Salivary molecules as indicators of hydration status

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Abstract

Introduction: Maintaining an appropriate level of hydration is essential to health, especially when doing physical exercise. Thus, the importance of monitoring the before, during and after exercise status. Therefore, knowing and studying the changes and indicators that may provide reliable information on fluctuations in a simple, quick manner, via non-invasive sampling techniques becomes essential. Material and Methods: The data analyzed and processed (α-amylase, K^+, Cl^-, cortisol, total protein, and osmolality concentration in saliva) in this study are available on the free access research platform for complex physiological signals (https://physionet.org/content/qde/1.0.0/). Ten male subjects voluntarily took part in this study. The median and standard deviation of height, body weight, and age were 179 ±7.5 cm. 79.3 ±9.0 kg. and 25.5 ±3.7 years, respectively. Results: The α-amylase, K^+, cortisol, total protein, and osmolality concentration in saliva showed significant variations for moderate dehydration levels. For the cases of Cl^- concentration changes in saliva, statistically significant changes can be observed at a much earlier stage (in mild dehydration conditions). Conclusions: The results suggest that Cl^- concentration in saliva might be used as an indicator for the hydration status. Therefore, a scale to monitor the hydration status is proposed.

Keywords

hydration status, saliva, electrolytes, aerobic exercise

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Abstract: Introduction: Maintaining an appropriate level of hydration is essential to health, especially when doing physical exercise. Thus, the importance of monitoring the before, during and after exercise status. Therefore, knowing and studying the changes and indicators that may provide reliable information on fluctuations in a simple, quick manner, via non-invasive sampling techniques becomes essential. Material and Methods: The data analyzed and processed (α-amylase, K^+, Cl^-, cortisol, total protein, and osmolality concentration in saliva) in this study are available on the free access research platform for complex physiological signals (https://physionet.org/content/qde/1.0.0/). Ten male subjects voluntarily took part in this study. The median and standard deviation of height, body weight, and age were 179 ±7.5 cm. 79.3 ±9.0 kg. and 25.5 ±3.7 years, respectively. Results: The α-amylase, K^+, cortisol, total protein, and osmolality concentration in saliva showed significant variations for moderate dehydration levels. For the cases of Cl^- concentration changes in saliva, statistically significant changes can be observed at a much earlier stage (in mild dehydration conditions). Conclusions: The results suggest that Cl^- concentration in saliva might be used as an indicator for the hydration status. Therefore, a scale to monitor the hydration status is proposed.

Keywords: hydration status, saliva, electrolytes, aerobic exercise.

1. Introduction

Water is one of the essential components of the human body. It represents 76% of skeletal muscle mass. The balance of total body water is homeostatically regulated by mechanisms that modify excretion routes (sweat, urine, breath, etc.) and stimulate its consumption and absorption [1].

Maintaining an appropriate level of hydration is essential to health, especially when doing physical exercise. Thus, the importance of monitoring the before, during and after exercise status. Therefore, knowing and studying the changes and indicators that may provide reliable information on fluctuations in a simple, quick manner, via non-invasive sampling techniques becomes essential.

It is a known fact that physical exercise may cause important water and electrolyte loss through sweat, mainly when performed in conditions of high temperature and high relative humidity. Under any of these circumstances, an appropriate strategy for replenishing fluids must be established. Otherwise, there is a risk of reaching a dehydration state, which affects athletic performance and health [2].

When physical exercise causes a bodyweight loss of 3 to 5% (severe dehydration), the following symptoms can be detected: cramps, apathy, weakness, and disorientation. In case of not interrupting the exercise, it is very likely that exhaustion and heat stroke are reached as a consequence of body thermoregulation loss [3].

Additionally, body fluid loss causes a decrease in plasma volume, which provokes a decrease in blood pressure and, consequently, poor blood flow towards muscles and skin. This decrease in blood flow to the tissues is compensated through a variety of mechanisms, such as an increase in the heart rate and alterations in gastrointestinal functions, which undergo changes that cause unbalanced gastric emptying, a decrease in thirst, nausea, vomit, and diarrhea [4].

Therefore, it is essential to obtain information about the fluctuations of biochemical elements that may work as biological markers of the body hydration status, especially if they include the possibility of being collected in an efficient, quick, non-invasive manner during the performance of a physical activity, with no significant interruption. Accordingly, the aim of this study was to determine the relation between electrolyte concentration, metabolic molecules and saliva osmolality, and the hydration levels in different subjects, while performing physical exercise on a treadmill.

2. Materials and Methods

The data analyzed and processed in this study are part of a series of experiments performed by a research group from two German universities: the Institute of Exercise Training and Sport Informatics of the Cologne Sports University, and the Digital Sports Group of the Pattern Recognition Lab of the Friedrich-Alexander University of Erlangen-Nürnberg [5]. These data are available on the free access research platform for complex physiological signals [6], administrated by the members of the Laboratory for Computational Physiology of the Massachusetts Institute of Technology (MIT). The study included the collection of data related to other bodily fluids and markers, apart from those considered in saliva. However, the following description refers to details related solely to salivary markers.

2.1. Subjects

Ten male subjects voluntarily took part in this study. The median and standard deviation of height, body weight, and age were 179 ± 7.5 cm. 79.3 ± 9.0 kg. and 25.5 ± 3.7 years, respectively. All the subjects gave their informed consent in writing after the research protocol was approved by the local ethics committee. In order to minimize confounding variables and side effects, people with considerable differences in age, body build and composition, or diseases that might affect their salivary composition were not considered for this study.

2.2. Preliminary examination

The subjects were submitted to a preliminary examination within one and seven days before data collection. The examination included the establishment of the ventilatory threshold and maximum oxygen consumption in each subject. The values were calculated based on individual performance in an incremental training test on a treadmill, which led to volitional fatigue [7].

2.3. Experimental procedure

Four previous conditions were established to ensure hydration conditions comparable among individual subjects. 1) The subjects were required to refrain from doing exhausting physical activity, drinking alcohol and caffeine the day before data collection. 2) The subjects were required to report to the laboratory at 6:30 a.m. on the day of data collection, after a 10-hour night fasting period. 3) Upon arrival, each subject was provided with an identical breakfast, which included 250 ml of apple juice with water. One hour later, the subjects were

provided with 312 ml of a meal replacement beverage. 4) The subjects were not allowed any food or beverage intake until the end of data collection.

Salivary marker collection and the total body water (TBW) loss reference values were started at 9:35 a.m. For this purpose, the subjects ran for a total of 120 minutes. In order to minimize confounding environmental effects, the exercise was performed on a treadmill. In order to minimize the confounding effects of clothes, the subjects wore identical t-shirts and shorts. In order to minimize the confounding effects of physical capacity, the subjects ran at individual speeds, appropriate to their minimum ventilatory threshold, 60% of maximum oxygen consumption.

In order to facilitate sampling, the 120 race minutes were divided into eight intervals. Each interval consisted of 15 minutes of activity and 8 minutes of rest. Sampling was performed during resting periods. The control samples were collected immediately after the first activity interval.

2.4. Salivary markers

The saliva was obtained using Salivette cotton swabs (Sarstedt, Nürnbrecht, Germany), which were placed under the subjects' tongues during the resting period [8]. No mouthwash was used before the saliva collection since it has been observed to modify salivary osmolality for approximately 15 minutes. The saliva samples were analyzed at the laboratory in search of six markers, namely: amylase, chloride, cortisol, osmolality, potassium, and protein.

The amylase (α-amylase) concentration was measured with the colorimetric enzymatic method, using the 4.6 ethylidene-p-nitrophenyl-α, D-maltoheptaosoid Roche Diagnostics (Mannheim, Germany) substrate. The diluted salivary samples were analyzed with a Roche integra 800 system. The chloride concentration was analyzed with a chloridemeter (CM20, Kreienbaum, Langenfeld, Germany). The cortisol concentrations were measured by liquid chromatography-tandem mass spectrometry. Potassium and protein were measured by Cobas Integra 800 (Roche Diagnostics, Mannheim, Germany). The osmolality was analyzed with a vapor pressure osmometer (Vapro, Wescor, Logan, UT, USA).

2.5. Total Body Water Loss

The reference values for total body water loss were obtained by establishing the body-weight loss after each activity interval. This method is based on an assumption of no food or beverage intake, and no urine and feces loss. Consequently, body weight changes during physical exercise were solely due to water loss [9]. Therefore, the subjects were required to remove their clothes and eliminate all body sweat using towels. Subsequently, nude body weight was measured by high precision equipment (± 5 g precision; DE 150K2D, Kern & Sohn, Balingen-Frommern, Germany) The difference in nude body weight (in kilograms) between two consecutive activity intervals was equal to the total body water loss (in liters), after the corresponding activity interval. No urine or feces loss took place during the procedure; therefore, they were not considered as an additional loss of total body water.

2.6. Data processing and statistical analysis

The data processing involved simple and conventional calculations. The average value of the series was included for non-existing data (which represents under 0.06% of the information). Deltas (difference in body weight and difference in Cl⁻ concentration per interval, for each subject) were used for the calculation of total body water (TBW) loss, and chloride concentration. For the purpose of normalizing data and reducing the inherent response to participants' individual characteristics, the total body water is presented as body weight percentage for each experimental subject (Figure 1).

The data were analyzed using the free access PAST Paleontological statistics software package for education and data analysis [11]. One-way ANOVA tests and Tukey multiple

comparison tests were performed owing to the fact that the data presented a Gaussian distribution (normal distribution). Significant differences were considered starting at a P value ≤ 0.05 (*); 0.01(**); 0.001(***). (Ns) is used for non-significant cases.
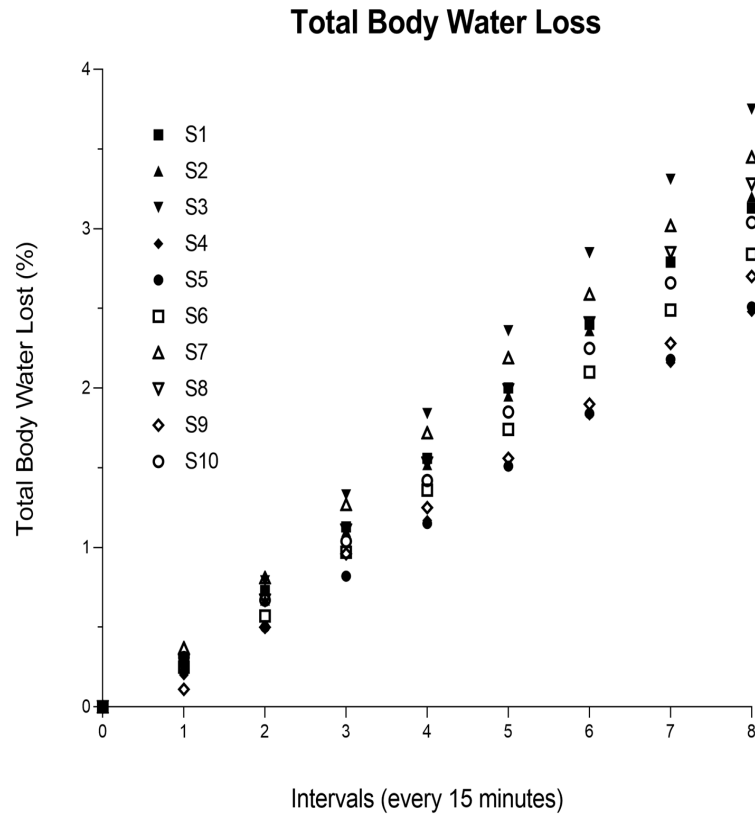

Fig. 1. The percentage of total lost body water accumulated (y-axis) for each participant in each interval (x-axis)

3. Results

Figure 1 shows the progressive total body water loss in the participants of the study, caused by physical exercise throughout the experimental sessions. Reaching all levels of moderate dehydration (between 2.5 and 3.5% of their body weight).

Alpha-amylase concentration in saliva showed low sensibility to total body water loss percentages equal to or under 2.25% (Figure 2).

For the cases of Cl- concentration changes in saliva, a certain tendency can be observed regarding the percentage of total body water loss. Notwithstanding, statistically significant changes become noticeable at mild dehydration levels (Figure 3).

The cortisol concentration in saliva shows a behavior similar to α-amylase (Figure 4), where significant changes can be noticed in a somewhat late phase, considering that total body water loss values that exceed 2% can be understood as moderate dehydration.

After the statistical analysis of the data obtained by measuring osmolality in saliva samples and potassium concentration, it is observed that significant differences appear once the subjects present moderate dehydration levels (Figures 5 and 6, respectively).

Protein concentration in saliva proved to be the least sensible parameter to detect changes in total body water loss, as statistically significant changes can be observed starting at 2.65%, and if we refer to time of exercise, appears after the seventh interval, that is, at 105 minutes of physical activity (Figure 7).
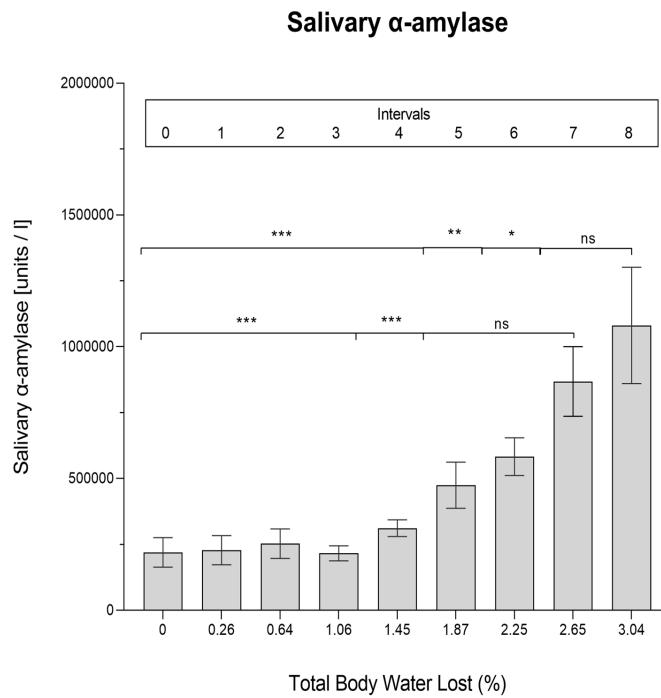
Salivary α-amylase

Fig. 2. Alpha-amylase concentration in saliva (y-axis) for each of the percentages of total body water lost and accumulated by interval (x-axis). Data analyzed with one-way ANOVA and Tukey's post test of multiple comparisons were considered significant at *P* values < 0.05 (*)
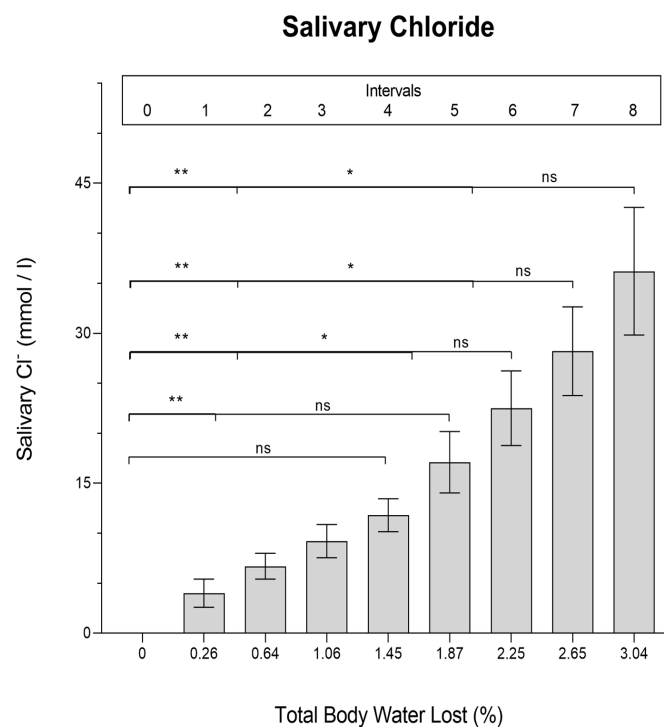
Salivary Chloride

Fig. 3. Chloride concentration in saliva (y-axis) for each of the percentages of total body water lost and accumulated by interval (x-axis). Data analyzed with one-way ANOVA and Tukey's post test of multiple comparisons were considered significant at *P* values < 0.05 (*)
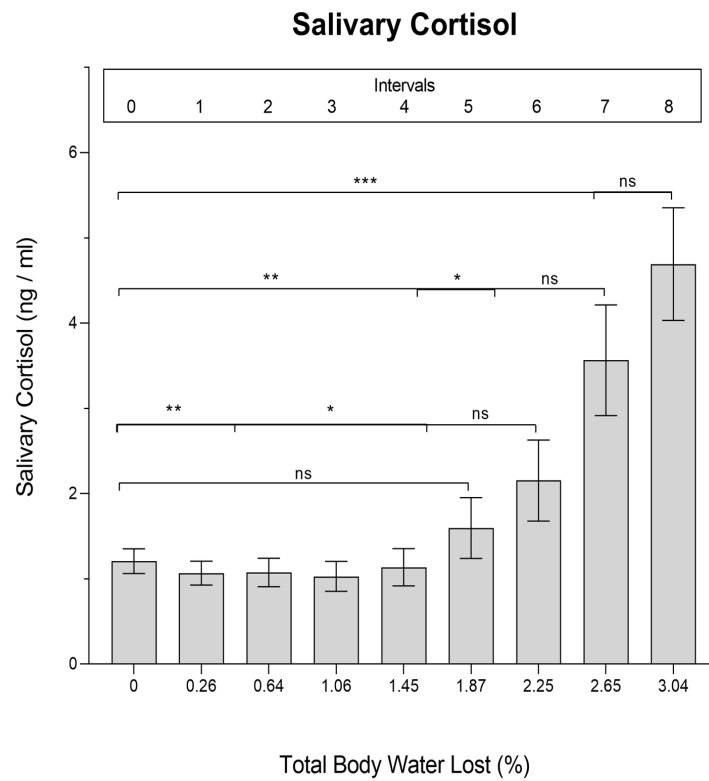
Salivary Cortisol

Fig. 4. Cortisol concentration in saliva (y-axis) for each of the percentages of total body water lost and accumulated by interval (x-axis). Data analyzed with one-way ANOVA and Tukey's post test of multiple comparisons were considered significant at *P* values < 0.05 (*)
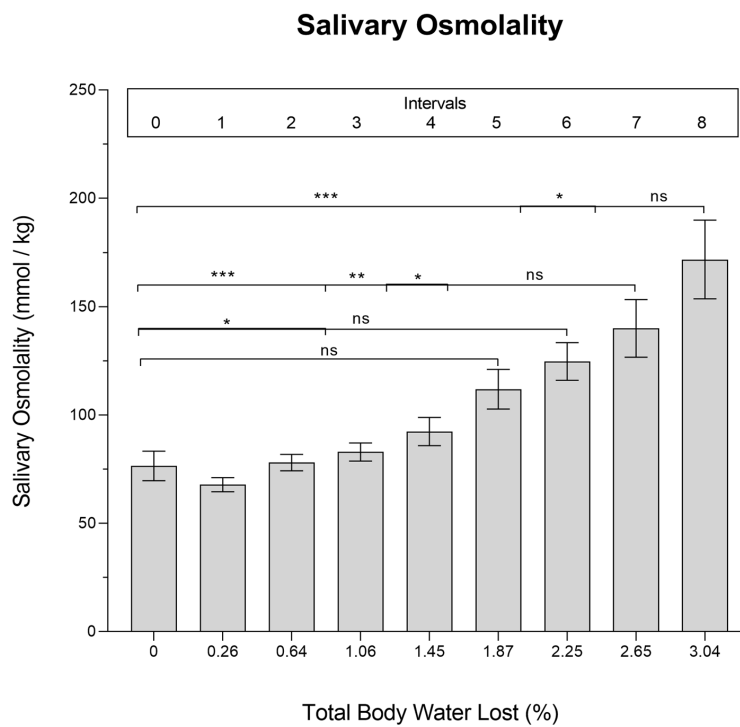
Salivary Osmolality

Fig. 5. Osmolality changes in saliva (y-axis) for each of the percentages of total body water lost and accumulated by interval (x-axis). Data analyzed with one-way ANOVA and Tukey's post test of multiple comparisons were considered significant at *P* values < 0.05 (*)

Salivary Potassium

Fig. 6. Potassium concentration in saliva (y-axis) for each of the percentages of total body water lost and accumulated by interval (x-axis). Data analyzed with one-way ANOVA and Tukey's post test of multiple comparisons were considered significant at P values < 0.05 (*)
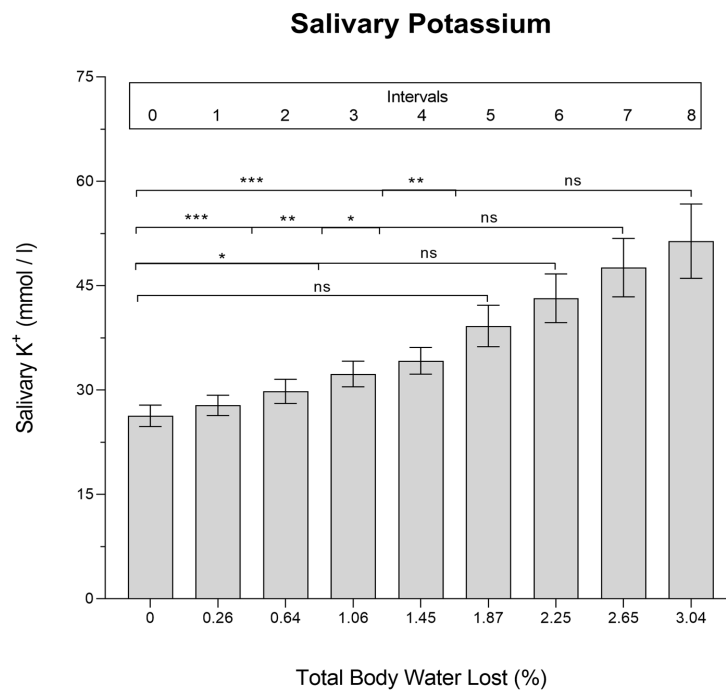
Salivary Protein Concentration

Fig. 7. Protein concentration in saliva (y-axis) for each of the percentages of total body water lost and accumulated by interval (x-axis). Data analyzed with one-way ANOVA and Tukey's post test of multiple comparisons were considered significant at *P* values < 0.05 (*)
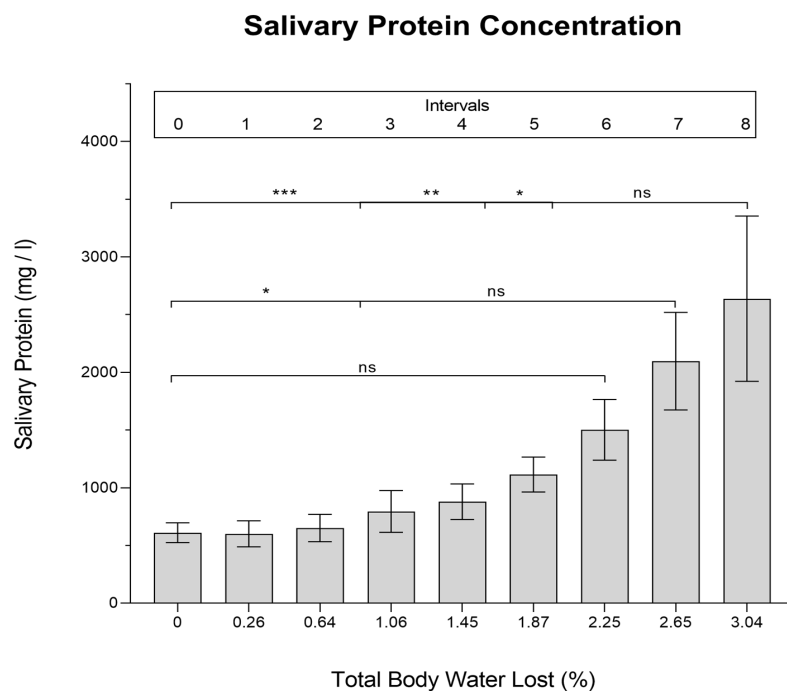
4. Discussion

The use of biomarkers in saliva to determine the hydration status represents a real possibility. Additionally, it offers a vast range of advantages from a technical, logistic, and procedural point of view. Notwithstanding, there is much yet to be clarified, mainly regarding the nature of the fluctuation of target molecule levels or concentrations (Cl^-, K^+,

cortisol, α-amylase, total protein and osmolality). On the other hand, standardizing sampling techniques is an aspect that requires more attention, which is probably one of the most sensible areas to improve. The experimental design used allows monitoring the progressive process of total body water loss in experimental subjects who reached moderate dehydration levels (Figure 1). Details such as a decrease in resting and sampling time might be necessary, even though in general they can be considered appropriate.

4.1. Alpha-amylase concentration as a hydration status indicator

Different conditions are involved in considering α-amylase as a potential marker for the hydration status, partly because of early reports on its increase after physical activity [11]. However, it shows no variation at moderate dehydration levels (Figure 2). If we consider that one of the main cellular α-amylase exocytosis mechanisms of acinar cells is mediated by the sympathetic nervous system through the liberation of catecholamines and that this is the most active system during physical exercise [12], it seems contradictory that this enzyme increases its concentration after 100 minutes of exercise. It would be more appropriate to consider the fact of coinciding this time-lapse with a moderate dehydration level, the volume of water in saliva is affected, and thus the concentration of α-amylase increases.

4.2. Chloride concentration as a hydration status indicator

Cl^- secretion from acinar cells during physical exercise is modulated by the activity of K^+ channels depending on Ca^+, which are concomitantly liberated through a signaling pathway elicited by the sympathetic nervous system [13]. The difference that it might present regarding the behavior of α-amylase and its showing changes at a much earlier stage is that primary salivary formation is isosmotic, even though when passing through the ductal system it is modified by reabsorption methods present there. Due to its ionic nature, it seems to have a stronger connection with water movement and, therefore, to show fluctuations. Based on these data, the Cl^- concentration could be used as a hydration status marker since it presents significant changes when reaching mild dehydration levels (Figure 3). In consequence, a scale to monitor hydration status (Table 1) is proposed based on the averages of Cl^- concentration fluctuations, related to the total percentage of total body water loss. However, further studies should be performed to learn more about this apparent relation, as an initial measure is considered for the Cl- concentration to be able to monitor the hydration status based on its changes throughout training or physical activity, in general.

Table 1. Proposed scale to monitor the hydration status during exercise

Cl$^-$ concentration changes (mmol/l)	Hydration level (% TBW lost)
17	Mild (1.5 %)
22	Moderate A (2%)
28	Moderate B (2.5%)
35	Moderate C (3%)

4.3. Cortisol concentration as a hydration status indicator

Even though cortisol increases its concentration levels in the blood as a response to stress induced by exercise and mainly after it [14], for the case of concentration in saliva and in the conditions created for this study, cortisol does not seem to be a molecule to consider in order to monitor the hydration status during physical exercise, as it shows significant changes when subjects reach moderate dehydration levels (Figure 4).

5. Conclusions

For future studies aimed at searching for saliva hydration status indicators, it is necessary to approach the phenomenon using non-linear data analysis methods, considering that the mechanisms and systems that modulate salivary secretion in training conditions apparently adjust best to this kind of mathematic method. The chloride concentration in saliva during exercise could be used to create a conversion table where the Cl⁻ mmol/l in saliva are related to the percentage of total body water loss in the subjects. However, it would be advisable to focus on this aspect, include as many subjects as possible, and thus submit it to evaluation under different experimental protocols.

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