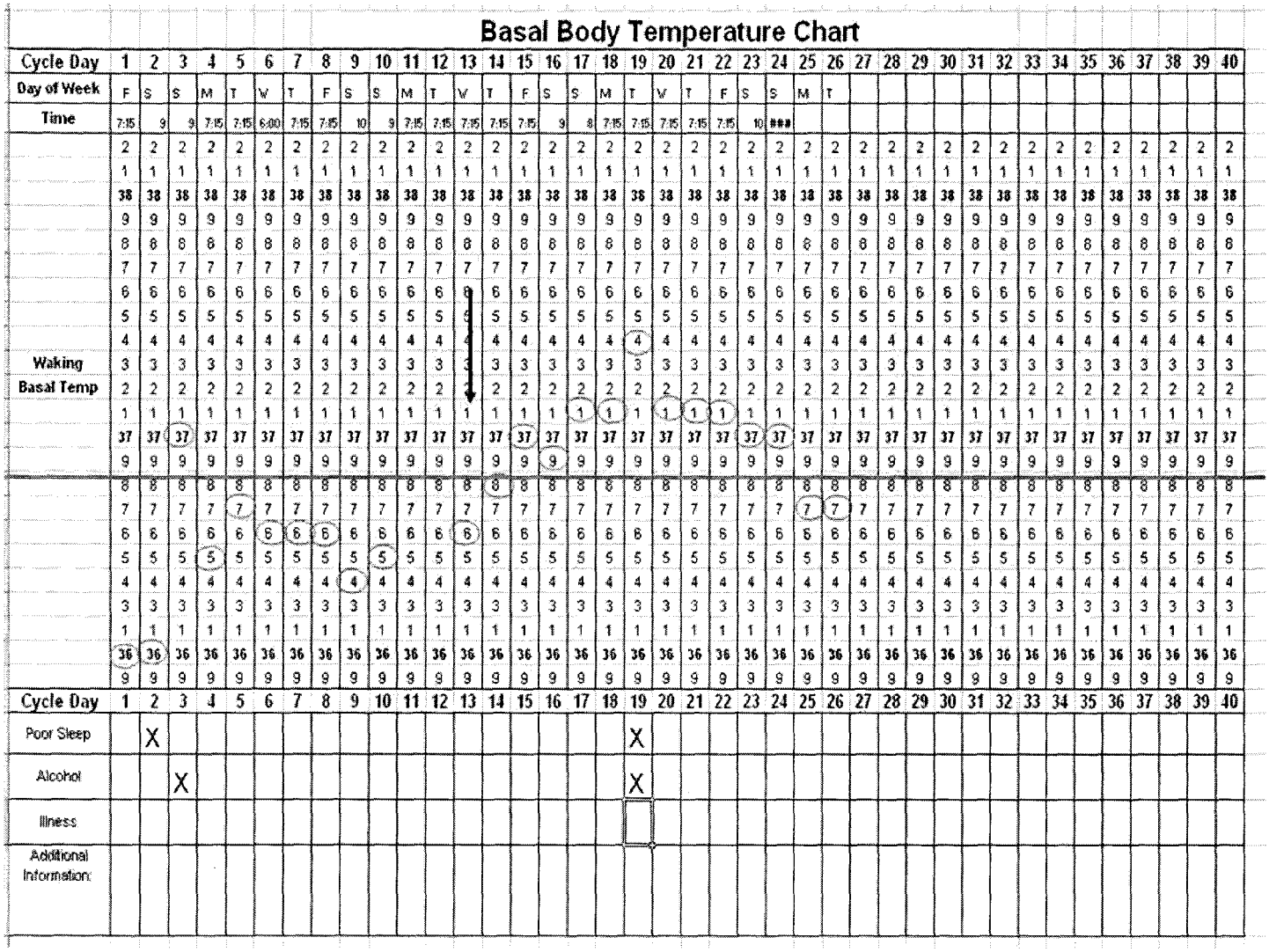
I agree to participate in this study.

Volunteering Subject (print): _____ Date: _____

Volunteering Subject (signature): _____ Date: _____

Signature of Researcher: _____ Date: _____

Appendix B: Example of Menstrual Cycle Log



Basal Body Temperature Chart

Cycle Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Day of Week	F	S	S	M	T	W	T	F	S	S	M	T	W	T	F	S	S	M	T	W	T	F	S	S	M	T														
Time	7:15	9	9	7:15	7:15	6:00	7:15	7:15	10	9	7:15	7:15	7:15	7:15	7:15	9	8	7:15	7:15	7:15	7:15	7:15	10	###																	
	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38
	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
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Waking	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Basal Temp	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
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	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37
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	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
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	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36
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Cycle Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Poor Sleep		X																	X																						
Alcohol			X																X																						
Illness																																								
Additional Information																																								