Sweat-from gland to skin surface – production, transport and skin absorption

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Running head: the passage of sweat through the skin

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Abstract

By combining galvanic skin conductance (GSC), stratum corneum hydration (HYD) and regional surface sweat rate (RSR) measurements at the arm, thigh, back and chest, we closely monitored the passage of sweat from gland to skin surface. Through a varied exercise-rest protocol, sweating was increased slowly and decreased in 16 male and female human participants (25.3 ± 4.7 yrs, 174.6 ± 10.1 cm, 71.3 ± 12.0 kg, 53.0 ± 6.8 ml·kg·min^{-1}). ΔGSC and HYD increased prior to RSR, indicating pre-secretory sweat gland activity and skin hydration. ΔGSC and HYD typically increased concomitantly during rest in a warm environment (30.1 ± 1.0°C, 30.0 ± 4.7% RH) and only at the arm did ΔGSC increase prior to an increase in HYD. HYD increased prior to RSR, before sweat was visible on the skin, but not to full saturation, contradicting earlier hypotheses. Maximal skin hydration did occur, as demonstrated by a plateau in all regions. Post exercise rest resulted in a rapid decrease in HYD and RSR but a delayed decline in ΔGSC. Evidence for reabsorption of surface sweat into the skin following a decline in sweating, as hypothesized in the literature, was not found. This suggests that skin surface sweat, after sweating is decreased, may not diffuse back into the dermis, but is only evaporated. These data, showing distinctly different responses for the three measured variables, provide useful information about the fate of sweat from gland to surface that is relevant across numerous research fields (e.g. thermoregulation, dermatology, ergonomics and material design).

New and Noteworthy

After sweat gland stimulation, sweat travels through the duct, penetrating the epidermis before appearing on the skin surface. We found that only submaximal stratum corneum hydration was required before surface sweating occurred. However,

full hydration only occurred once sweat was on the surface. Once sweating reduces, surface sweat evaporation continues but there is a delayed drying of the skin. This information is relevant across various research fields, including environmental ergonomics, dermatology, thermoregulation and skin-interface interactions.

Key words: epidermal hydration, galvanic skin conductance, sweat rate, eccrine sweat glands

Introduction

Eccrine sweat gland function, regulation and adaptation have been extensively investigated (6, 47, 48); typically measured using surface monitors in the form of ventilated or unventilated sweat capsules, technical absorbent pads or by direct sweat drop analysis (18, 20, 30, 34). However, the appearance of sweat on the skin surface stems from processes beginning earlier and deeper within the skin structures, which may go undetected by these measurement techniques. As a result, our understanding of sweat formation, how it traverses through the gland and reaches the skin surface is somewhat limited. It is the main intent of this paper to contribute knowledge to this area.

Whilst from a heat strain perspective mainly sweat appearing on the skin surface available for evaporation may be relevant, from a thermoregulatory control perspective the stages before the appearance of sweat are pertinent too. When studying basic thermoregulatory control, measurement of the initial activation of the sweat gland is relevant. When studying sensory function of the skin or sensory interaction of skin with clothing (37, 38), both relevant to behavioral thermoregulation (3, 15, 22, 45), sensation and discomfort have been reported to be affected by changes in skin properties. These occur with increased epidermal

hydration; most notably through epidermal swelling and increased surface friction (3, 4, 16). In addition, with recent aims to develop wearable sensor devices that monitor sweat and its contents (14), knowledge of how sweat penetrates the skin could be useful. Moreover, in clinical diagnostics, identifying deficiencies in the early stages of sweat formation are linked to a range of illnesses, such as hypohidrosis or anhidrosis that accompany diseases such as diabetes mellitus. Thus, understanding the stages of sweat production prior to the appearance of surface sweating adds important knowledge beyond the cooling aspect of surface sweat evaporation.

Sweat production begins by the secretion of an isotonic fluid into the secretory coil. This pre-secretory sweat gland activity can be detected by measuring galvanic skin conductance (GSC) (9); a measure of the skin's ability to transmit an electrical current that is enhanced by the presence of a weak electrolyte solution such as sweat. Sweat moves from the secretory coil into the straight re-absorptive duct that traverses the dermis of the skin. Here ions, namely Na^+ and Cl^- are reabsorbed so that a hypotonic fluid is released onto the skin surface, conserving electrolytes for the body. It has been suggested that epidermal hydration, i.e. moisture transfer from coil directly into the skin occurs prior to surface sweating (25). This process may be relevant for the delivery of important ions and peptides in maintaining epidermal barrier homeostasis and antimicrobial function of the skin (46, 49). Further to the surface, the stratum corneum, i.e. the outer layer of the skin is very hygroscopic, in that it can hold up to 70% of its own weight in water (23). Utilizing galvanic conductivity data, it has been postulated that the corneum hydrates first before sweat is released onto the skin surface (5). Given that stratum corneum saturation has been shown to suppress sweating (hidromeiosis) (7, 42), either by swelling of the keratin ring surrounding the sweat duct pore (35) or by compression of the last convolutions

of the excretory duct by hyperhydrated epidermal cells (13), it seems unlikely that maximum hydration of the stratum corneum would be achieved before surface sweating is visible (36). However, the extent to which the stratum corneum hydrates before sweat reaches the surface remains unknown. To the authors' knowledge this has only been studied with changes in relative humidity whereby the skin absorbs moisture from the environment rather than from sweat production, hence raising the first research question for the present study, whether this process also occurs as postulated (5) when sweating. In addition, stratum corneum thickness varies across the body (scapular: ~11 μm, dorsal forearm: ~20μm) (41) and so too does sweat gland size, density and sweat rate (43, 50, 51), leading to the second research question for this study, whether regional differences in epidermal hydration may be apparent.

In order to research the pathways of sweat, devices that can discriminate between the different locations of the fluid and the movement of sweat from the gland to the skin are required. As mentioned, pre-secretory sweat gland activity can be detected by measuring GSC and surface sweating can be detected using sweat capsules (20, 32, 34) or sweat absorbing patches (18, 50, 51). Recent developments in skin measuring devices, such as dielectric moisture meters (1), which have shallower measurement depths than galvanic skin conductivity devices means it is now possible to also investigate the extent at which sweat hydrates the epidermis and/or stratum corneum and also what happens to epidermis hydration once sweating has subsided. In 1970, Edelberg (11) suggested that the sweat on the surface, within the duct and acrosyringium will either slowly diffuse into the stratum corneum or be reabsorbed back into the sweat gland after sweating ceases. This leads us onto the third research question for this study: what happens to sweat once sweating ceases? More studies are required to confirm this finding as such data could be relevant in dermatological

research, such as the sweating associated exacerbating factors for atopic dermatitis (31, 54). Combining several of these technologies that measure different aspects of sweat production may provide insight into the movement of sweat from the secretory coil to skin surface. Therefore, the fourth research question for this paper is how these difference techniques reflect the different aspects of sweat movement to the skin surface.

In relation to our four aforementioned questions we hypothesized that after sweat gland activation, submaximal epidermal hydration will occur before surface sweating occurs. It is further hypothesized that due to the larger sweat gland size and sweat rate (SR) on the torso, sweat will traverse the gland more quickly than at the extremities and thus regional variations in the measurements will be evident. Once sweating declines, we hypothesize that sweat will diffuse into the stratum corneum or be reabsorbed back into the sweat gland. Finally, we hypothesized that GSC, HYD and RSR measurements can distinguish different phases of the sweating process and can detect regional differences.

Methods

Participants

Sixteen healthy human participants (eight males and eight females; 174.6 ± 10.1cm, 71.3 ± 12.0kg, 25.3 ± 4.7 yrs, 53.0 ± 6.8ml·kg·min^{-1}) were recruited from the staff and student population at Loughborough University. Participants were informed about the study purpose and procedures prior to providing verbal and written consent and completing a health screen questionnaire. Loughborough University Ethical Advisory Committee approved the study. Participants were asked to refrain from strenuous exercise, caffeine and alcohol intake in the 12 hour prior to all testing. Prior to the

main experimental trial, participants were allowed to shower as per their daily routine but were instructed not to use any moisturizing lotions 12 hours prior to the experiment.

Experimental protocol

Preliminary tests

During the first visit, participants' stature and body mass were recorded followed by a submaximal fitness test based on the Åstrand-Rhyming method (ACSM, 2006). The submaximal fitness test was completed on a treadmill (Woodway PPS Med, Woodway Inc., Waukesha, WI, USA) in 19°C, 40% RH. The test was comprised of four 5-min exercise stages that aimed to raise heart rate (Polar Electro Oy, Kempele, Finland) from 110 beats·min^{-1} to 85% of their age-predicted heart rate max (220-age). The work rate and heart rate during the last min of each stage was recorded, which was used to predict their maximal oxygen uptake ($\dot{V}O_{2max}$). A line of best fit was applied to the data and extrapolated to the value corresponding to the participants age predicted heart rate max (220-age). $\dot{V}O_{2max}$ was then predicted from the x-axis.

Main Experimental trial

Upon arrival to the laboratory, participants self-inserted a rectal thermometer 10 cm beyond the anal sphincter, which was used as an indication of core temperature (T_{re}). Participants dressed in prescribed running shorts, plus sports bra for females, and their own personal socks and athletic shoes. Participants then entered the preparation area where the ambient conditions were 23.4 ± 0.5°C, 50.0 ± 4.7% RH. Preparation involved cleaning the skin measurement areas and applying skin temperature sensors, GSC electrodes and sweat rate absorbent pads to four locations on the body (detailed

below). The chest, scapula, upper arm and mid anterior thigh were chosen as measurement sites based on regional sweat rate data from Smith et al. (50, 51), which shows these areas to be of distinctly different sweat rates.

To answer our research questions, we selected a protocol that would slowly increase sweat production through changes in ambient conditions and exercise intensities. The test was split into 3 main stages; rest (R), exercise (EX) and post exercise (PEX). Seated rest consisted of two 10 min periods whereby the first 10 min was in ambient conditions of 23.4 ± 0.5°C, 50.0 ± 4.7% RH (R1) and the last 10 min of rest was inside an environmental chamber set at 30.1 ± 1.0°C, 30.0 ± 4.7% RH (R2). Participants stayed in the chamber for the remainder of the experiment. The resting conditions were then followed by a stepwise exercise protocol: 20 min at 30% $\dot{V}O_{2max}$ (EX1), 10 min at 50% $\dot{V}O_{2max}$ (EX2) and 20 min at 70% $\dot{V}O_{2max}$ (EX3) on the treadmill. Following cessation of exercise, participants rested in the chamber for an additional 20 min (separated into two ten min blocks, hereafter referred to as PEX1 and PEX2, (see Figure 1)).

Measurements

Ambient temperature and relative humidity were monitored (Eltek/Grant 10Bit, 1000 series Squirrel data logger, Grant Instrument Ltd, Cambridge, UK) and recorded at 1-min intervals during the trial.

The four designated measurement sites were cleansed with deionized water and dried with sterile towels prior to the application of sensors or absorbent pads. The skin was not abraded, as per the application of electrodes for electromyography (EMG) measurement, as the removal of the keratin in the upper layers of the skin contributes to the skins conductance (11). Figure 2 shows the (back) measurement site

configuration, which covered an approximate surface area of 20cm^2 for all measures (galvanic skin conductance (GSC) epidermal/stratum corneum hydration (HYD), regional sweat rate (RSR) and T_{sk}). Skin thermistors (Grant Instrument Ltd, Cambridge, UK) were attached to the skin using 3M^{TM} Transpore^{TM} surgical tape, (3M United Kingdom PLC) located at the chest, scapular, upper arm and thigh. Mean skin temperature (mean T_{sk}) and mean body temperature (T_b) were calculated using the following equations (17, 39):

Mean T_{sk} = (0.3*Triceps) + (0.3*Chest) + (0.2*Quadriceps) + (0.2*Calf)

T_b = (0.8*T_{re}) + (0.2*mean T_{sk})

Adjacent to each skin thermistor a pair of pre-gelled disposable Ag/AgCl electrodes (EL507, Biopac System, Goleta, California, USA) were placed 3cm apart (from the medial edges of the electrodes) for the measurement of GSC (MP35 Biopac System, Goleta, California, USA). The system applies a direct constant voltage (0.5V) as an excitation source across the electrodes. The electrodes directly reflect the electrical signal of the skin and a transducer converts this physiological signal into a proportional electrical signal; expressed in micoSievert (μS). The GSC signal was recorded at a gain of 2000 and 35Hz using the Biopac software (Biopac Student Lab Pro); based on the manufacturers guidelines this resulted in an input resolution of 0.15μS. GSC was measured as a change from baseline, which was noted as the lowest value recorded during R1 (ΔGSC).

The space between the electrodes was used for the measurement of stratum corneum hydration (HYD), which was taken at intervals using a MoistureMeterSC Compact (Delfin Technologies Ltd., Kuopio, Finland) device with an operating frequency of 1.3 MHz and according to the manufacturers has a resolution of 0.1%. The output is

given in arbitrary units, related to the combined capacitance and dielectric constant of the stratum corneum (SC). The output value is low when water content of the SC is low and the dry SC layer is thick and will increase with increasing water content and a decreasing dry layer thickness. Whilst both parameters are expected to change together, the measurement principle makes it dependent on both (1). The typical penetration depth is approximately 50 microns (1). The unit contains an inbuilt force sensor to monitor the pressure of the probe application to the skin. With target pressures around 1.4 to 2 Newton. The MoistureMeterSC begins measuring as soon as it comes into contact with the skin and takes approximately 3sec to display the reading. The same investigator took all measures for consistency. The MoistureMeterSC was positioned in the central space between the GSC electrodes and approximately 1cm below the lower edges of the RSR measurement site. From pilot testing, these distances were deemed appropriate to provide local data, whilst preventing measurement interference between methods.

HYD was measured every 2.5 min during all resting periods and at 5-min intervals during exercise, for which the participants had to cease exercise temporarily (<30s) for an effective measurement. During this measurement, the appearance of sweat on the skin was confirmed visually by inspecting the measurement areas each time a HYD measurement was taken. This was done under the standard lighting of the chamber (650 Lux, which is well above office lighting requirements of 500 Lux). After identification the sweat was dabbed dried with a paper towel before the measurement of HYD.

The area just above the electrodes was designated as the location for the collection of surface regional sweat rate (RSR); based on a similar absorbent pad technique described by Havenith et al. (18). This technique has been shown to be highly

242 correlated with the ventilated capsule system (30). Individual absorbent pads, with an
243 absorbent surface area of 10 cm^2 and a 1.3 cm wide adhesive border (3M^{TM}
244 Tegaderm^{TM}, 3M Solutions, Bracknell, UK) were used for the measurement of RSR.
245 All patches were placed in labelled airtight zip-lock bags and weighed using
246 electronic scales prior to use (Sartorius, YACOILA, Sartorius AG, Goettingen,
247 Germany, Resolution 0.01g). One pad per location was applied to the skin for the full
248 duration of each respective stage (R1, and R2 for 10 mins each, EX1 for 20mins, EX2
249 for 10mins and EX3 for 20 mins). The area was wiped completely dry immediately
250 prior to application and individual stopwatches were used to measure the application
251 duration of each pad. Whilst the application durations were longer than previously
252 advised by Havenith et al. (18) the absorbent pads used could hold more liquid than
253 was actually absorbed in the present study. After removal, the pad was immediately
254 returned to its airtight bag, reweighed and the application period recorded. RSR was
255 calculated from the weight change of the pad, the pad surface area and the duration of
256 application using the following equation:

257 $$SR = \frac{\left[\frac{(w_w - w_d)}{SA}\right]}{t} \cdot 3600$$

258 Where,

259 SR sweat rate ($g \cdot m^{-2} \cdot h^{-1}$)

260 w_w wet weight of pad (g)

261 w_d dry weight of pad (g)

262 t time, duration of pad application (s)

263 *SA* surface area of pad (m^2) (based on dry pad weight and material weight per m^2)

Gloves were worn whilst handling the pads to prevent any contamination of water and oils from the researcher's hand.

Reliability and validity of HYD, GSC and RSR measurement

Whilst there is a wealth of publications on GSC, mostly on its use for determination of psychological stress, but also some on its link with higher sweat rates, clearly showing its validity for the determination of sweat gland activity (2, 26, 29, 53), the authors struggled to find publications on its reliability / reproducibility. A large number of factors play a role in its measurement, from the use of direct versus alternating current, different electrodes, polarization issues etc. (29). For the present application, the most important references to show the relevance of this measurement are those linking the GSC with the activation of sweat gland numbers, both in increasing and decreasing number of active sweat glands (53), where a strong correlation is demonstrated. Due to its high inter and intra variability, GSC is standardized relative to a baseline value (GSC), which was determined during R1.

Similarly, for HYD, validation studies are present in the literature, but very little information on repeatability / reliability exists. One study by Alanen et al. (1) reported that the relative standard deviation varied between 2% and 5% for repeated individual measures using the MoistureMeterSC. For the MoistureMeterSC, a number of papers show its validity for measuring skin hydration (1, 27), linking the results to other instruments, but it should be noted that in most cases instruments have not been validated against sweating, i.e. skin wetting, but more to responses of dehydration as well as to application of various skin hydration formulations.

Finally, for RSR, studies have compared the absorbent patch technique to the sweat capsule technique with excellent results showing the technique to have good internal

reliability and being able to detect differences in local sweat rate as small as 0.12 mg/min/cm^2 in a variety of conditions (30). The main points to keep in mind in comparing sweat absorbents versus capsules are the differences at low and very high sweat rates. In the former, no sweat may be absorbed at the surface by an absorbent patch, while vapor already could be drawn out of the skin by the capsules technique. In the case of high sweating, using the capsule technique, the skin remains dry while the absorbent patch may have more moist skin with a risk of hidromeiosis during long exposures.

In general, whilst for GSC and HYD measurements, the evidence on reliability may be limited; this should not have been a major issue in the present study where the focus was on comparative measurements obtained simultaneously, with less emphasis on absolute values.

Data Analysis

The physiological (ΔGSC, T_{re}, T_b, mean T_{sk} and all four local T_{sk}) data were averaged every 2.5 min during the resting period and every 5 min during the exercising periods to coincide with the measure of HYD.

To determine an increased sweat production from our three measures (RSR, ΔGSC and HYD) at each location, we used a two way ANOVA (stage x location) to analyze the effect of the protocol stages (R1, R2, EX1, EX2, EX3, PEX1 and PEX2) and location (chest, back, arm and thigh). A single RSR sample was collected for each stage of the protocol, whilst both ΔGSC and HYD had more frequent sampling times. Rather than analyze each sample time point, which increases the risk of Type II errors, or using the mean of each stage, which reduces the overall response measured for each stage, we used the final sampling time for HYD and the mean of the final 2.5

mins for ΔGSC from each of the 7 stages of the protocol in the analysis. This enabled us to determine if ΔGSC and HYD increased throughout each stage of the protocol. Where main effects were observed, Bonferroni post hoc comparison were used to identify if the variables were significantly different to the previous stage. Due to the exploratory nature of this research project, ΔGSC and HYD data that was not used in the statistical analysis was still monitored for relevant physiological changes, especially during R1, R2 and EX1 when sweating was initiated.

For each location, the relation between ΔGSC, HYD and RSR was analyzed using Pearson's correlation. The relation between ΔGSC and HYD was calculated from the mean of every 2.5 min during rest and every 5 min during exercise. As the sampling time of RSR differed to ΔGSC and HYD, the final measurement of each stage (for R1, R2, EX1, EX2, EX3) for ΔGSC and HYD was used to analyze the relation with RSR. The onset for sweat appearing on the skin surface is marked along the regression line and defined as the threshold for external sweating.

To analyze the effect of each stage of the protocol, the remaining physiological data (T_{re}, T_b, mean T_{sk} and all four local T_{sk}) were analyzed using a one-way ANOVA using the final time point of each stage. Where main effects were observed, Bonferroni post hoc comparison was used to identify if any of the measured variables were significantly different to R1.

All data were analyzed using GraphPad Prism 6 and checked for normality. Any data not normally distributed were analyzed using the Kruskal-Wallis non-parametric equivalent. Mean and standard deviations (± SD) are presented and significance was defined as $p<0.05$.

Results

Protocol effect

Table 1 summarizes the mean ± SD (n=16) T_{re}, T_b, mean T_{sk} and local T_{sk} responses measured during the different stages of the protocol. In summary, all physiological responses remained relatively stable during R1 and R2 and increased during the exercise protocols (EX1-EX3). All variables declined post exercise (PEX1-PEX2) but only T_{re} returned to baseline values. Statistical analysis revealed a significant main effect of the 'protocol stage' for T_{re}, T_b, mean T_{sk} and each local T_{sk} ($p<0.05$). Post hoc comparisons were used to detect if each variable was significantly different to R1 and the results are presented in Table 1. T_{re} did not increase from R1 to R2 ($p>0.05$), but began to increase from EX1 and remained elevated above R1 until the end of PEX1 ($p<0.05$). $T_{b,}$ mean T_{sk} and all local T_{sk} responses increased from R2 to EX3 and then declined post exercise. At all stages, T_b, mean T_{sk} and all local T_{sk} were all significantly higher than R1 ($p<0.05$, Table 1).

Figures 3A-C illustrates HYD, ΔGSC and RSR, respectively, during each stage of the protocol. In summary, RSR did not change during the rest periods (R1 and R2) but both HYD and ΔGSC began to increase at all locations (see additional smaller inserted graph for Figure 3A and B, respectively). All variables increased during the three exercise stages and thereafter declined post exercise. A significant main effect of protocol stage, location, and interaction effects were observed for RSR, ΔGSC and HYD ($p<0.05$). RSR, ΔGSC and HYD during the pre-exercise rest periods (from R1 to R2) did not significantly increase ($p>0.05$). Non-significant (due to inter-individual variability) increases were observed at all locations for ΔGSC and HYD data during R1 and R2 but it is important to note that the increases were physiological meaningful based on previous findings. Exercise initiated an increase in all three variables at all locations.

HYD at all locations increased from R2, to EX1 to EX2 ($p<0.05$) but then started to level off in EX3 with only the arm and thigh increasing further ($p<0.05$). ΔGSC significantly increased during exercise. RSR increased during exercise at all locations and peaked during the final exercise stage of EX3.

Ceasing exercise resulted in all sweat variables to decrease; RSR decreased significantly from EX3 to PEX1 but levelled off in PEX2 and was still slightly above baseline after the 20 min post exercise rest. ΔGSC also decreased from EX3 to PEX1 with some variation over locations, after which all started to level off but still remained substantially above baseline. HYD declined in the rest period over all zones though with different patterns. HYD remained significantly higher than baseline for all locations ($p<0.05$).

Regional differences

Two way repeated measures ANOVA revealed a significant main effect of location and a significant interaction between location and protocol stage ($p<0.05$). There were no regional differences reported during the resting phases for any of the measured variables. As exercise began all variables increased and regional differences were generally observed between the torso and the extremities. The torso showed the strongest increase in the warm-up/exercise periods and also remained highest post exercise.

HYD initially increased significantly faster for the torso during exercise, but towards EX3 HYD was similar for all locations. ΔGSC also increased faster on the torso compared to the extremities during exercise, but in contrast to HYD these regional differences remained present to the end of EX3. Further regional differences were observed for RSR during EX2 and EX3, with the torso (chest and back) producing

more sweat compared to the extremities (arm and thigh) ($p<0.05$). The arm SR was only different (i.e. lower) to the thigh during EX2 ($p<0.05$).

After the cessation of exercise, RSR decreased significantly from EX3 to PEX1 for all locations. The largest decrease occurred at the chest, followed by the back and smaller decreases were observed at the extremities but the regional patterns were still evident. RSR then only further decreased at the chest and back from PEX1 to PEX2 ($p<0.05$), being still slightly above baseline after the 20 min post exercise rest. ΔGSC decreased from EX3 to PEX1 but this only reached significance at the chest and back ($p<0.05$). Then, ΔGSC levelled off, at different levels for different zones but remained substantially above baseline. HYD also declined in the post exercise rest period, starting at similar values for all zones at the end of EX3. Declines were slow for the chest and back ($p>0.05$) but faster for the extremities ($p<0.05$) in PEX1, with the latter levelling off substantially below the torso values in PEX2. HYD remained significantly higher than baseline for all locations ($p<0.05$).

Interestingly, locations with the highest RSR or ΔGSC value did not always correspond with the highest HYD. While HYD values at the end of exercise were very similar for all zones, suggesting corneum hydration saturation (back 122 ± 21AU, thigh (124 ± 18AU, chest 110±25AU, arm 114±23AU) the RSR (back 1178 ± 466 $g \cdot m^{-2} \cdot h^{-1}$, chest 1065 ± 541 $g \cdot m^{-2} \cdot h^{-1}$, arm 780 ± 338 $g \cdot m^{-2} \cdot h^{-1}$, thigh 674 ± 322 $g \cdot m^{-2} \cdot h^{-1}$) and ΔGSC (back 18 ± 9 µS, chest 23 ± 22 µS, arm 10± 5 µS thigh 11 ± 6 µS) were largely different.

For RSR and ΔGSC, the general picture was that the higher the end exercise value, the larger the decrease from EX3 to PEX1 and PEX2. However, this was not the case for HYD, as values were very close for all regions at the end of exercise (~110-

123AU), and then during post exercise (PEX1 and PEX2) HYD decreased largely at the extremities (to ~45 AU) but remained elevated for the chest and back (~80AU).

Relation between variables

The relations amongst the three variables (ΔGSC and HYD and RSR) from rest to the end of exercise are illustrated in Figures 4A, B and C, respectively. An additional graph is included in Figure 4A to highlight the relation between ΔGSC and HYD during the pre-exercise rest periods, where internal sweating was likely to have been initiated and values started to change from baseline. Combining the regression analysis with the threshold for surface sweating was deemed important to understand each measurement and how internal and external sweat affects each of the measures. It was decided not to include post exercise data in these graphs, as a hysteresis in the response was observed indicating a change in the relations upon sweat reduction. The main factor to which this was attributed is the accumulation of sweat under the GSC electrodes, which will be discussed later.

From Figure 4A it is possible to observe an initial increase in ΔGSC with no change in HYD at the arms only. An exponential or bi-phasic relation was observed between ΔGSC and HYD (Figure 4A) and between RSR and HYD (Figure 4B), with an obvious threshold occurring allowing for the data to be separated into two phases. Strong and very similar significant relations are observed between ΔGSC and HYD in the first phase for all individual locations ($r^2 \geq 0.803$, $p<0.05$). The threshold between the two distinct portions of the relation coincided with the visible appearance of sweat on the skin surface and typically occurred at ~70AU for HYD and 4μS for ΔGSC. Above this threshold larger changes in ΔGSC compared to changes in HYD are observed for all individual locations ($r^2 \geq 0.839$, $p<0.05$) with some variation over

zones and the torso reaching the highest values. Similar relations for the different zones were observed between RSR and HYD (see Figure 4B) and the transition in the relation also coincided with the point at which sweat was first visible on the skin surface, again occurring at approximately 70AU for HYD (as indicated by the dotted lines). With fewer data points collected for RSR, a bi-phasic response was less evident in the RSR - ΔGSC relation (Fig 4C), and a single, strong, significant linear relation existed (all locations $r^2 \geq 0.71$, $p<0.05$) across the whole range. An exponential relation existed between RSR and HYD and as such the data were transformed to produce an approximate linear relation. Strong significant linear relations existed between HYD and RSR (all locations: $r^2 \geq 0.949$, $p<0.05$).

Discussion

By simultaneously measuring GSC, HYD and RSR, this study aimed to track sweat from its production in the secretory coil, to travelling through the re-absorptive duct, penetrating the acrosyringium to hydrate the epidermis/stratum corneum and finally being released onto the skin surface. For the interpretation of the results, HYD changes are interpreted as changes in stratum corneum hydration, GSC changes as changes across all layers from sub-dermis, including the actual gland and duct, to the skin surface, and RSR measurements as reflecting surface sweating only. The main findings of the present study revealed that, as hypothesized, epidermal/stratum corneum hydration does occur prior to the release of surface sweat, but only to a submaximal level and that maximal stratum corneum hydration does occur, but only later on when surface sweat is also present. In relation to our second hypothesis, the study demonstrated clear regional differences in the development of HYD, GSC and RSR. Thirdly, in the 20 min period after the cessation of exercise, sweat does not appear to diffuse into the stratum corneum, contrary to our hypothesis and what was

previously suggested (11). Sweat production does not cease completely in this period, and most likely exceeds epidermal/stratum corneum reabsorption. The speed of skin hydration while sweating is linked to local sweat production (highest on torso), but once the skin is wet for a while, hydration becomes uniform despite sweat production differences. However, once sweating declined HYD drops fastest in areas where absolute sweat production was lowest (extremities). Our final hypothesis was confirmed as it was shown that the three methods clearly measure different aspects of sweat formation, transport and absorption. These findings will be discussed in detail below.

As a side note, although both males and females were recruited, the aim of the present study was not to determine sex differences and this thus this did not form part of the analysis. In general however, there were no noteworthy differences between the sexes in any of the data reported.

Sweat gland stimulation

Sweat glands are stimulated in response to a rise in T_{re} and/or T_{sk} or non-thermal, mainly metabolic factors (24, 28, 32). According to our data T_{re}, T_b and mean T_{sk} remained stable during the initial rest period (R1) hence the lack of change in HYD, ΔGSC and RSR (see Figure 3B & C). Movement to a warm chamber resulted in an increase in ambient temperature of ~7.0°C, resulting in an initial drop in T_{re} and an increase in mean T_{sk}. The drop in T_{re} and rise in mean T_{sk} is typical of the core-to-periphery re-distribution of heat via a change in skin blood flow observed upon initial exposure to hot conditions or with exercise (21). Such thermophysiological changes stimulate the sweat glands to produce an iso-osmotic precursor fluid from the secretory cells after which sweat will travel towards the skin surface. HYD and ΔGSC

increases without RSR increases, indicate the initiation of sweat production, without any release onto the skin surface in R2. While increases in HYD and ΔGSC failed to reach statistical significance during R2 and EX1, mainly due to individual variations in responses, the magnitudes of the increases observed are physiologically meaningful as they are typical of pre-secretory sweat gland activity for ΔGSC and increases in epidermal hydration (1, 12, 26).

In order to discriminate pre-secretory sweat gland activity in the subdermis from the epidermis, an increase in ΔGSC prior to an increase in HYD was required. Technically this was difficult to determine but it was observed on the arms (see small inserted graph in Figure 4A). It is possible that the time between sweat gland stimulation in the subdermis and passage of sweat towards the stratum corneum, located in the epidermis, occurred more quickly than was detectable from the HYD measurement, which was taken every 2.5 mins for all zones except the arm (due to their higher sweat production rate).

For the arm, ΔGSC did increase at the start of R2 whilst HYD did not increase to significant levels until the end of R2. This may suggest that upon stimulation the passage of sweat through the coil and the duct towards the acrosyringium in the epidermis is faster in the chest, back and thigh compared to the arm and thus we were able to detect this only in the arm with our measuring devices. These regional differences may be associated with the structure or sensitivity of the sweat glands at different parts of the body. Sato & Sato (44) reported a strong significant relation between sweat rate and sweat gland size (3.14 * length * diameter) ($r=0.8109$, $p<0.005$) and between sweat rate and cholinergic sweat gland sensitivity ($r=0.806$, $p<0.001$) measured from self-diagnosed ‘poor’ and ‘good’ sweaters. Given that Smith et al. (52) found no regional differences in cholinergic sensitivity between the arm,

thigh or chest, the differences in the passage speed of sweat through the skin may be mainly attributed to a smaller peripheral sweat gland size compared to torso sites. It is possible that GSC and HYD can distinguish between sweat in the gland and sweat in the sub-dermis as evident by the responses observed at the arm. However, in order to study the production of sweat in the sub-dermis before it starts to hydrate the skin in higher sweat regions, future research should seek ways to continually measure skin hydration, and/or raise SR even slower.

Sweat within the stratum corneum

It has been suggested that before sweat is released onto the skin surface, a process known as corneal hydration occurs in which the sweat penetrates the acrosyringium due to a build-up of pressure and is absorbed by the stratum corneum in the upper layers of the epidermis (25). Once the sweat enters the stratum corneum, supposedly substantial corneal hydration occurs due to its hygroscopy. It has been shown to be able to hold up to 70% of its own weight in water (23). However, to the authors knowledge, this has only been shown in experiments involving changes in ambient relative humidity (23), submersion in water (36, 40) and dermatological studies of topical solutions for epidermal treatments (33), but not before in studies involving sweating. We hypothesized that sweating would be visible on the skin when the stratum corneum is only sub-maximally hydrated. Indeed, we can confirm this as the current study indicates that during sweating, the epidermis gradually hydrated with the speed related to local sweat production values towards a saturation plateau that was similar for all areas. Our data support previous findings that the corneum hydrates substantially before sweat is released onto the skin surface (5), with the threshold for the appearance of surface sweat occurring at approximately 70AU (58% of the maximum value) in HYD. Maximal hydration occurred during EX3 for all

532 locations, but was more evident on the chest and back. A saturated stratum corneum
533 has been shown to suppress sweating (i.e. in hidromeiosis) (36), which typically
534 occurs after substantially longer periods of heat exposure (> 90 min) and for higher
535 sweat rates than observed in the present study. Thus, given that a maximum hydration
536 seemed to be achieved in the present study, there also must be a time factor for the
537 development of the impact of this maximum hydration on sweat output; perhaps
538 pointing at a slow development of the skin and sweat duct swelling to which the
539 hidromeiosis is attributed.

540 **Sweat on the skin surface**

541 Visible sweating was typically first observed during EX1, which coincided with an
542 increase in RSR during this measurement period. During the exercise stages it is
543 assumed that sweat is present in the secretory coil, the re-absorptive duct, the
544 acrosyringium and on the skin surface. The HYD and ΔGSC thresholds for observed
545 external sweating are indicated in Figure 4A and B by vertical dotted lines. Prior to
546 this point we observed a strong significant relation for all locations (r^2>0.80, p<0.05),
547 which is representative of internal sweat; occurring typically up to 70 AU for HYD
548 and 4 μS for ΔGSC. There is a clear transition in the slope of the relation between
549 RSR and HYD around this point, while the transition in the slope of RSR and ΔGSC
550 is not as strong, if present at all. From Figure 4B it can be seen that before external
551 sweating was visually confirmed, the RSR measurement showed some small amounts
552 of external sweating to occur below this threshold. This, and the observation that
553 HYD was well below its maximum at the threshold (70 out of 120 AU, i.e. 58%),
554 supports our first hypothesis that the skin does not need to hydrate fully before
555 surface sweating and evaporation begins. Above the external sweat threshold, HYD
556 increased less per unit of increase in RSR, while ΔGSC shows a stronger increase per

unit of RSR increase compared to the slope below the threshold. This difference suggests that HYD is mainly driven by internal sweating, but saturates when moisture is added on the surface, while surface sweating has a slightly bigger magnitude of impact on ΔGSC than internal sweating. Arguably, the latter relation could be described by a single slope, however the slope change is small. As such, strong significant and similar linear relations were found between local RSR and ΔGSC for all locations (Figure 4C, $r^2>0.71$, $p<0.05$). This supports previous research which suggests that GSC is strongly related to increasing and decreasing number of active sweat glands (53). The chest and upper back have higher maximal ΔGSC and RSR, which coincides with the observed higher RSR at these sites and with literature reporting highest sweat rates at the torso in comparison to the extremities (8, 19, 50, 51). Nevertheless, in Figure 3B, ΔGSC during EX3 was highest at the chest and exceeded that of the upper back, yet RSR was similar between sites. In situations where the difference in ΔGSC between locations is not mirrored by differences in RSR this may be attributed to a higher sodium chloride (NaCl) content for a given sweat output. Despite these regional differences, Fig. 4C suggests that ΔGSC is a good overall indicator of sweat generation. HYD on the other hand would not be a good indicator across the range of sweat generation due to its clear saturation once surface sweating starts. The different observations for the different methods confirm our final hypothesis and support previous research that the different methods measure different parts of the sweating process.

Decline in sweat production

Once exercise was terminated, ΔGSC and RSR declined sharply despite T_{re} and T_{sk} remaining elevated, consistent with earlier observations (21), most likely due to a drop in non-thermal feedback to the brain. The decline was most notable (with more

582 significant differences) during the first 10 minutes (PEX1) but sweating was still
583 present after 20 minutes (PEX2). In the present study, the sharp drop in RSR
584 coincided with a sudden decrease in ΔGSC occurring immediately upon the cessation
585 of exercise with the magnitude of the drop linked to the absolute sweat rate in
586 exercise as well as to the size of the drop in RSR after exercise ceased, i.e. strongest
587 at the back and chest (see Figure 3B). Edelberg (10) suggested that after sweating
588 stops, sweat within the duct, and acrosyringium will either slowly diffuse into the
589 stratum corneum or will be reabsorbed into the sweat gland. If this were the case, we
590 might expect to see an increase in HYD. Though as the skin was already fully
591 hydrated across the areas tested before exercise ceased this is not a plausible
592 explanation. Indeed, HYD does not increase further, and at the extremities actually
593 decreased immediately. This, together with the lower RSR at the extremities during
594 exercise, suggests the skin surface sweat may dry up faster and thus HYD reduces
595 faster at these body regions accordingly, without an indication of a stratum corneum
596 reabsorption phase. It is plausible that more sweat will be present on the skin's
597 surface at the end of exercise for the chest and back due to their higher RSR, while
598 sweat rates on the torso remain higher after exercise, for longer than at the
599 extremities. Thus, while all skin sites were saturated, the higher RSR and surface
600 sweat layer may have kept HYD higher for the chest and back after exercise while the
601 skin starts to dry out earlier for the extremities.

602 Overall, the present data does not confirm a relevant role for the re-absorption of
603 sweat back into the skin and sweat glands as we hypothesized and previously
604 suggested (12). If this would take place at all in relevant quantities, the present data
605 suggest that this would only be observed if exercise ceases before skin hydration
606 reaches saturation, i.e. at quite low to moderate sweat rates. Hence, if these processes

607 are to be investigated further to add more certainty to our hypothesis, future studies
608 should consider recovery after periods with lower heat loads and lower sweat rates, as
609 well as using much longer post exercise rest periods that allow variables to return to
610 baseline. In terms of application of these findings, e.g. in clothing design, the data
611 indicates that for the torso, material that quickly wicks sweat away may be beneficial
612 from a thermoregulatory, behavioral and sensorial perspective. In addition, the data
613 provides useful information for those aiming to develop wearable sensors, monitoring
614 sweat and its contents to discern what occurs at the skin once sweating declines.

615 In terms of using the different methods to describe sweating in this stage, Figure 3a-c
616 shows that while RSR data converge after 20 min post exercise, HYD and ΔGSC still
617 discriminate between regions, separating those with high sweating during exercise
618 (torso) and early post exercise from those with lower values (extremities). For ΔGSC,
619 a practical limitation may be present that prevents it from returning to baseline in a
620 timely accurate manner. This will be discussed in the next section.

621 **Limitations**

622 A limitation to ΔGSC in its presently used method is that the metal electrodes cover a
623 section of, and remain in contact with, the skin and thus any sweat produced
624 underneath cannot be evaporated. As sweat production decreases, ΔGSC will
625 therefore not return to baseline, as any sweat formerly produced will be contained in
626 the skin under the electrodes' contact point, keeping skin hydration directly under the
627 electrodes high. After a quick decline post exercise, ΔGSC remained stable while in
628 contrast, HYD measured in an uncovered area with free evaporation continued to
629 decline. In this case, it is possible that the sweat within the epidermis was being
630 reabsorbed into the sweat glands; however, as no marked changes in ΔGSC were

noted, this process would be slow. Pilot testing using artificial sweat sprayed onto skin and electrode from the outside (i.e. without the moisture under the electrodes), confirmed that the issue is not with sweat on the skin but with sweat under the electrode. In general, for GSC, hysteresis effects from increasing to decreasing sweat have been described in the literature, even when the electrodes are placed outside the sweating region (53). Thus apart from the occlusion issue by the electrode, other factors may influence this too.

In the present experiment, absorbent pads were left on longer than previously advised (5 mins) (30, 50, 51) and therefore the T_{sk} underneath the absorbent pad may have been higher than measured by the neighbouring skin thermistor. Our longer pad application durations may have caused an elevated local T_{sk} and a subsequent increased sweat production. However the risk of hidromeiosis that could have been initiated by having longer applications of potentially wet pads, would be low as the patches used could hold substantially more moisture than was collected in any sample. In addition, previous studies utilizing the absorbent patch technique used plastic sheeting and tight fitting clothing to keep the absorbent patches affixed to the skin. This was not possible in the present study, as clothing would have interfered with epidermal/stratum corneum hydration, which also would have been difficult to measure. Therefore, we utilized a patch with an adhesive covering affixed to the absorbent material and a 1.3 cm wide border to affix to the skin. The adhesive tape does not absorb moisture but some sweat may have been present on the adhesive material when weighed, which may have resulted in a higher estimation of sweat rate. However, this would be rather consistent across all measurements.

Conclusion

Sweat gland activation before skin hydration changed was detected at the arms by changes in ΔGSC but no changes in HYD. As hypothesized, the epidermis hydrates prior to surface sweating being detected but did not do so to full saturation. Once surface sweating was visible, the skin continued to gradually hydrate to a maximum. At the cessation of exercise, sweat rate dropped, and surface sweat appeared to evaporate quickly, while ΔGSC and HYD trailed behind, indicating that surface sweating disappeared first whilst skin dried up slowly.

As hypothesized, regional differences were evident on all measured sweat variables, with the responses strongly linked to the absolute sweat productions at the different locations. Higher sweat productions on the torso led to distinctly higher values for HYD and ΔGSC, during and after exercise. Contrary to the hypothesis, no evidence was found for sweat re-absorption into the stratum corneum or the sweat gland, after sweating was reduced. The three different measurement techniques provided distinct information on different sweat stages and regions, but also had overlapping responses. The data from this study provides useful information for research pertaining to environmental ergonomics, dermatology, thermoregulation, skin-interface interactions and wearable physiological monitoring devices.

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Gerrett, Katy Griggs and George Havenith were fully responsible for the design and conduct of the trials, data analysis and write up.

Disclosures

The authors declare that they have no conflict of interest.

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Figure Title

Figure 1: Schematic diagram of the testing protocol indicting time periods for R1 (pre exercise rest in 23.4 ± 0.5°C, 50 ± 4.7% relative humidity), R2 (pre exercise rest in 30.1 ± 1.0°C, 30 ± 4.7% relative humidity). EX1, EX2 and EX3 corresponding to three different exercise intensities (30%, 50% and 70% of VO_{2max} on a treadmill, respectively), which were then followed by two post exercise recovery periods, labelled PEX1 and PEX2. R2 to PEX2 were all conducted 30.1 ± 1.0°C, 30 ± 4.7% relative humidity.

Figure 2: The measurement area of the upper back indicating the location of a) an electrode for galvanic skin conductance (ΔGSC), b) thermistor for skin temperature, c) absorbent patch for regional sweat rate (RSR), d) MoistureMeterSC for epidermal hydration (HYD), which was applied periodically.

Figure3: Mean (n=16) a) HYD, b) ΔGSC, c) RSR at the chest, back, arm and thigh during rest in temperate condition (23.4 ± 0.5°C, 50 ± 4.7%, R1), rest in a warm condition (30.1 ± 1.0°C, 30 ± 4.7%, R2), exercise at 30% VO_{2max} (EX1), exercise at 50% VO_{2max} (EX2), exercise at 70% VO_{2max} (EX3) and post exercise rest in a warm environment (PEX1 and PEX2). Additional smaller graphs are inserted to a) and b) to increase the resolution of HYD and ΔGSC (respectively) during the resting periods.

Note: RSR has a different measuring frequency to ΔGSC and HYD with only one sample per stage measured.

Figure 4: The relation between A) ΔGSC and HYD B) RSR and HYD, C) RSR and ΔGSC. Data points in A are the mean of all participants (n=16) measured during rest (samples taken every 2.5 min) and exercise (samples taken every 5 min). Data points in B for ΔGSC and C for HYD are the mean of all participants (n=16) of the final measurement of each stage. In Figure 3A the vertical and horizontal dotted line indicates the approximate means for HYD (~70AU) and ΔGSC (~4μS) at which sweat was noted as being visually present on the skin surface. Below the dotted lines a strong significant linear relation is observed for all locations (r^2>0.803, $p<0.05$). Above the dotted lines strong significant linear relation is observed for all locations (r^2>0.839, $p<0.05$). The smaller inserted graph (in A) highlights the relation between the two parameters measured from R1 to EX1 when internal sweating was likely initiated. In Figure 3B and C the vertical dotted line indicates the approximate mean for when sweat was noted as being visually present on the skin surface. Strong significant linear relations existed between HYD and RSR (all locations: r^2>0.949, $p<0.05$) and between RSR and ΔGSC (all locations r^2>0.71, $p<0.05$).

878 **Table 1:** Physiological responses (mean ± SD, n=16) measured during each stage of the protocol. Values for R1, R2, PEX1 and PEX2 are the
879 mean of the final 2.5 min whilst EX1, EX2 and EX3 are the mean of the final 5 min, * and ** indicates significant difference ($p<0.05$ and
880 $p<0.001$, respectively) to R1. T_{re} = rectal temperature, T_b = body temperature, T_{sk} = skin temperature. R1- rest in temperate condition (23C, 50%
881 RH), R2= rest in a warm condition (30ºC, 30% RH), EX1 =exercise at 30% VO_{2max}, EX2 = exercise at 50% VO_{2max}, EX3= exercise at 70%
882 $VO_{2max,}$ PEX1 and PEX2 = and post exercise rest in a warm environment.

	R1	R2	EX1	EX2	EX3	PEX1	PEX2
T_{re} (ºC)	37.3 ± 0.3	37.2 ± 0.3	37.3 ± 0.2*	37.6 ± 0.4**	38.1 ± 0.3**	37.7 ± 0.2**	37.5 ± 0.2
T_b (ºC)	36.2 ± 0.3	36.4 ± 0.3*	36.6 ± 0.2**	36.9 ± 0.3**	37.4 ± 0.3**	37.0 ± 0.3**	36.8 ± 0.2**
Mean T_{sk} (ºC)	31.5 ± 0.8	33.3 ± 0.6**	33.8 ± 0.5**	34.0 ± 0.5**	34.5 ± 0.8**	34.2 ± 0.7**	33.9 ± 0.8**
Chest T_{sk} (ºC)	32.5 ± 1.2	34.1 ± 0.8**	34.3 ± 0.7**	34.4 ± 0.7**	34.6 ± 0.9**	33.9 ± 1.1**	33.6 ± 1.2*
Back T_{sk} (ºC)	31.8 ± 1.1	33.7 ± 0.8**	33.8 ± 0.7**	33.6 ± 0.9**	33.8 ± 1.8**	34.4 ± 0.8**	34.0 ± 0.9**
Arm T_{sk} (ºC)	30.3 ± 1.2	32.4 ± 0.7*	33.1 ± 0.8**	34.0 ± 1.2**	35.1 ± 1.6**	34.3 ± 1.8**	34.2 ± 1.2**

Thigh T_{sk} (ºC)	31.0 ± 0.9	32.8 ± 0.6**	33.8 ± 0.5**	34.3 ± 0.6**	35.0 ± 0.7**	34.3 ± 0.8**	34.0 ± 0.7**

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