

Does Acute Heat Stress Modulate Human Milk Production in Lactating Postpartum Women?

By

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## Abstract

**Introduction:** With rising ambient temperatures globally, heat-vulnerable populations are at greater risk of negative health consequences. Of increasing concern is the potential impact on human milk production and potential subsequent infant growth and development in our warming climate. While evidence in animals suggests a negative impact on milk production during periods of heat stress, whether acute heat stress modulates human milk production in postpartum mothers remains largely unexplored.

**Method:** Ten lactating postpartum mothers ( $32 \pm 3$  years,  $24.6 \pm 3.5$  kg/m<sup>2</sup>,  $33 \pm 11$  weeks postpartum) completed a 48-h experimental protocol whereby 24-h lactation performance was assessed before and after a one-hour passive heat stress intervention using a water-perfused suit at 49°C. Throughout the 48-h intervention, mothers recorded their lactation performance as human milk production using a baby scale accurate to  $\pm 2$  grams, fluid intake, and wore a FitBit Charge5 to monitor activity levels. Heart rate, blood pressure, and oral temperature were recorded before the intervention, immediately before and after the acute passive heating, and at the end of the intervention. Whole-body sweat losses were measured during the acute passive heat stress.

**Results:** Passive heat stress resulted in  $343\text{mL} \pm 190\text{g}$  of whole-body sweat losses, and increased heart rate ( $+12 \pm 5$  BPM,  $p < 0.0001$ ) and oral temperature ( $+0.4 \pm 0.2^\circ\text{C}$ ,  $p = 0.021$ ). 24-h human milk volume was not different after ( $335 \pm 156\text{mL}$ ) compared to before passive heating ( $333 \pm 118$  mL) and did not alter milk production ( $p = 0.921$ ). Additionally, participants consumed more fluid during the 24h after ( $1565 \pm 70\text{ml}$ ) compared to before ( $1325 \pm 190\text{ml}$ ,  $p = 0.005$ ).

**Conclusion:** Acute whole-body heat stress does not significantly affect the 24-hour human milk production in breastfeeding mothers likely due to increase fluid intake

## Chapter 1: Introduction

Climate change has resulted in rises in mean ambient temperature globally with the expectation of increased frequency, duration, and intensity of heat waves into the 21<sup>st</sup> century (Marx et al., 2021). While heat waves threaten the health and wellbeing of all individuals, it is especially concerning for vulnerable populations including infants, older people, pregnant and breastfeeding women, people with chronic health conditions (e.g. cardiovascular, respiratory, kidney diseases, obesity, mental illness), and outdoor workers (Singh et al., 2019). During heat stress, thermoregulatory responses including cutaneous vasodilation and sweating attempt to mitigate catastrophic rises in core temperature (Stolwijk, 1977). If left unchecked, chronic heat exposure can accelerate dehydration and exacerbate cardiovascular strain, which may increase the risk of developing heat-related illnesses (Akerman et al., 2016, Bouchman et al., 2022). Recognizing the potentially unavoidable exposure to heat waves globally for many communities and the direct impact on human health, it is critically important to understand how heat stress affects individuals throughout the lifespan.

Gestation and postpartum is a unique period of a women's life comprising dynamic and significant physiological changes to support the growth and development of a fetus. Since the seminal animal-based work of Edwards (1969), hyperthermia during pregnancy has been identified as a possible teratogen (any agent that causes an abnormality following fetal exposure during pregnancy) (McMurray and Katz, 1990), although the risk for teratogenic hyperthermia via afebrile exposures in humans (i.e. heat stress or exercise) is relatively low (Smallcombe., et al., 2022). For example, recent studies have proposed that moderate intensity exercise in hot and humid conditions may not result in core temperature exceeding the teratogenic threshold (the level of exposure to a substance at which it begins to cause birth defects or developmental

abnormalities in embryos or fetuses) confirming that temperature regulation during pregnancy remains largely intact (Ravanelli et al., 2019, Smallcombe, et al., 2022). During the postpartum period, milk synthesis is regulated by hormones including prolactin and oxytocin, as well as feedback mechanisms. Additionally, milk production involves the secretion of various components such as water, fats, proteins (including casein and whey proteins), carbohydrates (primarily lactose), vitamins, and minerals (World Health Organization, 2009). Lactating women are recommended to increase fluid intake to support human milk production for healthy infant growth and development. Human milk is a specific source of essential nutrients for newborns including maintenance of hydration status (Ip et al., 2007). Indeed, animal models to date have observed reductions in lactation volume in animals exposed to high ambient temperatures over summer months (Gaafar et al, 2011). Theoretically, heat exposure in postpartum women could exacerbate fluid losses through sweating (reaching ~0.5 – 1.0 L/h fluid losses) thereby compromising human milk production. While supplementation with formula may be a viable alternative, barriers include accessibility, economic cost, unsafe drinking water needed to mix with formula and supply chain disruption (Kent., 2015). Further, the incidence of morbidity and mortality is lower among breastfed compared to formula-fed infants (Stuebe, 2009). Thus, understanding whether heat stress modulates lactation is necessary in our warming climate.

Human milk production may be a well-controlled physiological mechanism. For example, 24-h fluid restriction diets or fasting have shown to not alter breast milk production in lactating women (Rakicioğlu et al, 2006). Physiologically, heat stress augments blood flow and systemic vasodilation which may promote greater milk production through enhanced fluid delivery to mammary glands. The limited studies suggest heat therapy such as warm steam therapy and warm compress therapy to augment lactation capacity (Rosnani et al., 2018). This

plausible biological explanation here may support heat therapy as a novel nonpharmacological intervention for improving lactation capacity in new mothers who may have insufficient milk production to support infant growth and development. Due to the paucity of data and importance of the relationship, the present thesis will identify whether acute heat stress modulates 24-h human milk production in otherwise healthy lactating mothers. Based on the limited evidence in animal studies, it is hypothesized that human milk production will reduce during the 24-h period following acute heat stress.

## **Chapter 2: Literature Review**

In this section, a comprehensive literature review is provided on different areas concerning this research. First, a review of the physiological adaptations during pregnancy and the postpartum period will be given. Second, general literature regarding breast feeding and fluid intake as well as the effect on exercise on them will be provided. The subsequent sections will summarize prior studies regarding thermoregulation, heat stress during pregnancy and heat stress during postpartum period, respectively.

### **2-1 Physiological Adaptations during Pregnancy and Postpartum**

During pregnancy and postpartum, mothers undergo remarkable anatomical and physiological changes in order to support the developing fetus. For many women experiencing an uncomplicated pregnancy, these changes resolve after pregnancy with minimal residual effects within a week to 12 months after delivery (ACOG committee, 2021). A notable physiological change during pregnancy is the elevation in basal metabolic rate (BMR) due to the increased metabolic demand associated with maternal-fetal circulation, respiration, renal function, and increased tissue mass (Lof et al., 2005). Further, maternal plasma volume increases by 40% to 50% while red cell volume increases by only 15% to 20%, causing a fall in hemoglobin concentration, hematocrit and red blood cell count (Carlin and Alfirevic, 2008). Level of clotting factors I, VII, VIII, IX, X, and XII, and fibrinogen counts are elevated during pregnancy (Soma-Pillay et al., 2016). The increased blood volume supply and circulatory need of the enlarging uterus, as well as the needs of the fetoplacental unit, protects the parturient from bleeding at the time of delivery. Further, these changes in the coagulation system can put pregnant and postpartum woman at an increased risk of venous thrombosis (Katz and Beilin,

2015). However, this risk is short-lived, with most women returning to original pre-pregnant blood volume and hemoconcentration within 8 weeks after delivery (Soma-Pillay et al., 2016).

As well as volumes, the increase in metabolic demand also results in cardiac output rising by 20% from pre-pregnant levels (Soma-Pillay et al., 2016). The initial rise in cardiac output is primarily attributed to peripheral vasodilatation resulting in a reduction in systemic vascular resistance thereby increasing stroke volume and heart rate (Datta et al., 2010). Cardiac output, heart rate, and stroke volume decrease to pre-labor values 24- to 72-h postpartum and return to non-pregnant levels within 6 to 8 weeks after delivery (Datta et al., 2010). With the increased cardiac output, glomerular filtration rate rises during pregnancy due to increased renal plasma flow and slowly returns to nonpregnant values by the sixth week postpartum (Datta et al., 2010). Furthermore, respiratory rate and tidal volume increase by 15% and 40% respectively and all respiratory parameters return to normal status within 6 to 12 weeks postpartum (Datta et al., 2010).

Mammary gland development is stimulated by estrogen at puberty and further growth in the ductal and lobule-alveolar system occurs during pregnancy due to the large quantity of estrogen and progesterone. Although estrogen and progesterone are essential for the physical development of the breasts during pregnancy, the hormone prolactin impacts milk secretion (Alex et al., 2020). Prolactin is secreted by the mother's anterior pituitary gland, and its plasma concentration rises steadily (about 10 to 20 times the normal nonpregnant level) from early pregnancy until the birth of the baby (Guyton & Hall., 2015). Immediately after the baby is born, the lactogenic effect of prolactin stimulates the secretion of human milk (Biswas and Rodeck, 1976). Other hormones such as growth hormone, cortisol, parathyroid hormone, and insulin are necessary to stimulate the uptake of amino acids, fatty acids, glucose, and calcium by mammary



glands to begin the formation of milk (Hall, 2015). Human milk production is energetically demanding, consuming on average 630 kcal per day which represents approximately a quarter of the total energy intake of a lactating woman (Butte & King, 2005). Moreover, it is estimated that approximately 400 to 500 liters of blood are required to circulate through the mammary gland to produce 1 liter of milk (Geddes et al, 2012). Milk is secreted continuously into the alveoli of the breasts, but it does not flow easily from the alveoli into the ductal system. The milk must be ejected from the alveoli into the ducts which is caused by a neurogenic and hormonal reflex. Milk ejection (or let-down) occurs within 1 minute following the initiation of a baby suckling (Capuco & Akers, 2009).

The American Academy of Pediatrics encourages mothers to exclusively breastfeed their infants for the first six months and continue breastfeeding as long as both mother and baby desire (Meek & Noble, 2022). Human milk is uniquely adapted to the needs of the developing infant, and it is a complex fluid that contains more than 200 constituent nutrients. Human milk primarily contains 87% water, 7% carbohydrates (lactose, glucose, galactose, and oligosaccharides), 4.5% fat (triglycerides and fatty acids), and 1% protein (including caseins, immunoglobulins, albumin, lactoferrin, enzymes, hormones, and growth factors) with the remaining 0.5% including minerals, electrolytes, and vitamins that play a significant role in enhancing optimal infant well-being. The amount of milk transferred to the infant affects the infant's nutrient intake and the mother's nutrient requirements (Belitz, 2009; Kent, 2007; Jenness, 1979).

Milk volume is defined as the amount of milk that is consumed by infants and may also be referred to as milk intake. The most widely accepted method for measuring milk intake is test weighing, a procedure in which the infant is weighed before and after each feeding, preferably using a balance scale accurate to  $\pm 1$  g. In this method, milk intake is usually underestimated by

approximately 1 to 5% (Brown et al., 1982) because of evaporative water loss from the infant between weighing. The mean volume of milk secreted by healthy women up to 6 months postpartum is estimated to be 750 to 800 ml/day  $\pm$ 165 ml (Butte et al., 1984). Following the initiation of solid foods at the 6<sup>th</sup> month, milk intake varies widely as the nutritional requirements of the infant are satisfied by a combination of both food sources. For example, the amount of milk intake for the participants tested averaged 769 g/day at 6 months, 637 g/day at 9 months, and 445 g/day at 12 months (Dewey et al., 1991). During early postpartum, there is a positive relationship between nursing frequency and the amount of milk produced (Institution of medicine, 1991). Additionally, various infant and maternal factors affect milk volume. Infant characteristics such as birth weight, sucking strength, gestational age at delivery, and illness will affect breast milk production. Mature babies with normal birth weight usually have greater sucking strength, frequency and feeding duration which may increase milk volume (Prentice, 1986). Moreover, maternal factors include age, parity, stress, acute illness, substance use, nutrition and fluid intake influence human milk production (Institute of Medicine, 1991). Hytten (1954) showed that older women might encounter difficulties establishing and maintaining lactation because of "disuse atrophy". Multipara (a woman who has delivered more than one child) are more successful in breast feeding than primipara. This is probably due to multipara being more experienced receiving previous physiological adaptations to nurse (Miller, 1952). Stress and anxiety also influence milk production by inhibiting the milk-ejection reflex (Dozier et al., 2012). Lastly, factors such as smoking may reduce milk volume through an inhibitory effect on prolactin or oxytocin levels (Institute of Medicine, 1991).

## 2-2 Breastfeeding and fluid intake

Although it is commonly considered that milk production requires a substantial fluid intake on the part of the mother, data reveals that nursing women can tolerate a significant level of dehydration and that additional fluids have minimal influence on milk volume (Ndikom et al., 2014). Lactating women who did not eat or drink from 5:00 a.m. to 7:30 p.m. during Ramadan lost 7.6% of their total body water and experienced serum dehydration despite milk volume remaining unaffected (Prentice et al., 1984; Rakiciolu et al., 2006). Furthermore, a study reported no impact in milk volume production despite restrictions in fluid intake by up to 1,765 ml/day for 10 days (Oslen, 1991). These findings have been corroborated by others highlighting no change in milk volume and no relationship between fluid intake and milk volume among 21 lactating women in the US who consumed at least 25% more amount of fluid than the normal for 3 days (Dusdieker et al., 1985). A pilot study by Morse et al. (1992) examined the effects of increased and decreased fluid intake on the human milk supply of 10 mother-infant pairs and considered three treatments, each maintained for 72 hours. Treatment A, typical fluid intake, was calculated based on body weight of subject as follows: 40 mL/kg body weight (recommended intake) + 750 mL (for lactation loss) for 24 hours. Treatment B consisted of a 50% reduction of the recommended intake and treatment C, consisted of a 50% increase over recommended intake. Total breast milk intake was determined by both tests weighing the infants and the amount of milk yield measured by an electric breast pump. Again, increases or decreases in fluid intake do not jeopardize milk supply or yield (Morse et al,1992). Illingworth and Kilpatrick (1953) assigned 210 lactating women in the first 9 days of postpartum, in high-fluid intake group (2880 ml/d) and control group (as much as desired). Actual fluid consumption in the high-fluids group was around 3,200 ml/day, while in the other group it was around 2,100 ml/day. Neither infant

growth nor duration of breastfeeding differed between groups. While drinking excess fluid may mitigate dehydration which could arise from exercise or heat stress, fluid consumption in excess will not increase total milk production. Rather, maintaining sufficient body fluid levels has a vital role in supporting healthy human milk production. Indeed, body fluid levels are critical to human health and would be affected by medicines, diseases, diarrhea and sweating rate due to high temperature (Illingworth and Kilpatrick, 1953 and Hunt and Smith, 2005).

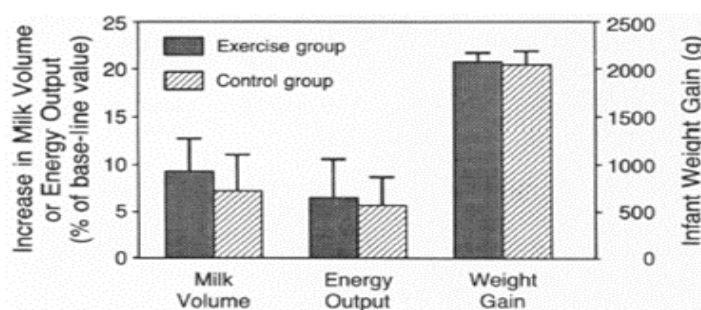
Yagil et al. (1986) studied the effect of dehydration on the milk water content of Bedouin women. Milk samples were collected from 80 women in each group in the winter (average temperature 18°C, 75%RH) and summer (average temperatures 40°C, 45%RH). The participants drank water, but not enough, as was indicated by the concentration of their urine and osmolality. Milk water, fat, lactose, and urine sodium and potassium were examined. The results showed that during the summer, there was a dilution of milk in the partially dehydrated Bedouin women. Yagil et al. (1986) concluded that the dilution of milk was a physiological response to heat, which lead to lower fat and higher water content without changing the total quantity of human milk. It remains unclear whether a similar response may be observed following acute heat stress.

### **2-3 Breastfeeding and exercise**

Exercise is recommended for pregnant and breastfeeding women due to its multifaceted health benefits, although women should increase their energy intake to accommodate their greater energy expenditure. It is widely assumed that maternal nutrition status, which is influenced by physical activity, is directly related to human milk composition and quantity. Severe malnutrition has been associated with a reduction in human milk volume and lower concentration of nutrients in milk (Lovelady et al., 1990). Additionally, exercise, especially if

conducted under heat stress, will induce greater cardiovascular strain, more sweating, and consequently incur greater dehydration, which may affect milk volume.

Dewey (1995) evaluated 33 early postpartum lactating women who were randomly assigned to an exercise group (18 women) or a control group (15 women). The exercise programs consisted of 45 minutes of aerobic exercise per day, 5 days a week for 12 weeks. Energy expenditure, maximal oxygen consumption, milk volume, and composition were measured at 6 to 8, 12 to 14, and 18 to 20 weeks postpartum. Milk volume was assessed by having mothers weigh their infants before and after each feeding with an electronic balance accurate to  $\pm 1$  gram. The results showed that there were no significant differences between the exercise and control group in the changes in breast milk intake by the infants, and all infants in both control and exercise groups gained weight throughout the 12 weeks of study (Dewey et al., 1995). Thus, the prescribed exercise program did not have any effects on human milk volume and composition. Figure 1 shows an increase in infant's weight in both groups during 12 weeks (Dewey 1995).



**Figure 1.** Increase in infant's weight during the exercise program (From Dewey 1995).

In a separate study carried out by Lovelady et al. (1990), 16 healthy breastfeeding women, who were exclusively breastfeeding their 9-to-24-week-old infants, were recruited and separated into an exercise and control group. The exercise group (8 women) was engaged in an

aerobic exercise program at an intensity of 70 percent of their maximal heart rate and had been exercising for more than 6 months before the study (swimming, jogging and bicycle riding). Thus, intensive exercise may not have an adverse effect on lactation. However, whether the independent effect of heat stress – a biological consequence of exercise or exposure – modulates the response remains unknown and warrants further investigation (Cary and Quinn, 2001).

## **2-4 Thermoregulation in humans**

One of the most tightly regulated elements of human physiology is core body temperature which is controlled by central and peripheral thermoreceptors integrated centrally and processed by the hypothalamus (Kurz, 2008). Thermoregulation represents the ability of an organism to keep its temperature within homeostatic limits across a range of environmental conditions (Periard et al., 2021). During heat stress, the rise in internal body temperature will result in the proportional activation of thermoeffector organs (i.e. sweating and cutaneous vasodilation) to mitigate excessive hyperthermia. If thermoeffector output is sufficient to blunt the rise in internal temperature, the conditions are defined as compensable. However, when thermoeffector output is insufficient to meet the net heat gain of the individual, internal temperature will persistently rise denoting uncompensable heat stress which may be fatal if left unchecked (Ravanelli et al., 2019). There are four avenues for heat transfer between the body surface and the surrounding environment including conduction, convection, radiation and evaporation (Parson, 2014). The largest modifiable avenue for heat dissipation is the evaporation of sweat (or water) from the skin surface (Wenger, 1972).

Humans attempt to maintain their core body temperature at  $\sim 37^{\circ}\text{C}$  through initiation of behavioral and physiological autonomic responses. Behavioural thermoregulation includes adjustment of clothing, moving to a more desirable location and turning on air conditioning or

opening windows would decrease the thermal stress. Physiological responses include peripheral cutaneous vasodilation and sweat secretion (Stolwijk, 1977). Cutaneous vasodilation occurs in an attempt to shunt heat from the core to the periphery for release to the surrounding environment. If insufficient to match the heat loss required, sudomotor activation will increase sweat secretion onto the skin surface for subsequent evaporation and heat liberation from the body (Parson, 2014). During heat stress in healthy individuals, cutaneous vasodilation results in reductions in total peripheral resistance which is counteracted by increases in heart rate and cardiac output (Marchand and Gin, 2021). Further, splanchnic vessels constrict alongside reductions in central circulating blood volume in order to maintain blood pressure, organ perfusion, and thermal homeostasis to cutaneous vascular beds (Rowells et al., 1971). Failure of neural thermoregulatory responses occurs with progressive hyperthermia due to extreme and sustained heat exposure with an inability to achieve heat balance causing central nervous system dysfunction (Cheshire, 2016). This phenomenon is defined as heat stroke which manifest complications such as endothelial cell injury, inflammation, thrombosis, oliguria, hypotension, and neurological dysfunction (Hunt and Smith, 2005) Additionally, extreme and prolonged heat exposure will lead to increased sweating to liberate heat from the body. This physiological process would decrease total body water causing electrolyte concentration imbalances which could exacerbate heat-related illnesses and central nervous system dysfunction (Mack and Nadel, 2011).

## **2-5 Heat stress during pregnancy**

In pregnancy and lactation, hormonal and metabolic alterations result in changes in maternal temperature and thermoregulatory mechanisms. The thermogenic effects of progesterone, changes in maternal metabolism, and the heat generated by the fetus cause

maternal core temperature to increase initially in the first trimester followed by a gradual fall, until 6 months after delivery in breast-feeding women (Wells, 2002; and Hartgill et al., 2010). Maternal hyperthermia is defined as core temperature exceeding 39.0°C and mothers are advised to avoid physical activity in hot and humid conditions due to the proposed teratogenic effects (McMurray and Katz, 1990). Hyperthermia during pregnancy most often occurs from a fever due to illness, extremely intense exercise or prolonged exposure to heat sources. Several studies and reviews have reported that maternal hyperthermia could influence cell division in the embryo, resulting in neural tube defects and congenital malformation in early gestation (Soultanakis-Aligianni, 2003). A prospective follow-up study investigated the association between the heat exposure to a hot tub, sauna, fever, or electric blanket and an increased risk for neural tube defects (NTDs) during early pregnancy. In the first trimester of pregnancy, exposure to heat in the form of a hot tub, sauna, or fever was linked to an elevated risk of NTD (Milunsky et al., 1992). Smallcombe et al. (2021) indicated that pregnant women can perform moderate-intensity exercise for 45 minutes in 32°C and 45% Relative Humidity with very low apparent risk of excessive maternal hyperthermia. Although some previous studies showed a negative physiological link between heat exposure and birth outcome during pregnancy, it may be unrelated to thermoregulatory dysfunction and more closely related to other external factors (Smallcombe et al., 2021).

## **2-6 Heat stress in the postpartum period**

To the best of our knowledge, limited work has focused on the maternal and infant response to heat stress during the postpartum period. However, some pilot evidence suggests that thermoregulatory control remains largely intact when mothers and child are exposed to acute heat stress (35°C and 70%RH, Tsuzuki and Tochiham, 1994). However, heat stress may result in

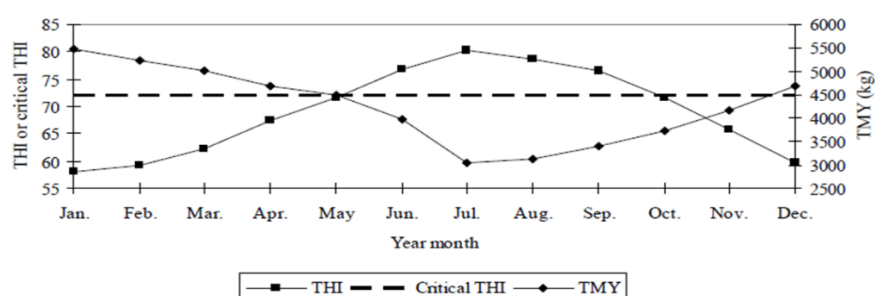


sweat-induced dehydration resulting in shifts in body water between the intra- and extravascular spaces which could potentially impact human milk production and/or composition (Van Loan et al, 1995).

Woodward and Cumming (1990) investigated the possible effect of ambient temperature and humidity on the breast milk intakes of 35 Australian infants aged 6-12 weeks. Mothers and babies were recruited in two climatically different cities with mean temperatures ranging from 14 - 28°C, and humidity from 48 - 97%. Milk intake at a feed is estimated by test-weighing before and after each feeding over 24-h (as explained above). The results demonstrated that in the short term, human milk intake does not seem to be influenced by ambient temperature or humidity (Woodward and Cumming, 1990). However, these conditions examined remained within the thermoneutral zone, requiring predominantly insensible heat loss mechanisms such as cutaneous vasodilation (Savage and Brengelmann, 1996). It remains unknown whether heat stress outside the thermoneutral zone resulting in sweating could modulate human milk production. While no empirical evidence exists in humans, animal models have suggested that lactation may be altered during chronic exposure to high ambient temperatures.

With respect to animal models, Brown et al. (1988) conducted two trials for two successive years in summer for sets of six Nubian and six Alpine goats. They were exposed to three air temperatures for 5 weeks with the same relative humidity (35%): 20°C (cool), 27°C (warm), and 34°C (hot). Collectively, increasing temperature depressed milk yield in Alpine more than in Nubians goats. Kadzerea (2002) states that heat stress increases the loss of body fluid due to sweating which leads to a reduction in milk production among lactating dairy cows. Increasing air temperature and temperature-humidity index can result in insufficient heat dissipation capacity in lactating dairy cows and therefore lead to a rise in core temperature above

critical thresholds which are related to decreased dry matter intake (DMI) and milk yield. Every 1°C rise in air temperature above a cow's Thermal Neutral Zone results in approximately 36% reduction in milk production (West, 2003). A study on 581 Friesian cows during an 8-year period from 1997 to 2004 investigated the effect of heat stress on milk production and composition. Average temperature humidity index (THI) values confirm the presence of heat stress during the summer season (June to September). They reported that with increases in THI from 59.82 in the winter season to 78.53 in the summer season, heat stress reduced total (305 days) and daily milk yield by 39.00%, and 29.84%, respectively. As Figure 2 implies total average milk production per cow was significantly ( $p < 0.05$ ) higher in the spring period ( $42.74 \pm 4.98$  L) compared to summer ( $39.60 \pm 5.091$  L) (Gaafar et al., 2011).



**Figure 2.** The effect of heat stress on total milk yield (From Gaafar et al., 2011).

However, not all animal studies have observed a reduction in milk yield due to heat stress. A study in 2013 evaluated 8 dairy goats housed in a different climate chamber for 2 periods of 35 days with 4 goats in each room, either a thermal neutral (20°C day-night) or heat stress (12-h day at 37°C and 12-h night at 30.5°C) with 40 % humidity. Although DMI reduced and body temperature increased, milk yield did not vary between HS (Heat Stress) and TN (Thermal Neutral) goats (Hamzaoui et al., 2013).

Interestingly, controlled heat stress may prove to be beneficial during the postpartum period in humans. Heat therapy is a non-pharmacological treatment approach that has been

shown to reduce the risk of various cardiovascular and metabolic disorders (Weber and Silver, 2007). An increase in tissue temperature leads to increased metabolism and vasodilation, which can initiate beneficial physiological adaptations. Vasodilation improves blood flow to the injured site, aiding healing by increasing the supply of nutrients and oxygen to the area, as well as removing pain-inducing and inflammatory mediators (Freiwald., 2021). Although breastfeeding is a natural process for feeding infants, breast engorgement, blocked ducts, and mastitis are common conditions affecting up to 50% of all women who select to breastfeed (Babakazo et al., 2022). Breastfeeding problems can lead to serious complications in breastfeeding, including the cessation of breastfeeding (Mangesi, 2016). Application of warm packs to the breast prior to feeding aid oxytocin uptake, frequent feeding, softening of the areola, widening of the ducts, increases blood circulation, stimulation for faster let-down and increased milk flow (Sweet and Vasilevski, 2022). A study investigated the impact of applying warm breast compress for 39 nursing mothers. A breast compress that was warmed up in a microwave oven for 1 minute at 180 W was applied to one of the breasts for 20 minutes, and both breasts were sucked by an electrical breast pump for 15 minutes. The amount of human milk obtained from the warmed breast was significantly higher than that obtained from nonwarmed breasts (Yiğit et al, 2012). Thirty-two postpartum women received the treatment of steam bath therapy and the result showed that the average human milk production increases after warm steam exposure (Rosnani et al., 2018).

## Chapter 3: Summary and Research Purpose

Previous animal studies indicated that heat exposure may reduce milk production through exaggerated body fluid loss and high core temperature in lactating animals, however whether these findings translate to humans remains largely unexplored. Due to the importance and benefits of breastfeeding, inevitable impacts of climate change and global warming and limited research elucidating the effects on lactating postpartum mothers, this thesis will study the effect of acute heat stress during the postpartum period on human milk production. Thus, the primary *research question* is outlined as below:

Does an acute bout of whole-body passive heat stress, which increases core temperature and induces sweating, modulate human milk production during the next 24-h in comparison to the previous 24-h period?

### 3-1 Hypothesis

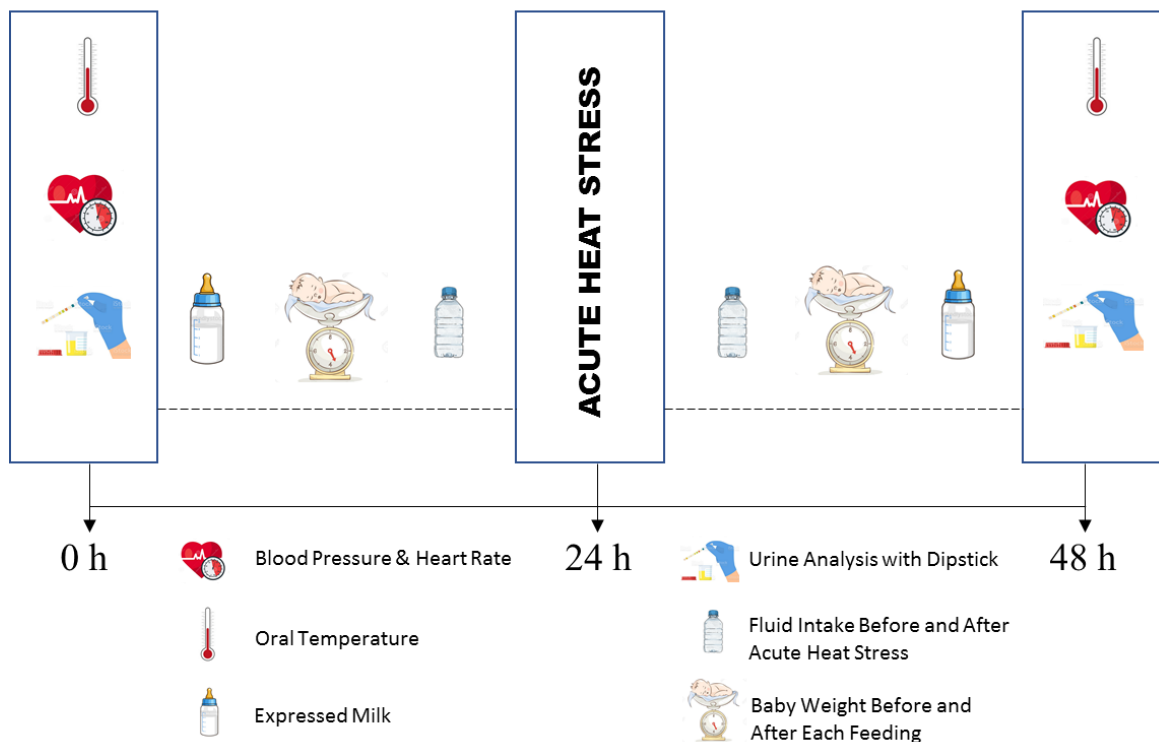
Based on the limited evidence in animals, it is hypothesized that the volume of human milk production will be lower during the 24-h period following acute whole-body heat stress relative to the preceding 24-h period. Additionally, it is expected that the reduction in total milk volume per feed will lead to an increase in feeding frequency in the subsequent 24-h following heat stress.

## Chapter 4: Methodology

### 4-1 Participants

Following ethical approval from the Lakehead University Research Ethics Board, 10 healthy mothers aged 18 – 40 y who were breastfeeding their 6-week to 12-month infants were recruited for this interventional study. Women who had a healthy pregnancy and delivered between 37 to 42 weeks of gestational age were included. Additionally, mothers with cardiovascular, metabolic, or neurological diseases, and/or had smoked tobacco products within the last 12 months were excluded from the study. Participant recruitment was conducted through partnerships with local lactation consultants, referrals from Maternity Care Midwives, and using social media platforms.

***Preliminary session:*** This initial contact with participants was established through email and then further information was provided via a phone call. The information sheet and consent form were sent to the participants by email. During this preliminary session, the researcher described the study details and answered any questions or addressed any concerns. Following verbal and written consent, the participant was asked to complete the Biographic Information Sheet which was obtained the participant's preliminary information, and the researcher and participant scheduled a time to complete the experimental protocol.



**Figure 3.** Graphical depiction of the 48-h data collection protocol.

**Experimental protocol:** Figure 3 graphically depicts the data collection procedure for the study. At the participants' convenience, the 48-h data collection period was coordinated. All instruments and data collection tools for the participant were provided and delivered to their primary residence in advance, and a member of the research team educated and trained the participant on proper utilization. Blood pressure & heart rate (A&D Instruments), and oral temperature (Kinsa Thermometer), were taken at rest before and after the 48-h data collection. During the 24-hr prior to and following the acute passive heat stress exposure (totaling 48-h), participants were asked to log their fluid intake volume, recorded total volume of breast milk produced through infant feeding (estimated by baby weight before and after each feeding) or pumping (using the provided document). Participants were provided with a water bottle with

measurement markings and a log sheet to record their fluid intake throughout the entire 48-h period. Additionally, all participants were provided a FitBit Charge 5 to track their number of steps over 48 hours.

Human milk production was estimated by test-weighing (as  $W_a - W_b$ ), where  $W_a$  is the baby's weight just after the feed and  $W_b$  its weight just before the feed or by weighing net expressed milk (Brown et al., 1982). Additionally, for mothers who were pumping, the volume of milk was recorded after breast expression. A clinical grade infant weighing scale accurate to  $\pm 2$  grams was used for measuring babies' weight (Doran DS4500). Participants were asked to maintain their usual feeding and activity habits throughout the 48-h period. Evaporative water losses from skin and lungs were accounted for during the feed by having the mother weigh her baby just before the feeding began ( $W_b$ ), right after the feed ended ( $W_a$ ) and again 20 min after the feed ended ( $W_e$ ), without changing the baby's clothing. The rate of evaporative loss was determined as  $(W_e - W_a)/20$ , and thus, the true milk intake at the feeding episode was  $(W_a - W_b) + (D * [(W_e - W_a)/20])$ . The duration,  $D$ , of the feed was defined as the interval elapsed between  $W_b$  and  $W_a$ .

***Acute passive heat stress:*** Following the initial 24-h baseline collection period, participants conducted a 1-h passive heat exposure session. This passive heat stress session occurred in the Environmental Physiology Lab (SB-1028E) at CJ Sanders Fieldhouse. Upon arrival, participants were asked to self-insert a flexible probe (~2mm diameter) through the anus into the rectum (10-12 cm) in order to measure internal body temperature continuously. Proper instruction was given to the participants on the placement of the rectal probe. Participants provided a urine sample in private to assess hydration status with a manual refractometer and weigh themselves with a balance scale accurate to  $\pm 2$  grams (GBK-333Ha, Adams).

Participants changed into a shirt and shorts and were instrumented with a 3-lead electrocardiogram (BioAmp, AD Instruments), an automated blood pressure cuff (Tango M2, SunTech), 4 wireless skin temperature sensors (iButton, Maxim Integrated). After all instrumentation was complete, participants donned a water perfusion garment (COOLTube suit, MedEng) which was connected to a circulating water bath (TC-102, Brookfield) and lay supine. Following a 10-minute baseline period, 49°C water began circulating water perfusion garment for a total of 60 minutes. Following the passive heating protocol, all instrumentation was removed, and the participants weighed themselves again using the balance scale. The participants then continued to record their fluid intake and human milk production for the next 24-h. All equipment and data log sheets were collected by the research team from the participant following the 24-h data collection period.

#### **4-2 Statistical analysis**

All statistical analysis was conducted using SPSS 25 (IBM). The main independent variable is time, along with descriptive variables including the age of the baby, age of the mother, and mothers' body mass index. The dependent variables were total fluid intake and total human milk production during the 24-h periods before and after heat stress. Paired sample t-test was used to compare dependent variables between the first (pre) and second (post) 24-h and Cohen's D was calculated to indicate effect size. Using a 95 percent confidence interval, a 1x4 repeated measures one-way analysis of variance (ANOVA) was used to independently assess the dependent variables of blood pressure, heart rate, and oral temperature at four time points (before 24h, before heating, after heating, after 24h).



## **Chapter 5: Results**

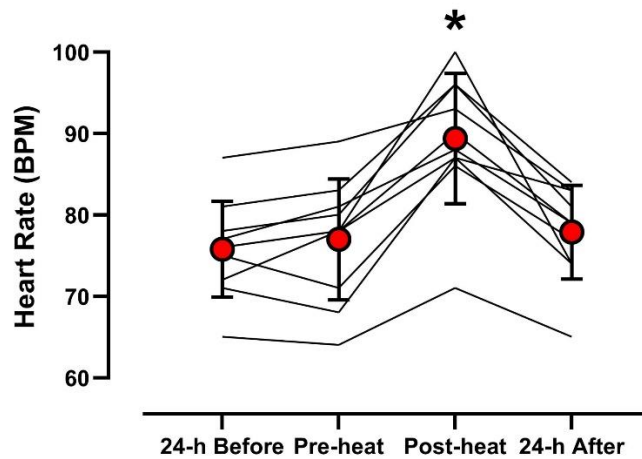
### **5-1 Participant's characteristic**

Of the 19 recruited for the study, 10 completed the 48-h intervention. The characteristics of 10 breastfeeding women included an average age of  $32.0 \pm 2.8$  years, a body mass index of  $24.6 \pm 3.5$  kg/m<sup>2</sup>, and a postpartum period of  $33.3 \pm 11.7$  weeks.

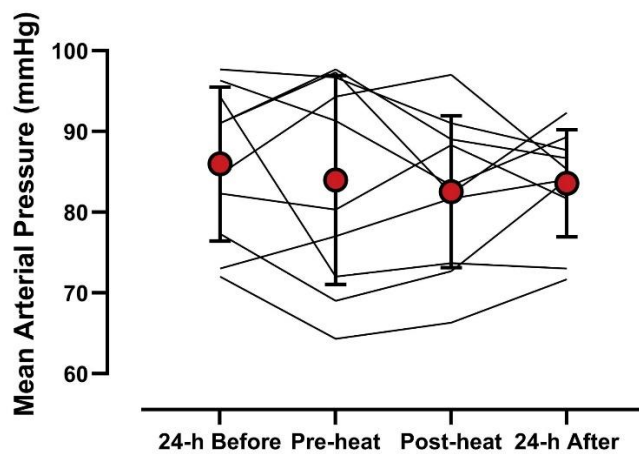
### **5-2 Cardiovascular, temperature, and sweating responses following heat stress**

Passive heating increased rectal temperature over the 60-minute period from  $36.8 \pm 0.6^\circ\text{C}$  to  $37.2 \pm 0.3^\circ\text{C}$  resulting in a mean rise in rectal temperature of  $0.4 \pm 0.3^\circ\text{C}$ , with  $p < 0.05$ . Mean skin temperature increased from  $33.8 \pm 1.1^\circ\text{C}$  to  $37.1 \pm 0.9^\circ\text{C}$  after 60 minutes of passive heating. Whole-body sweat loss was  $343 \pm 190$  grams following the passive heating protocol.

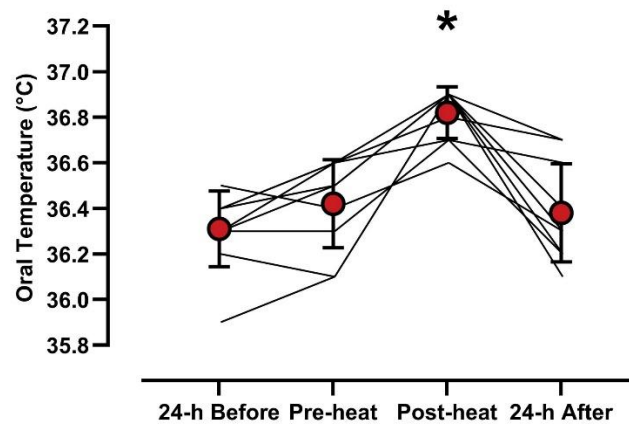
Over the 48-hour period (24 hours before, pre-heat, post-heat, and final 24 hours after), repeated measure ANOVA showed that there was a main effect of time for heart rate ( $F(1,9) = 8.514$ ,  $p < 0.001$ ). A Tukey post hoc tests revealed that heart rate was highest after passive heating ( $p < 0.001$ ) and not different at any other time point (Figure 4). Moreover, the mean arterial pressure was not different at any time point ( $p = 0.888$ ) (Figure 5). The oral temperature was significantly higher post heat stress trial ( $F(1,9) = 16.982$ ,  $p < 0.001$ , Figure 6).



**Figure 4.** Mean and individual resting heart rate measured at each time-point over the 48-h experimental protocol. BPM: beats per minute. \*Significantly higher than all other time points ( $P < 0.05$ ).



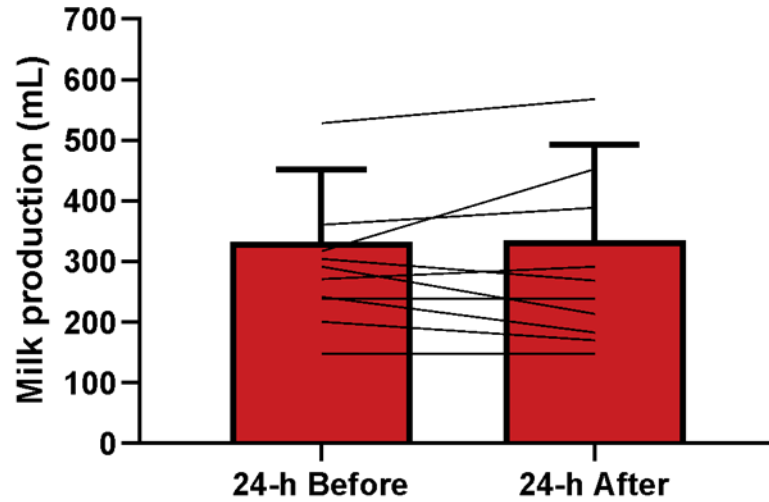
**Figure 5.** Mean and individual mean arterial pressure (MAP) at each time-point over the 48h experimental protocol.



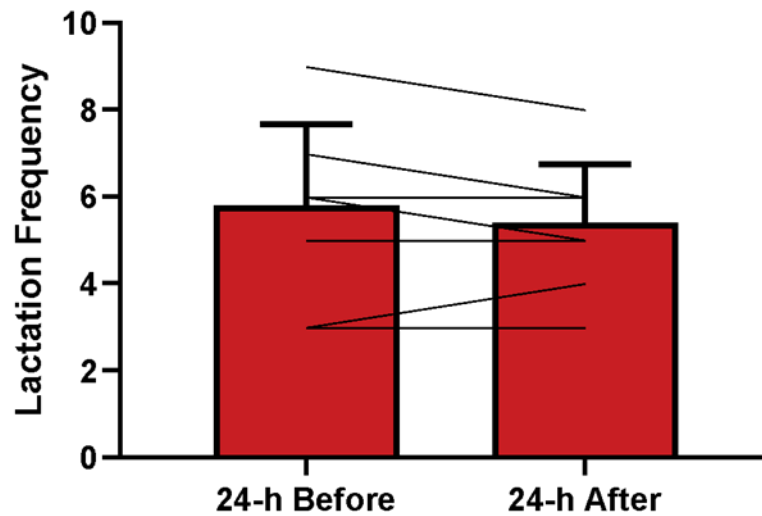
**Figure 6.** Mean and individual oral temperature (°C) measured at each time-point over the 48-h experimental protocol. \* Significantly higher than all other time points (\* $p < 0.001$ )

### 5-3 Lactation Performance

The average total milk production in the first 24 hours was  $333 \pm 118$  mL, with the post-heat volume at  $335 \pm 156$  mL (Figure 7) and no significant difference was observed in human milk volume after passive heating compared to before ( $p = 0.921$ ). Moreover, the data indicates that there is no significant difference between lactation frequency for the first day, which was  $5 \pm 2$ , compared to  $5 \pm 1$  for the second day, with a p-value of  $p = 0.103$  (Figure 8).



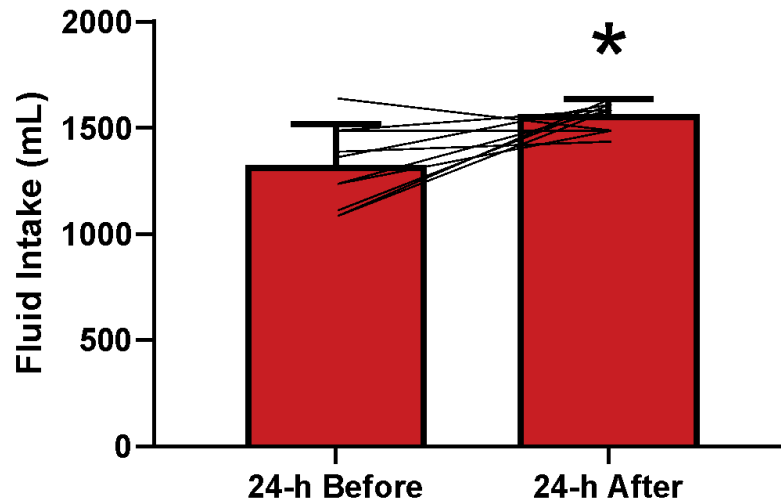
**Figure 7.** Mean and individual total human milk production (in mL) during the 24-h period before and after acute passive heat stress.



**Figure 8.** Mean and individual lactation frequency during the 24-h period before and after acute passive heat stress. Individuals' data demonstrated that 3 participants had identical results.

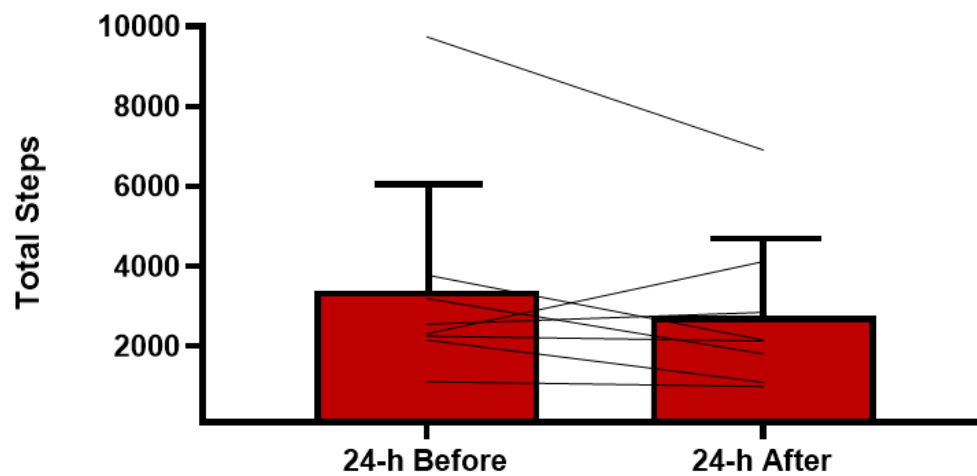
#### 5-4 Behavioural Modification

Fluid consumption significantly increased during the 24-h following whole-body passive heat stress to  $1565 \pm 70$  mL ( $p = 0.005$ ,  $d = 1.009$ ) in comparison to the pre-heating 24-h period ( $1325 \pm 190$  mL, Figure 9).



**Figure 9.** Mean and individual total fluid intake (in mL) during the 24-h period before and after acute passive heat stress. \*Significantly higher after heat stress ( $p < 0.05$ ).

Out of the initial 10 participants, data from 8 individuals were included in the Fitbit data analysis, with 2 participants excluded due to missing data. Data indicated no significant difference in physical activity levels before and after heat exposure. The number of steps before heat exposure was  $3390 \pm 2680$ , and after that, it was  $2759 \pm 1949$ , with  $p = 0.245$  (Figure 10).



**Figure 10.** Mean and individual total step count during the 24-h period before and after acute passive heat stress.

## **Chapter 6: Discussion**

This is the first study to investigate the effect of acute heat stress on human lactation performance. In this study, we explored whether an acute bout of whole-body heat stress, which increases core temperature and induces sweating to reduce total body water, modulates human milk production during the subsequent 24-hr period in comparison to the pre-heating 24-hr period. Based on present findings, 60 minutes of whole-body passive heat stress in lactating postpartum mothers does not alter 24-hr lactation volume and frequency.

Human milk contains about 87% water, and about 12 % solid components as macronutrients that may vary depending on the environmental and maternal factors (Ballard & Morrow, 2013). During heat stress, the body defends against hyperthermia by increasing sweat rate to dissipate the additional heat. Increased sweating can result in reductions in total body water, which may affect mammary gland function and hormonal regulation, resulting in negative consequences on lactation performance and a reduction in breast milk volume (Dewey et al., 2001; Lawrence & Lawrence, 2016). However, the findings presented in the current thesis do not support this hypothesis, rather lactation performance following acute whole-body heat stress remains undisturbed.

### **6-1 Heat stress and milk production**

Heat stress can have an impact on breastfeeding behavior and reduce milk production (Nzeyimana et al, 2023). The increase in sweat rate following heat exposure could potentially disrupt fluid balance as the body attempts to dissipate heat. Considering that milk comprises about 87% water, it's plausible that acute heat stress might affect milk volume due to the increased sweating rate and potential disturbance in fluid balance (Alex et al, 2020). However,

the acute whole-body heat stress imposed in the current study, which increased total body water loss through sweating, did not impact human milk production, which may likely be explained due to the proportional increase in fluid intake following heat stress (See Section 6-3 for more details). In contrast, Gaafar et al. (2011) found a negative correlation between milk production and the heat stress in dairy cows. Specifically, with a 20-point increase in temperature humidity index (THI) during the summer, heat stress resulted in a reduction of total and daily milk yield by almost 33 percent. Additionally, a recent study found that women self-reported spending less time breastfeeding during the hottest times of the year, with a decrease of approximately 2.3 minutes per day per 1°C increase in ambient temperature (22.6 – 33.7°C, Chérie et al, 2022). In contrast, a previous animal study exposed goats to 35 days in a thermal neutral chamber or heat stress (Hamzaoui et al., 2013). Despite an increase in body temperature observed in the heat-stressed goats, there was no significant difference in milk yield between the goats exposed to heat stress compared to the thermal neutral condition (Hamzaoui et al., 2013). Collectively, current evidence is mixed and particularly lacking in humans. It is hypothesized that the absence of any difference in the present study may be due to the acute heat stress imposed warranting further work in more chronic heat stressed conditions to confirm any heat-mediated effects on lactation in humans.

## **6-2 Heat stress and lactation frequency**

Frequency of feeding was not affected by the acute heat stress intervention. According to the World Health Organization (2009), infants who are feeding on demand based on their appetite obtain what they need for satisfactory growth. Infants of mothers with low milk supply may need to feed more often to ensure sufficient daily intake and production. In this study, it was observed that lactation frequency remained consistent during the first 24 hours compared to the



subsequent 24 hours. Nevertheless, it is important to acknowledge that irrespective of external factors such as acute heat stress experienced by mothers, their infants consistently require a specific amount of milk per day. When mothers can produce adequate amount of milk, they can meet the needs of their infants based on their feeding frequency and volume requirements. The absence of differences in nursing frequency noted in our study may be attributed to the fact that, during each feeding interval, mothers produced a sufficient milk supply to fully satisfy their infants' needs, regardless of the acute heat exposure. Therefore, it indicates the absence of a difference in the milk volume after acute heat stress condition.

### **6-3 Fluid intake and heat stress**

The mothers in the present study exhibited an increase in ad libitum fluid intake of approximately 240 ml, somewhat proportional to the amount of sweat lost during acute heat stress, despite lacking awareness of the extent of their water loss. The increased total body water loss following heat-induced perspiration increases the thirst response, and evidence has demonstrated a direct correlation between the level of hypohydration and the amount of fluid intake (Engell et al., 1985). Additionally, one animal study illustrated a significant increase in the water intake of individual cows when exposed to temperatures exceeding 22°C (Thompson, et al, 1949). Following acute heat stress, in response to elevated body temperatures, there is an increase in sweat loss, leading to changes in plasma osmolality and central blood volume, resulting in faster dehydration rates and a heightened feeling of thirst (Sawka and Montain, 2000). Consequently, individuals tend to consume more fluids to alleviate their thirst, aiming to prevent heat-related illnesses. Consuming water ad libitum after heat exposure partially replaces lost body water and effectively regulates water balance (Armstrong, 2006). Moreover, lactation further stimulates thirst in mothers due to the release of oxytocin during breastfeeding, which

acts as a powerful thirst stimulus (Bentley, 1997). This hormone is structurally like vasopressin, the antidiuretic hormone, which is involved in regulating fluid balance in the body. Moreover, prolactin, another hormone involved in milk production, also plays a role in stimulating thirst (Bentley, 1997). The increased thirst sensation experienced by lactating mothers after heat exposure is important for maintaining adequate fluid intake by drinking more, which is crucial for milk production and the health of both mothers and infants. The current evidence suggests that the thirst stimulus in postpartum mothers may be sufficient to maintain water balance and milk production following acute heat stress to protect the health of mothers and their breastfeeding infants. Future work is warranted to examine if chronic heat-mediated dehydration may impact human milk production, particularly among populations with water insecurity (Rosinger, 2020).

#### **6-4 Physical activity and heat stress**

We assessed the impact of acute heat stress on physical activity levels from the lactating mothers during the 48-hour intervention. It is important to note that two participants did not wear their activity tracking devices consistently due to parental responsibilities and as a result, were not included in data analysis. Our findings revealed that there were no significant changes in the number of steps over the 48-hour study period and the subjects maintained their regular activity levels even after experiencing the acute whole-body heat stress.

Heat stress imposes additional physiological strain on the body, including increased heart rate, elevated core temperature, and accelerated dehydration therefore individuals may reduce their physical activity levels (Baker & Ely, 2018). Donnan et al. (2021) explored the effects of heat on athlete performance and found a decrease in physical exertion under heightened heat conditions. Additionally, prolonged exposure to high temperatures increases physiological strain,

discomfort and fatigue in postpartum women that might lead to a decrease in physical work capacity, affecting activities such as bathing, dressing, playing, and caregiving with their infants (Part et al., 2022). It has also been consistently associated with increased feelings of irritability, fatigue, and decreased overall mood thereby leading to decreased motivation and willingness to engage in physical activities (Varghese et al., 2020). In our study, despite the acute whole-body heat stress, subjects displayed no significant changes in their number of steps over the 48-hour study period, indicating that they maintained their regular activity levels.

Additionally, heat stress can significantly impair the quality of life of postpartum mothers. Physical discomfort, fatigue, and dehydration associated with heat stress can exacerbate the challenges already faced during the postpartum period, including parental responsibilities, sleep deprivation and hormonal imbalances (Lovelady & Colby, 2013). The combination of heat stress and postpartum fatigue can exacerbate the negative impact on the daily activities of lactating women (Callahan et al., 2006) warranting further scientific inquiry.

### **6-5 Limitations, Considerations, and Future Prospectives**

The present study reports the physiological responses of a small sample of postpartum breastfeeding women and thus is not without limitations. Infant weight was used as a proxy for human milk volume production, however, estimates of evaporative water loss were used to enhance the accuracy of the results. In general, good compliance was observed among the participants, however 2 participants were unable to continuously wear the FitBit Charge 5 and thus alternative activity tracking devices should be considered for future work. The study was designed to assess whether moderate reductions in total body water following acute heat

exposure would alter 24-h human milk volume. It remains unclear whether greater heat stress, or greater reductions in total body water, may compromise lactation performance in postpartum mothers warranting further exploration. Additionally, most participants were 6 – 12 months postpartum where infants may be incorporating more solids in their diet. Thus, this limitation prevents a comprehensive understanding of the effects of heat stress during the earlier postpartum stages when infants are reliant on fluid nutrient delivery and a mother's milk production is highest. Another limitation stems from the homogeneity of our participant pool, which may not reflect broader populations of breastfeeding mothers. Specifically, future work should consider how the combination of heat stress and water insecurity may impact human milk production, particularly in developing countries. Additionally, the study's findings cannot be generalized to women with pre-existing health conditions such as hypertension, diabetes, or metabolic disorders, as all participants were healthy.

## **6-6 Conclusion**

In conclusion, this study provides the first evidence that acute whole-body mild passive heat stress resulting in moderate fluid losses does not modulate the 24-h lactation performance in postpartum women. Future work is needed to elucidate whether chronic heat stress or greater heat-mediated reductions in total body water, particularly during the early postpartum period, impacts lactation performance in postpartum mothers.

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Appendix A  
Biographic Information

Name:

Date:

Mother		Infant	
Age		Age	
Weight		Sex	
Height		Gestational age	
Parity		Birth weight	
Type of delivery		Current weight	

	Before 48-h trial	After 48-h trial
Weight of infant		
Weight of mother		
Blood pressure		
Heart Rate		
Hydration status		
Oral temperature		

