SWEAT ANALYSIS: A PAINLESS ALTERNATIVE TO REAL-TIME VITAL SIGNS ANALYSIS

PART 3: RELIABILITY OF SWEAT CONCENTRATION

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Thermoregulatory sweating is responsible for the loss of water and electrolytes when sweating. In some cases, protracted exercise periods, involvement in high-intensity exercise and working out in hot surroundings can lead to the loss of large quantities of sweat, which can cause dehydration and electrolyte imbalances, thus hampering physiological performance. In medical and sports settings, the sweating rate (SR) and the levels of electrolytes in sweat are known to differ significantly from one individual to the next due to numerous inherent or extraneous causes. Therefore, it is necessary to consider customised fluid replacement strategies as recommended by Baker (2017). Furthermore, before deciding to use sweat components as biomarkers for determining vital signs and disease diagnosis, it is imperative to bear in mind the dependability of sweat concentration. The quantity and concentration of sweat determine the testing outcomes. Therefore, any undue variations in sweat concentrations can interfere with the final outcomes.

Consequently, scientists and medical experts have performed sweat tests using different methods, which can also cause unwanted variability in sweat rate and concentration. For instance, sweat analyses can be done using sweat samples collected using whole-body methods or can be restricted to a particular body region. In addition, the analytical methods used to analyse sweat can vary in their modes of operation, scheduling and length of sweat collection, skin sanitising processes, and collection and storage of sweat samples as well as the actual analytical procedure. Using unacceptable or uneven methods of sample collection and analysis can alter outcomes substantially by introducing errors, background noise and misinterpretation of findings. Therefore, this section looks at the methodological considerations to guarantee the

reliability of sweat concentrations as well as various studies conducted to validate different sampling and analytical approaches. Even though sweating leads to the loss of several electrolytes, sodium is lost in large quantities, significantly affecting the body's fluid and electrolyte balance. Therefore, most studies reviewed herein tend to focus on sweat sodium content as an indicator of sweat concentration.

Influence of Analytical Methods on Sweat Concentration Validity

Different analytical procedures can be exploited to determine sweat sodium concentration, which could have varying implications for athletes and scientists. Therefore, the reliability of sweat concentration can be influenced by the laboratory methods used to analyse the sweat. A few studies have sought to determine the influence of this parameter on sweat concentration. For example, Baker *et al*. (2014) weighed field methods of extracting and analysing sodium and potassium in sweat against reference laboratory approaches for sweat samples collected using absorbent patches in a hot, moist environment. The field extraction method entailed using a syringe to suction sweat samples from the absorbent patch, whereas the laboratory approach involved centrifuging the patch to collect accumulated sweat. In contrast, the analytical method in the field used the HORIBA compact system, whereas laboratory analysis used ion chromatography and high-performance liquid chromatography (HPLC).

Sweat samples for both conditions were collected from seven body sites, including the forehead, right anterior mid-thigh, left posterior mid forearm, right posterior mid forearm and upper chest as well as the left and right scapula. A total of 30 athletes were involved in one-hour cycling activities in a heat chamber at 33°C. Skin areas at the specified anatomical sites were cleaned using deionised water and dried with gauze 10 minutes following the initiation of

exercise. Each anatomical site had two sweat patches, each of which was analysed in the heat chamber using the field system or laboratory approach.

The researchers noted that the sodium and potassium concentrations obtained using the syringe system fell within error measurements of ±15.4 and ±0.68 milliequivalents per litre, respectively (Baker *et al.*, 2014). These results did not differ significantly from those obtained in a typical laboratory setting. The findings of this study showed the reliability of sweat concentration when extracted and analysed using the field (syringe HORIBA) technique from local absorbent patches. Therefore, this method can be used successfully in instances where rapid estimates of sweat sodium are required in a hot, moist environment.

In a separate study, Goulet *et al.* (2017) compared the concentrations of sodium in sweat as measured using 5 analytical techniques: flame photometry, ion chromatography, ion conductivity, indirect ion-selective electrode and direct ion-selective electrode. A total of 14 participants were involved in the study, leading to the collection of 70 sweat samples that were subjected to analysis using various techniques. Ion chromatography was employed as the reference investigative instrument. Excellent relative and absolute reliabilities were demonstrated among the instruments with an intraclass correlation coefficient of 0.999 and a coefficient of variation (CV) of 2.6%. A high relative validity was also achieved using the five analytical techniques.

When determining the inter-technique absolute validity (using ion chromatography as the reference standard), similar standard errors of estimates were noted among the methods, ranging between 2.8 and 3.8 mmol/L. However, the lowest CV was observed with the direct ion-selective electrode (3.9%), whereas the highest CV was recorded with ion conductivity at 12.3%. These findings led to the conclusion that sweat sodium concentration varies with the type of analytical

technique used to determine it. Consequently, findings obtained using different techniques are not equivalent and should not be used as substitutes. Nonetheless, considering typical variations in sweat sodium levels, which Goulet *et al*. (2017) estimated at ±12%, the inexactness of the endorsements based on flame photometry, ion chromatography, ion conductivity, indirect ion-selective electrode and direct ion-selective electrode has negligible health and physiological effects. However, the impact of these differences in diagnostic situations is unknown.

Influence of Sampling Site on Sweat Concentration Validity

Different types of sweat glands are found in various body parts, which means that the levels of sweat excreted may differ from one body part to another. Therefore, the reliability of sweat concentration may be affected by the area of the body from which the sweat has been collected. In this regard, Baker *et al*. (2018b) determined the association between regional and whole-body sweating rate in addition to regional and whole-body sweat Na^+ concentration over the course of exercise. A total of 26 participants were involved in the study, out of which 17 were male and 9 were female. The subjects were engaged in recreational cycling for 1 hour and 30 minutes. Whole-body sweat was collected using the washdown method. In contrast, the regional sweating rate and sodium concentration were measured using absorbent patches from nine anatomical sites. A meaningful agreement was observed between the rate of sweat production at various body regions, sodium concentration and the whole-body measurements. Day-to-day variability had a noteworthy impact on the regression model to forecast whole-body sweat level from regional sweat rates at most body sites.

However, no effect was discernible on the regional and whole-body sweat sodium concentrations. The findings suggested that regional sweating responses cannot be handled as immediate substitutes for whole-body sweating rejoinders. However, using regression equations

to forecast whole-body sweat sodium from regional sweat sodium can estimate whole-body sweat sodium with satisfactory accuracy rates, particularly when using the thigh or forearm. Nevertheless, traditional whole-body mass balance computations remain the recommended method for measuring the speed of whole-body sweat production. This decision is informed by the fact that using the regional sweat rate to project the whole-body sweat rate when absorbent patches are used to collect sweat fails to satisfy precision or reliability requirements needed to guide fluid consumption endorsements.

Influence of Sample Handling and Storage on the Reliability of Sweat Concentration

As a biological fluid, sweat contains several molecules whose stability may be affected by storage conditions. These changes may affect the outcomes of sweat analysis. In this regard, Baker *et al*. (2018b) determined the impact of storage temperature on the concentrations of sodium, chloride and potassium in sweat samples tested 7 days post sampling. The sweat samples were collected by way of the absorbent patch method. A total of 845 sweat samples were obtained from 39 participants with a mean age of 32 years and average body weight of 72.9 kgs. Of the samples, 609 were tested on the same day (pre-storage) for sodium, potassium and chloride by ion chromatography, while 236 were analysed for sodium concentration using only a compact ion-selective electrode. The samples were subsequently stored at four different conditions: −20 °C, 8 °C, 23 °C or alternating between 8 °C and 23 °C for a week. The samples were then tested using the same techniques and labelled post-storage.

The researchers noted a high correspondence between pre-storage and post-storage sweat electrolyte concentrations. Mean differences between the two storage conditions were statistically significant. However, the difference did not have a substantial impact on the practical applications of sweat electrolyte concentrations. All storage conditions generated

reliable outcomes that did not differ significantly in the levels of sweat electrolytes obtained when the samples were tested immediately after sampling versus those assessed after holding the samples for 7 days.

Influence of Biological Factors on the Reliability of Sweat Concentration

Sweat sodium chloride concentrations have been employed in the identification of cystic fibrosis for several decades. The disorder is attributed to a dysfunction of the CF transmembrane conductance regulator (CFTR), which is a protein channel that oversees the conveyance of chloride and bicarbonate ions (Collaco *et al*., 2016). Impaired CFTR performance in the sweat gland results in high chloride levels in perspiration. Therefore, changes in the CFTR function can also interfere with the validity of sweat concentration when sampling sweat for the measurement of vital signs as well as other diagnostic or prognostic purposes.

Collaco *et al*. (2016) investigated the causes of discrepancies in sweat chloride concentrations among patients suffering from cystic fibrosis. The researchers took into consideration a number of biological factors, including demographic, environmental and distinct individual variations. Sweat chloride amounts were measured in 1,761 participants, including twins or family members. Transmutations in the CFTR gene were mainly responsible for disparities in the dilutions of sweat chloride, accounting for approximately 56.1% of the variation (Collaco *et al*., 2016). Other sources of variation included time (sweat testing on different days), which accounted for 13.8% of the differences; environmental attributes such as diet and climatic conditions contributed towards 13.5% of the variation, whereas other outstanding factors such as test inconsistency accounted for 9.9% of the variation. Distinct individual features such as genetic variations and exposure to specific environments led to 6.8% of the variability.

The evaluation of information from identical siblings showed that modifier genes had no substantial influence on outcomes because the heritability approximation was insignificant. Therefore, for a person with cystic fibrosis, while changes in the CFTR gene influence most of the deviations in chloride levels in sweat, the rest of the discrepancies are linked to random factors. The authors concluded that sweat chloride quantities were reliable biomarkers for evaluating patients' reaction to treatments meant to remedy a mutant CFTR gene if assay precision and exactitude can be augmented.

These studies show that sweat concentration is a stable biomarker for various medical purposes. However, its reliability can be affected by several biological and nonbiological factors as already described. These challenges can be circumvented by following a few best practices as recommended by Baker (2017). First, it is worth noting that to collect data that are true reflections of sweating during exercise, the application of sampling patches should be done following the initiation of physical activity. The rate of sweating usually increases gradually at the commencement of exercise until a stable rate is achieved. However, the most appropriate time to apply the patch has not been established and can differ based on various features such as the intensity of exercise, the surroundings and heat adjustment status among others. However, it is proposed that patches should be applied approximately 20 to 30 minutes after the commencement of exercise to yield reliable exercise sweating rates and sweat concentrations. Nonetheless, if the purpose of sweat collection is for diagnostic purposes where sweat rate values are not required, the patches can be applied earlier. Still, the need remains for additional research to ascertain the effect of patch application scheduling on sweat sodium concentration to inform best practices in the testing of sweat.

When preparing to collect sweat, the athlete's or patient's skin should be cleaned using alcohol, cleansed with deionised or distilled water and dried with paper towel or gauze that is free of any electrolyte. These processes should be done just before the application of the patch to avoid contaminating the collected sweat. Other researchers suggest that forceful cleaning and thorough cleansing of the skin is needed to eliminate skin surface contamination attributed to mineral deposits such as zinc, iron, magnesium, copper and calcium as well as skin desquamation. However, it has been demonstrated that these procedures are unnecessary when quantifying sodium and potassium levels (Buono, Stone and Cannon, 2016).

Another notable challenge is the shedding of patches in the course of exercise. To avoid this problem, the sampling anatomical site can be shaved before applying the patch. An arm wrapper consisting of breathable material can also be used to cover forearm patches to preclude the loss of sticking power. Another best practice is to monitor patches and remove them after an adequate sweat sample has been absorbed but before the patch becomes saturated. Visual assessment is required in this case. The absorbent pad should then be detached from the adhesive dressing using a sterile pair of forceps and kept in an airtight container before analysis. Furthermore, the researcher should don clean gloves free of any electrolyte when applying and removing the patch to avoid introducing contaminants to the sweat sample. Sweat can be removed from the pervious cushion by placing it in a sieve tube followed by centrifuging it at approximately 3000 rpm for about 10 minutes if the sample is to be taken to a test centre for additional analysis. Another alternative is to place the pad in a syringe and squeeze the sweat out if the analysis is to be conducted in a field setting.

Regarding the storage of sweat samples, it is necessary to ensure that absorbent pads or sweat samples are sealed in airtight containers to avoid evaporation, which could lead to

inaccurate assessment of electrolyte concentrations. Few investigations have been conducted to establish the influence of storage time and temperature on the integrity of sweat samples. In the case of sweat testing criteria meant for the identification of cystic fibrosis, samples are required to be stored at approximately -4°C for 72 hours at most to preserve the integrity of the sweat and avoid evaporation (Collie *et al*., 2014). However, the studies that informed the development of these guidelines did not consider the storage of sweat samples for longer durations, such as 7 days. Nonetheless, Baker *et al*. (2018b) filled this gap and ascertained that no practical differences were observable in sweat concentration values obtained from samples analysed immediately and after 7 days. Nevertheless, appropriate storage conditions are needed to ensure the integrity of sweat volumes and composition.

For the purpose of sweat analysis, numerous analytical techniques have been established to measure sweat electrolytes. Examples include mass spectrometry, ion-selective electrode, ion chromatography, and flame photometry (Baker et al. 2018). Modern-day laboratory reference methods for the investigation of sweat electrolytes include inductively coupled plasma mass spectrometry and ion chromatography. These methods need minute quantities of sweat samples and are associated with high levels of accuracy, sensitivity and reliability with CVs ranging from 1 to 5% (Doorn *et al*., 2015). In the case that the investigator cannot meet the required sample storage conditions and duration, it may be better to analyse samples in the field as opposed to transporting them to the laboratory for subsequent testing. Other benefits of field testing of sweat samples include reduced transport costs and delays in obtaining outcomes. During field analysis, commonly used techniques are ion-selective electrode and conductivity, which have demonstrated high reliability with CVs ranging from 1 to 4%; in addition, these techniques can generate sweat sodium concentrations within 2 to 4 mmol/L (Baker et al., 2014). However, more

studies are needed that will compare different analytical methods to come up with best practices in sweat sodium concentration analysis in field and laboratory setups.

Local sweat concentration is not an acceptable direct determinant for whole-body sodium concentration as shown by Baker *et al*. (2018a). The researchers attributed this observation to the formation of a microenvironment following skin coverage by a patch, which enhances local humidity and wetness of the skin. Furthermore, it is possible for the skin stratum corneum to interact with sweat accumulated within occlusive layers. It has also been shown that sweat sodium levels differ across various anatomical sites (Baker, 2017).

These three shortcomings and their confounding impact on sweat sodium concentration can be alleviated several ways. For example, absorbent patches are made up of occlusive layers that enhance the accumulation of moisture on the skin. As a result, sweat ducts block gradually, leading to withholding of sweat at the sweat sample collection site. This phenomenon is known as hydromeiosis (Baker *et al*., 2018b) and can be reduced by using patches that consist of substances with high absorbency. Reducing the duration that the patch rests on the skin can also lower this effect. Some authors propose that patches should be left on the skin for a maximum of 5 minutes, while others have left the patches on for as long as 90 minutes in field conditions. Prolonged patch times may be associated with the inability of the investigator to reach the subject during exercise or the need for large quantities of sweat samples. Information is limited regarding the effect of patch adherence time and how it affects the concentration of sodium in sweat. Therefore, future studies should focus on ascertaining best practices in the use of absorbent patches to collect sweat.

Another common problem in sweat analysis is obtaining falsely high electrolyte concentrations due to leakage of electrolytes to the samples from the skin using occlusive

dressings. It is also possible for the skin to absorb the water from sweat. This issue can be avoided by using sweat potassium concentrations as a quality control check. Given the physiology involved in the movement of sodium and potassium, it is expected that sweat potassium levels should remain constant even in the face of fluctuating sweat rates. Consequently, having sweat potassium levels that are not within the expected limit of 2 to 10 mmol/L is an indication of possible leakage, evaporation or contamination (Dziedzic *et al.*, 2014).

Studies have shown that the rate of sweating and sweat sodium concentrations vary across various parts of the body (Baker *et al.*, 2016; Baker, 2017; Baker *et al.*, 2017). Regional discrepancies in the sweating rate can be explained by anatomical differences. Similarly, interregional variations in the level of sweating and concentration of sodium follow a distinct pattern with the highest rate being observed on the forehead followed by chest, scapula, forearm and finally the thigh having the lowest proportion. The most frequently used sites in the quantification of sweat parameters usually overapproximate whole-body sodium concentration by 25 to 100%. These areas include the forearm, chest, forehead and scapula. However, the levels of sodium in specific anatomical sites have a high correlation with whole-body sodium amounts. For these reasons, it is possible to employ mathematical regression equations to predict total sodium levels using sweat obtained from specific anatomical sites.

Overall, substantial inconsistencies are have been observed in the pace of sweating and sodium concentrations in perspiration during exercise. These variations can be explained by factors such as disparities in the intensity of exercises, the state of the surroundings, the capacity to acclimatise to heat and genetic disposition among others. Unexpected variations can also occur because of unreliable methodology. Furthermore, small variabilities in sweat testing

outcomes can still be observed when an investigator adheres to recommended best practices. Changes in body mass prior to and following exercise can inform the estimation of the whole-body sweating rate. However, relevant corrections for other factors contributing to body mass that are unrelated to sweat are necessary. These include fluid input and output in the form of urine.

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