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Cutaneous microvascular response during local cold exposure - the effect of female sex hormones and cold perception

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ABSTRACT

It is generally known that differences exist between males and females with regard to sensitivity to cold. Similar differences even among females in different hormonal balance might influence microvascular response during cold provocation testing.

The aim of the present study was to measure sex hormone levels, cold and cold pain perception thresholds and compare them to cutaneous laser-Doppler flux response during local cooling in both the follicular and luteal phases of the menstrual cycle.

In the luteal phase a more pronounced decrease in laser-Doppler flux was observed compared to follicular phase during local cooling at 15 °C (significant difference by Dunnett's test, $p < 0.05$). In addition, statistically significant correlations between progesterone level and laser-Doppler flux response to local cooling were observed during the follicular ($R = -0.552$, $p = 0.0174$) and during the luteal phases ($R = 0.520$, $p = 0.0271$). In contrast, the correlation between estradiol level and laser-Doppler flux response was observed only in the follicular phase ($R = -0.506$, $p = 0.0324$).

Our results show that individual sensitivity to cold influences cutaneous microvascular response to local cooling; that microvascular reactivity is more pronounced during the luteal phase of the menstrual cycle; and that reactivity correlates with hormone levels. The effect of specific sex hormone levels is related to the cold-provocation temperature.

1. Introduction

Raynaud's phenomenon, which is characterized by intense cold-induced constriction of cutaneous arteries, is more common in women compared with men. The fluctuations of symptoms according to menstrual cycle were also observed which implies an important role of sex hormones in its pathogenesis (Bakst et al., 2008; Eid et al., 2007). The cold pressure testing where one hand is immersed in the cold water is commonly used as a diagnostics procedure. Researchers have also described the differences between men and women in the response of their cardiovascular system to cold pressure testing (Tousignant-Laflamme and Marchand, 2009a). In spite of the general knowledge that differences in sensitivity to cold exist between males and females (Cankar et al., 2000; Cankar and Finderle, 2003; Fillingim et al., 2009; Meh and Denislic, 1994; Tousignant-Laflamme and Marchand, 2009a) Despite above mentioned both sexes are regarded as a homogenous group during the test results interpretation.

Similarly women have greater sensitivity to pain with lower thresholds and tolerance to painful stimuli (Alabas et al., 2012). Although the pain conditions are more common in women within reproductive age and demonstrate cyclical exacerbations in accordance with menstrual cycle indicating the role of sex hormones in pain perception, the underlying mechanisms are unclear. The net effect of progesterone and estrogen on pain perception depends on the sum of their pronociceptive and antinociceptive effects at many levels of neuroaxis (Aloisi and Bonifazi, 2006; Iacovides et al., 2015; Nag and Mokha, 2014; de Tommaso, 2011; Traub and Ji, 2013; Wiesenfeld-Hallin, 2005). Nevertheless, it is still unclear whether the absolute hormone level values or their fluctuation determine the pronociceptive or antinociceptive direction of their action (Traub and Ji, 2013; Iacovides et al., 2015).

In addition, both estrogen and progesterone might directly influence microvascular reactivity involved in microvascular response to cold. First, both female sex hormones augment cold induced vasoconstriction mediated by α_2 adrenoceptors in vessel walls (Colucci et al., 1982; Eid et al., 2007; Mercuro et al., 1999; Sita and Miller, 1996). In addition, estrogen also regulates microvascular function via stimulation of non-genomic signaling, resulting in rapid nitric oxide release (Kim et al., 2014; Huang and Kaley, 2004) which might attenuate microvascular cold-induced vasoconstriction (Hodges, 2006). Despite that, the study of Matsuda-Nakamura and coworkers did not confirm an effect of body

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core temperature changes during the menstrual cycle phases on cold perception or on the cardiovascular system response to whole body cooling (Matsuda-Nakamura et al., 2015).

Our previous study showed that differences exist between males and females in their microvascular response to local cooling. In addition, females exhibit intra-menstrual cycle variability in their microvascular response to local cooling (Cankar and Finderle, 2003). In females, sensitivity to cold is presumed to depend on the phase of the menstrual cycle (Kowalczyk et al., 2006). Therefore, the perception of cold and cold pain might influence the results of local cold provocation testing.

In addition, there is no general consensus on the temperature that should be used for cold provocation testing. A great range of cooling temperatures is commonly used both for males and females (Minson, 2010), in spite of the well-known fact that at different cooling temperatures distinct cold and/or cold pain receptors are activated (Campero et al., 2001; Caterina, 2003; Lötsch et al., 2015; Morrison et al., 2008; Schepers and Ringkamp, 2009; Tominaga and Caterina, 2004). At cooling temperatures below 15 °C receptors other than thermoreceptors are activated (Adriaensen et al., 1983; Saumet et al., 1985).

To date there is no study comparing quantitatively determined individual thermal sensations with cutaneous microvascular response during cooling test in women who are in the follicular and luteal phases of their menstrual cycle.

The aim of present study is to determine specific differences in the microvascular response to local cooling in women who are in each of the two phases of the menstrual cycle and to determine the correlative relationship between quantitatively determined perception of cold stimuli and estradiol and progesterone levels on the one hand, and cutaneous microvascular response to local cold exposure at different cooling temperatures commonly used in cold provocation testing on the other. We hypothesized that both female sex hormones and cold perception threshold influence cutaneous microvascular response to local cold exposure in women with a regular menstrual cycle.

2. Methods

2.1. Subjects

The measurements were performed on eighteen healthy female volunteers (mean age 31.5 ± 5.7) with no chronic illnesses and no prescribed medication, including pharmacological contraception to insure normal progesterone and estrogen levels fluctuations. All participants kept dairies of their menstrual cycles to ensure that measurements were performed at comparable intervals of both menstrual cycle phases.

The subjects were not allowed to consume any drinks containing alcohol, caffeine, or theine 12 h prior to the test. To avoid the effect of ambient temperature on thermal perception (Strigo et al., 2000), the measurements were carried out in a quiet room at 24 ± 0.5 °C and 45% humidity, while subjects were at rest in a supine position. All measurements were performed in the springtime to avoid possible seasonal differences in arterial pressure (Tanaka et al., 1985) and in the morning to avoid diurnal variations in pain perception (Strian et al., 1989). All subjects were informed about the research procedure and gave their written consent. The study was approved by the Committee for Medical Ethics at the Ministry of Health of the Republic of Slovenia (approval number: 142/1/9).

2.2. Experimental protocol

All measurements were done once in the early follicular (day 4–5) and once in the middle of the luteal phase of the menstrual cycle (day 22–25). The phase of the cycle was confirmed with serum measurement of estradiol (normal range: 0–0.56 nmol/L in follicular phase, 0.1–0.9 nmol/L in luteal phase) and progesterone levels (normal range: 0–3.1 nmol/L in follicular phase, 19–76 nmol/L in luteal phase). The data are presented in Table 1.

2.3. Hormone levels measurements and thermal perception thresholds assessment

The first part of the experiment (Fig. 1) began with blood sampling and attachment of the probes. Afterwards, subjects were allowed to acclimatize for additional 10 min. To assess individual perception of thermal stimuli the Marstock method was employed. We used the test designed by Fruhstorfer and coworkers, which enables a quantitative assessment of cold perception threshold as well as cold pain perception threshold (Fruhstorfer et al., 1976). The principle governing the measurement of thermal perception thresholds with this technique has been described elsewhere (Music et al., 2011). The Somedic thermotest (Somedic AB Stockholm, Sweden) with a 25 × 50 mm thermode as the stimulator was used, cooling the skin on the left thenar (flat surface). Due to variation in thermal perception threshold values obtained on different cooling sites (Bartlett et al., 1998), measurements were performed on the same site that was used for measurements during cold pressure testing on the left hand.

The stimulation thermode was gently fixed to the left thenar using adhesive tape and was not moved during measurements, as it is known that dynamic tactile stimulation accompanying thermal stimulations attenuates temperature perception and its quality (Green, 2009). After the attachment of the thermode, and in a supine position, subjects first adapted to its neutral temperature set at 30 °C for 10 min. The subject was instructed to press her own switch (button) as soon as she perceived the thermode becoming cold (cold perception threshold measured) or as soon as she felt pain because of the cold (cold pain perception threshold detection). The researcher then triggered a change in the temperature without notifying the subject. Thermal flows of 0.5 °C/s and 1.5 °C/s were used to determine the threshold of cold perception and cold pain perception threshold, respectively. The difference between the initial neutral temperature and the temperature at which the test subject perceived a change in the temperature represents the cold perception threshold, while the absolute value of the temperature at which the test subject perceived cold pain represents the cold pain perception threshold. The cold perception threshold measurement always preceded the cold pain perception threshold measurement.

The temperature was measured to the nearest 0.1 °C, and the thresholds of perception were measured to the nearest 0.2 °C. Both measurements were repeated five times.

Each consecutive measurement began after at least a 5-minute period of the neutral thermode temperature at 30 °C.

2.4. Microvascular reactivity during local cooling

In the second part (Fig. 1) of our experiment, continuous non-invasive blood pressure, HR, and laser-Doppler measurements of cutaneous microvascular blood flow on the finger tip of the cooled hand were performed at two different cooling temperatures. A temperature of 15 °C induces exclusively cold sensation, while 10 °C induces both cold and pain sensation (Campero et al., 2001; Caterina, 2003; Lötsch et al., 2015; Morrison et al., 2008; Schepers and Ringkamp, 2009; Tominaga and Caterina, 2004).

Laser-Doppler flux, arterial pressure and HR measurements were recorded simultaneously during the entire experiment. Laser-Doppler

Table 1
Serum estradiol and progesterone levels in the follicular and luteal phases of the menstrual cycle.

	Follicular phase	Luteal phase
Estradiol (nmol/L)	0.196 ± 0.15	0.394 ± 0.24*
Progesterone (nmol/L)	1.221 ± 0.67	28.33 ± 17.20*

Values are means ± SD.
* Statistically significant difference between follicular and luteal phase at $p < 0.05$.