Title:

Reliability of a wearable sweat rate monitor and routine sweat analysis techniques under heat stress in females

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Abstract

Introduction: The aim of the study was to evaluate the reliability of five different sweat analysis techniques which measure; whole body sweat rate [WBSR], local sweat rate [LSR] (via technical absorbent [TA] method and KuduSmart® monitor), sweat conductivity [SC] and sweat gland activation [SGA] in a female population when exercising moderately under heat stress. **Methods:** Fourteen females (age; 26 ± 7 years, body mass; 66.5 ± 7.6 kg, height; 167.1 ± 6.4 cm) completed a preliminary threshold walking test (to determine exercise intensity) and two main trials, separated by 2 days. Main trials consisted of 30-minutes seated rest in the environmental chamber (35°C, 50% relative humidity) in an upper body sauna-suit, before its removal, and walking at a moderate intensity (4 metabolic equivalents) for 30-minutes (speeds ranged from 4.8-6.5 $km.hr^{-1}$). WBSR was measured via nude mass pre and post exercise. The TA and Tegaderm patches (for sweat sodium chloride) were placed on the back, forearm and chest for the entire 60-minutes, replicated for all participants for both trials. SGA was assessed following the 60-minute trial and the KuduSmart® monitor was placed on the left arm for the 30-minutes of exercise. **Results:** WBSR, LSR methods and SC demonstrated no difference between trials (p >0.05), good agreement (within limits), strong correlations ($r \geq 0.88$) and low typical error of measurements [TEM] (<0.04$L.min^{-1}$, 0.13 $mg.min^{-1}.cm^{-2}$ and 8 $mmol.L^{-1}$, respectively). SGA method showed moderate intra-class correlation (r=0.80), with high TEM (5 glands) and large limits of agreement. **Conclusion:** Sudomotor function is reliable, as demonstrated by good reliability, small TEM and strong correlations. The use of these sweat techniques is appropriate and practical in females who are exercising at moderate intensity under heat stress, and so, may aid future interventions. SGA shows larger variation and should be used with caution.

Keywords: Sweat analysis, heat stress, exercise, reliability, females.

1. Introduction

Heat tolerance describes the variations in thermoregulatory and sudomotor function to heat stress (Epstein, 1990). During exercise and with additional heat stress, the production and subsequent evaporation of sweat is vital for effective human temperature regulation (Smith and Havenith, 2011). As a result, measurements of sudomotor function, particularly local and whole body sweat rate (WBSR), are commonly used to assess thermoregulatory function across a range of environmental conditions (e.g. heat and humidity), interventions (e.g. heat acclimation) and populations (e.g. clinical – multiple sclerosis) (Morris et al., 2013). Havenith et al., (2008) reported no statistical differences in the sweat rate of anatomical positions between sexes, however strong trends were identified (whole body loss; females; 975g *vs.* males; 1171g). Although sweat analysis techniques have been used in reliability studies encompassing female participants, they have been underpowered with the ratio of males used (Goulet et al., 2017 [11 men, 3 women]; Gagnon and Kenny, 2012 [8 men, 8 women]; Kenefick et al., 2012 [8 men, 2 women]; Shirreffs and Maughan, 1997 [undefined participants]). Therefore, these studies alone cannot identify any differences in sex accurately, which may affect interventions used in a female population and so warrants investigation in a solely female population.

Humans have a substantial and flexible capacity for the active secretion of sweat (Taylor and Machado-Moreira, 2013), which can be assessed using whole-body techniques or methods localised to specific anatomical locations. One of the most accurate, simplest and cost effective methods of measuring WBSR is via changes in nude body mass (NBM) (Sawka et al., 2007). Furthermore, local sweat measures may offer a suitable alternative to establish regional sweat rates and assess electrolyte concentrations, especially in field situations (Baker, 2017) or if a greater understanding of sudomotor function is required for clinical, occupation or athletic purposes. There is a vast array of methods to assess local sweat rate (LSR), currently the most commonly cited technique is the ventilated capsule (VC) (Morris et al., 2013). Although highly reliable (coefficient of variation (CV) = 2%, (Kenefick et al., 2012)), capsules cover only < 0.02% of the body's surface area and so, a tendency towards other absorbency techniques have been utilised for better representation of local and WBSR (Havenith et al., 2008). Morris et al., (2013) assessed the accuracy and reliability of the technical absorbent (TA) method compared to the VC method among 40 cyclists. LSR values for the TA were highly correlated ($r > 0.9$) with the VC method across exercise time, anatomical position and sample surface area. The

TA method requires 20-minutes of steady-state exercise and sweating for accurate correlative analysis (Baker, 2017), and is a reliable, practical and cost-effective alternative.

With profuse sweating, electrolytes are lost and may disrupt fluid balance. This disruption, especially whilst exercising in heat stress, may increase the risk of heat-related illnesses (Coris et al., 2004). A reduction in sodium chloride ($Na^+ Cl^-$), providing more dilute sweat, attenuates the development of hyponatremia (Sawka and Montain, 2000). Sweat conductivity (SC), expressing composition from $Na^+ Cl^-$ molarity, was first measured by the '*gold standard*' whole body wash down technique. However, the Sweat-Chek^{TM} system, which is portable, fast and user friendly, is commonly used and indicates good reliability in males (Hammond et al., 1994). The Sweat-Chek^{TM} system has been reinforced as a reliable tool with variations of samples being 11 mmol.L^{-1} for $Na^+ Cl^-$ after 60-minutes of exercise (Boisvert and Candas, 1994). We are aware that females have a different temporal pattern to males (Mee et al., 2015) and therefore during interventions, such as heat acclimation, it is more difficult to accurately assess meaningful and statistical changes when reliability of these measurements is unknown in females.

With increasing advancements in wearable technology, developments in sensors for non-invasive and continuous analyte monitoring have been developed (Peake et al., 2018; Gao et al., 2017). These developments and wearable SMART monitors enable a wide range of personalised diagnostic and physiological monitoring applications for health and exercise (Gao et al., 2016a). With the delayed response in sweating after the onset of physical activity, wearable monitors that can analyse LSR and conductivity metrics in real-time are helpful for healthcare practitioners, nutritionists, physiologists and coaches for exercise and training purposes. For LSR, a few prototypes have been developed and utilised, which are described in the review by Gao et al., (2018), however, the reliability of these monitors remains unknown. A new wearable KuduSmart® monitor has been developed by Crossbridge Scientific Ltd., UK that provides the user with real-time information on LSR. However, no empirical data on this wearable KuduSmart® monitor exists to evaluate its reliability and this is required before its widespread-use, especially considering only 5% of the available technology has been formally validated (Koh et al., 2016; Peake et al., 2018).

Furthermore, the standard technique for assessing the number of sweat glands (SGA) is advocated by Gagnon et al., (2012) utilising the modified iodine technique. SGA methods are found to be accurate and reliable against computer software and manual counts, with a

moderately low CV (11 ± 10%), mean bias of 3 ± 29 glands.cm^2 and high correlation (r = 0.88) between tests. Whilst this method has been endorsed for use under experimental laboratory and field conditions, it has solely been used in a male population and thus further investigation is required for the female population.

Therefore, the aim of this study was to assess the reliability of a new wearable sweat rate monitor and an array of the most commonly cited sweat analysis techniques (WBSR, LSR, SGA and SC) between trials in females. It was hypothesised that each method of sudomotor function would show at least 3 out of 4 of the following criteria to be deemed reliable; good agreement, as demonstrated by acceptable limits of agreement [LoA] (> 90%), a low absolute TEM (< 0.2 mg.min^{-1}.cm^{-2}, 0.2 L.hr^{-1}, 10 mmol.L^{-1} or 3 glands.cm^2) or CV (< 10%), strong intra-class correlation reliability (ICC > 0.9) and no significant differences (p > 0.05) between trials.

2. Methodology

2.1 Participants

Following the approval of the experimental design by the Institution's Ethics Committee, written informed consent was obtained from 14 females (mean ± standard deviation [SD], age; 26 ± 7 years, body mass; 66.5 ± 7.6 kg, height; 167.1 ± 6.4 cm and body surface area [BSA]; 1.75 ± 0.12 kg.m^2). Participants had no prior health issues (screened by medical questionnaire), had not undergone specific heat acclimation within the last two months, were normotensive and non-smokers. The study was conducted in accordance with the guidelines of the revised Declaration of Helsinki (2013). Participants attended the laboratory on three separate occasions, taking place in the follicular phase of those females who were pre-menopausal. These timings were determined by self-reported menstrual cycle questionnaires. No verification of 17β-estradiol and progesterone concentrations were performed, however, the experimental session timings were determined optimal by Stachenfeld and Taylor (2014) and reinforced by hormone analysis in past studies performed alongside the self-reported questionnaire (Mee et al., 2015; Relf et al., 2017).

Participants abstained from strenuous exercise, alcohol and caffeine in the 24-hours prior to testing. Participants consumed 500 mL of water 2-hours prior to each trial to ensure adequate hydration levels (Sawka et al., 2007). All trials took place at the same time of day and outside of the summer months, to control for circadian rhythm (Kräuchi and Wirz-Justice, 1994) and

additional natural heat load (Shapiro et al., 1981), respectively. Participants were asked to replicate dietary intake on the day of all trials, however this was not analysed nor recorded.

2.2 Experimental protocol and pre-trial preparation

Participants completed one preliminary trial and two main trials, which all took place in an environmental chamber (TISS, Hampshire) set to 35°C, 50% relative humidity (RH). Anthropometric data was collected upon arrival; NBM was measured to the nearest 0.01 kg using digital scales (Adam, GFK 150, USA) and stature was measured using a stadiometer (Detecto, USA), which were then used to estimate BSA (Dubois and Dubois, 1916). On arrival to each trial, participants provided a fresh mid-flow urine sample. Euhydration was confirmed by the following criteria which was met (Sawka et al., 2007); 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). Following this analysis, NBM was recorded from pre and post exercise, with differences used to determine non-urine fluid loss (e.g. WBSR).

2.3 Preliminary test

The pre-programmed walking ventilatory threshold protocol was standardised for all participants to determine the exercise intensity for the main trials. It consisted of seven stages, which began at 3.5 $km.hr^{-1}$ and increased every 3-minutes by 0.5 $km.hr^{-1}$, at 1% gradient (Jones and Doust, 1996) on a treadmill. Rectal core temperature (T_{re}), heart rate (HR), ratings of perceived exertion (RPE), thermal sensation (TS), local thermal sensation limited to the perception of the torso only (LTS), and thermal comfort (TC) were recorded at the cessation of each 3-minute stage. At 2-minutes into each stage, 45-seconds of expired air was collected using open-circuit spirometry, and analysed using a gas analyser (Servomex International Ltd., UK). A two-point calibration was undertaken using a mixture of gases and pre-determined oxygen and carbon dioxide percentages (15 and 5%, respectively) (BOC, UK) prior to every trial. The temperatures and volumes of the gases were acquired using a dry gas flow meter (Harvard Apparatus Ltd., UK) and a fixed flow pump model Dymax 30 (Charles Austin Pumps Ltd., UK).

2.4 Metabolic Equivalent (MET) calculation

One MET is defined as the amount of oxygen consumed while sitting at rest and is equal to 3.5 $ml.kg.^{-1}min^{-1}$ (Jette et al., 1990). The energy cost of an activity can be determined by dividing

the relative oxygen cost of the activity ($ml.kg.^{-1}min^{-1}$) by 3.5. METs are a simple, practical and easily understood exercise intensity prescription method. For the two main trials, 4 METs was prescribed to replicate a moderate intensity of exercise for females (2.8-4.3 METs) (Jette et al., 1990; Park et al., 2014).

2.5 Main trials

The first 30-minutes required the participants to rest in a seated position within 35C, 50%RH with a commercially available upper-body sauna suit jacket (Everlast, London, UK), to restrict evaporative heat loss, elevate physiological strain and therefore, ensure adequate sweat production for analysis (Willmott et al., 2018). This was then removed and participants began the 30-minute walk at the predetermined speed replicating an intensity of 4 METs. The treadmill speed did not differ between the two main trials for each participant (5.6 ± 0.5 $km.hr^{-1}$).

2.6 Experimental measures

Participants were familiarised to perceptual scales prior to the trials; TS (0 *Unbearably cold* to +8 *Unbearably hot*) (Toner et al., 1986), RPE (6 = *Very, very light* to 20 = *Exhaustion*) (Borg, 1982) and TC (1= V*ery comfortable* to 6 = *Very uncomfortable*) (Guéritée and Tipton, 2015). A rectal probe (Henley, UK) was self-inserted 10 cm past the anal sphincter which provided continuous T_{re} measurement throughout trials. A HR monitor (Polar FT1, Polar Electro, Finland) was affixed to the chest. Skin temperature (T_{skin}) was recorded using skin thermistors (Eltek Ltd, Cambridge, UK) attached to four sites; the midpoint of the right pectoralis major (T_{chest}), midpoint of the right triceps brachii lateral head (T_{arm}), right rectus femoris ($T_{upper\ leg}$) and right gastrocnemius lateral head ($T_{lower\ leg}$), and connected to a temperature logger (Squirrel 1000 series, Eltek Ltd., UK). Mean skin temperature (T_{skin}) was calculated using the equation by Ramanathan (1964); mean $T_{skin} = (0.3 \times [T_{chest} + T_{arm}]) + (0.2 \times [T_{upper\ leg} + T_{lower\ leg}])$. Both physiological and perceptual measurements were taken at 5-minute intervals throughout the rest and exercise periods of the main trials. Expired air was collected at three-time points during the 30-minute walk (minutes 4-5, 14-15 and 24-25) to assess the accuracy of the MET prescription.

2.7 Sweat analysis methods

Five methods were analysed in this study; WBSR, LSR via the TA patches and the KuduSmart® monitor, SC via the Tegaderm absorbent patches and Sweat-Chek^{TM} analysis and SGA through the modified iodine technique. SGA, Sweat-Check^{TM} and TA methods were assessed at 60-minutes. Both Tegaderm and TA patches were placed on the dorsal surface and standardised for all participants for both main trials for the;

Arm; TA patch (Technical absorbents, Grimsby, UK) applied 2cm below the antecubital fossa and the Tegaderm (Tegaderm^{TM} + Pad, 3M Health Care, St Paul, MN, USA) placed exactly 1 cm below.

Chest; TA patch placed on the left side of the chest 1 cm below the clavicle, and with 4 cm separating the Tegaderm on the right side, also 1 cm below the clavicle.

Lower back; TA placed on the right side of the vertebral column, in line with the superior of the iliac crest, 5 cm lateral of the vertebral column. The Tegaderm was then placed 10 cm to the left of the TA.

TA method. The absorbent patches were cut to 8 x 4 cm and zinc oxide tape was applied to the outside. Prior to experimentation each patch was weighed with the zinc oxide tape in a separate impermeable plastic Ziploc bag to the nearest 0.1 mg using weighing scales (PS-60 II, Fisher Scientific UK, Loughborough, UK). The patches were held in place on the three sites by means of the zinc oxide tape for the whole 60-minute trial and weighed immediately post in its original plastic bag. LSR was calculated using the difference between pre and post mass, divided by the surface area of the patch (i.e. 32 cm^2) and the duration of application 60 minutes, yielding values in $mg.min^{-1}.cm^{-2}$.

KuduSmart®. The KuduSmart® monitor (Crossbridge Scientific Ltd., UK) was turned on and placed in the chamber for 10-minutes prior to collection for stabilisation and assessment of the offset number for future analysis. It was then placed, after the removal of the sauna suit, on the left arm, 6 cm above the antecubital fossa for the duration of the 30-minute walk. The tightness of the strap (how many holes remained) was recorded and replicated for both trials (Figure 1). During analysis, an average of the 30-minutes Kudu score was taken, multiplied by the conversion score and minus the individual offset (~0.4) to give LSR ([Kudu score – offset]*0.18]), yielding values in $mg.min^{-1}.cm^{-2}$.

***** INSERT FIGURE 1 HERE *****

SC method. Absorbent sweat patches of size 5 x 7cm were applied for the 60-minute trial period. The Tegaderm was then removed with gloves and placed into a 10 mL BD plastipak syringe (Becton & Dickinson UK, Oxford, UK). The Sweat-Chek^{TM}, sweat conductivity analyser (Wescor Biomedical Systems, Utah, USA) was calibrated with distilled cold water according the manufacturer guidelines. The patch filled syringe was the pressed through the Sweat-Chek^{TM} system, which has a range of 0-150 mmol.L^{-1} and all values were reported in mmol.L^{-1}.

SGA method. At 60-minutes, the number of activated sweat glands was assessed via the modified iodine technique (Gagnon et al. 2012). From pilot work in our laboratories, the amount of iodine placed in Tupperware boxes for the 48-hour saturation was 0.4 g.

2.8 Statistical analyses

Prior to experimentation, *a priori* sample size was determined using G*Power software (v3.1) (Prajapati et al., 2010) from published mean, SD and effect size data from similar experimental designs (e.g. Gagnon et al., 2012; Mee et al., 2015, Willmott et al., 2015; Relf et al., 2017]) and our own research studies (Gibson et al., 2015; Mee et al., 2015; Willmott et al., 2015, 2016, 2018). The subsequent number of participants required was based upon alpha (α: 0.05) and beta (β: 0.80) levels to ensure sufficient power. All data were first checked for normality using the Shapiro-Wilk method. Sweat analysis measures during the two main trials were examined using a battery of reliability statistics (Atkinson and Nevill, 1998). To identify differences between main trials for the sudomotor measures, a paired samples T-test was conducted. Effect size (d_z) was categorised as small (0.2), medium (0.5) and large (0.8) (Cohen, 1988). TEM was calculated (TEM=SD(diff)/√2) and was expressed as a percentage of its respective mean to form the CV (%). Intra-class correlation coefficient (ICC) with 95% confidence intervals (95% CI) were calculated as a measure of retest correlation between the two main trials using an absolute agreement, two-way mixed effects model. An ICC value of 0.7-0.8 was defined as moderate correlation, with >0.9 as high and <0.7 as low, in accordance with Vincent & Weir (1994). Bland-Altman plots with 95% LoA were created using Microsoft Excel (2013) to indicate individual differences between trials plotted in relation to respective individual means (Atkinson & Nevill, 1998). All data was analysed using a standard statistical package (SPSS version 20.0) and reported as mean ± SD. Statistical significance was accepted at the level of $p < 0.05$.

3. Results

Participants were in a similar resting physiological state for both trials, with no differences in Uosm (122 ± 90 vs. 221 ± 200 mOsm.kg^{-1}, p = 0.08), NBM (65.98 ± 7.58 vs. 66.02 ± 7.76 kg, p = 0.25), T_{re} (37.51 ± 0.34 vs. 37.44 ± 0.45°C, p = 0.41) or HR (75 ± 12 vs. 73 ± 9 beats.min^{-1}, p = 0.33). The average exercise intensity as expressed by METs for both trials, did not differ between trials (4.00 ± 0.44 vs. 3.93 ± 0.52 METs, p = 0.25). There was no difference in resting or exercising T_{re} and mean T_{skin}, p > 0.05 (Table 1). Reliability data and statistics are presented in Table 2.

***** INSERT TABLE 1 HERE *****

***** INSERT TABLE 2 HERE *****

3.1 Whole body sweat rate (WBSR)

There was no difference for WBSR between trials (Table 1); with low variability displayed and all data remained within LoA, as reinforced in Figure 2.

***** INSERT FIGURE 2 HERE *****

3.2 Local sweat rate (LSR)

There were no differences between the LSR in any of the three sites tested between trials, nor for the KuduSmart® monitor (Table 1). All sites and monitor displayed good LoA (Figure 3) and low TEMs.

*****INSERT FIGURE 3 HERE *****

3.3 Sweat conductivity (SC)

There were no differences between the SC on either of the three sites tested and strong agreement and ICC of the data between trials (Table 1). Figure 4 highlights mean bias and LoA for all sites.

***** INSERT FIGURE 4 HERE *****

3.4 Sweat gland activation (SGA)

There was no difference between number of active sweat glands between trials. However, there was moderate variability presented (Table 1) and larger LoA (Figure 5).

***** INSERT FIGURE 5 HERE *****

4. Discussion

4.1 Overview

This is the first study to examine the reliability of a wearable sweat rate monitor and other routinely used sweat analysis techniques when exercising under heat stress in a female population. In agreement with our hypothesis, the majority of the sweat analysis techniques assessed displayed good reliability with no differences between trials, strong correlations, low typical error and were within the LoA. This has important implications, ensuring these techniques are appropriate and practical when evaluating interventions in females. All participants were tested in the early follicular phase of the cycle (day 0-10) due to evidence displaying that luteal phase is associated with a delayed onset of sweating threshold (~0.3°C) and vasodilation and also a higher resting core temperature (~0.3-0.6°C) (Pivarnik et al., 1992; Marsh and Jenkins, 2002).

Using a battery of absolute and relative reliability statistics provides an overall view of the robustness of each measurement. When making conclusions from the results found it is important to note that the interpretation of CV (%) is typically classified as 'good' if CV is < 5% in most reliability literature. However, the CV is limited in interpretation, whereby a larger CV % may be observed when investigating a small sample size and, or low magnitudes in the measured values (Atkinson and Nevill, 1998). Therefore, the absolute measure of TEM offers a more meaningful indicator of reliability for sweat analysis where the data are smaller (Willmott et al., 2018), and as such each variable had to coincide with 3 out of 4 of the reliability measures to be determined as reliable. Acknowledging the different types of variation and reliability, the quantification of random biological and methodological noise in sweating techniques was exhibited, even after controlling for pre-analytical factors (e.g. menstrual cycle, time of day and season) (Fraser, 2001; Kenefick et al., 2012).

4.2 WBSR

Findings from this study determined the TEM (CV) for WBSR, as measured by NBM to be 0.04 $L.hr^{-1}$ (10.2%), when exercising moderately (4 METs) at the same relative intensity and duration on both trials. Previous studies have found lower CVs of 4.7% after 3 bouts of 20-minutes cycling in males (Brokenshire et al., 2009; Hayden et al., 2004), but similar findings in our laboratory with a TEM (CV) of 0.09 $L.hr^{-1}$ (8.5%) in males (A G B Willmott et al., 2015). Predefined limits for interventions to show meaningful changes for WBSR have been set to 0.2 $L.hr^{-1}$ (Willmott et al., 2017; Neal et al., 2015) and so this study shows the error to be well within these limits and therefore, reliable in females to determine changes following interventions (e.g. heat acclimation). Moreover, all data fell within the LoA and there were strong correlations found between trials (ICC = 0.939), this is similar to the findings of Mee et al., (2015); with an ICC of 0.95 and CV of 9% in the female population assessed. Finally, it is important to note the possibility of human error occurring in the efficiency and extent of towel drying prior to nude weighing post trials on each occasion, which may increase variability within the data.

4.3 LSR

LSR was evaluated via the TA technique, which has been identified as a reliable, cost-effective and practical alternative compared to the gold standard hygrometry VC technique (Morris et al., 2013). Previous literature has concluded that the sweat rate on the extremities is lower than that of the trunk due to reductions in skin blood flow (Havenith et al., 2008), which was also reinforced by this study on both trials, as forearm LSR was lower (Trial 1 and 2; 0.32 ± 0.10 and 0.35 ± 0.13 $mg.min^{-1}.cm^{-2}$) than the back (0.63 ± 0.37 and 0.66 ± 0.37 $mg.min^{-1}.cm^{-2}$) and chest (0.69 ± 0.39 and 0.72 ± 0.46 $mg.min^{-1}.cm^{-2}$).

Morris et al., (2013) assessed the reliability of the TA method under similar conditions (~33°C, 30% RH) with 38 male and 4 female participants cycling at 60% of peak oxygen consumption for 75-minutes, with 5-minute LSR measurements after 10, 30, 50 and 70-minutes. Absolute LSRs were lower in the current study compared to Morris et al (2013), for both arm (0.44 at 5 minutes *vs.* 0.98 $mg.min^{-1}.cm^{-2}$ at 70 minutes) and back (0.56 at 10 minutes *vs.*1.12 $mg.min^{-1}.cm^{-2}$ at 70 minutes), which may be due to the majority of the sample used being males (38 out of 42) compared to the all-female current study. However, duplicate samples showed

similar correlations (r = 0.96 by Morris (2013), and arm; r = 0.93, back; r = 0.94, chest; r = 0.95 for our study). Additionally, Morris et al. (2013) demonstrated the mean bias (± 95% LoA) was 0.07 ± 0.28 $mg.min^{-1}.cm^{-2}$ using the TA method with 100% of samples falling within the LoA. Moreover, a CV of 11% has been identified for LSR and 6.3% specifically for the forearm (Hayden et al., 2004). The present study identified a larger CV of 12.6% for LSR, although, this was lower (23.3%) than that presented by Kenefick et al., (2012),with differences in this study between trials being up to ~0.3 $mg.min^{-1}.cm^{-2}$ and without statistical differences, further reinforcing the results from our study. Furthermore, the smallest detectable difference was 0.12 $mg.min^{-1}.cm^{-2}$ using the TA method (Morris et al., 2013), which supports the TEM ranging from 0.04 to 0.13 $mg.min^{-1}.cm^{-2}$ for all sites in this study. Mean differences in LSR reported as a function of factors such as; heat acclimation (2.0 $mg.min^{-1}.cm^{-2}$ (Patterson et al., 2004)), sex (0.6 $mg.min^{-1}.cm^{-2}$ (Ichinose-Kuwahara et al., 2010)), and age (0.75 $mg.min^{-1}.cm^{-2}$ (Dufour and Candas, 2007)), greatly surpass the statistically derived smallest detectable difference for all anatomical locations using the TA method in this study, and that of Morris et al., (2013).

It has been commonly noted for patches to be placed on the skin for 5-minutes to reduce the effect of hidromeiosis, as the lack of ventilation increases moisture accumulation on the skin and therefore progressively blocks sweat ducts and sweat suppression (Baker, 2017). However, pilot work for this study raised the challenge of lower sweat rates in females (Gagnon and Kenny, 2012) and insufficient sweat collection during application durations of < 30-minutes. Therefore, the patches were applied for the entire 60-minutes, supported by applications of up to 90-minutes in field methodologies, with no significant differences found between times applied for (Duffield et al., 2012; Kilding et al., 2009). This study demonstrates lower absolute LSR from each of the three anatomical locations compared to aforementioned literature (Morris et al 2013;Baker, 2017), which is in line with literature demonstrating that females have a lower regional sweat output at different anatomical locations (Havenith et al., 2008), identifying a significant trend (13% lower). Previous research has reported a reduction in sweat output in females to be due to reduced sweat output per gland (Sato and Dobson, 1970) and a lower thermosensitivity of the evaporative heat loss response (Gagnon and Kenny, 2011).

4.4 KuduSmart® monitor for LSR

This is the first study to identify the reliability of the newly designed KuduSmart® wearable monitor to assess LSR. This SMART wearable monitor indicates moderate ICC correlation between data (r = 0.88) with a low TEM (0.08 $mg.min^{-1}.cm^{-2}$), but not CV (13.5%) owing to the limitations of this statistical approach to low numerical data sets. The absolute TEM of the KuduSmart® is lower to that of the TA method found on both the chest and back site (0.08 vs 0.13 $mg.min^{-1}.cm^{-2}$). Other consumer-wearable activity trackers have demonstrated similar correlations and reliability to the current monitor (r = 0.8); however, the range of confidence intervals was not as wide (0.61-0.96) as previous trackers (0.36-0.7) (Evenson et al., 2015) and therefore, indicates moderate to strong reliability. Further, this is reinforced by other activity based monitors that have been used in research that have declared similar moderate reliability (ICC range 0.60-0.8) (Welk et al., 2004) to findings of this study and so supports its use for research applications.

The larger absolute value and confidence intervals may be due to how the monitor is placed. Although the placement (e.g. 6 cm above the antecubital fossa on the left arm) and tightness (e.g. number of holes on the strap) is replicated the same for each participant, the relative tightness of the strap from individual to individual may vary and so may alter LSR. This may also explain why the sweat rates are significantly larger compared to the TA method at the arm site (Table 1). The tightness of the strap to ensure the monitor remains in the same place throughout the trial may enhance the temperature of the skin, increasing LSR (Nadel et al., 1971). It may be useful observation for Crossbridge Scientific Ltd., UK and other leading wearable technology equipment to incorporate a temperature gage in the monitor to establish any differences in local T_{skin}, and by how much, to establish the mechanisms behind this slight over-estimation of LSR at this site. However, the latest wearable monitor that provides real-time information linked directly to a tablet, demonstrates good reliability for LSR is exciting for upcoming research. The monitor itself is easily applied and has practical benefits which make it more accessible and user friendly to all populations compared to other methods of sweat rate analysis commonly used within the literature (absorbent methods). Consequently, the validity should be now be evaluated under both temperate and heat stress conditions to warrant world-wide use in future research studies and application in the field.

4.5 Sweat conductivity

The replacement of sweat Na^+ Cl^- losses plays an integral role in the restoration of fluid balance. Whilst the whole-body wash down protocol is the gold standard for assessing Na^+ Cl, the regional patch method is commonly used in the literature, especially when cycling protocols are not used (Baker et al., 2009). Maughan (1991) reported normal ranges of Na^+ Cl^- varying from 20-80 $mmol.L^{-1}$. Findings from the current study report trunk samples present higher Na^+ Cl^- concentrations than those from limb sites (Dziedzic et al., 2014). Moreover, this current study demonstrates lower Na^+ Cl^- values for all sites compared to Dziedzic et al (2014) when assessing trained male cyclists (~20 $mmol.L^{-1}$ less). These findings are in line with research that dictates sweat Na^+ Cl^- increases with sweat rate (Buono et al., 2018), therefore, the lower intensity of exercise and thus, lower sweat rates in this study are in line with lower Na^+ Cl^- (Maughan, 1991).

Reliability for all three sites was strong for both the chest and forearm measurements (ICC > 0.9, and TEM [CV] = 4 $mmol.L^{-1}$ [<10%]) which is consistent with measurements in our laboratories (unpublished findings); r = 0.98 and TEM (CV) of 2.4 $mmol.L^{-1}$ (3.1%) and other literature (11 $mmol.L^{-1}$[14%] (Boisvert and Candas, 1994)). The back measurements showed moderate reliability (r = 0.86) with a TEM (CV %) of 8 $mmol.L^{-1}$ (15.4%), as indicated in Figure 4. However, the study by Dziedzic et al., (2014), indicated non-significant changes of 8 $mmol.L^{-1}$ and therefore, the reliability and TEM in a female population is less than the change required for statistical significance.

The Sweat-Chek™ requires a sweat sample of ~6-10µL and is accurate to within 2 $mmol.L^{-1}$. It must be noted that of the 14 participants, for 3 participants there was insufficient sweat produced for the machine to report a reading for both the back and chest sites, an observed finding in itself. Moreover, only 6/14 samples were collected for the forearm, demonstrating the Sweat-Chek™ may not be suitable for lower intensity exercise and, or the female population, whose overall sweat production has been demonstrated to be less than males (Havenith et al., 2008). It would be interesting to assess these measurements during the luteal phase of females, where research has demonstrated sweat rates to be elevated and therefore, complete samples for all sites may be achieved (Marsh and Jenkins, 2002). The chest is not a site typically cited in the literature for sudomotor function and thus comparisons are not possible for previous reliability. However, the novelty of investigating this measure on the

chest site, as well as the commonly used forearm and back, gives opportunity for assessment of other diseased populations (e.g. breast cancer patients). The Sweat-Chek^{TM} technique, to the author's knowledge, has not been investigated in females and so the sensitivity of this device should be assessed in future studies.

4.6 SGA

Physiologically active sweat glands are most frequently identified using colorimetry or plastic impression techniques (Taylor and Machado-Moreira, 2013). This study utilised a previously validated method for assessment of SGA via the modified iodine technique (Gagnon et al., 2012). It has been demonstrated for different quantities of sweat glands to be activated during different intensities in the heat, up to 150 glands.cm^2 have been reported during moderate exercise in males and specifically ~132 ± 110 glands.cm^2 in the back in males (Taylor and Machado-Moreira, 2013).

During the experiment by Gagnon et al., (2012), it was indicated that the average SGA was 81 ± 30 glands.cm^2 with a range of 35-137, whereas SGA was lower in this study's female population (23 ± 9 glands.cm^{-2}). It must be noted that the intensity and duration of exercise (e.g. 3 x 30-minutes exercise periods at fixed metabolic heat production equal to 200, 250, and 300 W.m^2) was higher in the male population of Gagnon et al (2012) and may explain the higher amount of SGA. Gagnon et al (2012) reported a TEM (CV) of 3 ± 29 glands.cm^{-2} (11 ± 10 %), with an associated relationship (r = 0.88) which is lower than the findings of the current study (Table 1). However, as suggested by Gagnon et al., (2012), differences of less than 30 glands.cm^2 between trials or following interventions may not be worth considering, which may implicate future research where less heat sensitive females are investigated

Results of our study report an ICC of 0.8 (e.g. moderate reliability) between trials for SGA and the 95% confidence intervals displayed large variability between individuals (0.32-0.94) and therefore this variable is not determined reliable as it did not meet 3/4 criteria and should be used with caution in future research. Therefore, it is likely there is a higher variability between females in the amount of SGA during low-moderate exercise intensity (Figure 5). As limited information regarding the quantification of iodine for the two-day absorption period, we suggest 0.4 g of iodine provides suitable absorption and colour to the cotton paper for effective SGA methods. However, there are potential errors associated with the application of the cotton

paper (e.g. pressure and time), which can affect the amount of activated sweat glands indicated on the paper.

4.7 Conclusion

This is the first study to investigate a wearable sweat rate monitor and the routinely used sweat analysis techniques in a female population. All measures showed moderate to strong reliability between trials when exercising moderately under heat stress in females. For the WBSR, LSR and SC techniques, all sites presented strong reliability, and TEMs were all less than previous literature has informed for statistical significance. These findings warrant the use of these techniques for future research utilising these sudomotor function measurement methods in a female population. SGA via the modified iodine technique has only previously been researched for reliability in one other study (Gagnon et al., 2012) and in the current study demonstrated a high CV and wide range for ICC, and therefore should be used with caution in future research.

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List of Tables

	T_{re} (°C)		Mean T_{skin} (°C)	
	Trial 1	**Trial 2**	**Trial 1**	**Trial 2**
REST	37.37 ± 0.10	37.30 ± 0.10	34.91 ± 0.72	34.85 ± 0.78
EXERCISE	37.59 ± 0.17	37.59 ± 0.19	35.91 ± 0.31	36.15 ± 0.24

Table 2. Summary of sweat analysis techniques [WBSR = whole body sweat rate, LSR = local sweat rate, SC = sweat conductivity and SGA = sweat gland activation].

	Trial 1	**Trial 2**	**Mean bias**	**95% LoA**	***p***	***d_z***	**ICC**	**TEM**	**CV%**
WBSR ($L.hr^{-1}$)	0.42 ± 0.12	0.39 ± 0.14	0.03	(-0.09, 0.14)	0.09	0.49	0.94	0.04	10.2
LSR - back ($mg.min^{-1}.cm^{-2}$)	0.63 ± 0.3	0.66 ± 0.38	-0.03	(-0.40, 0.33)	0.51	0.18	0.94	0.13	20.5
LSR - chest ($mg.min^{-1}.cm^{-2}$)	0.69 ± 0.39	0.72 ± 0.46	-0.03	(-0.39, 0.33)	0.56	0.17	0.95	0.13	18.6
LSR - arm ($mg.min^{-1}.cm^{-2}$)	0.32 ± 0.10	0.35 ± 0.13	-0.03	(-0.14, 0.09)	0.11	0.46	0.93	0.04	12.6
KuduSmart® ($mg.min^{-1}.cm^{-2}$)	0.61 ± 0.19	0.58 ± 0.16	0.03	(-0.19, 0.25)	0.35	0.37	0.88	0.08	13.5
SC - back ($mmol.L^{-1}$)	51 ± 17	50 ± 13	0.64	(-20.82, 22.09)	0.85	0.06	0.86	8	15.4
SC - chest ($mmol.L^{-1}$)	57 ± 20	57 ± 21	-0.5	(-12.63, 11.63)	0.80	0.08	0.98	4	7.3
SC - arm ($mmol.L^{-1}$)	43 ± 6	45 ± 8	-2	(-13.23, 9.23)	0.43	0.38	0.8	4	9.3
SGA ($glands.cm^{2}$)	23 ± 7	23 ± 10	-0.26	(-14, 14)	0.94	0.04	0.8	5	22.0

95% LoA = limits of agreement, ICC = intra class correlation, TEM = typical error of measurement, CV = coefficient of variation as a percentage.

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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 Ashley Willmott

Ash completed his BSc (Hons.) in sport and exercise science in 2012 and Ph.D entitled "Optimising heat acclimation state and refining strategies for the acquisition of heat adaptations" at the University of Brighton in 2018. Ash is a member of the Environmental Extremes Laboratory (EEL), with current research themes aligned to investigating heat alleviation strategies, both acute (e.g. cooling) and chronic (e.g. heat acclimation) for occupational and athletic performance, whilst also considering immune function and health status. Ash is a sport and exercise science physiology lecturer and an accredited British Association of Sport and Exercise Science (BASES) physiologist.

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 Senior 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 60 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.