Title:

Validity of a wearable sweat rate monitor and routine sweat analysis techniques using heat acclimation

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Abstract

Introduction: the aim of this study was to assess the validity of a novel wearable sweat rate monitor against an array of sweat analysis techniques which determine sudomotor function when exercising moderately under heat stress. Construct validity was determined utilising a 5-day short-term heat acclimation (STHA) intervention. **Methods**: Nineteen healthy individuals (age: 41 ± 23 years, body mass: 74.0 ± 12.2 kg, height: 174.9 ± 6.9 cm) [male; n = 15, female; n = 4] completed nine trials over a three-week period, in a controlled chamber set to 35°C, 50% relative humidity for all sessions. The pre and post-trials were separated by five consecutive controlled hyperthermia HA sessions. Sweat analysis was compared from pre and post-trial, whereby whole body sweat rate (WBSR) was assessed via pre and post nude body mass. Local sweat rate (LSR) was determined via technical absorbent patches (TA) (weighed pre and post) and a novel wearable KuduSmart® (SMART) monitor which was placed on the left arm during the 30-minutes of exercise. Tegaderm patches, used to measure sweat sodium chloride conductivity (SC), and TA patches were placed on the back, chest and forearm for the 30-minutes cycling. **Results:** Sudomotor function significantly adapted via STHA ($p < 0.05$); demonstrated by a WBSR increase of 24%, LSR increase via the TA method (back: 26%, chest: 45% and arm: 48%) and LSR increase by the SMART monitor (35%). Finally, SC decreased (back: -21%, chest: -25% and arm: -24%, $p < 0.05$). **Conclusion:** All sweat techniques were sensitive to sudomotor function adaptation following STHA, reinforcing their validity. The real time data given by the wearable KuduSmart® monitor provides coaches and athletes instant comparable sudomotor function feedback to traditional routinely used sweat analysis techniques.

Key words: sweat analysis, short-term heat acclimation, sudomotor function, validity, heat stress.

1. Introduction

It is reported that evaporation of sweat is the most influential autonomic thermoeffector in humans (Gagnon and Crandall, 2018), accounting for 80% of heat dissipation during temperate exercise. Humans have an extensive and adaptive capacity for the active secretion of sweat (Taylor and Machado-Moreira, 2013), which is usually evaluated using whole-body techniques or methods localised to precise anatomical locations. Sudomotor function is most commonly reported to be via; body mass changes (whole body sweat rate; WBSR), local sweat rate (LSR) and sweat composition / conductivity (SC) (Gagnon and Crandall, 2018), in a plethora of literature whereby thermoregulatory function can be diagnosed, monitored and intervened (Morris et al., 2013).

The ability to measure analytes, specifically those related to sudomotor function, in real time via wearable physiological monitoring technologies is of growing interest to researchers and practitioners (Gao et al., 2017; Pham et al., 2020). Recent advances in technology have enabled an enhanced manufacturing of products that are non-invasive and continuously review and investigate analytes, for enhanced monitoring of health and exercise diagnostics and physiological assessment (Gao et al., 2016). A novel sweat rate monitor (KuduSmart®) was developed by Crossbridge Scientific^{LTD} which measures LSR ($mg.min^{-1}.cm^{-2}$) in real time linked to a computer tablet. Numerous wearable devices provide precise measurements that demonstrate sufficient reliability for experimental and clinical purposes. This was recently reinforced for the KuduSmart® (Relf *et al.* 2019), demonstrating an error of 0.08 $mg.min^{-1}.cm^{-2}$, coefficient of variation of 13.5% and intra-class correlation of 0.88.

Wearable devices for sudomotor function have been demonstrated to be placed in the same anatomical location for data collection (upper arm), as the KuduSmart (Guinovart et al., 2013; Pham et al., 2020). However, the focus of these sensors, to date, have been for electrolyte concentrations ((Rose et al., 2015; Koh et al., 2016; Choi et al., 2017; Alizadeh et al., 2018; Choi et al., 2018), not local sweat rate. Therefore, a complete wireless sensor with wearability that is low cost, robust and communicates in real-time to an iPad, which automatically enforces a maximum time-resolves readings of sweat rate, is vital in the current sports and health monitoring market.

Although an array of other sweat analysis techniques have been deemed reliable alongside the KuduSmart® monitor (Relf et al., 2019), limited evidence exists of their validity (Lemon et al., 1986; Boisvert and Candas, 1994). Whilst reliability of a measurement is important in research designs, it cannot guarantee valid conclusions (Krippendorff, 2008), and therefore the occurrence of Type II errors (Hayden et al., 2004). To ensure sweat analyses techniques are measuring what they claim to represent, three types of validity can be assessed; logical, criterion and construct validity (Currell and Jeukendrup, 2008). It is universally accepted that heat acclimation (HA) induces sweat adaptations, enhancing evaporative cooling (Armstrong and Maresh, 1991; Daanen et al., 2018). When investigating sudomotor

responses, considering the abundance of evidence surrounding HA and the adaptations associated with it, construct validity via pre and post intervention is most appropriate.

Humans have a vast capacity to adapt anatomically, physiologically and biochemically to a wide range of environmental states (Taylor, 2014). Physiological adaptation occurs through repeated stress application, whereby the regulatory mechanisms ensure stability of the internal environment and optimal functioning. Reponses to HA appear dependent upon the magnitude and rate of physiological change (Tyler et al., 2016). Although all methods of HA serve individual merit and purpose (Garrett et al., 2011), the isothermic method is becoming increasingly more popular owing to the forcing function that increases in proportion to adaptations to reach and sustain a target core temperature (frequently 38.5°C) (Garrett *et al.,* 2011) and more efficient work ratio (Gibson et al., 2015). Traditionally, HA protocols consist of at least 10 exposures, however, as little as four exposures have reported to confer partial heat adaptation (Sunderland *et al.,* 2008).

The commonly reported positive adaptations from HA include; increased WBSR (19 ± 21%), LSR and plasma volume (4.3 ± 4.7%), alongside reductions in sweat sodium (22 ± 16 $mmol.L^{-1}$), core temperature (0.18 ± 0.14°C at rest, 0.32 ± 0.24°C exercising) and heart rate (6 ± 5 bpm at rest, 16 ± 6 bpm exercising) (Tyler et al., 2016; Buono et al., 2018). However, as highlighted in the meta-analysis, adaptations to the heat are varied and individual, which are usually related to a number of factors; age, sex, training status, with the optimal approach still unknown (Tyler et al., 2016). Moreover, there is a lack of clarity on the time course for some sudomotor adaptations, particularly surrounding sweat rate, where improvements have been found in STHA (SR (+ 5 ± 11 %, n = 101); however, large increases were only observed following medium (+ 29 ± 29 %, n = 122) and long-term (+ 33 %, n = 11) approaches (Tyler et al., 2016). The consensus currently is that sweat rate is one of the last physiological mechanisms to adapt (Périard et al., 2015), however with reports that 75-80% of adaptation is induced in 4-7 days (Pandolf, 1998), there is growing popularity of STHA (Willmott et al., 2016; Tyler et al., 2016).

Following HA, initiating of sweating is shifted, inducing onset of sweating at a lower core temperature (-0.28 ± 0.21°C)(Tyler et al., 2016), which enhances the defence of the body with a lower core body and skin temperature rise during exercise (Gagnon and Crandall, 2018). Sweat is more dilute after HA, resulting in better conservation of salts and plasma volume and allowing evaporation to occur at a lower vapour pressure (Pryor et al., 2018). Na^+ in sweat can be conserved following repeated heat exposures (Buono et al., 2018), to as much as a 50% decrease following 10-days of heat acclimation (Kirby and Convertino, 2017). WBSR is the central sudomotor response reported in all HA literature, evidence surrounding LSR and sweat conductivity/composition is far less prevalent, especially for STHA. Sweat rate, and therefore, heat loss capacity is highly individualised, due to the quantity and size of sweat

glands among different people (Shibasaki et al., 2006; Tansey and Johnson, 2015). Thus, with the error of each technique and reliability now established for a range of different sweat analysis markers (Relf et al., 2019), enhanced data surrounding sudomotor alterations via HA, specifically LSR is warranted to provide validation in the literature.

Therefore, the aim of this study was to assess the validity of a novel wearable sweat rate monitor against an array of accepted sweat analysis techniques by means of 5 days STHA. It was hypothesised that sweat rates will increase and sodium chloride concentrations will decrease, demonstrating sensitivity to sudomotor adaptation, following STHA and acceptable validity of techniques. It was also hypothesised that each sweat analysis technique would exceed the technical error of measurement previously acknowledged by Relf *et al.* (2019); WBSR > 0.04L/hr^{-1}, KuduSmart® > 0.08 mg.min^{-1}.cm^{-2}, LSR > 0.13 mg.min^{-1}.cm^{-2}, SC > 8 mmol.L^{-1} and be significantly different ($p < 0.05$) between trials.

2. Methodology

2.1 Participants

Nineteen, physically active un-acclimated individuals (age: 41 ± 23 years, body mass: 74.0 ± 12.2 kg, height: 174.9 ± 6.9 cm) [male; n = 15, female; n =4] volunteered and provided written and informed consent for participating in this study. Experimental procedures conformed to the principles of the Declaration of Helsinki (2013) and approval of the experimental design and procedures was obtained from the Institution's Ethics Committee. Participants had not experienced hot air temperatures (>25°C) for > 3 months and were non-smokers with no prior health conditions. Moreover, the females were not on any form of oral contraception.

2.2 Pre-trial standardisation

Experimental trials were conducted at the same time of day, to account for changes due to circadian rhythms (Kräuchi and Wirz-Justice, 1994). Prior to testing all participants were required to abstain from alcohol, caffeine and exhaustive exercise for 48 hours. They were also requested to consume similar diets in the 48 hours before each trial and to arrive at the trial in a euhydrated state; determined by urine osmolality (UOsm) <700 mOsm.kg^{-1} (Advanced Micro Osmometer 3300, Vitech Scientific Ltd., UK) and specific gravity (Usg) <1.020 (URC-Ne handheld refractometer, ATAGO CO Ltd., Japan) (Sawka et al., 2007). Anthropometric data was collected upon arrival; stature measured using a stadiometer (Detecto, USA) and nude body mass (NBM) to the nearest 0.01kg using digital scales (Adam, GFK 150, USA).

2.3 Experimental design

Participants completed nine trials over a three week period (Figure 1). Two preliminary and two post-trials were separated by five consecutive days of short-term heat acclimation (STHA); all trials took place in a controlled environmental chamber (TISS, Hampshire, UK) set to 35°C, 50% relative humidity.

2.3.1 Pre and post-trial 1

The first pre and post-trial involved a ventilatory threshold test to determine the intensity for the 30-minutes exercise for pre and post-trial 2. Participants rested for 30-minutes in the chamber to allow for stabilisation before cycling on a recumbent bike (BC50; Cardiostrong, Germany) starting at 25W for those >30 years (increasing by 15W)(Waldock et al., 2018) and 50W (25W increments) for <30 years for 21-minutes, with each 3-minute stage increasing in intensity. At 2-minutes into each stage, ~45-seconds of expired air was collected using open-circuit spirometry. Gas samples collected were analysed using a gas analyser (Servonex Xentra 4100, Servonex International Ltd, Crowborough, UK and Buhler Gas Sample Dryer, Type PKE4, Buhler Technologies GmbH, Ratingen, Germany). The gas analyser was calibrated using Nitrogen (N) and a mixture of known gases of oxygen (O_2) and carbon dioxide (CO_2) quantities (British Oxygen Company, UK). Samples were zeroed with 100% N, then set with 80% N, 15% O_2 and 5% CO_2, followed by 80% N 18% O_2 and 2% CO_2. Gas temperature and volume were then sampled using a fixed flow pump model Dymax 30 (Charles Austin Pumps Ltd., UK) and dry gas meter (Harvard Apparatus Ltd, Kent, UK).

2.3.2 Pre and post-trial 2

Participants rested for 30-minutes, then completed 30-minutes steady-state cycling on the recumbent bike at a relative intensity of 3.5W.kg^{-1} metabolic heat production (using the equation by Nishi, (1981)). Sweat analysis and heat adaptations following STHA were determined by comparing the data collected from pre to post-trial 2. Post-trial 2 followed the same procedure, however with a re-prescribed intensity from post-trial 1, to account for any training effect from the STHA (Willmott et al., 2018).

2.3.3 STHA protocol

STHA followed an isothermic model (Gibson et al., 2015; Willmott et al., 2016). Participants cycled (Monark 620 Ergomedic, Varberg, Sweden) at differing intensities for males (2.3W.kg^{-1} if <30 years, 1.5W.kg^{-1} if >30 years) and females (2W.kg^{-1} if <30 years, 1.2W.kg^{-1} if >30 years) to achieve a target core temperature (T_{re}) rise (1.5°C above rest or absolute of 38.5°C) in the first 60-minutes of exercise. They then rested and/or cycled to maintain this target for the remaining 60-minutes. Participants were then cooled to within 0.5°C of resting T_{re} measurements.

INSERT FIGURE 1 HERE*

2.4 Physiological measurements

T_{re} was monitored continuously throughout trials using a single use rectal probe (449H, Henleys Medical, Hertfordshire, UK), self-inserted 10cm past the anal sphincter. A heart rate (HR) monitor (Polar FT1, Polar Electro, Finland) was affixed to the chest. Both HR and T_{re} were taken at rest and 5-minute intervals throughout trials.

Four methods of sweat analysis were assessed in this study; whole body sweat rate (WBSR), local sweat rate via the technical absorbent patches (TA) and the KuduSmart® monitor (Crossbridge Scientific Ltd., UK) (SMART) (Figure 2) and sweat conductivity (SC) to give composition via the Tegaderm absorbent patches and Sweat-Chek™ analysis. WBSR was assessed via NBM taken pre and post the trial, and reported in $L.hr^{-1}$. The Tegaderm (Tegaderm™ + Pad, 3M Health Care, St Paul, MN, USA) and TA patches (Technical absorbents, Grimsby, UK) were placed on the designated sites for the 30-minutes cycle during the pre and post-trial 2. Both Tegaderm and TA patches were placed on the dorsal surface of the three sites (lower back, chest and forearm) and standardised for all participants. Locations of both TA and Tegaderm patches are described in full detail by Relf *et al.* (2019).

The KuduSmart® monitor stabilised in the chamber for 10-minutes prior to collection. It was placed on the left arm, 6 cm above the antecubital fossa for the duration of the 30-minute cycle. The tightness of the strap (how many holes remained) was recorded and replicated for pre and post-tests. To determine LSR, an average of the 30-minutes Kudu score was taken, multiplied by the conversion score and minus the individual offset (~0.4) ([Kudu score – offset]*0.18]), yielding values in $mg.min^{-1}.cm^{-2}$.

*** INSERT FIGURE 2 HERE****

2.5 Statistical analysis

All data are reported as mean ± standard deviation and assessed for normality and sphericity prior to analysis. Between-trial comparisons for all sweat measures were analysed using a paired samples *t-test*. All data were analysed using SPSS (Version 25.0) and statistical significance was accepted as $p < 0.05$.

3. Results

INSERT TABLE 1 HERE*

INSERT TABLE 2 HERE*

3.1 Whole body sweat rate

WBSR significantly increased pre to post HA intervention by 24%, $p = 0.003$ (Figure 3).

INSERT FIGURE 3 HERE*

3.2 LSR

Local sweat rate increased for all three sites assessed post HA intervention; back (+ 26%, $p = 0.002$), chest (+ 45%, $p = 0.001$) and arm (+ 48%, $p = 0.001$) (Figure 4) and the KuduSmart® (+ 35%, $p = 0.004$) (Figure 5).

INSERT FIGURE 4 HERE*

INSERT FIGURE 5 HERE*

3.3 Sweat conductivity

Sweat sodium chloride significantly decreased on all sites examined post HA intervention; back (-21%, $p = 0.006$), chest (-25%, $p = 0.000$) and arm (-24%, $p = 0.001$) (Figure 6).

INSERT FIGURE 6 HERE*

4. Discussion

Reliability of any variable is critical as it indicates the biological and technical variation of the measure and can reduce the risk of a Type II error occurring (Atkinson and Nevill, 1998). Although this is important, without validity, a measure is deemed impractical. Therefore, the aim of this study was to assess the validity of a novel, wearable sweat rate monitor and an array of sweat analysis techniques by means of 5 days STHA. We can accept the alternate hypothesis, as sweat rates increased alongside decreased sodium chloride concentrations, demonstrating sudomotor adaptation following STHA (Chalmers *et al.*, 2014; Tyler *et al.*, 2016; Pryor *et al.,* 2018).

4.1 Wearable sweat rate monitor

Similar to previous research, our new device design presents a proof-of-concept approach for an inexpensive custom alternative to bulky devices for wearable non-invasive health monitoring (Pham et al., 2020). To our knowledge, there is no existing evidence surrounding the validity of a real time sweat rate monitor via the construct of HA. Following the appraisal of good reliability (Relf et al., 2019), the novel sweat rate monitor increased LSR from 0.61 to 0.83 $mg.min^{-1}.cm^{-2}$. When taking into account its error of 0.08 $mg.min^{-1}.cm^{-2}$, this concludes an interventional increase in LSR by 0.14 $mg.min^{-1}.cm^{-2}$. The previous concern of over-estimation of this monitor compared to the arm site (Relf *et al.*, 2019) is absent in the current study, however, a temperature gauge would be an important addition to the KuduSmart® to monitor skin temperature during studies.

This non-invasive device, likewise to others among the literature has the same anatomical location placement on the body (Pham et al., 2020), has been indicated to respond well and accurately to changes in skin conductance. However, this device has only been assessed in steady-state exercise and therefore the accuracy is still unknown and requires investigating in non-steady state exercise. Providing validity to this monitor paves the way for future research, as it is more user-friendly than other methods of sweat rate analysis commonly used within the literature (absorbent methods or ventilated capsule), whilst also giving real-time data to a tablet for athletic, healthy and clinical populations.

4.2 Whole body sweat rate

WBSR significantly increased from 0.90 ± 0.25 to 1.11 ± 0.27 $L.hr^{-1}$ (+ 24%), with recent findings from our laboratory reporting the typical error of measurement (TEM) to be $0.04L/hr^{-1}$ and a coefficient of variation (CV) of 10.2%, we are therefore confident in the changes from HA to be interventional and not from the error of the technique. This follows the same trend as findings from Willmott *et al.* (2018)'s 5 day HA protocol, in similar conditions (38°C, 20%RH) whereby T_{re} was unchanged but larger increases in WBSR (+35%) shows thermosensitivity was induced via higher sweat gain following HA. Current WBSR increases can be explained by using an isothermic model and matching exercise-heat dose (duration, intensity) to produce a more significant heat strain and maximising the time spent at the targeted T_{re} (60-minutes, 1.5°C above rest or an absolute of 38.5°C). It has been evidenced that a change of > 0.2 $L.hr^{-1}$ is seen to be meaningful amongst HA studies (Willmott et al., 2015), thus it can be confirmed that changes in WBSR presented here are meaningful and statistically different to reinforce the construct validity elicited.

Petersen *et al.* (2010) demonstrated no increase in WBSR (1.3 ± 0.3 to 1.2 ± 0.2 $L.hr^{-1}$) after 4 days of HA in 30°C, 60%RH amongst cricketers, similar to Sunderland *et al.,* (2008). However, the lack/absence of adaptation in these studies may be explained by the experimental design; with non-consecutive HA days (Sunderland *et al.,* 2008), a lower ambient temperature for HA sessions (30°C, 24-60%RH) and intermittent sprints lasting 30-45-minutes, resulting in a lack of stimulus (heat stress

and duration at increased core temperature) for adaptation to occur. This has been supported by others suggesting that eight sessions of HA is required for a plateau in sweat rate adaptation (Armstrong and Maresh, 1991), however again this may be limited by a non-adequate stimulus provided.

A meta-analysis from Tyler *et al.* (2016) indicated much smaller adaptations to WBSR following STHA (+ 5 ± 11%, n = 101) compared to this study, with much larger increases found with medium-term (+ 29 ± 29%, n = 122) and long-term HA (+ 33%, n =11). However, the studies included in this meta-analysis varied from ~4-7 sessions, conditions of 30-48C, 20-90%RH, but the majority lasted <60 minutes per session. It was concluded that strong positive relationships between SR and duration, ambient temperature and frequency of the HA regimen are prevalent. This study used an isothermic model, whereby participants remained at the target core temperature for 60-minutes, which may explain the greater adaptations found.

4.3 Local sweat rate

Unlike the abundance of evidence informing WBSR responses from HA, for LSR the evidence is sparse. Taking into account the issues surrounding the over reliance on a variable's error by its CV % of those with smaller units (Atkinson and Nevill, 1998), our current study clearly demonstrates changes that are larger than the CVs already demonstrated in past studies (Back; 26 vs 20.5% error, Chest; 45 vs 18.6% error, Arm; 48 vs 12.6% error and KuduSmart®; 35 vs 13.5% error). In absolute terms, the smallest detectable difference for TA method of LSR was 0.12mg.min^{-1}.cm^{-2} (Morris et al., 2013), and 0.04-0.13 mg.min^{-1}.cm^{-2} (Relf et al., 2019), which all sites have surpassed (Table 2). Findings were reinforced by Patterson et al., (2004) who concluded when exposed to a sufficiently powered and sustained acclimation, only 6 controlled hyperthermia sessions were required to increase LSR 33 to 69% (thigh-forearm), with no changes from day 8 to 22, highlighting a rapid sudomotor response, quicker than reinforced in the literature (Tyler et al., 2016).

4.4 Sweat conductivity

HA rapidly enhances sodium ion (Na^+) reabsorption from the duct of the eccrine sweat gland (Morgan *et al.,* 2004), playing an integral role in restoration of fluid balance (Buono et al., 2018). There is a plethora of information evidencing that a more dilute sweat can be more readily evaporated due to the resultant reduction in cutaneous water vapour pressure and as such induces heat adaptation via enhanced heat dissipation (Taylor, 2014). A recent meta-analysis (Tyler et al., 2016) informs that SC is not an essential measure in all studies *vs* WBSR, T_{re} or HR, however amongst the meta-analysis the average reductions are demonstrated to be ~ -22 ± 16 mmol/L1, n = 92, for Na^+, and -13 ± 13 mmol/L, n = 11, for Cl^-. It has been postulated that greater conservations in Na^+ in sweat can be elicited following

repeated heat exposures (Chinevere et al., 2008; Buono et al., 2018), to as much as a 50% decrease following 10-days of HA (Kirby and Convertino, 2017), which is thought to be a consequence of aldosterone responsiveness and increasing the expressions of the epithelial sodium channels (Buono et al., 2018).

There is a strong notion among the literature that longer term HA will reduce Na^+ SC more so, which is reinforced by Willmott *et al.* (2018) when comparing 5 days (-13 ± 13 mmol.L^{-1}) to 10 days HA (-27 ± 19 mmol.L^{-1}). However, there is evidence to support adaptations after short time frames such as STHA, where SC decreased by 19% after just two days of treadmill walking in 40°C, 40% (Buono et al., 2018). This study was amongst the first in the literature to express such quick responses after HA, however not unexpected, as Sato and Dobson (1970) reported that after a single intradermal injection of aldosterone, sweat Na^+ reduced by 15%. Similar reductions in SC of the forearm were observed by Petersen *et al.* (2010) employing only 4 days HA; 78 ± 12 to 63 ± 10 mmol.L^{-1}, *vs* 71 ± 15 to 54 ± 13 mmol.L^{-1} in this study. Enhanced sodium reabsorption via reduced concentrations in the sweat has also been evidenced by Racinais *et al.* (2012) with 18% reduction after 6 days and WBSR increase of 34% similar to the study presented here with no significant decrements in T_{re}, T_{skin} or HR. SC reductions for these STHA studies are greater than the associated error with the three sites (absolute 4-8 mmol.L^{-1} and relative ~10-15% CV (Relf et al., 2019)) and so we can endorse these as valid.

It is important to note the lack of investigation of the chest site among the literature in recent years (Kirby and Convertino, 2017), even though it correlates consistently to other body areas whilst representing mean sodium concentration of total body sweat (Peart, 1965). As a result, the authors are aware they are relying solely on the literature by Relf *et al.* (2019) demonstrating the error presented at this local site (4 mmol.L^{-1} and 7.3% CV). Consequently, the validity of this measurement at the chest can be confirmed after STHA due to the reduction of SC by 25% and > 4 mmol.L^{-1} (Table 2).

The lack of sensitivity of this sweat conductivity technique using the Sweat-Chek™ system was highlighted by Relf *et al.* (2019) who demonstrated (3/14 participants did not sweat enough to gain a sample for all three sites, and 8 for the forearm), this was also found in this current study whereby no samples were measurable for two participants (one for all 3 sites and one for 2/3 sites). It is interesting to note that these were males (>65 years) and so emphasises the lack of sensitivity for this technique for populations with reduced / challenged sudomotor function (Holowatz et al., 2010).

4.5 Limitations

It is plausible that the lack of adaptation found in T_{re}, but enhancements in sudomotor function, may be explained by the experimental design (Figure 1). With the post-trials occurring 1-2 days apart after the cessation of HA, this lead to a 4-5 days gap before the last post-trial where variables were assessed for

adaptation (post-trial 2). It is acknowledged that for every day without heat exposure ~2.5% of adaptations are lost (Daanen et al., 2018), therefore there was a potential ~10-15% loss. Moreover, it has been reported that the first adaptations to occur via HA are HR and core temperature, which are also typically found to decay fastest (Pandolf *et al.,* 1977).

Moreover, taking into account the age of some of the participants, it is important to note the difficulty of completing HA with an ageing population. Expressed in the first instance by Daanen and Herweijer, (2015), elderly participants reported that it was very difficult to complete the one hour acclimation program and with over half the cases the original 80W intensity had to be reduced by ~30W. Taking this into account and following extensive pilot work, it was apparent that the individuals >60 years in the current study could not work as hard as the 'young' group to gain a core temperature increase to 38.5°C, nor was it ethically viable (rise of 2°C above resting levels). However, greater HA adaptation was displayed in this current study due to potentially three factors when comparing designs; a higher age pool (> 75 years), just females and only 3 days of one hour stimulus (Daanen and Herweijer, 2015). As adaptation has been elicited in just 4 days by Sunderland et al., (2008) we can conclude that for further adaptation to occur a larger stimulus needs to be applied to those > 60 years (more HA days).

With four female participants included, the authors are aware there are potential limitations for not controlling for the menstrual cycle, however due to the longevity of the experimental design, crossover between phases was inevitable (Figure 1). It has been demonstrated that there is a different temporal pattern to HA between sexes, informing both sexes responded to STHA, though, females needed longer for thermoregulatory and cardiovascular stability (Mee et al., 2015). However, this study found similar adaptations when isolating sexes and comparing alterations in the four females to the males (Table 2). With the trend for a higher LSR on the chest site for females prevalent and lower LSR and WBSR compared to males, this is in line with previous research (Horstman and Christensen, 1982; Havenith et al., 2008). Moreover, there is controversy into the effects of menstrual cycle on core temperature, as to whether this would have affected pre or post-trial resting T_{re}. It has been reported that during the luteal phase of the menstrual cycle, resting T_{re} can be raised by as much as 0.3-0.6°C (Pivarnik et al., 1992; Marsh and Jenkins, 2002), yet the consensus of the implications remains equivocal (Notley et al., 2019), with evidence on females significantly under-represented in the literature (Sims and Heather, 2018). Nevertheless, there are a handful of studies investigating females whereby testing has been performed in the follicular phase (Sunderland *et al.,* 2008; Mee *et al.*, 2015; Relf *et al.*, 2017; Buono *et al.*, 2018), however due to the experimental design and length this was not implemented in the current study.

4.6 Conclusion

All sweat techniques are deemed valid as they successfully identified sudomotor function adaptations following STHA. The real time data given by the wearable KuduSmart® monitor provides instant and comparable sweat analysis detail than routinely used sweat analysis techniques and therefore may be of use to determine differences in populations and interventions in future research and be used in more practical, field-based settings.

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List of Tables

Table 1. Phenotypic data for rest and at the end of the exercise period of pre and post-trial 2 following STHA. Data are presented as Mean ± SD. * denotes significance between trials ($p < 0.05$).

		Pre-trial	Post-trial
T_{re} (°C)	**Rest**	37.08 ± 0.46	37.08 ± 0.41
	End exercise	37.44 ± 0.34	37.50 ± 0.33
HR (beats.min^{-1})	**Rest**	68 ± 13	62 ± 10*
	End exercise	120 ± 19	116 ± 18
T_{skin} (°C)	**Rest**	30.51 ± 0.79	30.49 ± 0.76
	End exercise	35.74 ± 0.68	35.49 ± 0.71
RPE	**Rest**	7 ± 1	7 ± 1
	End exercise	12 ± 2	11 ± 2*
TS	**Rest**	5 ± 1	5 ± 0
	End exercise	6 ± 1	5 ± 0*
TC	**Rest**	2 ± 0	2 ± 0
	End exercise	3 ± 1	3 ± 1
WBSR (L.hr^{-1})		0.90 ± 0.36	1.11 ± 0.54*

Table 2. Sweat analysis for all techniques pre and post HA intervention (Mean ± SD), for all participants and separated by sex. Data are presented as Mean ± SD. * denotes significance between trials for the whole data set ($p < 0.05$).

		Mean		Females		Males		Young		Elderly	
		Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**
WBSR (L.hr^{-1})		0.90 ± 0.25	1.11 ± 0.27	0.78 ± 0.37	1.15 ± 0.84	0.93 ± 0.36	1.10 ± 0.48	1.07 ± 0.35	1.33 ± 0.60	0.66 ± 0.21	0.81 ± 0.25
LSR (mg.min^{-1}.cm^{-2})	**Back**	1.74 ± 0.58	2.20 ± 1.01	1.74 ± 1.06	2.00 ± 1.24	1.74 ± 0.84	2.25 ± 0.83	1.86 ± 0.99	2.23 ± 0.94	1.58 ± 0.67	2.15 ± 0.90
	Chest	1.32 ± 0.72	1.92 ± 0.85	1.40 ± 0.96	2.28 ± 1.69	1.30 ± 0.75	1.82 ± 0.76	1.42 ± 0.95	2.06 ± 1.22	1.19 ± 0.47	1.73 ± 0.54
	Arm	0.76 ± 0.35	1.13 ± 0.77	0.66 ± 0.32	0.86 ± 0.31	0.79 ± 0.39	1.20 ± 0.67	0.75 ± 0.27	1.02 ± 0.55	0.77 ± 0.50	1.28 ± 0.72
	KuduSmart	0.61 ± 0.19	0.83 ± 0.33	0.54 ± 0.14	0.70 ± 0.12	0.64 ± 0.15	0.86 ± 0.35	0.62 ± 0.10	0.83 ± 0.36	0.61 ± 0.21	0.83 ± 0.27
SC (mmol.L^{-1})	**Back**	72 ± 16	57 ± 16	60 ± 19	60 ± 14	75 ± 17	56 ± 17	82 ± 14	62 ± 17	56 ± 12	49 ± 8
	Chest	92 ± 22	69 ± 18	95 ± 45	62 ± 31	91 ± 21	71 ± 16	102 ± 24	74 ± 20	73 ± 20	61 ± 17
	Arm	71 ± 15	54 ± 13	87 ± 27	58 ± 24	66 ± 19	53 ± 15	79 ± 20	57 ± 20	56 ± 10	49 ± 10
Age (yrs)		41 ± 23		34 ± 25		43 ± 23		22 ± 2		68 ± 3	
Height (cm)		175 ± 7		167 ± 6		177 ± 6		175 ± 6		174 ± 8	
Body Mass (kg)		74.0 ± 12.2		63.5 ± 14.3		77.0 ± 10.4		74.0 ± 13.5		74.1 ± 11.1	

List of Figures

Vitae

Miss Rebecca Relf

Rebecca completed her BSc (Hons.) undergraduate degree in Sport and Exercise Science from 2011-2014 at the University of Brighton. She was then awarded a scholarship to continue her academic studies at the university and completed her MSc degree in Applied Sport Physiology in 2015. Throughout her MSc degree, Rebecca underwent work experience with the Sport and Exercise Science Consultancy Unit (SESCU) as a Trainee Sport Scientist. In January 2016, Rebecca secured a job as a part-time Technical Instructor at the University of Brighton. She began her part-time PhD examining 'Heat sensitivity and alleviating strategies for female breast cancer survivors' in October 2016. Rebecca is a member of the Environmental Extremes Laboratory (EEL).

Dr Kirsty Waldock

Kirsty is a Scientific Officer within the Ministry of Defence as part of the Army Health and Physical Performance Research team. Dr Waldock's current research interest is mitigating musculoskeletal injury risk and optimising performance, within the British Army. Prior to joining the Ministry of Defence, Kirsty completed her PhD scholarship at the University of Brighton in 2019, where she examined the effects of heat alleviating strategies in the elderly population. Dr Waldock's other research interests include: extreme environmental physiology, improving sports performance and healthy ageing.

Mr Gregor Eichhorn

After completing his Bachelor's degree in 2012 in Sport and Exercise Sciences at the University of Konstanz, Germany, Gregor took up a position as an exercise physiologist working with athletes from various sports in Zurich (Switzerland). Gregor continued his studies by starting the MSc in Applied Exercise Physiology at the University of Brighton. After completing the MSc programme he continued working as an exercise physiologist and coach for endurance athletes in Cologne, Germany. He began his part-time PhD examining heat acclimation strategies to promote cellular resistance in an elderly population in 2017. He is a member of the Environmental Extremes Laboratory (EEL).

Dr Melanie Flint

Mel is a Reader in Cancer Biology University of Brighton and an adjunct Research Assistant Professor in the Department of Pharmacology, University of Pittsburgh. She is currently Co-leader of Brighton and Sussex Cancer Research Network and Translational Sciences Lead, Centre for Stress and Age-Related Disease. Dr Flint is also a member of the British Breast Group. Dr Flint has published over 30 papers related to stress and cancer and is the recipient of finding from numerous sources including National Institutes of Health, Cancer Research UK, Team Verrico and the Breast Cancer Trust. The focus of Dr Flint's laboratory is translational cancer research. Specifically, her research examines the mechanism of stress hormones on cancer and immune cell signalling.

Dr Louisa Beale

Louisa is Principal Lecturer in Sport and Exercise Science at the School of Sport and Service Management, University of Brighton, where she has worked since 2001. Dr Beale's research is concerned with how physical activity can prevent disease, improve wellbeing and health outcomes in the real world and she has related publications in international journals. Dr Beale works in collaboration with the NHS, charities, the leisure industry and local community and supervises PhDs in exercise cardiology, exercise and pregnancy and detraining and muscle function.

Dr Neil Maxwell

Neil is a Reader of Environmental Physiology within the School of Sport and Service Management at the University of Brighton, where he has worked since 1997. Dr Maxwell leads the Environmental Extremes Lab, supervising PhD students while also engaging with industry and national sports teams. He has over 70 publications in international journals allied to thermal and hypoxic stress and how the body tolerates each, particularly during exercise. Through his research and innovation, Dr Maxwell aims to inspire health, occupational and sporting communities to engage in safe and effective exercise in environmental extremes and reduce the incidence of illness.