INDOOR SWIMMING FOR 2.5 KM DECREASES NITRITE AND pH IN EXHALED BREATH CONDENSATE

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ORIGINAL

INDOOR SWIMMING FOR 2.5 KM DECREASES NITRITE AND pH IN EXHALED BREATH CONDENSATE

NADAR 2.5-KM INDOOR DISMINUYE EL NITRITO Y pH EN AIRE ESPIRADO CONDENSADO

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ABSTRACT

OBJECTIVE: to determine the effect of an aerobic swimming test in a chlorinated indoor swimming pool on the concentration of NO_2^-, H_2O_2 and pH in exhaled breath condensate. METHODS: ten amateur swimmers swam 2.5 km in a chlorinated pool. Samples were obtained before the test and four times in the eight hours after the test. Mixed models and the Spearman test were used for the statistical

analysis. RESULTS: the test was performed at 74.99 ± 10.10% of the cardiac reserve and lasted 50.80 ± 8.98 minutes. After the test, NO_2^- (p = 0.04) and pH (p = 0.02) in the exhaled air condensate decreased. Pre-exercise values were related to the absolute changes p=0.0002, p=0.047 and with the training volume p = 0.017, p = 0.077 for NO_2^- and H_2O_2 respectively. CONCLUSIONS: Swimming in a chlorinated pool decreases the NO_2^- concentration and pH in exhaled breath condensate.

KEYWORDS: swimming, oxidative damage, exhaled breath condensate

RESUMEN

OBJETIVO: determinar el efecto de una prueba aeróbica de natación en piscina clorada indoor sobre la concentración de NO_2^-, H_2O_2 y el pH en el condensado del aire espirado. MÉTODO: diez nadadores aficionados nadaron 2.5 km en piscina clorada. Se obtuvieron muestras antes y en cuatro oportunidades durante las ocho horas posteriores a la prueba. El análisis estadístico usó modelos mixtos y la prueba de Spearman RESULTADOS: la prueba se realizó a 74.99±10.10 % de la reserva cardíaca y duró 50.80±8.98 minutos. Posterior a la prueba disminuyó el NO_2^- (p=0.04) y el pH (p=0.02) en el condensado del aire espirado. Los valores pre-ejercicio se relacionaron con los cambios absolutos p=0.0002, p=0.047 y con el volumen de entrenamiento p=0.017, p=0.077 para NO_2^- y H_2O_2 respectivamente. CONCLUSIONES: la natación en piscina clorada disminuye la concentración de NO_2^- y el pH en el condensado del aire espirado.

PALABRAS CLAVE: natación, daño oxidativo, condensado del aire espirado.

INTRODUCTION

During exercise, the respiratory system undergoes considerable changes in its functionality. Both the blood arriving into the vascular compartment and the air into the airway are increased. This last zone, normally produces an environment protecting the epithelial cells, in terms of temperature and humidity, which can be affected during exercise, via accelerated evaporation, due to the drop in temperature and eventually due to an increase in inhalation of irritants such as cold air, pollen, particulate matter and environmental gases. Finally, this leads to localized inflammation, it alters the tissue redox status, and may affect lung function (Araneda & Tuesta, 2016). Over the last few years, our research group has been focused on the study of this phenomenon using the sample analysis of exhaled breath condensate (EBC). Thus far, we have reported increased H_2O_2 and malondialdehyde in climbers (Araneda et al., 2005) and subjects who train at medium altitude (Heinicke et al., 2009). Also, we have observed increased pro-oxidants in urban runners in distances over 21 km (Araneda et al., 2012). Subsequently, we reported localized increases in the concentration of nitrites and hydrogen peroxide in the EBC in active people who ran 10 km on the open-air

track (Araneda et al., 2014). Finally, under laboratory conditions, we have found that the longer the exercise time is, the greater the production of these same chemical species (Tuesta et al., 2016).

An environmental scenario widely used for exercising is swimming pools that use chlorine as a disinfectant for water. Considering the well-known effects that this environment produces on the skin and mucosa of the swimmers, it is relevant to study the pulmonary effects on its users (Davies et al., 2018; Cavaleiro et al., 2018). Previously, in subjects who swam for 40 minutes, no change was found in the concentration of 8-isoprostanes and cytokines measured in EBC, with an increase in the Clara cell secretory protein (CC16) in serum suggesting an increase in epithelial permeability (Font-Ribera et al., 2010). In a later report, an increase in the concentration of 8-isoprostanes in EBC was described after a 100-minute training session (Morissette et al., 2016). Taking this into account, we raise the following question: What is the effect of performing acute exercise in a chlorinated pool on the production of pro-oxidants and the pH in the EBC? To answer this, we performed a swimming test in an indoor chlorinated pool, under the usual environmental conditions of humidity concentration, temperature and chlorine concentration in swimmers with a follow-up of up to eight hours after the test.

MATERIAL AND METHODS

PARTICIPANTS

Ten (10) amateur swimmers were enrolled in the study, whose characteristics are detailed in Table 1. Participants were non-smokers, had no history of rhinitis or asthma, and no respiratory infections in the past month. Also, they did not use anti-inflammatory drugs, antioxidants, or any other nutritional supplement. They were informed orally and in writing of the protocol, before giving their written consent. This study was conducted following the ethical principles that govern Chilean national legislation and according to the ethical principles of the Declaration of Helsinki.

Parameter	Values
Sex (Male/Female)	7/3
Age (years)	27.9±13.1
Height (m)	1.64±0.51
Weight (kg)	62.6±9.2
BMI (kg/m^2)	22.3±2.8
Experience (years)	4.0±2.1
Training volume (hours/week)	12.6±10.7
Training frequency (days/week)	5.7±0.9

Table 1. General characteristics of the participants. Data are shown as average ± standard deviation.

PROCEDURES

Participants did not engage in intense physical activity in the 48 hours before the test. On the test day, they showed up at 7:00 AM having had breakfast an hour before the test. The resting heart rate was determined, while they were lying down and after 5 minutes of rest. Subsequently, the EBC samples were taken, at rest, after a mouthwash with distilled water and using a nasal clip. For this, the EBC equipment previously described was used (Araneda et al., 2005; Araneda & Salazar, 2009). The total collection time was approximately 15 minutes, period in which about 1.5 ml of sample was obtained. Once the sample collection was done, samples were stored in liquid nitrogen, and then transferred at -80 °C until analysis. The EBC samples and heart rate measurement were performed prior to exercise and after exercise at 20, 180, 360 and 480 minutes. After the first EBC sample collection, participants performed a warm-up that consisted of free swimming for 200 meters at 60% of the maximum heart rate calculated as 220 minus age. Once this period concluded, they carried out a maximum test of 2,500 meters of “front crawl” swimming. They stopped every 500 meters, so the examiner could determine their heart rate for 1 minute. The environmental conditions during the test were at a water temperature of 27.0 ± 1.7 °C and a relative humidity of 56.0±6.4%. Residual free chlorine was kept in the range of 0.5 to 1.5 ppm according to the standard of the aquatic center.

Hydrogen peroxide: this chemical was measured in the EBC using FOX_2 reagent (Nourooz-Zadeh et al., 1994). This reagent contains Fe^{+2} (250 µM), which in an acidic environment ($HClO_4$, 110 mM) is oxidized to Fe^{+3} due to the presence of H_2O_2. The amount of H_2O_2 was monitored by the reaction between the ferric ion

and the xylenol orange indicator (250 µM). Also, sorbitol was added to the original reaction (100 mM) according to Gay & Gebicki (Gay & Gebicki, 2002); this method has been previously used by our research group (Araneda et al., 2012; Araneda et al., 2014). For measurements, 350 µL of EBC and 150 µL of FOX_2 were taken and then the sample was incubated for 1h at room temperature; absorbance was read at 560 nm in a plate reader (EPOCHTM, BioTek Instruments, EE.UU.). Three calibration curves were performed for each group of measurements using H_2O_2 (Merck) as standard.

Nitrites (NO_2^-): Nitrite concentration was measured using the spectrophotometric assay based on Griess reaction (Green et al., 1982), and 300 µL of Griess reagent were added 0.1% of naphthylethylenediamine-dihydrochloride;1% sulfanilamide; 3% of H_3PO_4 at 300 µL of EBC. The mixture was incubated for ten minutes and the absorbance at 550 nm was measured using the previously mentioned plate reader. Three curves were performed for each measurement, with sodium nitrite as standard.

pH: It was measured using the Paget-Brown method (Paget-Brown et al., 2006). Thus, 100 µL of EBC were bubbled with Argon for eight minutes at a flow of 350 mL / min, and then the pH was measured using a microelectrode of 3 x 38 mm (Cole and Palmer) connected to a pH meter (Oakton® Acorn pH 6).

STATISTICAL ANALYSES

To study the trend of the parameters measured in the EBC over time, the following mixed model was designed:

$$y = \beta \text{ x } minute + \alpha$$

Where "y" is the value calculated for the parameter to be studied, "α" is the intercept, and "β" is the slope of the curve. Correlations were determined using Spearman's test, after applying the Shapiro-Wilk test to determine the type of distribution of the sample. Absolute changes were calculated in the correlations (Δ) of the parameters measured in the EBC, where the value obtained before the test was subtracted from the average of the four values obtained after the test. In addition, when dividing this parameter by the value before exercise and multiplied by one hundred, the percentage change was obtained (%Δ). The level of significance used was $p<0.05$. For the statistical analysis, the software Stata 14 and GraphPad Prism, USA, were used.

RESULTS

The test was performed at 161.56 ± 11.45 beats/min, which is equivalent to 74.99 ± 10.10% of the cardiac reserve, which in turn had a value of 123.50±9.9 beats/min. The total test time was 50.80 ± 8.98 minutes. Regarding the parameters measured in EBC, a high dispersion is observed, as shown in Table 2.

Table 2. Concentration of hydrogen peroxide, nitrite and pH for the EBC in participants performing a 2.5 km swimming test in an indoor chlorinated pool. Participants were measured before (pre) and after (post) the test was finished at 20, 180, 360 and 480 minutes. Values are expressed as median and interquartile range.

Parameter	pre	20min-post	180-post	360-post	480-post
$[H_2O_2]_{EBC}$ µmol · l-1	0.68 (0.20-1.08)	0.63 (0.27-1.72)	0.48 (0.22-0.83)	0.55 (0.32-1.09)	0.42 (0.24-0.78)
$[NO_2]_{EBC}$ µmol · l-1	2.00 (0.73-3.59)	2.22 (1.84-2.65)	1.50 (1.05-2.27)	1.91 (1.45-2.90)	1.42 (0.78-1.81)
pH_{EBC}	7.74 (7.43-8.00)	7.68 (7.42-8.03)	7.68 (7.47-7.87)	7.52 (7.28-7.81)	7.57 (7.38-7.88)

According to the tendency model obtained using the mixed model test, the $[NO_2^-]_{EBC}$ decreased in the period of time analyzed; α=-0.0019 and β=2.86; p=0.044 (see Figure 1b). The same behavior was found for pH_{EBC}; α=-0.00055 and β=7.69; p=0.021(see Figure 1a). As for $[H_2O_2]_{EBC}$, the model had a value of α=-0.0005 and β=0.98 without achieving statistical significance (p=0.17). As for the correlations, associations between the test duration and the average heart rate (r=-0.74; p=0.016) were found; training volume measured in hours per week (r=-0.77; p=0.010) as shown in Figure 2a, and percentage of cardiac reserve used during the test (r=-0.65; p=0.049). Age of participants showed a trend toward statistical significance with the training volume (r=-0.62; p=0.06) as shown in Figure 2b. Regarding the metabolites measured in EBC, values of $[NO_2^-]_{EBC}$ pre-exercise were correlated with the training volume (r=-0.74; p=0.017) as shown in Figure 2c, with Δ $[NO_2^-]_{EBC}$ (r=-0.94; p=0.0002), as shown in Figure 3b and with %Δ $[NO_2^-]_{EBC}$ (r= -0.88; p=0.002). Values of Δ$[NO_2^-]_{EBC}$ were correlated with the training volume (r=0.78; p=0.01). Regarding the %Δ, $[NO_2^-]_{EBC}$ was correlated with the training volume (r= 0.76; p=0.015) and age (r= -0.66; p=0.044). $[H_2O_2]_{EBC}$ pre-exercise was correlated with Δ $[H_2O_2]_{EBC}$ (r=-0.83; p=0.005) and %Δ $[H_2O_2]_{EBC}$ (r= -0.76; p=0.015) as shown in Figure 3a. Also, a trend toward statistical significance with the training volume (r=-0.58; p=0.077) was found, as shown in Figure 2d. Values of Δ$[H_2O_2]_{EBC}$ were correlated with Δ$[NO_2^-]_{EBC}$ (r=0.86; p=0.003), while %Δ $[H_2O_2]_{EBC}$ was correlated with %Δ $[NO_2^-]_{EBC}$ (r=0.79; p=0.009). The value of pH_{EBC} pre-exercise was correlated with the percentage of average maximum heart rate during the test (r=-0.75; p=0.015), while a trend toward statistical significance was observed with the percentage of the cardiac reserve (r=-0.62; p=0.060). The values of Δ pH_{EBC} (r=0.73; p=0.021) and the values of %Δ pH_{EBC} (r=0.69; p=0.035) during the test were associated with age. Finally, all values of the parameters measured in the EBC were correlated (n=50), and a direct relationship between $[NO_2^-]_{EBC}$ and $[H_2O_2]_{EBC}$ (r=0.78; p<0.0001) was found.

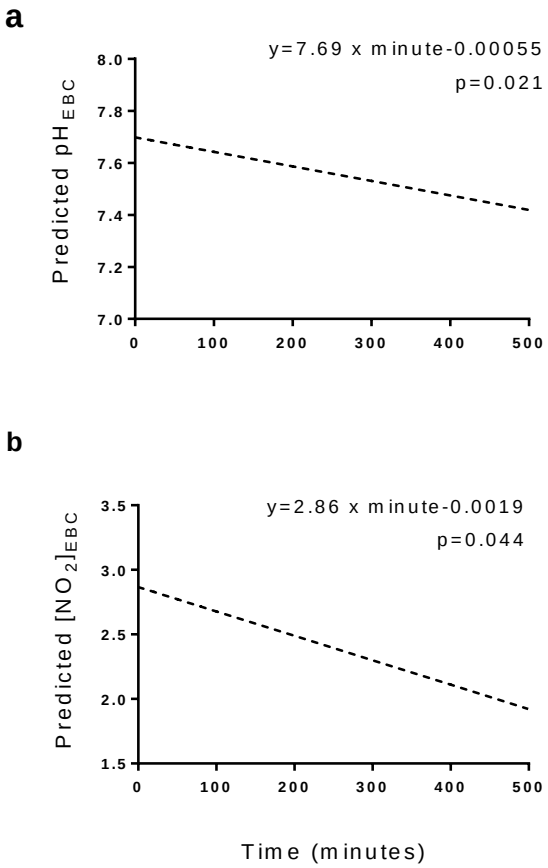

Figure 1. (a) Mixed models proposed for the variation of pH_{EBC}, and (b) $[NO_2]_{EBC}$ drawn up from the pre-values and the values obtained in the four post-exercise measurement stages for eight hours.

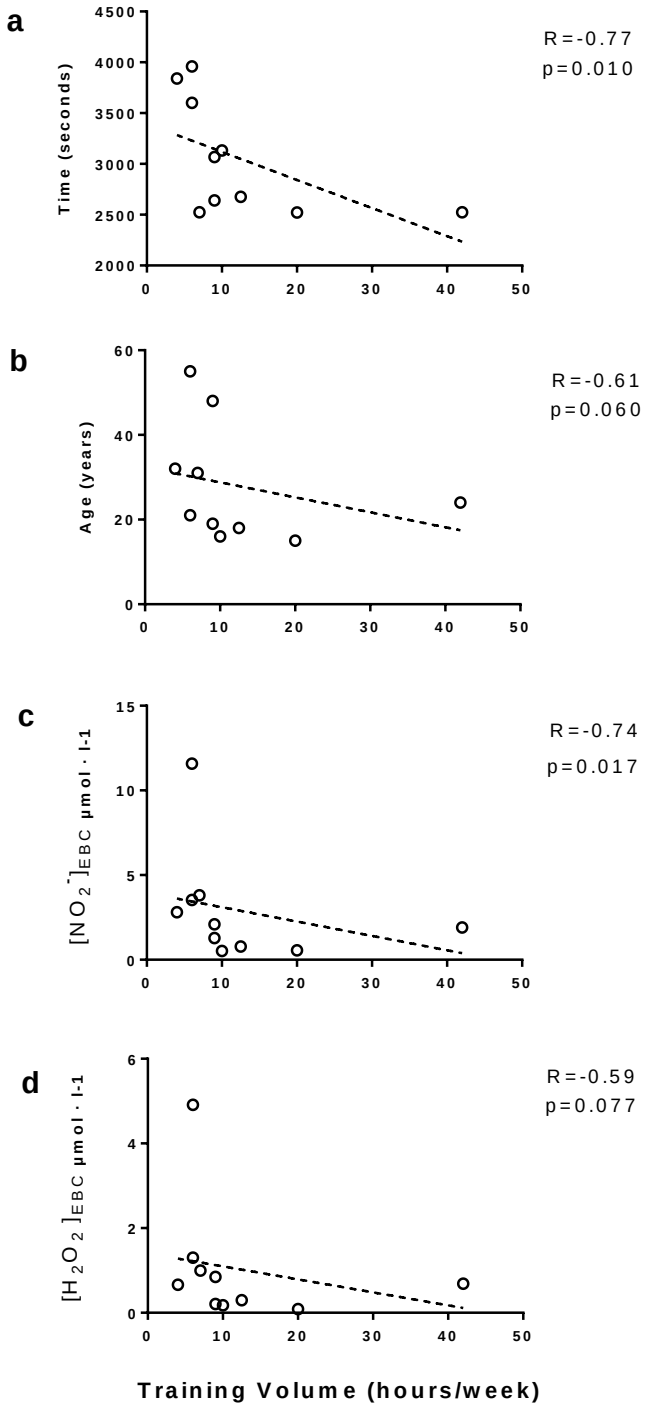

Figure 2. (a) Relationship between the volume of physical training in the pool and the time of the 2.5 km swimming test; (b) age; (c) $[NO_2]_{EBC\ pre}$ and (d) $[H_2O_2]_{EBC\ pre}$.

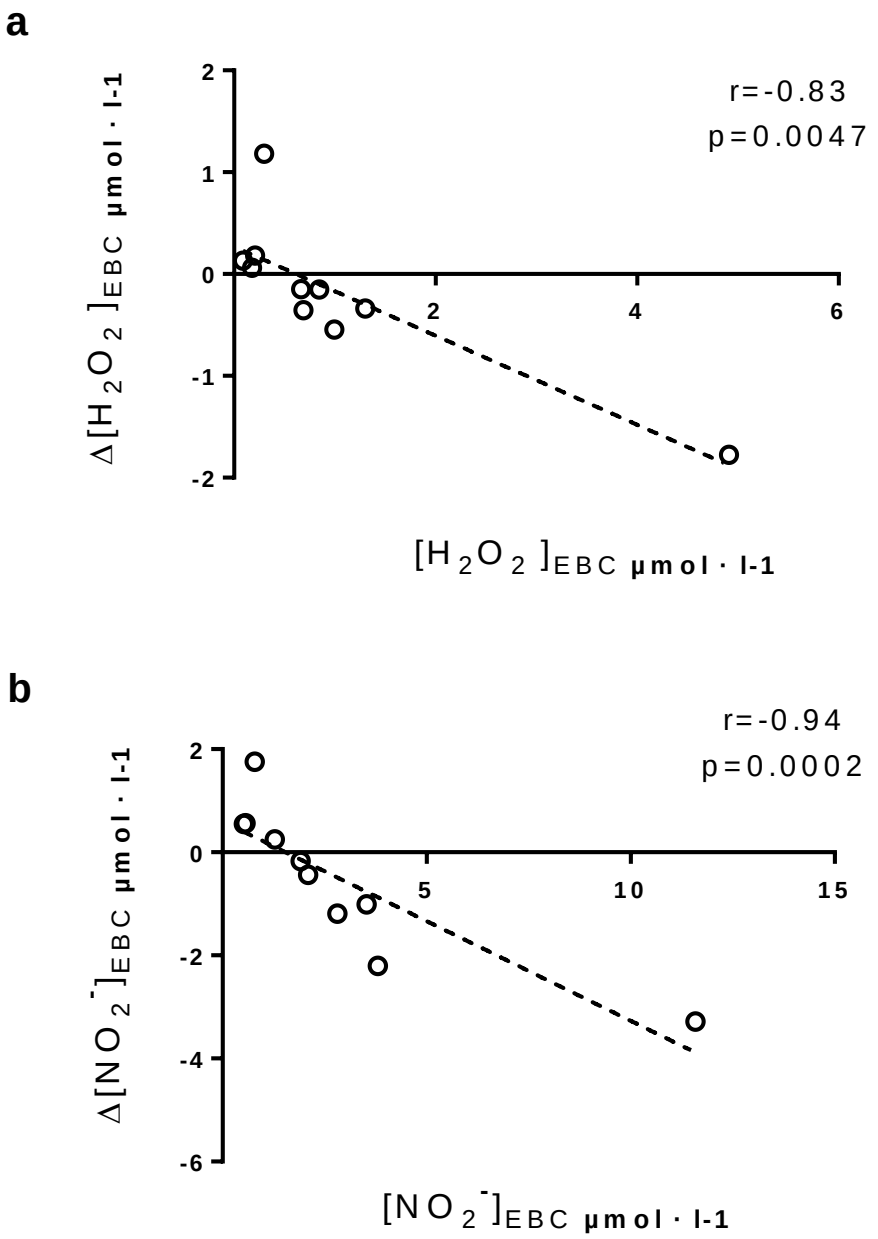

Figure 3. (a) Relationship between pre-exercise values and absolute changes in $[H_2O_2]_{EBC}$ and (b) $[NO_2]_{EBC}$ obtained by the test of swimming 2.5 km in an indoor pool. Post-exercise values correspond to the average of the four samples taken after the test.

DISCUSSION

In this report, swimmers performed a 2.5 km test in a chlorinated indoor pool. After the test, a decrease in $[NO_2^-]_{EBC}$ and pH_{EBC} was found, with no change in $[H_2O_2]_{EBC}$ for eight hours after the stimulus. If compared to our previous results,

this general trend is novel due to the decreased nitrite -a known pro-oxidant of the airway- and the promotion of a potential localized inflammatory condition measured as pH_{EBC}. In view of the above, our results are in part contrary to the initial hypothesis, since we expected that the combination of intense exercise (about 75% of the cardiac reserve for almost an hour) and the contact with chlorine and its derivatives, both vaporized and dissolved in the pool water, would be the origin of an increase in pro-oxidants and promoters of inflammation.

The pro-oxidants evaluated in the EBC (H_2O_2 and NO_2^-) are chemical species which participate in physiological functions and vascular reactivity, intracellular signaling processes (Magherini et al., 2019) and pathological functions, since they are mediators of the inflammatory process. Pro-oxidants have been found to be in higher concentrations in EBC in people with asthma, cystic fibrosis, chronic obstructive pulmonary disease or after smoking (Konstantinidi et al., 2015; Causer et al., 2020). During exercise, the short-term protocols have reported no changes in the $[H_2O_2]_{EBC}$ (Nowak et al., 2001*,* Marek et al., 2008*)*. When exercise is performed for more than two hours in outdoor foot races (Araneda et al., 2012) or more than ninety minutes on a cycle ergometer under laboratory conditions (Araneda et al., 2016) we have usually found increases in both $[H_2O_2]_{EBC}$ and $[NO_2^-]_{EBC}$ at 20 and 80 minutes after cessation of exercise. As already mentioned, these findings are contrary to those presented in this work, where the obtained model showed a decrease in $[NO_2^-]_{EBC}$ the eight hours after exercise. In one of the few experimental models, which is similar to the model presented in this work, Font Ribera*,* reported a trend towards decreased nitric oxide (from which nitrites are derived), measured directly in the exhaled breath, after a group of atopic subjects swam for 40 minutes in a chlorinated pool (*Font Ribera et al., 2010*). As for $[H_2O_2]_{EBC}$, we found great variability in the obtained values, which may be the reason that statistical significance was not achieved. However, this chemical species is likely to behave similarly to $[NO_2^-]_{EBC}$ due to the close correlations we have found between them (please refer to results). An explanation of our results forces us to look for differences with other exercise models and environmental conditions previously published. When swimming, breathing is developed in voluntary cycles, which implies less total pulmonary ventilation in comparison to foot races. This would imply a lower drop in the airway temperature and less epithelial dehydration. In addition, the test was carried out under conditions of higher temperature and humidity which is typical of indoor pools. So, a decrease in the difference between the epithelium and the environment is produced, causing less water loss and reducing cell damage.

Thus, a decrease in the difference between the epithelium and the environment is produced, leading to less water loss and, thereby, reducing cell damage. To this theoretical favorable scenario of decreased formation of pro-oxidants via irritation, the potential increase in (enzymatic / non-enzymatic) antioxidant systems in their activity or concentration after exercise is added (Powers et al., 2014). Taking into account both factors, they may contribute to explain the findings.

Regarding another point, lung inflammation has been studied by determining $pH_{EBC.}$ Thus, the decrease in pH_{BEC} value has been described in the case of pulmonary inflammatory processes (Hunt, 2006). In our current report, lower pH_{EBC} values up to eight hours after exercise have been found. This finding is pointing in the direction we were expecting. It is an interesting finding, given that previously, a trend towards post-exercise alkalosis had been described, as reported by Riediker and Danuser, with a treadmill exercise at 60% of maximum heart rate (Riediker & Danuser, 2007). In the same direction, we also found a trend towards an increase of pH_{EBC} in participants in two 10-km tests performed at maximum intensity, both in non-trained (Araneda et al., 2014) and in trained (habitual) runners (Araneda et al., 2012). This trend is of great interest, given that the time in the aforementioned distance is similar to the time invested in the swimming test currently reported. In this sense, our current finding is more comparable to the downward trend of pH_{EBC} in foot races for more than two hours, at distances of 21 and 42 km respectively. This suggests that a new factor was probably added to exercise as a promoter of the decrease in pH_{EBC}. Thus, it is possible that this factor would be the direct contact with the vaporized chlorine derivatives in the pool, since these species act as irritants of epithelial surface. Even though this hypothesis is plausible, our experimental model is not sufficient to verify it and it should be evaluated later.

Furthermore, in our sample, a wide dispersion in age (see table 1) is shown. It is also observed that most trained subjects showed better performance in the test, with a tendency for them to be younger subjects (as shown in Figure 2a and 2b, respectively). In the chemical markers measured in the EBC, it can be observed that the basal values of both pro-oxidants are closely related and inversely related with the absolute changes produced by exercise, as shown in Figure 3a and 3b. For this reason, it was relevant to characterize the factors with which pro-oxidants could be related. In our sample, higher concentrations of basal $[NO_2^-]_{EBC}$ (see Figure 2d) were found in swimmers with fewer hours of training per week, and were likely to be older. Regarding $[H_2O_2]_{EBC}$ (see Figure 2c), where significance is not achieved (p=0.077); the trend is similar to the previous one. This idea is further strengthened by the close correlation between the values of $[NO_2^-]_{EBC}$ and $[H_2O_2]_{EBC}$ as described in the results. In any case, it cannot be ruled out that age also has some influence on the basal values of pro-oxidants; however, the low number of participants prevents us from isolating this effect and it should be studied in the future. In the case of the basal value of pH_{EBC}, we have only indirect evidence that it could be related to the training volume, since it is inversely correlated with two parameters measured in the test; the percentage of the cardiac reserve used and the average heart rate, which are significantly associated with the time spent on the test. If so, it would imply that the best trained subjects would have a lower basal value of pH_{EBC}. This finding is similar to that described in long-distance runners, where it has been described that subjects with more training hours have a lower basal pH_{EBC} (Ferdinands et al., 2008).

Regarding post-exercise changes in pro-oxidants, subjects with the highest basal values (who train less) showed greater decreases, both in$[H_2O_2]_{EBC}$(see Figure 3a)

and in $[NO_2^-]_{EBC}$ (see Figure 3b). This result can be related to previous findings, where individuals who had a higher level of training show less changes in 10-km races, if compared to non-trained subjects (Araneda et al., 2104), although in the current report, the trend of post-test values is downwards. In addition, there are two correlations regarding age that may reaffirm that younger participants, who were more highly trained and who worked at a higher intensity during the test, showed less changes in %Δ pH_{EBC} and %Δ $[NO_2^-]_{EBC}$ respectively. Another novel point regarding our previous results is the lack of relationship between the production of pro-oxidants and the changes in pH_{EBC} with a downward trend observed in exercise (Araneda et al., 2014; Araneda et al., 2012). Therefore, in the absence of this effect, even with pro-oxidants with a downward trend as we have described, it is possible that other factors could explain the decrease in pH_{CAE}, such as the direct contact with chlorine derivatives, as discussed above. Finally, the low number of participants, considering the known high variability of the determinations in EBC, was a limitation of this study. Likewise, spirometric measurements that could contribute to elucidate eventual pulmonary functional changes are missing.

CONCLUSION

In a group of healthy swimmers, after a 2.5 km test in an indoor chlorinated pool, nitrite concentration and pH in the exhaled breath condensate are decreased up to eight hours after swimming. In part, the higher temperature and humidity typical of indoor swimming pools may be involved in this phenomenon. The results obtained in this research provide a glimpse into the potential usefulness of these markers to monitor swimmers in chlorinated pools.

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