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Research Article

Comparison of Resting Pilocarpine-Induced Sweat Sodium Concentration and Exercise-Induced Sweat Sodium Concentration in Highly Trained and Moderately Trained Athletes

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Abstract

Resting sweat sodium concentration is commonly estimated using pilocarpine iontophoresis and a commercial sweat sodium analyzer (SSA), yet it may not reflect changes in sweat sodium during exercise. We compared pilocarpine-induced (resting) sweat sodium concentration with exercise-induced sweat sodium concentration during a 90-minute treadmill run. Twenty-five adults (10 males [40%], 15 females [60%]; age 36 ± 12.2 yrs) completed the trials. Before exercise, a resting sweat sample was induced on the upper forearm via pilocarpine iontophoresis and collected with a Macroduct® device, then analyzed on the SSA. Participants then ran for 90 minutes at moderate intensity (~65% heart-rate reserve). Exercise-induced sweat was collected on the inner forearm with Macroduct® collectors that were replaced at 15–30 minutes and at 90 minutes and analyzed on the SSA. Participants were categorized into highly trained and moderately trained athletes based on their average treadmill speed during the trial (average speed < 6.5 mph = moderately trained, average speed >6.5 mph = highly trained). Mean (±SD) resting pilocarpine-induced sodium concentration was 51 ± 19 mM. Exercise-induced sweat sodium concentration averaged 50 ± 22 mM at 15–30 minutes and 57 ± 23 mM at 90 minutes, with no differences versus pilocarpine values (p=0.84 and p=0.09, respectively). Subgroup trends indicated greater divergence in moderately trained participants, particularly moderately trained females, who showed a difference between the pilocarpine-induced values and the 90-minute exercise-induced values (p=0.033). Although group means were similar at rest and during exercise, meaningful individual and subgroup variability was observed. Resting pilocarpine-induced sweat sodium may not reliably represent exercise-induced sweat sodium for all athletes, especially moderately trained females, highlighting the need for exercise-specific measurements when developing hydration and electrolyte replacement strategies.

Keywords: exercise, hydration, pilocarpine, sodium concentration

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Introduction

Sweat plays a crucial role in the body for regulating core temperature through heat dissipation and regulating electrolyte balance especially during exercise (Casa et al., 2000). Physiological and environmental factors such as heart rate, sweat rate, core temperature, and relative humidity can influence the rate and composition of sodium excretion through sweat during physical activity (Baker, 2017). Understanding these factors is essential for developing effective hydration strategies and optimizing athletic performance. Hydrating properly helps to regulate body temperature, support cardiovascular function, and maintains plasma volume (Murray, 2007). Imbalances in sodium levels can lead to health and performance issues in athletes, such as hyponatremia, characterized as abnormally low levels of sodium, or dehydration which could cause impairments in physical performance and pose serious health risks (Cheuvront et al., 2003).

Understanding an individual's sweat sodium concentration is crucial in developing a personalized hydration strategy which is shown to reduce hydration-related issues. One method to determine sweat sodium concentration is through pilocarpine-induced collection of a resting sweat sample that is then tested using a sweat sodium analyzer (SSA). However, this test does not allow a user to determine changes in sweat sodium concentration during exercise. Exercise-induced sweat sodium concentrations are typically determined by patch testing and subsequent sweat analysis (Baker, 2019). Discrepancies between the pilocarpine-induced and exercise-based methods have raised questions about the accuracy of assessing sodium loss during physical activity by using a pilocarpine iontophoresis test as a proxy for measured sodium loss during exercise.

There are three main sweat glands that mediate the physiological response of sweating. These sweat glands include eccrine, apocrine, and apoeccrine glands, with eccrine glands being the primary contributors to thermoregulatory sweating. Eccrine sweat is comprised of mainly water and sodium chloride, but it contains other electrolytes in smaller concentrations as well (Baker, 2019). Exercise-induced sweating starts from an increase in core temperature, which activates sympathetic cholinergic pathways that stimulate the eccrine glands (Baker, 2019). Unlike exercise-induced sweat, pilocarpine-induced sweat is activated by a cholinergic agent called pilocarpine nitrate that binds to the muscarinic receptors of eccrine sweat glands to induce sweat production through a small electric current that pushes the chemical agent into the skin (Basu et al., 2013).

When comparing pilocarpine-induced sweat and exercise-induced sweat, pilocarpine iontophoresis allows sweat secretion to only be induced from local cholinergic stimulation of sweat glands, while

exercise-induced sweat is stimulated from other local and central mediators such as adrenergic stimulation, neuromodulators, and the exercise pressor reflex (Baker, 2017). Previous research has shown differences between exercise-induced and pilocarpine-induced sweat when cycling at 75% functional power threshold power (Harris et al., 2024). The purpose of this study aims to compare the sodium concentration between exercise-induced sweat from a running trial and pilocarpine-induced sweat. Through analyzing and understanding the differences in sodium concentrations between these two conditions, the aim is to provide insights into the variability of sweat composition during exercise and to improve methods for assessing and managing electrolyte balance in athletes and physically active individuals.

Methods

Participants

This study recruited 25 healthy adults (10 [40%] males, 15 [60%] females; age 36 ± 12.2 yrs) from 19 years of age to 67 years of age through fliers and internet notices at Cleveland State University for a total of 25 trials completed. Participants were screened to ensure that a 90-minute treadmill running protocol could be safely performed. A PAR-Q+ questionnaire (Physical Activity Readiness Questionnaire) was used for screening. Study exclusion criteria included any positive answers to the PAR-Q+, where exercise was determined to be high risk for the subject. The protocol was approved by the Institutional Review Board (IRB; IRB-FY2022-75) of Cleveland State University and all subjects provided written informed consent; participant characteristics can be found in Table 1.

Table 1. Baseline information of participants

Characteristic	**Mean +/- SD**	**Range (Min to Max)**
Age (yr)	36 ± 12	19 to 67
Weight (kg)	73.4 ± 18.13	51.7 to 121.6
Height (m)	1.72 ± 0.09	1.57 to 1.85
%Male	44%	
%Female	56%	
Average Heart Rate (bpm)	148 ± 7	133 to 159
Average Body Temperature (F)	100 ± 2	96.1 to 102
Average Room Temperature (F)	74 ± 2	70.4 to 77.5
Average Room Humidity (%)	31 ± 13	17.5 to 65.1

Resting Sweat Collection

Before the onset of exercise, a resting sweat sample was collected from each participant using pilocarpine iontophoresis. The method of using pilocarpine iontophoresis involves applying a small

electrical current to drive the sweat-inducing agent, pilocarpine, into the skin to stimulate sweat production on the upper forearm. The pilocarpine sweat gels contain a concentration of 0.5% Pilocarpine Nitrate, Agar, Methylparaben, and Propylparaben (Precision Hydration, Christchurch, United Kingdom). The upper forearm was first cleaned with distilled water, then the pilocarpine sweat gel discs were added to the electrodes. The electrodes were attached to the subjects' upper forearm, and the sweat inducer was activated. The sweat induction was conducted at 1.5 mA for five minutes until iontophoresis was completed, and the electrodes could be taken off of the subject, per manufacturer guidelines. From this procedure, sweat was induced under the electrodes and was collected; the pilocarpine sweat gels, electrodes, and sweat inducer can be seen below in Figure 1.

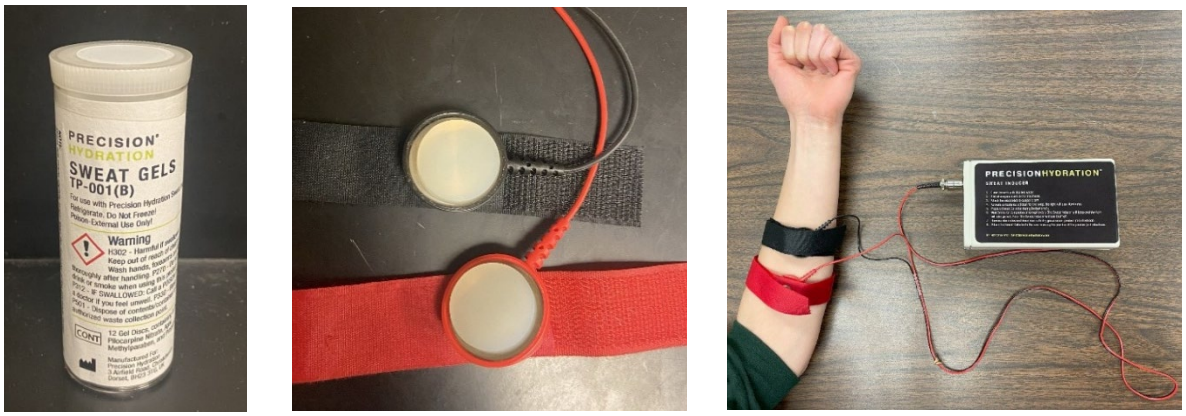

Figure 1. Precision Hydration sweat gels, pilocarpine sweat gel discs added to the electrodes, and electrodes attached to the sweat inducer and the forearm of the subject.

The sweat was then collected in a Macroduct® Sweat Collector (Wescor, Logan, Utah), which is solely used to collect sweat, and was applied firmly to the forearm to eliminate any air gaps. The Macroduct® Sweat Collector is a disposable plastic device with a concave undersurface and a small opening at the apex of the convex surface that leads to a 0.64 mm small-bore plastic tubing coiled into a spiral. Hydraulic pressure is created when sweat is secreted from the sweat glands which allows sweat to flow up and be collected in the plastic tubing. The volume of the spiral collection tube is approximately 85 microliters which is a suitable amount to analyze sweat. The subject's skin was wiped clean with sterile gauze and deionized water before placing the Macroduct®. Once the Macroduct® tubing had collected enough volume, it was removed through use of a blunt needle and cut from the subject's arm. The Macroduct® collector must remain on the forearm until the plastic tube is removed at its attachment point. The Macroduct® on a subject's forearm can be seen in Figure 2 below. Collected sweat was analyzed using a laboratory-quality sweat sodium analyzer (Precision Hydration, conductivity-based measurement) (Dyshko et al., 2024).

Figure 2. Macroduct® sweat collector on a subject's forearm. The clear cover was removed to display the spiral of the collecting tube.

Exercise Protocol

A Macroduct® sweat collector was placed on the inner arm of the subject, and the subject started running at a moderate intensity for 90 minutes on the treadmill, targeting to be at approximately 65% of their heart rate reserve (Chuna et al., 2010). The Macroduct® Sweat Collector was used to collect exercise-induced sweat. The sweat collector was taken off of the subject's inner forearm and replaced after 15 to 30 minutes and analyzed on the SSA to determine the sodium concentration. After 90 minutes, the second sweat collector was taken off of the subject and analyzed on the SSA. Heart rate, body temperature, room temperature, and relative humidity were also recorded during the exercise session. Heart rate was measured through a Polar Heart Rate Monitor (Polar H10, Hofwisenstr, Switzerland), body temperature was measured through a Core Body Temperature Monitor (CORE1, Hofwisenstr, Switzerland). Room temperature and humidity were measured with a ThermoPro Digital Hygrometer Thermometer and Humidity Meter (ThermaPro TP50, Lawrenceville, Georgia).

Data Analysis

All data processing, visualization, and statistical analyses were performed in R (RStudio, v4.5.2, R Core Team, Vienna, Austria). Participants were categorized into four characterization groups based on fitness level and sex: Highly Trained Female, Highly Trained Male, Moderately Trained Female, and Moderately Trained Male. The male and female groups were categorized based on the average treadmill speed during the trial. A treadmill speed greater than or equal to 6.5 mph was categorized

as a highly trained participant while a treadmill speed less than 6.5 mph was categorized as a moderately trained participant (Gordon et al., 2017). For each group, sweat sodium concentration was evaluated across three sweat collection conditions: pilocarpine-induced, 15–30-minute exercise-induced, and 90-minute exercise-induced.

For each group, descriptive statistics were calculated and reported as mean ± standard deviation (SD). To evaluate whether exercise-induced sweat sodium differed from pilocarpine-induced sweat sodium within each participant characterization group, paired-sample, two-tailed t-tests were conducted for the comparisons of pilocarpine-induced sweat versus 15–30-minute exercise-induced sweat and pilocarpine-induced sweat versus 90-minute exercise-induced sweat. These tests were performed separately within each group, treating each row as a paired observation across sweat collection conditions. Statistical significance was defined as $p < 0.05$.

Results

Average pilocarpine-induced resting sodium concentration was 51 ± 19 mM; after 15-30 minutes of exercise, the average exercise-induced sodium concentration was 50 ± 22 mM, and after 90 minutes of exercise, the average exercise-induced sodium concentration was 57 ± 23 mM. There was no difference in sweat sodium concentration at different exercise times compared to the pilocarpine-induced values (15–30-minute exercise-induced vs. pilocarpine-induced p-value= 0.84, 90-minute exercise-induced vs pilocarpine-induced p-value= 0.09) (Figure 3).

When comparing between fitness level and sex, differences were evident. After 90 minutes of exercise, the sweat sodium values in the Moderately Trained Female subgroup were higher than the pilocarpine-induced sweat sodium values (p-value = 0.033). No differences were observed in other subgroups when comparing pilocarpine-induced sweat sodium values and 15-30 minutes exercise-induced and 90-minute exercise-induced sweat sodium values. To assess the level of agreement between the pilocarpine-induced and exercise-induced measurements in each subgroup, intraclass correlation coefficients (ICC) were assessed. Additionally, the 95% confidence interval (CI) was evaluated (shown in Table 2).

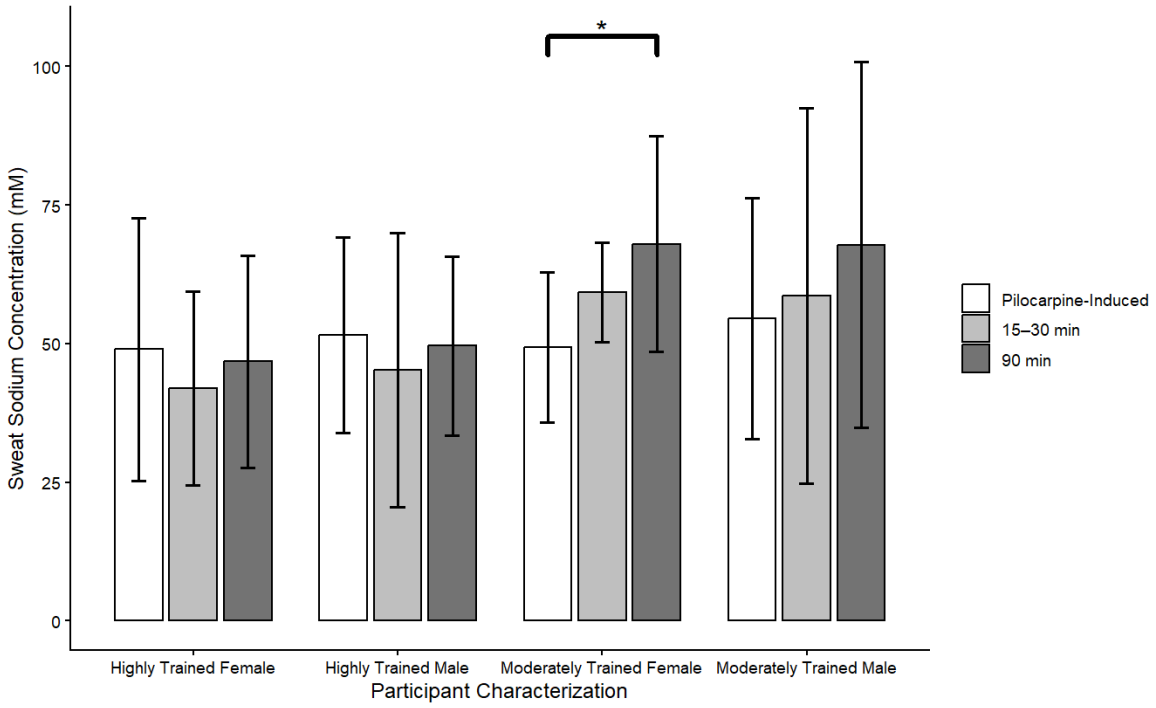

Figure 3. Comparison of pilocarpine-induced and exercise-induced sweat sodium concentrations between Highly Trained Females, Highly Trained Males, Moderately Trained Females, and Moderately Trained Males. * indicates $p<0.05$.

Table 2. Intraclass correlation coefficient (ICC), 95% confidence interval (CI), and p-values when comparing pilocarpine-induced and exercise-induced sweat sodium concentrations between Highly Trained Females, Highly Trained Males, Moderately Trained Females, and Moderately Trained Males.

	ICC	95% CI	P-value
Highly Trained Female Pilocarpine-induced vs 15-30 minutes	0.84	0.41 - 0.96	0.077
Highly Trained Female Pilocarpine-induced vs 90 minutes	0.94	0.78 - 0.99	0.4
Highly Trained Male Pilocarpine-induced vs 15-30 minutes	0.9	0.36 - 0.99	0.16
Highly Trained Male Pilocarpine-induced vs 90 minutes	0.9	0.36 - 0.99	0.61
Moderately Trained Female Pilocarpine-induced vs 15-30 minutes	0.42	-0.19 – 0.88	0.075
Moderately Trained Female Pilocarpine-induced vs 90 minutes	0.36	-0.17 – 0.85	0.033
Moderately Trained Male Pilocarpine-induced vs 15-30 minutes	0.68	-0.44 – 0.96	0.73
Moderately Trained Male Pilocarpine-induced vs 90 minutes	0.52	-0.38 – 0.93	0.33

ICC, intraclass correlation coefficient; CI, confidence interval.

Figure 4 evaluates the agreement between the pilocarpine-induced and exercise-induced sweat sodium concentrations by plotting each subgroup and timepoint. If the two collection methods yielded the same sweat sodium concentrations, the data would fall directly on the line of identity (y=x). Deviations from this line reflect disagreement between the pilocarpine-induced and exercise-induced measurements. Points located above the line indicate that exercise-induced sweat sodium exceeded the corresponding pilocarpine-induced value, while points below the line indicate that exercise-induced values were lower than pilocarpine-induced values. In the highly trained subgroups, the data cluster closely along the line of identity, suggesting stronger agreement between these methods. However, larger deviations from the line of identity are observed in the moderately trained subgroups, particularly the moderately trained females. This indicated a poorer agreement between these methods. This shows how pilocarpine-induced sweat sodium concentrations may not represent exercise-induced sweat sodium concentrations in moderately trained athletes.

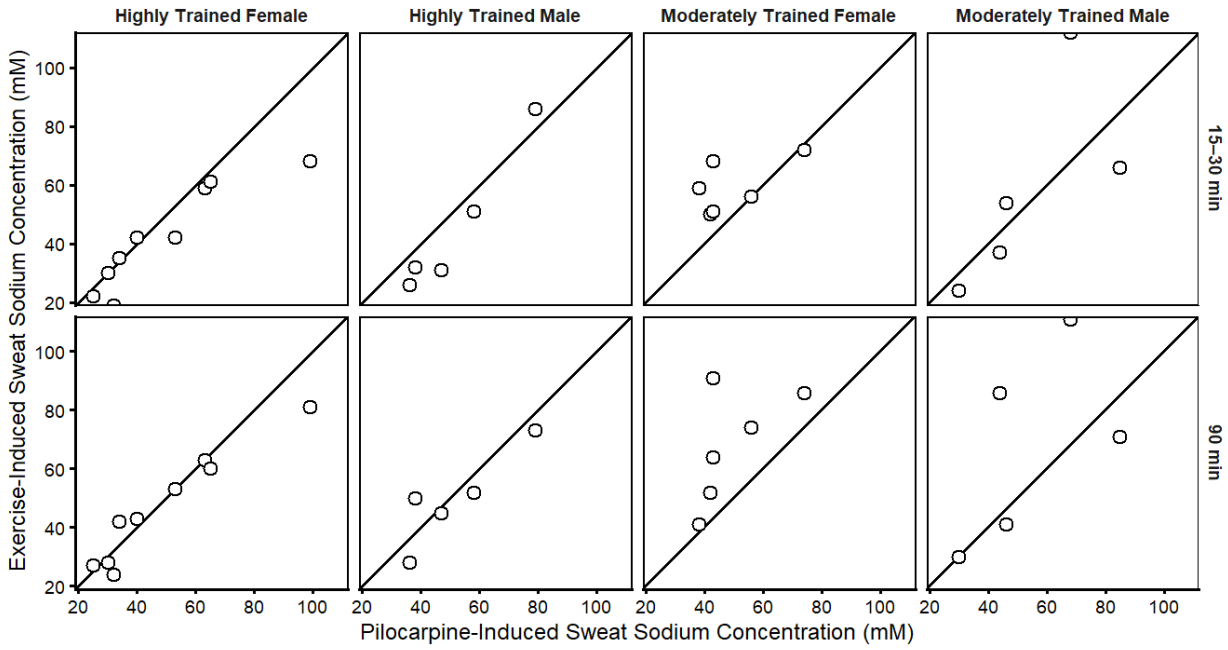

Figure 4. Pilocarpine-induced and exercise-induced sweat sodium concentrations agreement between Highly Trained Females, Highly Trained Males, Moderately Trained Females, and Moderately Trained Males. The line of identity (y=x) is shown as a reference.

Discussion

The present study examined the differences between pilocarpine-induced and exercise-induced sweat to assess whether resting sweat measurements could reliably be used to predict sweat sodium levels during a 90-minute treadmill run. Among the entire trial, pilocarpine-induced values were similar to exercise-induced values at 15-30 minutes and at 90-minutes, showing no statistical significance when all participants were considered together (p-value = 0.84 and p-value = 0.09, respectfully). These findings indicate that pilocarpine iontophoresis may approximate average sweat sodium concentration correctly across an entire population of runners but may not capture differences between subgroups of fitness level and sex.

Despite the lack of significant differences in group means, subgroup analyses indicate that pilocarpine-induced to exercise-induced sweat sodium concentrations are not uniform across all fitness levels. In particular, moderately trained females demonstrated the greatest divergence, with a statistically significant difference between pilocarpine-induced values and 90-minute exercise-induced values (p-value = 0.033). This supports the conclusion that pilocarpine-induced sweat sodium may be less reliable for exercise-induced sodium loss in moderately fit individuals. Agreement analyses further reinforce this. Intraclass correlation coefficients (ICC) were used to qualify agreement between pilocarpine-induced and exercise-induced measurements within each fitness level and sex. In the highly trained subgroups, ICC values were generally high (0.84 – 0.94 in females and 0.90 in males), suggesting a stronger agreement between resting and exercise-induced values in these participants. In contrast, agreement between moderately trained athletes were substantially lower (0.36 – 0.42 for females and 0.52 – 0.68 for males). This is consistent with greater variability and poorer correspondence between the sweat collection methods. Collectively, these results indicate that the utility of pilocarpine-induced sweat testing methods depends on athlete characteristics and exercise duration.

Differences between pilocarpine-induced and exercise-induced sweat sodium concentrations have meaningful performance implications when sweat testing is used as a guide to individualized hydration strategies. If a pilocarpine-induced test underestimates an athlete's exercise-induced sweat sodium concentration, sodium losses during training or competition may be under-replaced. In this case, an athlete who replaces primarily with water or low-sodium fluids may develop a progressive dilution of extracellular sodium and become susceptible to hyponatremia, characterized by a low amount of sodium in the bloodstream (O'Neal et al., 2020). Additionally, hyponatremia can result in symptoms such as headache, nausea, dizziness, and impaired performance (Oracio et al., 2000). Conversely, when

pilocarpine-induced values exceed exercise-induced values, athletes may over-replace sodium, which can cause negative effects such as hypertension if the sodium intake is chronically high (Stachenfeld et al., 2008). These findings support the recommendation that exercise-specific sweat sampling may be warranted when individualized sodium replacement recommendations are needed, especially for moderately trained athletes and for longer-duration sessions.

This study used a single moderate-intensity running protocol (65% heart-rate reserve) and did not take into consideration how results may differ across intensities, exercise type, or environmental conditions (Harris et al., 2026). Additionally, the relatively small sample size (n = 25) limits the statistical power of the analyses and restricts the generalizability of the findings, particularly for subgroup comparisons based on sex and fitness level. Future studies should investigate larger, more diverse populations and account for these additional factors along with menstrual cycle phase and hydration status. Tracking these factors may give more insight into how the sweat sodium concentration changes over the course of the trial and compares to the pilocarpine-induced sweat. Having a larger sample size would allow for the study to be validated.

Conclusion

This study evaluates whether a resting pilocarpine test can be used in place of an exercise-induced sweat sodium test through evaluating pilocarpine-induced sweat sodium against exercise-induced sweat sodium collected during a 90-minute moderate intensity treadmill run, with additional categorization between fitness level and sex. While mean values across the full study were comparable between pilocarpine-induced and exercise-induced at both 15–30 minutes and 90 minutes, subgroup analyses demonstrated that agreement is not uniform, with a significant difference observed in the moderately trained female subgroup at 90 minutes. These discrepancies indicate that pilocarpine-induced sweat testing may not accurately reflect dynamic physiological responses during prolonged physical activity. The results emphasize that relying solely on resting pilocarpine-induced sweat sodium concentrations could lead to either underestimation or overestimation of sodium loss during exercise, which would impact hydration and electrolyte replacement strategies. Therefore, exercise-induced sweat should be used to accurately determine sweat sodium values to optimize performance and recovery. Future research should aim to validate these findings in larger and more diverse populations while controlling additional physiological and environmental factors.

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