Thermoregulation in exercising horses:
Aspects of temperature monitoring during field exercise

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Hij kende en berekende zijn idealen en durfde.
Wat een stoer karakter.

Voor papa Henk
Voor iedereen
For everyone

List of abbreviations

AERA Australian Endurance Riders Association
BM body mass
BOM Bureau of Meteorology
BSA body surface area
CI confidence interval
CNV Copy Number Variants
COT cost of transport
EHI Exertional Heat Illness
EHS - Exertional Heat Stroke
ER exertional rhabdomyolysis
FEI Fédération Equestre Internationale
GI gastrointestinal
GPS Global Positioning System
H metabolic heat production
IRT infrared thermography
M metabolic rate
MH metabolic heat
GI gastrointestinal
RH relative humidity
RR respiratory rate
RTD resistance temperature detector
SDF synchronous diaphragmatic flutter
SDS synchronous diaphragmatic flutter
SET standard exercise tests
SESOI smallest effect size of interest
SIRS systemic inflammatory response syndrome
T_a ambient temperature
T_c core body temperature
T_{re} rectal temperature
T_{sk} skin temperature
TNZ thermo-neutral zone
WBGT Wet Bulb Globe Temperature

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Chapter 1

1.1 General introduction: Thermoregulation in exercising horses

Heat stress is a major problem for sport horses and its prevalence is expected to continue to increase in the context of global warming, hence the need to validate robust minimally invasive monitoring techniques. From an animal welfare point of view, it is of great importance to prevent heat stress using real-time monitoring of thermoregulatory processes during work in the field. Exertional heat illness (EHI) associated with various kinds of exercise affects the wellbeing of all mammals including humans. It is one of the oldest medical syndromes known to sports science, and whilst physiological differences exist between species, heat-related syndromes are similar amongst human and equine athletes. In human athletes, exertional heat stroke (EHS) is among the top three causes of sudden death during competition, and in summer, it is the number one cause of athlete death in the USA (Casa et al., 2015). Similarly, equine hyperthermia and exertional heat illness (EHI) are ongoing welfare issues for all exercising horses (Geor and McCutcheon, 1998; Brownlow et al., 2016; Brownlow and Mizzi, 2021). All equine sport disciplines are under increasing criticism by the general public and animal welfare associations. When fatalities occur, audiences are quick to condemn sport and racing events and increasingly call for safeguarding wellbeing in sports horses.

The type of competition that the horses perform has various important impacts. Racehorses perform high intensity exercise for a few minutes whereas endurance horses perform submaximal intensity exercise over several consecutive hours. In between these two extreme examples, a whole range of exercise types exists, each challenging the thermoregulatory wellbeing of horses. When this challenge exceeds the physiological coping capacity of the horse, fatalities can occur. In the past 20 years, several epidemiological studies have investigated sport-related horse fatalities. Reported prevalence of fatalities in these studies is high, and the overall fatality rate in racehorses ranges from 0.06-1.9% deaths in race starts (Boden et al., 2005; Boden et al., 2007; Lyle et al., 2012; Georgopoulos and Parkin, 2016; Hellings et al., 2022).

Most injuries are of musculoskeletal origin, and it is reasonable to assume that ongoing heat stress predisposes a horse to developing musculoskeletal injuries (Williams et al., 2001; Lyle et al., 2012; Hellings et al., 2022). At present, the association between EHI and racehorse injuries is not well understood. For example, Williams et al (2001) reported an overall fatality rate of 0.29% of all starts including hurdle races in Britain. Allied to that, a group of 4.7% non-fatalities included cases with exhausted horse syndrome or heat exhaustion. Lyle et al. (2012) and Hellings et al. (2022) in turn, identified a higher incidence rate of sudden death in thoroughbred (TB) racehorses and standardbred (SB) racehorses respectively during the summer months indicating that heat exhaustion played a significant role.

Reported overall fatality rates in eventing horses competing in cross-country events in the UK varied from 0.18-1.5% (Murray et al., 2004; Comyn et al., 2017; Bennet et al., 2021).

For horses competing in 160 km endurance rides in the USA, a fatality rate of 0.15% has been reported (Balch et al., 2014). Very recently, a large-scale study investigated which risk factors predispose horses for elimination from endurance rides organized by the Féderation Équestre Internationale (FEI). In that study however, fatality rates were not included (Zuffa et al., 2022). The FEI is the governing organization for equestrian sport and is responsible for international competitions worldwide. These horse injuries and fatalities represent an emotional loss to the owners and a major financial loss to the horse industry. They are a direct threat to horse welfare, as well as to jockey and rider safety.

Finally, it is critical to realize that the economic impact of the horse industry in various countries should not be underestimated. For example, the thoroughbred (TB) racing industry in Australia is worth $5 billion AUD per year with an estimate of $427 million AUD prize money (Agrifutures.com.au). As another example, FEI endurance rides in the Middle East offer an estimated $3.6 million USD prize money and $40,000 USD prize money per endurance ride completion (FEI). Understandably, this economic impact and the inherent pressure for profit could be potentially hazardous to horse welfare. Currently, attempts are being made to create awareness for the welfare of equine athletes. For example, there is a growing consensus that a decrease in horse injuries and fatalities is required to maintain the social license to operate in equestrian sports (Heleski et al., 2020).

Unfortunately, due to global warming, it will become more and more challenging to safeguard thermoregulatory wellbeing in horses competing in the open air. It is expected that the world's average ambient temperature (T_a) will continue to increase and sudden heat waves will rise in prevalence (Perkins-Kirkpatrick and Lewis, 2020, Raymond et al., 2020; Trancoso et al., 2020). Consequently, the risk of horses developing heat stress and heat-related illness during exercise or post-exercise is expected to dramatically intensify (Leyk et al., 2019). Both human and equine sports events occur in various climatic environments including hot and humid conditions such as the Olympics in Atlanta (1996), Hong Kong (2008), Rio de Janeiro (2016) and Tokyo (2020/2021). Just as with human athletes, it is impossible to completely adapt competition calendars and locations to climate change due to the global aspect in which equine international level competition takes place. Therefore, there is a great need for developing reliable real-time core body temperature monitoring devices that allow for taking preventive measures in order to swiftly intervene at early stages when coping boundaries are being crossed in a competing horse. Furthermore, there would be application for such technologies in all competing mammals.

1.2 Thermoregulation

1.2.1 Thermoregulation: core versus shell temperature

Thermoregulation is the process of regulating body temperature to balance the metabolic heat (H) load and the exchange of heat with the environment.

Body temperature in mammals is divided into an inner core body temperature (T_c) and an outer shell temperature. The T_c in endothermic species is regulated within a narrow range (37.4 – 38.0°C) while shell temperature varies depending on ongoing thermoregulatory processes (Ewart, 2020). The inner core temperature refers to deep-body temperatures, while shell temperature includes intramuscular, subcutaneous and surface skin temperatures. Heat is moved between core and shell areas to equilibrate heat production and heat loss and thus regulate the body temperature. As a consequence, temperature is not uniform at different locations in and on the body. In addition, a diurnal variation in T_c of up to 1°C may occur, with horses measuring their lowest T_c in the morning (Ewart, 2020). However, when monitoring thermoregulation during exercise, the inner T_c provides an adequate indicator of body temperature response.

1.2.2 Thermoregulatory control

For optimal functioning of physiological processes, it is necessary that body temperature is closely regulated. Efficient thermoregulation maintains the T_c within a narrow range to prevent the onset of heat-related problems. Thermoregulation is continuous, however fluctuations occur both short term and, through acclimatization, long-term. To achieve this, a wide array of physiological effectors can be activated via endocrinological pathways (thyroid and adrenal glands) and sympathetic pathways, resulting in changes to resting metabolic rate, sweat gland activity, metabolism of brown adipose tissue and influencing cycles of sleep, dietary input and physical activity. Several conditions influence thermoregulation, such as environmental conditions (temperature, humidity and solar radiation), exercise intensity (high, short duration versus low intensity long duration), acclimatization, temperament, hydration status, anhidrosis, infections and inflammation. For example, under resting conditions, the liver produces the greatest amount of body heat, whereas under exercising conditions, the skeletal muscles perform this function.

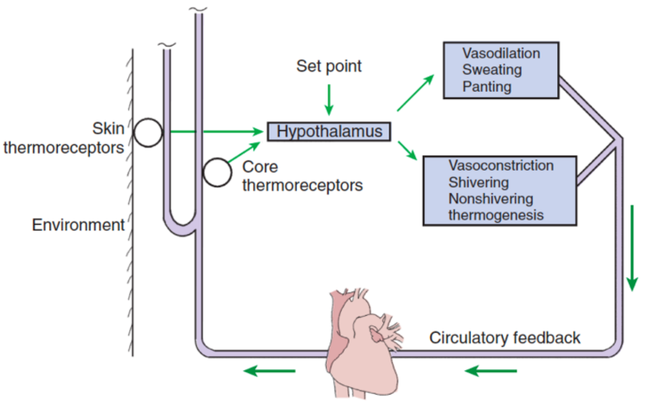

Figure 1. Thermoregulation and feedback control mechanisms include skin thermoreceptors in the skin and the core which provide information to the hypothalamus, which adjusts the responses either to produce or to lose heat. (Reproduced with kind permission from Elsevier Inc. Ewart, 2020).

Thermoregulatory processes are controlled via neurophysiologic control centers in the hypothalamus. Both peripheral and central thermoreceptors are nerve endings which respond to changes in T_c and the T_a, all in communication with the hypothalamus (**Figure 1**). The peripheral thermoreceptors in the skin detect the peripheral shell temperature and respond to a range of external temperatures from cold to hot (5 - 60°C), with cold thermoreceptors being most numerous (**Figure 2**). The sensors transduce temperature into neural activity by using transient receptor potential (TRP) ion channels (Guthrie and Lund, 1998; Ewart, 2020). Recently, in 2021, the Nobel Prize for Physiology was jointly awarded to David Julius and Ardem Patapoutian for revealing how nerve impulses are initiated so that temperature and pressure can be perceived. They identified the cDNA encoding of a novel ion channel for the heat-pain receptor TRPV1 using a gene in capsaicin (https://www.nobelprize.org/prizes/medicine/2021/advanced-information/) (**Figure 2**).

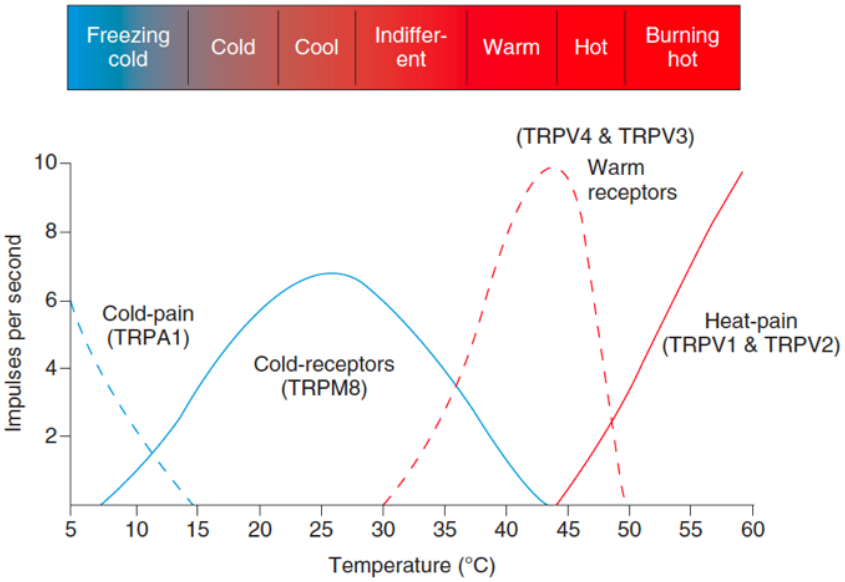

Figure 2. Skin thermoreceptors shown are temperature-specific discharge frequencies, as well as the various transient receptor potential (TRP) ion channels, as substrates for those thermoreceptor responses. (Reproduced with kind permission from Elsevier Inc., Ewart, 2020).

This essential research provides the molecular basis for the mammalian ability to sense temperature and respond to high environmental temperatures.

The central thermoreceptors of the body measure the inner T_c and include thermoreceptors located in large blood vessels, the dorsal horn of the spinal cord and abdominal viscera. The thermoreceptors detecting temperature changes in the blood mainly comprise heat-sensitive receptors. Some heat-sensitive thermoreceptors are located in the preoptic area of the anterior hypothalamus (POA) near the organum vasculosum of the lamina terminalis (OVLT). The blood brain barrier in this region is very thin or even non-existent, allowing for easy diffusion of cytokines as part of a fever response activated by endogenous pyrogens, thus resulting in a higher temperature set-point in the hypothalamus. By acting as a thermostat, the hypothalamus is the primary site to coordinate the homeostasis of thermoregulation. The POA integrates and converges all afferent information from both peripheral and central thermoreceptors into efferent signals to the posterior hypothalamus. This efferent output will activate different physiological autonomic responses and behavioral responses.

During exercise, metabolic heat originating from contracting muscles will be transported via the blood to the skin surface by convection. The cholinergic system activates skin capillary vasodilation, increases cutaneous blood flow and consequently, skin temperature. The sympathetic nerve control system of the skin also activates the sweat glands resulting in evaporation of sweat from the skin surface to its surroundings (Guthrie and Lund, 1998; Hodgson 2014; Mota-Rojas et al., 2021a). The behavioral responses of the horse to internal temperature rise include preference for shade and wind in addition to standing in water (Holcomb et al., 2014; Holcomb et al., 2016; Mota-Rojas et al., 2021a). However, under constrained sport event conditions, the onus is on owners and sport event officials to monitor and prevent heat stress and EHI.

1.3 Thermoregulation during exercise

1.3.1 Metabolic heat production

In the body, heat is generated by tissue metabolism. This heat is also referred to as metabolic heat production (*H*, IUPS Thermal Commission, 2001). Exercise is characterized by a high internal heat production when chemical energy is converted to mechanical energy by contracting muscles. Muscle contraction is very inefficient and about 75-80% of the energy is produced as heat (Hodgson, 2014; Marlin and Nankervis, 2002; McCutcheon and Geor, 2008). This heat load is conveyed towards the core of the body via the conductive heat exchange between working muscles and blood as well as to the surrounding tissue and body compartments (Guthrie and Lund, 1998; Hodgson, 2014). Once a certain thermoregulatory set-point is surpassed, the body will engage thermo-homeostatic corrective mechanisms.

Efficient thermoregulation maintains the T_c within a narrow range to prevent the onset of heat-related problems. The metabolic rate (*M,* IUPS Thermal Commission, 2001) in mammals includes the basal amount of energy per unit of time required to function at rest and is associated with body mass (BM) and body surface area (BSA). The smaller the animal, the larger the relative amount of BSA. This results in greater heat loss and correspondingly higher mass-specific MR (Ewart, 2020). The horse, in particular, has a higher muscle percentage of total BM compared to other species including humans. For example, 50% of the BM of a thoroughbred (TB) horse is skeletal muscle (McCutcheon and Geor, 2008; Ewart, 2020). Therefore, the rate of *H* in horses exercising at high intensities may increase to 40 – 60 times *M* (Hodgson, 2014). Other additional factors increasing the thermoregulatory workload such as the body weight of the rider, the weight of the tack and the racetrack conditions, need to be considered as well (Pagan and Hintz, 1986). Factors to be considered during field exercise are the type of terrain such as soil conditions of the track, obstacles and footing during training and competition (Murray et al., 2004; Bennet et al., 2021).

Finally, the dietary thermic effect is of importance, especially in horses being herbivorous and hind gut fermenters. The thermic effect of food is the amount of heat generated by food digestion above the resting metabolic rate. Compared to humans, horses have an enormous additional thermal load due to fermentative activity in the large colon and caecum (Ewart, 2020). Grain, chopped fibers and high-fat diets have a lower thermic effect and heat production compared to other equine diets (Warren et al., 1999; Ewart, 2020). However, an advantage of the voluminous hind gut of horses is its enormous water holding capacity which can be used for thermoregulatory purposes (Warren et al., 1999).

1.3.2 Equine thermoregulation in various equine sport disciplines

Each sport discipline represents a specific challenge for the thermoregulatory system. Endurance on the one hand and racing and eventing on the other can be regarded as extremes of an exercise spectrum. Due to the inefficiency with which energy is converted to muscular contraction, both intensity and duration of a specific type of exercise and the performance capacity of each specific horse have a great impact on *H*. The rate at which oxygen is consumed in an exercising horse provides a direct indication of its exercise intensity, evident in the *M* and subsequent heat production (Hodgson et al., 1993). Besides exercise intensity, the duration of exercise varies greatly for horses performing in sporting disciplines, and consequently the *H* production.

The relationship between these factors is illustrated in the following formula:

$$H = \dot{V}O_2 \text{ x k x duration of exercise}$$

— in which *H* represents metabolic heat production expressed as kcal min^{-1} or kJoule min^{-1}; $\dot{V}O_2$ represents the oxygen consumption rate expressed in liters per min, k stands for heat liberated per liter of oxygen consumed (5 kcal; 21 kJ) (Hodgson et al., 1993; McCutcheon and Geor, 2008) and duration of exercise is expressed in minutes.

The oxygen intake may be calculated by the following equation which includes additional workload determining factors such as BM of horse and rider and the weight of the tack:

$$\dot{V}O_2 = \text{COT x speed x body mass (BM)}$$

— in which cost of transport (COT) is expressed in ml oxygen per kg body mass per m (ml O_2 kg^{-1} m^{-1}), speed is expressed in m min^{-1} and body mass in kg. The COT takes into account additional factors associated with varying terrain such as footing and the incline and decline of terrain (Schroter and Marlin, 2002).

During maximal exercise intensity, the *H* is approximately 50 times higher compared to *M* and is directly related to rate of oxygen use. Thus, the higher the speed, the higher the rate of *H* generation (Hodgson, 2014; Marlin and Nankervis, 2002). For example, a racehorse exercising at 58 km h^{-1} with a VO_{2max} of 80 L min^{-1} equates to *H* production of 400 kcal min^{-1} (approximately 1.3 MJ min^{-1}) (McCutcheon and Geor, 2014). Without adequate heat dissipation in a 500 kg horse, T_c would increase by 1°C min^{-1}, and thus approximately by 3°C when exercising for three minutes.

On the other hand, although oxygen consumption is lower during submaximal exercise (for example at a speed of 15 km h^{-1}), the overall *H* production (100 kcal min^{-1}; 0.33 MJ min^{-1}) is also high due to the greater duration of the exercise. For example, endurance competition typically encompasses multiple consecutive 40 km loops for a total of 40 to 160 km (**Figure 3**, **Table 1**) (Hodgson et al., 1993). The calculated *H* production for a 160 km ride is approximately 200 – 400 MJ of *H* which could result in an overall T_c increase of 2.5 - 5°C (**Table 1**).

The relation between exercise intensity and duration can also be illustrated by monitoring speed and heart rate. An overview of different exercise intensities and the associated heart rate and speed is provided in **Table 1**.

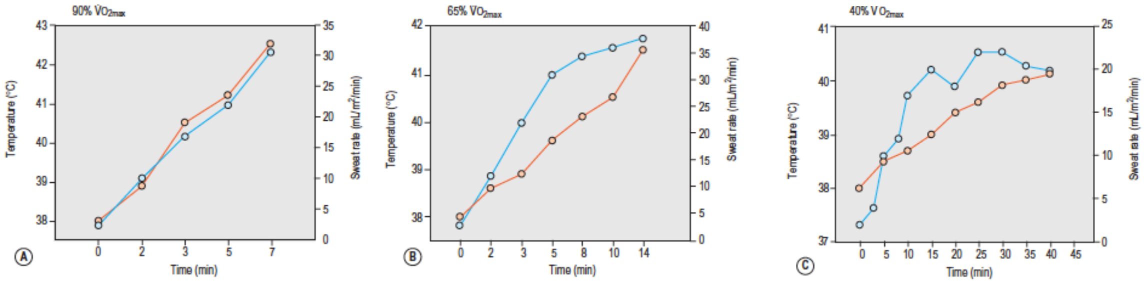

Figure 3. The graph shows T_c (°C, left Y-axis) evolvement (red line) and sweating rate (ml m 2^{-1} min^{-1}, right Y-axis, blue line) for different exercise intensities, indicated by a VO_{2max} of respectively 90% (panel A), 65% (panel B) and 40% (panel C) over time (minutes, X-axis)–High intensity exercise (A) results in high T_c in short time while T_c in submaximal exercise (C) tends to increase more slowly. Although the oxygen consumption is lower during submaximal endurance exercise, the overall heat load is higher due to the longer duration of the endurance exercise. Reproduced with permission from Am. Phys. Society (Hodgson et al., 1993, adapted in Chapter 41, book Equine Sport Medicine & Surgery by Hinchcliff et al.).

Variable	**Endurance (400 kg)**	**Eventing (cross-country) (500 kg)**	**Racing (450 kg)**
Distance	160 km (40 km loops)	~4000 m & obstacles	~800 - 3200 m
Speed (km h^{-1}) [m s^{-1}]	15 [4.1]	32 [8.9]	58 [16.1]
Heart rate range (bpm) [average or peak]	80 – 180 [130]	170 – 200	>180 [240]
VO_2 (L min^{-1})	25	42	80
Theoretical heat production (kcal min^{-1} [MJ min^{-1}]	100 [0.3]	200 [0.6]	400 [1.3]
Theoretical increase in T_c (°C min $^{-1}$)	0.25	0.5	1
Average duration of exercise (min)	600 - 1200	7-12	3.2

Table 1. Variables related to metabolic heat production in horses during different athletic events together with the associated rate of metabolic heat production: heart rate, speed and duration of exercise to enable calculation of theoretical increase in core body temperature per minute. Kg, kilograms body mass; min, minutes; kcal, kilocalories; kJ, kilojoule. Adapted from Hodgson, 2014; McCutcheon and Geor, 2014 and Allen et al., 2016.

Submaximal exercise – endurance

The main equine sport discipline that is performed at submaximal intensity over a period of several hours is endurance competition which tests the stamina of horses over varying terrain, similar to marathon athletes. Endurance horses compete over distances of 80 to 160 km divided into exercise loops (sections) of 30–40 km. In endurance rides, each rider must safely manage the capabilities of their horse, considering its fitness with emphasis on the horse's welfare (FEI). After each endurance exercise loop, a recovery rest period is imposed to allow the heart rate (HR) to return to below values of 60 or 64 beats per minute (bpm) (Nagy et al., 2012; FEI; AERA). The range of mean winning speeds over 100–120 km FEI endurance rides around the world varies between 14.1 – 24.8 km h^{-1} while reported maximum winning speeds range from 17.2 to 26.2 km h^{-1} (Nagy et al., 2010).

Other disciplines with submaximal exercise include jumping and dressage exercise with a varying intensity from submaximal to high. Isometric dressage exercise such as the collected canter and piaffe are especially demanding. Though competition itself only entails a maximum of 10 minutes, horses are subjected to warming-up exercise that adds to the thermal load.

Because endurance horses challenge their thermoregulatory system for several consecutive hours, metabolic disorders such as dehydration often coincide with heat stress in these horses. Also, electrolyte disbalances are often encountered, together with a wide array of organ failure pathologies (Fielding et al., 200; Misheff, 2020).

Moderate to high intensity exercise - eventing and racing

Sport disciplines involving high-intensity, short duration exercise levels include thoroughbred (TB) racing, standardbred (SB) trotting, eventing, western riding and polo (Hodgson, 2014; Allen et al., 2016). The intensity and duration of these exercise levels vary and are summarized in **Table 1**. For horses undertaking such high-intensity, short-duration exercise, thermoregulatory processes to dissipate their heat load are being challenged. Polo exercise typically involves repeated acceleration and deceleration combined with short powerful turns during which large muscle groups especially of the hindquarters perform strenuous exercise (Marlin et al., 1999; McGwan et al., 2002; Innes and Morgan, 2015). Polo competitions are generally organized in the summer season and therefore represent an increased risk for occurrence of EHI. Eventing is a challenging competition which entails three phases: jumping, cross-country and dressage on the last day; all phases are undertaken during one or over three consecutive days when competing at national and international levels. The cross-country phase is performed at a high intensity level (mean HR 195 bpm and mean blood lactate 10.2 mmol liter^{-1}) and includes jumping obstacles on the course (Serrano et al., 2002). Eventing horses may suffer from EHI in particular after the cross-country phase of eventing competitions, which are also commonly held during the summer (personal observation, Elliot 2021). While fatigue is often identified as a risk factor when competing over a longer distance, environmental conditions and heat exhaustion were not been investigated in a recent epidemiology study (Bennet et al., 2020).

In summary, efficient thermoregulation during exercise is paramount to ensure the horses' high performance and health. Excessive elevation in T_c limits performance capacity, contributes to fatigue and can impair health (Geor et al., 1995; Hodgson et al., 1993; Kohn et al.,1999; McConaghy et al., 2002; McCutcheon and Geor, 2008).

1.3.3 Mechanisms of heat loss

An increase in muscle temperature has several benefits for athletic performance, however, preventing an excessive rise in T_c requires transfer of heat from the active muscles and body core to the skin followed by dissipation into the environment from the body surface. Therefore, the surface area (cm^2) of the body is a critical factor in determining rates of heat exchange. Because heat exchange with the environment is proportional to the relative size of the body surface area (BSA), the rate of heat exchange per unit of BM is the largest in the smallest animal (if other variables are equal). That is, smaller animals with a lower BM have a greater BSA to volume ratio. For example, the BSA to volume ratio is 50% less in a 500 kg horse when compared to humans (Hodgson, 2014; Ewart 2020). This lower BSA-to-mass ratio of horses results in greater demands being imposed on the thermoregulatory system during exercise (Hodgson et al., 1993; Hodgson et al., 1994; Wallsten et al., 2012). If the skin temperature is higher than that of the surroundings, the body can lose heat by both radiation and conduction.

Conduction is the direct heat transfer between surfaces of different temperatures in contact with each other. The conduction mechanism is most optimal in areas with a high BSA-to-volume ratio, such as the head and extremities. Heat transfer by conduction is improved by a wet surface and because of this, conduction is the main mechanism involved when cooling horses with cold water.

Convection is the movement of heat through air or water (‘heat flow’). Convection between particles occurs in all fluids including the circulatory convection of the heat collected by blood from perfused muscles and transferred to a cooler skin. Forced convection takes place in the upper respiratory tract especially during exercise due to existing air pressure differences. Furthermore, forced convection is applied during cooling strategies post-exercise when using fans with colder air to exchange heat between the horse and surrounding air. Clipping of the hair coat enhances convection whereas a thick longer hair coat will insulate the skin.

Radiation is the movement of heat between objects without physical contact via electromagnetic radiation. The heat load from solar radiation is a direct heat load on the skin, with absorption depending on coat colour and structure. Solar radiation will heat the skin, contributing up to 15% of the total heat gain (Guthrie and Lund, 1998). Indirect radiation is the heat load due to a reflection of radiation from any surrounding surfaces. During cooling strategies, a decrease of direct solar radiation is achieved when horses stand in the shade.

Evaporation is the endothermic process during which liquid turns into vapor. The evaporation of sweat at the skin surface removes heat from the body in this way. Evaporation also depends on the skin-to-ambient-vapor pressure difference, and therefore, occurs when skin temperature is higher than T_a. Because of the dependence of the evaporation process on the temperature and the vapor gradient between the skin and the immediate surroundings, the process will have a limited capacity in hot and humid weather. Finally, a low amount of water diffusion through the skin and some water evaporation from the respiratory tract is the continuous low amount of ‘hidden’ loss of water (Ewart, 2020).

Medium to large mammals mainly use panting to evaporate heat. While true panting as seen in the dog and sheep has not been defined in the horse, McConaghy et al.

(1996) described an “atypical form of panting” characterized by an increased blood flow to the nose, but not to the tongue (as occurs in dogs). This form of panting is seen post-exercise in endurance horses and racehorses recovering in hot and humid environments (McConaghy et al., 1996; Brownlow and Mizzi, 2021).

Sweating

The horse is one of the few species, besides humans, camels and kangaroos, able to use sweating as a heat dissipation pathway due to the presence of many sweat glands in the skin (Hodgson, 2014; Hodgson et al., 1994; Marlin and Nankervis, 2002; McCutcheon and Geor, 2014). Horses have apocrine sweat glands in the skin with varying densities of glands over the entire skin surface, producing sweat containing the protein latherin. Latherin has a surfactant-like mechanism to promote evaporation of sweat. Humans produce isotonic sweat relative to plasma while the sweat of horses is mildly hypertonic. Once the thermoregulatory balance is challenged, evaporative cooling via sweat glands is activated. Dissipation of heat is mainly achieved by evaporation of sweat from the skin surface (70- 85%) but also evaporation of heat from the respiratory tract (15-30%) (Hodgson, 2014; Jones and Carlson, 1995; Marlin and Nankervis, 2002; McCutcheon et al., 1995; McCutcheon and Geor, 2014). One liter of sweat evaporated equates to the dissipation of approximately 2428 kJ of heat (McCutheon and Geor, 2014).

The capacity to sweat relies on an active vasodilatory and vasoconstrictor mechanism controlled by the autonomic nervous system in horses during exercise (McConaghy et al., 1996; McCutcheon and Geor, 2014; Ewart, 2020; Mota-Rojas et al., 2021a). The control of sweating is an integrated process including neural input (sympathetic nervous system) and humoral input via activation of β_2 adrenoreceptors on the sweat glands by epinephrine (Jones and Carlson, 1995; McCutcheon et al., 1995; Scott et al., 2001; Jenkinson et al., 2006; McCutcheon and Geor, 2008; Hodgson, 2014; Ewart, 2020; Mota-Rojas et al. 2021a; Brownlow and Mizzi, 2022). Overall, the increase in T_c is the main stimulus to activate sweating (**Figure 3**) (Hodgson et al., 1993; McCutcheon and Geor, 2014). Furthermore, the production of catecholamines associated with stress and exercise intensity also contributes to active sweating. Sweating rates in horses are two-to-three-fold times higher than human sweating rates; for example, a horse with BSA of 5 m^2 may sweat between 6 - 15 L h^{-1} (McCutcheon and Geor, 2014).

Sweat-related research has mainly involved TB horses while one study investigating Arabian endurance horses revealed the breed to have a lower sodium concentration in their sweat (Spooner et al., 2010). Estimated sweating rates are based on measured percentage BM loss by comparing the BM pre- and post-endurance exercise (Barnes et al., 2010). Reported estimated sweat loss is responsible for approximately 5 - 10% loss of equine BM depending on the environment (Hodgson et al 1994; Barnes et al., 2010; McCutcheon and Geor, 2014). Sweat production rate in the horse also varies by body location; rates measured on the neck and back of horses during laboratory-based exercise studies using ventilated capsules on the skin or absorbent pads showed that the sweating rate at the neck is double that at the quarters (Hodgson et al., 1993; McConaghy et al., 1995; Guthrie and Lund, 1998; Hodgson, 2014; McCutcheon and Geor, 2014). Other studies have determined sweating rate based on an objective visual sweat scoring system ranging from zero to five, based on qualitative descriptions ranging from localized sweating areas to profuse sweating (Zeyner et al., 2014). Quantitative measures of sweat used ventilated capsules attached to the skin surface and include analysis of ion concentrations. Equine sweat contains chloride as the major ion, followed by sodium, with low potassium and very low calcium (McCutheon et al., 1995). Ion concentration in sweat is dependent on the sweating rate and hence on the exercise intensity and environment, particularly the relative humidity (RH) (**Figure 3**) (Hodgson 1993; McCutheon et al., 1995; McCutcheon and Geor, 2000). Besides ions including ammonium, many other biomarkers are present in sweat such as cortisol, urea, lactate and interleukin 6 (Sakharov et al., 2010; Sonner et al., 2015; Klous et al., 2021)

As a result of the mechanism involved in the evaporative process, sweating as a cooling pathway becomes ineffective when the vapor pressure at the skin surface is at a maximum value in hot and humid weather conditions. In that case, more sweat is produced than can evaporate and sweat drips off the skin without cooling the skin surface. The inability to cool the skin can contribute to the development of hyperthermia.

1.4 Hyperthermia and Exertional heat illness (EHI)

Hyperthermia occurs when thermoregulatory homeostatic processes fail to compensate for increased heat production or in case of a resting situation, to dissipate normal resting metabolic heat output. Currently, hyperthermia has no international consensus definition; in some cases, it has been defined as the T_c above the upper limit (> 38.2°C) while other researchers classify upper limit T_c > 39.0°C or 40°C as hyperthermia. Hyperthermia represents a clear risk for development of EHI. A T_c of 42˚C is considered as severe hyperthermia in human studies (Bouchama and Knochel, 2002; Adams, 2020). McConaghy et al. (1995) reported that the muscle temperature is 2.5—3°C higher than T_c while the hypothalamus temperature is 0.5°C lower (41.5ºC). Risk of injury due to hyperthermia is associated with exposure to a high T_c and the risk increases when a high T_c exists for longer periods of time. Horses may be more susceptible to hyperthermia associated with exercise than humans for several reasons: horses are larger and engage a large percentage of active muscles during exercise while also having a lower BSA-to-volume ratio to dissipate heat. It is unclear at which upper T_c the pathophysiological events are triggered that eventually lead to EHI in horses (Hodgson, 2014).

1.4.1 Pathogenesis of EHI

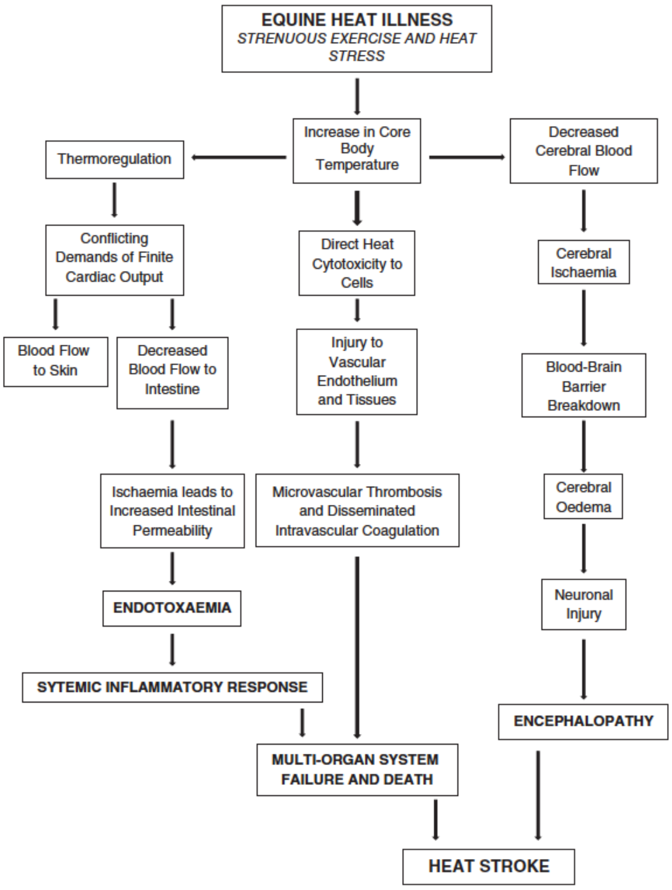

Figure 5. From Brownlow et al., 2016. A flowchart of the development of the equine Exertional Heat Illness (EHI).

Hyperthermia can affect the brain and muscle function in addition to manifestation of compromised gastrointestinal, respiratory and cardiovascular function.

Levels of severity are associated with the degree of damage at the level of the central nervous system and possibly coincide with systemic organ failure (Geor, 1998; Lindinger, 1999; Ewart, 2020). At the ultrastructural level, these high temperatures will denature many proteins and many enzymatic systems outside their optimal operational temperature will shut down, followed by apoptosis of cells, eventually resulting in multi-organ failure. While hyperthermia and heat stress are more commonly associated with execution of prolonged exercise, horses performing strenuous exercise over short time intervals may also suffer from heat stress (Brownlow and Mizzi, 2021).

Direct effects of hyperthermia

A T_c above 40°C can be life-threatening for exercising horses due to impaired cellular function and consequently, an inflammatory response of the body. This widespread cytotoxicity is a direct effect of the heat and may eventually lead to a systemic inflammatory response syndrome (SIRS) (**Figure 5**, Brownlow et al., 2016; Brownlow and Mizzi, 2021). Part of the SIRS cascade involves the injury of endothelium that progresses to coagulopathy, disseminated intravascular coagulation (DIC) and formation of microthrombi. These systemic inflammatory processes associated with EHI cause injury at the level of multiple organs such as heart, kidneys, liver and lungs (Casa et al., 2015; Brownlow et al., 2016, Brownlow and Mizzi, 2021). The hyperthermic status causes cerebral ischemia followed by increased blood-brain barrier permeability and the entrance of proteins and other osmotic active substances resulting in cerebral oedema. When EHI further progresses to exertional heat stroke (EHS), a complete failure of the body's thermoregulatory system occurs. EHS is commonly associated with neurological injury and eventually with multi-organ dysfunction/failure and death in extreme cases (Brownlow et al., 2016; Brownlow and Mizzi, 2021).

Effects of long lasting challenging the thermoregulatory system

Long term intensive thermoregulatory coping has its consequences at the ultrastructural level at many locations such as the neuronal circuitry, chemical reactions, and fluid exchange via cell membranes. Endurance horses, for example, rely to a large extent on hypertonic sweat loss to cool down and may sweat approximately 15 to 20 liters per hour when exercising in cool, dry conditions and up to 30 liters per hour in warm and humid climates. Keeping in mind that approximately 10% of the BM represents the circulating blood volume, these are astonishing amounts. During an endurance ride in a moderate T_a with normal water intake during rest periods, the estimated dehydration is 6% of BM but in a hot environment, dehydration may be up to 10% (Hodgson et al., 1994; Marlin and Nankervis, 2002; Barnes et al., 2010). During endurance rides, sweating rate is highest throughout the first two to three hours over a distance of approximately 32 km into a 160 km ride. The horses' anxiety and horse-specific temperament might be considered a potential cause of increased sweat loss during the first endurance loop (McCutcheon and Geor, 2010). As a result, the highest body weight loss occurs during the first half of the ride (Barnes et al., 2010; McCutcheon and Geor, 2010; Munoz et al., 2017).

Due to their mildly hypertonic sweat, endurance horses may lose large amounts of electrolytes in each liter of sweat that is produced. As a consequence, the overall loss of key electrolytes may lead to low blood sodium concentrations and a lower plasma osmolality, failing to stimulate central osmotic receptors in the hypothalamus to trigger the secretion of antidiuretic hormone (ADH) also known as vasopressin. As a result of the lack of ADH secretion, the thirst response is not activated in severely dehydrated horses and eventually discouraging water intake just when it is most needed. Dehydration and electrolyte loss in turn result in a decreased sweating response (Muñoz et al., 2017). In addition, the redistribution of the blood flow to the skin to favor sweating will decrease effective circulating volume to the gastro-intestinal (GI) tract and muscular compartment. This intestinal ischemia due to redistribution of the blood flow may cause intestinal barrier break down and consequently a high intestinal permeability and endotoxin translocation adding to the progress of SIRS. The change in cardiac output is triggered by thermoregulatory-induced physiological feedback failure and exhaustion which also affect other body systems ultimately resulting in "exhausted horse syndrome". In addition, the combined effects of energy, fluid and electrolyte depletion may also result in exhausted horse syndrome including GI and central nervous system (CNS) dysfunction (Foreman, 1998; Muñoz et al., 2017).

1.4.2 Clinical signs of EHI in endurance horses versus in racehorses

Clinical signs of EHI in horses that are manifested typically depend on the type of sport discipline. Endurance horses develop metabolic disorders and heat exhaustion due to long lasting effects of thermoregulatory stress such as dehydration and electrolyte loss while high-intensity exercise horses such as racehorses develop EHI with predominantly neurological signs. Both EHI and EHS involve extensive organ and tissue injury, the pathophysiology of which is still not completely unravelled and understood.

Metabolic disorders in horses generally are mainly governed by dehydration and electrolyte loss, leading to reduced thermoregulatory coping capacity and associated injury to various body systems. All these biological changes can additionally lead to a decreased cardiac output. The electrolyte disbalance may alter membrane potential and contribute to the onset of GI ileus, manifest cardiac arrhythmia and synchronous diaphragmatic flutter (SDF). Common debilitating metabolic disorders in endurance horses include abdominal discomfort (colic), dehydration, SDF, exertional rhabdomyolysis (ER) and hyperthermia (Fielding et al., 2009; Misheff, 2020).

An SDF is a spasmodic contraction of the diaphragm synchronous with the heartbeat. The movement produces an audible thump; hence SDF is commonly known as “thumps”. Changes in electrolyte levels such as calcium, magnesium and potassium alter the membrane potential of the phrenic nerve which is then discharged at the time of atrial depolarization. This condition is reported in endurance horses exercising in warm conditions and is related to electrolyte imbalances. However, it is also anecdotally reported in eventing and racehorses. The cause and prevalence of SDF in racehorses is currently unknown and the association of SDF with warm conditions and other risk factors requires further investigation.

Cardiac abnormalities due to electrolyte loss are more commonly reported in human endurance athletes than in endurance horses. When metabolic disorders progress, GI permeability increases, leading to translocation of endotoxins into the blood circulation and triggering a systemic inflammatory response (SIRS) that can lead to shock. Eventually exhausted horse syndrome or ‘heat exhaustion’ develops with clinical signs varying from mild to more severe. This heat exhaustion condition is not a failure of the thermoregulation, it is more a product of the combined presence of dehydration, hypovolemia and hypotension due to fluid evaporation in response to heat production (Foreman, 1998; Muñoz et al., 2017; Ewart, 2020).

Clinical signs of exhausted horse syndrome and EHI include depression, increased T_c, dehydration (slow capillary refill times, decreased pulse pressure), increased respiratory rate (RR), ileus and colic, weakness and fatigue (Fielding et al., 2017; Muñoz et al., 2017). Tachycardia related to an increased T_c will continue to rise to compensate for the low plasma volume in order to guarantee cardiac output. The RR increases in an attempt to increase evaporation from the respiratory tract and can double its normal rate when exercising in hot and humid environment and with heart rate up to 80 -100 bpm (Brownlow et al., 2016; Fielding et al., 2017; Brownlow and Mizzi, 2021). Eventually clinical signs may progress to intestinal ischemia, exertional rhabdomyolysis, renal failure, liver dysfunction, and neural ischemia. A post-exhaustion syndrome up to 48 hours post-exercise describes signs of severe complications of SIRS such as laminitis, myonecrosis, colic, renal and hepatic failure. Signs related to endotoxemia and SIRS may further be complicated by laminitis.

The clinical manifestations of EHI include neurological signs varying from abnormal mentation with irritability, depression, head shaking and random kicking, and progressing to disorientation, unpredictable springing forward and ataxia. The neurological signs occur more typically in racehorses and commonly progress to EHS which can include dry skin, loss of consciousness, delirium, pupil dilation, muscle rigidity, seizures and collapse and death. Brownlow et al. (2016, 2021) categorized the clinical signs in racehorses by levels: level 1—vague signs of irritability, restlessness and agitation; level 2—continuous or spasmodic uncontrolled “kicking”; level 3—various bizarre neurological signs from altered mentation to ataxia including an abnormal gait referred to as “broken leg syndrome” (often misdiagnosed as such); level 4—disorientation and collapse.

1.4.3 Prevalence of EHI in various equine sport disciplines

Several studies have been performed in the past looking into prevalence, however, currently there is as a consensus amongst researchers has become clear that many cases go unnoticed, and thus, only severe cases are being recognized. Secondly, it is expected that because of global warming, this problem will inevitably continue to grow for all sport horses and human athletes.

EHI and equine endurance exercise

Metabolic disorders in endurance horses lead to the elimination of the horse from an endurance ride. The prevalence of metabolic disorders in endurance horses ranges from 4.2 - 15% (Barnes et al., 2010; Nagy et al., 2010; Fielding et al., 2011; Nagy et al., 2014; Younes et al., 2015; Fielding et al., 2017; Muñoz et al., 2017; Bennet and Parkin, 2018; Legg et al., 2019). More research is needed to map out the exact prevalence of metabolic disorders, heat exhaustion and EHI in order to decrease the elimination rate during rides.

EHI and equine high intensity exercise

Recently, the prevalence of EHI in racehorses has been reported by two studies. One from Japan stated a prevalence of 0.09% during summer with a clear increase over the past few years (Nomura et al., 2019; Takahashi and Takahashi, 2020). Another study performed in eastern Australia focused on selected EHI cases post-exercise at the racetrack and suggested an EHI incidence of up to 9.5% during hot summer months (Brownlow and Brotherhood, 2021). The latter study used the four severity levels of EHI reported by Brownlow et al. (2016) and concluded that 96% of exercising horses could be categorized as level 1. This suggests that low level and thus discrete EHI cases may have been overlooked in the past. Further investigation into the prevalence in EHI in different states in Australia is required and importantly, into risk factors related to EHI in racehorses to prevent EHI in the future.

1.4.4 Treatment of hyperthermia

Treating hyperthermia by increasing evaporative heat loss can be accomplished using several cooling techniques (Kohn and Hinchcliff, 1995; Marlin et al., 1998; Takahashi et al., 2020). The first treatment of hyperthermia involves aggressive cooling by applying cold to ice cold (0-12 °C) water over the entire body to encourage conductive transfer of body heat (Elliot, 2021). Concerns that water would function as an insulator especially in horses with a longer haircoat is counteracted by the fact that water is a better conductor of heat than air. Pouring water over the entire body also cools the body surface through evaporation. However, evaporative cooling is slower than the conductive transfer of H. To enhance the temperature gradient, a sweat scraper was commonly used to remove water warmed-up by the skin. However, this seems to be a waste of time when dealing with cases of severe heat stress and collapse in humans, horses and dogs. Also, in the past, cooling of the large back muscles (gluteal and biceps muscles) was discouraged due to the assumption this would cause muscular problems. In fact, cold water only affects skin temperature and not deeper layers such as the muscles.

Cooling management in horses is targeted to achieve a T_c of 39°C (preferably 38.5°C) or lower in order to lower the risk of developing EHI and deterioration to EHS. Firstly, treatment of a hyperthermic horse should be performed in a shaded, ventilated area. Additionally, large misting fans or evaporative mobile air-conditioning cooling units support the convection of heat from the hyperthermic body by enhancing circulation of surrounding air to enable a lower T_a and immediate removal of evaporated sweat.

Treatment by a combination of aggressive cooling methods may prevent more serious complications associated with heat exhaustion. If the T_c does not fall below 39°C or even further increases, more aggressive, invasive treatment is indicated. Such aggressive treatment includes large volumes of IV isotonic fluids, providing at least 20-30 liters through a large bore (12-gauge) IV catheter. The total fluid load needed ranges from 20-80 liters per horse, commonly administered until the horse urinates. The use of hypertonic saline (3%), similar to use in cases of cranial and brain injury, may be effective in reducing cerebral oedema if horses are unresponsive to initial fluid therapy.

To facilitate handling horses with EHI and resultant unpredictable behavior such as kicking, medication with alpha-2 adrenoreceptor agonists is recommended to provide sedation, muscle relaxation and analgesia. The careful use of anti-inflammatories is advised in dehydrated horses to inhibit synthesis of cytokines, prostaglandins and thromboxane involved in cases with SIRS. To date, the use of corticosteroids seems controversial. Seizure events may require IV treatment with anti-seizure medication such as benzodiazepines until the seizure is under control.

1.4.5 Prognosis of horses with EHI

Horses with hyperthermia tend to be depressed, tired and weak and treatment by aggressive cooling methods can prevent more serious complications associated with heat exhaustion. With adequate therapy, T_c may reduce by 3 - 4°C within 30 - 60 minutes, along with lower HR and RR and improved mentation. A positive clinical response to treatment will be associated with a good prognosis and a T_c below 40 °C. If the T_c does not decrease or even increases, complications may develop. A T_c above 42-43 °C can result directly in physical cell damage such as in the muscles, the GI tract and central nervous system function (Hodgson et al., 1994; McConaghy et al., 2002; Brownlow et al., 2016). In severe cases, multiple organ system collapse and endotoxemia can occur, often complicated by renal failure and rarely cardiac arrhythmias. These cases have a potentially unfavorable prognosis for overall survival. Some horses develop complications 2 - 4 days post exhaustion. These include myopathy, progressive laminitis, renal failure secondary to muscle necrosis, myoglobinuria, gastrointestinal ulceration, hepatic dysfunction, impaction colic and central nervous system disorders (Foreman, 1998; Muñoz et al., 2017).

1.4.6 Risk factors

The thermo-neutral zone (TNZ) or comfort zone is the T_a at which a warm-blooded animal can maintain its T_c with minimal effort and metabolic rate. In the horse this is between 5 °C and 25 °C (Ewart, 2020). When compared to humans, the range and upper critical temperature of TNZ is different (20 – 30 °C) hence in a T_a of 25 °C, humans are still within their comfort zone as opposed to horses being outside of their thermo-comfort zone (Ewart, 2020). Once the critical upper limit of the TNZ is reached in all mammals, thermoregulation processes are intensified.

Environment

The heat dissipation capacity of the environment is not only determined by surrounding air or T_a. Other factors such as solar radiation, RH and air velocity (wind speed) are essential aspects of real-time field exercise in different climate conditions. Consequently, the location of where the exercise is performed is part of the heat loss capacity assessment. For example, heat loss capacity may vary at different racetrack locations (Brownlow and Mizzi, 2022).

The TNZ for horses is further influenced by the number of days exposed to the environment involving acclimatization along with individual variables such as age, breed, skin and hair thickness, body condition, diet and living conditions (indoor or outdoor).

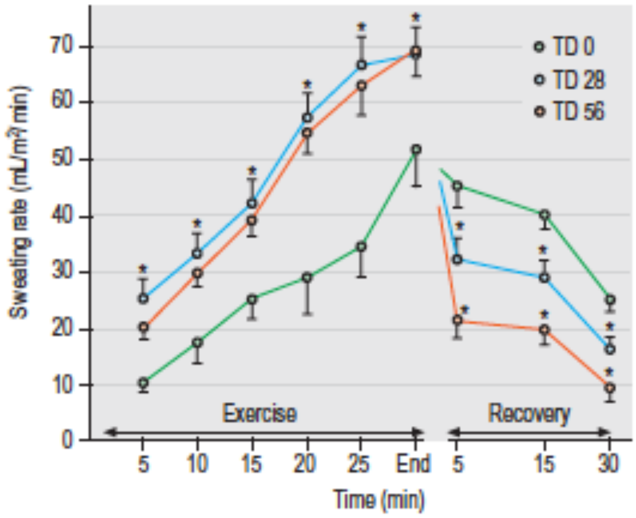

Figure 6. Shows the sweating rate response during exercise at hot, dry conditions (32-34°C and 45-55% relative humidity [RH]) and during recovery. Green curve shows these results on day 0, blue on day 28 and orange on day 56 of an 8-week exercise training protocol in moderate conditions (19-21°C and 45-55% RH). From: McCutcheon and Geor, 2000.

Overall, thermoregulation is especially challenged in hot (greater than 25°C) and humid (70-90% RH) weather conditions (Guthrie and Lund, 1998; Marlin et al., 2001). These environmental conditions can compromise heat loss leading to an elevated T_c. For example, a T_a over 35 °C reduces the thermal gradient between the skin and T_a and consequently, lowers the rate of heat transfer. **Figure 6** demonstrates the sweating rate response in a hot environment with a time related linear increase of the sweating range during standard exercise tests and an acclimation over 56 days of exercise training in a moderate environment. There appears to be a better equilibrium in heat production and heat loss due to increased ability to sweat. The benefit is seen during recovery when the sweating ratio drops faster, indicating no or less heat storage.

If the humidity rises, the vapor gradient between skin and the nearby environment impairs the evaporation of sweat. This will result in sweat dripping off the skin which is called ‘over sweating’. Over sweating is a waste of fluid as dripping only removes approximately 10% of the heat compared to evaporation. The normal sweating rate and over-sweating are not directly related to each other but depend on the effect of RH, radiation and convection on *H*. For example, the rate of *H* production doubles in a hot and humid environment compared to a cool and dry environment (Geor et al., 1995; Geor et al., 1998; Lindinger, 1999).

A considerable volume of research was carried out in preparation for the 1996 Olympic Games in Atlanta and the 2008 Olympic Games in Hongkong, to investigate if conditioning and heat acclimatization would improve tolerance of the equine athletes to exercise in hot circumstances. Evidence suggests that after acclimatization, the equine body is better able to maintain cardiovascular homeostasis in hot temperatures and conserve salt while sweating profusely (McCutcheon & Geor, 2000; Marlin et al.,1999; Geor et al., 2000; Lindlinger et al., 2000). McCutcheon et al. (1999) investigated the sweating rate after varying training sessions and showed that acclimatization over 21 days decreased the total sweat loss especially during recovery. Such an effect is also suggested by **Figure 6**. Notably, many riders train their horses in the morning or evening when it is cool, while the competition or race is often held during the hottest time of the day. This means that when training for a competition, horses require a few weeks of acclimatization by training at the same time of day as the competition depending on the actual temperature. If training takes place at the cooler periods of the day, acclimation will occur to but will probably require a few weeks more.

Projected influence of global warming

Global warming is a generally accepted phenomenon and the scientific community is warning the world about its consequences (Bouchama, 2006; Luber and McGeehin, 2008; Poumadere et al., 2005; Solymosi et al., 2010; Summers et al., 2009). Changing environmental conditions are the dominant risk factor for the occurrence of heat stress and EHI for both human and equine athletes. These cases are expected to rise in prevalence due to global warming (Brownlow et al., 2016; Nomura et al., 2019; Raymond et al., 2020; Takahashi and Takahashi, 2020). Likewise, for sporting events in the open air, a robust thermoregulatory welfare monitoring protocol needs to be created in order to prevent occurrence of heat stress. Effective monitoring is essential to safeguard horse welfare.

Many regions around the world already record a Wet Bulb Globe Temperature (WBGT) above 28°C during approximately 2 to 3 months annually. These regions include the southern part of North America and Europe, Oceania, parts of Africa, South America and Asia (Raymond et al., 2020). The horseracing industry and equine sport competitions are well-established in those continents with warm to hot climates. For example, countries with a very hot climate such as Saudi Arabia, Qatar and Dubai (United Arab Emirates) are amongst the world’s top 10 leading countries hosting horseracing, often with the highest prize money. Those countries are also highly popular for their long-distance endurance rides (Nagy et al., 2010). Obviously, local horses there have been reasonably acclimatized to their hotter circumstances, although global warming is very likely to even put this adaptation under threat.

In the face of climate change, heat stress will become an increasingly challenging issue for a wide array of equine sports disciplines, especially during field competitions. Over the past century, for example, the average temperature in Australia increased by 1°C. In addition, the number of very hot days during each year has increased and extreme heat waves are becoming more frequent (Australian BOM, CSIRO; Hanna, 2011). Another hazard related to sudden heat waves are the bush fires occurring worldwide affecting all mammals including horses.

Horse-related risk factors

Armstrong et al. (2007) reported risk factors in human athletes in their consensus statement in order to prevent EHS. In addition to hot and humid environmental conditions, the most common risk factors were unfitness, higher BM, age, lack of acclimation and presence of dehydration. Similar risk factors are reported in equine athletes. It is also of major concern that a previous EHI experience will increase the risk of recurrence based on one study in South African racehorses (Brownlow and Miszzi, 2021) and the evidence from studies involving human athletes (Adams, 2020). The prediction of EHI risk in an individual horse is difficult due to the individual variation in heat dissipation capacity, which in its turn is associated with intrinsic horse-related factors. Factors that affect this ability include genotype, breed, sex, body condition score, morphological differences, age and their character or temperament (stress, nervousness, motivation). Skin-related properties such as sweating rate, skin thickness, blood vessel density, hair coat length, clipping and coat color all influence the efficacy of evaporation (Mostert et al., 1996; Morgan et al., 2002; Wallsten et al., 2012; Wilk et al., 2020; Maśko et al., 2021; Mota-Rojas et al., 2021a; Mota-Rojas et al., 2021b; Domino et al., 2022). The individual variation in heat dissipation capacity is associated with genotype and breed. In this respect some interesting developments in the genetics influencing heat tolerance are reported by Wang et al., (2022). The Jinjiang horse population seems to be unique in its adaptation to high temperature and humidity and for instance in terms of molecular functions, many copy number variants, regions which are enriched in nucleic acid binding, receptor activity and heat shock protein binding. Thus, the identification of specific genes conferring thermo-tolerance in heat-tolerant breeds may be an additional strategy to improve the genetic background in sport breeds. The study in the Jinjiang suggest that candidate genes may be the nuclear factor-kappa B (NF-κB) gene and the heat shock gene (HspA1A). The rationale is that by a rapid activation of NFκB by hyperthermia, induction of heat shock protein (Hsp) takes place which confers protection against various forms of cellular and tissue injury.

The effect of breed relates to the ratio of BSA to BM—the higher the relative BSA in relation to the BM, the higher the heat dissipation capacity (Morgan, 1995). The effect of breed relates to the low BSA-to-BM ratio of horses. In particular, heavy breeds result in greater demands being imposed on the thermoregulatory system (Hodgson et al., 1993; Hodgson et al., 1994; Wallsten et al., 2012). Horses are prey animals and when the autonomic sympathetic nervous system is activated in a fight-or-flight response situation, the H and HR are significantly increased (Ewart, 2020; Brownlow and Mizzi, 2021; Mota-Rojas et al., 2021a). Therefore, in nervous and stressed horses, the release of catecholamines is associated with increased sweat and metabolic rates (Mota-Rojas et al., 2021a). McKeever et al. (2010) reported that aging horses reached a T_c of 40 °C twice as fast during exercise compared to young horses. Notably, this experiment included only five horses with an average age of 26 years, an age at which horses are normally retired from sport activities.

Other horse-related risk factors include unfitness, overweight and failure to rehydrate. Dehydration prior to exercise could be due to water withholding practices or long duration transportation. The use of furosemide in racehorses may be associated with varying degrees of dehydration and a 5.5 times higher risk for developing EHI (Warren et al., 1999). Dehydration before and during the competition affects the ability to lose heat and dehydrated horses develop fatigue at lower levels of hyperthermia.

Horses competing in tropical climates for a longer time may develop anhidrosis, better known as the inability to sweat and thus lose heat via evaporation. Reported prevalence of partial to complete anhidrosis varies from 6% up to 25% of horses living in subtropical and tropical regions (Mayhew and Ferguson II, 1987; Johnson et al., 2010). The cause of the anhidrosis in horses is currently unknown and therefore defined as acquired idiopathic anhidrosis. Horses with anhidrosis have an impaired functioning of the β_2-adrenoceptor pathway resulting in a decreased functioning of the sweat glands which may lead to their degeneration (Wilson et al., 2007). Breuhaus (2009) showed that anhidrotic horses have a normal thyroid function compared with normal horses. Anhidrosis is associated with poor hair coat, reduced performance and a high risk of developing hyperthermia (Mayhew and Ferguson II, 1987; Johnson et al., 2010; Hodgson, 1994). In addition, a horse may present with poor performance and respiratory disease (tachypnoea, cough, pleural abnormalities and neutrophilic lung cytology) (Sullivan et al., 2015). Diagnosis is confirmed by an intradermal terbutaline test and skin biopsy. Treatment is not available, apart from moving horses to a cooler environment. Future research is required to identify the pathogenesis of anhidrosis especially in association with the current global warming.

Malignant hyperthermia (MH) is a more extreme example of genetic heat intolerance largely due to a mutation in the ryanodine receptor (RYR) causing excess release of calcium from the sarcoplasmic reticulum in muscle cells leading to cell death (Manley et al., 1983; Aleman et al., 2009). Malignant hyperthermia is most common in pigs and humans. An inherited autosomal dominant single point mutation in the RYR1 gene is linked to Quarter horses and Paint horses (Aleman et al., 2009; McCue et al 2009). A more severe clinical phenotype occurs in horses also affected with polysaccharide storage myopathy (McCue et al., 2009). Interestingly, EHS and ER have some similarities to a MH-like syndrome in humans related to exercise and a hot environment (Capacchione and Muldoon, 2009; Hosokawa et al., 2017; van den Bersselaar et al., 2021). In that respect, Wilberger et al. (2013) did not detect RYR1 mutations during genetic testing of hair samples in 101 endurance horses with and without ER.

Exercise-related risk factors

Exercise factors potentially influencing thermoregulation include terrain condition, ground surface, weight of the rider, long warming-up periods, long distance transportation prior to exercise, time of the day combined with equine circadian rhythms and the surrounding environment as described earlier (Hodgson, 2014). For example, Schroter and Marlin (2002) modelled the oxygen “cost of transport (COT)” in an equation to include exercising on various terrain conditions such as flat, uphill and downhill. Specific predisposing risk factors in racehorses include racing on sand tracks (2.25 times higher risk than racing on turf tracks), longer distance races, higher age, gender (geldings) and racing during the day (MacDonald et al., 2008; Takahashi and Takahashi, 2020).

Horses may already have an unrecognized increased T_c following transportation prior to an event on warm to hot days especially in poorly ventilated vehicles. A study in Australia showed that a minimum of 10% of the problems occurring in long distance transport was related to heat stress (Padalino et al., 2015). Marlin et al. (2001) reported that the recovery from transport and an additional acclimatization for 16 days prior to competition in a hot T_a (WBGT index 27.6°C) provided sufficient time if HR, rectal temperature (T_{re}) and plasma volume were monitored.

In conclusion, the influence of the difference in breeds, BM and coat colors in livestock, dogs and horses related to the genotypic profile is well reported. A reliable approach to monitoring thermoregulation of individual equine athletes will provide essential information on the thermal response of a specific horse to its metabolic heat load and its environmental challenges (Marlin et al., 1999) similar to recent reports in human athletes (Adams, 2020; De Korte et al., 2021).

1.4.7 Prevention of heat stress and EHI

Prevention of heat stress at competitions, races and events during warm weather is essential. In that respect, it’s important to realize that many subtle cases go unnoticed, which is all the more reason be very vigilant. Preventative measures at competitions or racing organizations include providing adequate facilities for application of cooling strategies post-exercise, including water hoses, ice, fans and shade. In addition, availability of suitable drinking water tap points in proportion to the number of horses is essential. Riders should monitor physiological responses of their horses including HR and RR as well as observing the general mentation of the horse and its willingness to continue exercise.

Stimulation of water and food uptake is another key to EHI prevention, especially in endurance, polo and eventing horses. Additional supplementation with electrolytes is a preventative strategy to balance hypertonic sweat loss due to thermoregulatory demands (Warren et al., 1999; McCutcheon and Geor, 2010). However, currently more research is required to provide data on which electrolyte combination needs to be given and even more importantly at which time points. Water uptake is stimulated by electrolyte supplementation and can be facilitated by offering water preferably at 20 °C with electrolytes and good quality hay (Düsterdieck et al., 1999; Butudom et al., 2002; 2004; Sampieri et al., 2006). However, in general, the thirst response is not activated in these dehydrated horses, therefore spontaneous water uptake remains a challenge, according to the common saying, “You can lead a horse to water, however, you can’t make it drink”. Other strategies include a moderate warm-up for the competition, pre-cooling of the body and hyperhydration before exercise in warm conditions (Korte et al., 2017; Klous et al., 2021).

To safeguard the welfare of horses worldwide, hot weather policies have been developed, for example, in Australia by Equestrian Australia (EA policy, 2012). These policies contain useful recommendations and rules for all equestrian sport competitions that compete under different weather circumstances. For example, equine athletes can benefit from changes of start times (early morning or at night) and shortening or changing a cross-country or endurance course. These policies also determine whether or not the temperature is suitable to start or to continue the competition or race. Perhaps not surprisingly however, these policies differ not only across sports disciplines but also amongst states and countries around the world. Nevertheless, hot weather policies should be evidence-based and based on a consensus of heat stress supported by experts involved.

The primary significant outcome from the 1996 “Atlanta Project” studies was that the WBGT index was identified as a measure for conditions conducive to the development of heat stress in horses. The WBGT combines dry bulb temperature, humidity, wind speed, solar radiation (sun angle and cloud cover). The WBGT is used as a proxy to indicate risk of heat stress in direct sunlight and is commonly published on national meteorology organization websites. The risk of EHI is especially relevant in warm climates where the WBGT index can vary from 32°C up to 39°C. The WBGT index is commonly used in hot weather policies to prevent EHI during competitions in the field: above 28°C, precautions are required while 32–33°C is considered to be hazardous and cancellation of competitions is recommended particularly when WBGT is over 33°C. Recently, Brownlow and Brotherhood (2021) included the measurement of vapor pressure as an additional tool for monitoring the potential for occurrence of heat stress.

An example of how weather conditions can challenge top level international equestrian competition is the cancelation of the Endurance ride during the 2018 World Equestrian Games. This decision was made by the organization, while horses were already 80 km into the ride because over 50% of participating horses were treated for metabolic disorders. The decision to cancel was in line with the FEI Code of Conduct for the Welfare of the Horse which states competitions must not take place in extreme weather conditions (WBGT index above 31°C) that may compromise welfare or safety of the horse and rider.

Additionally, transport guidelines for safeguarding horses during transportation on warm days are also essential. Transport regulations such as the European Union and IATA transport rules specify rules such as maximal T_a, maximal total travel hours, rest time including walking, ventilation, providing food and water (IATA rules: https://www.iata.org/en/publications/store/live-animals-regulations/).

However, transport rules specifically for horses are not in place in many countries such as Australia. For example, horses affected by ER, exhausted horse syndrome or EHI should not be transported for 12 – 24 hours. Moreover, all EHI prevention recommendations should be driven and augmented by the education of the riders, trainers and horse owners, basically everyone who takes part in the equestrian industry.

1.5 Different temperature monitoring methods: invasive versus non-invasive

To prevent episodes of hyperthermia in exercising horses, it is critical to assess thermoregulation by monitoring the T_c during real-time competition exercise. In addition, it can be informative to monitor other physiological parameters such as speed, heart rate and electrolyte balance during competition exercise. An accurate assessment and continuous T_c monitoring will provide customized and vital oversight of the thermal response of an individual horse to metabolic heat load and environmental challenges. This knowledge is crucial to identify nonlethal and lethal heat exhaustion markers (Marlin, et al., 1999). The pace at which problems that are associated with global warming manifest is the driving force to quickly develop and validate solid continuous monitoring techniques to safeguard thermoregulatory wellbeing (Smith et al., 2006; Klous et al., 2020; Brownlow and Smith, 2021). To this end, many wearable devices have recently come to market and the temptation is great to apply all these wearables designed for humans directly onto horses. These wearables provide useful and varied data output for athletes, however, the physiological significance of these data for horses has not been characterized.

To date, most equine studies have been performed indoors in laboratory settings with invasive methods of T_c recording. For example, several methods for monitoring T_c in horses include applying a rectal sensor, blood catheterization, measuring surface skin temperature, or an internal (deep body) sensor. Although restricted to controlled indoor conditions, these laboratory-based studies have provided essential information on horses exercising in hot and humid environments.

1.5.1 Laboratory-based thermoregulation research - invasive monitoring

Most research investigating thermoregulation has been performed in TB horses during the 1990s. A large body of this research was published in preparation for the Olympic Games namely “The Atlanta project”. Those thermoregulation studies were all laboratory-based. Horses were exercising on a treadmill in a controlled environment during standardized exercise testing (SET) (Hodgson et al., 1993; Marlin et al., 1996; Courouce-Malblanc, 2013; Hodgson, 2014). While those findings resulted in significant changes being applied to the management of those equine sport events, the results may have minimal relationship to thermoregulatory monitoring during real-time exercise in the field. Monitoring of the T_c in those laboratory-based studies was predominantly conducted by continuously measuring the arterial (pulmonary) blood temperature, proven to be ‘the gold standard’ (Hodgson et al., 1993; Marlin et al., 1996; Courouce-Malblanc, 2013; Hodgson, 2014). However, during field exercise, this approach is not feasible and is too invasive. One field study did use thermistors to measure blood and brain temperature in three free-running horses in the field (Mitchell et al. 2006). Essentially, the laboratory conditions cannot replicate the exact situations of horses competing in open air over varying terrain and being additionally challenged by weather conditions, interaction with other horses, to name a few factors

1.5.2 Field based thermoregulation research – non-invasive monitoring

Field exercise monitoring is essential to provide real-time data of individual horses exercising in various outdoor circumstances. An obvious example of differences between laboratory and field exercise is the physical location of the exercise. The outdoor location circumstances may vary widely for example on terrain such as soil, turf, grass, irregular surfaces, or an inclining or declining surface. Another aspect related to the location in the field is the ambient environment: shade versus solar radiation and wind versus an enclosed area without wind. All these aspects must be accounted for when monitoring body temperature in the field and for that purpose, monitoring thermoregulation during field exercise is required.

To date, little is known about thermoregulation in real circumstances during field exercise and recovery in different equine sports disciplines. One reason is the previous lack of effective temperature monitoring equipment that can be safely and comfortably applied during field exercise. Monitoring equipment must be reliable, practical with minimal animal handling, safe for the horse and the rider, and ideally non-invasive.
Currently in existing field studies, the most common method to record T_c in field competition is serial measurements of the T_{re} pre- and post-exercise (Kohn and Hinchcliff, 1995; Kohn et al., 1995; Hargreaves et al., 1999; Jeffcott et al., 2009; Wallsten et al., 2012). These are “snapshots” of the temperature evolvement and practice has proven that this approach doesn’t allow remedial intervention at early

stages. By the time an increased T_{re} is measured, the thermoregulatory system is already under substantial pressure. In addition, taking the T_{re} can be dangerous in heat-affected horses and can record incorrectly due to the loss of anal tone. Hence, there is a need to monitor equine core body temperature response accurately and reliably in the field to avoid potential heat-related harm.

The principal aim of monitoring core body temperature is to function as a ‘whistle blower’ for changes in temperature that indicate the horse’s wellbeing is under threat during or after exercise. Only continuous real-time checking during actual field exercise will meet this aim. When evaluating temperature monitoring methods, it is essential to appreciate that from a physiological standpoint, a time-lag exists between exercise-induced H output and T_c evolvement. The T_c evolvement is subsequently translated into an additional temperature time-lag evolvement expressed at several different anatomical locations such as the rectum, the muscular compartment, and the skin surface. Evolvement can be additionally complicated by environmental factors such as hot and humid weather (Kohn and Hinchcliff, 1995; Marlin et al., 1996; Jeffcott and Kohn, 1999; Kohn et al., 1999; Jeffcott et al., 2009; Wartzek et al., 2011; Hodgson, 2014; Raymond et al., 2020; Mota-Rojas et al., 2021a).

A few studies focus on continuous monitoring of equine thermoregulation and the T_c during exercise and recovery in real-time field competitions (Smith et al., 2006). One of those studies used intra-uterine temperature loggers as a nonsurgical, minimally invasive method (Smith et al., 2006). This method has the advantage of collecting data over several competitions, however, data were downloaded only after removal of the logger. In contrast, continuous telemetric recording methods provide real-time results throughout and after the competition, thus allowing early detection of hyperthermia which does not require subsequent time-critical analysis.

1.6 Overview of gastrointestinal pill monitoring in mammals

A novel monitoring method applicable during exercise training and competition in the field has been investigated, namely a telemetric gastrointestinal (GI) temperature pill. In human athletes, the use of GI pills to measure and monitor T_c has been reported to be a common practice. The GI pill allows for accurate continuous recording of the GI temperature during field exercise, for example in marathon, football, military, tennis exercising. The GI temperature pill is a non-invasive method and was evaluated in several athletic temperature studies during exercise. It was found to be highly practical, as it was easily swallowed with drinking water, and a reliable method to monitor the body's thermal response to exercise. Importantly, the GI pill has proven to be a more accurate and precise tool to monitor thermal response than serial T_{re} measurements.

To date, the GI pill has been used during field exercise in other mammals such as elephants and dogs, and was used to monitor T_c in resting cattle in the field. One study evaluated the pill in horses at rest and during 158 km transport using the CorTemp® system (Green et al., 2005; Green et al., 2008). An overview of past and current GI pills and their technical details is presented in **Table 2**.

Another effective approach to monitoring the thermoregulatory response in exercising horses in the field can be continuous monitoring of surface skin temperature (T_{sk}) as a reliable proxy for monitoring thermoregulatory wellbeing. Advantages of using tools such as infrared thermography (IRT) to assess T_{sk} include the non-invasive nature and easy collection of temperature data (Mota-Rojas et al. 2021b; Rojas-Valverde et al., 2021).

Name GI pill, country	Horse Y/N	Transfer distance	Manufacturer accuracy***	Bias** (°C)	Reliability ** (bias) (°C)	Inertia: response time (seconds, s)**	Remarks
Jonah pill (Vital sense, Phillips), USA	Y	Excellent, > 1m	0.17	-0.017°C ± 0.023°C	0.002°C ± 0.014°C#	39 ± 6 s	Connect with Equivital manager Discontinued
Smartpill, USA	Ponies only (Stokes et al. 2012)	Only transfer T_c in ponies with antenna	…	-	-	-	Measures pH and intraluminal pressure
CorTemp, USA	Y, horse at rest (Green et al., 2006)	0.65 m	0.27	0.077°C ± 0.040°C	0.017°C ± 0.083°C	25 ± 4 s	Connect with CorTrack manager *Unpractical
Anipill (e-Celsius), France	? * Unsuccessful (unpublished information)	?	0.23	-0.081°C ± 0.055°C	-0.007°C ± 0.033°C	21 ± 13	Connect with Equivital and e-Performance manager
MyTemp, the Netherlands	N	1m		-0.003°C ± 0.006°C	0.001°C ± 0.008°C#	19 ± 2 s	Connect with MyTemp manager Interest to evaluate in horses

Table 2. Overview of gastrointestinal (GI) pills and their use. *Authors' opinion after investigating in several horses in the field; **Comparison in vitro study based on temperature-controlled water baths from Bongers et al. (2018); ***manufacturer details; # values did not differ when GI temperature recording was repeated (reliability).

An overview of the T_{sk} equipment used in a number of equine exercise studies shows that 19 studies used IRT, five studies used thermocouples and seven studies used thermistors including two studies using the i-Button®.

1.7 Conclusion

Monitoring the thermoregulation of individual sport horses is vital to ensuring their ongoing physical wellbeing and athletic performance. A horse's core temperature can function as a 'whistle blower' for temperature rises that indicate the horse's health and performance are under threat during or after exercise. Consequently, monitoring a horse's core body temperature response accurately and reliably during competition can prevent heat illness and avoid potential harm to exercising horses. The current approaches to monitoring thermoregulatory responses such as T_{re}, and T_{sk} cannot fully reflect core temperature during real-time field conditions. Therefore, the hypothesis of this PhD is that equine core body temperature can be most successfully monitored continuously, precisely and in real-time during exercise and recovery by a telemetric gastrointestinal temperature measuring device.

1.8 References

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Chapter 2

Aims of thesis

Hyperthermia and Exertional Heat Illness (EHI) are of increasing worldwide concern impacting various disciplines in the horse industry including racing and competitive sports events. A further increase in the incidence of hyperthermia and EHI is expected due to global warming.

To date, very few studies have investigated thermoregulation during field exercise. Field-based research is of crucial importance to further evaluate the impact of weather conditions. Continuous monitoring of core body temperature during field exercise and recovery can provide a valuable baseline to assess and validate practical methods, and more importantly, enable prompt interventions. With a better understanding of equine heat stress responses, the prevention of hyperthermia can enhance the welfare and athletic performance of all sport horses.

The main aim of the current PhD research was to create a baseline for continuous core body temperature monitoring, to compare core body temperature evolvement between different types of exercise and to take the first steps towards identification of practical proxies for monitoring equine thermoregulation towards ensuring wellbeing in the field. For this purpose, the following specific aims and strategies were formulated for this PhD research:

1. Evaluate and validate the reliability of a telemetric gastrointestinal (GI) pill to monitor the core body temperature of horses during real-time field exercise. For this purpose, the GI pills were submerged in water baths and their output was compared to that of a certified thermocouple device (part A). Thereafter, an *in vivo* training study was performed during which the GI pills were evaluated during field exercise and their output was compared to that of a rectal probe thermocouple device (part B).
2. Evaluate the reliability of monitoring the thermoregulatory response using the GI pill as a proxy for core body temperature (T_c) in horses during real-time field exercise. For this purpose, trotter and endurance horses were equipped with

non-invasive monitoring devices (GI pill, skin temperature [T_{sk}] device, heartrate [HR] monitor and global positioning system [GPS]). A large dataset of T_c (GI core temperature) recordings and other physiological responses was analyzed (T_c, heart rate, speed and blood parameters). The T_c profiles were established during exercise and recovery. This study was the first study to be conducted using a telemetric GI pill in equine field competitions. The next objective was to compare the equine thermoregulatory response to different types of exercise under normal weather conditions. For this purpose, two types of exercise were chosen that are at the outer boundaries of the exercise intensity spectrum in sport horses, namely endurance versus trotter racing. The study focused on comparing endurance horses exercising at submaximal intensity during 80 km endurance rides with trotters exercising high intensity over 1540 m under normal weather conditions.

3. Continuous monitoring of skin surface temperature could be an effective and practical approach to monitoring thermal wellbeing in sport horses. To evaluate this hypothesis, endurance horses were equipped with both GI pills and an infrared skin surface temperature device during real-time field exercise. The skin temperature recordings were compared to the continuous GI pill temperatures (T_c) to determine if skin temperature could be a reliable proxy for the thermoregulatory response during exercise and recovery.

Chapter 3

Evaluation of a telemetric gastrointestinal pill to continuously monitor gastrointestinal temperature in eight horses at rest and during exercise

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3.1 Abstract

Objective

To evaluate use of a telemetric gastrointestinal (GI) pill to continuously monitor GI temperature in horses at rest and during exercise and to compare time profiles of GI temperature and rectal temperature.

Animals

8 Standardbred horses.

Procedures

Accuracy and precision of the GI pill and a rectal probe were determined in vitro by comparing temperature measurements with values obtained by a certified resistance temperature detector (RTD) in water baths at various temperatures (37°, 39°, and 41°C). Subsequently, both GI and rectal temperature were recorded in vivo in 8 horses over 3 consecutive days. The GI temperature was recorded continuously, and rectal temperature was recorded for 3.5 hours daily. Comparisons were made between GI temperature and rectal temperature for horses at rest, during exercise, and after exercise.

Results

Water bath evaluation revealed good agreement between the rectal probe and RTD. However, the GI pill systematically underestimated temperature by 0.14°C. In vivo, GI temperature data were captured with minimal difficulties. Most data loss occurred during the first 16 hours, after which the mean ± SD data loss was 8.6 ± 3.7%. The GI temperature was consistently and significantly higher than rectal temperature with an overall mean temperature difference across time of 0.27°C (range, 0.22° to 0.32°C). Mean measurement cessation point for the GI pill was 5.1 ± 1.0 days after administration.

Conclusions and clinical relevance

This study revealed that the telemetric GI pill was a reliable and practical method for real-time monitoring of GI temperature in horses.

3.2 Introduction

The effect of exercise on body temperature in horses has been evaluated mainly indoors with controlled experimental conditions by use of treadmill exercise tests with body temperature recorded as blood temperature (obtained by inserting a thermal sensor into the pulmonary artery) (Hodgson et al., 1993; Hodgson et al., 1994; Geor et al., 1995; McConaghy et al., 1995a; McConaghy et al., 1995b; Marlin et al., 1999; Scott and Schroter, 1999; Geor et al., 2000). However, to better understand the mechanisms by which exercising horses regulate body temperature in real-life conditions, there is a need to explore new techniques that can possibly be applied in field settings. Currently, intermittent measurement of rectal temperature of horses at rest is common practice throughout training and competition. Continuous monitoring of body temperature during exercise may have several important advantages over intermittent measurements obtained from horses at rest. These include determining and monitoring body temperature patterns and maximum body temperature limits for individual exercising horses, allowing early intervention during competition, and evaluating body temperature patterns and effectiveness of postexercise cooling. Such data may improve well-being and performance of exercising horses. Skin (Wallsten et al. 2012), intrauterine (Smith et al, 2006) and eye (Johnson et al., 2011; Bartolome et al., 2013) temperature has also been explored as methods for assessing thermo-regulation and for monitoring horses during competition. Eye temperature can only be measured intermittently in horses at rest whereas skin and intrauterine temperature recordings can be obtained continuously during exercise (Smith et al., 2006; Johnson et al., 2011; Wallsten et al., 2012; Bartolome et al., 2013). However, there are disadvantages with these techniques because skin temperature is not reflective of core body temperature and intrauterine temperature measurements can only be retrieved following removal of the device from the uterus and obviously can only be obtained from mares (Smith et al., 2006; Wallsten et al., 2012).

A nondigestible temperature-sensitive pill that passes through the GI tract has been used for continuous monitoring of body temperature. This technology is minimally invasive and wireless, and it enables real-time display on a mobile device by use of Bluetooth technology. A GI pill has been evaluated and used for continuous monitoring of thermoregulation in human athletes during field and exercise studies (Kolka et al., 1993; McKenzie et al., 2004; Byrne et al., 2006; Casa et al., 2007; Easton et al., 2007; Lim et al., 2008; Duffield et al., 2009; Ganio et al., 2009; Domitrovich et al. 2010; Darwent et al., 2011; Teunissen et al., 2012; Liu et al., 2013). In addition, GI pills have been tested in resting and exercising dogs (Angle et al., 2011), elephants (Weissenboeck et al., 2010), cattle (Reid et al., 2012), and horses (while at rest and during transportation) (Green et al., 2005; Green et al., 2008).

To our knowledge, there have been no reports of continuous body temperature monitoring of horses by use of a GI pill during field exercises, and the accuracy of tracking temperature changes in these circumstances by use of a GI pill currently is unknown. Therefore, the aim of the study reported here was to evaluate the use of a GI pill in exercising horses. Our objectives were to perform an in vitro assessment of the accuracy, precision, and agreement of results for a GI pill with those of a certified RTD as the reference method by use of a water bath system; to assess the feasibility for use of the GI pill as a means of continuous monitoring of GI temperature in resting and exercising horses; and to compare the time profile of GI temperature provided by use of the GI pill with rectal temperature of horses while at rest and during exercise.

3.3 Material and methods

Animals

Eight unconditioned Standardbred horses (7 mares and 1 gelding) were used in the study. Mean ± SD body weight was 465 ± 9.5 kg, mean body condition score was 4 (scale, 1 to 9), and age range was 4 to 10 years. Horses were examined and deemed to be healthy prior to the study. The horses were housed in individual yards (3.5 X 3.5 m) with ad libitum access to oat hay and water. The daily exercise protocol consisted of 30 minutes of exercise (10 minutes of walking followed by 20 minutes of lunging exercise at a fast trot). After exercise was completed, horses were provided drinking water (within 3 minutes after end of exercise) and walked by hand for 10 minutes. The study was approved by the University of Adelaide Animal Ethics Committee and was conducted at Roseworthy campus during the fall.

GI pill and rectal probe

A commercially available telemetric GI pill[a] was used in the study. The pill was 8.7 mm in diameter and 23 mm in length, and it weighed 1.6 g. According to the manufacturer specifications, the GI pill had a temperature sensing range of 25° to 50°C, accuracy of ± 0.1°C between 32° and 42°C, and resolution of ± 0.01°C. The transmission range was reported as 1 m, with data recordings at 5- or 15-second intervals, depending on whether the older or updated version of the system was used. A rectal probe[b] was also used. Measurement range for the rectal probe was reportedly –40° to 100°C, with accuracy of ± 0.18°C and response time of 3 minutes. The rectal probe recorded data at 5-second intervals.

In vitro evaluation

Temperature recordings for 8 GI pills and the rectal probe were compared with those obtained by use of a certified RTD[c]. The GI pills were suspended in an open small plastic container. The GI pills, rectal probe, and RTD[d] were submersed sequentially in water baths with recirculating water at 3 temperatures (37°, 39°, and 41°C); temperature for the 3 baths ranged from approximately 37° to 45°C. Once the sensors were activated, they were allowed to remain in each water bath until a plateau temperature was reached and held for a minimum of 3 minutes.

In vivo evaluation

A GI pill in 0.5 L of water was administered at 5 pm to each horse by nasogastric intubation. The GI temperature data recorded by the GI pill were transmitted at 40.68 MHz, and the signal was recorded every 5 to 15 seconds by a receiver located in a sensor belt system[e] placed on each horse **(Figure 1)**. Data from the GI pill were recorded continuously over 3 consecutive days. In addition, temperatures were measured with a rectal probe for 3.5 hours each day to enable us to make comparisons between GI temperature and rectal temperature recordings of horses at rest and during exercise. The rectal probe was inserted to a depth of

Figure 1. Photograph of a sensor belt adapted for use in horses. The belt has been placed around the girth with the receiver positioned ventrally at the sternum, protected by padded covers.

approximately 30 cm proximal to the anal sphincter. Rectal temperature was recorded for 120 minutes while horses were at rest, for 30 minutes during exercise, and for 1 hour after exercise. If it was expelled, the rectal probe was immediately replaced. A temperature logger[f] was used to measure the ambient temperature and relative humidity, which were confirmed by recordings obtained at a local Bureau of Meteorology station[g].

Presence of each GI pill in the GI tract of each horse was evaluated daily with the data logger. The time point at which no temperature signal output could be detected (i.e., the pills were expelled from the rectum) was recorded as the data log cessation time point. The GI pills were not retrieved from the faeces.

Statistical analysis

Feasibility of use of the GI pill and of the quality of data retrieved was evaluated. The evaluation involved assessing the proportion of usable data collected by use of the GI pill with the receiver system (after removal of erroneous data), calculating data loss (total and 4-hour increments), and recording the cessation time of the GI pill.

The nature and magnitude of accuracy and precision (International Organization for Standardization, 1994) were assessed during the in vitro evaluation. Temperature for the GI pill and rectal probe were compared with RTD temperature. The Lin concordance correlation coefficient was calculated by use of a statistical package[h]. The Bland-Altman method was used to calculate the 95% limits of agreement (mean difference ± 1.96•SD) and the bias[i] (Bland and Altman, 1986; Bland and Altman, 1995). Potential within-pill correlation (repeated measures) was investigated with a mixed linear regression model[h]. and estimation of the intraclass correlation. Variability explained by between-pill variation was negligible (intraclass correlation = 4.960×10^{-20}), and measurements for each pill were deemed independent. Mean response time of the 8 GI pills was determined by recording the time required to reach equilibrium temperature in 2 water baths (39°C and 41°C) (Kolka et al., 1993; Teunissen et al., 2012).

To determine whether the GI pill was an accurate measuring device in horses in a field setting, data from periods of rest and exercise were analyzed. The GI temperature data were adjusted for bias calculated during the in vitro water bath evaluation and then used for statistical analysis. One-minute values (mean and 95% CI) were calculated for GI and rectal temperature data of horses at rest (120 minutes), during exercise (30 minutes), and after exercise (60 minutes) for 3 days. Recorded temperatures were considered erroneous and excluded from analysis when values were associated with rectal probe expulsion, were outside the lower or upper limits of the body temperature range for horses (< 36.5°C to 41°C), or changed by > 0.5°C in 30 seconds (i.e., exceeded the sensor response rate) (Green et al., 2005; Green et al., 2008). Measurements obtained during the first 15 minutes after insertion of the rectal probe and > 60 minutes after exercise were not included in the analysis. To account for repeated measures, a mixed effects linear regression model[h] was used to investigate differences between GI and rectal temperatures over time, with horse and trial iteration within horse as random effects. Dependence among residual values (2 temperatures measured with a brief interval between measurements are more similar than 2 temperatures measured with a large interval between measurements) was accounted for by adding an autoregressive residual covariance matrix in the model. Difference between GI and rectal temperatures was reported as predicted mean values with 95% CIs and compared across activity periods; values were considered significant at $P < 0.05$, with Bonferroni correction to account for multiple pairwise comparisons. The time for the mean GI temperature and rectal temperature to return to a pre-exercise cut-off value of 38.5°C after exercise was also recorded.

The time profile of total data loss for the GI pill was calculated for each horse as the number of missing data points divided by the total number of data points. Mean data loss for the 8 horses was calculated at 4-hour intervals, and total cumulative loss was calculated for the entire recording period (76 hours). Total data loss was analyzed by use of a univariate 1-way ANOVA with post hoc testing with the Fisher least significant difference test. Values were considered significant at $P < 0.05$.

3.4 Results

In vitro evaluation

The in vitro evaluation revealed excellent correlation between measurements for the GI pill and RTD and between the rectal probe and RTD throughout the water bath trials. Calculated concordance correlation coefficients between GI temperature and

RTD temperature and between rectal probe temperature and RTD temperature were 0.996 (95% CI, 0.993 to 0.999) and 0.999 (95% CI, 0.999 to 1.0), respectively, which illustrated good precision of the methods.

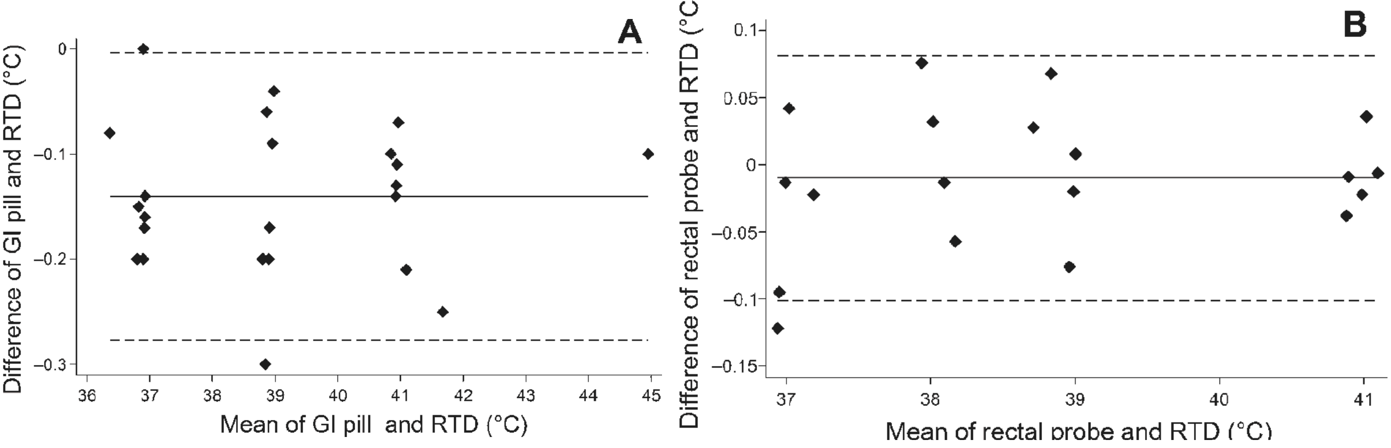

Figure 2. Bland-Altman plots comparing temperature measured concurrently in 3 water baths (37°, 39°, and 41°C) by use of a certified RTD with that measured by use of 8 GI pills (A) and a rectal probe (B). Each symbol represents the mean of each paired sample against the difference between samples. The mean relative bias (dotted line) and 95% CI (mean ± 1.96•SD; dashed lines) are indicated. Limits of agreement for the GI pill and RTD were –0.277° to –0.004°C with a systematic bias of 0.140°C. Limits of agreement for the rectal probe and RTD were –0.101° to 0.082°C with a systematic bias of 0.010°C. The bias correction factors for the GI pills (0.997) and rectal probe (1.0) reflected excellent accuracy, and the calculated concordance correlation coefficient between GI temperature and RTD temperature was 0.996 (95% CI, 0.993 to 0.999) and between T_{re} and RTD temperature was 0.999 (95% CI, 0.999 to 1.0), which illustrated good precision.

Similarly, bias correction factors for the GI pill and rectal probe revealed excellent accuracy (0.997 and 1.0, respectively). The calculated systematic bias for the GI pill measurements was 0.140°C (95% limits of agreement, –0.277° to –0.004°C) and for the rectal probe measurements was 0.010°C (95% limits of agreement, –0.101° to 0.082°C; **Figure 2**). Variability explained by between-pill variation was negligible (intraclass correlation, 4.960×10^{-20}), and measurements for the GI pills were deemed to be independent. Mean ± SD total time to equilibrium temperature (response time) in the 39° and 41°C water baths was 75 ± 72 seconds and 83 ± 95 seconds, respectively.

In vivo evaluation

One GI pill was inadvertently dropped on the floor and subsequently damaged during administration; thus, a second GI pill was administered to that horse 48 hours later. The rectal probe was regularly expelled by all horses and required replacement. Output data collected at those time points were considered erroneous and excluded from analysis. No adverse events were detected in the horses during the in vivo evaluation.

Mean ± SD total data loss for the 8 horses was 16.5 ± 4.1% with a large interindividual variation (range, 3.2% to 37.3%). Data loss was mainly during the first 12 to 16 hours after GI pill administration, which was evident in the 4-hour data **(Figure 3)**. Data loss significantly (*P* = 0.014) decreased as time progressed and reached a plateau at 16 hours after GI pill administration. Total data loss did not differ significantly (*P* = 0.056) between 16 and 72 hours after pill administration. Elimination of the first 16 hours of the recordings resulted in a mean data loss of 8.6 ± 3.7% (range, 1.1% to 32.6%).

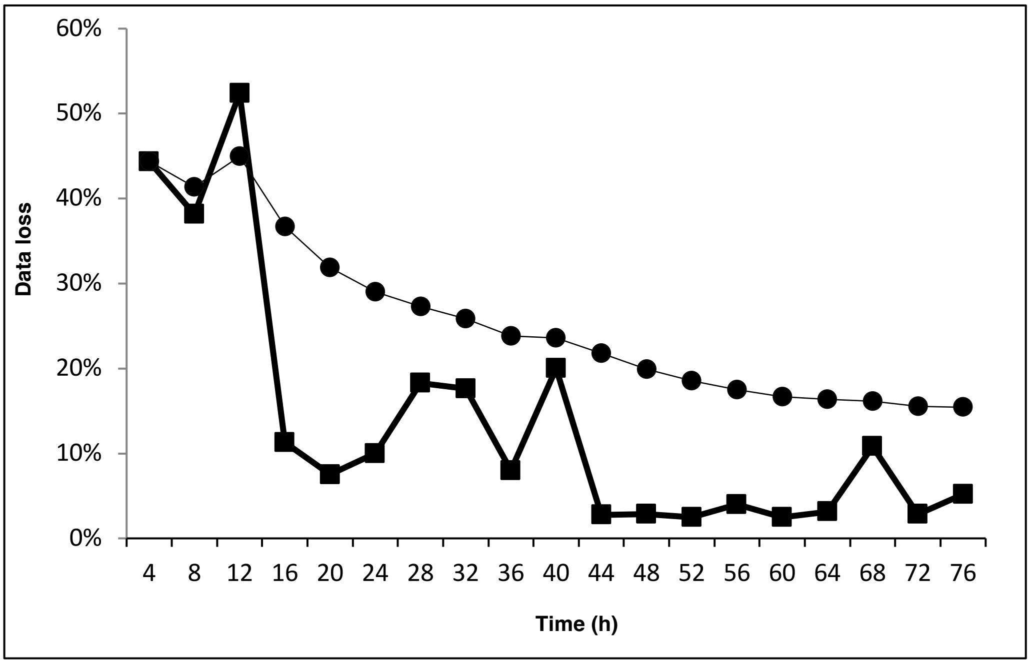

Figure 3. Mean percentage total data loss for 76 hours (circles) and for 4-hour intervals (squares) after administration of the GI pill. Results represent data for 8 horses for hours 4 to 28 and 7 horses for hours 32 to 76. Total data loss represents the number of missing data points divided by the total number of data points, and 4-hour loss represents the percentage of data loss within each 4-hour period.

In vivo GI temperatures were adjusted by adding the correction factor of 0.14°C (i.e., bias obtained during the *in vitro* evaluation). Ambient temperature (mean ± SD minimum and maximum temperatures, 6.62 ± 0.7°C and 19 ± 0.9°C, respectively) and mean relative humidity (minimum and maximum values, 62.2 ± 3% to 90.7 ± 2.7%) were similar during the 3 days.

Differences between GI and rectal temperatures were compared for horses at rest, during exercise, and after exercise **(Figure 4)**. Data were recorded for 7 horses, with differences in the number of trial iterations per horse (range, 1 to 3). The GI temperature consistently was significantly (*P* < 0.001) higher than the rectal temperature, with an overall mean temperature difference across time of 0.27°C (range, 0.22° to 0.32°C). This temperature difference did not differ significantly among the 3 time periods (at rest, during exercise and after exercise).

During the 120-minute rest period, mean GI and rectal temperatures were 37.94°C (95% CI, 37.82° to 38.06°C) and 37.63°C (95% CI, 37.50° to 37.77°C), respectively. Both GI and rectal temperatures increased during the exercise period and continued to increase after the end of exercise (**Figure 4**). Mean maximum GI temperature was 38.88°C (95% CI, 38.76° to 38.93°C) at 6 minutes after completion of exercise, whereas the mean maximum rectal temperature was 38.58°C (95% CI, 38.45° to 38.71°C) at 4 minutes after completion of exercise.

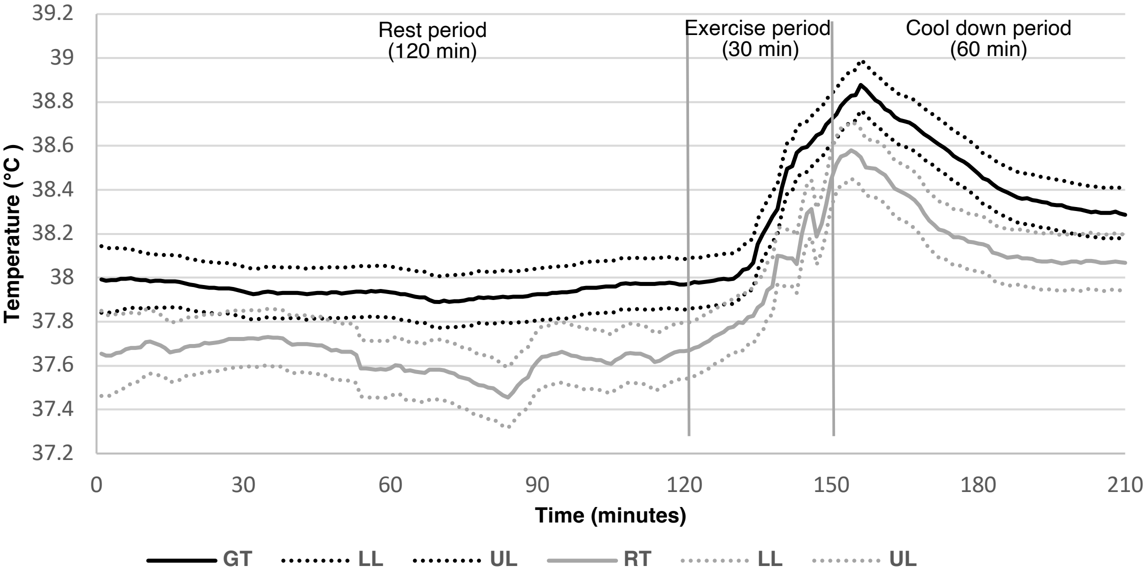

Figure 4. The 1-minute mean values for GI temperature (solid black line) and T_{re} (solid gray line) measured by use of a GI pill in 7 horses while at rest (120-minute period), during exercise (30-minute period that consisted of 10 minutes of walking followed by 20 minutes of lunging exercise at a fast trot), and after exercise (60-minute period). The lower and upper limits for GI temperature (black dotted lines) and T_{re} (gray dotted lines) are indicated. The GI temperature differed significantly ($P < 0.001$) from the T_{re} across time (mean difference, 0.27°C; 95% CI, 0.22° to 0.32°C).

Mean ± SD cessation time point was 5.1 ± 1.0 days after administration. For 7 horses, the GI pill could not be detected after 5 days; however, the remaining horse expelled the GI pill at 12 days after administration.

3.5 Discussion

To our knowledge, the study reported here was the first that has been conducted to evaluate the feasibility of a GI pill for continuous monitoring of GI temperature in horses during exercise in a field setting. The GI pill and data logger system was found to be a minimally invasive and practical method for real-time monitoring of GI temperature during lunging exercise and has the potential to be adapted for use during exercise performed with a rider[j].

Neither the use of a rectal probe nor pulmonary artery catheterization (the criterion-referenced standard) are suitable for use in measuring core temperature of horses in field settings because of expulsion of the rectal probe during defecation and the invasive nature of the catheterization procedure, respectively (Hodgson et al., 1993; Marlin et al., 1996; Kohn et al., 1999a; Marlin et al., 1999; Green et al., 2005). Other methods (e.g., implantable microchips) have provided no correlation with rectal temperature in steers (Reid et al., 2012) or with rectal temperature or blood temperature in dogs (Greer et al., 2007). Measurement of intrauterine temperature by insertion of a temperature logger into the uterus has been used successfully to continuously monitor temperature in 9 mares during rest and exercise (Johnson et al., 2011). Researchers of that study reported a similar but slightly lower temperature profile to that described for the present study. Disadvantages of the intrauterine method are that it is not a real-time measurement, it can only be used in mares, and there are no data on possible adverse effects on future reproduction. Additionally, similarly to rectal temperature, uterine temperature may lag behind body (core) temperature because of its peripheral location.

For the present study, the adjusted GI temperature was consistently higher than rectal temperature in horses at rest, which is in accordance with results of other studies. Investigators of 1 study reported small differences in the hourly mean temperature when comparing GI temperature with other temperature measurements; GI temperature was 0.5°C higher than rectal temperature and 1.0°C higher than blood temperature (Green et al., 2005). Similarly, GI temperature and rectal temperature reported for the present study were significantly different during exercise, with only a small but uniform difference throughout the measurements, which suggested that random error between methods was acceptably small at GI temperatures. The GI temperature reached a higher maximum temperature than did the rectal temperature, which may suggest that GI temperature may be more reflective of changes in body temperature during exercise than is rectal temperature. Similarly, studies of humans conducted to compare GI temperature with other temperature measurements revealed an acceptable level of agreement with a consistent and significant bias existing between GI temperature and rectal temperature (Byrne et al., 2007; Casa et al., 2007; Easton et al., 2007; Duffield et al., 2009; Ganio et al., 2009; Pearson et al., 2012). Investigators of 1 study reported that pulmonary artery blood temperature and GI temperature measurements at the end of heat stress were similar, there was an extremely small bias for limits of agreement, and blood temperature and GI temperature were highly associated but the early GI temperature data were lower than blood temperature data (Pearson et al., 2012). Similarly, there is a slower response of rectal temperature relative to GI and intra-esophageal temperatures (Kolka et al, 1993; Byrne et al., 2006). In addition, there is a slower response of rectal temperature and GI temperature relative to the response of intra-esophageal temperature during high-intensity exercise in humans.24

In the study reported here, GI temperature continued to increase after exercise, which is consistent with results of other studies conducted to evaluate rectal temperature of horses during treadmill exercise (Hodgson et al., 1993; McConaghy et al., 1995b; Marlin et al., 1996; Kohn et al., 1999; Marlin et al., 1999). This emphasizes the importance for continuous recording of temperature after exercise (McConaghy et al., 1995b; Green et al., 2005).

Bland-Altman evaluation of results for the GI pill in water baths revealed reasonable precision with low variation and justified the use of the small bias as a reasonable correction factor to adjust GI temperature in the in vivo evaluation. Although reproducibility and repeatability were not directly assessed during the in vivo evaluation in the study reported here, an acceptable level of GI temperature reliability for humans has been reported (Byrne et al., 2007; Pearson et al., 2012). It has been suggested that an acceptable limit of agreement is 0.4°C (when comparing GI temperature and rectal temperature in humans), and an acceptable bias is < 0.1°C (Pearson et al., 2012). Further investigations with higher water bath temperatures and higher-intensity exercise, long-term exercise, or exercise during hot conditions would be valuable to confirm reliable temperature measurement at higher body temperatures (e.g., 42°C and 43°C) (Pearson et al., 2012).

The GI pill response time was acceptable and confirmed the suitability for continuous monitoring of temperature patterns over time, especially when temperature patterns are more important than absolute values. However, the response time and rate of change for the GI pill at the start of exercise or during periods of acceleration and deceleration may be less rapid than changes in blood temperature, which is a result similar to that reported for humans Byrne et al., 2007; Pearson et al., 2012; Teunissen et al., 2012). Evaluation of the response time during rapid changes of body temperature is recommended (Kolka et al., 1993; Teunissen et al., 2012), especially for animals that require a rapid response time (e.g., racehorses). In the study reported here, data obtained during the first 15 minutes after insertion of the rectal probe (which was lubricated with cold gel) were removed because this would have falsely influenced the correlation between rectal and GI temperatures. In the present study, data loss predominantly occurred during the first 16 hours after GI pill administration; therefore, it is recommended that the GI pill be administered to a horse approximately 16 hours before the time at which monitoring is required. Data loss (after elimination of the first 16 hours of data) was within acceptable limits and without extreme outliers, except for 1 horse. We have no explanation as to the reason that this occurred, specifically for that 1 horse. Possible reasons included damage to the GI pill or interference in the transfer of data from the GI pill. In general, data loss for the GI pill most likely occurs because of electromagnetic interference, the fact that the sensor may have a limited transfer range during movement of the GI pill through the GI tract, or temporary failure of the data logger (Byrne et al., 2006). The use of GI pills in horses has been evaluated in other studies (Green et al., 2005; Green et al., 2008; Stokes et al. 2012). Although the GI pill system used in those studies has not been evaluated during exercise, data loss in horses at rest was considerably higher than that recorded for the present study (Green et al., 2005; Green et al., 2008; Stokes et al. 2012). Investigators of 1 study evaluated another GI pill system in 8 adult horses at rest (Green et al., 2005). The GI pills were administered 17 hours before data collection, and mean data loss during the subsequent 6 hours of monitoring was 13.3 ± 4.7% for GI temperature, compared with mean data loss for the blood temperature (16.4 ± 12.5%). However, a much higher mean data loss (48.5 ± 4.7%) was reported during 2 days of monitoring of horses in stables and trailers (Green et al., 2008). Furthermore, downloading of data with the system used in those studies is an extremely tedious task (in our experience), which suggests that the system is less practical (Green et al., 2005; Green et al., 2008). The pH, GI temperature, and luminal pressure were measured in 7 ponies by use of a modified GI pill system equipped with an extra antenna for the external receiver to enhance the transmission range. This intervention was needed to overcome the larger body size of the ponies, compared with the body size of humans. Data loss in the ponies with the modified receiver was approximately 15%, but the exact duration of data collection was not specified (Stokes et al., 2012). In addition, other systems do not allow real-time viewing of temperature data.

The cessation time cannot be known exactly without collecting the GI pill from feces. Battery life of the device during active transmission is approximately 10 days. In the study reported here, we assumed that monitoring the signal from the GI pill was a sufficient method to determine whether the pill was still within the GI tract. The data log cessation time point differed greatly among horses, which is in accordance with results of other human (McKenzie et al., 2004; Darwent et al., 2012) and equine studies (Green et al., 2005; Green et al., 2008), and could have been attributable to factors such as interindividual differences in GI tract motility. Cessation time in other studies29,30 of horses ranged from 33 hours to > 1 week. Cessation time in studies of humans ranged from 12.5 hours up to 5.6 days (McKenzie et al., 2004; Darwent et al., 2012).

In the study reported here, real-time temperature monitoring by use of the GI pill in combination with a data logger in horses during exercise was found to be feasible. There was little data loss > 16 hours after administration of the GI pill, and there was good correlation between GI and rectal temperatures. These findings may provide many avenues for future research on thermoregulation and exercise physiology of horses in field conditions. Better understanding of thermoregulation in horses will help to improve training protocols and to prevent exertional heat illness, which would be a major step with respect to well-being of horses.

Footnotes

a. Jonah core body temperature capsule, Philips Respironics, Murrysville, Pa.

b. Onset HOBO Pro V2 logger U23-004, Onset Computer Corp, Bourne, Mass.

c. Thermal water bath, Model NBTII-P, Labec Laboratory Equipment, Marrickville, NSW, Australia.

d. Resistance temperature detector certified by the National Association of Testing Authorities, Instrument Choice, Adelaide, SA, Australia.

e. Equivital EQ-01 sensor electronics module, Equivital Hidalgo Ltd, Cambridge, England.

f. Onset HOBO Pro V2 logger U23-001, Onset Computer Corp, Bourne, Mass.

g. Roseworthy weather station, Bureau of Meteorology, Roseworthy, SA, Australia.

h. Stata, version 14.1, StataCorp LLC, College Station, Tex.

i. SPSS software, version 19.0, IBM-SPSS, Chicago, Ill.

j. Verdegaal E, Jonas S, Caraguel C, et al. Real-time monitoring of the core body temperature of endurance horses during field exercise (abstr). Equine Vet J 2014;46:19–20

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Chapter 4

Continuous monitoring of the thermoregulatory response in endurance horses and trotter horses during field exercise: Baselining for future hot weather studies.

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4.1 Abstract

Establishing proper policies regarding the recognition and prevention of equine heat stress becomes increasingly important, especially in the face of global warming. To assist this, a detailed view of the variability of equine thermoregulation during field exercise and recovery is essential. Thirteen endurance horses and 12 trotter horses were equipped with continuous monitoring devices (gastrointestinal (GI) pill, heartrate (HR) monitor and global positioning system) and monitored under cool weather conditions during four endurance rides over a total of 80 km (40 km loops) and intense trotter track-based exercise over 1540 m. Recordings included GI temperature (T_c), speed, HR and pre- and post-exercise blood values. A temperature time profile curve of T_c was constructed, and a net area under the curve was calculated using the trapezoidal method. Metabolic heat production and oxygen cost of transport were also calculated in endurance horses. Maximum T_c was compared using an independent samples t-test.

Endurance horses (mean speed 14.1 ± 1.7 km h^{-1}) reached mean maximum T_c (39.0 ± 0.4°C; 2 x 40 km in 8 horses) *during exercise* at 75% of completion of T_c exercise and T_c returned to baseline within 60 minutes into recovery. However, the mean T_c was still 38.8 ± 0.4°C at a HR of 60 bpm which currently governs ‘fit to continue’ competition decisions. Trotters (40.0 ± 2.9 km h^{-1}) reached a comparable mean max T_c (38.8 ± 0.5°C; 12 horses) *always during recovery*. In 30 % of trotters, T_c was still > 39°C at the end of recovery (40 ± 32 minutes).

The study shows that horses are individuals and thermoregulation monitoring should reflect this, no matter what type of exercise is performed. Caution is advised when using HR cut-off values to monitor thermal welfare in horses since we have demonstrated how T_c can peak quite some time after finishing exercise. These findings have implications for training and management of performance horses to safeguard equine welfare and to maximize performance.

4.2 Introduction

Exertional heat illness (EHI) in horses is characterized by severe central nervous system dysfunction, such as physical collapse, and has been described in detail (Brownlow et al., 2016; Brownlow and Mizzi, 2020). Recently, a Japanese study identified a wet-bulb globe temperature (WBGT) index above 28°C to be responsible for a 28.5% higher risk for development of EHI when compared to the index below 20°C (Takahashi and Takahashi, 2020). A progressive increase in the prevalence of human EHI casualties is anticipated due to global warming (Raymond et al., 2020) and a similar effect can be anticipated for animals in general. In continents with warm climates and established horseracing industries, a WBGT above 28°C is often recorded during approximately 2 to 3 months annually. These regions include the southern part of North America and Europe, Oceania, parts of Africa, South America and Asia (Raymond et al., 2020). It is a common misconception that EHI affects horses in only very hot and humid weather conditions, however EHI casualties are also reported to occur on warmer pre-season days. To date, EHI has been underreported most probably because only the more severe and overt clinical cases are being recognized, leaving many mild cases unnoticed with possibly deleterious effects (Brownlow et al., 2016; Brownlow and Mizzi, 2021).

Consequently, there is great need to further fine-tune early detection of hyperthermia and to obtain a detailed view of the flexibility of equine thermoregulation in the field under different exercise and climatic conditions. Such knowledge will allow for identification of predisposing factors that may lead to the formulation of evidence-based hot weather policies and recommendations for the post-exercise and recovery period to prevent hyperthermia, EHI and EHS (Hosokawa et al., 2019; MOOC heat illness). Another critical aspect that needs to be clarified is the optimal rest and recovery period that is required before ‘return to exercise' is allowed after an EHI event. For example, human athletes who have experienced an EHI episode are required to rest and rehabilitate for a certain period, varying from 3-6 weeks to a year (Adams and Jardine, 2020). Similarly, various regulations govern eliminated endurance horses who are given rest periods to recover following a competition (A.E.R.A., 2020; (F.E.I., 2021).

The way the thermoregulatory system is challenged greatly depends on the speed and distance that are realized by the exercising horse (Hodgson et al., 1994). Additionally, important breed differences are to be expected (Fielding et al., 2011). For example, trotter horses are bred for harness racing over distances ranging from 1540 m (short distance races), 2140 m (medium distance races), 2640 m (long distance races) and rarely to 3140 m (ultra-long-distance races), with average race speeds of 47.3 - 48.6 km h^{-1} (Bertuglia et al., 2014). In comparison, endurance horses compete over distances of 80 to 160 km divided over exercise loops (sections) of 30–40 km under the regulations of either the National Endurance Riding Associations or the Fédération Équestre Internationale (FEI) (F.E.I., 2021). The range of mean winning speeds at FEI 100–120 km endurance rides around the world varies between 14.1 – 24.8 km h^{-1} while reported maximum winning speeds range from 17.2 to 26.2 km h^{-1} (Nagy et al., 2010). After each exercise loop, a recovery rest period is imposed to allow the HR to return to below values of 60 bpm under the regulations of the Australian Endurance Riding Association or 64 bpm as per FEI regulations (Nagy et al., 2012; F.E.I., 2018; A.E.R.A., 2020). Once this is achieved, a follow-up inspection including metabolic and gait assessment is performed by the certified endurance veterinarian to determine whether the horse is deemed fit to continue with the next exercise loop or qualifies for completion of the competition. Clinical assessment during endurance rides is essential to ensure sufficient recovery in all horses since a high elimination rate (nearly 50%) is reported—more specifically, the elimination rate due to metabolic disorders in endurance exercise varies between 4.2–15% (Barnes et al., 2010; Nagy et al., 2010; Fielding et al., 2011; Nagy et al., 2014; Younes et al., 2015; Fielding et al., 2017; Bennet and Parkin, 2018; Legg et al., 2019). Common debilitating metabolic disorders include abdominal discomfort, dehydration and exertional rhabdomyolysis (Fielding et al., 2009; Verdegaal et al., 2018; Misheff, 2020).

At present, little is known about the core body temperature evolvement in real time during different types of exercise performed by racehorses and endurance horses under field conditions. The vast majority of thermoregulatory studies have been conducted under indoor laboratory conditions using a treadmill and subjecting the horses to performance of specific standardized exercise tests. To this end, monitoring of arterial pulmonary blood temperature has proven to be 'the gold standard' (Hodgson et al., 1993; Marlin et al., 1996; Courouce-Malblanc, 2013; Hodgson, 2014). However, this approach is generally not feasible and too invasive under field conditions; only one study reported similar invasive methods in three horses during free field exercise using thermistors to measure blood and brain temperature (Mitchell et al. 2006). A few studies are available that focus on continuous monitoring of equine thermoregulation during exercise and recovery in the field (Smith et al., 2006; Verdegaal et al., 2017). Smith and colleagues used intra-uterine temperature loggers as a nonsurgical, minimally invasive method to measure evolvement of intra-uterine temperature during field exercise (Smith et al., 2006). This method has the advantage of collecting data over several competitions, however, it does not monitor real-time core body temperature as data were downloaded after removal of the logger. Obviously, the intra-uterine method is only applicable in mares. Furthermore, no data are available on the long-term effect of these loggers on uterine function. In contrast, the telemetric recording method in the current study is able to provide real-time results and does not require subsequent time-critical analysis. This could be undertaken at any time after completion of the exercise period (Verdegaal et al., 2017).

It is important to note that most existing field studies compare rectal temperature (T_{re}) before and after exercise (Kohn and Hinchcliff, 1995; Kohn et al., 1995; Hargreaves et al., 1999; Jeffcott et al., 2009; Wallsten et al., 2012). However, T_{re} is reported to significantly lag behind the core body temperature both during and after exercise (Hodgson et al., 1993; Geor et al., 1995; Verdegaal et al., 2017). This means that the heating of the core of the body is always reflected in the T_{re} at a much later stage and at a lower temperature. Recently, the researchers reported and validated a novel real-time temperature monitoring method namely a telemetric gastrointestinal (GI) pill that allows for accurate continuous recording of the GI temperature in exercising horses in the field (Verdegaal et al., 2017). The GI pill proved to be a more accurate and precise tool to monitor thermal response than serial T_{re} measurements. Importantly, the GI temperature (T_c) was consistently and significantly higher than the T_{re} (mean difference 0.3°C, with a range of 0.2 – 0.3°C) and T_c increased earlier than T_{re} on all occasions (Verdegaal et al., 2017).

The aim of the current study was to continuously monitor and compare the time profile of the dynamic thermoregulatory response of endurance horses in real-time competitions and trotter horses in the field. The GI temperature profiles were established during exercise and recovery under cool weather conditions to create a baseline thermal response profile for future field studies performed under hot and humid weather conditions. For this purpose, trotter and endurance horses were equipped with several non-invasive monitoring devices (GI pill [T_c], heartrate [HR] monitor and global positioning system [GPS] monitor) to continuously monitor their physiologic responses to field exercise.

4.3 Materials and methods

Horses

In the first study group, 13 trained endurance horses (Horses 1-13) were enrolled, competing at either 40, 80 or 100 km distances. The experimental design is presented in a flowchart in **Figure 1**. The characteristics of the horses such as breed, age, sex, body mass, coat color, together with competition distance are presented in **Table 1**. The body mass of endurance horses was determined using an equine mass tape (Horse and pony weight tape®) (Wagner and Tyler, 2011). When calculating the energy expenditure of the horses, the estimated mass of the rider was considered (Pagan and Hintz, 1986) based on 'rider riding division criteria' determined in the AERA rules (A.E.R.A., 2020). These criteria encompass: 1) HM division—heavy mass rider of 91 kg or greater, estimated average 103 kg; 2) MM division—middle mass of 73 kg or greater, estimated average 83 kg; 3) LM division—low mass, no minimum mass, estimated average 63 kg. The trotter horses were weighed on a mass scale (Ruddweigh 700®, Gallagher Group Limited, New Zealand).

All 13 endurance horses (Horses 1-13, **Table 1**) were sourced on a voluntary basis through the South Australian Endurance Riders Association (SAERA) at a maximum of 4 horses per event. All horse owners signed a written consent form before enrolment. The horses remained under the care of their owners for the duration of the events and owners could withdraw their horse at any time during the study. Before competition, all horses were subjected to a health inspection conducted by endurance veterinarians according to AERA riding rules (A.E.R.A., 2020).

The second study group consisting of 12 unconditioned trotter horses (Standardbreds) (Horses 14-26, **Table 1**) was randomly selected from a university-based teaching herd at a maximum of 4 horses per event. Trotters were kept in large paddocks without additional exercise or training. Before enrolment, trotters were subjected to a standard clinical health check. The present study was approved by the University of Adelaide Animal Ethics Committee (project number S-2011-224), which conformed to the Australian Code of Practice for the care and use of animals for scientific purposes, Canberra, Australia, 2004.

Study design

The study design is illustrated in **Figure 1**. Endurance horses (**Table 1**) were competing at distances of either 40 km (n=2) or 80 km (n=11) and 2 of those horses continued to cover 100 km. Data collection for endurance horses was performed during 4 endurance events during the cooler South Australian winter months (May, June, July) and event locations varied from -34° 28' 5.70" S to -34° 32' 3.08" S). The horses exercised under solar radiation through farming land of varying terrain with altitudes ranging from 4 m to 462 m.

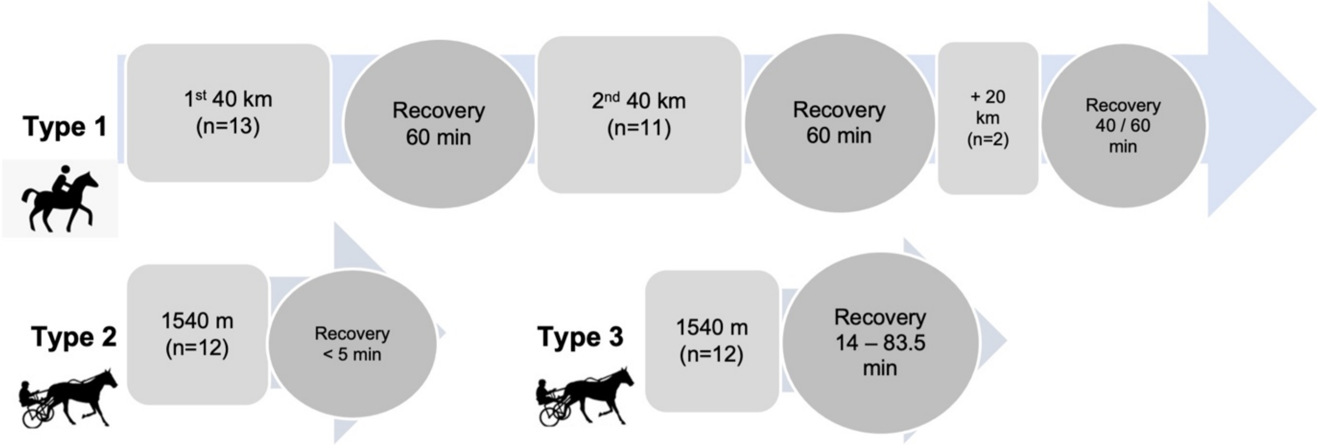

Figure 1. Flow chart of study design describing two types of exercise and the distance; km, kilometers; m, meters; n, number of horses; min, minutes; endurance exercise; moderate trotter warm-up exercise; fast trotter exercise

Horse number	**Sex**	**Age (y)**	**Breed**	**Body mass (kg)**	**Total mass incl rider**	**Coat color**	**Distance (km)**	**Horse experience (years)**	**Age start (years)**	**GI Pill Y/N**	**GPS/HR Y/N**	**BOM (°C) (T_a), min - max**	**PCV (Hct), lactate, TS, pH Y/N**	**Blood gas Y/N**	**Biochemistry (electrolytes, glucose) Y/N**	**Hematology**
1	G	11	Arab	669	752	Gr	80	6	4	Y	Y	13-26	Y	Y	Y	N
2	G	9	Arab	484	587	B	80	2	7	Y	Y	13-26	Y	Y	Y	N
3	M	13	Arab	426	489	C	80	3	10	Y	Y	6-19	Y	Y	Y	N
4	M	7	QH x TB	470	573	C	100	1	6	Y	Y	6-19	Y	Y	Y	N
5	M	11	Arab	450	533	C	80	2	9	Y	Y	6-19	Y	Y	Y	N
6	M	8	Arab	370	453	Gr	100	4	4	Y	Y	6-19	Y	Y	Y	N
7	M	9	Arab	450	523	C	80	2	7	Y	N^	3-22	Y	N	Y**	Y
8	G	11	Arab	470	553	Gr	80	1	9	Y	Y	3-22	Y	N	Y**	Y
9	G	7	QH	490	573	C	80	1	6	N	Y	7-13	Y	Y	Y	N
10	G	10	Arab x TB	484	587	C	80	5	5	N	Y	7-13	Y	Y	Y	N
11	G	5	Arab	458	541	B	40	0	5	N	Y	7-13	Y	Y	Y	N
12	G	7	Arab	525	608	B	80	NK	NK	N	Y	3-22	Y	N	Y**	Y
13	M	15	Arab	480	563	Gr	40	NK	NK	N	N^^	3-22	Y	N	Y**	Y
14	G	10	SB	484	-	B	1.54	-	-	Y	Y	5 -13	Y	N	N	N
15	G	5	SB	610	-	B	1.54	-	-	Y	Y	5 -13	Y	N	N	N
16	G	17	SB	482	-	B	1.54	-	-	Y	Y	5 -13	Y	N	N	N
17	G	5	SB	604	-	B	1.54	-	-	Y	Y	5 -13	Y	N	N	N
18	G	7	SB	554	-	B	1.54	-	-	Y	Y	3-18	Y	N	N	N
19	G	11	SB	482	-	B	1.54	-	-	Y	Y	6-14	Y	N	N	N
20	G	18	SB	480	-	B	1.54	-	-	Y	Y	6-14	Y	N	N	N
21	G	6	SB	602	-	B	1.54	-	-	Y	Y	6-14	Y	N	N	N
22	M	4	SB	508	-	B	1.54	-	-	Y	Y	14-20	Y	N	N	N
23	G	11	SB	482	-	B	1.54	-	-	Y	Y	14-20	Y	N	N	N
24	G	18	SB	480	-	B	1.54	-	-	Y	Y	14-20	Y	N	N	N
25	G	6	SB	602	-	B	1.54	-	-	Y	Y	14-20	Y	N	N	N

Table 1. Study population characteristics and monitoring devices. **Horse 1-13:** 13 endurance horses: G (*n* = 7), M (*n* = 6). Arab include part Arabian horses, QH, Quarterhorse; TB, Thoroughbred, endurance exercise; **Horse 14-25**: 12 untrained trotters, all Standardbred (SB): G (*n* = 12), fast trot exercise; G, gelding; M, mare; Gr, grey; C, chestnut; B, bay; The riders' and horses' performance history includes: Age start, indicates age (years) when horse started competing; Horse experience, indicates number of years in competition (40 km or more); GI pill, gastrointestinal pill; GPS, global positioning system; HR, heartrate monitor (Polar); B.O.M; Bureau of Meteorology, local stations closest to location of exercise at varying km distance from the actual event (in total 4 endurance locations and 1 trotter racetrack, distance ranged from 5.3 to 53 km; B.O.M stations were: Roseworthy 27.9 km distance from ride at Sandy Creek; Rosedale station 5.3 km from ride at Tarlee; Meningie station 53 km from ride at Coorong; Nuriootpa station 28.8 km from ride at Sedan; Roseworthy station at 2.2 km from trotter racetrack; N, no; Y, yes; NK, not known; ^indicates HR only; ^^indicates second 40 km only; blood gas analysis included pH, pCO_2, pO_2, HCO_3^-, base excess and lactate levels; **indicates biochemistry by VETSCAN (utilizes *dry* and liquid reagents); TS, total solids; TP, total protein; AST, Aspartate amino transferase; CK, creatinine kinase

Following each exercise loop of 40 km, endurance horses were required to rest for 60 min according to AERA riding rules (A.E.R.A., 2020) and were subsequently inspected by independent endurance veterinarians before competition could be continued. Following every exercise loop, blood samples were collected. Subsequently, horses were cooled by pouring buckets of water over their bodies and then scraping it off. Horses were allowed to drink water and eat hay *ad libitum*. The trotters were harnessed to a jogger and exercised under full sunlight over a 770 m sand training track in South Australia (-34° 32' 3.08" S) during Australian winter months (June, July). The racetrack was harrowed and watered on a regular basis to keep the track conditions as constant as possible. Trotters were subjected to 3 consecutive phases of exercise: slow warm-up phase (770 m at 25 km h^{-1}, not included in data analysis); moderate warm-up phase: 1540 m at a speed of 35 km h^{-1} (9.7 m s^{-1}); and the more intense fast trotter exercise: 1540 m at a speed of 42 – 50 km h^{-1} (11.7 – 13.9 m s^{-1}) (**Figure 1**). During the last phase, trotters were encouraged to exercise at their individual maximum speed. Post-exercise, blood samples were collected followed by cooling with a water hose for approximately 10 min and horses were allowed to drink water *ad libitum*. The T_c recovery period varied from 32 to 83 min depending on the duration of the individual T_c recovery.

Continuous monitoring of GI temperature (T_c) over time

All horses were equipped with monitoring equipment (**Table 1**). The GI temperature (T_c) representing the core temperature was recorded continuously during exercise and recovery using the ingestible telemetric GI pill (Jonah pill®; Phillips, USA). The night before undertaking exercise, GI pills were successfully administered to 8 endurance horses and 12 trotters using nasogastric intubation as previously described (Verdegaal et al., 2017). The timing of the GI pill administration was random, unrelated to the horses' feeding regime.

T_c was recorded by an external receiver, Equivital® Sensor Electronics Module (SEM, EQ02 Equivital data Logger®, Hidalgo, UK). The receiver was positioned in a pocket of the Equivital Sensor Belt®. This system was modified to accommodate a sturdy strap fitted closely around each horse's saddle girth (**Figure 2**). Equivital T_c data were recorded every 15 seconds and were uploaded from the SEM to the Equivital Software Manager® for data processing.

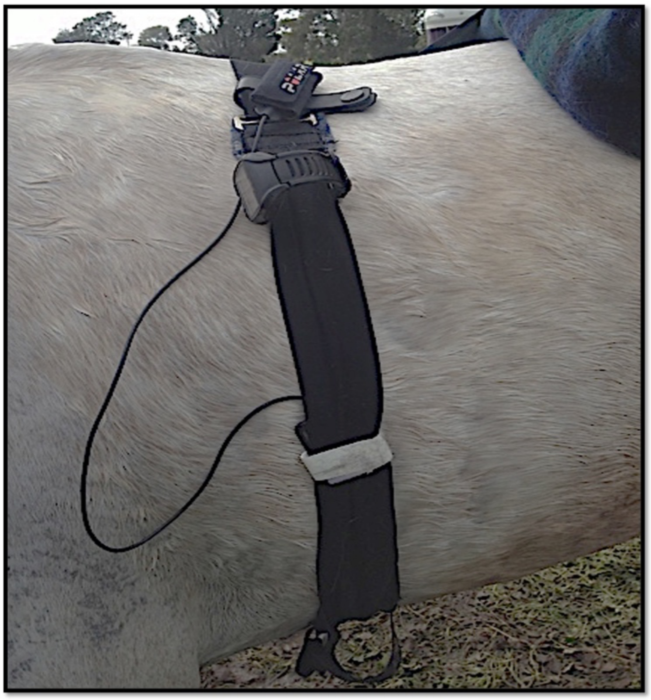

Figure 2. Monitoring equipment: A modified Equivital belt for use on horses with the external receiver device (Sensor Electronics Module) located inside of the belt at of the ventral part of the thorax to monitor the T_c by GI pill while the Garmin GPS watch and the Polar heartrate electrodes were attached at the upper part of the belt.

Monitoring of distance, speed, inclination and heartrate (HR) over time

Travelled distance, speed and inclination of the course were recorded every second using a GPS monitor (Garmin Forerunner 910XT GPS Watch®; Garmin Ltd., Schaffhausen, Switzerland) attached to the gullet of the saddle. The HR was continuously monitored telemetrically by the Garmin Watch using Polar electrodes (Polar Electro®, Kempele, Finland) and recorded every second (Parker et al., 2010). The GPS and HR data were uploaded from the Garmin Watch to the Garmin Connect and Garmin Training Centre[1].

Ambient environment

On each data collection day, ambient temperature (T_a) was recorded continuously in a shaded area of the rest area (post-exercise) using a data logger device (Onset HOBO Pro V2 logger temp U23-00®, Onset Computer Corporation, Bourne, Maine, USA). In addition, T_a data were obtained from the Bureau of Meteorology as presented in **Table 1**.

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[1] https://connect.garmin.com/

Blood sampling and analysis

Blood samples from endurance horses were analyzed using portable equipment in the field and data are presented in **Table 1**. Venous blood was collected (by SJ, E-LV or LF) from the left jugular vein by needle divided in two separate syringes within 3 min after exercise. The first blood sample was collected in a 3 ml syringe containing heparin and closed with an airtight seal for venous blood gas analysis using a portable blood gas analyzer (EPOC, Epocal®; Ottawa, Canada) (Averay et al., 2014; Kirsch et al., 2019). The second venous blood sample was collected in a 20 ml syringe and distributed into a lithium heparin blood tube and a plain blood tube and stored on ice before samples were centrifuged at 3000 x *g* for 10 min (Hematocrit centrifuge 200®; Hettich Lab, Tuttlinger, Germany). In trotters, venous blood samples were collected from the left jugular vein by a vacutainer technique in lithium heparin tubes (presented in **Table 1**).

Hematocrit was determined with the microhematocrit method and plasma total solids (TS) via a refractometry meter (Refractometer, RHC-200 [ATC] ®). Plasma lactate concentration was analyzed with a hand-held lactate analyzer (Accutrend Plus®, Roche, USA). Blood pH recording in trotters was measured using approximately 2 ml of blood and a pH meter (pH Cube®; TPS Pty Ltd, Brendale, Australia) with a pH calibration prior to every measurement by a voltage plot against the specified pH value of the buffer solution followed by introduction of the probe into the blood for approximately 30 s to read the pH. The biochemistry profiles were analyzed using a blood gas analyzer EPOC® (n = 9; sodium [Na^+], potassium [K^+], calcium [Ca^{2+}], glucose) or a dry biochemistry analyzer (Vetscan VS2®; Chemistry Analyzer, Abaxis, USA) (n = 4; Na^+, K^+, Ca^{2+}, glucose, creatinine, urea, aspartate amino transferase [AST], creatinine kinase [CK]). Hematology tests were performed in 4 horses (VETSCAN HM5 Hematology Analyzer®, Abaxis, USA). An overview of the blood analysis performed in individual horses is presented in **Table 1**.

Data processing

Each exercise period of 40 km (endurance) or 1540 m (trotter) will be referred to throughout the study as the T_c exercise period. Each recovery period (post-exercise) following 40 km (endurance) or 1540 m (trotters) will be referred to as T_c recovery period. Recordings performed during each T_c period included GI temperature (T_c), speed, HR and post-exercise blood values. Hyperthermia was defined as T_c above 39°C based upon the results of the researchers' previous study (Verdegaal et al., AJVR 2017) in which the mean maximum T_c was 38.9°C (95% CI, 38.8° to 38.9°C) at 6 min post-exercise. A T_c time profile curve of each individual horse was constructed for the endurance exercise, warm-up exercise (trotters) and (fast) trotter exercise. This included assessment of the value and time point of the maximum T_c (max T_c) during the T_c exercise or recovery period (40 km, total 80 km or 1540 m), the HR and speed at max T_c, recovery time (min) from max T_c to a T_c of 38.5°C, 38.3°C, and 38.0°C, respectively and recovery to the baseline T_c and number of horses with hyperthermia. In addition, the delta T_c (°C change post-exercise) and the recovery time from max T_c to a HR of 60 bpm were calculated as per Australian regulations (A.E.R.A., 2020).

To quantify the T_c response, the net area under the T_c curve (net AUC) was calculated for T_c and summated for the total T_c exercise period and the total T_c recovery period (Horswill et al., 2008; Datta et al., 2021). The net AUC (baseline set at rest T_c) was calculated using the trapezoidal method of T_c over time (min) expressed as °C x min. In this study, the net AUC is summated to present the cumulative T_c – time distribution (Datta et al., 2021). The net AUC provided an estimate of the dynamic thermal response to thermal load. The thermal load considered exercise intensity, T_a including solar radiation, and environmental evaporative power during exercise and recovery (IUPS Thermal Commission, 2001). Further processing of the recorded data included calculation of the metabolic heat production (H, kJ min^{-1}) (IUPS Thermal Commission, 2001) and the oxygen cost of transport (COT—the oxygen consumption necessary to travel 1 m) for each endurance horse (Schroter and Marlin, 2002). Calculation of both H and COT provides a multi-perspective view on thermoregulatory challenges represented by different types of exercise. The following formula was used:

$$H = \dot{V}O_2 \times k \times \text{duration of exercise}$$

— in which $\dot{V}O_2$ represents the oxygen consumption rate expressed in liters per min, k stands for heat liberated per liter of oxygen consumed (5 kcal; 21 kJ) (Hodgson et al., 1993; McCutcheon and Geor, 2008) and duration of exercise is expressed in min. The rate at which oxygen is consumed in an exercising horse provides a direct indication of its metabolic rate and subsequent heat production (Hodgson et al. 1993).

In the current study, $\dot{V}O_2$ was estimated by calculating the COT, speed and body mass as follows:

$$\dot{V}O_2 = \text{COT} \times \text{speed} \times \text{body mass}$$

— in which COT in ml oxygen per kg body mass per m (ml O_2 kg^{-1} m^{-1}), speed is expressed in m min^{-1} and body mass in kg.

The COT was calculated based on an incline, decline or flat terrain using the following equation (Schroter and Marlin, 2002):

Uphill (incline) and flat terrain: $\text{COT} = 0.123 + 1.561(\text{gradient})$

Downhill terrain (decline): $\text{COT} = 0.123 + 1.591(\text{gradient}) + 9.762(\text{gradient})^2 + 14.0(\text{gradient})^3$.

The gradient of the terrain was recorded as meters incline or decline indicated by the GPS device. The overall H per 40 km T_c exercise period was calculated by cumulating the H and presented as cumulated H.

Statistical analysis

All data are presented as mean ± SD (range). Comparison and correlation analyses were performed using IBM SPSS Statistics 26.0 software or using GraphPad Prism version 9.1.2 for MacOS, GraphPad Software, San Diego, California USA[2]. The net AUC (°C x min) and max T_c (°C) between the first and second 40 km exercise loops were compared using a paired t-test and a general linear model ANOVA approach which included horse ID as a random variable. The mean max T_c was then compared between endurance and trotter exercise using an independent samples t-test. The correlation between time to HR ≤ 60 bpm (dependent) and the T_c (°C) at end of exercise (independent) was assessed by a scatterplot and analyzed using a regression model. Blood values were compared using a one-way ANOVA *post hoc* Tukey's test. Statistical significance was set at $\alpha < 0.05$.

4.4 Results

All horses completed their exercise trial without any adverse events. No horses were withdrawn by owners. The owner of horse 13 erroneously removed the GPS

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[2] www.graphpad.com

equipment during the first 40 km. The GI pills were administered successfully to all horses resulting in complete T_c recordings. Unfortunately, an exceptional event of a malfunctioning batch of GI pills prevented activation of the GI pill in 5 of the 13 endurance horses. The GPS data were not recorded (only the HR) in 1 endurance horse (horse 7) and in 1 trotter (horse 19) while HR was not recorded in horse 21 (**Table 1**). Unphysiological and unrealistic values (artifacts) for T_c, GPS and HR (below 10 bpm and above 220 bpm) were removed prior to analysis. The endurance horse group encompassed 5 mares and 7 geldings of different breeds (although mainly Arabians) with an age of 9.5 ± 2.8 years and body mass of 479 ± 68 kg (**Table 1**). The mean age of starting endurance competitions was 6.1 ± 2.1 years with a mean of 2.5 ± 1.9 years of experience in endurance ride competitions. The T_c was recorded in 8 horses over a total of 80 km T_c exercise separated in loops of 40 km and associated T_c recovery periods of 60 min. The Equivital belt became dislodged in horse 1 during the first loop causing data loss at the end of a 40 km loop. As a result, the belt underwent additional modification for the subsequent recordings by fitting sturdy straps sandwiched into the belt to stabilize the girth position. The T_c was not recorded during recovery following the first loop in horses 1 and 2 due to premature removal of the belt by the owners. The group of 12 trotter horses included 1 mare and 11 geldings with an age of 9.8 ± 5.3 years and body mass (BM) of 537 ± 60 kg (**Table 1**).

Ambient environment

On all occasions, the T_a was relatively cool: mean minimum 6.7 ± 0.4°C, mean maximum 18.4 ± 2.9°C (**Table 1**). More specifically, the T_a on the four separate days of endurance exercise showed a minimum T_a of 13.4, 6.3, 2.8, 6.6°C, and maximum T_a of 26.3, 19.0, 22.0, 18.8°C, respectively (HOBO data). The T_a recorded by Bureau for Meteorology stations at varying distances from the event location are presented in **Table 1**.

Individual GI temperature (T_c), heartrate (HR) and speed data during endurance and trotter exercise over time

The individual T_c parameters and descriptive analysis of T_c are presented in Supplementary **Table S1**. The descriptive analysis of speed, HR, H and COT (endurance only) and duration of exercise of individual horses is presented in Supplementary **Table S2**.

Overall T_c profiles during endurance exercise

The overall mean speed of endurance horses was 14.0 ± 1.4 km h^{-1} over the first 40 km (n = 11) and 14.2 ± 2.1 km h^{-1} over the second 40 km (n = 11) with a mean HR of 114 ± 13 bpm (Supplementary **Table S1** and **S2**). There was no significant correlation between end T_c of the first 40 km endurance exercise loop and recovery time to HR ≤ 60 bpm and (p = 0.646) but the end T_c at the second 40 km showed a significant correlation with HR recovery (p = 0.045). The cumulated H of the total 80 km was 120000 ± 18000 kJ. The T_c profiles of endurance horses during exercise and recovery are presented in **Table 2**, **Figures 3A** and **4**, and Supplementary **Table S1** and **S2**. The mean max T_c, absolute max T_c and net AUC of the first 40 km exercise were not significantly different from the second 40 km exercise ($F_{1,7}$=2.436, p = 0.163 [for max T_c], p = 0.66 [for max absolute T_c] and p = 0.56 [for net AUC], respectively) (**Figure 3A** and **6**). Mean duration of all 40 km T_c endurance exercise was 191 ± 43 min. The mean max T_c during exercise was 39.0 ± 0.4°C and time to max T_c was 143 ± 60 min at a mean distance of 29.5 ± 11.6 km. Hyperthermia was recorded in 50% of the T_c exercise periods. The total dynamic thermal response (net AUC, T_c °C x min) was 106.4 ± 44.3°C x min per 40 km and was approximately 0.5°C per min (**Figure 6**).

Overall T_c profiles during endurance recovery

Post-exercise, only 25% of all T_c recovery periods returned to their base T_c while 50% decreased to or lower than 38°C within 39.6 ± 14 min. The time that T_c returned to 38.5°C varied between 0 to 57 min (14/15 T_c recovery periods). Horse 7 showed a different exercise T_c time profile with an end of exercise T_c of 39.4°C while the max T_c (39.9°C) occurred at 57 min post-exercise (**Figure 4**). The dynamic thermal response during T_c recovery period demonstrated a heat loss by a negative value (-18.1 ± 25.9°C x min) and a large variation amongst individuals (range 23.7 - 59.7°C x min) with a net AUC min^{-1} of -0.3 ± 0.4°C.

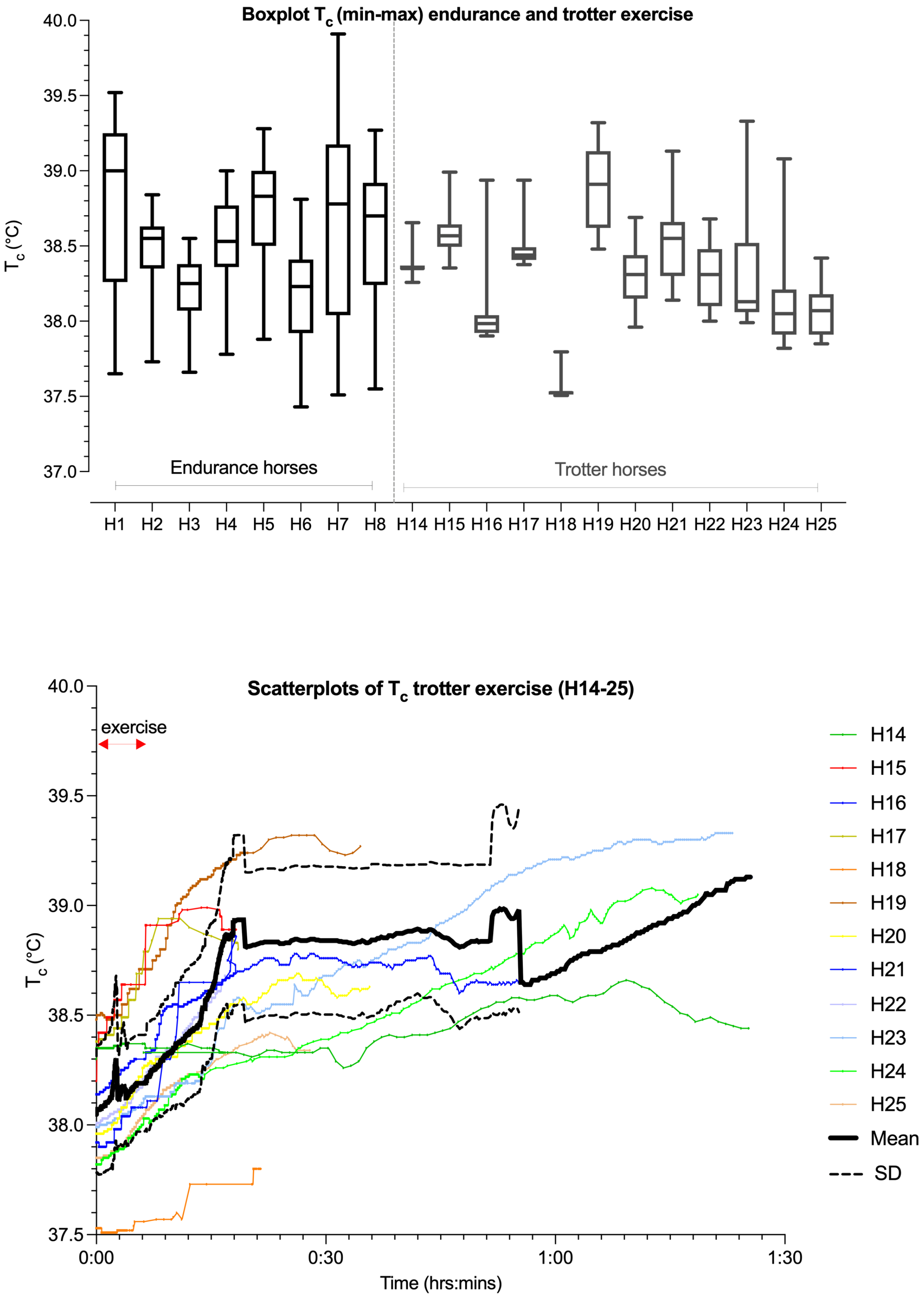

Figure 3. Box and whisker plots of T_c in endurance and trotter exercises demonstrating median (solid line), interquartile range (identified by rectangular area) and minimum to maximum (max) ranges to show entire range of data (whiskers or error bars) of boxplots of T_c (°C) in horses (H; horse number) endurance (H1 – H8) and trotter (H14- H25) exercise (**A**). Scatterplots of T_c over time with T_c (°C) (left y-axis) over time (duration of exercise in hours [h] and minutes [min]) (x-axis) in all trotter horses (H14-25) and the mean (thick black line) ± SD (dotted black line, calculated with n > 3 horses) during high intensity trotter exercise over time (**B**).

Overall T_c profiles during moderate warm-up trotter exercise and recovery

The T_c profiles of trotter horses during exercise and recovery are presented in **Table 2**, **Figures 3** and **5**, and Supplementary **Table S1** and **S2**. The mean speed of the trotters during 1540 m distance trotter exercise was 27.3 ± 5.3 km h^{-1}) with a mean HR of 140 ± 24 bpm (Supplementary **Table S2**). The T_c profile of trotter moderate exercise revealed a minor T_c increase and a minor dynamic thermal response during exercise and recovery.

Overall T_c profiles during trotter exercise

The mean speed of the trotters during 1540 m distance trotter exercise was 40.0 ± 2.9 km h^{-1} with a mean HR of 147 ± 17 bpm (Supplementary **Table S2**).

Post-exercise, HR recovered to 60 bpm in 5 of the 12 trotters and was coupled with a T_c lower than 39.0°C in all those trotters. The T_c profile of trotters reached their mean max T_c of 38.8 ± 0.5°C during recovery within a mean time of 40.2 ± 30.2 min. The overall increase in mean T_c (delta) varied (0.5 ± 0.5°C). Hyperthermia occurred in more than 40% of the trotters. Indeed, four trotters still had a T_c higher than 39°C at the end of recovery, mean time of 68.6 ± 24.6 min duration. Only one T_c returned to 38°C baseline during recovery. The T_c profiles of trotter horses during exercise and recovery are presented in **Table 2**, **Figures 3** and **5**, and Supplementary Table S1. The dynamic thermal response was low during trotter exercise with a mean of 0.2 ± 0.4°C x min. However, thermoregulation in trotters occurred during recovery with a mean dynamic thermal response of 18.6 ± 22.1°C x min with a wide range (**Figure 6**) and net AUC min^{-1} of 0.5 ± 0.3°C.

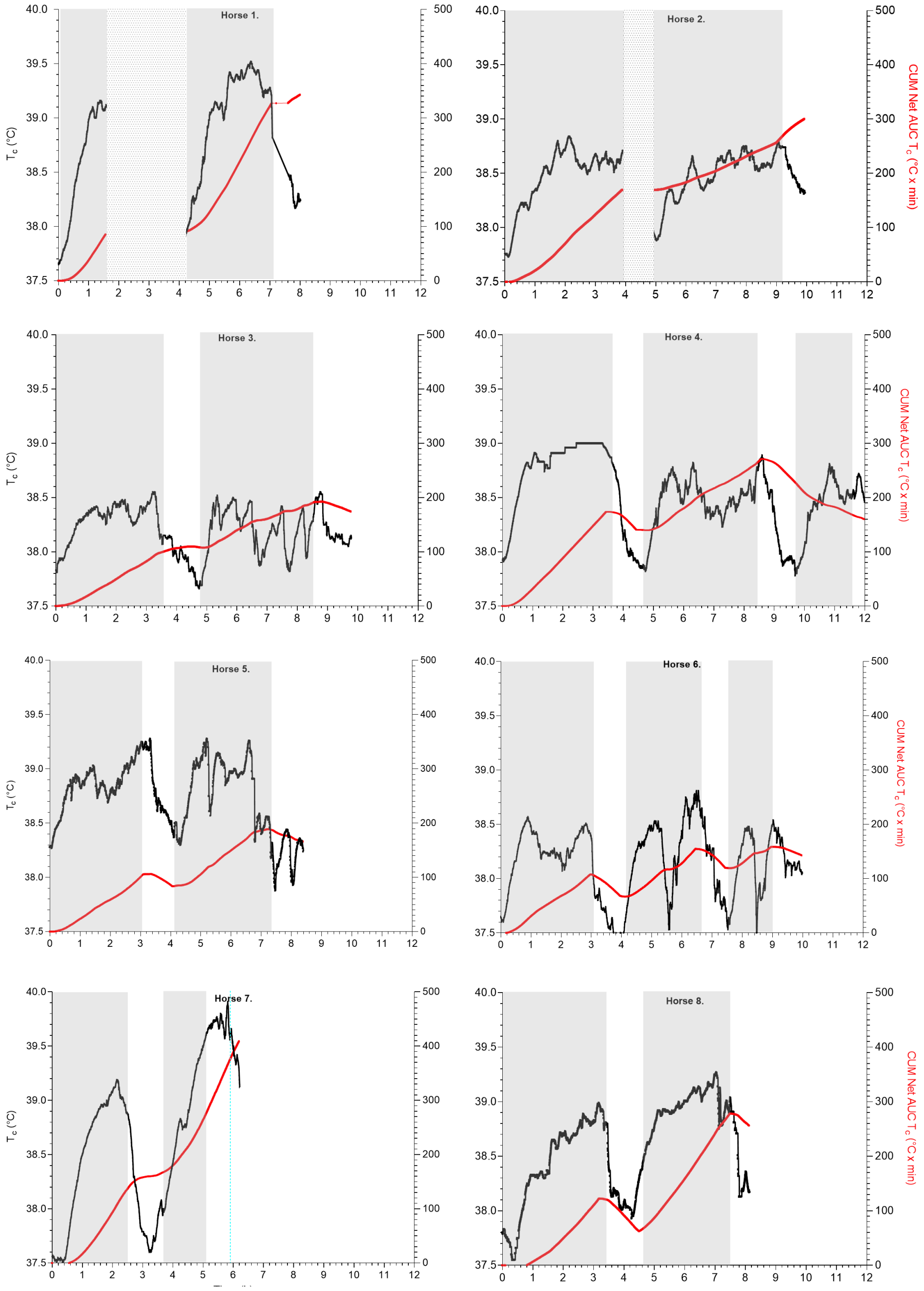

Figure 4. Scatterplots of T_c (°C) (left y-axis, black line) and cumulated net AUC (T_c °C x min) (right y-axis, red line) over time (hours, h) during T_c exercise periods (identified as grey blocks) and recovery in endurance horses H1-8, H1 data loss at end of leg 1 and H1, H2 without T_c recording during the first recovery (identified as spotted blocks); H4, H6 continued to 100 km total.

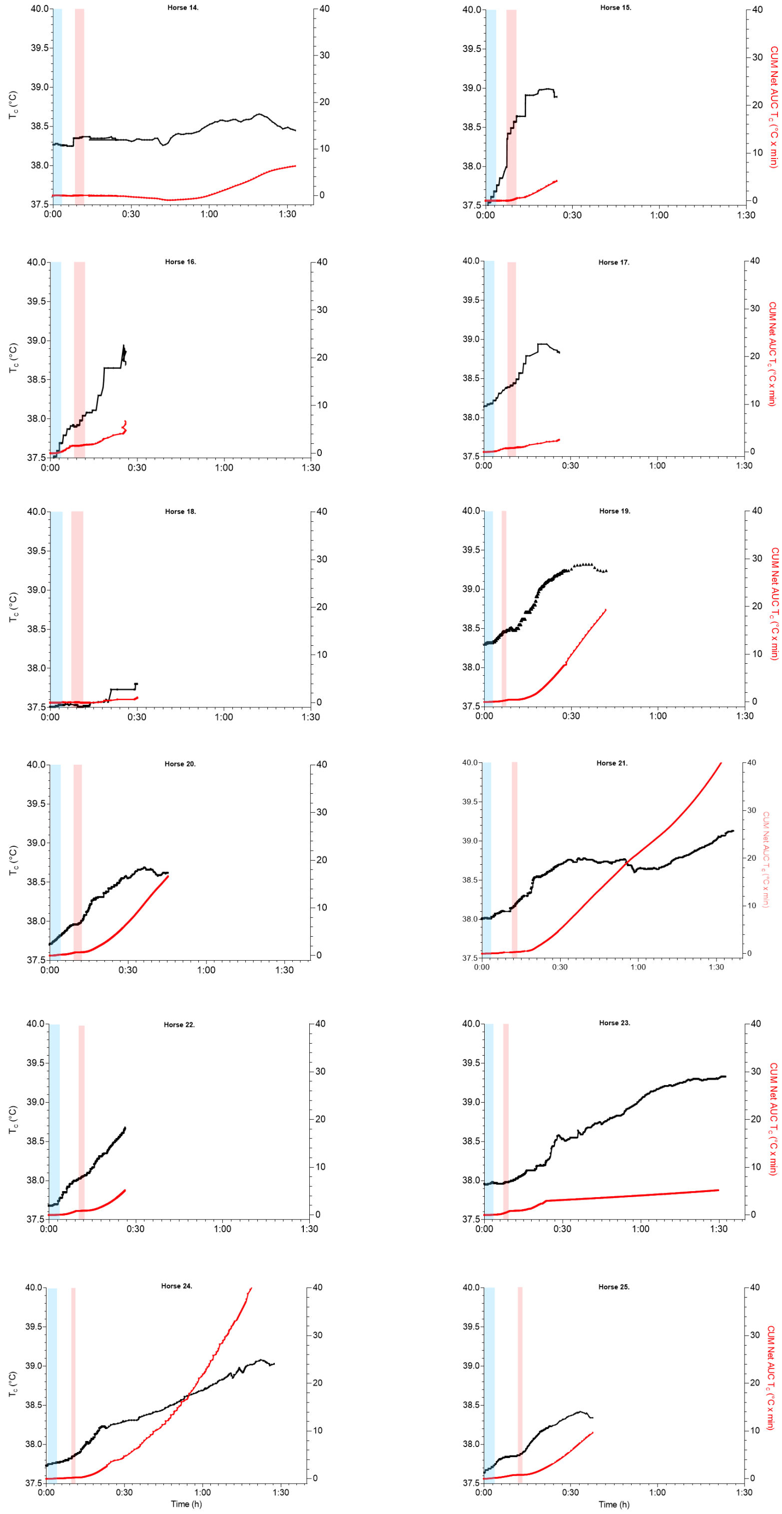

Figure 5. Scatterplots of T_c (°C) (left y-axis, black line) and cumulated net AUC (T_c, °C x min) (right y-axis, red line) over time (hours, h) during exercise and recovery in trotter horses H14-25, warm-up exercise (moderate intensity, identified as blue blocks) and trotter (high intensity, identified as light-red blocks).

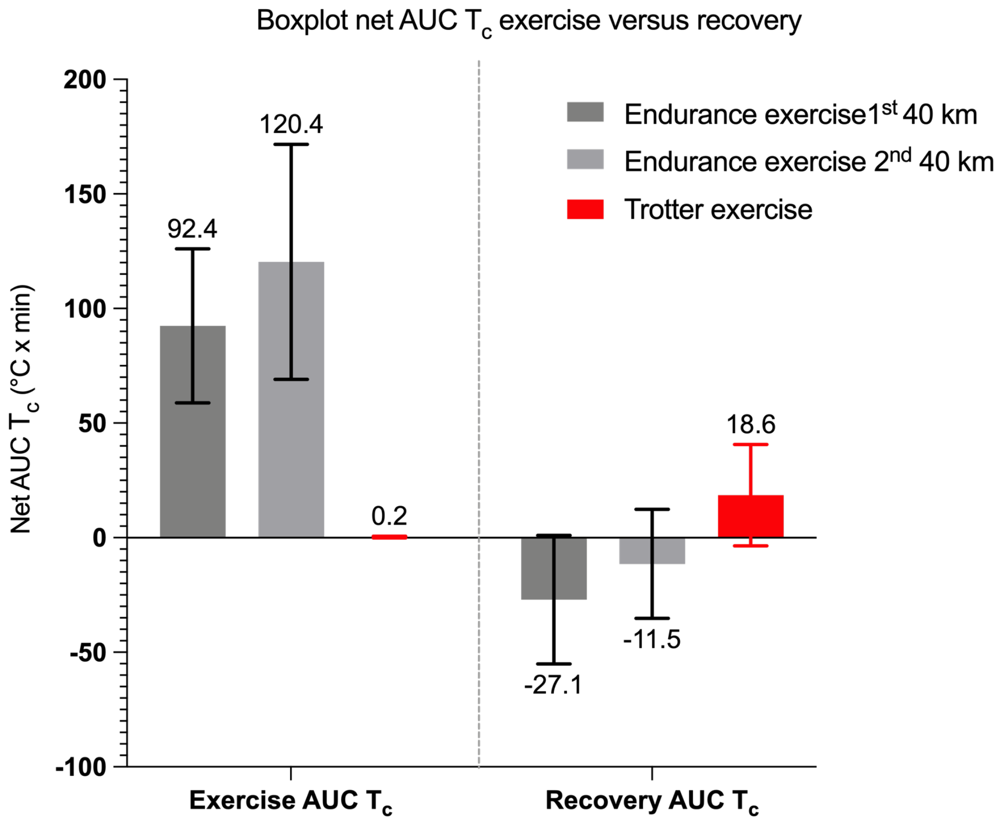

Figure 6. Boxplot of the dynamic thermal response presented by net AUC T_c (°C x min) during T_c exercise period of endurance and trotter exercises (presented with mean net AUC T_c on top bar). The 1st 40 km of endurance exercise was not significantly different to the 2nd 40 km. Net AUC T_c of exercise and recovery from full speed trotter exercise over 1540 m was minor during exercise while T_c increased post-exercise as represented by positive net AUC T_c.

Comparison of the T_c profiles during endurance and trotter exercise

The mean overall values of all data during the two different types of exercise are presented in Supplementary **Table S1**. The max T_c in endurance exercise did not significantly differ from max T_c in trotter exercise ($p = 0.19$). In addition, the max T_c and absolute max T_c of the first 40 km and second 40 km endurance exercise loops did not significantly differ from trotter exercise when analyzed separately using an independent sample t-test ($p = 0.49$ and $p = 0.26$, respectively).

After 60 min recovery, hyperthermia persisted in only one endurance horse performing endurance exercise. This is in contrast to trotter exercise during which hyperthermia occurred during recovery in 5 of the 12 trotters.

Type exercise	Endurance (n = 16; 40 km periods)	Trotter 2nd warm-up (n = 12, 1,540 m)	Trotter (n = 12, 1,540 m)
Duration (min) exercise	198 ± 63 (83-247) (40 km, n =25); T_c only: 191 ± 43 (n = 16)	3.7 ± 0.4	2.9 ± 1.4. (1.5 – 6.1)
Duration (min) recovery	60	4.9 ± 1.4 (2.6 – 7)	40.2 ± 30.2 (11.5 – 83)
T_c (°C)	38.5 ± 0.3 (38.2 - 39.0)	37.9 ± 0.3 (37.5 – 38.3)	38.1 ± 0.3 (37.5 - 38.5)
Max T_c (°C) (during exercise or recovery)	39.0 ± 0.4 (38.5 – 39.9**)	38.0 ± 0.3**	*38.8 ± 0.5** (37.6 – 39.3)
Time to max T_c (min)	143 ± 60 (54 – 245, n = 15)	NA**	34.3 ± 28.4** (6.5 – 79)
Exercise distance (km) to max T_c	29.5 ± 11.6 (11 – 40, n = 15)	NA	NA
Max $T_c \geq$ 39°C	8/16	0	4/12
Max $T_c \geq$ 38.5 °C	all	0	5/12
Speed at max T_c (km h^{-1})	16.1 ± 4.3 (4.7 – 21)	22.5 ± 8.3	337.7 ± 10.0 (18.6 – 51.9)
HR at max T_c (bpm)	105 ± 29 (63 – 152)	135.1 ± 39.0	145 ± 36 (80 - 204)
Return T_c to 38.5°C	15/ 16	6/12	1/12 (and 1 T_c never above 38)
Time max T_c to 38.5°C (min)	21.2 ± 22.9 (0 - 80)	NA	NA
Return T_c to 38.3°C	13/16	2/12	1/12 (and 1 T_c never above 38)
Time max T_c to 38.3°C (min)	31 ± 24.6 (0 - > 77) (n = 13/16)	NA	NA
Return T_c to 38.0°C	10/16	8/12	8/12 (1 T_c never above 38)
Time max T_c to 38.0°C (min)	39.6 ± 14 (n = 8/16)	0 (6//12) (2 T_c never above 38)	NA
Return to base T_c (Y/N)	4/16	8/12	1//12 (and 1 T_c never above 38)
Time max T_c to baseline (min)	52 ± 37 (n = 4/16)	0 (4/12); (1 T_c never above baseline)	NA
Net AUC T_c exercise (endurance: 2nd 40 km from base T_c)	7116 ± 1997 (7203 ± 3125)	7.9 ± 11	4.8 ± 8.5
Net AUC T_c exercise 1st 40 km	7421 ± 1940	NA	NA
Net AUC T_c exercise 2nd 40 km	6811 ± 2138	NA	NA
Net AUC T_c recovery	-1516 ± 1326 (n=15)	5.4 ± 6.8	1821.3 ± 4434.0 (-14.3 – 15769)
Net AUC T_c recovery 1st 40 km	-1979 ± 1555 (n=7)	NA	NA
Net AUC T_c recovery 2nd 40 km	-1169 ± 1102 (n=8)	NA	NA
Delta T_c first 10 min recovery (°C)	-0.24 ± 0.34 (-0.24 – 0.15, n = 15)	NA	+0.36 ± 0.16 (0-0.58) (n = 11)
Net AUC min^{-1} (°C min^{-1}) exercise	37.3 ± 9.9	2 ± 2.7	1.9 ± 3.7
Delta T_c end exercise to end recovery	-0.71± 0.4 (-1.16 - 0.23)	0.1 ± 0.1 (0 – 0.2)	0.6 ± 0.4 (0 – 1.3)
Delta T_c end exercise to end recovery min^{-1}	-0.01± 0.01 (-0.02 – 0.02)	0.02	0.04 ± 0.02 (0 – 0.06)
Time to HR ≤ 60 bpm (min)	6.3± 6.5 (0 - 24)	2.3 ± 1.2 (0.5 -4)	23.1 ± 20.9 (5 - 51) 4/12
T_c > 39°C at end recovery	1/16 (39.13 after 90min)	NA	4/12
Net AUC min^{-1} (°C min^{-1}) recovery	-23.7 ± 20.4 (-55 - 13.8)	2.9 ± 3.3	54.5 ± 143.6
Speed (km h^{-1})	14.1 ± 1.7 (10.5– 17.4) (n=22, without horse 7)	34.1 ± 3.5 (30.4 – 38.0) (n=7)	38.2 ± 0.3 (38.0 – 38.8) (n=7)
Max speed (km h^{-1})	23.4 ± 2.8 (19.2 - 30) (n=22)	34.1 ± 7.3 (25.0 - 42.3) (n=7)	49.7 ± 3.9 (42 – 52.7) (n=7)
HR (bpm)	114 ± 13 (89 - 143) (n=24)	130 ± 32 (75 – 177) (n=8)	151 ± 17 (130 – 169) (n=8)
H (kJ) total 80 km exercise #	71000 ± 12000 (52000– 94000) (n=22)	-	-
COT (ml O_2 kg^{-1} m^{-1}) #	0.13 ± 0.01 (0.11 – 0.15) (n=22)	-	-
$\dot{V}O_2$ (LO_2 min^{-1}) #	15.0 ± 2.3 (11.2 – 20.5) (n=22)	-	-

Table 2. Overall variables during exercise and recovery of endurance and trotter exercise. Data are presented as overall mean ± SD. T_c, GI pill temperature; n, number identified only if different than 16 or 12 exercise periods; max T_c, maximum T_c; AUC, area under the curve; # indicates calculated; HR, heartrate; H, metabolic heat production; COT, cost of transfer; $\dot{V}O_2$ L min^{-1}, oxygen consumption, liters per minute; *indicates during exercise; **indicates during recovery; NA, not applicable; -indicates not available.

Blood analysis data in endurance and trotter exercise

Blood results and the delta of lactate, pH and Hct values of all horses (endurance and trotter exercise) are presented in **Figure 7**, Supplementary **Table S3** and Supplementary **Figure S1**. An ANOVA revealed a significant difference in post-exercise delta lactate value between endurance (1.2 ± 0.6 mmol L^{-1}) and trotter (9.4 ± 4.0 mmol L^{-1}) as well as between warm-up trotter (1.6 ± 1.0 mmol L^{-1}) and fast trotter exercise with a higher lactate value in the last exercise ($F_{(2,38)} = 37.63$, $p < 0.0001$). No significant difference was identified between first and second 40 km endurance exercise ($p = 0.984$), however, comparing both first and second 40 km endurance horses to trotter horses separately confirmed a highly significant difference ($p < 0.001$). In addition, mean delta lactate in the 40 km endurance exercise was significantly different to trotter exercise using an independent samples t-test ($p < 0.001$). The delta Hct and delta pH values did not reveal significant differences between endurance and trotter exercise ($p = 0.60$ and $p = 0.26$, respectively).

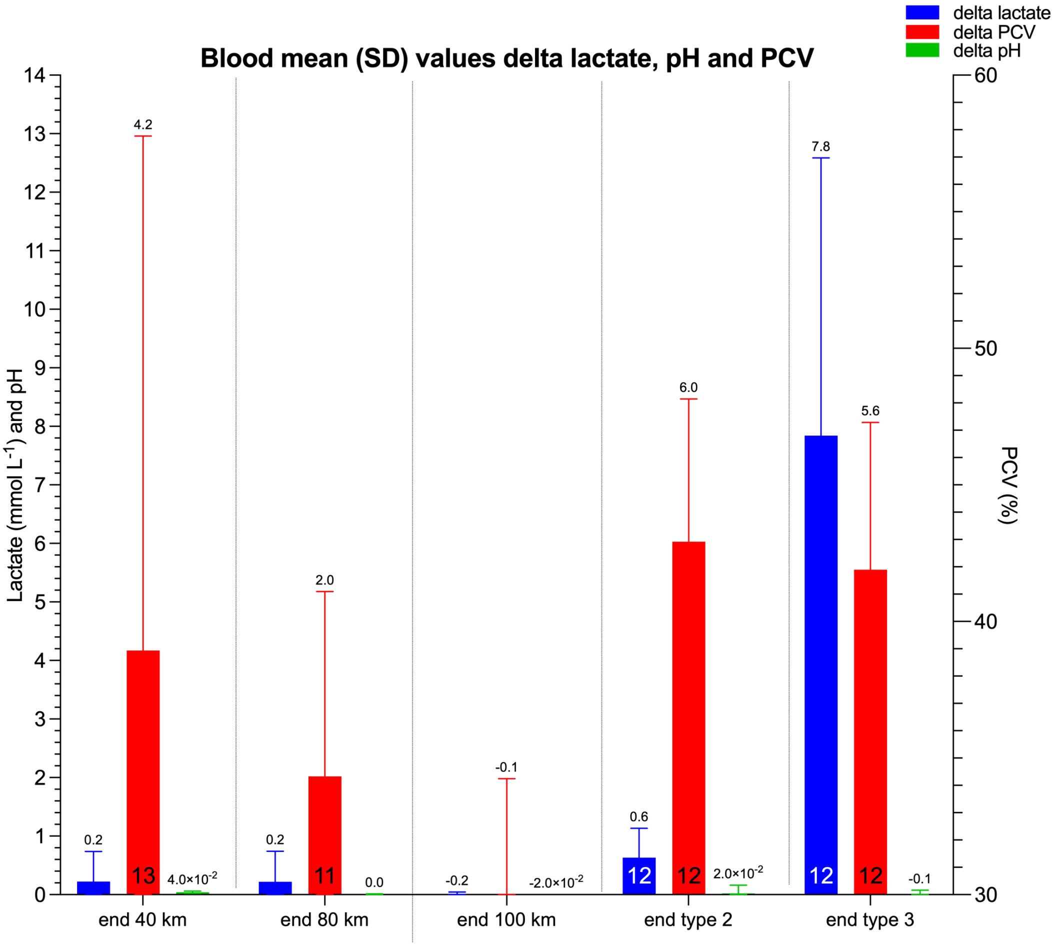

Figure 7. Bar graph of blood parameters: lactate and pH on the y-axis presented as mmol L^{-1} and the number respectively, and the PCV (Hct) on the right y-axis presented as %. The type of exercise is presented on the x-axis as pre-, and post-ride in endurance and trotter exercises. The mean value is presented on top of the bar while sample size at the bottom in column.

4.5 Discussion

This was the first study to be conducted using a telemetric GI pill in field competitions to compare the equine thermoregulatory response to different types of exercise under normal weather conditions during the cooler South Australian months. For this purpose, a large dataset of T_c recordings and other physiological responses was analyzed and compared between two types of exercise that differed greatly in duration and intensity (endurance compared with trotter exercise). As a result, an integrative real time view was obtained on how the equine body copes with heat load production during different types of exercise in the field. The GI pill proved to be a reliable tool to continuously monitor individual thermoregulatory well-being in sport horses.

There was an important difference in the time profile of the thermoregulatory response between endurance and trotter horses. Endurance horses reached their mean max T_c during exercise towards the end of each leg of 40 km (after ± 30 km) and almost all returned to a T_c lower than 38.5°C during 60 min into recovery. Trotter horses, however, always reached their mean max T_c post-exercise at least 30 min into recovery. Moreover, in a third of the trotters, T_c was still higher than 39°C and HR was higher than 60 bpm at the end of recovery. When HR is at 60 bpm, horses may still have a high T_c (38.8 ± 0.4°C, range 38.0 – 39.7°C). Continuous monitoring of T_c by a telemetric temperature-sensitive pill that passes through the GI tract has been evaluated in horses previously during rest and 158 km transport using the CorTemp® system (Green et al., 2005, 2008). While the Equivital system was validated in the researchers' previous study similarly during rest periods (Verdegaal et al., 2017), we applied this monitoring method in equine athletes specifically for the first-time during field exercise.

The current study showed that the dynamic thermal response calculated by the net AUC proved to be a more useful presentation of the metabolic response rate than the calculated H (based on calculated values). Despite the individual variation of the max T_c in endurance and trotter horses, it is of interest that the overall max T_c in endurance horses (39.0 ± 0.4°C; range 38.5 – 39.9°C) was not significantly different to the max T_c in trotters (38.8 ± 0.5°C; range 37.6 – 39.3°C). Another important finding was that persisting hyperthermia was recorded in four trotters after a mean of 69 min post-exercise while only 1 endurance horse recorded 39.9°C after 60 min of recovery.

Most importantly, the current study has demonstrated that horses show important individual differences in their thermoregulatory response to different types of exercise. This result was obtained due to the continuous monitoring of the T_c instead of performing serial measurements of T_{re}. For example, there was a large variation (range 11 – 40 km) between endurance horses with respect to the distance at which max T_c was reached. It is noted that while the moment-to-moment location of the GI pill is unknown, based on a previously performed study it can state that T_c changes are mainly attributed to exercise and less likely to the movement of the GI pill along the GI tract (Verdegaal et al., 2017). Both the individual variation in max T_c and dynamic thermal response underline that individual continuous T_c monitoring is essential for reliable oversight. This reliability is especially important since many horse-specific factors contribute to the thermoregulatory response, such as the genetic make-up of a horse, its temperament, and its basic performance capacity level. However, for endurance horses, their performance capacity levels should have been quite similar for all studied horses, keeping in mind their similar competition experience levels for the 80 km distance (**Table 1**).

Continuous individual monitoring studies of the thermoregulatory response to date

Based on recent equine and human studies, it is important to review different tools that allow for continuous reliable monitoring of the thermoregulatory response to exercise in the field with a minimal data loss. In that respect, the GI pill has proven to be a practical, accurate and precise tool to monitor thermal response during field exercise both in human and equine athletes (Edwards and Clark, 2006; Verdegaal et al., 2017; Olcina et al., 2019).

A recent alternative method is the use of intra-muscular microchips embedded in different locations to continuously record muscle temperature (Kang et al., 2020). Although the study was performed under laboratory conditions, this technique could eventually be employed in the field. There was a positive correlation between muscle temperature and central venous temperature (CVT) only when combining all exercise phases including recovery. Interestingly during the recovery phase, the muscle temperature continued increasing and lagged behind the CVT. This non-alignment is in accordance with previous studies reporting on the lack of direct correlation between muscle temperature and core temperature (Byrd et al., 1989; McCutcheon et al., 1992; Hodgson et al., 1994). Therefore, more research is needed to obtain a better understanding of the associations between muscle temperature and core body temperature. One possible disadvantage of the technique is the requirement of invasive surgery to place the microchip.

Comparison of the T_c profiles during endurance and trotter exercise

The results of the current study are important for formulation of future competition and hot weather policies (MOOC heat illness; MOOC endurance). The study results show that both endurance and trotter horses reached comparable max T_c, although at different time points. Endurance horses reached their mean max T_c (39.0 ± 0.3°C, 38.5 – 39.5°C) during endurance exercise towards the end of each leg of 40 km (after ± 30 km) and almost all T_c returned to lower than 38.5°C during 60 min into recovery. In contrast, after the completion of trotter exercise, trotter horses reached their max T_c (38.8 ± 0.5°C, 37.6 – 39.3°C) at an average of 34 min post-exercise with a mean T_c increase of 0.6 ± 0.4°C (0 – 1.3°C). Moreover, in a third of the trotters, T_c was still higher than 39°C and HR was higher than 60 bpm at the end of recovery. We found that within the 60-min rest time, all endurance horses achieved an overall recovery to 38.5°C in 15 out of 16 T_c 40 km exercise periods, and 50% of these horses reached lower than 38°C. This supports the mandatory rest periods of 60 min as determined in the AERA and Federation Equestrian International (FEI) endurance regulations when exercising in a cool environment (F.E.I., 2018; A.E.R.A., 2020). The present study was not able to demonstrate a correlation between the end T_c first 40 km and recovery time to HR of 60 bpm, however the end T_c second 40 km was related to HR recovery. According to the AERA competition regulations, horses with a HR of 60 bpm at the end of their recovery period would meet all criteria to be labelled as ‘fit to continue’ the competition. Interestingly, the current study revealed that the mean overall 40 km T_c was still higher than normal (38.8 ± 0.4°C; range 38.0 – 39.7°C). This means that at the time that the HR recovered to 60 bpm, most horses were still actively coping with increased core body temperature. FEI regulations are stricter than AERA rules regarding the duration of the HR recovery time and require the HR to return below 64 bpm within 15 min post-exercise (F.E.I., 2021). The current study demonstrated that in the majority of T_c periods (20 out of 22, T_c 40 km exercise periods) the HR recovered to below 64 bpm within 15 min, although again, the mean T_c was still 38.7 ± 0.4°C (range 38.0 – 39.6°C) (F.E.I., 2021). The HR recovery regulations in endurance horses are based on studies demonstrating that not only increased speed and distance but also a longer than 11-13 min cardiac recovery time is associated with higher risk of metabolic elimination after endurance exercise, more specifically with a 70% probability of elimination at the next veterinary check (Nagy et al., 2010; Younes et al., 2015; Bennet and Parkin, 2018). More field research is needed to investigate when heat accumulation in such horses can become problematic during follow-up exercise. The difference in thermoregulatory responses between endurance exercise and racing exercise is well-known and the current study provides exercise-specific core thermal response features which need to be addressed when formulating future equine competition and hot weather policies.

Hyperthermia post-exercise

Overall, 50% of the endurance horses developed hyperthermia during the standardized exercise loops, which underlines the importance of an effective T_c recovery period of sufficient duration. The persistence of hyperthermia in exercising endurance horses is one of the several factors causing metabolic disorders and fatigue due to the decreased supply of blood and fuel to the exercising muscles, brain and GI tract, and increased muscle and brain temperature (McCutcheon et al., 1992; Hodgson et al., 1993; Jones and Carlson, 1995; Geor and McCutcheon, 1998; Gonzalez-Alonso et al., 1999; McConaghy et al., 2002; Gerard et al, 2013; Brownlow et al., 2016; Brownlow and Mizzi, 2021).

While hyperthermia and heat stress are more commonly associated with prolonged exercise, the current study revealed that horses performing short bouts of strenuous exercise in the field may suffer increasing from hyperthermia during recovery (1/3 of the trotters, **Figure 3B** and **5**, **Table S1**).

Hyperthermia in racehorses post-exercise can be explained by the greater amount of metabolic heat generated by their larger muscle mass (compared to endurance horses) during their shorter exercise duration but higher intensity exercise (Hodgson et al., 1994; Geor et al., 1995; Jones and Carlson, 1995; Schott et al., 1999). In addition, the high intensity trotter exercise represents a short but acutely pronounced challenge to a multitude of organ systems at the same time such as the respiratory, cardiovascular, musculoskeletal and sympathetic nervous systems, of which the latter may be one of the driving forces behind the hyperthermia (Hetem et al., 2013). The "acuteness" may also explain the hyperthermia that was encountered in more trotter horses compared to endurance horses as well as the more significant interindividual variation seen at the level of the thermoregulatory response in trotter horses. Finally, the current study results underline the importance for formulating guidelines for the management and welfare of all equine sports disciplines. These physiological factors, coupled with environmental factors in the field, can overwhelm the capacity of the heat loss mechanisms of sport horses. It is important to point out that there is no scientific evidence to date suggesting that horses experience heat injury when their T_c reaches and is sustained at 39°C. However, it should be kept in mind that if horses, and especially trotters, engage in a subsequent exercise bout too soon, their core body temperatures would be starting at an elevated level.

In most trotters (7/12), the first signs towards lowering and normalizing core body temperature became visible after a minimum of 20 min into the recovery. Problems may arise when the hyperthermia in racehorses (with or without clinical signs) is not recognized. The delayed onset of hyperthermia may go unnoticed when trotters are transported shortly after completion of the race. Hyperthermia coupled with transport during hot weather is likely to increase heat loading. The identification of ongoing hyperthermia during recovery is essential information with respect to equine welfare and emphasizes the need for extended continuous monitoring post-exercise. The findings of the current study may explain personal anecdotal evidence expressed by trotter trainers noticing that some horses show discomfort the day after an otherwise uneventful competition day (Courouce et al., 2002; Richard et al., 2010; Bertuglia et al., 2014) due to unrecognized post-racing hyperthermia (McConaghy et al., 2002; Brownlow and Mizzi, 2020; Brownlow and Mizzi, 2021). The current findings also highlight that the habit of withholding water prior to racing events is not an ideal practice. Indeed, several studies involving endurance or trotter horses report that dehydration and electrolyte loss are significant predictors for early elimination due to metabolic reasons (Barton et al., 2003; Fielding et al., 2009; Barnes et al., 2010; Munoz et al., 2010; Trigo et al., 2010; Waller and Lindinger, 2010). Likewise, a recent meta-analysis in human athletes demonstrated the importance of fluid ingestion to counteract hyperthermia and to improve performance capacity (Alhadad et al., 2019). In the current study, no fluid intake restrictions were applied and blood values showed that the studied horses were not dehydrated.

As in endurance horses, the recovery for trotters to HR 60 bpm was not associated with a recovered T_c. This is important knowledge for the monitoring of trotters during recovery when using HR as guidance. At a HR of 60 bpm point in time, the mean T_c was still 38.7 ± 0.5°C (range 38.0 – 39.3°C) while 8/12 trotters still measured a T_c higher than 38.5°C. A post-exercise peak T_{re} was earlier reported in other studies (Hodgson et al., 1993; Kohn and Hinchcliff, 1995; Marlin et al., 1996; Marlin et al., 1998; Kohn et al., 1999; Foreman et al., 2006; Verdegaal et al., 2017) and supports the conclusion that it is necessary to continue monitoring racehorses during recovery. Based on the findings in the current study, coupled with those reported by previous studies, continuous monitoring of racehorses and sport horses for hyperthermia using the GI pill or T_{re} during a period of rest for at least 60 min post-exercise is highly recommended.

The dynamic thermal response (net AUC T_c)

The continuous T_c monitoring allowed for the construction of detailed temperature-time profiles and the subsequent application of the AUC approach. The present study measured net AUC T_c to assess the dynamic thermal response to the metabolic thermal load, a product of the thermoregulatory mechanisms that increase temperature over time (Hodgson et al., 1993; Schroter and Marlin, 1995; Schott et al., 1999; Hodgson, 2014). As expected, the current study demonstrated that the mean dynamic thermal response was much higher during endurance exercise compared to trotters due to higher H over time. The core thermal response during recovery in endurance horses was negative due to metabolic heat dissipation. Alternatively in trotters, both heat load and heat loss mechanisms were in place during recovery, represented by a relatively high dynamic thermal response compared to the short duration of the exercise. The thermal response index using area under the fever curve has been applied in several mammal studies comparing the fever response over time (Feng and Lipton, 1987; Murakami and Ono, 1987). Only a few equine research groups report the use of AUC to assess temperature response over time. One equine study used AUC to demonstrate changes in muscle and tendon tissue temperature over time during therapeutic ultrasound treatments at different tissue depths (Montgomery et al., 2013). Studies involving human athletes have used AUC to compare AUC temperature response in two study groups. One study used AUC to quantify the body temperature response over time (60 min cycle exercise at 65% VO_2max) by continuous recording of T_{re} and demonstrated that T_{re} AUC response was not different between ingesting a carbohydrate drink (47.7 ± 11.6 [°C x min]) and a placebo (50.1 ± 11.1 [°C x min])—unfortunately recovery data were not reported (Horswill et al., 2008). Another study reviewed comparisons of different cooling methods using AUC (T_{re} to time) to assess cooling rates over time and argued that a larger AUC was associated with an increased risk of tissue and organ injury (Casa et al., 2007). A recent human study used AUC to quantify the thermal response during bladder cancer treatment to assess its use as a prognostic parameter and concluded AUC was a simple calculation to predict the medical outcome (Datta et al., 2021). Similarly, we can argue that in the current study a high net AUC (high dynamic thermal response) may be associated with a higher risk of thermally based injury due to insufficient thermoregulatory mechanisms. When comparing the values of the calculated H and calculated COT in the current study across endurance exercise (according to Schroter and Marlin, 2002), the H calculations were not discriminative for endurance horses with high max T_c. Moreover, these calculations did not consider anaerobic exercise and therefore could not be applied to trotter exercise for comparison. Another concern is that the H equation is formula-based using several calculated values such as $\dot{V}O_2$. When comparing this calculated H method to the AUC method which can only be used in association with a continuous monitoring technique, the latter seemed a much more accurate approach.

Blood values in response to exercise

Blood lactate values showed only a mild increase in endurance horses and were not different between the first and second 40 km endurance exercise. These values were similar to an earlier report which identified no difference in blood lactate values between eliminated endurance horses and finishers (Fielding et al., 2009).

Limitations

The current study has various limitations that should be considered when assessing those findings. As applies in all field studies, not all conditions could be 100% controlled, such as perfectly equal conditions of competition tracks, T_a, solar radiation, and the fact that the endurance part involved privately owned horses which obviously received different diets and followed different trainings protocols. In retrospect, scraping off water from the horses during cooling down was not the most optimal approach since recent research comparing 5 cooling methods in racehorses favored continuous reapplication of cold water without subsequently scraping it off (Takahaski et al. 2020). Those researchers did not seek to investigate individual external horse-related influencing factors such as diet, supplements, health history, duration of transport prior to competition nor processes such as the circadian rhythms which may have caused a diurnal variation in T_c. Future research is needed to investigate these variables to augment the current understanding of heat load production in sport horses in varying ambient conditions.

4.6 Conclusion

Continuous monitoring of T_c using the GI telemetry pill is a reliable, non-invasive method of assessing the thermoregulatory response in exercising horses. It provides vital individual recording and monitoring of important interindividual differences in T_c to time profiles. Clearly, seeking a universal thermoregulatory response pattern that will apply to all exercising horses will prove futile. Consequently, continuous monitoring allows for applying an AUC approach which is a more robust parameter when compared to calculated H. In the current study, endurance horses reached their max T_c on average when completing over 75% of a 40 km exercise leg; this returned to baseline within the mandatory 60 min recovery time. However, several horses still had T_c values above 38.5°C when their HR had already returned to 60 bpm which is used in endurance (AERA) as 'fit to continue' competing. Trotter horses reached their peak T_c during their recovery period on average at 34 min after exercise. Here again, several horses had T_c values above 38.5°C with a HR already returned to 60 bpm. The current study results have shown that T_c monitoring should continue in trotter horses at least until 60 min post-exercise.

Future research involving strenuously exercising horses should focus on the possible impact of intrinsic characteristics such as genetics and training, as well as more challenging environmental conditions. The findings of the current study have implications for improving the overall welfare of sport horses and future management of equine welfare at sport events such as the Olympic Games (Hosokawa et al. 2020; Elliott, 2021). The current study provides reliable supporting evidence for the need for industry-wide, temperature-monitoring guidelines to prevent EHI in endurance horses and racehorses when exercising and recovering in the field.

Conflict of Interest Statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethics statement

The present study was approved by the University of Adelaide Animal Ethics Committee (project number S-2011-224) Australia. The owners of the participating horses provided their written consent to participate in this study.

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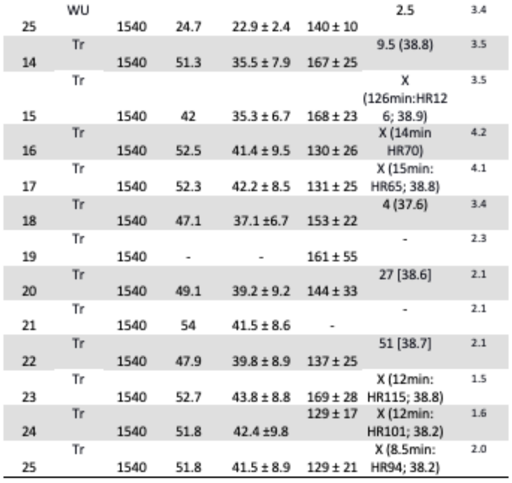
4.7 Supplementary Tables

Horse number	Type exercise	Distance (km)	Duration exercise (min)	Duration recovery (min)	Baseline Tc (°C)	Max Tc (°C) exercise	Max Tc (°C) during recovery	Speed (km) at max Tc during exercise	HR (bpm) at max Tc during exercise or /recovery	Distance (km) at max Tc exercise	Time (min) to max Tc exercise	Time (min) to max Tc recovery	Time (min) max Tc to 38.5°C	Time (min) max Tc to 38.3°C	Time (min) max Tc to 38.0°C	Time (min) max Tc to base T$_c$	Tc (°C) end exercise	Delta Tc (°C) 40 km (total from base Tc)	Tc (°C) end recovery	Delta Tc (°C) recovery: from end exercise to max Tc during	Tc (°C) at 10 min post exercise	Delta Tc (°C) change 10 min^{-1} recovery	Delta Tc (°C) 10 min rec. min^{-1}	Delta Tc (°C) end exercise to end recovery min^{-1}	Time (min) to HR < 60 bpm	Return to base Tc Y/N	Return to 38.0°C Y/N	Net AUC Tc (°C x min) during exercise (2nd 40 km only: net AUC from base Tc)	Net AUC Tc (°C x min) recovery (recovery to HR 60 bpm)	Net AUC from start 40 km (°C x min))/min duration exercise	Net AUC (°C x min))/ min duration recovery
1	End	40	165	60	37.7	39.2**	NA	11.5	150	17	82	-	- (Y)	-	-	X	39.1**	1.5	38.0	-1.2	-	-	-	0	14	N	N	85.3	-	0.5	-
1	End	2nd 40	182	60	38.0	39.5**	NA	17.2	91	31	167	-	14	48	X	X	39.3**	1.6 (1.9)	38.3	-1.0	38.7*	-0.5	-0.1	0	22	N	N	231.2 (176.7)	23.7	1.0	-0.4
2	End	40	236	60	37.8	38.8*	NA	15.9	133	21	126	-	NA	3	55	X	38.7*	1.1	37.9	-0.8	37.9	-1.2	-0.1	0	NA	N	Y	69.3	-	0.7	-
2	End	2nd 40	242	60	37.9	38.8*	NA	4.7	80	40	245	-	24	27	X	X	38.8*	0.8 (1.0)	38.3	-0.4	38.7*	0	0	0	16	N	N	135.2 (87.1)	-11.9	0.6	-0.2
3	End	40	207	60	37.8	38.6*	NA	20	132	39	192	-	57	77	44	57	38.1	0.7	37.8	-0.3	38.2	0	0	0	4	Y	Y	97.7	11.8	0.5	0.2
3	End	2nd 40	247	60	37.8	38.5*	NA	20	127	11	54	-	0	16	X	X	38.5*	0.7 (0.7)	38.1	-0.3	38.5*	+0.1	0	0	5	N	N	82.2 (82.2)	-17.4	0.3	-0.3
4	End	40	207	60	37.9	39.0**	NA	12	78	30	146	-	24	6	42	53	38.9*	1.1	37.9	-1.0	38.8*	-0.1	0	0	3	Y	Y	23.6	-33.1	0.8	-0.6
4	End	2nd 40	247	60	37.9	38.9*	NA	14	68	40	244	-	31	33	36	39	38.9*	1.0 (0.9)	37.8	-1.1	38.6*	-0.2	0	0	4	Y	Y	132.7 (38.6)	-43.9	0.5	-0.7
(4)	End	Extra 20	144	(40)	37.8	38.8*	NA	18	127	16	67	-	17	X	X	X	38.5*	1.0 (0.9)	38.4	-0.1	38.6*	0	0	0	11	N	N	90.6 (-70.8)	-1.5	(0.6)	(-0.04)
5	End	40	186	60	38.3	39.3**	NA	21	63	40	181	-	55	X	X	X	39.2**	1.0	38.4	-0.8	39.2**	0	0	0	0	N	N	105.8	-22.3	0.6	-0.1
5	End	2nd 40	188	60	38.4	39.3**	NA	19	102	16	67	-	4	60***	X	106	38.6*	0.9 (1)	38.3	-0.3	38.2	-0.3	0	0	2	N	Y	92.1 (105.3)	-3.5	0.5	-0.2
6	End	40	179	60	37.7	38.6*	NA	13	114	12	54	-	NA	9	31	5	38.4	0.9	37.5	-0.9	37.8	-0.6	-0.1	0	5	Y	Y	107.7	-4.0	0.6	0.7
6	End	2nd 40	149	60	37.5	38.8*	NA	21	82	39	145	-	18	20	35	X	38.8*	1.2 (1.1)	37.7	-1.1	38.6*	-0.2	0	0	2	N	Y	110.5 (86.6)	-35.1	0.5	-0.6
6	End	Extra 20	92	60	37.7	38.5*	NA	17	89	20	150.5	-	3	22	X	X	38.5*	0.8 (0.9)	38.0	-0.5	38.4	-0.1	0	0	0	N	N	34.3 (38.9)	-15.7	(0.4)	(0.3)
7	End	40	148	60	37.6	39.2**	NA	N	152	-	129	-	11	13	14	X	38.9*	1.6	37.8	-0.8	38.6*	-0.3	0	0	5.5	N	Y	127.7	-55.0	0.9	-0.9
7	End	2nd 40	83	60	37.8	39.4**	39.91**	N	83	-	82	57	X >80	X	X	X	39.4**	2.1 (2.4)	39.6**	+0.2^	39.5**	+0.2	0	0	24***	N	N	62.5 (84.8)	18.5	0.8	0.2
8	End	40	209	60	37.8	39.0**	NA	16.5	120	40	148.5	-	7	60***	X***	X	39.0**	1.2	38.0	-0.9	38.8*	-0.2	0	0	11	N	N	122.3	-59.7	0.6	-0.7
8	End	2nd 40	187	40$^{\&}$	38.3	39.3**	NA	17.4	110	37	158	-	16	19	X	X	39.0**	1.0 (1.5)	38.2$^{\&}$	-0.8$^{\&}$	38.8*	-0.3	0	0	2	N	N	116.4 (215.8)	-22.1$^{\&}$	0.6	-0.6$^{\&}$
14	WU	1,54	4.12	3.39	38.3	38.3	38.3	NA	49	-	0	-	N/A	0	X	0	38.3	0	38.3	0	-	-	-	-	-	-	-	0	0	0	0
15	WU	1,54	4.09	2.55	37.0	37.8	38.0	NA	185	-	4	-	0	0	N/A	X	37.8	0.8	38.0	0	-	-	-	-	-	-	-	0	0.1	0	0
16	WU	1,54	4.12	3.5	37.5	37.8	37.9	NA	119	-	4	-	0	0	0	X	37.8	0.1	37.9	0	-	+0.1$^{\&}$	0	0	-	-	-	0.7	0.3	0	01
17	WU	1,54	4.14	3.5	38.1	38.2	38.4	NA	123/63	-	3	-	N/A	X	X	X	38.3	0.1	38.4	0	-	+0.1$^{\&}$	0	0	-	-	-	0.2	0.4	0.1	0.1
18	WU	1,54	4.23	5	37.5	37.5	-	NA	135/-	-	3	-	N/A	N/A	N/A	N/A	37.5	0	37.5	0	-	0	0	0	N/A	-	-	0.1	0	0	0
19	WU	1,54	3.51	4.5	38.	38.4	38.5	-	139/56	-	4	-	0	X	X	X	38.4	0.1	38.5	+0.1^	-	+0.2$^{\&}$	0	0	6	-	-	0.1	0.5 (0.1)	0	0.1
20	WU	1,54	3.52	5.1	37.1	37.8	38.0	NA	144/42	-	4	-	N/A	N/A	0	X	37.8	0.8	38.0	+0.1^	-	+0.1$^{\&}$	0	0	0.7	-	-	0.2	0.5 (0)	0.1	1.0
21	WU	1,54	3.46	6.9	38.0	38.0	38.1	NA	-/-	-	4	-	X	X	X	X	38.0	0	38.1	+0.1^	-	-0.1$^{\&}$	0	0	0.5	-	-	0.1	0.1 (0)	0	0
22	WU	1,54	3.41	6	37.7	37.8	38.0	NA	123/71	-	0	-	0	0	0	X	37.8	0.1	38.0	+0.2^	-	+0.2$^{\&}$	0	0	1	-	-	0.06	0	0	0
23	WU	1,54	3.38	6	38.0	38.0	38.0	NA	213/112	-	3	-	N/A	N/A	0	0	38.0	0	38.0	0^	-	0$^{\&}$	0	0	2.5	N	N	0	0	0.1	0
24	WU	1,54	3.46	5	37.7	37.8	NA	NA	128/-	-	2	-	N/A	N/A	0	0	37.8	0	37.8	+0.1^		-0.1$^{\&}$	0	0	0.8	N	N	0.1	0.1 (0)	0	0
25	WU	1,54	3.42	7	37.6	37.7	NA	NA	128/-	-	3	-	N/A	N/A	0	0	37.7	0.1	37.9	+0.1^	-	-0.1$^{\&}$	0	0	2.2	N	N	0.2	0.6 (0.1)	0.1	0.2
14	Tr	1,54	3.52	82	38.3	38.3^^	38.7	NA	142/142	-	4^^	65	N/A	0	-	-	38.3	0	38.4	+0.3^	38.3	0	0	0	9.5	N	N	0	6.4	0	0.8
15	Tr	1,54	3.51	14.5	38.4	38.6^^	39.0**	NA	152/72	-	12^^	7	X	-	-	-	38.6	0.9	38.9	+0.3^	39.0	+0.3	0	0	10	Y	Y	0.6	3.8	0.2	1.1
16	Tr	1,54	4.18	14	37.9	38.1^^	38.9*	NA	169/75	-	5^^	12	X	X	X	X	38.1	0.2	38.7	+0.9^	38.7	+0.6	0	0	X	N	N	0	5.0	0	0.4
17	Tr	1,54	4.14	15	38.4	38.5^^	38.9*	NA	139/67	-	4^^	6.5	X	X	X	X	38.5	0.1	38.8	+0.5^	38.9	+0.4	0	0	X	N	N	0.2	0.2	0.1	0.1
18	Tr	1,54	3.39	18	37.5	37.5^^	37.6	NA	NA/ 58	-	0^^	17	NA	NA	NA	NA	37.5	0	37.5	+0.1^	NA	NA	NA	0	5	N	N	-0.1	0.1	0	0
19	Tr	1,54	2.34	32	38.5	38.5^^	39.3**	-	84/73	-	2^^	22	X	X	X	X	38.5	0	39.3**	+0.4^	39.1	+0.6	+0.1	0	X	N	N	0	18.7	0	0.6
20	Tr	1,54	2.06	33.5	38.0	38.0^^	38.7*	NA	161/-		2^^	24	X	X	X	X	38.0	0	38.6	+0.7^	38.4	+0.4	0	0	27	N	N	1.2	16.1	0	0.5
21	Tr	1,54	2.06	83.5	38.1	38.2^^	39.1**	NA	80/-	-	2^^	72.5	X	X	X	X	38.2	0.1	39.1**	+0.9^	38.5	+0.3	0	0	X	N	N	0.5	44.1	0.2	0.5
22	Tr	1,54	2.07	15	38.0	38.1^^	38.7*	NA	149/-	-	3^^	14.5	X	X	X	X	38.1	0.1	38.7	+0.6^	38.5	+0.5	+0.1	+0.1	51	N	N	0.1	4.3	0	0.3
23	Tr	1,54	1.54	82	38.0	38.0^^	39.3**	NA	204/-=	-	2^^	79	X	X	X	X	38.0	0	39.3**	+1.3^	38.3	+0.2	0	0	X	N	N	0	66.3	0	0.8
24	Tr	1,54	1.57	77	37.8	37.9^^	39.1**	NA	158/-	-	3^^	71	X	X	X	X	37.9	0.1	39.1**	+1.2^	38.2	+0.4	0	0	X	N	N	0	48.8	0	0.6
25	Tr	1,54	2.00	26	37.9	37.9^^	38.4	NA	154/-	-	2^^	20.5	NA	NA	X	X	37.9	0	38.3	+0.5^	38.2	+0.4	0	0	X	N	N	0	9.3	0	0.4

Supplementary Table S1. Table presenting T$_c$ parameters in endurance (End) exercise (per 40 km) (H1-13) and trotters in warm-up (WU) and trotter (Tr) exercise (H14-25) and their recovery (rec.). T$_c$, gastrointestinal temperature; HR, heartrate; min, minutes; max, maximum; *italic* indicates extra 20 km exercise (n = 2 horses); delta (°C change), T$_c$ change during exercise and recovery periods including first 10 min recovery period; Y/N, yes or no; NA, Not applicable; $^{\&}$ indicates recovery 40 min (H8 2nd 40 km) instead of 60 min in endurance horses (H1-8); – indicates no data collected/ not available; X indicates not reached T$_c$ or HR; * indicates 38.5°C or higher; ** indicates 39°C or higher; ^ indicates increase of T$_c$ during recovery; ^^ indicates max T$_c$ during recovery; delta T$_c$, T$_c$ change over time; *** indicates unstable (increase or decrease) over time; net AUC, area under the curve expressed as (°C x min); $^{\&\&}$ indicates short recovery of varying duration (3.5 – 7 min).

Horse number	Type exercise	Distance (km)	Max speed (km h^{-1})	Mean speed (km h^{-1})	Mean HR (bpm)	Time to HR < 60 bpm (min)	Duration exercise (min)	Total H (kcal) exercise #	Total H (kJ) exercise #	Mean H (kcal) exercise min^{-1} #	Mean H (KJ) exercise min^{-1}#	Mean VO$_2$ (L O$_2$ min^{-1}) exercise #	Mean COT (ml O$_2$ kg^{-1} m^{-1}), exercise #
1	End	1st 40	26.1	13.8 ± 3.2	113 ± 31	14	165	12000	52000	110 ± 50 (10 -240)	450 ± 230 (30 – 1010)	19.8 ± 6.7 (1.4 – 30.5)	0.15 ± 0.09 (0.05 – 0.53)
1	End	2nd 40	26.1	15.3 ± 2.9	103 ± 15	22	182	22000	94000	110 ± 40 (20 – 240)	460 ± 170 (70 – 1000)	20.5 ± 6.3 (3.2 - 38.1)	0.13 ± 0.05 (0.05 – 0.37)
2	End	1st 40	22	12.9 ± 3.2	116 ± 21	NA	236	21000	87000	70 ± 40 (10 – 200)	300 ± 150 (30 – 850)	14.2 ± 6.8 (1.3 – 31.1)	0.15 ± 0.09 (0.05 – 0.53)
2	End	2nd 40	23.5	10.5 ± 4.3	110 ± 19	16	242	15000	63000	70 ± 40 (90 – 220)	290 ± 170 (90 – 930)	13.7 ± 7.5 (0.1 – 29.9)	0.13 ± 0.05 (0.05 – 0.38)
3	End	1st 40	28	12.8 ± 4.2	111 ± 24	4	207	14000	57000	60 ± 40 (0 – 220)	240 ± 170 (2 - 910)	11.2 ± 10.0 (0.1 – 30.0)	0.15 ± 0.22 (0.05 – 0.95)
3	End	2nd 40	30	13.6 ± 5.6	98 ± 23	5	247	19000	80000	70 ± 40 (0 – 210)	300 ± 180 (2 - 871)	13.7 ± 7.1 (0.1 – 29.9)	0.14 ± 0.08 (0.05 - 0.93)
4	End	1st 40	21	13.6 ± 3.3	110 ± 21	3	207	18000	740000	60 ± 30 (10 – 120)	260 ± 120 (40 – 500)	15.6 ± 6.8 (2.3 – 29.9)	0.14 ± 0.08 (0.05 – 0.89)
4	End	2nd 40	22	12.6 ± 4.4	114 ± 36	4	247	21000	88000	60 ±30 (0 – 120)	240 ± 120 (0 – 500)	13.5 ± 7.0 (0.1 – 29.6)	0.14 ± 0.07 (0.05 – 0.81)
4	End	20*		10.4 ± 4.4	106 ± 27	11	144	10000	40000	20 ± 120 (0 – 70)	70 ± 04 (0 – 270)	12.1 ± 6.9 (0.2 – 29.9)	0.13 ± 0.08 (0.05 – 0.94)
5	End	1st 40	24	16.6 ± 2.7	108 ± 21	0	186	18000	74000	80 ± 40 (20 – 340)	340 ± 170 (80 – 140)	15.4 ± 6.6 (3.9 – 30)	0.13 ± 0.07 (0.047 – 0.687)
5	End	2nd 40	27	15.3 ± 5.2	110 ± 26	2	188	17000	71000	70 ± 50 (0 – 270)	310 ± 190 (0 – 1120)	13.7 ± 7.1 (0.1 – 29.8)	0.131 ± 0.069 (0.05 – 0.70)
6	End	1st 40	24	16.3 ± 3.2	91 ± 21	5	179	14000	60000	90 ± 50 (20 – 230)	390 ± 190 (100 – 980)	17.3 ± 6.8 (4.7 – 30.0)	0.11 ± 0.07 (0.05 - 0.69)
6	End	2nd 40	25	17.4 ± 3.5	89 ± 22	2	149	13000	55000	80 ± 60 (0 – 270)	350 ± 240 (0 – 1140)	14.5 ± 7.7 (0.1 – 29.3)	0.13 ± 0.07 (0.05 – 0.70)
6	End	20*	23	15.8 ± 2.4	99 ± 21	0	92	8000	33000	30 ± 40 (0 – 210)	110 ± 150 02 – 900)	5.3 ± 6.9 (0.1 – 29.5)	0.13 ± 0.04 (0.04 – 0.46)
7	End	1st 40	-	-	123 ± 24	5.5	148	-		-	-	-	-
7	End	2nd 40	-	-	122 ± 9	24	83	-		-	-	-	-
8	End	1st 40	21	14.3 ± 4.8	123 ± 30	11	209	15000	64000	70 ± 30 (0 – 180)	300 ± 1230 (10 – 760)	14.0 ± 5.5 (0.4 – 29.9)	0.13 ± 0.07 (0.05 – 0.91)
8	End	2nd 40	23.5	16.2 ± 3.2	130 ± 29	2	187	18000	74000	80 ± 30 (20 – 210)	340 ± 130 (70 – 870)	15.8 ± 5.1 (3.1 – 29.9)	0.13 ± 0.05 (0.05 – 0.90)
9	End	1st 40	21.1	14.4 ± 2.1	107 ± 17	2	219	19000	80000	80 ± 29 (10 – 240)	360 ± 120 (50 – 990)	16.5 ± 4.7 (2.2 – 297)	0.13 ± 0.05 (0.05 – 0.91)
9	End	2nd 40	22.4	13.6 ± 3.8	109 ± 18	5	224	20000	84000	80 ± 40 (0 – 220)	340 ± 150 (10 – 930)	15.6 ± 6.1 (0.5 – 44.3)	0.14 ± 0.08 (0.05 – 0.90)
10	End	1st 40	21.1	13.3 ± 3.6	106 ± 24	2	215	14000	60000	70 ± 30 (10 – 180)	280 ± 110 (30 – 750)	13.2 ± 4.9 (0.1 – 0.1)	0.12 ± 0.04 (0.05 – 0.81)
10	End	2nd 40	23.8	11.4 ± 5.8	107 ± 27	1.5	215	13000	53000	60 ± 330 (0 -160)	260 ± 140 (10 – 660)	12.2 ± 6.5 (0.3 – 28.7)	0.13 ± 0.04 (0.04 – 0.54)
11	End	1st 40	19.2	12.2 ± 4.3	123 ± 46	0	218	15000	64000	60 ± 30 (0 – 210)	260 ± 130 (0 – 880)	12.2 ± 5.5 (0.1 – 30.0)	0.13 ± 0.05 (0.05 – 0.41)
12	End	2nd 40	20	14.3 ± 3.7	124 ± 26	5	185	17000	71000	80 ± 30 (0 – 220)	330 ± 140 (10 – 9220)	15.3 ± 0.5 (0.5 – 29.8)	0.13 ± 0.05 (0.05 - 0.59)
12	End	40	21	14.9 ± 2.1	139 ± 34	6	209	20000	85000	90 ± 30 (10 – 230)	380 ± 130 (20 – 950)	17.5 ± 4.5 (1.1 – 29.9)	0.13 ± 0.05 (0.05 – 0.64)
13	End	1st 40	-	-	143 ± 32	2	184	-		-	-	-	-
13	End	2nd 40	22	15.0 ± 4.2	112 ± 28	5	206	19000**	78000	80 ± 30 (0 – 30)	320 ± 100 (10 – 820)	15.2 ± 4.4 (0.5 – 29.8)	0.13 ± 0.06 (0.05 – 0.90)
14	WU	1540	36.9	30.4 ± 6.3	163 ± 36	3	4.1						
15	WU	1540	39.8	30.9 ± 5.6	165 ± 32	3	4.1						
16	WU	1540	42.3	31.9 ± 7.0	116 ± 20	3	4.1						
17	WU	1540	40.0	32.1 ± 6.2	116 ± 21	3	4.1						
18	WU	1540	38.4	37.1± 6.7	153 ± 22	3	4.2						
19	WU	1540	-	-	119 ± 25	6 (38.4)	3.5						
20	WU	1540	25	22.2 ± 2.9	126 ± 13	48 s (37.8)	3.5						
21	WU	1540	25	23.4 ± 2.1	-	-	3.5						
22	WU	1540	26.3	23.5 ± 2.9	145 ± 12	1 (37.8)	3.4						
23	WU	1540	28.1	23.3 ± 2.9	177 ± 24	2.5 (38.0)	3.4						
24	WU	1540	25.2	22.5 ± 2.5	104 ±77	1	3.5						



25	WU	1540	24.7	22.9 ± 2.4	140 ± 10	2.5	3.4
14	Tr	1540	51.3	35.5 ± 7.9	167 ± 25	9.5 (38.8)	3.5
15	Tr	1540	42	35.3 ± 6.7	168 ± 23	X (126min:HR126; 38.9)	3.5
16	Tr	1540	52.5	41.4 ± 9.5	130 ± 26	X (14min HR70)	4.2
17	Tr	1540	52.3	42.2 ± 8.5	131 ± 25	X (15min: HR65; 38.8)	4.1
18	Tr	1540	47.1	37.1 ±6.7	153 ± 22	4 (37.6)	3.4
19	Tr	1540	-	-	161 ± 55	-	2.3
20	Tr	1540	49.1	39.2 ± 9.2	144 ± 33	27 [38.6]	2.1
21	Tr	1540	54	41.5 ± 8.6	-	-	2.1
22	Tr	1540	47.9	39.8 ± 8.9	137 ± 25	51 [38.7]	2.1
23	Tr	1540	52.7	43.8 ± 8.8	169 ± 28	X (12min: HR115; 38.8)	1.5
24	Tr	1540	51.8	42.4 ±9.8	129 ± 17	X (12min: HR101; 38.2)	1.6
25	Tr	1540	51.8	41.5 ± 8.9	129 ± 21	X (8.5min: HR94; 38.2)	2.0

Supplementary Table S2. Table presenting Global positioning system (GPS), heartrate (HR) values and metabolic heat production (H, calculated in H1 – 13 only) in: endurance horses (H1-13) (End) per 40 km, and trotters (H14-25) during warm-up (WU) and trotter (Tr) exercise and recovery. Data are presented as mean ± SD. Min, minutes; km, kilometers; H, metabolic heat production expressed in kcal of kJ; VO_2 L^{-1}, oxygen per liters; COT, cost of transport, oxygen (ml) per kg bodyweight per meter (m) expressed in ml O_2 kg^{-1} m^{-1} exercise; – indicates no data collected/ not available/ not recorded; * indicates extra 20 km in italic; ** indicates speed only recorded during second 40 km; # indicates calculated values; X indicates HR 60 bpm was not reached at the time (min) of last recording.

	Rest endurance exercise*	Post 40 km (n=13*)	Post 80 km (n=11*)	Post 100 km (n=2*)	Rest warm-up exercise (n=12)	Post warm-up exercise (n=12)	Post trotter exercise (n=12)
Lactate (mmol L^{-1}, n=13*)	1.02 ± 0.32	1.24 ± 0.6 (0.22)	1.46 ± 0.35 (0.22)	1.27 ± 0.86 (-0.19)	0.96 ± 0.5	1.59 ± 1.03 (0.32)	9.43 ± 4.02 (7.98)
PCV (%, n=13*)	42.45 ± 11.26	46.62 ± 4.84 (4.17)	48.64 ± 5.7 (2.02)	48.50 ± 0.71(-0.14)	41.5 ± 5.3	47.5 ± 2.45 (3.33)	53.08 ± 2.47 (5.17)
pH (n=9*)	7.38 ± 0.02	7.42 ± 0.03 (0.04)	7.43 ± 0.03 (0.01)	7.41 ± 0.03 (-0.02)	7.27 ± 0.12	7.28 ± 0.14 (0.02)	7.24 ± 0.17 (-0.06)
TP/TS (g dL^{-1}, n=13*)	5.99 ± 0.75	6.39 ± 0.95 (0.4)	7.03 ± 0.84 (0.64)	5.75 ± 0.21 (-1.28)			
PCO_2 (mmHg, n=9*)	49.44 ± 3.77	48.18 ± 3.07 (-1.03)	46.51 ± 4.31 (-1.6)	50.00 ± 2.69 (3.5)			
PO_2 (mmHg, n=9*)	31.99 ± 6.24	31.09 ± 3.77 (-0.9)	32.64 ± 6.68 (1.55)	27.30 ± 6.22 (-5.34)			
HCO_3^- (mmol L^{-1}, n=9*)	29.44 ± 2.27	31.59 ± 1.89 (2.1)	30.41 ± 2.89 (-1.18)	32.35 ± 0.35 (1.94)			
BE (mmol L^{-1}, n=9)	4.36 ± 2.41	7.19 ± 2.21 (2.8)	6.10 ± 3.06 (-1.09)	7.90 ± 0 (2.8)			
SO_2 (%, n-9*)	62.57 ± 18.13	61.11 ± 13.74 (-1.40)	62.24 ± 13.66 (1.1)	50.30 ± 13.72 (-11.9)			
Na^+ (mmol L^{-1}, n=13*)	136.55 ± 2.25	138.31 ± 2.66 (1.76)	135.64 ± 3.23 (-2.67)	135.50 ± 2.12 (-0.14)			
K^+ (mmol L^{-1}, n=13*)	3.71 ± 0.29	3.55 ± 0.3 (-1.6)	3.45 ± 0.43 (-0.1)	3.30 ± 0 (-0.15)			
Ca^{++} (mmol L^{-1}, n=9*)	1.25 ± 0.11	1.21 ± 0.13 (-0.04)	1.20 ± 0.14 (-0.01)	1.18 ± 0.2 (-0.02)			
Ca Tot (mmol L^{-1}, n=4*)	3.2 ± 0.21	3.16 ± 0.16 (-0.04)	3.07 ± 0.09 (-0.08)				
TCO_2, (n=13*)	30.34 ± 1.75	31.96 ± 2.42 (1.7)	29.49 ± 4.82 (-2.47)	33.90 ± 0.42 (4.41)			
Glucose (mmol L^{-1}, n=13*)	6.33 ± 1.7	5.86 ± 0.81(-0.47)	4.58 ± 1.01 (-1.28)	3.75 ± 1.63 (-0.83)			
CK (IU L^{-1} (n=4*)	367 ± 13	563 ± 416 (196)	2586 ± 3395 (2023)				
AST (IU L^{-1} (n=4*)	549 ± 271	444 ± 247 (-105)	580 ± 377 (136)				
BUN (mg dL^{-1}, n=4*)	19.0 ± 0	18.8 ± 0.5 (-0.02)	21.7 ± 1.2 (2.9)				
Creat (mg dL^{-1}, n=4*)	0.75 ± 0.07	1.08 ± 0.15 (0.33)	1.23 ± 0.25 (1.5)				
Tbil (mg dL^{-1}, n=4)	1.85 ± 0.07	1.98 ± 0.48 (0.08)	2.43 ± 0.55 (0.45)				
Alb (g dL^{-1}, n=4*)	3.5 ± 0	3.4 ± 0.3 (-0.1)	3.4 ± 0.4 (0)				
Glob (g dL^{-1}, n=4*)	3.2 ± 0.7	4.4 ± 1.5 (1.2)	3.3 ± 0.1 (-1.1)				
WBC (10^9 L^{-1}, n=4*)	4.7 ± 1.7	5.9 ± 1.3 (1.2)	6.5 ± 2.4 (0.6)				
Lymph (10^9 L^{-1}, n=4*)	0.8 ± 0.2	0.6 ± 0.3 (-0.2)	0.6 ± 0.1 (0)				
Neutr (10^9 L^{-1} (n=4*)	2.1 ± 0.2	5.2 ± 2.3 (3.1)	5.8 ± 2.4 (0.60)				

Supplementary Table S3. Table presenting blood parameters of endurance horses and trotters (only lactate, PCV, pH) pre- and post-ride. Data are presented as mean ± SD (delta, difference with previous value); n=, indicates number of samples; * indicates number of sampled endurance horses; PCV, packed cell volume (Hct); TS, total solids; TP, total protein; PCO_2, partial pressure of carbon dioxide; PO_2, partial pressure of oxygen; HCO_3^-, bicarbonate; BE, base excess; SO_2, oxygen saturation; Ca Tot, total calcium; tCO_2, total CO_2; CK, creatinine kinase; AST, aspartate amino transferase; BUN, blood urea nitrogen; Creat, creatinine; Tbil, total bilirubin; Alb, albumin; Glob, globulin; WBC, white blood cell count in horses 7,8,12,13; Lymp, lymphocyte count; Neutr, neutrophil count.

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Chapter 5

Is continuous monitoring of skin surface temperature a reliable proxy to assess the thermoregulatory response in endurance horses during field exercise?

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5.1 Abstract

Hyperthermia is a performance and welfare issue for exercising horses. The thermoregulatory stressors associated with exercise have typically been estimated by responses in the laboratory. However, monitoring surface skin temperature (T_{sk}) coincident with core temperature (T_c) has not previously been investigated in horses exercising in the field. We investigated the suitability of monitoring surface T_{sk}, as a metric of the thermoregulatory response, and simultaneously investigated its relationship with T_c using gastrointestinal (GI) temperature. We evaluated T_{sk} in 13 endurance horses competing during four endurance rides over 40 km (n=1) or a total of 80 km (n=12) distance. Following each 40 km loop, horses were rested for 60 minutes. T_{sk} and T_c were continuously recorded every 15 seconds by an infrared thermistor sensor located in a modified belt and by telemetric GI pill, respectively, and expressed as mean ± SD. Net area under the curve (AUC) was calculated to estimate the thermoregulatory response to thermal load of T_{sk} over time (°C x minutes) using the trapezoidal method. The relationship between T_{sk} and T_c was assessed using scatterplots, paired t-test or generalized linear model ANOVA (delta T_{sk}) (n = 8). Ambient temperature ranged from 6.7 - 18.4°C. No relationship was found between T_{sk} and T_c profiles during exercise and recovery periods, and no significant difference between delta T_{sk} results was detected when comparing exercise and rest. However, time to maximum T_{sk} (67 minutes) was significantly reduced compared to T_c (139 minutes) ($p = 0.0004$) with a significantly lesser maximum T_{sk} (30.3°C) than T_c (39.0°C) ($p = 0.0002$) during exercise. Net AUC T_{sk} was 1164 ± 1448 and -305 ± 388°C x minutes during periods of exercise and recovery, respectively. The current research demonstrated that T_{sk} monitoring does not provide a reliable proxy for the thermoregulatory response and horse welfare, most probably because many factors can modulate T_{sk} without directly affecting T_c. Those factors such as weather conditions, applicable to all field studies, can influence the results of T_{sk} in endurance horses. The study also reveals important inter-individual differences in T_{sk} and T_c time profiles, emphasizing the importance of an individualized model of temperature monitoring.

5.2 Introduction

In the face of climate change, hyperthermia and heat stress have become increasingly challenging issues for a wide array of equine sports disciplines, especially during field competitions (Raymond et al., 2020; Verdegaal et al., 2021). An increase in core body temperature (T_c) leading to hyperthermia may cause a widespread cytotoxicity as a direct effect of heat, while indirect effects related to decreased cardiac output cause neural and intestinal ischemia. If unchecked, these systemic inflammation processes eventually lead to exertional heat illness (EHI) (Casa et al., 2015; Brownlow et al. 2016, Brownlow and Mizzi, 2021). The clinical manifestations of EHI include neurological signs varying from irritability, depression, ataxia, collapse, and may further progress to exertional heat stroke (EHS) with multi-organ dysfunction and death (Casa et al., 2015; Brownlow et al. 2016; Brownlow and Mizzi, 2021). In human athletes, EHS is among the top three causes of sudden death, and in summer, it is the number one cause of athlete death in the USA (Casa et al., 2015). Similarly, both EHI and EHS are problematic conditions in equine athletes (Geor and McCutcheon, 1998). The prevalence of metabolic disorders in, for example, endurance horses, triggered by thermoregulatory induced physiological feedback failure and exhaustion, ranges from 4.2 - 15% (Barnes et al., 2010; Nagy et al., 2010; Fielding et al., 2011; Nagy et al., 2014; Younes et al., 2015; Fielding et al., 2017; Bennet and Parkin, 2018; Legg et al., 2019). Recently, the prevalence of EHI in racehorses has been reported: two studies from Japan state a prevalence of 0.09% during summer with a clear increase over the past few years (Nomura et al., 2019; Takahashi and Takahashi, 2020). Moreover, a study in Eastern Australia focused on selected EHI cases post-exercise at the racetrack and suggested an EHI incidence of up to 9.5% during hot summer months (Brownlow and Brotherhood, 2021). The latter study used the four severity levels of EHI reported by Brownlow et al. (2016) and concluded that 96% of horses could be categorized as level 1. This suggests that low level and thus discrete EHI cases may have been overlooked in the past.

Environmental conditions are the dominant risk factor for heat stress events and EHI cases are expected to further increase in prevalence due to global warming (Brownlow et al. 2016; Nomura et al., 2019; Raymond et al., 2020; Takahashi and Takahashi, 2020; Brownlow and Brotherhood, 2021). That worrying reality drives the ongoing efforts of research groups worldwide to develop reliable approaches to monitor and safeguard thermoregulatory wellbeing in horses (Smith et al., 2006; Verdegaal et al., 2017; Klous et al., 2020; Brownlow and Smith, 2021; Verdegaal et al., 2021). The researchers have previously reported on the continuous monitoring of the thermoregulatory response in endurance and trotter horses using a telemetric gastrointestinal (GI) pill (Verdegaal et al., 2021). Briefly, the GI temperature pill is a non-invasive method to monitor T_c during exercise in the field. The GI pill was administered the night before the endurance competition to allow recording of a large temperature data set to establish T_c profiles of exercising horses. The data were telemetrically transferred to a device located in a belt under the saddle girth and supported continuous real-time monitoring. It was demonstrated that the GI telemetry pill is a reliable method to monitor T_c and assess the individual dynamic thermal response in exercising horses in the field. The study revealed important inter-individual differences in T_c time profiles, despite the horses performing the same exercise protocol. This finding emphasizes the importance of an individualized model of temperature monitoring. It can be concluded that the continuous monitoring approach allows for intervention at early stages of heat accumulation and the possibility to take prompt and effective preventative measures.

Another elegant approach to monitoring the thermoregulatory response in exercising horses in the field could be continuous monitoring of surface T_{sk} as a reliable proxy for monitoring thermoregulatory wellbeing. Advantages of using tools such as infrared thermography (IRT) to assess T_{sk} include the non-invasive nature and easy collection of temperature data (Soroko and Howell, 2018; Mota-Rojas et al. 2021b; Rojas-Valverde et al., 2021). Notably, most exercise studies use such monitoring methods to assess T_{sk} only pre- or post-exercise, not during exercise. To illustrate, a recent systematic review of T_{sk} studies performed in human endurance athletes reported that only a few studies involved the continuous monitoring of T_{sk}; only two out of the 45 exercise studies monitored T_{sk} every 30 seconds, one study every 180 seconds, and four studies every 5 minutes (Rojas-Valverde et al., 2021). Also, these human studies highlight the existence of essential inter-individual differences with respect to the T_{sk} response time profile (Rojas-Valverde et al., 2021). Important inter-individual T_{sk}

differences have been reported in all mammals (Mota-Rojas et al., 2021a; Mota-Rojas et al., 2021b). For example, an equine study comparing T_{sk} in 21 horses at rest revealed significant inter-individual differences (Meisfjord Jorgensen et al., 2020). Most existing equine thermoregulatory exercise studies (n = 12 studies) involving T_{sk} have focused on single point post-exercise measurements using a handheld IRT camera, leaving both the pre-exercise and intra-exercise periods out of their scope (Morgan et al., 2002; Simon et al., 2006; Jodkowska et al., 2011; Wallsten et al., 2012; Yarnell et al., 2014; Soroko and Howell, 2018; Redaelli et al., 2019; Soroko et al., 2019a; Soroko et al., 2019b; Takahashi et al., 2020; Wilk et al., 2020; Brownlow and Smith, 2021). An overview of all T_{sk} studies in horses at rest and during exercise is presented in **Supplementary Table 1**.

To date, only a few equine studies have focused on continuous monitoring of T_{sk} during exercise and recovery (n = 7, **Supplementary Table 1**). All were conducted in laboratory conditions, for example on a treadmill (Geor et al., 1995; Marlin et al., 1996; Marlin et al., 1998; Marlin et al., 1999b; Geor et al., 2000; Soroko et al., 2018). Only one study has recorded T_{sk} continuously during a short bout of field exercise of 4.5 minutes duration to investigate the effect of pre-exercise cooling in 10 horses (Klous et al., 2020). The study recorded surface T_{sk} using a microchip (i-Button®) attached to the skin with removable glue and simultaneously monitored rectal temperature (T_{re}) using a rectal probe, both T_{sk} and T_{re} reduced over time (3°C and 0.3°C respectively).

From a physiological standpoint, it is essential to appreciate that a time lag exists between exercise-induced metabolic heat (MH) output and T_c evolvement. The T_c is subsequently translated into an additional temperature time-lag evolvement, expressed at several different anatomical locations such as the rectum, the muscular compartment and the skin surface whether or not additionally complicated by environmental factors such as hot and humid weather (Kohn and Hinchcliff, 1995; Marlin et al., 1996; Jeffcott and Kohn, 1999; Kohn et al., 1999; Jeffcott et al., 2009; Wartzek et al., 2011; Hodgson, 2014; Raymond et al., 2020; Mota-Rojas et al., 2021a). Most importantly, T_{re} evolvement has been reported to significantly lag behind the T_c both during and after exercise (Hodgson et al., 1993; Geor et al., 1995; Verdegaal et al., 2017), which renders the T_{re} less suitable as a “whistle blower” for thermoregulatory instability. In the researchers’ previous studies, the current study demonstrated that GI temperature is a more reliable proxy for the thermoregulatory response and T_c when compared to T_{re}, and that continuously monitoring GI temperature evolvement demonstrated how the equine body copes with exercise challenging the thermoregulatory system (Verdegaal et al., 2017; Verdegaal et al., 2021). Endurance horses, for example, reached their mean maximum T_c (39.0 ± 0.4°C) during exercise at 75% of completion of exercise, and T_c returned to baseline within 60 minutes into recovery (Verdegaal et al., 2021). However, the mean T_c was still 38.8 ± 0.4°C at a heart rate (HR) of 60 bpm, which currently governs “fit to continue” competition decisions (A.E.R.A., 2020), thus questioning the use of HR values to make such important decisions. However, contrary to this finding, trotter horses reached a comparable mean maximum T_c (38.8 ± 0.5°C) during recovery. Moreover, in 30% of trotters, T_c was still > 39°C at the end-of-recovery period (40 ± 32 minutes) following exercise in a cool environment, findings that may have post-exercise management implications.

In order to identify a reliable proxy for thermoregulatory response in the field, a solid correlation must exist between that specific proxy and T_c evolvement, despite the existence of a time-lag (Wartzek et al., 2011; Rojas-Valverde et al., 2021). However, currently, very few equine studies have involved the simultaneous continuous monitoring of T_c (either using carotid artery temperature, or an GI pill, or the T_{re}) together with an additional temperature monitoring device during field exercise (Mitchell et al., 2006; Verdegaal et al., 2017; Klous et al., 2020). On the other hand, with the ongoing development of new wearables and sensors, there is an increasing number of exercise studies investigating continuous T_{sk} monitoring wearables (Geor et al., 1995; Marlin et al., 1996; Marlin et al., 1998; Marlin et al., 1999a; Geor et al., 2000; Soroko et al., 2018; Klous et al., 2020) (**Supplementary materials Table 1**). These devices all provide data output although the physiological meaning of these data is not always clear.

The MH produced during exercise needs to be dissipated from the horse to the surrounding environment through four main pathways**,** namely radiation, conduction, convection and evaporation, the last being the most essential and pivotal pathway in horses (Hodgson et al., 1994; McCutcheon and Geor, 2008; Hodgson, 2014; Bertoni et al., 2020; Mota-Rojas et al., 2021b; Brownlow and Mizzi, 2022). Evaporation from the body surface is mainly achieved by increased blood flow, cutaneous vasodilation followed by evaporation of sweat from the skin (70- 85% of the MH load) (Jones and Carlson, 1995; McCutcheon et al., 1995; McCutcheon and Geor, 2008; Hodgson, 2014; Mota-Rojas et al. 2021a; Mota-Rojas et al. 2021c; Brownlow and Mizzi, 2022). Heat loss by evaporation can be enhanced by cooling techniques (Kohn and Hinchcliff, 1995; Marlin et al., 1998; Takahashi et al., 2020). When focusing on T_{sk} as a temperature monitoring method, it is vital to keep in mind that all these pathways to dissipate MH to the environment may influence the T_{sk} data output.

Monitoring T_{sk} simultaneously with T_c using the GI temperature pill during field exercise has not yet been investigated. The relationship between T_{sk} and T_c is not well understood due to physiological, endocrine, or vasomotor influences on both temperatures (Mota-Rojas et al., 2021a; Mota-Rojas et al., 2021c; Brownlow and Mizzi, 2022). Some studies have tried to correlate both T_c and T_{sk} (Marlin et al., 1996; Marlin et al., 1998; Marlin et al., 1999b; Geor et al., 2000). The current study aimed to evaluate the usefulness of continuous monitoring of T_{sk} by means of a surface IR sensor device as a proxy for the thermoregulatory response. For this purpose, the T_{sk} relationship with T_c was investigated by simultaneous and continuous telemetric measurements during real-time field competitions under cool weather conditions. Endurance horses were equipped with several non-invasive telemetric monitoring devices—a T_{sk} device positioned in a girth belt, an orally administered GI pill (T_c), a global positioning system (GPS) and a HR monitor.

5.3 Materials and methods

Horses

Thirteen mainly Arabian (n = 10) endurance horses participated in the study: 7 geldings, 6 mares, age 9.5 ± 2.8 years, body mass (BM) 479 ± 68 kg, body condition scores varied from 2-3 out of 5. Two cross-Arabians and one crossbred (Quarter Horse – Thoroughbred) were also involved (**Table 1**). Coat color included bay (n = 3), chestnut (n = 6) and grey (n = 4) and color was scored as follows: dark (bay and chestnut, n = 9) compared to light (grey) (**Table 1**). Relevant rider and horse performance history and the Bureau of Meteorology (B.O.M.) station output information were recorded (**Table 1** and **Supplementary materials S1**). All horses were deemed to be fit and healthy based on the veterinary inspection conducted before the competition and following each 40 km loop according to AERA riding rules (A.E.R.A., 2020). Horses were sourced on a voluntary basis through the South Australian Endurance Riders Association (S.A.E.R.A.) and all owners signed a written consent form. The study was approved by the University of Adelaide Animal Ethics Committee (project number S-2011-224).

Horse number	Sex	Age (y)	Breed	Body mass (kg)	Coat color	Distance (km)	GI Pill Y/N	GPS/HR Y/N	B.O.M. (°C) (T_a) min – max	Sweating score post-exercise, 1 to 3
1	G	11	Arab	669	Gr	80	Y	Y	13-26	2
2	G	9	Arab	484	B	80	Y	Y	13-26	1
3	M	13	Arab	426	C	80	Y	Y	6-19	2
4	M	7	QH x TB	470	C	80	Y	Y	6-19	1
5	M	11	Arab	450	C	80	Y	Y	6-19	1
6	M	8	Arab	370	Gr	80	Y	Y	6-19	2
7	M	9	Arab	450	C	80	Y	^	3-22	2
8	G	11	Arab	470	Gr	80	Y	Y	3-22	3
9	G	7	QH	490	C	80	-	Y	7-13	3
10	G	10	Arab x TB	484	C	80	-	Y	7-13	3
11	G	5	Arab	458	B	40	-	Y	7-13	1
12	G	7	Arab	525	B	80	-	Y	3-22	1
13	M	15	Arab	480	Gr	80	-	^^	3-22	2

Table 1. Study population characteristics and monitoring devices. Horse 1-13: 13 endurance horses: G (n = 7), M (n = 6). Arabian including part-Arabian horses, QH, Quarter horse; TB, Thoroughbred; x, crossbred; G, gelding; M, mare; Gr, grey; C, chestnut; B, bay; The riders' and horses' performance history includes: Age start, indicates age (years) when the horse started competing; Horse experience, indicates number of years active in competition (40 km or more); GI pill, gastrointestinal pill; GPS, global positioning system; HR, heartrate monitor (Polar); B.O.M; Bureau of Meteorology, local station closest to location of exercise at varying km distance from the actual event (in total 4 endurance locations, distance ranged from 5.3 to 53 km; -, no; Y, yes; ^indicates HR only; ^^indicates second 40 km only.

Study design

Horses competed over distances of 40 km (n = 1), 80 km (n = 10) or 100 km (n = 2), with each 40 km loop followed by a 60-minute recovery period. Endurance horses exercised at four different locations with altitudes ranging from 4 to 462 meters above sea level. Following each 40 km exercise loop, the sweating response was graded scoring from 1 – 3 (1: mild wet and white foam areas around head, neck, saddle and inside hindlimbs, 2: moderate dripping sweat from body, 3: extensive dripping sweat from body; **Table 1**). In addition, horses were immediately cooled down for an average duration of 10 minutes by pouring buckets of tap water (estimated average 20°C) over their bodies and subsequently scraping it off. Following each loop, a recovery period of 60 minutes was allowed during which inspection of the horses for 'fitness to continue' was performed, including checking for the presence of an HR below 60 beats per minute (bpm) by independent endurance veterinarians under the regulations of the Australian Endurance Riding Association (A.E.R.A., 2020). Horses were allowed to drink water and eat hay *ad libitum* during the 60 minutes rest period in a shaded area.

Simultaneous continuous monitoring of skin temperature (T_{sk}) (°C) and core GI temperature (T_c) (°C)

The T_{sk} (°C) was continuously recorded using an infrared (IR) sensor measuring 78 mm x 53 mm located in the Sensor Electronics Module (**Figure 1B**) (SEM, EQ02 Equivital data Logger®, Hidalgo, UK) with a 0 – 60°C temperature range, an emissivity of 1.0 and ± 0.3°C accuracy according to the manufacturer's specifications. The SEM device was located ventrally in a pocket of a modified Equivital Sensor Belt® fitted around the saddle girth (**Figure 1C**). The GI temperature (T_c) (°C) was continuously telemetrically recorded using the ingestible GI pill (n = 8) (**Figure 1A**) as previously described (T_c data are to be found in the **Supplementary materials S1)**. The T_{sk} and T_c data were recorded every 15 seconds and uploaded and processed in the Equivital Software Manager®.

Figure 1. T_{sk} and T_c monitoring equipment: Jonah® gastrointestinal temperature pill (**A**); an external receiver Equivital® Sensor Electronics Module (SEM) with infrared sensor to measure T_{sk} (**B**); modified belt for use on horses with the GPS Garmin® Watch and Polar® electrodes (identified by the blue pointer) with the red pointer indicating the SEM device including the T_{sk} thermistor position (**C**).

Monitoring of travelled distance, speed and heart rate (HR) over time

For each horse, the distance travelled and speed achieved were recorded telemetrically using GPS monitoring equipment (Garmin Forerunner 910XT GPS Watch®; Garmin Ltd., Schaffhausen, Switzerland) attached to the gullet of the saddle (**Figure 1C**). In addition, the HR was recorded by the Garmin Watch using Polar electrodes (Polar Electro®, Kempele, Finland) (**Figure 1C**) (Parker et al., 2009). The GPS and HR data were recorded every second and uploaded from the Garmin watch to the Garmin Connect and processed in the Garmin Training Centre[3].

Ambient environment

Horses exercised under varying degrees of solar radiation during the Australian winter months (June – August). On each data collection day, the ambient temperature (T_a, °C) and relative humidity (RH, %) were recorded continuously every 30 seconds in a shaded section of the rest area using a data logger device (Onset HOBO Pro V2 logger temp/RH U23-00®, Onset Computer Corporation, Bourne, Maine, USA). In addition, T_a data were obtained from the nearest B.O.M. weather station, presented in **Table 1**. The estimated wet bulb globe temperature (WBGT) was derived drawn from a WBGT chart (B.O.M).

Data processing

Recordings of each exercise period of 40 km and each recovery period following that exercise loop were processed. The net AUC (baseline set at rest T_{sk} and T_c) was

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[3] https://connect.garmin.com/

calculated using the trapezoidal method of T_{sk} (and T_c) over time expressed as °C x minutes. The net AUC was summated to present the cumulative T_{sk} – time distribution (Datta et al., 2021). The net AUC T_{sk} provided an estimate of the dynamic thermal response to the thermal load on the skin. This thermal load on the skin during exercise and the recovery included the T_c and T_a together with solar radiation.

Statistical analysis

All data are presented as mean ± SD (range). Comparison and correlation analyses were performed using IBM SPSS Statistics 26.0 software or GraphPad Prism version 9.3.0 for MacOS, GraphPad Software, San Diego, California USA[4]. Different approaches were taken to evaluate the potential of the T_{sk} data as a reliable proxy to assess the thermoregulatory response. The relationship between T_{sk} and T_c was assessed using scatterplots (8 horses each performing two subsequent 40 km loops). In addition, maximum T_{sk} and T_c and the time to reach maximum T_{sk} and T_c were compared using the paired t-test. Delta T_{sk} during exercise and recovery periods was compared. The association between T_{sk} and T_c at different points in time and the association with HR or coat color were analyzed using a general linear model ANOVA (when no significant effects of horse identity and treatment interaction were indicated and subsequently removed using backwards model selection). Statistical significance was set at $\alpha < 0.05$.

5.4 Results

All horses completed their exercise trials without any adverse occurrences. The Equivital belt became dislodged in horse 1 at the end of the first 40 km loop causing T_{sk} and T_c data loss. As a result, additional modifications were applied to the belt for the subsequent recordings by fitting sturdy straps sandwiched into the belt to stabilize the girth position (**Fig 1C**). During recovery after the first loop, T_{sk} was not recorded in horses 1 and 2 due to the owners' premature removal of the belt. The sweating response varied from 1 to 3 out of a score of 3 for all horses (**Table 1**). The T_c was

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[4] www.graphpad.com

recorded in 8 horses over 80 km (previously published, **Supplementary file S1**) (Verdegaal et al., 2021).

Horse	**Distance 40 km – 1st or 2nd**	**Net AUC T_{sk} (°C x min) exercise 40 km**	**Net AUC T_{sk} (°C x min) recovery 40 km**	**Base T_{sk} (°C) at start of exercise**	**Mean ± SD T_{sk} (°C) exercise**	**Min - max T_{sk} (°C) 40 km exercise**	**Delta T_{sk} (°C) exercise**	**T_{sk} (°C) at start of recovery (end exercise)**	**Delta T_{sk} (°C) first 10 min recovery**	**Delta T_{sk} (°C) recovery**	**Delta T_{sk} (°C) end exercise to end recovery min^{-1}**	**Mean ± SD T_{sk} (°C) recovery**	**Min –max T_{sk} (°C) recovery**	**Time to max T_{sk} exercise (min)**	**Time to min T_{sk} in recovery (min)**	**T_{sk} (°C) at end of recovery**	**T_{sk} (°C) at end of recovery**
1	1st	-743.7	-	28.5	20.7 ± 2.4	17.8 - 28.4	10.6	21.9	-	-	-	-	-	0	-	23.2	23.2
1	2nd	587.3	-0.78	23.2	26.4 ± 2.6	21.2 - 31	10.8	24	-0.3	3.7	0.06	23.7 ± 1.1	22.1 - 25.8	74	21	26.4	22.8
2	1st	-1328.6	-	26.4	20.9 ± 7.2	15.3 - 36.2	20.9	24.2	-1	6.6	-	23.3 ± 1.7	-	45	-	24.7	24.3
2	2nd	-515.3	54.9	24.3	22.6 ± 1.4	20.4 - 26.1	5.7	22.3	-1	5.1	0.09	23.3 ± 1.3	21 - 26.1	76	20	26	25.9
3	1st	838.1	-624.6	29.4	33.5 ± 0.8	29.4 - 34.2	4.8	32.5	-9.3	13.9	0.23	21.8 ± 3.5	18.8 - 32.7	39	29	22.1	22.5
3	2nd	2820.5	-597.3	22.5	33.9 ± 2.0	22.5 - 35.4	12.9	34.8	-6.4	11.5	0.19	26.4 ± 2.7	23.4 - 34.9	72	23	24.6	24.4
4	1st	1382.4	-85.5	27.7	21.0 ± 1.3	27.8 - 35.4	7.7	34.2	-12.3	18.4	0.31	21.8 ± 3.5	18.8 - 32.7	110	28	18.2	18.2
4	2nd	1422.9	169.2	18.2	24.0 ± 3.2	15.8 - 30.5	14.7	24.7	-0.6	7.5	0.13	22.1 ± 1.8	19.3 - 26.5	79	16	26.8	26.8
4	*Extra 20*	*(1051.1)*	*(-468.1)*	*26.9*	*34.4 ± 1.6*	*26.9 - 35.5*	*8.5*	*34.7*	*-8.6*	*17.2*	*0.29*	*22.8 ± 4.7*	*17.6 - 34.7*	-	*27*	*19.5*	*19.6*
5	1st	570.8	-635.5	27.6	30.7 ± 1.5	26.1 - 32.5	6.4	28.3	-10	15.4	0.26	16.7 ± 3.5	14.3 - 29.7	56	26	16.2	16.1
5	2nd	2965.8	-997.3	16.1	31.9 ± 3.8	15.8 - 34	18.2	33.5	-12.9	16.9	0.28	19.2 ± 3.3	16.6 - 33.4	79	16	18.2	18.2
6	1st	2736.5	-408.7	13.7	29.0 ± 4.0	12.4 - 32	19.6	29.7	-0.8	14.5	0.24	22.8 ± 5.3	15.3 - 29.5	74	12	19.3	19.5
6	2nd	995.4	251.0	19.5	26.2 ± 2.8	19 - 30.5	11.5	24.6	0.8	6.5	0.11	23.2 ± 2.2	20.1 - 26.6	55	19	20.1	20.1
6	*Extra 20*	*(809.8)*	*(-427.0)*	*20.1*	*29.0 ± 4.1*	*18.1 - 32.7*	*14.5*	*31*	*1.2*	*15.1*	*0.25*	*23.8 ± 5.9*	*17.2 - 32.3*	*36*	*46*	*20.1*	*20.1*
7	1st	960.6	-3	11	17.7 ± 6.3	10 - 32	22	13.3	-0.7	15	0.25	16.4 ± 3.9	12.1 - 27.1	26	10	18.9	19.2
7	2nd	80.4	97.5	19.2	20.4 ± 1.0	18.2 - 23.9	5.7	19.7	0.8	9.5	0.16	20.8 ± 1.2	15.8 - 25.3	64	18	20.2	20.2
8	1st	4170.6	-547.4	10	30.4 ± 4.1	10 - 34.5	24.5	25.9	1	11.4	0.19	19.3 ± 3.1	15.8 - 34.5	75	10	20.3	20.2
8	2nd	884.2	110.8	20.2	24.8 ± 2.1	19.2 - 29.4	10.2	26.7	-1	12.2	0.20	23.8 ± 3.1	15 - 27.2	150	19	18.3	18.8
9	1st	535.8	133.2	18.8	19.8 ± 1.7	15.6 - 23.5	7.8	23.4	-2.8	8.9	0.15	18.0 ± 2.6	14.6 - 23.4	179	16	22.6	22.6
9	2nd	-68.3	-172.4	22.6	22.3 ± 0.9	18.7 - 24.4	4.7	23.4	-3.2	10.4	0.17	17.6 ± 2.8	13 - 32.3	141	20	14.3	14.5
10	1st	934	-628.4	29.8	34.2 ± 1.1	29.9 - 36	6	34.7	0.3	17	0.28	23.9 ± 5.9	17.7 - 35	44	30	26.1	26.2
10	2nd	1825.3	-852.4	26.2	34.7 ± 1.5	26.2 - 36.1	10	34.8	-15.3	17	0.28	20.4 ± 2.3	17.5 - 32.3	63	26	21.2	20.9
11	1st	1691.6	-944.0	26.5	34.3 ± 1.4	26.6 - 35.5	9	35.4	-15.9	21	0.35	18.5 ± 5.7	14.5 - 35.4	179	27	17.7	17.7
12	1st	4748.6	-597.2	32.3	30.9 ± 7.6	9.5 - 37.5	29	35.3	-10.4	19	0.32	22.8 ± 3.5	17.9 - 35	152	26	28.4	28.7
12	2nd	1356.4	-524.7	28.7	37.4 ± 1.7	28.7 - 38.7	10	38.1	-19.4	22.2	0.37	19.6 ± 4.9	16.3 - 38.2	90	29	16.8	16.8
13	1st	-204.5	-169.3	29.5	24.2 ± 4.4	17.9 - 33.8	16	25.5	4.6	17	0.28	22.6 ± 4.6	15.5 - 32.8	84	18	26.8	26.4
13	2nd	453.9	-45.1	26.4	28.6 ± 2.5	21.6 - 35.2	14	27.9	2.6	11	0.18	27.1 ± 2.2	22.6 - 33.4	196	22	24.1	26.2

Table 2. T_{sk} (°C) parameters during 40 km exercise (or extra 20 km in 2 horses exercising over a total of 100 km) and recovery. Data are presented as mean ± SD. T_{sk}, skin temperature; AUC, area under the curve; min, minutes; min-max, minimum to maximum; *italic* indicates extra loop of 20 km exercise (total 100 km, n = 2 horses); delta (°C change), T_{sk} change during exercise and recovery periods including first 10-minutes recovery period; – indicates no data collected/ not available.

Environmental field conditions

The T_a and RH were successfully recorded between 5.00 am and 3.00 pm on all occasions. The T_a was relatively cool with a mean minimum of 6.7 ± 0.4°C and mean maximum of 18.4 ± 2.9°C (B.O.M.) **(Table 1)**. More specifically, the T_a on the four separate days of endurance exercise showed a minimum value of 13.4, 6.3, 2.8 and 6.6°C respectively, and a maximum value of 26.3, 19.0, 22.0 and 18.8°C respectively (HOBO data). The minimum RH ranged from 47.1 – 61.7% to a maximum of 84.8 – 100% value. Overall mean calculated values were 15.3°C (T_a) and 75.6% (RH) respectively, while the approximate WBGT was less than 20°C. In summary, all endurance horses competed in a cool environment.

Speed and heart rate (HR) data

All horses executed their endurance competition at a mean speed of 14.0 ± 1.4 km h^{-1} over the first 40 km (n = 11) and 14.2 ± 2.1 km h^{-1} over the second 40 km (n = 11) loop, with a mean HR of 114 ± 13 bpm. An overview of recorded speeds and HR data for individual horses can be found in the Supplementary file S1.

Individual T_{sk} and T_c recordings during endurance exercise over time

An overview of the simultaneously recorded individual T_{sk} (°C) and T_c (°C) time profiles is provided for all horses in **Figure 2**. All individual T_{sk} parameters, their respective descriptive analysis and specific T_{sk} points in time during the 40 km endurance loops are presented in **Table 2**.

Overall T_{sk} profiles and comparison to T_c

The overall T_{sk} profiles during endurance exercise and recovery and their associated parameters are presented in **Table 3** showing a mean time to maximum T_{sk} of 88 ± 51 minutes (n = 13). The mean maximum T_{sk} during exercise was 32.4 ± 4.3°C, and the mean minimum T_{sk} during recovery was 17.3 ± 3.1°C (n = 13). The mean overall response of T_{sk} was 1164 ± 1448°C x minutes for each 40 km exercise period. During recovery, the T_{sk} response was -305 ± 388°C x minutes (**Table 3**).

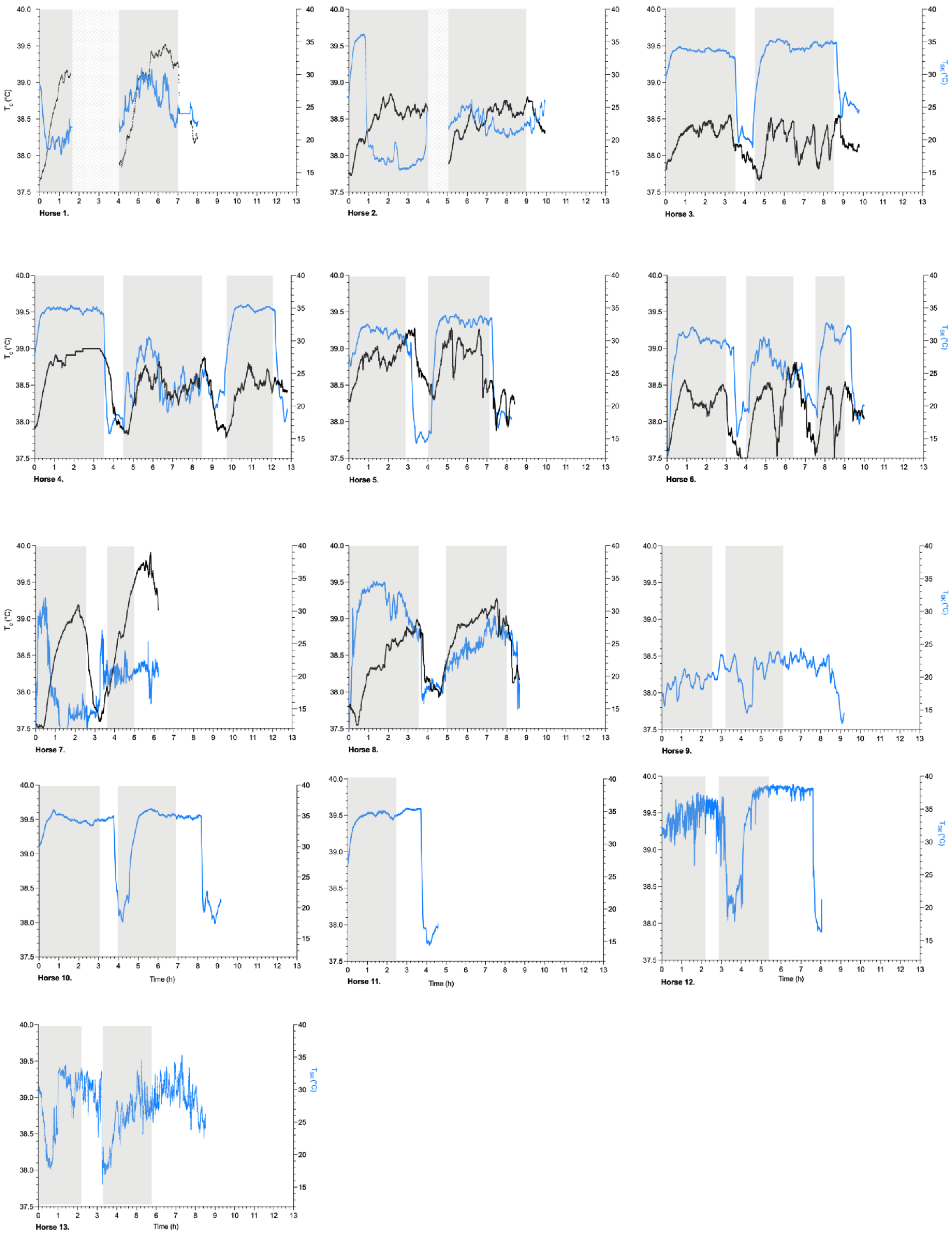

Figure 2. Scatterplots of T_{sk}, skin temperature (°C, identified as blue) and T_c, gastrointestinal temperature (°C, identified as black) (left y-axis) per subsequent exercise loop of 40 km (grey blocks) (h, hours, x-axis) in endurance horses Horse 1-13; dotted blocks identify blocks of no data recording; after each exercise loop, horses were cooled down by pouring buckets of water over their bodies, followed by scraping water off the body for a period of approximately 10 minutes.

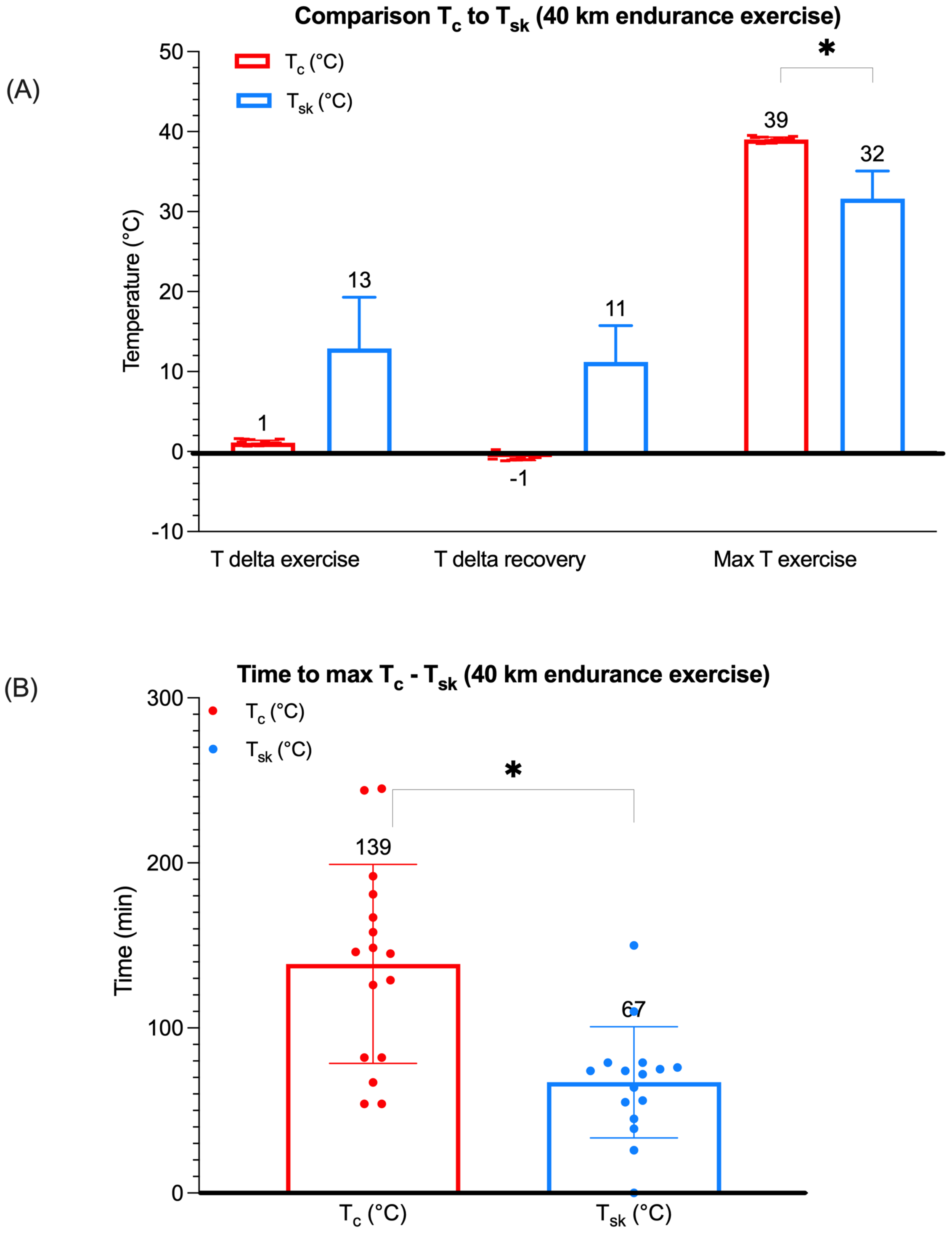

Figure 3. Boxplot diagram depicting T_c and T_{sk}, respectively in endurance horses. Mean (horizonal line) ± SD (error bars) of individual maximum T_c (°C, n = 8) and maximum T_{sk} (°C, n = 8) values. There was no significant association between T_{sk} and T_c; no significant difference between delta T_{sk} exercise and recovery ($p = 0.41$); a significantly greater maximum T_c when compared to maximum T_{sk} ($p = 0.0002$) (**A**); and a significantly increased time to maximum T_c than T_{sk} ($p = 0.0004$) **(B)**; *indicates a significant difference.

The T_{sk} and T_c profiles over time were compared in 8 horses and no relationship was found (**Figure 2**). Different T_{sk} and T_c points in time were compared to assess associations. Interestingly, the only significant correlation found was between the T_{sk} (°C) at end-of-exercise period and the T_c (°C) at the end-of-recovery period ($F_{1,14}=5.519$, $p = 0.034$). More precisely, a higher T_{sk} at the end-of-exercise period was associated with a lower T_c at the end-of-recovery period. The additional analyses revealed no significant correlations between T_{sk} (°C) and T_c (°C) including no correlation between T_{sk} at the start-of-exercise period (baseline T_{sk}) and the maximum T_c ($F_{1,14}=0.127$, $p = 0.727$). The study could not identify a significant effect of time to maximum T_{sk} (67 minutes) during exercise on the maximum T_c (39.0 °C) ($F_{1,14}=0.001$, $p = 0.978$, n = 8). On all occasions, peak T_c values (39°C) were significantly greater than peak T_{sk} values (32°C) ($p = 0.0002$) (**Figure 3A**). In addition, in all cases, there was a significantly shorter time to maximum T_{sk} (88 minutes) compared to the time to maximum T_c (139 minutes) ($p = 0.0004$) (**Figure 3B**).

Delta T_{sk} data were compared and when comparing exercise and recovery periods, no significant difference could be found between the two delta T_{sk} (°C) (mean 13°C and 11°C respectively) ($p = 0.41$) (**Figure 3A**). The delta T_{sk} was greater when compared to delta T_c on all occasions (**Figure 3A**). Also, delta T_{sk} and T_c points in time were compared to assess associations. A correlation between the delta T_{sk} during cooling in the first 10 minutes of the recovery period and the T_c at the end of the recovery period was not identified ($F_{1,13}=1.348$, $p = 0.266$).

Additionally, there were no significant effects of coat color on the maximum T_c ($F_{1,14}=0.010$, $p = 0.920$) nor on the maximum T_{sk} ($F_{1,22}=0.015$, $p = 0.904$). Furthermore, coat color was not associated either with delta T_{sk} during exercise (12.5 ± 6.6) ($F_{1,22}=1.098$, $p = 0.306$) or delta T_{sk} during recovery (13.0 ± 5.1) ($F_{1,21}=1.246$, $p = 0.277$).

Evaluation of HR in relation to T_{sk}

Additional analysis to evaluate the relationship between the T_{sk} and HR < 60 bpm revealed no significant correlation between the T_{sk} at the end of exercise and the duration to HR < 60 bpm ($F_{1,13}=4.019$, $p = 0.066$). In conclusion, the study did not identify an association between HR recovery and the recorded T_{sk} during endurance exercise and recovery.

Variables	Endurance 40 km n = 13 (n = 8*)
Duration (minutes) exercise	198 ± 63
Duration (minutes) recovery	60
T_{sk} (°C) overall	27.8 ± 5.6 (17.71 – 37.37)
Base T_{sk} (°C) (at start-of-exercise)	23.1 ± 6.1 (10 – 32.3)
Min T_{sk} (°C) exercise	19.8 ± 6.2 (9.5 – 29.9)
Max T_{sk} (°C) exercise	32.3 ± 4.3 (23.5 - 38.7); 31.6 ± 3.5°C*
T_{sk} (°C) exercise	27.2 ± 5.7 (17.7 – 37.4)
Time to max T_{sk} exercise (minutes)*	88 ± 51 (0 – 196); 67 ± 34*
Delta T_{sk} (°C) exercise	12.5 ± 6.6 (4.7 – 29); 12.9 ± 6.4*
Net AUC T_{sk} exercise (°C x minutes)	1164 ± 1448 (-1329 – 4749); 1114 ± 1469*
T_{sk} (°C) at end-of-exercise	28.0 ± 6.1 (13.3 – 38.1)
Min T_{sk} (°C) recovery	17.3 ± 3.1 (12.1 – 23.4); 17.7 ± 3.3*
Max T_{sk} (°C) recovery	30.9 ± 4.1 (23.4 – 38.2)
T_{sk} (°C) recovery	21.5 ± 2.8 (16.4 – 27.1
Delta T_{sk} (°C) recovery	13.0 ± 5.1 (3.7 – 22.2)
Delta T_{sk} (°C) first 10 minutes recovery	-4.7 ± 6.7 (-19.4 – 4.6)
Delta T_{sk} first 10 min recovery/ minute (°C min^{-1})	-0.5 ± 0.7 (-1.9 – 0.5)
Number of horses T_{sk} > 39°C**	None
T_{sk} (°C) at end-of-recovery 40 km (n = 25)	21.8 ± 3.8 (14.5 – 28.7)
Number 40 km periods T_{sk} returned to base T_{sk} at the end of 60 minutes recovery	14/25
Net AUC T_{sk} recovery (°C x minutes)	-305 ± 388 (-997 – 251); -230 ± 392*

Table 3. Overall T_{sk} and T_c variables during exercise and recovery of endurance exercise in a cool environment. Data are presented as overall mean ± SD. T_{sk}, skin temperature, AUC, area under the curve; T_c, GI pill temperature; max T_c or T_{sk}, maximum T_c or T_{sk}; n, number identified only if different 40 km exercise periods. *Indicates total 8 horses (comparison T_{sk} to T_c in 8 horses, total 16 x 40km-periods); ** T_{sk} > than 39°C based on (Brownlow and Smith, 2021).

5.5 Discussion

This is the first study to simultaneously monitor both T_{sk} and T_c continuously by means of several telemetric temperature recording devices on exercising horses in the field. The thermal sensors functioned correctly throughout the study, hence there was little to no data loss. Consistent with the researchers' previous field study (Verdegaal et al., 2021), the current study confirmed a substantial inter-individual variability in the T_{sk}

time profiles despite execution of the same exercise protocol. Similar findings have been reported in human athlete studies and underline the physiological complexity of the mammalian thermoregulatory response which is governed by a plethora of individually intrinsic variables (Fenemor et al., 2020; Foster et al., 2020; De Korte et al., 2021; Westwood et al., 2021).

With respect to T_{sk} and T_c monitoring in the current study, there was a lack of correlation between continuous monitoring of T_c and T_{sk}. Nevertheless, the association of a higher single point T_{sk} at the end-of-exercise period with a lower T_c at the end-of-recovery period is an interesting finding in the current study.

Up until now, a limited number of studies, almost all of which were treadmill based, monitored the T_{sk} continuously in horses during exercise and compared the T_{sk} to T_c (Geor et al., 1995; Marlin et al., 1996; Marlin et al., 1998; Marlin et al., 1999a; Geor et al., 2000). Only one recent field study has been performed monitoring T_{sk} and T_{re} (Klous et al., 2020). The treadmill studies confirmed the lack of correlation between T_{sk} and T_c. For example, two submaximal exercise studies using arterial blood temperature compared the effect of different environments on thorax surface T_{sk} and showed T_{sk} was different to T_c (Geor et al., 1995; 2000). Two high-intensity studies reported tail surface T_{sk} responses to cooling methods and acclimation respectively, and both studies showed T_{sk} recordings were different to the T_c recordings (Marlin et al., 1998; Marlin et al., 1999b). Apart from those studies, only one laboratory-based high-intensity exercise study comparing exercise in four horses in a cool versus hot environment suggested that the tail T_{sk} evolvement pattern seemed to follow the T_c pattern (using arterial blood temperature) although a statistical correlation was not investigated (Marlin et al., 1996). A recent laboratory equine exercise study using implantation of microchips measuring muscle temperature (defined as ‘outer shell temperature’ in that study) which may be extrapolated to field exercise in the future (Kang et al., 2020). There was a good correlation between central venous temperature (CVT) evolvement and outer shell temperature during a short bout of exercise (8 – 11.5 minutes) until CVT reached 41°C, though the outer shell temperature was reported to lag behind CVT during the recovery phase. While most of these experimental studies did not reveal a statistical correlation between T_{sk} and T_c, efforts to further investigate T_{sk} continue as the technique could easily be employed in the field. Consequently, wearable thermo-sensor techniques are being upgraded at an ever-increasing pace.

Continuous T_{sk} sensor recording during field exercise

Ongoing efforts to identify a reliable proxy for continuously monitoring the thermoregulatory response in horses during field exercise are not always successful or practical. A more invasive method with thermistors placed in blood and brain was used in three horses during free field exercise and reported a good correlation, however, for obvious reasons this is not easily applicable in practice (Mitchell et al., 2006). Several field studies have investigated less invasive continuously monitoring approaches such as the intra-uterine temperature (two mares) or the GI temperature; both approaches (intra-uterine and GI) showed a good correlation with the T_c (Green et al., 2005; Smith et al., 2006; Verdegaal et al., 2017). A recent study continuously monitored surface T_{sk} using i-Button® and simultaneous T_{re} during two canter bouts of 4.5 minutes of field exercise at a speed ranging from 6.7 to 7.5 meters per seconds (Klous et al., 2020). The T_{sk} was continuously monitored at the level of the rump and shoulder regions in 10 eventing horses using a cross-over study design. The study showed that pre-exercise cooling resulted in a delta T_{sk} ranging from -2.3°C to -3.3°C and a reduced median T_{re} of 0.3°C which peaked at 9 minutes into recovery, compared to the control group (Klous et al., 2020). Although the study did not investigate correlations between T_{sk} and T_c, the effect of lower T_{sk} pre-exercise on a reduced T_{re} is of interest, and consistent with previous human sport studies (Bongers et al., 2017; Racinais et al., 2021).

In brief, the current reliance on continuous T_{sk} sensor recordings during field exercise has been proven to be inconsistent and unreliable as a proxy for the thermoregulatory response. This is consistent with a study comparing the effects of precooling in 10 human athletes to estimate the T_c (using GI pill) (Faulkner et al., 2015).

Comparing T_{sk} with T_c

When comparing the delta T_c with delta T_{sk} in the current study, the delta T_{sk} was greater during endurance exercise as depicted in **Figure 3A**. However, a laboratory-based high-intensity equine exercise study using thermocouples attached to the skin with tape and located in pulmonary artery blood to continuously monitor T_{sk} and T_c in six horses revealed a delta T_{sk} of 2.5°C (Geor et al., 2000) similar to the delta T_c value in the current study (3°C). Associations between T_{sk} and T_c were not evaluated in that study, however, the difference in the exercise duration may indicate a difference in thermoregulatory activity over time, namely 200 minutes of endurance exercise in the current study versus average 40 minutes in the former study evidenced by the end-of-exercise $T_c > 41$°C (Geor et al., 2000).

Another interesting finding was the significantly higher time to maximum T_c when compared to time to maximum T_{sk} (**Figure 3B**). This finding suggests that the endurance horses in the current study performing in a cool environment were efficiently thermoregulating during exercise without the development of hyperthermia ($T_c > 39$°C) as has been documented previously (Verdegaal et al., 2021). On the other hand, a short duration high-intensity exercise in more challenging environmental conditions may trigger $T_c > 41$°C and consequently requires dissipation of excess *H* to occur post-exercise (Hodgson et al., 1994; Geor et al., 1995; 2000).

Despite all these ongoing efforts to practically incorporate surface T_{sk} monitoring into thermoregulation and wellbeing monitoring in the field, researchers should always keep in mind the possible factors that challenge a potential correlation between T_{sk} and T_c. In addition, the monitoring device used must be able to correctly function and cope with the practical conditions under which horses exercise and compete.

Important factors are environmental variables amongst which are weather conditions and whether or not additional cooling is applied. Furthermore, the type of temperature sensor equipment and the anatomical site at which the T_{sk} equipment is placed have their influence, together with individually intrinsic horse-related factors.

Environmental factors

Factors influencing T_{sk} and T_c evolvement and how they relate to each other

The T_{sk} at any site on the skin surface reflects a balance between heat being delivered to the skin by arterial blood, body and local skin metabolism, and heat exchange with the environment by convection, radiation and evaporation. Any factors that interfere with this balance can change the T_{sk}. Many factors that modulate T_c evolvement during exercise simultaneously influence T_{sk}, such as a plethora of performance capacity parameters as well as environmental conditions. Environmental factors can easily and quickly change T_{sk} without directly affecting T_c (Morgan, 1995; Robinson et al., 2008; Wartzek et al., 2011; Holcomb et al., 2013; Raymond et al., 2020; Mota-Rojas et al. 2021c). These factors include the T_a, solar radiation, soil radiation, humidity, shade and wind speed (air movement). For example, a varying T_a ranging from 20°C to 30°C was directly related to the onset of skin vasodilation and sweat evaporation (Morgan, 1995) while on the other hand, a low T_a was shown to induce a lower sensitivity (50%) of percutaneous T_{sk} microchips in 52 foals and 30 adult horses to identify fever compared to measuring T_{sk} in a hotter T_a (29°C) (Robinson et al., 2008). That would mean that in case of fever (also known as an increased T_c set-point) a cool sunless environment renders T_{sk} monitoring using microchips less representative for T_c monitoring. In addition, Holcomb et al. (2013) demonstrated that T_{sk} and T_{re} were highest at the peak solar radiation during the day. The T_{sk} sensor in the current study was located ventrally on the chest of the horse covered by the belt and the girth, thus avoiding T_a effects such as solar radiation.

It is common practice to cool down endurance horses during the recovery period between subsequent exercise loops. Cooling down was also applied in the current study design in real-life competition context. The goal was to challenge the temperature monitoring devices with real circumstances in which they would be required to function. With respect to cooling down approaches, the mechanism by which the thermoregulatory systems are challenged greatly depends on how the loss of heat counteracts *H* production through non-evaporative pathways as well as evaporative methods. The evaporative exchange of heat of the skin with the environment depends on the thermal gradient between T_{sk} by local skin perfusion and its immediate environment, including vapor pressure, airflow and solar radiation, especially during field exercise (Hodgson et al., 1993; MacRae et al., 2018; Brownlow and Brotherhood, 2021). At the end-of-exercise period, the cooling of sport horses is standard especially in endurance and 3-day eventing competitions. However, post-race cooling methods are not standardized in the racing industry. The duration of cooling endurance horses in the field is on average 10 minutes, based on each owner's judgment which could include HR monitoring. The mean end-of-exercise T_{sk} in the current study with endurance horses exercising in a cool environment (mean 15.3°C) was 28°C and no horses developed a T_{sk} higher than 39°C.

On the other hand, exercise studies in warmer environments documented a post-exercise T_{sk} higher than 39°C. For example, a recent report revealed that 28 out of 38 horses exercising in a hot, dry environment (mean T_a 38.8°C), and 6 out of 37 horses exercising in a warm, humid environment (mean T_a 31.1°C) showed a post-exercise IRT T_{sk} higher than 39°C. These researchers suggested horses recording T_{sk} higher than 39°C were at risk of developing heat stress and EHI and used this T_{sk} response as an indicator for racehorses requiring cooling (Brownlow and Smith, 2021). The association between T_{sk} and EHI risk could be physiologically explained by a low T_c -to- T_{sk} gradient, therefore decreased capability to transfer *H* to the skin thus compromising the dissipation of *H* by evaporation (Brownlow and Smith, 2021; Brownlow and Mizzi, 2022). A similar mean IRT T_{sk} of 40°C was recorded at the end-of-exercise in a recent study evaluating cooling methods in racehorses in a warm environment (mean T_a 31.8°C) (Takahashi at al., 2020). A T_{sk} higher than 39°C is consistent with earlier laboratory-based studies in a warm T_a (29.1°C and 31.1°C respectively) (Marlin et al., 1998; Kohn et al., 1999). In retrospect, scraping off water from the horses during cooling down was not the most optimal approach since Takahashi et al. (2020) favored continuous application of cold water without subsequently scraping it off.

T_{sk} equipment related features and location

Equipment to measure T_{sk}

Within the rapidly expanding wearable digital device industry, surface T_{sk} monitoring devices are constantly being upgraded to provide data output. However, in that respect, the critical question remains: how should we interpret those data? Overall, three different types of temperature sensor equipment are reported: thermistors (such as microchips), thermocouples and IRT devices, with IRT being the most studied device recently in horses (Soroko et al., 2018; Soroko and Howell, 2018; Redaelli et al., 2019; Soroko et al., 2019a; Soroko et al., 2019b; Brownlow and Smith, 2021; Giannetto et al., 2020; Meisfjord Jorgensen et al., 2020; Takahashi et al., 2020; Wilk et al., 2020; Maśko et al., 2021; Mota-Rojas et al., 2021d; Zielińska et al., 2021;

Domino et al., 2022). It is essential to understand that those sensors use different physical processes to obtain data, which may result in significant differences in data output. These sensor surface T_{sk} differences due to the type of equipment may show only a minor bias which may prove to be clinically meaningful (MacRae et al., 2018). For example, a study comparing IRT and thermocouples at single pre-exercise, intra-exercise and post-exercise points in 12 human athletes revealed a poor Bland-Altman agreement and low reliability between the different methods (Fernandes et al., 2014). In order to produce IRT imaging to picture surface T_{sk} of different parts or the whole-body, a remote IRT camera positioned at 30 cm proximity to the skin surface has been recently evaluated with varying results (Ramey et al., 2011; Soroko et al., 2018; Meisfjord Jorgensen et al., 2020). For example, a study compared IRT T_{sk} to T_{re} in 40 adult horses and concluded T_{sk} was not an accurate method to determine the T_c (Ramey et al., 2011). The remote position of the IRT held far from the skin has the advantage of not interfering with the local T_{sk} balance, although the remote T_{sk} measurement will be partly affected by the adjacent environment surrounding the skin (MacRae et al., 2018; Soroko and Howell, 2018; Mota-Rojas et al., 2021b). The temperature sensors that were used in the current study were in direct contact with the skin and covered by a belt. This belt might have interfered with the local thermal conductivity and the local evaporative cooling capacity and thus might have delayed equilibration of the local T_{sk} with the surrounding skin. On the other hand, an adequate and essential sensor-to-skin contact was ensured by the position of the sensor in the belt. Furthermore, the skin surface covered by the sensor was small enough to prevent causing local skin changes (MacRae et al., 2018; Soroko and Howell, 2018).

The current study was unable to calibrate and validate the IR sensor prior to the study, however, studies of different T_{sk} recording methods and comparisons with a certified thermocouple in a thermo-statically controlled water-bath are extremely rare. One study evaluated sensor systems in human athletes during rest and exercise in a hot environment and revealed a good agreement for employing a telemetric thermistor system when compared to the standard hard-wired thermistor system and a poor agreement for using a thermal camera (James et al., 2014).

In summary, IRT techniques differ widely in human and equine medicine, including positioning of the camera and environmental control measures (MacRae et al., 2018;

Soroko and Howell, 2018; Rojas-Valverde et al., 2021). A consensus guideline has been developed only recently addressing the multiple data collection methods of the human T_{sk} using IRT (Moreira et al., 2017), while Soroko and Howell (2018) described a protocol using IRT in equine medicine.

T_{sk} equipment location

The anatomical location of the sensor on the horse to record T_{sk} measurements has been shown to influence T_{sk} results (Jodkowska et al., 2011; Wartzek et al., 2011; Soroko et al., 2018; Soroko and Howell, 2018; Meisfjord Jorgensen et al., 2020; Mota-Rojas et al., 2021b). For example, remote IRT was used to evaluate differences between 10 locations on the body during two seasons in the year with the highest T_{sk} recorded at the level of the chest (22.5°C) and shoulders (20.4°C) in horses at rest in a cool T_a (mean 6.7°C) (Meisfjord Jorgensen et al., 2020). In another study, the IRT T_{sk} was greatest at the shoulder area when compared to three other T_{sk} locations measured at the start and the end of 20 minutes exercise (32.3°C and 34.2°C, respectively) in a moderate T_a (mean 23°C) (Soroko et al., 2018). The results of the current study in a cool T_a (mean 15.3°C) revealed a mean T_{sk} measured at the lower chest area of 23.1°C during an average of 200-minutes exercise and a mean T_{sk} of 21.8°C during recovery. The different T_{sk} values between the current study and Soroko et al. (2018) illustrate the effect of exercise intensity (submaximal versus maximal) and duration (long versus short). The differences in T_{sk} over several body areas may relate to the varying network of blood vessels in those body regions and its vasodilation in order to exchange thermal heat with the proximal environment (Bertoni et al., 2020; Mota-Rojas et al., 2021b; Mota-Rojas et al. 2021c; Brownlow and Mizzi, 2022). It is essential to note that both monitoring methods share some vasomotor or endocrine mechanisms, though they present differences depending on the degree of heat dissipation or retention that the organism needs. Consequently, monitoring of T_{sk} in the current study revealed a physiological response of the local T_{sk} to the changes of T_c during endurance exercise over time, though the responses were not correlated.

While IRT cameras are increasingly used in equine sport medicine, this method involves a single point in time measurement. One exception is the study by Soroko et al. (2018) who reported dynamic IRT monitoring every 15 seconds during treadmill exercise. To be precise, a review of the use of IRT in human endurance athletes reported that 25 of the 45 studies were conducted over the last five years as (2017 – 2021) but up until now, only five real-life field endurance studies have been performed (Rojas-Valverde et al., 2021). The latter review concluded that further analysis is required to assess whether T_{sk} could be used as a reliable proxy to describe real-time thermoregulation (Rojas-Valverde et al., 2021). Another important relevant finding is that surface T_{sk} may be low in human athletes with EHI and hence provide misleading information (Belval and Armstrong, 2018). A different IRT method approach is measuring eye surface temperature; that study revealed no relationship with T_c in horses (Soroko et al., 2016).

Individual horse-related factors

Horse-related factors include breed, body condition score, age, character (such as nervousness) and skin related properties such as sweat rate, skin thickness, blood vessel density, hair coat properties, clipping and coat color (Mostert et al., 1996; Morgan et al., 2002; Wallsten et al., 2012; Bertoni et al., 2020; Wilk et al., 2020; Maśko et al., 2021; Mota-Rojas et al., 2021b; Mota-Rojas et al., 2021d; Domino et al., 2022). The sweat loss in the current study was subjectively scored from 1-3 by E-L.V., and in retrospect, a more accurate sweating scoring based on objective specific phenotypic descriptions would have been a better approach (Zeyner et al., 2014). The effect of breed on T_{sk} relates to the ratio of BM to body surface area—the higher the body surface area in relation to the BM, the higher the heat dissipation (Morgan, 1995). The low surface area-to-mass ratio of the horse results in greater demands being imposed on the thermoregulatory system during long-term submaximal exercise (Hodgson et al., 1993; Hodgson et al., 1994; Wallsten et al., 2012). The current study included mainly Arabian horses, known to have a lower BM and hence a higher surface area-to-mass ratio.

The hair coat length in the current study was similar (all clipped) which is essential as clipping the winter coat resulted in improved heat dissipation during and after exercise resulting in decreased T_{sk} and T_{re}, as reported in previous studies (Morgan et al., 2002; Wallsten et al., 2012). One of those previous studies used a thermistor probe to evaluate the effect of coat clipping in three horses on both the surface T_{sk} and T_{re}. That study reported no effect of clipping on post-exercise T_{sk} while T_{re} was approximately 0.2°C higher in unclipped horses (Wallsten et al., 2012). Indeed, a longer haircoat length limited the thermal imaging in a study assessing T_{sk} in mares (Maśko et al., 2021). Furthermore, coat colors may be relevant (Mostert et al., 1996), however the current study of 13 horses revealed that light or dark coat color had no significant effect on T_{sk}, which is consistent with a previous study (Robinson et al., 2008). Individual horse-related character differences may exist such as nervousness that triggers sympathetic nerve activity associated with vasoconstriction of skin blood vessels. This neurophysiological response may explain varying reduced local T_{sk}, decreased heat loss and hyperthermia (Hetem et al., 2013; Bertoni et al., 2020; Mota-Rojas et al., 2021b; Mota-Rojas et al., 2021d).

Modeling using T_{sk}

While generally T_{sk} can be easily monitored, the T_{sk} in the current study did not provide data suitable for extrapolating to similar changes in the T_c. Consequently, the development of integrative models using T_{sk} to determine the heat balance during exercise has been investigated in human studies and in one equine study (Mostert et al., 1996; Eggenberger et al., 2018; Tanda, 2021). However, no regression model could predict physiological stress load using single point IRT T_{sk} in 17 human marathon runners in the field (Pérez-Guarner et al., 2019). A recent approach in human exercise research investigated the application of models and algorithms using data and variables such as HR and HR variability to successfully estimate T_c (Wartzek et al., 2011; Cuddy et al., 2013; Eggenberger et al., 2018; Welles et al., 2018; Hillen et al., 2019). Physiologically, HR reflects the blood flow rate to the muscles (MH production) and blood flow to the skin (heat loss). For example, recent studies concluded that combining continuous insulated T_{sk} and HR monitoring in 13 and 8 human athletes in a hot (35°C) and warm (25°C) environment respectively, could provide a predictive model of T_{re} or T_c (using GI pills) (Eggenberger et al., 2018; Welles et al., 2018). In contrast in the current study HR recovery in the endurance horses was not directly related to T_{sk}. Further investigation is required into the potential association of T_{sk} and HR for accurate predictive modelling of T_c in equine athletes.

Association between single point T_{sk} at the end-of-exercise period compared with T_c at the end-of-recovery period

An interesting finding of the current study performed with endurance horses was the association of a greater T_{sk} at the end-of-exercise with a significantly lesser T_c at the end-of -recovery (60 minutes). Several theories could be considered to explain this association between T_{sk} and T_c: firstly, the raised T_{sk} indicates the launch of an active thermoregulatory response to anticipate the increased T_c and once the *H* is successfully dissipated, the T_c decreases. This argument can be coupled with the effect of cooling post-exercise, which may be more prominent when T_{sk} is greater and ultimately results in higher dissipation of MH and a reduced T_c. Several other field exercise studies in horses have investigated correlations between single point T_{sk} and other variables (Redaelli et al., 2019; Brownlow and Smith, 2021). For example, a recent equine study involving 8 endurance horses investigated the association between endurance training intensity (1 hour at 19 km/h versus 2 hours at 16 km/h versus 3 hours at 20 km/h) and T_{sk} using an IRT camera measured at different locations and at different time points. The study identified that the T_{sk} at the coronary band increased with training intensity unlike the maximum T_{sk} (Redaelli et al., 2019). Aside from the variance in hot versus cool T_a in these studies, the differences in exercise intensity could explain the dissimilarity between the racehorse study results of Brownlow and Mizzi (2021) and the current study involving endurance horses. For racehorses undertaking high-intensity, short-duration exercise, the dissipation of *H* occurs post-exercise as opposed to endurance horses which manage their *H* throughout their submaximal long-duration exercise (Brownlow and Mizzi, 2021; Brownlow and Mizzi, 2022; Brownlow and Smith, 2021; Verdegaal et al., 2021). For example, the T_{sk} and its evolvement pattern can be related to acute blood flow variances associated with a different type of exercise intensity (Hillen et al., 2019; Mota-Rojas et al., 2021a). Overall, in the current study monitoring endurance horses conducting exercise during cooler months, the mean T_{sk} at the end-of-exercise was 28°C while none of these horses had a T_{sk} higher than 39°C.

The end-of-recovery period T_{sk} showed a considerable individual variation (range 14.5 °C – 28.7 °C) despite application of a uniform cooling protocol. The T_{sk} during the 60-minute recovery period revealed that the T_{sk} returned to baseline only in over 50% of the 40 km recovery periods. This is in contrast to other studies which found that after 20 minutes of treadmill exercise in a hot (32 - 34°C) and dry T_a condition, all T_{sk} values returned to baseline T_{sk} after 60 minutes, and after 45 minutes in a T_a of 20°C (without cooling) respectively (Geor et al., 2000; Simon et al., 2006). The main difference between the current study and other laboratory-based studies was the continuous T_{sk} monitoring during field exercise in an uncontrolled T_a.

Limitations

As in any study, there are several limitations that should be considered. Obviously, throughout this "in-the-field" study, not all research conditions could be controlled for 100% of the time, such as weather conditions involving T_a and the degree of solar radiation, the training and the dietary management of participating horses. These factors may have affected the individual T_{sk} and T_c time profiles. However, this applies to all "in-the-field" competition studies and under ideal conditions should not interfere with the reliability of a solid thermoregulatory monitoring proxy suitable for assuring the thermoregulatory wellbeing of competition horses in the field (Holcomb et al., 2013). Endeavors to assess the thermal environmental variables were limited to BOM and HOBO recordings of the T_a and the RH, with the HOBO device placed at one location. Other essential external variables such as wind speed were not included in the T_a measurements (Brownlow and Brotherhood, 2021). The current study involved only one type and location of wearable T_{sk} sensor based on IR technology. In future, other thermo-physical measuring approaches will prove to be more robust. However, on all occasions the involvement of a validated 'gold standard' against which the performance of new individual monitoring devices is set should be an essential part of future studies (MacRae et al., 2018).

5.6 Conclusions

While the method of monitoring T_{sk} may be non-invasive and straightforward, the current study results have clearly shown that T_{sk} monitoring on its own does not reliably estimate the T_c evolvement during field exercise in endurance horses since a correlation between T_c and T_{sk} could not be identified. Notably, a high T_{sk} at a single point during field exercise in a cool T_a did not identify endurance horses with an increased T_c. Further research into T_c monitoring in different equine sports and under differing weather conditions must be undertaken to create a baseline for further fine-tuning hot weather policies. Accordingly, veterinarians, trainers and owners can be advised to continuously monitor T_c to ensure the health and welfare of all horses.

5.7 Supplementary table

Monitoring skin temperature in endurance horses

Summary of a total of 30 selected studies measuring the surface T_{sk} in horses.

- 11 studies at rest
- 19 studies related to exercise including:
    - Seven studies continuous monitoring using T_{sk} **contact sensors**:
        - One field study using i-Button® (Klous et al., 2020)
        - Five laboratory-based studies in the 1990's: Marlin et al. (n = 3, using thermistor probe) & Geor et al. (n = 2, using thermocouple)
        - One recent study using dynamic infrared thermography (IRT) camera (Soroko et al., 2018)
    - 12 studies measured T_{sk} post-exercise at a single point including one study with T_{sk} both pre- and post-exercise

Monitoring continuous or not continuous

A total of nine studies monitored T_{sk} continuously (C) (including two studies at rest) and 21 studies monitored T_{sk} not continuous (N/C) (T_{sk} at a single point).

Overview of the T_{sk} equipment

- 19 studies used IRT
- Five studies used thermocouples
- Seven studies used thermistors including two studies using the i-Button®

Publication	**Thermo-sensor type**	**C N/C**	**T_c Y/N;**	**Number of locations- ROIs**	**Delta T_{sk} exercise**	**Hottest location & conclusion**	**Exercise / Rest Aim of the study Number of horses (n=)**
(Domino et al., 2022)	IRT camera	N/C	N	15	NA	horse T_{sk} > then donkey due to difference *skin thickness*	R - compare donkey & horse *skin*; n=18 & n=16
(Maśko et al., 2021)	IRT camera	N/C	N	10 ROIs: e.g., chest, hoofs	-	No T_{sk} difference in mares nor *coat length*	R - compare pregnant and non-pregnant mares & effect of *coat length*; n=40 mares
(Zielińska et al., 2021)	IRT camera	N/C	N	1: Legs	NA	*Non-pigmented* > T_{sk}	R - compare effect of laser TX on T_{sk} *pigmented vs non-pigmented*
(Brownlow and Smith, 2021)	IRT camera	N/C	N	3: neck, shoulder, thorax	-	> 39°C = higher risk EHI	E - **field,** post exercise T_{sk}; n=260
(Giannetto et al., 2020)	IRT camera	N/C	Y, single point T_{re}	5 ROIs	~5°C	T_{sk} not aligned with T_{re}	R - assess daily rhythm; n=5
(Klous et al., 2020)	i-Button® & glue	C	Y: C T_{re} N	2: Shoulder, rump	-3°C	Pre-cooling -> T_{re} median 0.3°C difference & T_{sk} mean -3°C difference	E - **field,** evaluate 8 min pre-cooling to 8.5 min eventing; n=10 eventers
(Meisfjord Jorgensen et al., 2020)	IRT camera	N/C	N	10 ROIs: incl chest, hoofs	-	Abdomen and flank; individual difference	R- compare 2 seasons and *coat length*; n=21
(Takahashi et al., 2020)	IRT camera	N/C: 1x prior cooling	Y: T_{PA} N – only single point	1: left thorax	- At T_{PA} 42°C, mean T_{sk} > 40-41°C	When T_{PA} 42°C, best T_{sk} at 17^e ICS > 40°C Aim until T_{PA} < 39°C: Shower is best	E - treadmill – run to 42°C T_{PA} – compare difference post-cooling methods; n=5 TB
(Wilk et al., 2020)	IRT camera	N/C	Y, single point T_{re} N	7 ROIs	~ 6°C	> 20% of BM -> higher T_{sk}	E - ridden – compare BM riders; n=12
(Redaelli et al., 2019)	IRT camera	N/C	N	7		Crown T_{sk} correlation intensity	E - correlate endurance intensity with T_{sk} & stress markers; n=8
(Soroko et al., 2019a)	IRT camera	N/C	N	7 muscle regions	~1-2°C	Higher T_{sk} in ridden horses	E - treadmill pre & post T_{sk} compare ridden/ non ridden & correlation blood parameters; n=9 ponies
(Soroko et al., 2019b)	Dynamic use IRT camera q15s	N/C	N	Saddle area: 6 ROIs		Saddle 'pressure'	E – field, saddle thermal pattern; n=18 racehorses
(Soroko, 2018)	Dynamic use IRT camara q15s	C	N	4: SH, neck, croup, chest		Neck	E – 25 min treadmill, 15 min trot (9.1 km/h); n=5 ponies
(Soroko et al., 2017)	IRT camera	N/C	N	2: joints	~25°C and T_a delta ~20°C	-	R - influence of T_a on T_{sk} difference between joints; n=64
(Edner et al., 2015)	Thermistors and IRT camera	N/C	N	Local T_{sk} under blanket – IRT overall thermography	-	No T_{sk} difference	R - magnetic blanket effect; n=10
(Yarnell et al., 2014)	IRT camera	N/C	N	Semitendinosus muscle	~20°C	T_{sk} during Dry treadmill ~ muscle activity	E - compare water treadmill; n=8
(Holcomb et al., 2013)	Thermocouple - held	N/C	Y, single point T_{re}	2-L+R triceps	~1°C in sun	Sun: T_{re} & cortisol WNL (sun 37.8°C vs 37.5°C), more sweating	R - compare T_{sk}, sweating in shade vs non-shade; n=12
(Wallsten et al., 2012)	Thermistor probes with sensors on skin	N/C	N	2: neck, biceps, tail	~10-20°C	*unclipped* & blanket higher thermoregulation based on RR & T_{re} (38.2°C)	E - ridden outside several trot/canter periods over 1000m, effect of *clipping &* blanket in cold T_a; n=3

(Ramey et al., 2011)	IRT camera	N/C	Y, single point T_{re} N	2: mucous membranes, trunk	-	Significant variation in T_{sk} readings	R - IRT vs T_{re}; n=40
(Jodkowska et al., 2011)	IRT camera	N/C	Y, single point T_{re} N	36 ROIs to cover whole BSA: and 25 ROIs post-exercise	~5°C (post exercise: 25-35°C)	Head, neck, trunk. T_{re} WNL	E - T_{max} on BSA compare before and after jumping; n=35
(Robinson et al., 2008)	implantable microchip		C at rest	1: nuchal ligament	-	Correlation influenced by T_a; only 55% sensitivity at lower T_a	R - compare implant T_{sk} to T_{re} to detect fever; N=52 foals, n=30 QH
(Simon et al., 2006)	IRT camera	N/C	N	2: FL & HL	~5-6°C	None – all return to base T_{sk} in 45min	E - treadmill, determine time to return to base T_{sk}; n=6
(Morgan et al., 2002)	IRT thermometer	N/C	N	1	-	*Clipping* result in better heat loss	E - treadmill, effect of *coat clipping* on T_{CV}, T_{sk}, T_{sk} used to calculate heat loss; n=6 SB
(Geor et al., 2000)	Thermocouples with sticky tape	C	Y: T_{PA} N	1- Shaved at thorax	~2.5°C	No difference in delta T_{sk} in HH, HD, CD (no T_{PA} correlation & always ~3°C difference)	E - submax. & heat storage post acclimation under HH comp to CD, HD; n=6
(Marlin et al., 1999a)	Thermocouple with glue	C- not exercise!	N	2: neck & gluteal	~ 2-6°C	T_{sk} significant correlation sweating onset (35°C) and rate at neck	R - adrenaline anhidrosis; n=10 compared to control
(Marlin et al., 1999b)	Thermistor probe	C	Y: T_{RA} N	1: tail skin (T_{RA}, T_{re})	~7°C	T_{sk} indicates sweating rate	E - treadmill training acclimation & sweating rate at T_{sk}/T_{RA}; n=5
(Marlin et al., 1998)	Thermistor probe	C	Y: T_{RA} N	2: tail skin, coat	~5°C	Response of T_{sk} and T_{RA} to cooling	E - treadmill cooling study; n=5
(Marlin et al., 1996)	Thermistor probe	C	Y: T_{RA} Y	1: tail skin (T_{RA}, T_{re})	~6°C	T_{sk} follows T_{RA} pattern	E - treadmill Cool compared to hot T_a; n=4
(Geor et al., 1995)	Thermocouples sticky tape	C	Y: T_{RA} N	1- Shaved lat thorax		T_{sk} diff from T_c	E - response to submax. (50% Vmax) under HH comp to CD, HD; n=5
(Morgan, 1995)	i-Button® taped	C - at rest	N	5 ROIs: spread over body	NA	Extra energy at lower T_a	R - lab study climate demand at different T_a

Supplementary Table S1. Overview of selected equine studies measuring T_{sk} (°C) at rest (R) or during exercise (E), non-continuous data points (N/C, at a single point) or monitoring continuously (C). Only essential data are presented. T_{sk}: skin temperature; T_{CV}: central venous blood temperature; T_{re}: rectal temperature; T_{RA}: right atrial blood temperature; T_{PA}: pulmonary artery blood temperature; T_a: ambient temperature; max.: maximum; min: minutes; IRT: infrared thermography; ROIs: regions of interest; *italic*: data related to the skin and coat characteristics; n =: = number of horses involved in the study; field: field studies; - : data not available; BSA: body surface area; QH: Quarter Horse; TB: Thoroughbred; BM: body mass; TX: therapy; HH, HD, CD: hot-humid, hot-dry, cool-dry T_a.

Conflict of Interest Statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethics statement

The present study was approved by the University of Adelaide Animal Ethics Committee (project number S-2011-224) Australia. The owners of the participating horses provided their written consent to participate in this study.

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Chapter 6

General discussion

This is the first PhD thesis to include multiple studies focusing on continuous monitoring of body temperature of sport horses during work and competition in open air (“in the field”), using minimally invasive monitoring devices that can withstand the test of practice and real competition. To date, there have been several laboratory-based studies of thermoregulation in sport horses during exercise, mostly conducted using treadmill tests (Hodgson et al., 1993; Marlin et al., 1996; Courouce-Malblanc, 2013; Hodgson, 2014) and two experimental field studies. One such study was performed under field conditions using an intra-uterine temperature sensor (Smith et al., 2006) and the second applied more invasive temperature measurement methods using thermistors placed in the blood and brain during free field exercise (Mitchell et al. 2006). However, the monitoring method being validated and extensively tested in the current thesis is easily applicable in practice and can function as a baseline for development and execution of future hot weather studies.

Equine competition is being performed at ever-increasing frequency and intensity, both at the professional and semi-professional level. Horses are being increasingly transported over the road and by air, and this happens globally. In other words, the equestrian industry is expanding. However, there are still many knowledge gaps to be filled in order to steer these developments in the right direction and above all, ensure equine welfare while maximizing performance. It is well known that training of horses is still performed predominantly based on empirical evidence which entails that the efficiency and effectivity of training levels of sport horses does not always match the competition level at which they need to perform. This partly explains the current and ongoing high prevalence of sports injuries in different disciplines (Hellings, 2022; Bennet et al., 2021; Zuffa et al., 2022).

The reality of global warming imposes additional stress on the physiological coping mechanisms that engage when horses compete. This PhD research is unique and breaks new ground because it includes the first existing studies focusing on continuous monitoring of core body temperature of sport horses during work and competition in the open air ("in the field"). Earlier laboratory-based studies have employed a treadmill and more invasive temperature measurement methods. For this doctoral research however, a gastrointestinal (GI) temperature pill was validated that provides continuous data output on core body temperature. The research has shown that this is a sound method that can withstand the test of practice and therefore holds great promise for monitoring and ensuring thermoregulatory well-being in horses during real competition. The results of this thesis provide the blueprint to support execution of future hot weather studies and lead to the formulation of effective and evidence-based guidelines and management rules to prevent the occurrence of heat stress in sport horses.

An increased body temperature is the result of accumulated metabolic heat generated by exercising muscles and is directly related to exercise/racing intensity, and to the performance capacity of the individual horse and the environmental conditions. The process of dissipating of metabolic heat in response to strenuous exercise is even more challenging during high environmental temperatures and high humidity (Guthrie and Lund, 1998; Marlin et al., 2001). Human athletes may voluntarily discontinue or decrease exercise intensity when they perceive high heat and/or humidity to prevent occurrence of exertional heat illness (EHI). Research has already shown that heat stress often goes unnoticed by horse owners due to the lack of awareness of the clinical signs (Nomura et al., 2019; Brownlow and Brotherhood, 2021). Most often, only the overt EHI cases are being recognized. Horses with overt EHI signs show restlessness and eventually collapse due to neurological injury. In the terminal stage of EHI, a progressive cerebral oedema and fatal exertional heat stroke (EHS) may develop.

It is a common misconception that heat illness affects horses only in hot and humid weather conditions (Hosokawa et al., 2019). Many cases also manifest themselves after competition during “normal climatic” days. This means that the weather per se can’t be used as single guideline to stimulate alertness in horse owners and organizers of competitions for the occurrence of heat stress (Brownlow et al., 2016; Brownlow and Mizzi, 2021). It also illustrates that thermoregulation is a very complex physiological process (Hodgson et al., 1994; Hodgson, 2014).

Therefore, there is great need to further fine-tune early detection of hyperthermia in individual horses, and to obtain a detailed view of the flexibility of equine thermoregulation in the field under different exercise and climatic conditions. Another critical aspect that needs to be clarified is the optimal rest and recovery period that is required before 'return to exercise' is allowed after an EHI event. To illustrate, human athletes are required to rest and rehabilitate for a certain period after an EHI event, varying from 3-6 weeks to a year (Adams and Jardine, 2020).

By extension, the threat posed by global warming applies not only to horses, but to all other mammals that compete in open air. Therefore, research groups worldwide are investigating the reliability of invasive and non-invasive devices that may provide in early detection of risk for development of EHI under different exercise and climatic conditions (Hodgson et al., 1993; Marlin et al., 1996; Courouce-Malblanc, 2013; Mitchell et al. 2006; Smith et al., 2006). This PhD researcher's work was explicitly chosen as not to work under laboratory conditions nor use invasive devices as reported in previous studies (Hodgson et al., 1993; Marlin et al., 1996; Courouce-Malblanc, 2013), but rather to focus on exploring and validating on a device that can withstand the test of real-time practice. In that respect, a gastrointestinal (GI) temperature pill was validated and subsequently tested in exercising horses in the field. The evaluation of the GI pill was performed in two types of exercise that are at the outer boundaries of the exercise intensity spectrum in sport horses, namely endurance versus trotter racing.

Finally, with the advent of all kinds of "wearables" and sensors developed for use in humans, there is a great temptation to blindly use these in equestrian sports. However, it is important to understand the physiological significance of the data outputs generated by these devices. In that respect, the continuous monitoring of surface skin temperature (T_{sk}) could be an elegant tool and therefore was explored in the current PhD thesis.

The first study (chapter 3) evaluated the reliability of an ingestible telemetric gastrointestinal (GI) Jonah® pill (Phillips, USA) to monitor the core body temperature of horses during real-time field exercise. Prior to use *in vivo* in competition, the pills were validated in water baths with three different temperatures relevant to horse body temperatures at rest and during exercise (37°, 39° and 41°C) and their output was compared to a certified resistance temperature detector (RTD). A field study was then conducted comparing temperature output values and time profiles of data output between the telemetric GI pill and a rectally inserted temperature probe. For this purpose, eight standardbred horses were involved in the study. The pill was administered by nasogastric intubation and data were telemetrically recorded by means of an external receiver in a sensor belt (Equivital®). This is the first study to describe both *in vitro* and *in vivo* evaluation of the suitability of a telemetric GI pill to continuously monitor T_c in horses, both at rest and during exercise. The RTD output showed that the GI pill was accurate and precise over the range of water bath temperatures which was based on Bland-Altman analysis. The authors evaluated the accuracy (validity) based on the findings depicted in the Bland-Altman graphs with the bias and the limits of agreement to assess the agreement between the two methods (GI pill and RTD sensor). The Bland-Altman analysis was chosen to allow for easy graphical display of the statistical analysis output, in particular the display of the bias: the more the mean line is away from zero, the smaller the degree of accuracy (presence of more bias or systematic error). The advantage of LOA is that it gives you an idea whether there is a bias, depending on the magnitude of the temperature. To explain this further, the RTD output showed that the GI pill was accurate and precise over the range of the recorded water bath temperatures, based on Bland-Altman analysis output. On top of that, a Lin concordance correlation analysis was performed. The Lin concordance correlation analysis demonstrated a calculated concordance correlation between GI temperature and RTD temperature and that the pill was precise ($r = 1.000$) with some inaccuracy (bias = 0.998), and systematically underestimating the true temperature by only 0.14°C.

Also, under practical conditions, the GI pill proved to be a reliable and user-friendly method to continuously monitor gastrointestinal temperature in exercising horses. The authors consider the smallest effect size of interest (SESOI) of ± 0.5°C clinically and biologically acceptable bias. This SESOI is within the manufacturer's specifications reporting an accuracy of ± 0.1°C between 32°C and 42°C. An essential advantage of this system is that the rider is able to access the data in real-time on a digital device such as their mobile phone which enables them to readily respond to signs of hyperthermia or even better, to reduce exercise speed at an early stage to prevent occurrence of hyperthermia. Unlike previous studies executed with other GI capsule devices, this GI pill showed minimum data loss (mean 16.5% ± 4.1 over three days). The excellent functioning of the Jonah pill may be due to the longer data transfer distance capability of 1 meter compared to other capsules. This large distance is essential, considering the larger dimension of horses being the limiting factor in wireless signal transduction. The data was sent with a frequency of 40.68 MHz to a receiver placed in the Equivital® sensor in the belt system under the girth. This is considered an advantage based on the fact that the receiver remains at a fixed proximity to the moving position of the GI pill through the digestive tract.

Besides administration of the GI pill via nasogastric intubation, an alternative administration method using a modified 'drenching' gun was tested. For this purpose, a drenching gun was adjusted to enable loading with the GI pill, together with 25 ml of water and was inserted into the caudal portion of the oral cavity, inducing a swallowing reflex. This approach worked well for the horses, however, when using the drenching gun in the field, some horses were able to bite and damage the pill prior to swallowing and therefore this administration method requires further optimization. The period that the pill remained inside the horse varied, with a mean of 5.1 days ± 1.0 (elimination time). The ideal GI pill would be present for a longer duration to enable monitoring during consecutive multiple training and competition events. It is a future goal of our research group to work towards such a device.

It is important to emphasize the fact that the GI temperature was consistently higher than T_{re} with a mean difference of 0.21°C at rest and 0.36°C during exercise. In addition, there was a significant lag of the T_{re} compared to the T_c. This means that the heating of the core of the body is always reflected in the T_{re} at a much later stage. Both findings confirm that the serial measurement of T_{re} is not suitable as an acute marker of hyperthermia and this is consistent with previous studies (Hodgson et al., 1993; Geor et al., 1995). Apart from that, measuring the T_{re} by the rectal probe proved impractical as it was often expelled and consequently required regular replacement. This impracticality shows the value of T_{re} monitoring during competition to safeguard thermoregulatory wellbeing in horses should be reconsidered.

The second study (Chapter 4) involved the next step to test the efficacy and reliability of the GI pill under field conditions during real-time competitions. It was deliberately chosen to compare two extremely different types of exercise with each other, with regard to the thermoregulation, namely endurance competition versus trotter harness racing. For this purpose, the T_c profiles of the individual horses were established during two different types of exercise and recovery periods over consecutive loops of 40 km (for endurance exercise) or 1540 m (for trotter harness racing). Because of the GI pill's continuous monitoring capability, it was possible to calculate the net AUC (with the baseline set at rest T_c) using the trapezoidal method of T_c over time to estimate the thermal load. Further analysis of the recorded data included calculation of the metabolic heat load (*H*, kcal min^{-1}) and the oxygen cost of transport (COT—the oxygen consumption necessary to travel 1 meter, the gradient) for each horse. The COT calculated the gradient including a negative number for downhill terrain.

This is the first study conducted using a telemetric GI pill in real-time field competitions to compare the equine thermoregulatory response to different types of exercise under cool weather conditions. Endurance horses reached their max T_c at 75% of a 40 km exercise leg. However, trotter horses reached their peak T_c at a mean of 30 minutes into their recovery period. Mean T_c was still 38.8 ± 0.4°C at HR of 60 bpm which is currently used in endurance (AERA rules) as a marker to deem a horse as 'fit to continue' competition. Similarly, several trotters had T_c values above 38.5°C at a HR of 60 bpm. The results showed that owners of trotters (and racehorses alike) should be aware that T_c peaks quite some time (mean 60 minutes) after finishing exercise. Therefore owners/riders should be very attentive to the thermal well-being of their horse long after competition, especially in the context of transport following events. This needs further attention in future research.

The study approach involving continuous temperature monitoring, allowed for grading the thermal load in exercising horses by calculating the area under the curve (AUC, °C-over-time) by using the trapezoidal method. Continuous monitoring allows for applying an AUC approach which is a more robust parameter when compared to calculating metabolic heat load by using variables in an equation, such as cost of transport per meter over inclining or declining terrain to calculate the oxygen consumption. The calculation of the total thermal load using AUC is an important and useful parameter as it sums up all the factors which tend to increase core body temperature. This thermal load is the build-up of temperature that cannot be dissipated immediately (so a net result of production and loss) determined by exercise intensity, environmental temperature, and environmental evaporative power. A horse experiences its highest thermal load when exercising intensively in warm, humid environments with little or no wind, and is therefore at the greatest risk of developing heat exhaustion under these conditions. Although most figures are described as scatter plots, in retrospect a better description for the time plots especially temperature versus time would have been “line graphs". Another remark is the word ‘surgery’; even though the implantation of standard identification microchips is routine and has been reported to be a stress-free procedure however, the microchipping procedure itself is invasive (Erber et al., 2012; Lindegaard et al., 2009).

The study clearly showed that different types of exercise trigger completely different thermo-evolvement time profiles, which was to be expected, however, more importantly, significant inter individual differences in thermo-reaction patterns were recorded between horses. The latter is a finding that strongly underlines the importance of individual monitoring. Recently, in human athletes, the importance of individual monitoring was also emphasized based on study results using GI pill recordings (de Korte et al., 2021). This highlights the physiological complexity of the mammalian thermoregulatory response which is governed by a plethora of individual intrinsic variables (Fenemor et al., 2020; Foster et al., 2020; De Korte et al., 2021; Westwood et al., 2021). A recent study investigated the genetic fingerprint of thermotolerance in indigenous horse breeds in China to identify specific genes in breeds well known for their pronounced heat-tolerance (Wang et al., 2022). The study results may further facilitate future mapping out of Copy Number Variants (CNVs) on the whole genome and provide valuable insights into the molecular mechanisms that govern adaptation of the body to high temperature and humidity. However, still, despite the fact that the horse genome has been unraveled, still, the exact function of many horse genes remains unclear. Therefore, it’s important to remain critical when labelling certain genes as “responsible for heat tolerance capacity.

Furthermore, the finding that T_c has inter-individual differences explains the difficulty to report a particular T_c as a cut-off T_c and thus as a EHI or EHS ‘risk zone’. The cause of EHS is the inability to dissipate metabolic heat during exercise and may not necessarily relate to a specific general cut-off Tc. For comparison, human scientific literature reports the following definitions: exertional heat exhaustion (fatigue) manifests at < 40.6°C while exertional heat stroke > 40.6°C ('SIRS-associated EHS'). Heat stroke is defined as a high T_c combined with the expression of neurological signs. Importantly, it was demonstrated that the EHS prognosis decreased when T_c did not decrease below 39°C within 30 minutes of cooling and if T_c > 42°C (Casa et al., 2015). A high T_c (> 42-43°C) was measured during SET on treadmills with horses exercising at high intensity for a short duration (Hodgson et al., 1993; McConaghy et al., 2002). The inter-individual variation may be associated with different the overall cellular response to higher T_c. Lepock (2005) stated that this response is the result from summation of molecular inactivation and activation. Inactivation is followed by cytotoxicity and other inactivating responses due to denaturation of thermolabile proteins and indirect inactivation of proteins due to aggregation. Activation response is due to the induction of thermotolerance via heat shock factors. In summary, the cause of physical cell injury is multifactorial, for example the denaturation of proteins at T_c over 46°C might be more critically relevant than T_c > 42-43°C. Moreover, the duration of hyperthermia is a critical aspect of a biologically induced hyperthermia.

A third study investigated another method to measure the temperature as a proxy of the core body temperature which involved the continuous monitoring of skin temperature in exercising horses (Chapter 5). This was achieved by continuous monitoring of the thermoregulatory response using an infrared skin surface temperature device. While it has been demonstrated to be a practical method to use, there is high variability depending on the body site measured, whether the temperature is assessed outdoors, and the position of the camera. Most notably, it has not been compared with T_c. This study was the first to compare simultaneous measurements of T_{sk} with T_c using a GI pill during 40 km endurance loops in real-time field competitions. For this purpose, large datasets of T_{sk} and T_c recordings were analyzed and compared. As a result, an integrative real-time view was obtained on how the T_{sk} responded during endurance exercise and during recovery. In this study, 13 endurance horses were equipped with non-invasive monitoring devices (GI pill, T_{sk} device, heart rate [HR] monitor and global positioning system [GPS]). Recordings performed during each 40 km loop exercise period included T_c, T_{sk}, speed and HR. A temperature time profile curve of T_{sk} for each individual horse was constructed for each exercise and recovery period. The net AUC (baseline set at rest T_{sk}) was calculated using the trapezoidal method of T_{sk} over time (minutes).

Significantly, the studies demonstrated no direct correlation between the T_{sk} and T_c time profiles. On all occasions peak T_c values were significantly higher than peak T_{sk} values and, in all cases, there was a significantly shorter time to max T_{sk} than the time to max T_c. No significant difference could be identified between delta T_{sk} when comparing exercise and recovery periods. The conclusion of the study was that continuous monitoring of T_{sk} was not a reliable tool to monitor the thermoregulatory response in horses. More research is needed in that respect. An interesting finding of the current study was the association of a greater T_{sk} at the end of exercise with a significantly lesser T_c at the end of recovery (60 minutes). Several hypotheses could be considered to explain this association between T_{sk} and T_c. For example, it could be hypothesized that the raised T_{sk} indicates the launch of an active thermoregulatory response to anticipate the increased T_c and once the metabolic heat is successfully dissipated, the T_c decreases. This argument can be coupled with the effect of cooling post-exercise, which may be more prominent when T_{sk} is greater and ultimately results in higher dissipation of metabolic heat and a reduced T_c. Considering that additional factors such as air movement and sweating can easily and quickly change T_{sk} without directly affecting T_c, further research is needed into reliable monitoring methods. Further research could include the application of algorithms integrating T_{sk} data with other data such as T_c, to develop a reliable predictive model of T_c. However, currently, we are far from that point. It is important that horse owners are informed and are not misled by solely relying on these T_{sk} sensors.

The findings of these thesis studies open many promising avenues for future research in equine thermoregulation and exercise physiology under field conditions in more challenging weather circumstances and in different sport disciplines. These current findings have implications for improving the overall welfare of horses and future management of equine welfare at sport events such as the upcoming Summer Olympic Games 2024 in Paris. This study provides reliable supporting evidence for the need for industry-wide temperature monitoring guidelines to prevent EHI in endurance horses and racehorses when exercising in the field. Better understanding of thermoregulation in horses will help to develop proper training protocols and implement strategies to reduce incidences of EHI and ensure the welfare of all competing horses is promoted and safeguarded.

Additional studies were initiated providing future endeavors to evaluate several aspects of thermoregulation during field exercise. The first pilot study was a prospective study to evaluate the effects of thermoregulatory processes in endurance horses during a national 160 km endurance ride by using biomarkers in several body fluids and comparing endurance horses that completed the ride to all horses with metabolic disorders (Verdegaal et al., 2018). The second pilot study was a retrospective study aimed to identify risk factors of EHI in racehorses in Australia. A third pilot study applied an online questionnaire to survey the use of electrolyte supplementation in endurance horses amongst Australian endurance riders (Verdegaal et al., 2015). Data from these studies are currently being processed and will further fine tune the approach for execution of future in-the-field studies.

Several limitations of the current PhD research should be kept in mind when interpreting the data. Among these limitations are those typically associated with execution of field studies such as differences in competition tracks, diet including supplements, and training protocols of privately owned endurance horses as well as transport prior to competition. Also, the drench gun to administer the GI pill should be further optimized. This would prevent the need for nasogastric intubation. Furthermore, only one type of cooling approach was tested in the current PhD.

Currently, little is known about the short-term and long-term effects of racing on horses' wellbeing, and the impact of EHI in racehorses, namely which body systems are impacted and whether the injury is temporary, permanent or recurring. In human athletes, research has shown that those who have suffered EHI have a higher risk of EHI in the future. Similarly, the recurrence of EHI has also been reported in racehorses as EHI cases showed EHI signs on more than one occasion (MacDonald et al., 2008). Other predisposing risk factors include racing on sand tracks (2.25 times higher risk than racing on turf tracks), longer distance races, higher age, gender (geldings) and racing during the day (MacDonald et al., 2008; Takahashi and Takahashi, 2020).

In addition, assessment of the thermoregulatory response during post-exercise recovery is essential to monitor the process of heat dissipation through thermoregulation and avoid a longer duration of hyperthermia. Monitoring post-exercise T_c, preferably using the GI pill, is essential to prevent horses being transported while still hyperthermic. Cooling post-exercise with water over the body is the treatment of choice which is commonly implemented for endurance horses and during FEI eventing competitions. The results of the current studies emphasize that all horses exercising even under cool conditions have an increased T_c at the end of exercise compared to their base temperature. The post-exercise hyperthermia was more evident in horses undertaking high-intensity exercise and indicates that post-exercise cooling is essential. The timing at which the cooling is applied is equally important. More research is needed in that respect. The post-exercise hyperthermia data also warrant the need for introducing high standards for the transportation of horses after competition.

The thesis study was not able to identify a relationship between the base T_c, prior to exercise and the maximum T_c or the time to return to base T_c during recovery. This is not consistent with studies in human athletes, based on a recent review of cooling methods that demonstrated that most pre-cooling techniques are effective in removing heat and improving exercise performance in a hot T_a (Bongers et al., 2017). In horses, only one study investigated pre-cooling in eventing horses in the field and demonstrated a minor decrease in T_{re} (0.3 °C) (Klous et al., 2021). Future studies should investigate if pre-cooling has an effect on the T_c, using GI pills, and compare different pre-cooling methods to evaluate effectiveness and practicality (Bongers et al., 2017).

The research presented in this thesis was conducted under cool weather conditions and provides a baseline for future hot weather studies. Field research is by nature subject to variable weather conditions, and hence a better understanding of the thermoregulatory responses of exercising horses across a wider range of conditions is necessary. In the future, additional aspects of the environment could be included such as the WBGT using a handheld tool and the wind speed. For endurance rides, these weather condition recordings will still be limited to the veterinary check points unless a wearable device for weather recording can be invented. In the view of the current global warming, future prospective and retrospective studies should evaluate the specific risk of warmer weather conditions affecting all sport disciplines such as racing, trotting, eventing, polo, western riding and endurance.

The pilot studies in this thesis warrant further investigations into several aspects of exercise in the field under practical and real-life circumstances. The fact that heat stress also manifests itself on “normal climatic” days triggers several questions. Maybe dietary load plays a role at those times or the weather conditions in the days prior to the competition. More research is needed in that respect.

There is a vital need for a large-scale study to establish the prevalence and risk factors for the development of EHI and EHS in Australia and globally. The results of such a study will allow for early detection and efficient management of EHI and EHS in horses. As a follow up from the pilot study investigating risk factors for EHI in racehorses in Australia, large-scale retrospective epidemiological research will further identify the prevalence and risk factors of EHI in racehorses, endurance horses and similarly in other sport disciplines. Several strategies can be designed to identify equine athletes at risk for EHI, similar to those employed in human sport medicine (Eberman et al., 2011). Besides the valuable outcome of analysis of retrospectively collected data, the way forward is to collect more evidence-based data by executing prospective studies in all sport disciplines.

Future thermoregulatory response analysis including biomarkers

Additional evaluation of the equine thermoregulatory response through the identification of potential biomarkers will increase thermoregulation knowledge and further establish the importance of early recognition of heat illness symptoms. An example from human medicine could involve analyzing several sweat biomarkers including lactate, cytokines, and ammonia and monitoring those biomarkers by non-invasive, wireless electrochemical sensing wearable skin patches (Zhang et al., 2021). The research into non-invasive diagnostic methods will continue using novel technology data devices for further investigation of poor performance and fatigue involving muscle or neural injury due to hyperthermia.

An important aspect of equine thermoregulation is the redistribution of blood to the skin to evaporate metabolic heat, however at the same time decreasing blood flow to visceral organs such as the intestine. Emerging evidence in human athletes highlights that gut health is an essential factor for performance capacity and exercise-induced gastrointestinal syndrome is a critical element in EHI. Acute impairment of the digestive system can progress to absorption of bacterial toxins from the gut into the bloodstream causing severe inflammatory response syndrome. This inflammation may occur in different body systems such as the muscles (leading to myopathy) and the heart (leading to cardiac arrhythmias). Ultimately, multi-organ failure and collapse can occur resulting in a fatal EHS. Little is known about the short-term and long-term effects of racing and endurance exercise and exercise-induced repetitive GI injury on GI inflammation and the microbiome ("gut health"). Compromised gut function is commonly reported in human athletes during long-distance submaximal exercise (Costa et al., 2017). Recently, short-duration high-intensity exercise in humans has been shown to change the composition of the gut microbiota and increase gut permeability ("leaky gut") (Pugh et al., 2017). For this purpose, sampling was performed during real endurance competitions, analyses of which are pending and planned within the research group.

The ultimate goal should be to create a kind of thermal passport for every individual sport horse. The study results of the current PhD research can be used to build a scientific, evidence-based risk profile in the different disciplines valid for each horse. The combination of those thermoregulatory field exercise study results along with national and international epidemiological studies can establish a potential risk profile. This risk profile could be developed for each equine athlete prior to entering a competition, based on the individual history, biomarkers, individual temperature profile and novel technological data. An individual horse's risk profile could inform athletes, trainers and governing bodies and contribute to data-driven decisions whether the individual horse is at risk of developing EHI and is allowed to engage in competition that specific day.

Finally, the effects of global warming will further challenge the equine industry to formulate effective and evidence-based management rules. Improved welfare outcomes are needed to maintain the social license to operate. The general public expects the equine sport industry as a whole needs to start being seen as taking horse welfare seriously as their fundamental ethic. Regulating bodies such as the FEI and racing associations are aware of the need for reducing horse injuries and fatalities in the equine industry, and the management of heat stress is an important part of welfare associated strategies. The results of these and future studies in different sport disciplines and environments can support data-driven evidenced-based policies. In addition to the increase in prevalence of EHI and EHS in horses (Takahashi and Takahashi, 2020), there are only a few recommendations in place to prevent EHI. A continuing update on current findings and evidence can be used to institute a consensus with the aim of preventing heat stress and exertional heat illness in horses, for example through establishing an International Heat-stress Safety Committee. A consensus is required regarding including pre- and post-exercise temperature monitoring guidelines based on the current evidence to establish recommendations and regulations for the transportation of horses prior to and after competitions and races. These guidelines must include transportation under different weather circumstances, distances, and vehicles (trailer/float, truck and plane).

To conclude, a greater understanding of EHI and EHS in equine athletes will lead to improved welfare outcomes for all sport horses and a more positive public perception of the welfare aspects of the horse industry.

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Appendix I

Incidence and description of exertional rhabdomyolysis in endurance horses at 160 km National Endurance Cup in Australia.

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Abstract presented at the 10th *Conference of International Conference Equine Exercise Physiology (ICEEP)* 2018, *Comparative Exercise Physiology*, 14, 1; S27.

Appendix I, abstract study.

Objective: To determine the incidence and underlying mechanisms of exertional rhabdomyolysis (ER) at the Tom Quilty Cup 160km endurance ride in a cool environment. Data collection included: a questionnaire, clinical examination and blood sampling from all hospitalized horses (HH) and 12 non-metabolically compromised horses (NH) (finishers n=9, lame n=3).

Of the 164 entries, 106 (65%) horses completed the ride. Reasons for non-completion included lameness (n=39; 24%), metabolic disorders (n=8; 5%) and voluntary withdrawal (n=11;7%). The ambient temperature ranged from 6.4-10.6°C and RH 92-97%. Mean speed of finishers was 10.1 ± 1.99 km/h.

Ten (6.1%) horses were hospitalized including 6 (3.7%) horses with ER (3 Arabians, 3 part-Arabians; mean age 12.8 years) which were eliminated or withdrawn between 60-100km. All but one, travelled from interstate. Clinical signs included a tired appearance, pain/stiffness or lameness. Serum CK and AST activities in ER cases (at hospital entry, range 4-21h post-start) (median 16021iu/l; range 2149-157440 and 2365iu/l; range 735-6976, respectively) were significantly different to NH (median 561iu/l; range173-3580 and 385iu/l; range 213-1149, respectively). Three ER horses had CK exceeding 10,000iu/L. All mean serum electrolytes, lactate and creatinine concentrations were within normal limits, while urea concentration was mildly elevated in HH only (mean ± SD, 8.38 ± 2.5 mmol/L).

Conclusions: Incidence of myopathy was similar to that reported in horses completing 80km rides while the CK and AST activities were variable. Further research is needed to investigate the possible effects of long-distance travel and muscle fatigue on ER development during endurance competition.

Appendix II

A retrospective study of the incidence and associated risk factors of Exertional Heat Illness (EHI) in Thoroughbred racehorses in different states in Australia, preliminary results.

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Abstract submitted to 10th *Congress of the European College of Equine Internal Medicine (ECEIM)* 2022 – pending acceptance.

Appendix II, abstract study.

We have previously reported on the continuous monitoring of the thermoregulatory response in endurance and trotter horses. Results showed that an individual monitoring approach is of key importance, together with vigilant awareness for occurrence of delayed onset of corporeal heat build-up, especially in racehorses. An increased prevalence of heat stress, especially in racehorses, is forecasted in the face of global warming. If heat stress further progresses to Exertional Heat Illness (EHI), a fatal outcome is likely. The main objective of this retrospective study was to map-out and phenotype EHI cases in racehorses in different Australian States.

Data were obtained from online steward reports (Racing Authorities Australia). Racetrack-, horse-related- and environment-related variables, and heat-stress case data were evaluated to determine risk factors associated with EHI in identified EHI cases (1) and suspected EHI cases (2) based on inclusion criteria. A descriptive statistical analysis was performed.

During 2019-2020 racing season, 6,648 races were evaluated across 151 clubs in the four States with a total of 61,064 flat race starters. The EHI prevalence per starter (and % per race) was 0.158%, n=5 suspected (1.26%) in Northern Territory (NT), 0.049%, n=1 and n=1 suspected (0.43%) in Tasmania (TAS), 0.039%, n=2 and n=3 suspected (0.36%) in South-Australia (SA) and 0.054%, n=20 and n=2 suspected (0.50%) in Queensland (QLD). Queensland recorded the highest number in November (n=7) and January (n=4). An EHI history was recorded in four, seven, two and one case in NT, TAS, SA and QLD respectively. Poor recovery cases recorded 31(0.979%), 42(1.025%), 13(0.094%) and 96(0.234%) in NT, TAS, SA and QLD.

The wide variety in reported prevalence’s potentially reveals differences between States in recognizing and diagnosing EHI and race management. Further research in all Australian States is required to formulate evidence-based and efficient future hot weather policies to minimize occurrence of EHI.

Appendix III

Owner's survey of electrolyte supplementation in Australian endurance horses, preliminary results.

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Appendix III, abstract study.

The use of electrolyte supplementation for endurance horses is subject to discussion and evidence for their use is conflicting. The aim of this study was to investigate electrolyte supplementation amongst endurance riders in Australia. An online questionnaire was made available to riders via the Australian Endurance Riders Association (AERA) website and social media over a period of two weeks. The survey included 41 questions related to the type, dose, method, frequency and timing of electrolyte supplementation level of competition and performance data including causes of elimination. Descriptive analysis was performed and potential associations explored using Mann Whitney tests (at $P<0.05$). Endurance horses were mainly purebred Arabian horses (67.9%) aged between 5-14 years. 100% of responders (88) supplemented with electrolytes at different times: during training (67.9%), pre-competition (60.7%), during competition (85.7%) and post-competition (78.6%). Supplements were mainly used to replace electrolytes losses (37.5%) and were primarily supplied in food (78.9%) or in paste form (45.8%) while only 5.7% used salt blocks. Median dose rates were higher during competition (47.5 g) compared with training (30 g). Elimination rate was 14.3%, mainly due to lameness (75%), myopathy (7.1%) and high heart rate (3%). This pilot study suggests wide variation in electrolyte supplementation during endurance riding in Australia. However, larger numbers are required to investigate their use in more detail.

Summary

Hyperthermia is an ongoing welfare and performance issue for all horses exercising in racing and other competitive sport events. At present, little is known about the influence of core body temperature evolvement on hyperthermia in real time during different types of exercise performed in field conditions such as racing and endurance events. Consequently, it is becoming increasingly important to establish appropriate policies regarding the detection and prevention of all types of heat stress. To achieve this, a detailed view of the variability of equine thermoregulation during field exercise and recovery is essential.

To date, the vast majority of thermoregulatory studies have been conducted in indoor laboratory conditions using a treadmill and subjecting horses to specific standardized exercise tests. However, this approach cannot successfully reflect real-time field conditions. Hence, there is a need to accurately and reliably monitor equine core body temperature responses to avoid potential harm due to increasing heat load.

Chapter 1 provides a review of current research into thermoregulation, hyperthermia and exertional heat illness (EHI) in exercising horses. In addition, several temperature monitoring methods in horses are described along with some relevant methods in human athletes. However, no studies have investigated the promising continuous monitoring method involving a telemetric gastrointestinal pill (GI) that can be applied in the field in all conditions.

Chapter 2 outlines the scientific aims of the thesis.

Chapter 3 describes a study designed to evaluate the efficacy of continuous monitoring of core body temperature using the novel telemetric GI pill during real-time field exercise for the first time. The results showed that the continuous recording of the GI core temperature in exercising horses in the field using the GI pill was non-invasive, practical and accurate. Temperature fluctuations experienced during exercise and recovery are reliably recorded, and tendencies toward EHI will be easily observed during field exercise. Importantly, the GI pill has proven to be a more accurate and precise tool to monitor core thermal response when compared with serial T_{re} measurements in the field.

Chapter 4 describes the application of this novel thermoregulation monitoring method in detail. The study involved measurements conducted in both endurance horses and trotters in order to compare exercise types in real-life competitions in the field. Not only were the core body temperatures (T_c) continuously monitored during exercise and recovery, the thermoregulatory responses to the different exercise intensities were also compared. The findings of this study reported real-time temperature evolvement during real-time competition in the field. More specifically, endurance horses reached peak temperature at 75% of completion of 40 km of exercise. However, trotters reached peak temperature *always during recovery*. In addition, the T_c in endurance horses returned to baseline within 60 minutes into recovery while in 30% of trotters, T_c was still higher than 39°C at the end of recovery.

Since endurance horses are considered as 'fit to continue' competition when the heartrate (HR) is 60 beats per minute or below, the finding that the mean T_c was still 38.8 ± 0.4°C at a HR of 60 bpm is of importance. Overall, the study showed that horses have very individual thermoregulatory responses which require highly accurate monitoring no matter what type of exercise is performed in the field.

Chapter 5 investigated the usefulness of monitoring skin temperature in endurance horses. A large array of skin temperature methodologies recently used in the field is reviewed, mainly pre- and post-exercise at time points,

In this study, to evaluate if skin temperature could be used as a proxy for core temperature, the skin temperature was continuously monitored and evaluated using an infrared monitor during a real-life endurance competition. The skin temperature was compared to the GI temperature and importantly, there was no correlation between skin and GI temperature.

Appendices

Metabolic disorders in endurance horses are commonly due to fluid and electrolyte loss and may be avoided by electrolyte supplementation (**Appendix I**). The use of electrolyte supplementation for endurance horses is subject to ongoing discussion and evidence for their use is conflicting. An online questionnaire revealed that 100% of the Australian riders that responded used electrolyte supplements at different times during training, as well as before, during and after competition. Median dose rates varied and were higher during competition. Additional survey questionnaires amongst Australian and worldwide endurance riders are required for further insight into electrolyte supplementation and its impact on equine welfare.

An overview of the extent of the elimination of endurance horses due to metabolic disorders in the National Australian Cup over 160 km in a cool environment is described in **Appendix II**.

Since the prevalence and risk factors of exertional heat illness (EHI) in Australian racehorses is currently unknown, a pilot study investigating EHI is described in an abstract (**Appendix III**). Research is continuing to investigate EHI in all Australian states to further identify risk factors related to EHI in racehorses especially in the face of future unfavorable environmental changes.

Samenvatting

Hyperthermie bij paarden is een voortdurend welzijns- en prestatie probleem voor alle racepaarden en sport paarden in competitieve sportevenementen. Op dit moment is er weinig bekend over de invloed van hyperthermie op de ontwikkeling van de kernlichaamstemperatuur in real-time tijdens verschillende soorten arbeid die worden uitgevoerd in veldomstandigheden, waaronder racen en endurance training. Het opstellen van een goed beleid met betrekking tot de preventie en behandeling van hitteberoerte (EHS) en inspanningshitteziekte (EHI) wordt steeds belangrijker, vooral ook met het oog op de komende Olympische Spelen. Als gevolg van de opwarming van de aarde wordt een geleidelijke toename van de prevalentie van EHI-slachtoffers verwacht. Even zorgwekkend is het toenemende bewijs voor het feit dat EHI te weinig wordt gerapporteerd omdat alleen de meer ernstige en openlijke klinische gevallen worden herkend, waardoor veel milde gevallen onopgemerkt blijven. Aan de andere kant is het een veel voorkomende misvatting dat hitteziekte alleen paarden treft in zeer hete en vochtige weersomstandigheden.

Om de voorgenoemde uitdagingen het hoofd te kunnen bieden, is het noodzakelijk een gedetailleerd beeld te krijgen van de variabiliteit waarmee het thermoregulatoir systeem van sportpaarden reageert op enerzijds inspanningen in standaard laboratory omstandigheden, maar anderzijds, belangrijk, op inspanningen gedurende wedstrijd omstandigheden uitgevoerd in open lucht.

Tot op heden is de overgrote meerderheid van thermoregulatie onderzoeken uitgevoerd in laboratoriumomstandigheden met behulp van een loopband en het onderwerpen van paarden aan specifieke gestandaardiseerde inspanningstesten. Deze benadering kan echter niet met succes real-time veldomstandigheden nabootsen. Daarom is het nodig om de lichaamstemperatuur van paarden nauwkeurig en betrouwbaar te monitoren om mogelijke schade en arbeids-gerelateerde hittestress ziekten te voorkomen.

Hoofdstuk 1 geeft een State of the Art overzicht dat relevant is voor onderzoek naar thermoregulatie, hyperthermie en inspanningshitteziekte (EHI) bij sport paarden. Daarnaast worden verschillende methoden voor temperatuurbewaking bij paarden

beschreven, samen met enkele relevante methoden die worden toegepast bij menselijke atleten.

Hoofdstuk 2 schetst de wetenschappelijke doelstellingen van het proefschrift. Het hoofddoel van het huidige doctoraatsonderzoek was om een basislijn te creëren voor continue monitoring van de lichaamstemperatuur, om de evolutie van de kernlichaamstemperatuur tussen verschillende soorten inspanning te vergelijken en om de eerste stappen te zetten in de richting van identificatie van praktische proxy's voor het monitoren van thermo-welzijn in open lucht.

Hoofdstuk 3 beschrijft een studie die de betrouwbaarheid evalueert van een telemetrische gastro-intestinale (GI) Jonah®-pil (Phillips, VS) om de lichaamstemperatuur van paarden te monitoren tijdens real-time veldoefeningen. Voorafgaand aan gebruik in vivo in competitie, werden de pillen gevalideerd in waterbaden met drie verschillende temperaturen die relevant zijn voor de lichaamstemperatuur van het paard in rust en tijdens inspanning (37°C, 39°C en 41°C) en hun output werd vergeleken met een gecertificeerde weerstandstemperatuur detector (RTD). Vervolgens werd een veldonderzoek uitgevoerd waarbij de temperatuur data output en het tijdprofiel van de gegevensuitvoer tussen de telemetrische GI-pil en een rectaal ingebrachte temperatuursonde werden vergeleken. De pil werd toegediend via nasogastrische intubatie en de gegevens werden telemetrisch geregistreerd door middel van een externe ontvanger bevestigd in een sensor singel (Equivital®). Dit is de eerste studie die zowel in vitro als in vivo evaluatie beschrijft van de geschiktheid van een telemetrische GI-pil om kernlichaamstemperatuur (T_c) continu te monitoren bij paarden, zowel in rust als tijdens inspanning. De resultaten toonden aan dat de continue registratie van de GI-kerntemperatuur bij trainende paarden in het veld met de GI-pil praktisch en nauwkeurig was. Er was weinig tot geen dataverlies. De RTD-output toonde aan dat de GI-pil nauwkeurig was over het bereik van de waterbadtemperaturen. Ook onder praktijkomstandigheden bleek de GI-pil een betrouwbare en gebruiksvriendelijke methode om de GI-kerntemperatuur bij trainende paarden continu te monitoren. Een essentieel voordeel van dit systeem is dat de jockey in real-time toegang heeft tot de gegevens output op een digitaal apparaat zoals zijn mobiele telefoon, waardoor hij of zij gemakkelijk kan reageren op tekenen van hyperthermie of, beter nog, de trainingssnelheid in een vroeg stadium kan aanpassen.

Een belangrijke bevinding in deze studie was ook het feit dat de GI-kerntemperatuur constant hoger was dan de rectale temperatuur (T_{re}) met een gemiddeld verschil van 0,21 °C in rust en 0,36 °C tijdens inspanning. Bovendien was er een aanzienlijke vertraging van de T_{re} evolutie in vergelijking met de T_c. Dit betekent dat de opwarming van de kern van het lichaam pas in een veel later stadium gereflecteerd wordt door de waarde van de T_{re}. Het laat zien dat we onze visie op de waarde van herhaalde metingen van de T_{re} tijdens wedstrijden, wat nu klassiek gedaan wordt in de controlerende dierenartsen posten, moet herbekeken worden. Wellicht is de T_{re} geen geschikte fysiologische parameter om thermisch welzijn van sport paarden te kunnen bewaken.

In **hoofdstuk 4** wordt beschreven hoe de GI-pil onder praktijk omstandigheden is getest, met name gedurende open lucht competitie. Er is bewust gekozen om twee zeer verschillende soorten inspanningen met elkaar te vergelijken op het gebied van thermoregulatie, namelijk endurance competitie versus draver racen. Hiertoe werden de T_c-profielen van de individuele paarden geregistreerd tijdens twee verschillende soorten inspannings- en recovery periodes gedurende opeenvolgende lussen van 40 km (voor endurance competitie) of 1540 m (voor draver races). Niet alleen werd de T_c continu gemeten tijdens inspanning en herstel, maar werden ook de thermoregulerende reacties op de verschillende inspanningsintensiteiten vergeleken. Voor elke T_c -inspanning- en herstelperiode werd een temperatuur-tijdprofielcurve van T_c van ieder individueel paard geconstrueerd. De netto AUC (basislijn ingesteld bij rust T_c) werd berekend met behulp van de trapezoidale methode. Verdere analyse van de geregistreerde gegevens omvatte de berekening van de metabole warmtebelasting (H, kcal min^{-1}) en de zuurstofkosten van transport (COT - het zuurstofverbruik dat nodig is om 1 meter te verplaatsen) voor elk paard.

Een eerste belangrijke bevinding van de studie was de substantiële individuele variabiliteit in de geregistreerde thermische reacties, wat het belang onderstreept van continue en individuele monitoring van trainende paarden. Door continue monitoring kan een AUC-benadering worden toegepast die een robuustere parameter is in vergelijking met berekende HP. Endurance paarden bereikten gemiddeld hun maximale T_c bij het voltooien van meer dan 75% van een lus van 40 km; T_c keerde terug naar de basislijn binnen de verplichte hersteltijd van 60 minuten. De gemiddelde T_c was echter nog steeds 38,8 ± 0,4 °C toen de hartslag (HR) al was teruggekeerd naar 60 slagen per minuut, wat momenteel wordt gebruikt in endurance (AERA-regels) als een beslissende indicator om een paard te beschouwen als 'fit to continue'-competitie. Dravers bereikten op hun beurt hun piek T_c tijdens hun herstelperiode gemiddeld 34 minuten na het beëindigen van de race. Ook hier hadden verschillende paarden T_c-waarden boven 38,5°C met een HR die al was teruggekeerd naar 60 slagen per minuut.

Onze resultaten hebben aangetoond dat T_c-monitoring bij draverpaarden moet worden voortgezet tot ten minste 60 minuten na de race. Het laat ook zien dat paardeneigenaren die aan competitie doen in disciplines waar veel korte periodes van intense acceleratie betrokken zijn, zich bewust moeten zijn van het feit dat de lichaamstemperatuur in veel gevallen pas geruime tijd na het beëindigen van de inspanning piekt en dat paardeneigenaren daarom zeer alert moeten zijn op het thermisch welzijn van hun paard, lang na het beëindigen van de wedstrijd. Dat laatste kan van cruciaal belang zijn in de beslissing wanneer je een paard na de wedstrijd gaat transporteren.

Hoofdstuk 5 onderzocht het nut van het monitoren van de huidtemperatuur bij endurance paarden. Een praktische en niet-invasieve benadering om de thermoregulatoire respons te meten, zou de continue monitoring van de oppervlaktehuidtemperatuur (T_{sk}) kunnen zijn. Binnen de snelgroeiende wearable-industrie worden temperatuur huid sensoren voortdurend geüpgraded en leveren ze steeds data output. Wat dat betreft blijft echter de kritische vraag: hoe moeten we die data output interpreteren? Deze studie was de eerste die gelijktijdige metingen van T_{sk} met T_c heeft vergeleken met behulp van een GI-pil. Het onderzoek werd uitgevoerd bij endurance paarden tijdens competitie.

De T_{sk}- en T_c-profielen werden vastgesteld tijdens de inspanning en tijdens herstel. Hiervoor werden 13 endurance paarden uitgerust met niet-invasieve meetapparatuur (GI-pil, T_{sk}-sensor, hartslagmeter (HR) en globaal positioneringssysteem [GPS]). Volgende parameters werden geregistreerd tijdens elke lus van 40 km: T_c, T_{sk}, snelheid en HR. Een temperatuur-tijdprofielcurve van T_{sk} en T_c werd geconstrueerd voor elk individueel paard voor elke inspannings- en herstelperiode. De netto AUC (basislijn ingesteld bij rust T_{sk}) werd berekend met behulp van de trapezoidale methode. De belangrijkste bevinding was de substantiële individuele variabiliteit in de geregistreerde T_{sk}-reacties, wat het belang benadrukt van individuele monitoring van trainende paarden. De studie toonde ook geen directe correlatie aan tussen de T_{sk} - en T_c-tijdprofielen. Een interessante bevinding van de studie was de associatie van een grotere T_{sk} aan het einde van de inspanning met een significant lagere T_c aan het einde van het herstel (60 minuten). Verschillende theorieën kunnen worden overwogen om deze associatie tussen T_{sk} en T_c te verklaren: ten eerste geeft de verhoogde T_{sk} de start aan van een actieve thermoregulerende reactie om te anticiperen op de verhoogde T_c en zodra de metabole warmte (H) met succes is afgevoerd, neemt de T_c af. Dit argument kan worden gekoppeld aan het effect van afkoeling na de training, dat prominenter kan zijn wanneer T_{sk} groter is en uiteindelijk resulteert in een hogere dissipatie van metabole hitte en een verminderde T_c.

Het gebrek aan correlatie tussen T_{sk} - en T_c-tijdprofielen benadrukt dat paardeneigenaren niet op T_{sk} mogen vertrouwen bij het monitoren van trainende paarden in een koele omgeving. In alle gevallen waren de piek- T_c-waarden significant hoger dan de piek- T_{sk}-waarden, en in alle gevallen was er een significant kortere tijd tot piek- T_{sk} dan tot piek- T_c. Er kon geen significant verschil worden vastgesteld tussen delta T_{sk} bij het vergelijken van inspannings- en herstelperioden. Het is daarom de vraag of continue monitoring van T_{sk} een betrouwbare proxy is om de thermodynamische respons bij trainende paarden te monitoren. Aangezien aanvullende factoren zoals luchtbeweging en zweten T_{sk} gemakkelijk en snel kunnen veranderen zonder T_c direct te beïnvloeden, is verder onderzoek nodig naar betrouwbare monitoringmethoden.

Het werk dat in het huidige doctoraat werd uitgevoerd, fungeert als blauwdruk voor het uitvoeren van toekomstige studies bij warm weer en voor het formuleren van effectieve en wetenschappelijk onderbouwde management richtlijnen om het thermowelzijn bij sportpaarden te waarborgen. Een probleem dat steeds uitdagender zal worden in het licht van de opwarming van de aarde.

Tijdens dit doctoraatswerk werden ook bijkomende pilootstudies uitgevoerd, waarvan de analyses en data-output nog aan de gang zijn. Een overzicht van die onderzoeken is te vinden in de bijlage.

Bijlagen

Stofwisselingsstoornissen bij endurance paarden zijn vaak te wijten aan vocht- en elektrolytenverlies en kunnen worden vermeden door het gebruik van elektrolyten supplementen (**bijlage I**). Het gebruik van elektrolyten supplementen voor endurance paarden is onderwerp van voortdurende discussie en het bewijs voor het gebruik ervan is tegenstrijdig. Uit een online bevraging bleek dat 100% van de Australische endurance paardeneigenaren elektrolyten supplementen gebruikten op verschillende momenten tijdens de training, maar ook voor, tijdens en na de wedstrijd. De mediane dosering varieerde en was hoger tijdens competitie. Aanvullende enquêtevragenlijsten onder Australische en endurance ruiters wereldwijd zijn nodig om meer inzicht te krijgen in elektrolyten supplementering en de impact ervan op het welzijn van endurance paarden.

Een overzicht van de omvang van de uitschakeling van endurance paarden uit wedstrijden, als gevolg van stofwisselingsstoornissen in de Nationale Australische Cup over 160 km in een koele omgeving (op basis van bureau van meteorologie), is beschreven in **bijlage II**.

Aangezien de prevalentie en risicofactoren van hitteziekte (EHI) bij Australische renpaarden momenteel niet bekend zijn, werd een piloot studie naar EHI opgestart (**bijlage III**). Onderzoek naar EHI in de verschillende Australische staten is belangrijk om risicofactoren gerelateerd aan EHI bij renpaarden verder te identificeren, vooral in het licht van toekomstige ongunstige veranderingen in het klimaat.

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Curriculum Vitae

Curriculum Vitae – English

Lidwien (Elisabeth) E.J.M.M. Verdegaal, MVM, DVM, Dip ECEIM, Spec RNVA Eq Int Med, registered Equine Internal Medicine Specialist in The Netherlands, Europe and Australia.

Lidwien-Elisabeth Verdegaal obtained her Bachelor of Veterinary Medicine (MVM) in 1993 and obtained her Doctor of Veterinary Medicine (DVM) at Utrecht University, The Netherlands, in 1996. Once qualified, she worked in a mixed practice in the Southern part of the Netherlands (Diessen) for two years before returning to Utrecht University to specialize in Equine Internal Medicine. Lidwien completed her residency in Equine Internal Medicine at Utrecht University's Faculty of Veterinary Sciences. She qualified as an Equine Medicine Specialist with the Royal Dutch Association of Veterinary Specialists and is a Diplomate of the European College of Equine Internal Medicine (ECEIM) accredited by passing the ECEIM Board exam in 2009.

Lidwien's focus on equine medicine led to 8 years of valuable experience as an Equine Medicine Specialist in private clinics working for the Jordanian Royal Family as well as establishing and working at a new equine referral hospital in Kuwait. She was a member of endurance and jumping FEI committees in Jordan and Kuwait where she developed her deep ongoing interest in thermoregulation and heat stress.

Since 2011, Lidwien has held tenure as a Senior Lecturer in Equine Medicine with the University of Adelaide's School of Animal and Veterinary Science in South Australia. She has contributed significantly to the development of the new veterinary medicine School at the University of Adelaide. She was part of endurance and jumping FEI veterinary committees and also an official FEI endurance and eventing competition veterinarian in Australia. Her lectures and clinic sessions are noted for her passion for her subject and enthusiasm in encouraging the next generation of veterinarians. As a firm believer in a healthy work-life balance, she ensures having quality time for family, friends, and the occasional gallop.

With over 25 years of experience to draw on, Lidwien Verdegaal is dedicated to performing high quality research and improving the welfare of all sport horses. Her current PhD research is the first joint-PhD agreement between the Ghent and Adelaide University, supervised by Prof. Catherine Delesalle of the Research group of Comparative Physiology, and Prof. Gordon Howarth and Dr. Todd McWhorter, both from the School of Animal & Veterinary Sciences at the University of Adelaide. The PhD research focuses on thermoregulation and metabolic disorders and is likely to have a worldwide impact on how heat stress is managed in real-life equine sport and racing conditions. This research work is the blueprint for further cooperation with the Research Group of Comparative Physiology of Prof. Delesalle.

Lidwien Verdegaal is the author of 13 peer reviewed publications of which 10 as first author. She was invited speaker at 19 conferences.

Curriculum Vitae – Dutch

Lidwien (Elisabeth) E.J.M.M. Verdegaal, MVM, DVM, Dip ECEIM, Spec RNVA Eq Int Med, is geregistreerd als Equine Internal Medicine Specialist in Nederland, Europese college en Australië.

Lidwien behaalde haar Doctor of Veterinary Medicine (DVM) in Diergeneeskunde in 1996 aan de Universiteit Utrecht, Nederland. Eenmaal afgestudeerd werkte ze gedurende 2 jaar in een gemengde praktijk in het zuiden van Nederland (Diessen) voordat ze terugkeerde naar de Universiteit Utrecht om daar een residency interne geneeskunde paard te volgen. Ze is Europees Diplomate Inwendige Ziekten Paard: European College of Equine Internal medicine (ECEIM) geaccrediteerd door het behalen van het ECEIM Board examen in 2009. Lidwiens' focus op paardengeneeskunde heeft geleid tot het opbouwen van 8 jaar ervaring als specialist in inwendige ziekten paard in privéklinieken voor onder andere de Jordaanse Koninklijke familie en het opzetten van een nieuwe paardenkliniek in Koeweit. Ze was lid van de FEI- commissie voor endurance en jumping wedstrijden in Jordanië en Koeweit waar ze haar interesse voor thermoregulatie en hittestress ontwikkelde. Sinds 2011 is Lidwien werkzaam als Senior Lecturer in Equine Medicine aan de Universiteit van Adelaide in Australië. Ze heeft een belangrijke bijdrage geleverd aan de ontwikkeling van de nieuwe diergeneeskundige opleiding aldaar. Ze was onderdeel van het veterinaire FEI endurance en eventing team en behandelend dierenarts tijdens talrijke endurance events. Haar colleges staan bekend om de passie waarmee ze gegeven worden. Lidwien is zeer enthousiast om de volgende generaties dierenartsen op te leiden. Daarnaast is er ruimte voor quality time voor familie, vrienden en een occasionele galop.

Het huidige promotieonderzoek is de eerste joint PhD die werd gecreëerd tussen de Universiteit Gent en de Universiteit van Adelaïde Faculteit Diergeneeskunde, en begeleid door Prof Catherine Delesalle van de Onderzoeks groep Comparatieve Fysiologie, en Prof Gordon Howarth en Dr Todd McWhorter, beiden van de School Animal and Veterinary Science, Universiteit van Adelaide. Haar promotieonderzoek richt zich tot het in kaart brengen van de thermoregulatie bij sportpaarden in verschillende sporttakken “in the field”, en dus niet in een laboratorium setting. De resultaten van dit onderzoek hebben geleid tot nieuwe inzichten op het vlak van correct monitoren en waarborgen van thermo-welzijn van sportpaarden, zowel voor, tijdens als na wedstrijden en vormen de blauwdruk voor verdere samenwerking met de Research Group of Comparative Physiology van Prof. Delesalle.

Lidwien Verdegaal is auteur van 13 wetenschappelijke publicaties waarvan 10 als eerste auteur. Zij was spreker op 19 symposia.

Publications Elisabeth-Lidwien (EJMM) Verdegaal

2022

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2018

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2017

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2003

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Verdegaal E-L.J.M.M., Howarth G.S., McWhorter T.J., and Delesalle C.J. Delesalle. Continuous Monitoring of the Thermoregulatory Response in Endurance Horses and Trotter Horses During Field Exercise: Baselining for Future Hot Weather Studies. Oral presentation at Meeting, *Australian Society for Medical Research (ASMR),* Adelaide Convention Centre, Adelaide, Australia, June 2022. Selected as a one of the four finalists for the Ross Wishart award.

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2020

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Gehring, R., Florschütz, T., Theelen, M. J. P., **Verdegaal, E-L.J.M.M.**, and Vendrig, J. C. Towards a population pharmacokinetic model of gentamicin in septicaemic neonatal foals. Oral presentation at International *Antimicrobial Agents in Veterinary Medicine (AAVM)* Congress, 2020.

2018

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2017

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2016

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2015

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2014

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2004

Verdegaal E.J.M.M. and van der Kolk J.H. Salivary cortisol concentration in healthy aged horses, horses with hirsutism and horses with colic. Oral presentation at International at *British Equine Veterinary Association Congress (BEVA)*, Sept. 2004.

2003

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