Heat tolerance of Female Firefighters:

Thermoregulatory responses to menstrual cycle phase, oral contraceptive use and menopause

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A report for The Worshipful Company of Firefighters

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THE WORSHIPFUL COMPANY OF FIREFIGHTERS

SERVICE, FELLOWSHIP, HARMONY AND HONOUR

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Lay Summary

This study sought to evaluate how females are affected by severe heat stress while wearing full firefighter personal protective equipment during a walking test of a similar intensity, humidity and temperature to that of a standard live fire training scenario. Females undertook tests in two different phases of their menstrual cycle and were split into groups of those taking or not taking oral contraceptives. A final group of females who were reporting as menopausal or peri-menopausal also undertook the heat tolerance testing. The research question was whether there were differences in how quickly females in these different groups heated up during the test or whether there was a difference in their perception or physiological response to the heat. The study demonstrated no differences in physiological or perceptual responses during menstrual cycle phases, with use of contraceptives or during menopause. We therefore propose that greater focus should be on methods to improve heat tolerance, such as consistent hydration, maintained heat acclimation, cooling methods, clothing and training status.

Review of Literature

Role of a Firefighter

Firefighting is one of the most hazardous civilian occupations requiring strength, power, aerobic and muscular endurance (Perroni et al., 2014). As well as fire emergencies, firefighters may respond to natural disasters, hazardous material incidents and road traffic accidents (Gnacinski et al., 2016). Firefighters can experience strenuous workloads in dangerous and chaotic conditions for irregular durations with ambient temperatures of 67 - 190°C leading to cardiovascular and thermal strain (Eglin, 2007; Barr et al., 2010; Perroni et al., 2014; Petruzzello et al., 2009). Fire suppression and search and rescue work has been seen to induce near maximal heart rates (90% of heart rate reserve) and oxygen consumption of 80 - 100% $\dot{V}O_2max$ (Dreger et al., 2006; Eglin et al., 2004). During a fire emergency, firefighters will often carry out multiple bouts of 20 minute fire suppression work with short breaks of 10 – 20 minutes to recover in between each exposure to the fire (Horn et al., 2011; Horn et al., 2013). Physical activity while wearing PPE even in temperate conditions, but especially in hot environments can result in uncompensable heat stress (UHS) (Annaheim et al., 2015). A rapid increase in average core temperature of 0.7°C has been recorded following one 20 minute fire exposure while after 4 such bouts, an average increase in Core temperature of 1.8°C was seen (Horn et al., 2013; Horn et al., 2018).

Firefighting is a worldwide activity which involves physically demanding tasks alongside external stress placed on the body (Holmer and Gavhed, 2007). This physiological demand is placed on all fire service personnel including the fire service instructors. With an elevation in metabolic heat production and high ambient temperatures, it has resulted in high core temperatures (38.0–39.0 °C), maximal heart rates (119–189 $b.min^{-1}$), high levels of sweat loss (0.5–2.0 $L.h^{-1}$) and high rates of energy expenditure (oxygen consumption 2.3–3.55 $L.min^{-1}$)(Eglin, 2007). The physiological strain can vary due to the temperature of the fire, the physical fitness of the individual or the time spent in the fire (Eglin, 2007). The duration of heat exposure often depends on the breathing apparatus but is usually 20 minutes but can be as long as 12 to 18-hours when dealing with a wildfire (Eglin and Tipton, 2005; Ruby et al., 2002).

Before becoming fully qualified each firefighter must undergo an initial training course to learn procedures such as fire rescue and control and this is renewed every two years to ensure skills are to the correct standard and new techniques can be learnt (Eglin and Tipton, 2005; Eglin et al., 2004). During these training sessions the firefighters participate in wears, which are live fire drills including fire behaviours, search and rescue and flashovers (Watt et al., 2016). Wears are typically performed for up to 4 days, 2 times a day and can range from 15-120 minute, with an average of 40 minutes per session, but vary dependent on the task being carried out (Watt et al., 2016; Eglin et al., 2004). The external stress caused during a wear can vary due to the fire size and proximity of the fire although, it has been reported to have an average temperature of 174.0 ± 83.9°C (Eglin et al., 2004).

Due to trained fire instructors running all training courses, they will enter live fires numerous times a day leading to extensive fire exposure in one week (Eglin, 2007). This high level of heat exposure alongside physical activity is likely to cause greater risk of accumulation of heat stress in comparison to standard firefighters (Watt et al., 2016). This in turn can impede cognitive function and mental performance which could result in injury due to poor decisions being made (Smith et al., 1996). However, the repeated heat exposure may lead to improved performance due to acclimation, if suitable preparation and safeguarding is followed (Gibson et al., 2015).

Working in Personal Protective Equipment

Many occupations require the use of personal protective equipment (PPE) to guard against hostile environmental conditions, such as; extreme temperatures, toxic chemicals and physical hazards (Smith, 2011; Holmer, 2006). Firefighting, ordnance disposal and nuclear, biological and chemical (NBC) containment require PPE to be worn in a variety of ambient conditions (Eglin, 2007; Stewart et al., 2014; Selkirk & Mclellan, 2001). Some sports also require protective clothing to be worn such as motor racing and American football (Armstrong et al., 2003; Benisek et al., 1979). PPE comprises thermal protective clothing (TPC); a helmet, safety shoes, turnout coat, undergarment, gloves, and self-contained breathing apparatus (SCBA) which together can weigh as much as 26kg (Lee et al., 2014). As well as being heavy, PPE is bulky which can affect mobility, efficiency and gait leading to an increased energy expenditure compared to light clothing (Coca et al., 2010; Cheung et al., 2000). SCBA can increase the work required to breathe, which combined with the added weight, leads to increased respiratory demands and metabolic heat production ($\dot{H}_{prod}$) (Cheung et al., 2010). NBC has been seen to increase metabolic rate by 13 – 18% compared to light clothing (Aoyagi et al., 1994; Cheung et al., 2000). TPC typically covers the head and includes multiple layers with thick and impermeable outer materials, in turn creating an encapsulated hyperthermic microenvironment, significantly reducing the amount of heat loss possible through sweat evaporation. Only a small air gap layer is seen between PPE and skin so little heat can dissipate by convection due to low air circulation (Holmer, 2006). This increases physiological strain, which can lead to a decline in cognitive function and a greater risk of accident or injury (Barr et al., 2010; Cheung et al., 2000). Although this equipment increases strain on the individual it is needed to protect the individual from hazardous ambient and radiant temperatures which in turn prevents burns, scalds and breathing in toxins caused by the fire (Kesler et al., 2018; Pascoe et al., 1994). The use of full PPE also protects the respiratory system and prevents the ingestion of toxic fumes (Joo-Young Lee et al., 2014).

Thermoregulation

Humans are endothermic homeotherms meaning that body temperature is maintained within a narrow margin around approximately 37°C by balancing $\dot{H}_{prod}$ with heat loss mechanisms (Mrowka & Reuter, 2016; Kenny et al., 2010). This is possible during various activities in different environments due to the interaction of several systems reliant on autonomic mechanisms (Charkoudian & Stachenfeld, 2016).

The cardiovascular system (CVS), central nervous system (CNS) and skin act together to maintain a Core temperature set by the primary thermoregulatory centre in the preoptic region of the anterior hypothalamus (PO/AH; Casa et al., 2015; Charkoudian & Stachenfeld, 2016). The hypothalamus is sensitive to change as little as 0.018°C and when this differs from the range then it will attempt to restore normal levels by generating effector mechanisms, such as sweating, shivering or arteriovenous shunt vasoconstriction or vasodilation, to maintain thermoregulation (Sessler, 2016). By thermoregulating within these margins, it allows mammals to be physically active with little dependence on the environment. This is due to essential mechanisms of the central nervous system and kidney functioning with high precision (Mrowka and Reuter, 2016). At rest, the human body can be thought of as having a heat producing core and a heat regulating shell with around 50% of an average adult's approximately 60 – 70 kcal.h^{-1} basal $\dot{H}_{prod}$ generated by internal organs. However, during exercise, $\dot{H}_{prod}$ can reach 1000 kcal.h^{-1} with around 90% of $\dot{H}_{prod}$ occurring in skeletal muscles (Casa et al., 2015; Krauchi, 2002).

Heat can be transferred through radiation, conduction, convection and evaporation and will travel down temperature and humidity gradients (Binkley et al., 2002; Brotherhood, 2008). Radiation of electromagnetic waves can occur from the body to the atmosphere or from high energy objects such as the sun to the body, heat will flow from a high energy surface to a lower energy surface. Convection occurs between the body and the fluid surrounding it. The direction of heat transfer depends on various properties of the fluid and the temperature gradient between the body and fluid. Convection can also dissipate heat from the core to the shell of the body if core temperature is higher than peripheral temperatures. The rate of heat exchange by convection is determined by the temperature gradient and the movement of the fluid which forms the boundary layer (Stevens and Fuller, 2015). A strong convection current is formed by warm air, as it is less dense then cold air, and a fast moving fluid (Stevens and Fuller, 2015). Conduction transfers energy from a hot object to a cooler object through direct contact. Evaporation of sweat from the skin is the most efficient heat loss mechanism for humans and depends on the velocity and water saturation (humidity) of the surrounding air (Brotherhood, 2008; Casa et al,. 2015; Charkoudian, 2016). Heat storage or loss can be calculated using the following equation from Havenith (2002) and Cheung et al. (2000).

$$S = \dot{M} \pm W \pm Cv \pm Cd \pm R \pm Resp - E$$

Where S = rate of heat storage, $\dot{M}$ = metabolic heat produced, W = external work done, Cv = convection, Cd = conduction, R = radiation, Resp = respiration and E = evaporation. A positive value for S would indicate heat gain.

When the temperature of the climate exceeds core temperature then heat loss by convection and conduction is reduced and is taken over by the secretion of the eccrine sweat glands; the heat is then lost via evaporation (Maughan and Shirreffs, 2010) Evaporation is the loss of water by passive diffusion through the skin and provides the largest amount of heat dissipation (Gunga, 2014; Havenith et al.,

2013). Once environmental temperatures rise or when heat loss is limited by protective clothing, then sweat evaporation will occur by transporting sweat from the skin surface into the atmosphere (Havenith, 2003; Gagnon et al., 2013). Sweat gland secretion is triggered due a rise in Core temperature which is stimulated by an increase in exercise or environmental temperatures, and a higher average skin temperature (Tanda, 2016). However, the amount of evaporation is determined by the positive pressure gradient between the skin, environment and the surface area exposed (Gagnon et al., 2013). Therefore, permeability of clothing can affect evaporation rate and shows a reduction in heat loss when clothing thickness increases and therefore impedes heat exchange (Havenith et al., 2013).

At rest in normothermic conditions, heat production closely matches heat loss and the primary mechanism used to regulate body temperature is skin blood flow controlled by sympathetic noradrenergic nerves of the cutaneous vasoconstrictor system (Smith & Johnson, 2016; Charkoudian & Stachenfeld, 2016). Afferent signals from the skin, muscles and core are integrated by temperature sensitive neurons in the thermoregulatory centre in the PO/AH (Charkoudian, 2016; Kenny et al., 2017). Body temperature increases result in increased firing rates of warm-sensitive neurons which activate the major heat loss mechanisms of cutaneous vasodilation to increase skin blood flow and sweating, elicited by sympathetic cholinergic nerves (Weinert & Waterhouse, 2007; Charkoudian & Stachenfeld, 2016). Skin blood flow may also be increased by inhibitory signals sent to the cutaneous vasoconstriction system. Autonomic control of thermoregulation allows homeostasis to be maintained simultaneously in different physiological systems (Charkoudian, 2016; Morrison, 2016).

Body temperature shows a circadian rhythm, being at the lowest point in the early morning and a peak in the evening with a variance of around 1°C (Drust et al., 2005; Minors & Waterhouse, 1981). This is thought to occur due to a rhythmic variance of input from the suprachiasmatic nucleus to the hypothalamic thermoregulatory centre causing the body temperature setpoint and thresholds for cutaneous vasodilation and sweating to vary over 24 hours (Weinert & Waterhouse, 2007).

Heat stress

Heat stress may be caused by hot environments, physical activity or a combination of both environmental factors and exertion. Thermoregulation, body fluid balance and the cardiovascular system are challenged by environmental heat stress which can result in aerobic performance decrements (Cheuvront et al., 2010). Even in moderate ambient temperatures, exertional heat stress can arise from strenuous activity placing significant strain on the cardiovascular system as active muscles, the brain and skin demand blood flow to maintain energy metabolism, central nervous system function and heat dissipation to maintain core temperature (Cheuvront et al., 2010; Annaheim et al., 2015). During exercise in the heat, core temperature increases, driven by the intensity of exertion while skin temperature rises as a result of the environmental conditions, followed by an increase in sweat rate (Brotherhood, 2008). If steady state exercise is carried out in a clement, low humidity environment in

light clothing, then heat balance can be achieved (there is no further rise in core temperature) and heat stress is said to be compensable (Kenny & Mcginn, 2017).

UHS arises when the body is unable to dissipate heat at the same (or higher) rate as heat is stored within the body, causing core temperature to continue rising and can lead to physiological and cognitive impairment and increased injury or accident risk (Wingo, 2015; Cheung et al., 2000). In ambient temperatures over around 35°C, air temperature is typically higher than skin temperature so heat cannot be lost through convection and cooling can only occur by evaporation of sweat (Eglin, 2007). If the environment is humid (over around 60% relative humidity, RH), the water vapour pressure gradient between the air and skin can become negative and sweat no longer evaporates. Sweating continues even if evaporation does not thus dehydration can occur, especially if physical activity continues for more than one hour (Casa et al., 2015; Brotherhood, 2008). Sweat rates of up to 1.5 $L.h^{-1}$ can be sustained with adequate rehydration, but firefighter sweat rates of 2 $L.h^{-1}$ have been measured while using PPE (Eglin, 2007; Cheuvront et al., 2010). Layers of clothing create microenvironments between skin and the first layer and between subsequent layers as air is trapped. TPC is frequently multi-layered therefore numerous microenvironments are created and heat must dissipate through all of these before reaching the external environment (Cheung et al., 2010). Temperatures between the outer jacket and underclothing of firefighters have been measured as high as 62°C and sweat evaporation is prevented, severely limiting heat dissipation (Eglin, 2007).

For individuals working in PPE in hot environments, UHS is likely due to the lack of heat dissipation combined with an increased $\dot{H}_{prod}$ generated by exercise and the added weight of the clothing and equipment (Cheung et al., 2000). This can lead to core temperatures rising to over 40°C which can result in exertional heat illnesses such as heat syncope, muscle cramps and heat exhaustion if Core temperature > 40.5°C (Casa et al., 2015; Eglin, 2007). As core and skin temperatures rise, $\dot{V}O_2max$ is limited due to hyperthermic factors causing an individual to experience an increase in relative work intensity and reduced aerobic performance. Skin blood flow increases to up to 60% of cardiac output in an attempt to dissipate heat which can result in reduced blood flow to muscles and the brain. In an attempt to prevent this, stroke volume, heart rate and therefore cardiac output increase further (Cheuvront et al., 2010; Charkoudian & Stachenfeld, 2016; Charkoudian, 2003). High intensity or extended periods of exercise in the heat (and attendant dehydration) can lead to a reduced stroke volume and cardiac output even as heart rate increases due to blood being shunted away from the core to the periphery and reduced plasma volume (Cheuvront et al., 2010; Hargreaves, 2008; Eglin, 2007).

Heat tolerance

Interindividual differences in heat tolerance can arise from a variety of factors which may include acclimatisation status, body composition, aerobic fitness, age or chronic illnesses (Cheung et al., 2000; Kenny et al., 2010; Marsh & Jenkins, 2002). It is unclear whether a critical core temperature exists at which fatigue would cause exercise to cease. Possibly the change in core temperature or the core to skin

temperature gradient is more important in determining heat tolerance than the absolute core temperature reached (Armstrong et al., 2003; Hargreaves, 2008; Janse De Jonge et al., 2012; Ely et al., 2009; Cuddy et al., 2014). The determining factor controlling exercise tolerance in the heat may alter in different conditions, although, it is unclear why (Cheung et al., 2000).

Improved aerobic fitness results in an improved physiological response to heat stress and increased heat tolerance (Cheung et al., 2000; Eglin, 2007; Lisman et al., 2014; McLellan et al., 2012). Higher core temperature are tolerated by those with a higher $\dot{V}O_2max$, possibly due to an expanded plasma volume giving superior protection from gut endotoxin leakage as thermal strain intensifies (Cheung et al., 2000; McLellan et al., 2012). Aerobic training also lowers the temperature at which vasodilation and sweating begin, increasing heat dissipation (Cheung et al., 2000). Increased body heat storage and lower resting core temperatures are seen in those with higher $\dot{V}O_2max$ (Cheung et al., 2000; Jay et al., 2011). In situations of UHS, this may not be beneficial as an increased sweat rate will not increase heat dissipation due to evaporation being limited, but may exacerbate dehydration and discomfort (Cheung et al., 2000; Eglin, 2007). Increased body fat percentage reduces body heat capacity, as adipose tissue has a lower heat capacity than lean tissue, and this can lead to a greater rate of core temperature increase and reduced heat tolerance in compensable environments (Selkirk & Mclellan, 2001; Marsh & Jenkins, 2002; Lisman et al., 2014). With UHS, it is less clear how body composition affects heat tolerance. Armstrong et al. (2010) found that increased lean body mass resulted in a greater increase in Core temperature when wearing full American Football League uniform. Selkirk and McLellan (2001) state that heat tolerance while exercising in NBC clothing depended in part on the heat capacity of the body with a lower body fat percentage leading to an increased exercise time tolerance. This may be related to increased fitness levels allowing greater tolerance of increases in Core temperature (McLellan et al., 2012). Wearing PPE will reduce an individual's heat tolerance as seen by shorter tolerance times and greater increases in core temperature (Barr et al., 2010). It is believed that ageing per se does not cause heat intolerance, rather it is the decrement in physical fitness associated with ageing that can result in reduced heat tolerance (Cheung et al., 2000).

Heat acclimation may not improve exercise tolerance times in UHS situations. Similar to increasing physical fitness, heat acclimation produces changes in plasma volume, heart rate and core temperature which increase work ability in the heat (Eglin, 2007). Heat acclimation also lowers the body temperature at which sweating begins while increasing sweat rate. Aoyagi et al. (1994) found that although tolerance times to UHS where not improved by heat acclimation, core and skin temperatures were lower following acclimation suggesting there may be some health and safety benefits for those working in TPC.

Dehydration causes reductions in stroke volume, cardiac output, mean arterial pressure and blood flow to muscles, exacerbating the effects of hyperthermia (Hargreaves, 2008). A body water deficit of over 2% usually results in performance decrements in temperate or hot conditions due to increased cardiovascular strain (Sawka et al., 2015; Cheuvront et al., 2010). It is believed that physical fitness and hydration status are the most important factors affecting heat tolerance and job performance of those

working in UHS conditions (Cheung et al., 2000; Eglin, 2007; Perroni et al., 2014). However, as heat tolerance is multi-factorial it is hard to predict from predisposing factors alone (Watkins et al., 2018).

Various heat tolerance tests (HTTs) have been designed to assess acclimation status, the risk of heat illness and determine when it is safe to return to work following heat illness (Moran et al., 2007; Watkins et al., 2018). The most commonly used HTT in occupational comprises a 2 hour walk at 5km.h^{-1} and 2% gradient in 40^{o}C and 40% RH. Participants wear light workout clothing (Moran et al., 2007). Heat tolerant individuals are defined as those whose Core temperature plateaus at less than 38 ± 0.3^{o}C with a heart rate of 120 ± 15 bpm. A Core temperature > 38.5^{o}C or heart rate > 145 bpm would indicate heat intolerance (Moran et al., 2007). By using no PPE during this test, heat dissipation is allowed by evaporation and with the length being 2h, which is unlike firefighters exposure due to BA usually lasting up to 40 minutes, this test has little ecological validity (Eglin, 2007; Watkins et al., 2018). More recently a test has been designed specifically for firefighters wearing PPE in a UHS environment which involves walking for 40 minutes at 6 W.kg^{-1} metabolic heat production, in 50^{o}C and 10% RH (Watkins et al., 2018). It has been suggested that heat tolerance may be more of a continuum with colour coded zones of green (Core temperature < 38^{o}C), yellow (38^{o}C ≤ Core temperature ≤ 38.5^{o}C) and red (Core temperature > 38.5^{o}C) (Mee et al., 2015; Watkins et al., 2018).

Menstrual cycle and thermoregulation

The median length of the menstrual cycle is 28 days although this can vary from 21-40 days (Figure 1; NHS, 2016; Reed & Carr, 2000). The luteal phase is fairly consistent in most individuals at around 14 days, while the follicular phase varies more widely but commonly lasts from 10-16 days during which time body temperature is typically around 0.6^{o}C lower than the luteal phase (Reed & Carr, 2000; Marsh & Jenkins, 2002). The cycle consists of various hormones being released by the anterior pituitary and ovary gland to coordinate the female reproductive system (Wadikar and Bhandarkar, 2017; Marsh and Jenkins, 2002). These hormones include oestrogen which increases after ovulation into the luteal phase and progesterone which increases during the luteal phase (Marsh and Jenkins, 2002). Typically the early follicular (EF) phase (day 1-5 of a 28 day cycle) and the mid luteal (ML) phase (day 18-24 of a 28 day cycle) are compared in studies of female thermoregulation as these are the hormonal extremes of the menstrual cycle (Grucza et al., 1993; Tenaglia et al., 1999; Marsh & Jenkins, 2002).

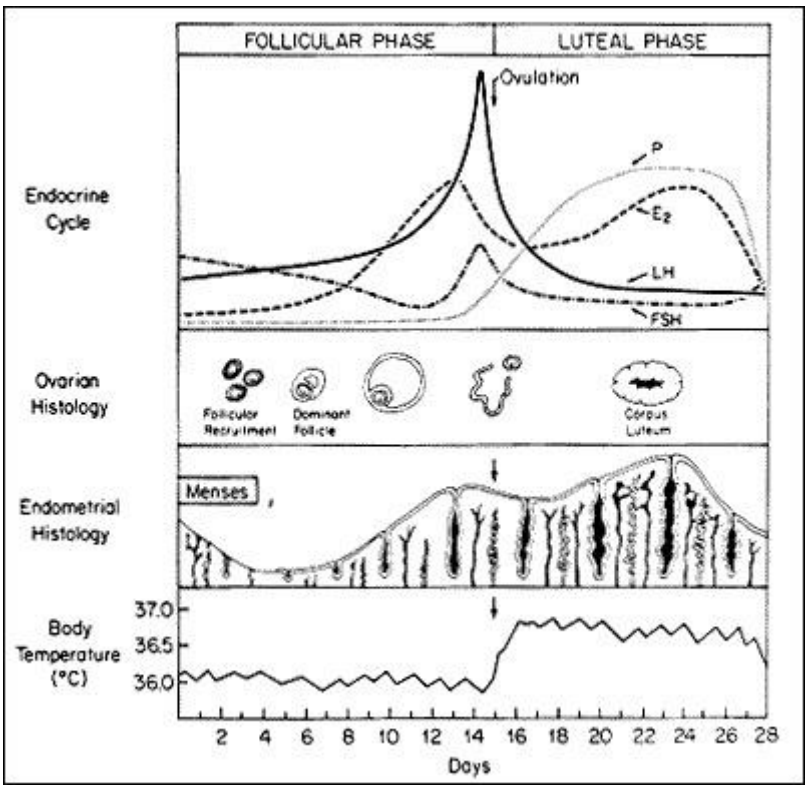

Figure 1. Hormonal, Ovarian, endometrial, and basal body temperature changes and relations throughout the normal menstrual cycle. *E_2= Estradiol, P=Progesterone, LH= Luteinising hormone, FSH= Follicle stimulating hormone* (Reed & Carr, 2000).

In a 2002 review, Marsh and Jenkins stated that there was 'significant evidence' that the increase in progesterone in the luteal phase (Figure 1) caused increased resting Core temperature. Progesterone alters the firing rate of neurons in the PO/AH resulting in a higher setpoint for resting core temperature during the luteal phase (Marsh & Jenkins, 2002). Evidence of an effect of menstrual cycle phase on the rate of increase in Core temperature during exercise (ambient and hot conditions), T_{sk} and temperature at which sweating starts in hot environments was deemed inconclusive. Oestrogens alone cause a reduction in body temperature due to increased vasodilation and changes in central autonomic control of skin blood flow and sweating therefore are not implicated in the increased Core temperature seen in the ML phase despite an increase in production rate (Charkoudian, 2003; Charkoudian & Stachenfeld, 2016). Progesterone causes reduced cutaneous vasodilation, increased vasoconstriction and is thermogenic altering thermoregulatory control across the menstrual cycle (Charkoudian, 2003). The increase in progesterone could result in increased resting heart rate and basal core temperature which can increase the subjective feeling of exertion resulting and reduced performance (Sims & Heather, 2018). Charkoudian and Stachenfeld (2016) state that increased progesterone and reduced estradiol during the luteal phase results in around a 0.5°C increase in threshold temperature for vasodilation and sweating. Forearm skin temperatures have been seen to be higher in the luteal phase during exercise and also at rest in 50°C while skin blood flow was increased in the luteal phase at 35°C. It was suggested this was due to increased estradiol and progesterone altering the neuroendocrine, endocrine or paracrine

influence on cutaneous vasculature resulting in an altered vasoconstriction/ vasodilation response (Kolka & Stephenson, 1989; Kolka & Stephenson, 1997). This may also be due to changes in hypothalamic control of sudomotor and vasomotor activity (H. Lee et al., 2014). Heart rate, rating of perceived exertion (RPE), and the increase in core temperature during exercise may be higher in the ML phase compared to MF (Pivarnik et al., 1992). Whereas a recent study by Notley et al. (2018) showed no significant difference in dry and evaporative heat loss or body heat storage between EF and ML phases and no difference in the change in heart rate, core or skin temperature. Using the HTT, 67% of women and only 26% of male soldiers were found to be heat intolerant (Druyan et al., 2012). The authors suggest that finding 'normal' values and redefining heat tolerance for women may be necessary.

Oral contraceptives

Combined oral contraceptives replace endogenous hormones with a reduced, non-fluctuating circulating level of exogenous progestins and oestrogens. This results in lower fluctuations of core temperature with menstrual cycle phase but a regular menstrual cycle is maintained. Progesterone only contraceptives result in disruption to the menstrual cycle and regular menses cease (Sims & Heather, 2018). Grucza et al. (1993) found that contraceptive use did not significantly alter resting Core temperature or the threshold temperature for sweating but both values were significantly higher during the ML phase compared to the follicular phase of users and non-users of OC. Minahan et al. (2017) report that while the final core temperature reached by OC users was no different than non-users during the EF phase, increased RPE and prolonged increase in skin blood flow occurred in OC users. In well trained but unacclimated individuals, sweat rate, RPE and heart rate were unaffected by menstrual cycle phase or OC use during exercise (Sunderland & Nevill, 2003). Moreover, the effect that oral contraceptive has on hormonal changes is seen to be at its lowest in the break phase (Gagnon and Kenny, 2011).

Menopause

Menopause occurs when the supply of ovarian follicles is exhausted and is inevitable in all women (Barlow et al., 2005). Menopause has been reported to occur at the average age of 51 with 17% of female firefighters now reporting to either be perimenopausal or menopausal, with 39% indicating it has impacted their work (Watkins et al., 2019). When entering peri-menopause, ovarian functions begin to decline with large variation of abrupt increase or decrease of oestrogen and a rise in follicle-stimulating hormone, indicating a degree of feedback failure from ovarian hormones (Bachmann, 2005; Barlow et al., 2005). Due to this instability of oestrogen, it is thought to contribute to effect females physically with vasomotor symptoms (VMS), sleep disturbance, psychologically (mood disturbance, anxiety, irritability, memory, low libido) and somatically (fatigue, aches, pains) (Nelson et al., 2005; Cohen et al., 2006; Shaver and Paulsen, 1993). When oestrogen levels are depleted and consequently menstruation stops, the final menopause has occurred (Barlow et al., 2005).

VMS such as hot flushes and night sweats are the most common symptom of the climacteric and occurs in 80% of women (Deecher and Dorries, 2007). These flashes tend to be associated with an acute rise

in skin temperature, peripheral vasodilation, a transient increase in heart rate (7–15 b.min^{-1}) and fluctuations in electrocardiographic baseline (Sturdee et al., 1978; Freedman, 2014). Hot flushes begin with significantly higher Core temperature (36.82 ± 0.04°C) when compared to non-flush periods (36.70 ± 0.005°C) and it is recognised that hot flushes are preceded by a significant increase in Core temperature (Freedman et al., 1995). When Core temperature increases a heat dissipation response is trigger due to acting outside the thermoregulatory neutral zone (TNZ) (Freedman and Blacker, 2002). In symptomatic women, the temperature limits narrow from 0.4°C to 0.0°C due to the shivering threshold increasing and the sweating threshold lowering (Freedman et al., 1995; Freedman, 2001). Therefore, if Core temperature reaches the threshold, hot flushes (sweating and peripheral vasodilation) occur until Core temperature returns to the TNZ (Freedman, 2014). This is supported further with hot flushes having a circadian rhythm where flushes peak at 1825h, which is on average when core temperature peaks (Freedman et al., 1995; Waterhouse et al., 2005). Although Core temperature is seen to fluctuate during the menopause it has been identified that core temperature is significantly lower ($p < 0.001$) in post-menopausal women (0.25 ± 0.06 °C) when compared to pre-menopausal women

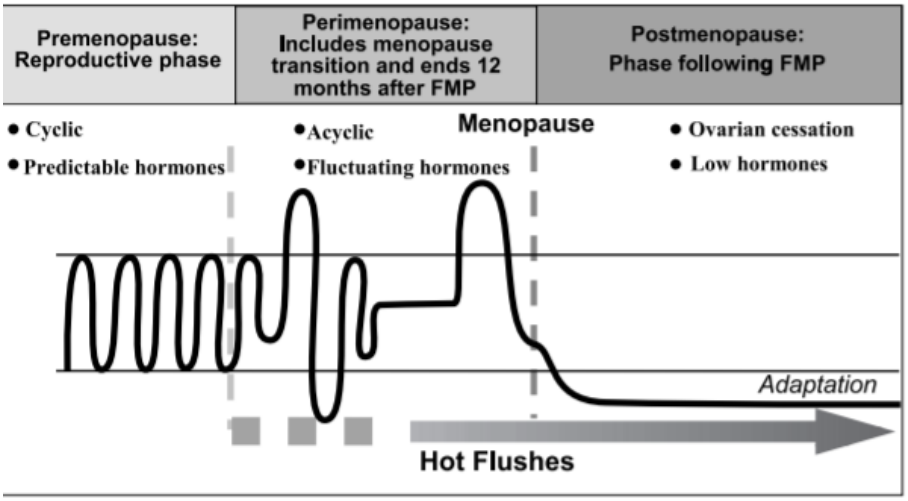

Figure 2: *Relationship between a woman's reproductive phases, oestrogen and the occurrence of hot flushes (Deecher and Dorries, 2007).*

VMS has been seen to decrease by 85% when using hormone replacement therapy (HRT; oestrogen/progestogen) (Bcerug et al., 1998). Moreover, it is has been shown that heart rate and Core temperature (~0.5°C) can be significantly reduced when using HRT (oestrogen) (Tankersley et al., 1992). HRT has also shown significant increase in Core temperature sweating threshold (37.98 ± 0.09°C to 38.14 ± 0.09°C) along with reduced hot flushes (1.4 ± 0.5 to 0.6 ± 0.6) due to the significant increase in oestradiol and decreased level of follicle stimulating hormone (Freedman and Blacker, 2002). Therefore, by increasing the Core temperature sweating threshold, the fluctuations in core temperature do not reach the threshold, and in turn do no cause heat dissipation responses associated with hot flushes (Freedman and Blacker, 2002).

Females and UHS

There is limited and conflicting data on women in UHS situations. With changes in core temperature being greater in the ML phase, no different or greater in the EF phase (Janse De Jonge et al., 2012; Lei et al., 2017; Tenaglia et al., 1999). Heart rate has been reported as higher during the ML phase or no different between phases during exercise in UHS (Lei et al., 2017; Tenaglia et al., 1999). Lei et al. (2017) and Tenaglia et al. (1999) found no difference in sweat rates between MC phases or with OC use while the same authors report no difference in skin temperatures and an increased skin temperature in the ML phase respectively. Tenaglia et al. (1999) report no significant difference in heart rate between phases, but Lei et al. (2017) recorded higher RPE during the ML phase. OC users appear to have more uniform thermoregulatory responses and heat tolerance times across the phases despite elevated baseline Core temperature during the quasi-ML phase (Tenaglia et al., 1999). Overall, it remains inconclusive what impact menstrual cycle phase, contraception or the menopause may have on heat tolerance in UHS.

1. **Table 1. Non-exhaustive summary of studies on female thermoregulation and heat tolerance**

STUDY	PARTICIPANTS	PROTOCOL	GROUPS	MAIN FINDINGS
CHARKOUDIAN & STACHENFELD, 2016 REVIEW	No oral contraceptives.	Light clothes.	Follicular v Luteal	•Baseline T_{re} L > F • T_{re} Threshold for sweating and vasodilation L > F
GRUCZA ET AL., 1993	n = 20. Oral contraceptives and no oral contraceptives. Mean age 21.3 ± 0.6 yrs and 22 ± 1.7 yrs.	20 min rest + 45 min cycle at 50% VO2 peak. 24°C, 50% RH. Light clothes.	Follicular (5-8) v Luteal (18-24) and Oral contraceptives v No oral contraceptives	•OC - T_{re} baseline L > F - ΔCore temperature no difference L v F - ΔT_{sk} no difference L v F - Sweating gains L > F •NOC - T_{re} baseline L > F - Δ T_{re} F > L - - T_{sk} baseline no difference L v F - ΔT_{sk} F > L
JANSE DE JONG ET AL., 2012	n = 12. No oral contraceptives. Mean age 23.7 ± 4.1 yrs.	60 min cycle at 60% VO2 peak + incremental test to exhaustion. 20°C, 45% RH and 32°C, 60% RH. Light clothes.	Hot v Temperate and Early-follicular (3-6) v Mid-luteal (19-25)	• T_{re} baseline & HR baseline L > F •Temperate - No difference in performance L v F - T_{re} submax exercise L > F •Hot - Time to fatigue F > L - T_{re} submax exercise L > F - Δ T_{re} no difference L v F - final T_{re} no difference L v F - HR & RPE submax exercise L > F - HR at exhaustion no difference L v F

KOLKA & STEPHENSON 1989	n = 7. No oral contraceptives. Mean age 26.6 ± 3.6 yrs.	180 min passive exposure + ~ 9 min cycle at 80% VO2 peak in 50°C, 14% RH and 20 min passive exposure + 35 min cycle at 85% VO2 peak in 35°C, 25% RH. Light clothes.	Follicular (4-7) v Luteal (19-22)	• Toes baseline L > F • Toes threshold for sweating L > F • T_{sk} at 50°C L > F
KOLKA & STEPHENSON 1997	n = 5. No oral contraceptives. Mean age 27.6 ± 4.4 yrs	30 min cycle at 80% VO2 peak. 35°C, 22% RH. Light clothes.	Early-follicular (3-6) v Mid-luteal (19-22)	• Toes at rest and during exercise L > F • T_{sk} at rest L > F • HR baseline no difference
KUWAHARA ET AL., 2005	n = 17. No oral contraceptives. Trained and untrained. Mean ages 22.1 ± 0.3 yrs and 20.0 ± 0.4 yrs.	30 min cycle at 50% VO2 peak. 25°C, 45% RH. Light clothes.	Trained v Untrained and Mid follicular (6-9) v Mid-luteal (19-22)	• Untrained & Trained - Toes baseline and exercising during L phase U > T - HR and Tsk no difference L v F • Trained- Toes, ΔToes and sweat response no difference L v F • Untrained- Baseline Toes L > F - Toes during exercise and ΔToes L > F - Sweat response F > L
LEE ET AL., 2014	n = 8. No oral contraceptives. Inactive. Mean age 25.0 ± 1.9 yrs.	40 mins passive heat exposure. 41°C, 21% RH. Light clothes.	Follicular (6-9) v Luteal (21-24)	• T_{sk} baseline & through heat exposure L > F • SR L > F

LEI ET AL., 2017	n = 10. No oral contraceptives. Well trained. Mean age 34 ± 9 yrs.	12 mins fixed intensity + 30 mins self-selected intensity cycle. 29°C, 81% RH. Light clothes.	Early-follicular (3-6) v Mid-luteal (18-21)	• T_{sk}, local SR, exercise performance, TS, RPE no difference L v F • T_{re} at rest & exercising L > F • Δ T_{re} F > L
MARSH & JENKINS, 2002 REVIEW	No oral contraceptives.	Light clothes.	Follicular v Luteal	• T_{re} baseline & exercising in ambient & hot L > F • SR L > F • T_{sk} baseline and exercising L ≥ F • HR, RPE baseline & exercising no difference L v F
MINAHAN ET AL., 2017	n = 16. Oral contraceptives and no oral contraceptives. Recreationally active. Mean age 22 ± 4 yrs.	52.5 mins passive exposure + 90, 135 & 180% lactate threshold. 22°C and 35°C. RH not given. Light clothes.	Oral contraceptives (active pill phase) v No oral contraceptives (2-6)	•Toes baseline OC > NOC • Final Toes no difference OC v NOC • HR no difference OC v NOC • Final RPE OC > NOC • Skin blood flow elevated for longer in OC v NOC
NOTLEY ET AL., 2018	n = 12. No oral contraceptives. Active. Mean age 21 ± 3 yrs.	30 min at 40, 55 & 70% VO2 peak + 15 mins rest. 40°C, 15% RH. Light clothes.	Early-follicular (~4) v Late follicular (~10) v Mid-luteal (~22)	• Toes baseline L > F • ΔToes, T_{sk} & HR no difference L v F
PIVARNIK ET AL., 1992	n = 9. No oral contraceptives. Active. Mean age 27.2 ± 3.7 yrs.	60 min cycle at 65% VO2 peak. 22°C, 60% RH. Light clothes.	Mid follicular (~7) v Mid-luteal (~21)	• T_{re} baseline & final L > F • HR & final RPE L > F • SR & T_{sk} no difference L v F

STEPHENSON & KOLKA, 1985	n = 4. No oral contraceptives. Mean age 29.5 ± 4 yrs.	30 min cycle at 60% VO2 peak. 35°C, 1.73kPa H2O vapour pressure. Light clothes.	Follicular (4-7) v Luteal (18-24)	• Toes & HR baseline L > F • HR exercising no difference L v F • Toes threshold for sweating & vasodilation L > F
SUNDERLAND & NEVILL, 2002	n = 15. Oral contraceptives and no oral contraceptives. Well trained. Mean age 20.3 ± 0.4 yrs.	High intensity intermittent running to exhaustion. 31°C, 23% RH. Light clothes.	Mid follicular (~7) v Mid-luteal (~21) and Oral contraceptives v no oral contraceptives	•NOC - Core temperature, HR, SR, RPE no difference L v F • OC - Core temperature L > F - HR, SR, RPE no difference L v F
TENAGLIA ET AL., 1999	n = 18. Oral contraceptives and no oral contraceptives. Recreationally active. Mean ages 23.3 ± 1.9 yrs and 23.4 ± 0.7 yrs.	75 - 225 minutes intermittent light exercise. 40°C, 30% RH. NBC clothing.	Oral contraceptives v no oral contraceptives and Early-follicular (2-5) v Mid-luteal (19-22)	•OC & NOC - Core temperature baseline & exercising L > F - HR, SR no difference L v F • NOC - ΔT_{re} F > L - Final T_{re} no difference L v F - T_{sk} baseline & exercising L > F • OC - ΔT_{re} no difference L v F - T_{sk} baseline & exercising no difference L v F

Bracketed numbers denote days of the menstrual cycle. L = luteal phase, F = follicular phase, OC = oral contraceptives, NOC = no oral contraceptives, HR = heart rate, SR = sweat rate, RPE = rate of perceived exertion, TS = thermal sensation, U = untrained, T = trained, Core temperature = T_{re}, T_{oes} = oesophageal temperature, T_{sk} = mean skin temperature, NBC = nuclear, biological and chemical

Study Aim

The aim of the study was to assess differences in heat tolerance between females who taking oral hormone contraception or not, and at different phases (early follicular and mid luteal) of the menstrual cycle. The second part of the study investigated thermoregulatory responses in menopausal women.

METHODS

Participants

Thirty females (age: 33 ± 13 years, height: 1.66 ± 0.05 m, body mass: 66.6 ± 8.7 kg) participated in the study. Thirteen were active firefighters, 17 were staff and students recruited from the University of Brighton. Nine participants were taking oral contraceptives (OC), whilst five participants self-reported as peri-menopausal or menopausal (MP). Of those in the MP group, two were using HRT. See table 1 for participant characteristics. Participants were required to complete a menstrual cycle questionnaire, a medical questionnaire and provide written consent prior to testing. The study was approved by the University of Brighton Life Sciences Ethics Committee. Participants were requested to avoid caffeine for 12 hours prior to testing and alcohol, exhaustive exercise and heat exposure > 25^{o}C for 24 hours before testing.

Table 2. Participant characteristics for each group: menopause (MP) (n = 5), those not using (NOC) (n = 16) and using (OC) (n = 9) oral contraceptives. *denotes significant difference from NOC.

	NOC	**OC**	**MP**
Age (years)	33 ± 12	23 ± 6 *	51 ± 6 *
Height (m)	1.67 ± 0.05	1.64 ± 0.06	1.66 ± 0.06
Body Mass (kg)	69.2 ± 8.2	64.2 ± 7.2	62.5 ± 11.2
Body Mass Index	24.8 ± 3.1	23.9 ± 2.9	22.6 ± 3.2

Experimental Design

Participants with regular menstrual cycles completed the menstrual cycle questionnaire and were subsequently invited for testing during days 1-6 (EF phase) and days 18-22 (ML phase) of a 28 day cycle (Grucza, et al., 1993; Marsh & Jenkins, 2002). Order of attendance was randomised. Menopausal participants were required to attend on one occasion at their convenience. Participants were provided with full personal protective equipment (PPE) or could bring their own in the case of firefighters. All trials took place in the morning between 7.30am and 11.30am. Those completing two visits did so at the same time of day to control for alterations in body temperature due to circadian rhythms (Drust et al., 2005). Each trial consisted of an implementation phase followed by a Heat Occupational Tolerance

Test (HOTT). Those with regular cycles who were not using oral contraceptives (NOC) also provided a venous blood sample prior to the HOTT for confirmation of menstrual cycle phase. Participants were required to be sufficiently hydrated prior to each trial. Euhydration was determined as urine colour ≤ 3, osmolality < 700 mOsm.kgH_2O^{-1} (Pocket Pal-Osmo, Vitech Scientific Ltd., UK) and specific gravity < 1.020 (hand refractometer, Atago Co., Tokyo, Japan) (Sawka et al., 2007).

Blood Sampling

A venous blood sample (10mL) was withdrawn from the anti-cubital fossa by a trained phlebotomist into EDTA tubes. Samples were centrifuged at 5000rpm and aliquoted into 1.5mL microtubes for storage at -80°C. Samples were subsequently analysed for Progesterone and 17β-Estradiol via enzyme-linked immunosorbent assays (Abcam, Cambridge, UK).

Heat Occupational Tolerance Test

Participants donned firefighter PPE consisting of a boiler suit (or similar), trousers and jacket (Ballyclare Special Products Ltd.), fire hood (MSA Gallet, Bellshill, UK) and boots (9005 Jolly Scarpe, USA). A 10-minute seated rest period in ambient temperature (23.5 ± 1.2ºC, 26 ± 4% RH) followed at the end of which baseline measures were taken. Participants then donned the helmet (F1SF, MSA Gallet, Bellshill, UK) and gloves (Firemaster 3, Southcombe Brothers Ltd., Somerset, UK) and entered the heat chamber at 50.0 ± 0.4ºC and 12 ± 2 % RH to walk continuously for 40 minutes. The initial speed was set at 4.5 km.h^{-1} and increased every 5 minutes for the first 15 minutes to achieve a metabolic heat production (H_{prod}) of 6 W.kg^{-1} to limit the effects of interindividual variances in body composition and aerobic fitness (Watkins et al., 2018). For the remaining 25 minutes speed was maintained with small adjustments of 0.2 km.h^{-1} used to maintain the required H_{prod}. Ventilatory gases were collected continuously throughout the 40-minute heat exposure using 30 second averaging of a Metalyzer Sport analyser (Cortex, Leipzig, Germany). Oxygen consumption ($\dot{V}O_2$) and respiratory exchange ratio (RER) were recorded each minute and used to calculate H_{prod} (Equation 1) (Cramer and Jay, 2014).

Physiological Measurements

Participants were weighed nude (Adam GFK 150 Body Scales, Connecticut, USA) before and after each trial to calculate whole body sweat rate. Participants were weighed in full kit prior to testing to allow for calculation of H_{prod}, PPE weighed 6kg. Core temperature (T_{re}) was measured using a Henley single use rectal temperature probe (449H, Henleys Medical, Hertfordshire, UK) inserted 10cm past the anal sphincter and recorded via a logging monitor (4600 series, YSI, Hampshire, UK). Skin temperature was measured using contact skin thermistors attached to the belly of the pectoralis major, triceps brachii, rectus femoris and gastrocnemius and recorded on a Squirrel Data Logger (1000 series, Grant Instruments, Cambridgeshire, UK), with mean skin temperature (T_{skin}) calculated as per Equation 2 (Ramanathan, 1964). A heart rate monitor was also affixed to the chest (FT1, Polar Electro, Kempele,

Finland). T_{re} and heart rate (HR) data were used to calculate Physiological Strain Index (PSI), as per Equation 3 (Moran, Shitzer and Pandolf, 1998). Temperature measurements and heart rate were recorded following the 10-minute rest period and every 5 minutes throughout exercise.

Equation 1

$$\dot{M}(Watts) = \dot{V}O_2 \frac{(\frac{RER - 0.7}{0.3} e_c) + (\frac{1 - RER}{0.3} e_f)}{60} \text{ x } 1000$$

$$\dot{H}_{prod}(W.kg^{-1}) = (\dot{M} - \dot{W})/\text{BodyMass}$$

Equation 2

$$\mathrm{T_{skin}}(°\mathrm{C}) = 0.3(\mathrm{T_{chest}} + \mathrm{T_{upperarm}}) + 0.2(\mathrm{T_{upperleg}} + \mathrm{T_{lowerleg}})$$

Equation 3

$$\mathrm{PSI} = 5(\mathrm{T_{re}t} - \mathrm{T_{re0}}) \cdot (39.5 - \mathrm{T_{re0}})^{-1} + 5(\mathrm{HRt} - \mathrm{HR_0}) \cdot (180 - \mathrm{HR_0})^{-1}$$

Where $T_{re}t$ and HR_t were concurrent measurements taken every 5 min during the protocol, and T_{re0} and HR_0 represent baseline states.

Perceptual Measures

Perception of effort was measured using the Borg rating of perceived exertion (RPE) on a scale of 6-20 (1982). Thermal sensation was assessed using a scale (TS) from 0 'unbearably cold' to 8 'unbearably hot' (Young et al., 1987). Measures were recorded after the 10-minute rest period and every 10 minutes throughout exercise. A Heat Illness Symptoms Index (HISI) was completed before and after exercise. The HISI contains 13 heat illness symptoms rated by the participant on a scale from 0 'no symptoms whatsoever' to 10 'so bad I have to stop', with a maximum score of 130 (Coris et al., 2006).

Statistical Analysis

Data were analysed using IBM SPSS 26. Parametric data presented as mean ± standard deviation, non-parametric data presented as median ± interquartile range. Significance was set at $p < 0.05$. Analysis was conducted in three stages: difference between phases within the oral contraceptive (OC) group) and within the non-oral contraceptive (NOC) group, difference in phases between NOC and OC, and difference between those in the NOC EF and those who were menopausal.

Stage 1: EF vs ML

A paired samples t- test was used to identify differences in sweat rate between phases. A two-way repeated measures ANOVA was implemented to determine significant differences in physiological measures between menstrual cycle phases over time. Non-parametric data (RPE, TS, HISI) was analysed for differences over time using Friedman's ANOVA. Bonferroni corrected paired samples t-test (parametric data), or Wilcoxon's signed rank test (non-parametric) were conducted as follow up tests to identify where differences occurred. Differences between phases for RPE, TS and HISI were assessed using Bonferroni corrected Wilcoxon's signed rank tests, with significance set at $p < 0.01$ for RPE and TSS and $p < 0.025$ for HISI. Analysis was repeated comparing NOC EF to NOC ML.

Stage 2: NOC vs OC & Stage 3: Menopause vs NOC EF

Independent samples t tests were utilised to determine if significant differences were present in participant characteristics for NOC vs OC groups and Menopause vs NOC EF groups. Two-way mixed methods ANOVA were conducted to determine differences in physiological measures between NOC and OC over time in each phase, and between Menopause and NOC EF. Perceptual measures were compared between group pairs using Bonferroni corrected Mann-Whitney U tests, with significance set at $p < 0.01$ for RPE and TSS and $p < 0.025$ for HISI.

RESULTS

Menstrual cycle phase was confirmed in 14/19 NOC participants via blood sample analysis. Levels of 17β-Estradiol and progesterone were greater in the ML phase in comparison to the EF phase ($p = 0.003$ and $p < 0.001$, respectively).

Table 3. Estradiol and progesterone hormone levels measured in the early follicular and mid luteal phases of the menstrual cycle. Reference ranges sourced from Stricker *et al.*, (2006). * denotes a significant difference between the two phases $p < 0.05$.

	17β-Estradiol (pmol/L)		**Progesterone (nmol/L)**	
	Reference Range	**Measured**	**Reference Range**	**Measured**
Early Follicular	50 – 359	124 ± 70	0.3 – 4.8	4.2 ± 2.7
Mid Luteal	252 - 1163	319 ± 175*	14.6 – 71.9	38.3 ± 22.4*

OC EF vs ML

There was no difference in sweat rate between EF and ML phases ($p = 0.208$). Over time core temperature, HEART RATE and PSI all significantly increased ($p < 0.001$). However, this variation over time was not affected by menstrual cycle phase ($p = 0.281$, $p = 0.913$, $p = 0.786$, respectively). Skin temperature also increased throughout the duration of the HOTT ($p < 0.001$), with an interaction effect between time and phases indicated ($p = 0.008$), however follow up tests were unable to identifying where differences occurred ($p > 0.005$).

RPE increased over time ($p < 0.001$) with all time points being greater than resting levels ($p < 0.001$) other than at 10 min in both the EF and ML phases ($p = 0.065$ and $p = 0.442$, respectively). There was no difference between phases at any time point ($p > 0.05$). TS followed a similar pattern, with increases over time ($p < 0.001$) and all time points being greater than baseline ($p < 0.05$) other than at 10 min in EF and ML phases ($p = 1.00$ and $p = 0.442$, respectively). In addition, there was no difference between phases for any time point ($p > 0.05$). HISI increased in both phases from pre to post the HOTT ($p < 0.001$), however there were no differences between phases at rest ($p = 1.00$) or post trial ($p = 0.782$).

NOC EF vs ML

Whilst core temperature, heart rate, PSI and Skin temperature increased over time ($p < 0.001$), this change was not different between NOC EF and NOC ML ($p = 0.073$, $p = 0.171$, $p = 0.778$ and $p = 0.981$). There was also no difference in sweat rate between the phases ($p = 0.089$).

In both the NOC EF and ML phases, RPE increased throughout the HOTT ($p < 0.001$), rising above baseline from 30min onwards ($p < 0.05$). There was no difference between the phases at any time point ($p > 0.05$). TSS also increased over time ($p < 0.001$), being greater than baseline from 20min onwards

($p < 0.05$) for both EF and ML in the NOC group. No differences were noted in TSS between the NOC EF and ML phases at any time point ($p > 0.05$). HISI increased in both the NOC EF ($p = 0.008$) and ML ($p = 0.008$) phases, but there were no differences between the phases ($p > 0.05$).

NOC vs OC

Participants were significantly younger in the OC group ($p = 0.009$), however there were no differences in height ($p = 0.141$), weight ($p = 0.152$) or body mass index ($p = 0.488$) (see Table 2).

EF

ANOVA results indicated group had a significant effect on core temperature increase over time ($p = 0.013$), however Bonferroni follow up tests were unable to identify any differences ($p > 0.05$). Changes in HR, PSI and Skin temperature were not affected by group ($p = 0.512$, $p = 0.372$ and $p = 0.347$). Sweat rate was also not different between the NOC and OC groups ($p = 0.256$). There were also no differences between groups for RPE ($p > 0.01$) or HISI ($p > 0.025$). TSS was greater in the OC group at 10 min only ($p = 0.003$).

ML

Similar to the EF phase, in the ML phase group had a significant effect on core temperature increase over time ($p = 0.019$) with follow up tests unable to identify differences ($p > 0.05$). There were also no differences between groups for heart rate ($p = 0.509$), PSI ($p = 0.561$) and Skin temperature ($p = 0.667$). In addition, sweat rate was similar between NOC and OC in the ML phase ($p = 0.130$). There were also no differences in RPE ($p > 0.01$), TSS ($p > 0.01$) or HISI between the groups.

Menopause vs NOC EF

Participants were significantly younger in the NOC group ($p = 0.001$), with no differences in height ($p = 0.160$), weight ($p = 0.640$) or body mass index ($p = 0.226$) (see Table 1). One menopausal participant withdrew from the HOTT early at 34 min due to the onset of a hot flush. The participant who experienced a hot flush had a core temperature at 30min (38.04°C) similar to the mean core temperature exhibited by the rest of the menopause group (37.98 ± 0.42°C) and the NOC EF group (38.18 ± 0.34°C). Data analysis comparing the remainder of the participants to the NOC EF group revealed no impact of group on the increase in core temperature ($p = 0.66$),heart rate ($p = 0.538$), PSI ($p = 0.455$) and Skin temperature ($p = 0.740$). There was also no difference in sweat rate ($p = 0.456$). In addition, RPE, TS and HISI followed a similar pattern of increase between the groups ($p > 0.0$, $p > 0.01$ and $p > 0.025$).

Heat Tolerance Classification

Participants were classified according to the heat tolerance continuum in green, yellow and red zones as set out by Watkins et al., (2018) and displayed in Figure 3. In the EF phase, of the 16 NOC participants, only one was classified in the green zone ($T_{re} < 38.0$°C), with the majority (13/16 [81%]) in the red zone ($T_{re} > 38.5$°C). The number of OC participants in the green zone was similar to the NOC group (1),

however the split of the remaining participants between the red and yellow classification was equal (4/9 [44%]). The menopause group also had an equal split between red and yellow zones (2/4 [50%]).

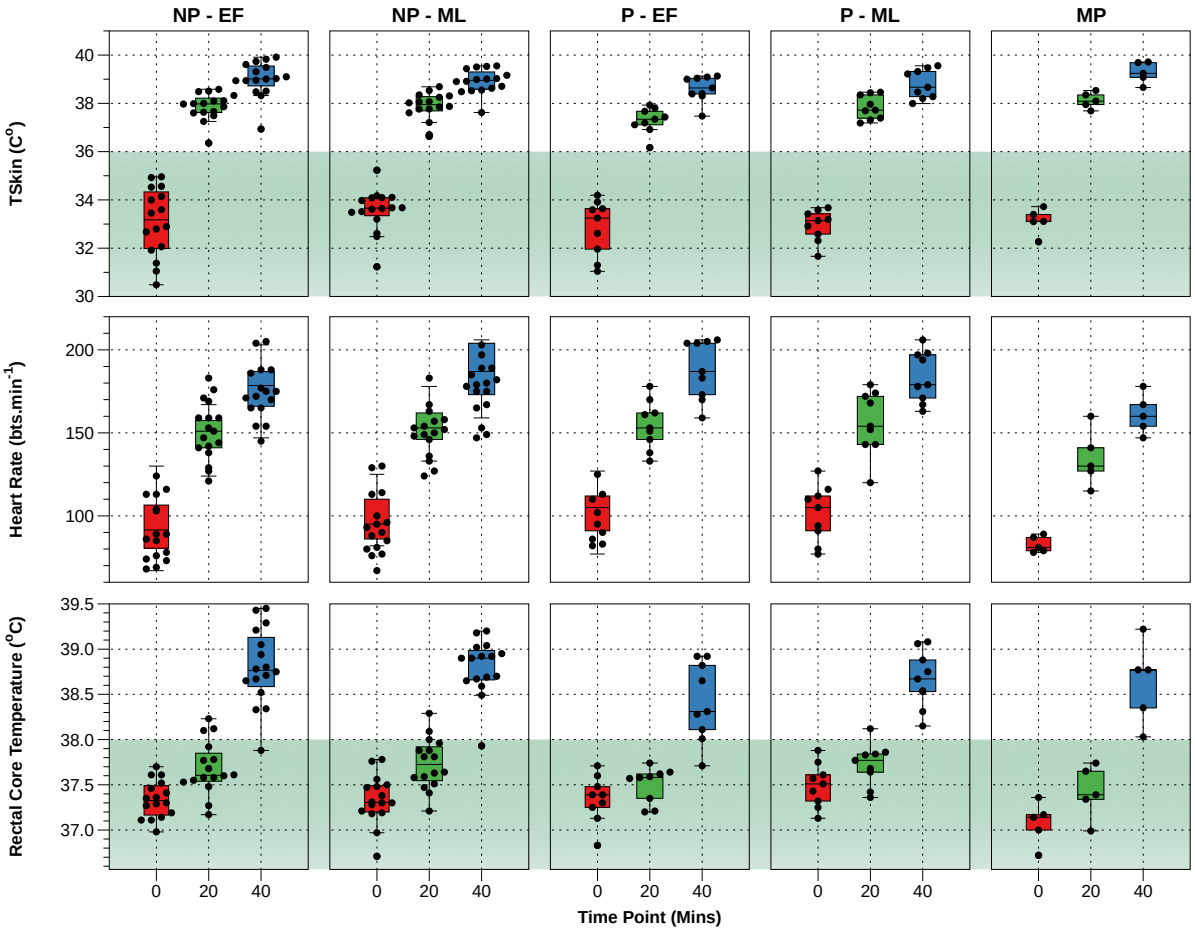

Figure 3: Rectal core temperature, heart rate and skin temperature at 0, 20 and 40 min time points for all four groups [NP-EF (No oral contraceptive pill – Early Follicular phase), NP-ML (No oral contraceptive pill – Mid-Luteal phase), P-EF (Oral contraceptive pill - Early Follicular phase), P-ML (Oral contraceptive pill – Mid-Luteal phase), MP (Menopause)].

Discussion

Menstrual Phases and Oral Contraceptive Pill

The aim of this study was to determine whether there was any variation in thermoregulation and heat tolerance responses in females between the EF and ML phases in both users and non-users of oral contraceptives, and between users and non-users. Overall, the results indicated no substantial difference in physiological and perceptual measures between both EF and ML groups in users and non-users, or between NOC and OC which did not support the initial predictions.

Physiological measures

There was no significant difference found for baseline core temperature in this study between EF and ML in both OC and NOC, which is opposing to the bulk of aforementioned studies (Grucza et al., 1993; Inoue et al., 2005; Janse et al., 2012; Lei et al., 2017; Pivarnik et al., 1992; Tenaglia et al., 1999). In NOC, resting core temperatures are anticipated to be higher (~0.2-0.5ºC) during the luteal phase due to greater plasma concentrations of progesterone released after ovulation occurs, this hormone has a central thermogenic effect elevating body temperature compared to the follicular phase (Charkoudian and Stachenfeld, 2016; Constantini et al., 2005). This study parallels Sunderland and Nevill (2003) indicating no differences in resting core temperature between MC phases in non-users.

There are comparatively fewer studies including OC use and menstrual cycle phases, but in users akin to endogenous sex hormones, exogenous oestrogen and progestins have shown to influence thermoregulatory processes, expecting similar higher ML phase baseline rectal temperatures compared to EF as non-users (Charkoudian and Johnson, 1997; Grucza et al., 1993), found in studies by Grucza et al (1993) (Follicular - 37.52 v Luteal - 37.81ºC) and Tenaglia et al (1999) (Follicular - 37.14 v Luteal - 37.36ºC). However, Tenaglia et al (1999) and Gruzca et al (1993) indicated that resting core temperature was not different between OC users and NOC, in accordance with this study. Minahan et al (2017) displayed resting core temperature was higher in OC than NOC, however oesophageal temperature was utilised rather than core temperature and only the follicular phase was investigated. The present study's findings may be explained by study design variation, resting measures were noted after a 10-minute rest in full PPE, with only one previous study utilising NBC clothing (Tenaglia et al., 1999), which may have influenced core temperature. A longer period of passive rest prior to exposure may be required to prevent the potential masking of differences between phases especially with the usage of PPE at rest (McLellan, 1993; Sunderland and Nevill, 2003). The universal agreement that baseline core temperature is higher in ML phase may not be as robust as believed. With no differences found in baseline core temperature between phases or between NOC and OC, it could imply no group had a thermoregulatory disadvantage initially.

In accordance to previous research, no significant difference was found in final core temperatures irrespective of menstrual cycle phase or OC use (Janse et al., 2012; Kuwahara et al., 2005; Minahan et al., 2017; Tenaglia et al., 1999), contradicting others that final core temperature persists higher in the

ML phase (Lei et al., 2017; Pivarnik et al., 1992) (+ ~0.2-0.3ºC in both studies). Kuwahara et al (2005) highlights that extreme activity or heat stress could mask the MC effect on core temperature and other physiological measures. This could be of particular interest in this study due to the UHS environment exposed by extreme heat and PPE; the effect of increased dehydration, heat storage and lack of heat dissipation mechanisms exposed in all groups may produce similar end core temperatures regardless of MC phase or OC use (Cheung et al., 2000).

Literature is contradicting regarding core temperature change in menstrual cycle phases of NOC, with studies finding no differences (Notley et al., 2018), larger increases in ML (Janse et al., 2012) or greater increases in EF (Grucza et al., 1993; Lei et al., 2017; Tenaglia et al., 1999), with no difference in core temperatures change found between ML and EF in non-users in this study. No differences were found between ML and EF in OC, supporting previous literature (Grucza et al., 1993; Tenaglia et al., 1999). This may be due to the assumption that OC use makes thermoregulatory responses during UHS more uniform across phases, it may not result in resetting of the thermoregulatory setpoint as believed to happen during ML phase of non-users (Cheung et al., 2000; Grucza et al., 1993). Sunderland and Nevill (2003) highlighted a greater increase in core temperature in NOC compared to OC (3.4ºC v 2.6ºC h^{-1} respectively).

In accordance with a range of present literature, no significant differences were present for baseline, final or heart rate change between any groups (EF v ML and OC v NOC) (Kuwahara et al., 2005; Kolka and Stephenson, 1997; Marsh and Jenkins, 2002; Minahan et al., 2017; Notley et al., 2018; Sunderland and Nevill, 2003; Tenaglia et al., 1999). It may be expected that all measures of heart rate would be higher in the luteal phase compared to the follicular, as seen in a few studies (+ ~10bpm) (Janse et al., 2012; Pivarnik et al., 1992), due to the increased plasma volume brought about by progesterone, this can increase cardiovascular strain by increased core temperatures and minute ventilation, consequently increasing heart rate (Godbole et al., 2016). No differences have been documented in research between OC and NOC for any measure of heart rate. As previous studies were conducted in relatively lower heat than the present study, the UHS environment could explain similar heart rates observed in all groups due to the excessive cardiovascular demand placed on the body from extreme heat and PPE regardless of OC use or MC phase (Cheung et al., 2010).

No significant differences were discovered in sweat rate between groups (EF v ML and NOC v OC) in agreement with previous research (Kolka and Stephenson, 1997; Sunderland and Nevill, 2003; Tenaglia et al., 1999). Literature is contradicting regarding this variable, a higher sweat rate during the follicular compared to luteal phase is documented in both NOC and OC (Charkoudian and Stachenfeld, 2016; Grucza et al., 1993; Kuwahara et al., 2005) with some documenting higher rates in the luteal phase in NOC (Petrofsky et al., 2017; Marsh and Jenkins, 2002) with no differences found between NOC and OC (Tenaglia et al., 1999). It would be expected that a greater sweat rate would be observed during the follicular phase due to a lower threshold temperature for the onset of vasodilation and sweating, caused by reduced progesterone concentrations operating on the warm-sensitive neurons in the PO/AH (Kolka

and Stephenson, 1989). Evaporation is the primary avenue for heat loss whilst exercising, in UHS, sweat does not readily evaporate with much of this being absorbed into the PPE, thus an increased sweat rate is not beneficial to performance in these situations compared to cooler temperatures (Petrofsky et al., 2005). Elevations in metabolic heat production from added weight and decreased evaporative efficiency impacts the thermoregulatory system by the inability to dissipate heat generated (Cheung et al., 2000), suggesting reason for the observed similar strain found between all groups regardless of phase or OC use.

No significant differences were found in either baseline or final skin temperatures between any groups. It would be expected that both resting and final skin temperature would be elevated during the luteal phase concurrent with core temperature as the onset of vasodilation and sweating is delayed (Marsh and Jenkins, 2002). This has been shown in some studies in NOC and OC for baseline skin temperature (Petrofsky et al., 2017; Tenaglia et al., 1999; Kolka and Stephenson, 1997), and some documenting no phase differences (Avellini et al., 1980; Grucza et al., 1993; Tenaglia et al., 1999). Final skin temperatures have shown no differences between phases in OC and NOC (Grucza et al., 1993; Kolka and Stephenson, 1997; Lei et al., 2017; Notley et al., 2018; Pivarnik et al., 1992) and higher temperatures in the luteal phase (Kolka and Stephenson, 1989; Marsh and Jenkins, 2002; Petrofsky et al., 2017; Tenaglia et al., 1999). With no differences reported in research between NOC and OC groups. The lack of difference in this study could possibly be due to UHS, the rise in core temperature causes a vasodilation response causing increased skin temperatures due to an increase in skin blood flow to the periphery for heat loss mechanisms (Charkoudian and Johnson, 2000). Sympathetic cholinergic nerves elicit sweating from the eccrine sweat glands, with sweating being the most effective way to remove heat from the body during hyperthermia which cools the skin as it evaporates (Petrofsky et al., 2017). However, due to PPE causing an obstructive barrier between the skin and the external environment, sweat is not dissipated sufficiently (Cheung et al., 2010). This could cause similar skin temperatures regardless of MC phase and OC use due to the lack of heat dissipation. It's suggested the sensitivity of heat loss responses during moderate exercise varies according to MC phase to a larger degree in a temperate environment than under excessive heat stress, shown in this study that intense environmental conditions could mask menstrual cycle or OC use effects on thermoregulatory responses usually found in moderate/compensable heat stress (Kuwahara et al., 2005).

Perceptual measures

There were no significant differences in final RPE between MC phase or group, which is in accordance with previous research (Lei et al., 2017; Marsh and Jenkins, 2002; Sunderland and Nevill, 2003). It might be expected that in both NOC and OC, final RPE would be higher in the ML phase compared to EF due to the proposed thermoregulatory disadvantage throughout heat exposure eliciting a greater perceived exertion (Constantini et al., 2005). Pivarnik et al (1992) and Janse de Jong et al (2012) both reported higher RPE scores in the luteal compared to the follicular phase. Minahan et al (2017) reported a significantly higher RPE in OC users compared to NOC. Previous studies evaluating perceptual

measures have differing protocols, including light clothing, cooler temperatures and various intensities compared to this study. Participants were not confined to a fixed metabolic heat production thus aerobic fitness and body composition could have affected results (Cramer and Jay, 2014). Overall, no difference was found in final RPE in this study indicating no group experienced greater perceived exertion, which could be due to the UHS environment exposure from PPE and excessive heat (50ºC) as this may induce similar excessive thermoregulatory strain in line with perceptual stress irrespective of MC phase and OC use (Cheung et al., 2000).

No difference was found for baseline, final TSS or TSS change between OC and NOC groups or ML and EF phase, in accordance with Lei et al (2017). Similar to RPE, it may be expected that due to different concentrations of progesterone and Estradiol would instigate a higher TSS in the luteal phase due to progesterone inducing a thermogenic effect, causing a higher subjective perceptual response (Charkoudian and Stachenfeld, 2011). Importantly again, the design of this study inducing a state of UHS could potentially mask any variation in thermal sensation that could be experienced between groups of MC phase and OC use that may be found at lower temperatures. UHS causes an incapable maintenance of the body's thermal steady state, possibly causing similar perceptual scores (Montain et al., 1994). The TSS used in this study is more sensitive that other popular scales used (Wang and Hu, 2016), thus a difference would be expected to be found if significant.

Menopause and non-menopause comparison

Separately, the study aimed to evaluate the differences in menopausal and non-menopausal individuals. The findings demonstrated no physiological or perceptual differences in response to severe heat stress.

Due to low availability of participants to undertake the testing, comparisons could only be made from a small sample of menopausal and perimenopausal individuals. The tolerance test is particularly challenging so to find individuals willing to undertake it and at this stage in their lives is difficult. When comparing the menopause group findings against that of the main cohort, we must be conscious of the sample size and conclusions made from it.

Physiological measures

There were no differences in physiological responses between the menopause and non-menopausal participants. Physiological increases were in line with that seen and reported above. A previous study demonstrated core temperature to be lower in post-menopausal women at rest (0.28± 0.08°C) and similar differences were seen during exercise (p<0.05) when compared to pre-menopausal women of equal aerobic fitness levels (Neff et al., 2016). This was different in the present investigation; results indicated no difference in core temperature between pre and post-menopausal groups. Firstly, this difference can be down to the variation of core temperature seen during a menopausal hot flush, which may have occurred in the post-menopausal group due to the extreme heat. Hot flushes begin with significantly

higher core temperature (36.82 ± 0.04°C) then non-flash periods and core temperature can be seen to drop low after the flush (Freedman et al., 1995). However, due to one individual on HRT having reported that their hot flushes were reduced by 95%, this oscillation in core temperature would not be seen in these individuals (Freedman, 2014). HRT has also been reported to cause a decreased core temperature at rest and during exercise (Stephenson et al., 1993). This decrease in core temperature (0.44°C, $p = 0.004$) through an earlier onset of cutaneous vasodilatory system (0.29°C, $p = 0.01$) may increase the individual's tolerance to heat (Brooks-Asplund et al., 2000). As half of the participants included in statistical analysis in the post-menopausal group are on HRT this could cause a large variation in the group and be an indicator of the non-significant difference seen in this study.

No difference in heart rate was noted in this study between groups. In previous research it has been identified that heart rate declines with age (heart rate $_{max}$= 209.39-0.776age, $r = 0.71$) indicating that those who are post-menopausal should have a lower heart rate than those who are pre-menopausal (Stephenson et al., 1993). However, it has been seen that highly trained individuals have a significantly lower heart rate $_{max}$ by 5 $b.min^{-1}$, than sedentary individuals of the same age (Whyte et al., 2008). Whilst all participants regularly completed physical activity (≥3 times a week), aerobic fitness was not directly measured and therefore may be a confounding variable in this study.

Skin temperature was shown to increase from pre to post test, yet no differences were reported between groups. Little research shows the difference in Skin temperature between pre and menopausal women. Although in a study looking at the increase of age and final Skin temperature when exercising in 35°C for 25 minutes, a significant increase was found (Drinkwater and Horvath, 1979). However, when the temperature is raised to 48°C, which is similar temperatures to our study, no significance was found, and Skin temperature remained similar across all ages. Nevertheless, there is seen to be an acute rise in skin temperature during menopausal hot flushes which coincided with the increase in sternal skin conductance which is used when defining hot flushes (Freedman, 2001). Although this increase is seen during hot flushes, studies have shown no significant difference in Skin temperature between individuals who take HRT (34.16 ± 0.15 °C) compared to individuals who do not (34.35 ± 0.15°C) (Freedman and Blacker, 2002). However, in scenarios of similar exogenous hormone use (high oestrogen phase of oral contraceptive use) vasodilation in response to heat is enhanced. This in turn may suggest higher Skin temperature due to greater vasodilation in HRT users (Fillingim and Edwards, 2001). Again, like core temperature and heart rate this indicated a large variation in the menopausal group and is an indication to why there is no significant result.

Sweat rate was seen to have no significant change between groups. Due to activation of eccrine sweat glands and sweat being secreted to promote heat loss by evaporation, high sweat rates (>1 $L.h^{-1}$) are commonly seen by all when exercising in the heat (Wendt et al., 2007). Menopausal women who have symptoms of hot flushes have been identified to have a significantly small thermoneutral zone in which they regulate their temperature (Freedman et al., 1995). It has been observed that brain norepinephrine release is greater in individuals who suffer from hot flushes and in animal studies this increase has

narrowed the width of the thermoneutral zone, therefore leading to a decreasing sweating threshold (Brück and Zeisberger, 1987). This indicates that those in the menopausal group should have a greater sweat rate, which is not seen in the present study. However, when individuals are on HRT is has been shown that the sweating threshold and in turn their thermoneutral zone is increased (37.98 ± 0.09°C to 38.14 ± 0.09°C), without changing basal levels of core temperature and Skin temperature (Freedman and Blacker, 2002). This is thought to be due to clonidine which ameliorates hot flushes by reducing norepinephrine and widening the thermoneutral zone (Freedman, 2014). Due to this increase in sweating threshold, it would indicate that those individuals on HRT in the post-menopausal group would cause a large standard deviation, leading to no significant differences.

Perceptual measures

RPE showed no significant difference between groups. Currently little research has been performed to show the difference between perceptual measures in pre and menopausal groups. Therefore, research on age must be considered. Research has been shown to support our finding with no significant effect of age on RPE (Aminoff et al., 1996; Groslambert and Mahon, 2006). Alternatively, some research states that RPE is seen to significantly decrease between the ages of 21 – 46 years when carrying out exercise in 49°C, 20% RH (Pandolf, 1997). This is also contradicted in the literature with RPE increasing with increased age (Borg and Linderholm, 1967; Bar-Or et al., 1972). Therefore, no clear evidence can be seen relating RPE to age. However, it has been concluded that the menopause does not have an impact on fitness levels (pre-menopause = 33.9 ± 6.3 mlO2 kg^{-1} min^{-1}, menopausal= 32.7 ± 6.6 mlO_2 kg^{-1} min^{-1}) (Abdulnour et al., 2016). Due to a strong correlation between RPE, heart rate and physical fitness, it would be unlikely that a difference would be seen between the two groups when looking at RPE at different time points during the HOTT. Therefore, supporting our findings in the present study.

TS and skin humidity is seen to be significantly higher (p<0.05) when individuals wear firefighter PPE compared to other light weight forms of PPE (Wang et al., 2013). Due to the low permeability of PPE a higher percentage of sweat accumulation is seen corresponding with high skin humidity. This in turn leads the body to sufferer uncompensated body heat strain and causes TS to increase at a significant rate throughout the test (Wang et al., 2013). However, TS showed no differences between groups, which is unlike previous studies. It is thought that TSS decreases with age for both cold and hot environments (p < 0.05) (Tuomaala et al., 2013; Inoue et al., 2016). With studies indicating a significant decrease in TS in an older population (46.4 ± 2.6 years) compared to younger population (21.2 ± 2.4 years) when exercising in 49°C, 20% RH (Pandolf, 1997). Although, this may be due to the generally decreased activity level and thus metabolic activity in older adults. The regular physical activity conducted by the menopause group may therefore negate these alterations.

Limitations

The study was undertaken by a range of individuals, not just firefighters, therefore it could be assumed that the heat tolerance of these individuals could be less than that expected of an entirely firefighter population. The small sample size of the menopause group made comparisons difficult. Further, with

this comparison of groups the consequence of age is also impossible to ignore or elucidate. In this instance we are most interested in the consequence and application of findings, so we must consider them together. We also acknowledge that the menopause generates fluctuating issues such as hot flushes, while we might not see these within the testing, these could well occur during other firefighting activities and effect the physiological and perceptual responses very differently. Control of menstrual phases is challenging, but the reporting and measurement of hormones minimised the potential limitations here.

The use of a heat chamber allowed control of the heat exposure but may lack ecological validity and the variable severities of a live fire exposure. The use of metabolic heat production as a test intensity allows comparison between interventions but can be argued against as it does not consider the comparison of exercise intensity and required cardiopulmonary fitness between groups.

Conclusion

Overall, the findings of this study indicate there are no substantial variations in thermoregulatory responses and heat tolerance between early follicular and mid luteal phases in both users and non-users of contraceptives, and menopausal individuals. There are likely to be daily variations to these responses, as a result of hormonal changes and these would become more common during menopause. From the data here, there is little to suggest female cycles have a significant regular influence on thermoregulation. Therefore, individuals should focus on preparation for heat exposure activities to achieve greatest meaningful improvements in heat tolerance, such as consistent hydration, maintained heat acclimation, cooling methods, clothing and training status.

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