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# Sweat Electrolyte Analysis for Health Monitoring: Investigating Ion Dynamics Using COBAS Pure 6 Analyzer

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**ABSTRACT:** Analysis of the electrolytes in sweat provides useful information about hydration, body temperature regulation and physiological responses to physical exertion in a human body. An examination of electrolytes in sweat collected after morning and afternoon workouts was conducted using the COBAS Pure 6 Analyzer. Results indicated that there were large differences in the composition of the electrolytes in the sweat samples collected after the morning versus afternoon workout. Sodium and Chloride loss were found to be very high after the morning workouts, while the loss of these two electrolytes was found to be larger in the sweat samples collected after the afternoon workouts. Losses of Potassium were found to be larger in the sweat samples collected after the afternoon workout, and this may have been caused by the greater muscle activity during the afternoon workout, and possibly by increased metabolism during this time.

The study examined the distribution of the electrolytes in the sweat of individuals in different blood types, and found evidence that genetics may influence the rate of secretion of ions by the glands of sweat. These results suggest that each person has their own unique system for regulating fluids, and controlling exercise performance and recovery. This study followed the ethical guidelines of World Health Organization (WHO) and Council for International Organizations of Medical Sciences (CIOMS) to ensure the safe participation and accuracy of the samples procured. Future investigations shall examine the impact of genetic markers, environmental factors and long-term trends to comprehend the dynamics of sweat electrolytes for diagnostic purposes in sports science and personal health management.

## INTRODUCTION

Sweat serves as an essential thermoregulatory mechanism for humans, allowing the body to cool itself and maintain electrolyte balance while exercising and when exposed to higher temperatures. During this process, electrolytes such as sodium ($Na^+$), chloride ($Cl^-$), and potassium ($K^+$) are released into the sweat at various rates. In addition, the quantity of these electrolytes is rarely consistent; rather they vary greatly based upon the intensity of the exercise, the environmental temperature, the hydration status of the individual, and the time of day that the exercise occurs [1,2]. As researchers explored the development of noninvasive biomarkers to assess the physiological condition of athletes, interest in the constituents and composition of sweat grew significantly within both the fields of sports science and clinical diagnostics [3].

The process of sweating is a very regulated physiological response in humans where the eccrine sweat glands are dispersed throughout the body and produce an initial fluid when stimulated by cholinergic input from the sympathetic nervous system [4]. The initial secretion produced is isotonic to the plasma, however the primary secretion then travels through the sweat gland ducts and as it does so, $Na^+$ and $Cl^-$ ions are reabsorbed through ion channels and transport proteins. Once the $Na^+$ and $Cl^-$ ions are reabsorbed, the resulting final sweat composition is typically hypotonic when compared to blood plasma [5]. The degree of reabsorption of $Na^+$ and $Cl^-$ ions is directly related to the flow rate of the sweat and varies greatly among individuals. Moreover, physiological adaptations that reduce sweat sodium concentrations occur as a result of acclimatization to prolonged periods of heat exposure and increased aerobic fitness [6,7].

Furthermore, numerous investigations have shown large distinctions in electrolyte loss at local sites and throughout the body by employing two types of sweat sampling: regional (such as sweat patches), or whole-body (such as a perspiration collector). Therefore, the use of regional sweat sampling must be applied with considerable caution prior to utilizing this type of information to draw conclusions regarding total hydration status and electrolyte loss [11].

In addition to the aforementioned considerations regarding variability in electrolyte loss based upon the type of sweat sampling employed, circadian rhythms add another layer of complexity to determining sweat electrolyte concentrations. The activity of sweat glands influences ductal reabsorption and there is evidence that hormone fluctuation follows a diurnal rhythm [12-13]. As an example, cortisol and aldosterone levels exhibit measurable differences between early morning and late afternoon hours. Such diurnal fluctuations in hormone levels would therefore result in measurable differences in sweat electrolyte concentrations while engaging in physical activity at varying times of the day. Therefore, this potential exists to evaluate differences in the composition of early morning versus late afternoon sweat samples to assess if such differences justify adjustments to individualized hydration strategies [14].

The ability to accurately measure sweat electrolyte concentrations has increased as a result of improvements in analytical techniques. For an instance, ion-selective electrodes combined with laboratory analyzers provide high precision and repeatable determination of small differences in electrolyte concentrations [15]. Thus, this enhanced capability shall be incorporated to assess hydration and electrolyte balance in athletic and clinical investigations. Furthermore, wearable biosensors have the ability to analyze the composition of sweat in real time. This would assist athletes and clinicians to adjust their strategies for hydration and electrolyte replenishment in response to the analysis [16].

Although investigations have been carried out from scientific communities to improve sweat collection methodologies, but standardization of sweat collection remains as a limiting factor. Variations in sweat rates, contamination of the sample during collection, and evaporation of the sample during collection may all be factors dependent on the methodology used that can affect the results and therefore diminish the comparability of the studies [17-18].

The objective of this study is to determine the extent to which there are interindividual differences in electrolyte concentration of sweat, as well as the degree to which those differences can be explained by physiological or environmental factors. As previously reported, individuals who consume higher amounts of sodium will produce sweat containing higher concentrations of sodium; thus, the loss of electrolytes due to dehydration contributes to disturbances in the body's balance of fluids. Differences in sweat production and electrolyte composition among genders provide the possibility for developing individualized hydration strategies. While several studies have investigated these variables, the field of research is still young with much yet to be understood about how changes in workload and circadian rhythms affect sweat electrolyte composition [20,21].

Understanding the factors that influence the electrolyte composition of sweat is important in order to develop hydration practices and performance strategies in an athletic or clinical setting. Additionally, in order to explore the relationship between workload in any time of day and electrolyte composition of sweat, this investigation proven with the results on the method in which sweat can be analyzed to monitor physiology in a safe and effective manner. In this study, sweat samples were collected from the participants and examined with the help of Ion Selective Electrode module (ISE) integrated into a Roche COBAS Pure 6 analyzer, for precise and reliable electrolyte analysis. The clinical study of sweat analysis was carried out in a controlled environment at JSS Medical College and Hospital, Mysuru, India ensuring that the collection and analysis of sweat samples would be free from contamination and would allow for consistent results.

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## METHODOLOGY

To acquire accurate and reliable sweat analysis findings each sample must be meticulously collected and analyzed in a systematic manner. In order to avoid the existence of any impurities that would potentially impede the analysis, participant's sweat was initially obtained using containers that had been cleansed and sterilized prior to the study. When enough sweat had been collected to fulfill the criteria of the analysis, the container was closed, tagged with the participant's identifiable number, and transferred in a timely way to the laboratory, where the sweat was evaluated using a Roche COBAS Pure 6 analyzer.

Ethical Approval

The investigations followed the international ethical standards and norms established by the World Health Organization (WHO) and the Council for International Organizations of Medical Sciences (CIOMS). Ethical integrity and participant safety were given top emphasis throughout the study procedure. All potential volunteers were first extensively consulted on the goal of the study, the procedures used to carry it out, and any potential dangers involved, allowing them to make an informed decision about participating. Written consent has been taken from all the study participants, with the utmost respect to the afore mentioned ethical procedures.

Collection of Sweat Samples

Secretion of sweat can be induced either by passive or active stimulation. Passive sweating shall occur when an individual at rest and also be induced pharmacologically and by exposure to environmental heat (e.g., sauna bathing)., on the other hand, active sweating shall be induced during the time of exercise [22,23]. In this study, active stimulation method was employed to ensure physiological stimulation of sweat secretion.

The study participants were comprised of 39 males in between the ages of 15 and 43 years, with their heights from 140 to 178 cm and weights in between 45 to 76 kg. Participants were refrained from consuming alcohol and doing anything exhausting for 24 hours before the sweat collection. Also, they had abstained from caffeine for about 12 hours prior. In order to maintain better hydration levels, participants were advised to drink atleast 500 mL of water in the previous night and another 500 mL just before the beginning of exercise. They all were non-smokers, was not taking any medications, and free from any illnesses, cardiovascular diseases, or thermoregulation disorders. This constrained selection criteria had influenced the physiological variability, assuring consistent electrolyte measurements, unaffected by other metabolism and health data of the participants.

The study had collected 0.2 mL of sweat from 24 participants in the morning batch and 15 samples from individuals from the evening batch after each had completed a controlled workout session on a treadmill. Each individual had worked at moderate to high intensity and the details were noted under careful monitoring to insure both the accuracy of the data and the safety of the participants. Sweat sample was collected by placing test tubes (PINACAL EXIM 1.8 mL graduated plastic clear container) on each subject once they began to sweat to prevent contamination and evaporation. The duration of time it took to collect the sweat sample varied from 20 to 40 minutes of working time on the treadmill based on the individual's sweat rate. All samples were labelled with participant details such as ID number, date, and time prior to being analyzed for tracking purposes.

Key Considerations for Sweat Sample Collection

Skin Preparation:

The process of removing residue from your skin prior to sampling is imperative to obtain reliable electrolyte measurements. There are numerous residues present on the skin and most are from lotions, oils, dirt, dead skin cells and residuals from previous sweating that will skew your electrolyte concentrations such as sodium, potassium and chloride. Most common contaminates are removed using either a deionized water rinse or an alcohol wipe to prevent bacterial competition and assure biochemically pure samples [24,25].

Environmental Factors

Factors related to the environment can affect both sweat composition and the balance of electrolytes in sweat. These are important to consider when collecting and analyzing data [26,27]. Some of the main environmental factors include:

- **Temperature & Humidity:** High temperature and high humidity will increase the rate of sweat and decrease the concentration of electrolytes in sweat. As a result, the rate of sweat loss will vary among individuals due to temperature and humidity.
- **Ventilation:** Inadequate ventilation may cause an increase in the body's thermal stress response and therefore modify the sweat secretion response. Adequate ventilation will minimize the amount of heat generated and therefore reduce variability in sweat response to the heat stimulus.
- **Altitude:** Changes in altitude will affect the hydration status of the body and the mechanism for producing sweat. Individuals at high altitudes will experience a greater loss of fluids due to lower humidity and increased respiratory water loss, and therefore may have altered electrolyte balances.
- **Sun Exposure:** Increased exposure to the sun will cause dehydration rapidly and therefore alter the composition of sweat. Additionally, increased metabolic activity resulting from excessive heat exposure will affect the secretion of electrolytes in sweat.

Storage of Sweat Samples

To preserve sample integrity, sweat samples were held at controlled temperatures until analyzed. Samples stored for short periods of time were maintained at a temperature of 2°C to 8°C and those stored for longer periods of time were frozen at a temperature of -20°C to -80°C depending on what type of analysis was being performed. In order to preserve the integrity of the biochemical components of sweat samples proper storage is crucial to minimize biochemical degradation. By freezing sweat samples microbial growth and enzymatic activity are minimized and the chemical composition of the sweat remains stable. Also, multiple freeze-thaw cycles should be avoided because each cycle can compromise the stability of critical biomarkers such as electrolytes, proteins, and metabolites [28]. To achieve optimal preservation following the following steps have been taken:

- Samples were placed in airtight containers to prevent contamination and loss due to evaporation.
- The storage environment was continuously monitored to provide consistent temperature conditions and to minimize temperature fluctuations.
- Cryoprotectant measures were used in studies where prolonged sample viability was required.

As a result of implementing standard storage procedures, the biochemical composition of sweat samples remained stable and were appropriate for biochemical assessment in physiologic, sports science and clinical research.

Determination of Electrolytes Using COBAS Pure 6 Analyzer

The COBAS Pure 6 Integrated Solution, developed by Roche Diagnostics, is a compact, self-contained analyzers designed to improve laboratory efficiency in small to medium-sized laboratories. The COBAS system combines three principal analytical modules into one single unit: the Clinical Chemistry module analyzes biochemical parameters associated with liver function, kidney function and metabolic profiles, the Immunochemistry (IC) module conducts immunoassay-based analyses of hormone levels, markers for infectious diseases, and tumor biomarkers; and the Ion-Selective Electrode (ISE) module determines the concentration of several essential electrolytes ($Na^+$, $K^+$, and $Cl^-$) necessary for accurate and critical diagnostic evaluations.

Electrolyte Analysis Using the ISE Module

This research utilized an Ion-Selective Electrode (ISE) Module of a COBAS Pure 6 analyzer to measure the electrolytes in the sweat samples with great precision and automatically as depicted in Figure 1.

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**Figure 1.** The COBAS Pure 6 Analyzer: A small laboratory device developed by Roche Diagnostics to provide very accurate results in clinical chemistry and electrolyte analysis. It includes automatic workflow systems for the efficient execution of various diagnostic tests.

A variety of specialized ion-selective electrodes are operated within the ISE System to evaluate the major electrolytes [29]:

- **Glass Membrane Electrode** - It has selective detection for sodium ions with high specificity.
- **Valinomycin-Based Membrane Electrode** - Used for the detection of potassium concentrations.
- **Silver/Silver Chloride (Ag/AgCl) Electrode** - Measures chloride components in biological fluids.

Sweat Sample Processing

After collection and storing participant's sweat samples, they were processed in accordance with the manual standard method and placed in the COBAS Pure 6 analyser. In order to obtain the highest level of accuracy, all the samples were spun in a centrifuge to remove suspended particles and any possible contamination sources were eliminated [30]. The COBAS Pure 6 analyser with ISE module allowed rapid processing of sweat samples with its automated workflow for minimizing errors.

This testing equipment measured the concentration of electrolytes contained in 0.2 mL of sweat sample at high levels of precision, yielding the results to assess hydration status, electrolyte regulation and individual variability in sweat composition. The results could be employed for applications in sport science, clinical diagnosis and personal health monitoring. By incorporating high sensitivity and high accuracy analytical techniques, this study has further enhanced the accuracy and reliability of sweat electrolyte measurements to provide a enhanced understanding of the physiological responses and mechanisms associated with hydration [31].

**Results and Discussions**

Sweat samples are examined through the ISE module of COBAS Pure 6 analyser where the electric potential of each type of ions was selectively measured. These methods provide higher reliability and reproducibility, making it suitable for measuring electrolyte concentrations in clinical diagnostics, evaluating kidney function, assessing hydration states and metabolism.

Roche COBAS Pure 6 Analyzer has been employed to carry out electrolyte analysis of sweat samples in test tubes as shown in Figure 2 for $Na^+$, $K^+$ and $Cl^-$ concentrations. This biochemical assessment supports clinical research and hydration evaluation.


**Figure 2.** COBAS Pure 6 Analyzer having sweat sample in test tubes.

Sodium ($Na^+$) Ion Concentration Analysis

The data collected for sodium ion concentration in a subject's sweat after several different exercise sessions is shown in Table 1 to demonstrate how the values are distributed among the three ranges of sodium ion concentration.

**Table 1:** Analysis of $Na^+$ Ion Concentration in Sweat Samples

| Session | Total Samples | Sodium Concentration (mmol/L) | No. of Samples | Percentage (%) |
|---|---|---|---|---|
| Morning and Evening | 39 | 10-90 | 14 | 35.90 |
| | | 91-145 | 12 | 30.77 |
| | | >145 | 13 | 33.33 |
| Morning | 15 | 10-90 | 5 | 33.33 |
| | | 91-145 | 7 | 46.67 |
| | | >145 | 3 | 20.00 |
| Evening | 24 | 10-90 | 9 | 37.50 |
| | | 91-145 | 5 | 20.83 |
| | | >145 | 10 | 41.67 |

The sodium ion ($Na^+$) concentrations in the 91 – 145 mmol/L concentration range were greater in the morning exercise session than they were in the evening exercise session; this indicates that the subject retained more sodium and/or lost less sodium during the morning sessions. On the other hand, sodium ion concentrations in the 10-90 mmol/L and >145 mmol/L ranges were greater in the evening sessions than they were in the morning sessions, which suggests an increase in sodium ion excretion/secretion as well as changes in sweat composition due to physiological or environmental conditions.

Potential Factors Influencing Sodium Ion Variability

Physiological and environmental factors can provide possible explanations for altered sweat composition between the morning and evening workouts.

1. **Circadian Rhythms for Electrolyte Regulation:** The body's circadian rhythms of regulating water balance and electrolyte levels can affect sweat chemical composition over the course of a day. In fact, there has been research suggesting that sodium retention is greater in the morning due to hormonal fluctuations associated with renal function and electrolyte excretion.

2. **Core Body Temperature Differences:** the temperature of the body's core, which is lower in the morning and rises as the day goes on, can also influence the amount of sweat produced in the evening, leading to potentially larger differences in sodium losses particularly when individuals participate in prolonged or strenuous exercise.
3. **Hydration Status and Fluid Intake Patterns:** Physiological hydration status and daily intake patterns can also significantly impact the chemical composition of sweat. For example, athletes who choose to do morning training sessions may be well-hydrated before they begin their workout, while athletes who choose to train in the evening may lose significant amounts of hydration throughout the day, which can also have effects on the chemical composition of sweat.
4. **Variations in Metabolic Rate:** Physiological metabolic rates can also differ between morning and evening workouts. Specifically, metabolism is slower in the morning and builds as the day progresses, influencing both thermoregulatory processes and the activity of sweat glands. Therefore, the potential for a greater sodium output in the evening compared to the morning, based on the metabolic demands of the individual, may also be influenced by the time of day in which the individual chooses to perform their exercise.
5. **Changes in Environmental Temperature and Humidity:** Temperatures in the morning are cooler than in the evening workouts, and humidity levels tend to be higher in the warmer and more humid conditions of the afternoon and/or evening. This can result in a faster rate of sweating, which can increase the loss of electrolytes and alter the chemical composition of sweat relative to the thermal stress experienced by the individual.
6. **Intensity and Duration of Exercise:** The intensity and duration of exercise may differ between morning and evening workout sessions, and these differences in exercise may result in greater sodium excretion in the sweat collected in the evenings compared to that collected in the mornings.

Chloride ($Cl^-$) Ion Concentration Analysis

The results of the analysis of sweat chloride concentration are presented in Table 2 and provide information regarding the potential for electrolyte changes due to varying amounts of physical activity throughout the day.

**Table 2:** Analysis of $Cl^-$ Ion Concentration in Sweat Samples

| Session | Total Samples | Chloride Concentration (mmol/L) | No. of Samples | Percentage (%) |
|---|---|---|---|---|
| Morning and Evening | 39 | 10-90 | 16 | 41.03 |
| | | 91-145 | 10 | 25.64 |
| | | >145 | 13 | 33.33 |
| Morning | 15 | 10-90 | 7 | 46.67 |
| | | 91-145 | 5 | 33.33 |
| | | >145 | 3 | 20.00 |
| Evening | 24 | 10-90 | 9 | 37.50 |
| | | 91-145 | 5 | 20.83 |
| | | >145 | 10 | 41.67 |

Data analysis indicated a significant variation between $Cl^-$ ion concentrations from the morning to the evening as well as within the evening exercise sessions. As Cl- ion levels were higher in the afternoon than in the evening with values of 10-90 mmol/L and 91-145 mmol/L, this indicates moderate chloride loss levels in the morning. Conversely, Cl- ion values greater than 145 mmol/L (evening) show a significantly increased amount of chloride loss compared to all other time periods, thereby supporting the conclusion that chloride loss is higher in the late evening.

Potential Factors Influencing Chloride Ion Variability

1. **Hydration Status and Fluid Balance:** Participants may be in a well-hydrated state at the start of the morning workout, with less chloride being lost as a result of fluid retention. On the other hand, participants completing an afternoon or evening workout may have experienced fluid loss throughout their entire day of physical activity and will also experience increased concentration of Cl- through progressive fluid loss.
2. **Sweat Loss and Thermal Charges:** As the heat from the sun increases, there is an increase in sweat rate which can result in a more rapid loss of sodium and chloride ions. The increased rates of sweat loss can result in a loss of more sodium and chloride ions; this loss of ions can explain why chloride levels were found to be elevated in the >145 mmol/L range.
3. **Circadian Electrolyte Regulation:** The circadian rhythm affects electrolyte excretion through regulation of reabsorption in sweat glands, with a resultant increase in sodium and chloride in the body that will lead to increased sodium retention in the sweat glands; therefore, sodium levels could be expected to be at their lowest concentration in the sweat during the morning session, and Cl- levels could also be expected to be lower in the afternoon due to diminished sweat gland function.
4. **Exercise Duration and Intensity**: Greater Cl- ion loss can occur if a person engages in longer or more intense physical activities during the evening, as these activities result in a greater amount of sweat being produced. In contrast, lower Cl- concentrations in the sweat are likely to occur when a person engages in shorter or less intense physical activities in the morning.

Potassium ($K^+$) Ion Concentration Analysis

Potassium ion concentrations in the sweat of participants who participated in both a morning session and an evening session are shown as a summary in Table 3 for each concentration range.

**Table 3:** Analysis of Potassium ($K^+$) Ion Concentration in Sweat Samples

| Session | Total Samples | Potassium Concentration (mmol/L) | No. of Samples | Percentage (%) |
|---|---|---|---|---|
| Morning and Evening | 39 | 2-10 | 2 | 5.13 |
| | | 11-15 | 5 | 12.82 |
| | | >15 | 32 | 82.05 |
| Morning | 15 | 2-10 | 0 | 0.00 |
| | | 11-15 | 3 | 20.00 |
| | | >15 | 12 | 80.00 |
| Evening | 24 | 2-10 | 2 | 8.33 |
| | | 11-15 | 2 | 8.33 |
| | | >15 | 20 | 83.33 |

Table 3 shows that there is a significant difference in the concentration of potassium ions between the morning and evening sessions. Potassium ion concentrations in the range of 11-15 mmol/L were higher during morning sessions than they were during the evening sessions, showing an increase in the level of potassium secreted in the body's sweat earlier in the day. Conversely, potassium ion concentrations in the >15 mmol/L range were significantly higher during the evening sessions than they were during the morning sessions, which would indicate that the amount of potassium lost in the sweat was greater late in the day than it was early in the day. Additionally, the number of sweat samples with potassium ion concentrations in the 2-10 mmol/L range was higher during the evening sessions, possibly due to a combination of differences in sweat rate and the physical state of the individual at the time of the sweat sample collection.

Potential Factors Influencing Potassium Ion Variability

1. **Muscle Activity and Potassium Release**: Potassium is crucial for muscle function, and as exercise intensity increases, it will increase with the amount of potassium lost through the process of sweating during the sessions in the evenings, as compared to the mornings; therefore, there was likely an increase in the amount of potassium lost through the process of sweating in the evening, possibly because the evening sessions were longer, or perhaps they had a higher level of demand.

2. **Sweat Gland Function and Circadian Rhythms:** There are physiological changes in the secretion of electrolytes that occur over the course of the day. Potassium retention from morning exercise could be better regulated at the cellular level than potassium loss in the evening; and potassium loss in the evening may be due to physiological changes that result in the sweat glands losing potassium more quickly.

3. **Hydration and Electrolyte Balance:** The participants in this study were likely better hydrated in the morning, which would explain lower levels of potassium lost through sweating. Conversely, by evening, a greater amount of total body water is lost as a result of cumulative fluid loss, which results in higher levels of potassium being lost through sweat due to less dilution.

4. **Environmental Temperature and Sweat Rate:** During the warmer conditions of the evening, individuals will produce more sweat, resulting in higher levels of potassium loss as a result of increased sweat rates; ultimately contributing to levels > 15mmol/L.

Comparative Analysis of Sweat Electrolyte Levels Across Sample IDs

The Sweat Electrolyte Data can be seen graphically in Figure 3 with Sample ID's represented by the x axis and the electrolyte concentration presented in (mmol/L) on the y axis. The dots in the figure represent the actual concentration levels of $Na^+$, $K^+$, and $Cl^-$ for each sample. The graphic is an easy method to compare and show how different electrolytes from one sample may differ from another sample while showing the differences in ion concentration in sweat among different people. This type of analysis aids in providing a better understanding of the physiological diversity that exists in sweat electrolyte composition and also encourages additional research to develop individualized methods for hydration and electrolyte management.

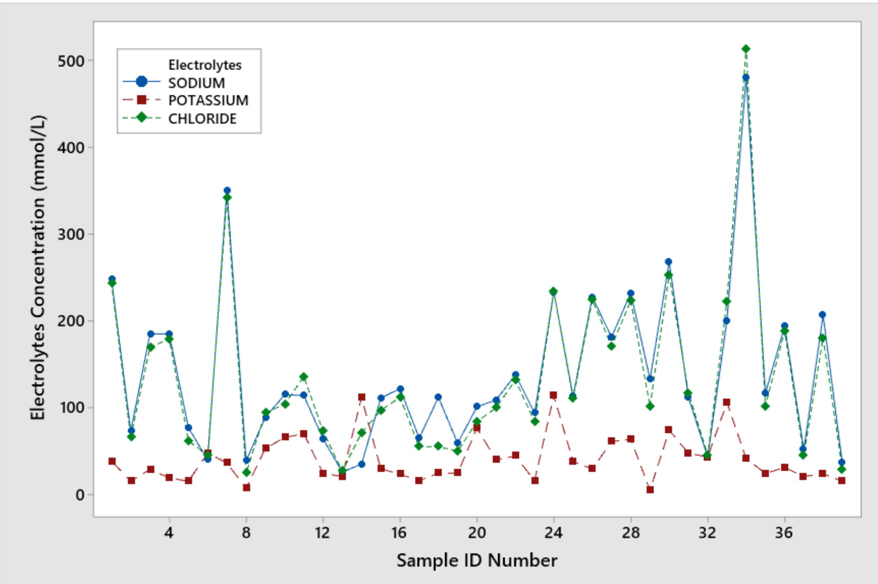


**Figure 3.** Electrolyte Profile of Sweat Samples

As per Figure 3, it is evident that the concentration of sodium and chloride levels in sweat samples are relatively identical. Samples from midrange shows that the sodium level in range of 91-145 mmol/l and chloride ions are also matched. The equality in these range is due to physiological mechanism of sweat glands where both sodium and chloride are co-transported and reabsorbed together to balance electroneutrality. In contrast, the concentration of potassium is relatively higher and often greater than 15 mmol/l reflecting the release of $K^+$ from active muscle tissue during exercise. Thus, the graph provides insight into variation of sweat composition along with the consistent balance of sodium and chloride created by intrinsic regulatory processes. These observations can support the design of personalized hydration and electrolyte replacement strategies to optimize outcomes for exercise performance and recovery.

Electrolyte Concentration Analysis by Blood Group

The haematological distribution of $Na^+$, $Cl^-$ and $K^+$ concentrations in subjects of different blood groups are shown in Table 4.

**Table 4:** Distribution of $Na^+$, $Cl^-$ and $K^+$ concentrations among participants of different blood groups.

| Blood Group | Total Samples | $Na^+$ Concentration (%) | $Cl^-$ Concentration (%) | $K^+$ Concentration (%) |
|---|---|---|---|---|
| **O+** | 17 | 41.18 (10-90 mmol/L)<br>35.29 (91-145 mmol/L)<br>23.53 (>145 mmol/L) | 52.94 (10-90 mmol/L)<br>23.53 (91-145 mmol/L)<br>23.53 (>145 mmol/L) | 5.88 (2-10 mmol/L)<br>17.65 (11-15 mmol/L)<br>76.47 (>15 mmol/L) |
| **A+** | 12 | 33.33 (10-90 mmol/L)<br>41.67 (91-145 mmol/L)<br>25.00 (>145 mmol/L) | 33.33 (10-90 mmol/L)<br>41.67 (91-145 mmol/L)<br>25.00 (>145 mmol/L) | 0.00 (2-10 mmol/L)<br>16.67 (11-15 mmol/L)<br>83.33 (>15 mmol/L) |
| **B+** | 9 | 22.22 (10-90 mmol/L)<br>11.1 (91-145 mmol/L)<br>66.7 (>145 mmol/L) | 22.22 (10-90 mmol/L)<br>11.11 (91-145 mmol/L)<br>66.67 (>145 mmol/L) | 11.11 (2-10 mmol/L)<br>0.00 (11-15 mmol/L)<br>88.89 (>15 mmol/L) |
| **AB+** | 1 | 100.0 (>145 mmol/L) | 100.0 (>145 mmol/L) | 100.0 (>15 mmol/L) |

Sodium ($Na^+$) Distribution

O+ individuals when considering sodium showed an almost equal distribution across all three concentration ranges, with relatively less loss, compared to B+ individuals, where more than 66% of the cases were in the >145 mmol/L range. The concentration between 10 and 90 mmol/L was highest among A+ and O+ participants, indicating moderate sodium retention..

Chloride ($Cl^-$) Distribution

O+ individuals showed the highest $Cl^-$ concentration between 10 and 90 mmol/L range, whereas B+ individuals had a much higher proportion (>66%) at the >145 mmol/L range. A+ participants were balanced across all $Cl^-$ concentrations, suggesting intermediate $Cl^-$ control.

Potassium ($K^+$) Distribution

B+ and AB+ blood groups exhibited the highest potassium concentration in sweat, with >88% and 100% of the sweat potassium concentration, respectively, falling into the >15 mmol/L category. This indicates more potassium loss through sweat. The potassium concentration in O+ participants covers a wide range, with very few in the 2-10 and 11-15 mmol/L categories.

Potential Implications

Electrolyte variability across blood groups may be due to genetic and physiological differences in sweat composition and ion regulation. Individuals who lose higher quantities of potassium and chloride through sweat may have to follow specific fluid and electrolyte supplementation strategies to maintain proper fluid balance to perform physical activity. Further investigations could explore whether genes associated with blood groups influence sweat gland performance, electrolyte retention and thermoregulation.

## CONCLUSION

This investigation analyses the variation in sweat electrolyte composition depending on duration of exercise, environmental conditions and physiological characteristics of the participant. The ion concentrations of sodium ($Na^+$), chloride ($Cl^-$) and potassium ($K^+$) were determined using Roche COBAS Pure 6 Analyzer for biochemical assessments of sweat samples.

The results revealed significant distinctions in morning and evening sweat electrolyte levels which depict the circadian aspects of hydration and thermoregulatory adaptations. Retention of sodium and chloride ions were relatively higher in the morning workouts and electrolyte losses was more during evening workouts which are due to increased sweat rates and thermal stress throughout the day. The loss trend for potassium was found similar as sweat samples exhibited greater drainage during evening workouts, which may relate to muscle activity and metabolic shifts as the day advances.

Additionally, the variation of electrolytes within various blood groups would allude to a potential genetic influence on the function of sweat glands and ion regulation. Thus establishing the need for planning individual hydration and electrolyte management strategies. The results of this investigation would promote sports science, clinical diagnostics, and personal health monitoring, thereby aiding the future evaluation of hydration and electrolyte balance concerning exercise time and genetic predisposition.

This study was carried out with strict adherence for ethical standards, ensuring participant safety and data integrity, along with WHO and CIOMS guidelines. Further investigations with genetic markers, environmental effects and longitudinal monitoring shall provide a holistic view of sweat electrolyte dynamics to improve health performance and recovery.

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