Is airway damage during physical exercise related to airway dehydration? Inputs from a computational model.

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Running title

Airway dehydration threshold determines exercise-induced epithelial damage

Author contribution :

AB, FD, JK and VB participated to all the steps of the experimental study. CK, BH, GB, VB, BM participated to the mathematical modelling part of the study. All the authors have read and approved the final version of the manuscript.

Abstract

In healthy subjects, at low minute ventilation ($\dot{V}_E$) during physical exercise, the water content and temperature of the airways are well regulated. However, with the increase in $\dot{V}_E$, the bronchial mucosa becomes dehydrated and epithelial damage occurs. Our goal was to demonstrate the correspondence between the ventilatory threshold inducing epithelial damage, measured experimentally, and the dehydration threshold, estimated numerically. In 16 healthy adults, we assessed epithelial damage before and following a 30-min continuous cycling exercise at 70% of maximal work rate, by measuring the variation pre- to post-exercise of serum club cell protein (cc16/cr). Blood samples were collected at rest, just at the end of the standardized 10-min warm-up, and immediately, 30 min and 60 min post-exercise. Mean $\dot{V}_E$ during exercise was kept for analysis. Airway water and heat losses were estimated using a computational model adapted to the experimental conditions and were compared to a literature-based threshold of bronchial dehydration. Eleven participants exceeded the threshold for bronchial dehydration during exercise (group A) and 5 did not (group B). Compared to post warm-up, the increase in cc16/cr post-exercise was significant (mean increase ± SEM: 0.48 ± 0.08 $ng.l^{-1}$ only in group A but not in group B (mean difference ± SEM: 0.10 ± 0.04 $ng.l^{-1}$). This corresponds to an increase of 101 ± 32% [range: 16 - 367%] in group A (mean± SEM). Our findings suggest that the use of a computational model may be helpful to estimate an individual

dehydration threshold of the airways that is associated with epithelial damage during physical exercise.

New and noteworthy:

Using a computational model for heat and water transfers in the bronchi, we identified a threshold in ventilation during exercise above which airway dehydration is thought to occur. When this threshold was exceeded, epithelial damage was found. This threshold might therefore represent the ventilation upper limit during exercise in susceptible individuals. Our results might help to prevent maladaptation to chronic exercise such as exercise-induced bronchoconstriction or asthma.

Keywords

Serum cc16, exercise ventilation, airway dehydration threshold, healthy participants, computational modelling

Glossary

A: Age

ASL: Airway surface liquid

b: Blood

cc16: Club cell protein

c_p: heat capacity at constant pressure of humid air

cr: Creatinine

$C_{sat}(T)$: Saturation concentration of humid air at a given temperature (T)

f_b: Breathing frequency

FEV_1: Forced expiratory volume in one second

FRC: Functional residual capacity

H: Height

HR: Heart rate

J_{evap}: Evaporation flux in the bronchial tree

Q_b: Blood flow rate in the bronchial circulation

RH: Relative humidity

RH_T: Relative humidity of the air in the trachea

T: Temperature

T_b: Body temperature

T_T: Temperature of the air in the trachea

t_e: Expiration time

t_i: Inspiration time

t_{tot}: Total time of the ventilation cycle

V_{cap}: Volume of the connective tissue involved in thermal transfers

$\dot{V}_E$: Ventilation

V_T: Tidal volume

δ_{cap}: Thickness of the connective tissue involved in heat transfer towards the lumen

Ξ: Dimensionless parameter of energy exchange between the mucosa and the blood

ρ: Volumetric mass of humid air

Introduction

Under physiological conditions, inspired air is heated and saturated with water vapor as it passes through the nasal cavities and upper airways (1-3). With the increase in ventilatory demand during exercise, breathing becomes mainly oral. The onset of oronasal breathing varies considerably between individuals and averages around 35-45 $l.min^{-1}$ (4-7). With oral breathing, the air entering the tracheobronchial tree is potentially colder and dryer than with nasal breathing (8). At low-level ventilation, a water replenishment mechanism, provided by the epithelium and submucosal glands, allows to maintain the water balance of the mucus (9,10) and compensates for the evaporation in the first airway generations (11). However, when ventilation is high, as during endurance exercise, a disbalance between water loss and replenishment occurs, known as airway dehydration (8). This phenomenon is considered to be the main stimulus of exercise-induced bronchoconstriction (EIB) (12).

Recent data suggest that exercise ventilation can cause airway inflammation (13-15) and epithelial damage (16-20). While the extent of post-exertion airway inflammation is determined by the presence or absence of EIB or asthma in young adults (14,15), epithelial damage is likely to be dependent on the level of ventilation but not on EIB (18,19). The integrity of the epithelial barrier may be assessed through the measurement of the level of club cells (cc) in the extrapulmonary fluids, and in particular the cc16 (16kD) in serum. This anti-inflammatory protein is secreted by non-ciliated epithelial cells, mainly club cells, in the terminal bronchioles. When the airway epithelium is compromised., cc16 is produced in the lung and diffuses into the blood through the bronchoalveolar barrier (21). An increase in cc16 concentration in the blood therefore reflects a loss of epithelial integrity, as observed in patients with lung disease or in healthy subjects exposed to air pollution or after exercising (21).

The relationship between ventilation, dehydration and airway damage is still misunderstood. We hypothesize that when mean ventilation maintained during a continuous exercise reaches

the dehydration threshold of the bronchi, serum cc16 increases post-exercise in healthy participants.(22)

Our aim is to compare the epithelial integrity before and after a 30-min continuous exercise in healthy subjects, depending on whether or not they have exceeded the theoretical ventilatory threshold of bronchial dehydration throughout the effort. An original aspect of our study is the determination of an individual theoretical threshold of dehydration using a computational model adjusted to our experimental conditions (11).

Methods

Subjects description

Healthy active young males (n = 16) aged 24±5 years participated in the study (Table 1). Sex was not an exclusion criterion because it does not influence exercise-induced changes in cc16 (20,23). However, no women volunteered to participate in our study. None was a current smoker, and all were free of any known cardiac or respiratory disease. None complained of respiratory symptoms during exercise. All had baseline lung function within normal limits i.e., forced expiratory volume in one second (FEV_1), forced vital capacity (FVC), forced expiratory flow from 25 to 75% of FVC ($FEF_{25-75\%}$) and FEV_1/FVC ratio above the lower limit of normal (LLN), corresponding to the fifth percentile (24). All the participants provided written informed consent prior to participation. The protocol was approved by the University of Brighton Ethics Committee, and the study was conducted according to the 2008 version of the Declaration of Helsinki.

Experimental data

The detailed methodology and the relevant variables for this cohort have been previously described, as part of our dataset has been published in a previous work (25). Briefly, participants performed an incremental exercise test to exhaustion (75W + 25W every 2 minutes) on a cycle ergometer (SRM, SRM GmbH, Germany).The maximal work rate and the first and second ventilatory thresholds (VT_1 and VT_2) were thus determined. Club cells 16kD (cc16) protein were sampled from the venous blood (catheter) of the sixteen healthy active young men at rest, after a 10-min standardized warm-up and immediately, 30 and 60 min after a 30-min continuous exercise on a cycle ergometer (620 Ergomedic, Monark, Varberg, Sweden) at 70% of the participant's maximal work rate. The maximal increase in cc16 weighted by creatinine from baseline to post-exercise (Δcc16/cr) was calculated as follows: ((cc16/cr post-exercise – cc16/cr baseline × 100/cc16/cr baseline)) and used as a marker of epithelial damage (20). Baseline corresponds to the post-warm-up period, and the cc16/cr post-exercise to the maximum cc16/cr value from blood samples performed immediately, 30 min or 60 min post-exercise. The ventilation ($\dot{V}_E$) and heart rate (HR) during the 30-min exercise were continuously measured and averaged over the entire duration of the exercise ($\dot{V}_E$: Medisoft, Germany, and HR: RS800, Polar, Kempele, Finland). Spirometric parameters were obtained through a flow-volume loop at rest, after warm-up and at 5 min, 30 min and 60 min post-exercise to confirm that the subjects had no airway obstruction (Easy One spirometer, Dyn'R, France). If any fall in forced expiratory volume in one second (FEV_1) of 10% of more (i.e. exercise-induced bronchoconstriction) was observed at 5 min, the spirometry was performed every 5 min until recovery. Theoretical maximum heart rate was calculated as 220-age (years). The room temperature (T_{room}) and relative humidity (RH_{room}) were kept constant (19±1°C and 55±3%, respectively).

Blood sample measurements

cc16 were measured by an automated latex immunoassay, which was validated by comparison with a fluorescence enzyme immunoassay using monoclonal antibodies (21). Serum creatinine was quantified by the Beckman Synchron CX5 Delta Clinical System (Beckman Coulter Inc., Fullerton, CA, USA). All the measurements were performed in duplicate by the same person who was blinded to the coded samples.

Computational model

To characterize whether each subject has exceeded the theoretical threshold of dehydration, we used a computational tool based on a mathematical model of heat and water exchanges in the airways (11). This tool uses a set of parameters as input, which are listed in Table 2. These parameters characterize the ventilation dynamics and the inspired air conditions (11) by using experimental data from Haverkamp *et al*. (26). These parameters were integrated in the lung model using a correlation between the room temperature (T_{room}) and the tracheal air temperature (T_T) (27). In parallel, the relative humidity at the entrance of the trachea (RH_T) was derived using an original phenomenological model specifically developed for this study (refer to *Supplemental data* steps 1 and 2 for complete methodology (URL version 3: https://hal.archives-ouvertes.fr/hal-03282571)). The computational tool was run up for various functional residual capacities (FRC), as described below. The computational model also integrated experimental data such as the controlled temperature and humidity of the room (19 °C and 55% relative humidity, respectively), as well as the calculated FRC of each individual (27). The individual functional residual capacity (FRC) has been used to determine the average diameters and lengths of the airways of our morphometric model (11,29) (*Supplemental data* step 4 (URL version 3: https://hal.archives-ouvertes.fr/hal-03282571)). Hence, this procedure leads to a bronchial geometry adapted to each individual. Then, the temperature and water content are computed in theses geometries using our computational tool. The computational

procedure is described in the *Supplemental data* steps 3 and 4 (URL version 3: https://hal.archives-ouvertes.fr/hal-03282571).

Determination of groups A and B

We used the mean $\dot{V}_E$ during the 30-min steady-state exercise to calculate the corresponding evaporation flux in the bronchial tree for each subject, in the temperature and humidity conditions in which the participants performed the exercise. We compared this evaporation flux to the threshold value at which water replenishment of the airway surface fluid is insufficient (dehydration threshold), which is estimated to be around $0.32 – 0.35 \times 10^{-3}$ $\mu l.mm^{-2}.s^{-1}$, based on the value of 120 $\mu l.cm^{-2}.h^{-1}$ derived by Widdicombe (10), and taking into account the uncertainty for the last significant digit of this value. According to the individual results of the model, two groups were constituted: participants exceeding the upper value of the threshold of 0.35×10^{-3} $\mu l.mm^{-2}.s^{-1}$ for bronchial dehydration (group A "above") and those who did not (group B "below").

Statistical analysis

The sample size (n=16) was calculated according to the change in cc16 in the first 8 subjects. Data are expressed as mean ± SEM. When the conditions of normality and homogeneity of variances were met, a parametric test was used, otherwise the equivalent non-parametric test was chosen. Two ways ANOVAs with repeated measures were carried out to identify significant differences in cc16/cr over time (baseline *vs* post-exercise) and between groups (group A *vs* group B). A Holm-Sidak post-hoc test for multiple comparisons was applied to detect any difference. In two participants (both in group B), the Δcc16/cr ratio was negative and both values were replaced by 0 (but the significance was not different with negative value). The effect size (Cohen's d) was calculated as [(mean group A-mean group B)/SD], SD being the standard deviation of the whole group of 16 participants (30). The effect is considered weak if

the d-value is below 0.2, moderate if between 0.2 and 0.8 and strong if greater than 0.8. The comparison between the Δcc16/cr ratio (%) and $\dot{V}_E$ data was analyzed using an unpaired sample t-test or was performed using a Mann-Whitney test. A p-value < 0.05 was considered as significant. Data were analyzed using Sigmastat (Software version 3.5)

Results

The individual evaporation flux in the bronchial tree relatively to the generation index and $\dot{V}_E$ values are presented in Figure 1. Accordingly, eleven participants constituted the group A and 5 the group B. The characteristics of both groups are presented in Table 1. The mean heart rate (HR) during 30-min exercise was significantly higher in group A (172±3 bpm and 88±1% of maximum theoretical HR) compared with group B (156±16 bpm and 80±3% of maximum theoretical HR, p=0.01, d=1.28).

The mean $\dot{V}_E$ during exercise was significantly higher in group A compared to group B ($p < 0.001$, d=1.62 in $l.min^{-1}$) and averaged 96.9±4.2 $l.min^{-1}$ in group A, ranging from 78.5 to 121.8 $l.min^{-1}$, and 68.3±3.1 $l.min^{-1}$ in group B, ranging from 60.1 to 74.8 $l.min^{-1}$. Thus, it was of 147±5% and 123±15% of $\dot{V}_E$ at VT_1 (p=0.005, d=1.34), of 93±15% and 89±6% of VT_2 (p=0.60, d=0.30), and of 61±2% and 55±4% of maximal $\dot{V}_E$ measured during maximal exercise test (p=0.14, d=0.80), respectively in group A and B.

Individual FRC varied from 3.09 l to 3.54 l in the 16 participants. Hence, we used the computational model to estimate the theoretical ventilation corresponding to the dehydration threshold for every subject, but also for two FRCs of 3 l and 3.5 l (Figure 2a and b), to evaluate the importance of FRC according to various $\dot{V}_E$ on dehydration. For example, the dehydration of the 4^{th} bronchial generation occurs at $\dot{V}_E$ between 70 and 75 $l.min^{-1}$ and between 75 and 80 $l.min^{-.1}$ for a FRC of 3 l and 3.5 l, respectively.

The Δcc16/cr ratio correlated with $\dot{V}_E$ during effort in the whole group (r=0.69, p=0.003) (Figure 3). The comparison of Δcc16/cr ratio of both groups of participants is represented in Figure 4. The change in cc16/cr ratio from baseline to post-exercise period is presented in Figure 5. There was a significant interaction time × group for cc16/cr (p=0.003). Compared to baseline, the increase in cc16/cr was significant (mean increase: 0.48±0.08 $ng.l^{-1}$, i.e. 101±32%, $p < 0.001$) only in group A but not in group B (mean difference: 0.10±0.04 $ng.l^{-1}$, i.e. 13±7 %, p = 0.79) (d=0.95 for absolute changes and d=1.30 for relative values). Individual values for Δcc16/cr ratio are presented in Figure 1. No difference in theoretical FRC (p=0.65, d=0.47) was observed between the groups (data not shown).

In group B, the maximum cc16/cr change was observed immediately post-exercise for 3 participants (60%) and 30 min post-exercise in two (40%). In group A, the maximum values were obtained immediately after exercise in 3 participants (27%), 30-min post-exercise in 4 participants (36%) and 60-min post-exercise in 4 participants (36%).

Discussion

Using our computational model, we were able to link the degree of bronchial dehydration to the FRC and $\dot{V}_E$ level during endurance exercise . Our results showed that the group with the lowest $\dot{V}_E$ did not reach the dehydration threshold during exercise and did not present significant epithelial damage following exercise. The level of ventilation maintained during exercise in this group was at maximum 74.8 $l.min^{-1}$. The computational simulation of dehydration of the bronchial generations shows that a ventilation rate above 80 $l.min^{-1}$ would have been necessary to cause dehydration. The participants with a ventilation above this level actually showed epithelial damage. The greater the ventilation, the greater the epithelial damage. This suggests

256 that the analysis of exercise ventilation with a computational model could help to identify a
257 threshold for airway dehydration and to prevent potential airway damage.

258 Increase in cc16 at exercise has been found in the literature to occur above 75% of maximum
259 heart rate during 6 min to 90 min of exercise (16,17,20). To the contrary, no change in cc16 has
260 been observed at lower intensities during 90 to 120 min of exercise (23,32), suggesting a link
261 between exercise intensity and the occurrence of epithelial damage (25). Few studies have
262 linked ventilation to bronchial damage. We observed a significant correlation between mean $\dot{V}_E$
263 during a 30-min exercise and Δcc16/cr ratio. Bolger et al. (19) also showed that a mean $\dot{V}_E$ of
264 87±2 $l.min^{-1}$ during a 8-min isocapnic hyperventilation challenge at a of dry air induced an
265 increase in cc16/cr in women. In parallel, the mathematical model of heat and water loss in the
266 human respiratory tract of Daviskas *et al.* (32) showed a link between the level of $\dot{V}_E$ and airway
267 dehydration in the bronchial tree, independently of asthma or exercise-induced
268 bronchoconstriction. Therefore, dehydration of the bronchial tree is likely to be concomitant
269 with bronchial epithelial damage during exercise (22).

270 In our study, the workload was set fixed percentage of each participant's maximal capacity, so
271 that exercise was either intense or moderate, depending on the participant's fitness level. In that
272 sense, $\dot{V}_E$ was higher in the group of participants that exceed the theoretical dehydration
273 threshold, compared with the group for which it was not exceeded. Despite good correlation
274 between $\dot{V}_E$ and heart rate during exercise (33), the use of heart rate is not ideal to assess the
275 dehydration and epithelial damage threshold. While the measurement of $\dot{V}_E$ was mainly limited
276 to laboratory setup, the development of new wearable devices capable of estimating ventilation
277 on the field (34) opens new perspectives in using ventilation to prescribe physical exercise in
278 health and disease. Indeed, the determination of an individual ventilation threshold for airway
279 dehydration could help to quantify and determine the pathophysiological consequences of
280 chronic exercise training above the threshold. Our analyses are thus the first step towards the

development of innovative training programs. Such programs should be based on the level of ventilation, and of epithelial damage for both athletes and patients with respiratory diseases. In particular, avoiding airway dehydration during exercise in patients with asthma is crucial to prevent exercise-induced asthma (EIA). Indeed, in asthmatic subjects with EIA and athletes with EIB, evaporative water losses from the airway surface is the key factor driving the release of local mediators and the subsequent development of bronchoconstriction (12). For the same level of $\dot{V}_E$, airway inflammation is greater in those with EIA and EIB, compared with those without (14). It has been also suggested that dehydration-induced epithelium injuries are responsible for a part of the inflammatory mediators process, even though shear stress plays probably also a role (22). Indeed, the transient reduction in volume/depth of airway surface lining fluid during high-level of $\dot{V}_E$ may be responsible for the detachment of the epithelial cells (22). As airway dehydration is difficult to assess through physiological measurements, its evaluation by numerical modelling appears promising. In our study, the estimated dehydration threshold corresponds well to the ventilatory threshold for the appearance of the epithelial damage indicator in the blood. This reinforces the validity of our model to assess the dehydration threshold $\dot{V}_E$.

Epithelial damage can be assessed using different biomarkers and sampling techniques. We chose a serum marker (i.e. cc16) rather than more invasive techniques, and we sampled blood before and at three different time-points following exercise (i.e., immediately at the end, 30- and 60-min post-exercise). Blood cc16 is thought to be the most valid and sensitive marker to detect lung epithelial damage in response to exercise (23). It is significantly related to alveolo-capillary membrane permeability measured by ^{99m}TC-labeled diethylenetriamine pentaacetic acid (DTPA) clearance rate (model estimates [95% confidence interval]: 0.24 [0.12-0.36]) after cycling exercise (90 min cycling at 60-75% VO_2max) in healthy subjects (31). It is also a quite an accessible marker, with validated methods of analysis. The time-points were selected

306 according to the study from Tufvesson *et al*. (20), who showed an increase of cc16/cr in plasma
307 after exercise until 60 min post-exercise. Using ^{99m}TC-DTPA, some studies also showed a peak
308 of epithelial permeability around 25-30 min post-exercise and a normalization of epithelium
309 permeability within 120 min (35). Therefore, we chose to collect blood immediately, 30- and
310 60-min post-exercise. In 4 participants in group A, the maximum cc16/cr value was obtained at
311 the last measured time point (i.e. 60-min post-exercise), and we cannot exclude that this value
312 could have increased after one-hour post-exercise among them. However, the 30-min and 60-
313 min values were within a close range in 3 of the 4 participants, suggesting that cc16 tended to
314 plateau 30-min after the end of exercise.

315

316 Altogether, our results may help to understand and control the epithelial damage, and to avoid
317 the development of respiratory disorders in athletes. In healthy endurance athletes, allowing
318 time for the epithelium to regularly repair might prevent the further development of EIB (22).
319 Air conditioning capacity during hyperpnea did not appear to differ between athletes and non-
320 athletes, with and without EIB either (36), suggesting the absence of coping mechanism. Thus,
321 young healthy subjects performing exercise present airway epithelial damage and dehydration,
322 both depending mainly on the level of ventilation. To avoid repeated airway injuries during
323 training, our study suggests controlling the parameters of exercise ventilation in relation to the
324 epithelial damage and dehydration threshold, calculated beforehand by the proposed model.
325 Moreover, the monitoring of the temperature and humidity content of the inspired air could
326 constitute a promising tool for the development of specific training methods, especially for
327 highly sensitive athletes such as long-distance runners, cyclists, triathletes or winter sports
328 athletes. Indeed, according to the change in atmospheric conditions, the computational model
329 allows to recalculate the maximal possible $\dot{V}_E$ to avoid airway dehydration and damage.
330 Therefore, atmospheric conditions would have a minor influence on the hydration status of the

airways, and on the magnitude of the evaporation compared to the replenishment threshold. A previous approach to protect the airways of people sensitive to cold dry air or with exercise-induced asthma consisted in wearing a face mask to humidify and warm the inspired air. This approach has focused on the control of inspired air conditions (37-40) to protect the airways. However, the exercise in these studies did not exceed 13 min 15 sec, $\dot{V}_E$ were not reported and to wear a mask during longer periods of time may not be tolerated by every athlete. It would be probably more convenient to monitor the intensity of the effort by measuring $\dot{V}_E$, being careful not to exceed the dehydration intensity for too long. More generally, our results provide new perspectives on the understanding and control of exercise-induced asthma and on the development of future exercise recommendations to prevent exercise-induced pathologies.

Regarding modelling, we selected the upper limit of the epithelium replenishment threshold at 0.35 10^{-3} $\mu l.mm^{-2}.s^{-1}$ based on the reference evaluation presented by Widdicombe (10). More precisely, this value is obtained based on several experimental results presented in the literature. First, Iovannitti *et al*. (41) evaluated the density of tracheal submucosal glands to be around 1 gland per square millimeter. From the work of Quinton (9), it appears that the maximal secretion rate is about 20 $nl.min^{-1}$ per gland. Combining these two pieces of information, the maximal flux of replenishment in the trachea can be calculated as high as 120 $\mu l.cm^{-2}.h^{-1}$ or 0.32-0.35 10^{-3} $\mu l.mm^{-2}.s^{-1}$. It is to be noted that the density of submucosal glands observed by Iovannitti *et al*. (41) presents a strong level of uncertainty. Furthermore, Quinton (9) suggests a replenishment rate that is close to a maximal value rather than an average value of secretion (see Fig 1 in (9)). These considerations imply that the value of the replenishment flux used in our work must be considered as a relatively high value rather than an average one, although the exact nature of the mechanisms underlying the replenishment/absorption remains to be elucidated. Furthermore, this replenishment threshold might be dependent on the bronchial air conditions. It is not certain that this value is always sustained, for example in cold and dry air

conditions in comparison with warm and humid air. Thus, the net evaporation flux, which is the actual evaporation rate minus the replenishment rate, might be underestimated in our results in the case of a lower replenishment. However, our results do not overestimate it. This approach ensures that we can detect and calculate the evaporation flux, at least in its lower limit.

The question of the value of the replenishment rate is complex, as air conditions seem to influence the osmolality of the airway surface lining (ASL), and thus the water fluxes (see for example the work of Freed and Davis (42)). They observed that the variations of the ASL osmolality during hyperventilation in cold dry air – probably due to strong evaporation – are related with the development of symptoms of bronchoconstriction induced by hyperventilation. In the framework of our study, this confirms that the modifications of the ASL properties occur depending on the ventilation and the air temperature and humidity conditions. It can be hypothesized that these modifications trigger the mechanism of ASL replenishment, in order to maintain the bronchial mucus balance (11,42).

The use of an upper value for the replenishment threshold is also consistent with the observation that small airways contain less, if any, secretory glands compared to larger airways (41,43,44). This suggests that the density of secretory glands decreases along the bronchial tree. In our work, we assume that the replenishment flux per square millimeter is constant, no matter the generation. However, it is rather expected that it should be lower in small airways than in large ones. This leads to another potential underestimation of the net evaporation flux in our analysis, especially in the intermediate bronchi. In this part of the lungs, the evaporation flux is still important (see Figure 2) as the air is not totally conditioned. However, this does not impact our conclusions; a more refined threshold evaluation could actually lead to a greater number of generations in which the evaporation is not compensated by the replenishment. Moreover, the high-replenishment approach leads to predictions in accordance with the observed behaviors.

The occurrence of EIB triggered by water losses implies to evaluate the magnitude and distribution of evaporation in the bronchial tree. In Figure 2, our computational results indicate that the evaporation is still non-negligible in the central lung i.e., as far as the 10^{th} to 12^{th} generations. This observation raises the question of whether the potential evaporation in these generations can be a trigger of EIB, especially if the replenishment threshold is lower than the one selected here. Indeed, Anderson *et al.* (12) suggested that high evaporation rates in these generations can be critical for the onset of EIB symptoms. This problematic is complex because several phenomena need to be taken into account.

In particular, the magnitude of the evaporation in this part of the lungs is *a priori* sensitive to the room air conditions and ventilation rates. To verify this hypothesis, we performed additional computations for two subjects from our pool of data: one with a value of $\dot{V}_E$ leading to a dehydration threshold below the fixed upper value of 0.35 10^{-3} µl.mm^{-2}.s^{-1} and one with a value of $\dot{V}_E$ that leads to a dehydration threshold reaching this upper value. We calculated their profile of evaporation in cold air conditions (T_{room} = 8 °C and RH_{room} = 24% (Figure ESM4, URL version 3: https://hal.archives-ouvertes.fr/hal-03282571). We observed that the evaporation rates in generations 10 to 12 are close to the rates in experimental conditions (T_{room} = 19 °C and RH_{room} = 55%) (Figure 1) for the corresponding minute ventilations, although slightly higher. Indeed, the conditioning of air occurs mainly in the first generations, after which the evaporation still occurs, but to a smaller extent.

Karamaoun *et al*. evaluated the replenishment rates needed for maintaining the mucus balance in the bronchi in case of evaporation (11). Their results include an important aspect of the mucus balance mechanism: by mass conservation, the volume of displaced mucus should increase when progressing up the bronchial tree, as the bronchial lateral surfaces decrease exponentially from the distal to the proximal generations (11). However, this does not seem to occur in healthy conditions. Thus, Karamaoun *et al*. (11) suggested that some mechanisms of control should

take place i.e., water fraction reabsorption and replenishment. In particular, in the central generations, in which the magnitude of evaporation is lower than in the proximal ones, the reabsorption mechanism should have a greater magnitude than in the upper bronchi (11). This implies that the central generations could compensate for larger evaporation rates by triggering a decrease in the rate of replenishment, which modulates the reabsorption consequently. Of course, this putative mechanism should be verified experimentally, especially at the beginning of exercise in cold conditions, which could amplify the onset of EIB.

Our computational results are based on several simplification hypotheses on the physical processes involved in the heat and water transfers in the airways. Among those, the physics of the transfers in the upper respiratory part have been simplified to produce a compact version of the phenomenological model. In addition, the idealized geometry of the morphometric model used does not represent accurately some specific features of the bronchi, namely the branching and size asymmetry of the physiological bronchial tree (45). Nevertheless, our computational approach can reproduce the dynamics and orders of magnitude of heat and water exchanges in the bronchi (11). As the present study constitutes an analysis based on orders of magnitude, our results can be interpreted with a strong level of confidence, in terms of both the dynamics of heat and water exchanges in the airways and of the magnitude of the calculated outputs.

Conclusion

Using a computational model for heat and water transfers in the bronchi, we assessed the level of dehydration in the bronchial tree in 16 healthy subjects compared to their pulmonary capacity (FRC) and ventilation level during exercise. We identified a threshold in ventilation during exercise above which airway dehydration is thought to occur. When this threshold of ventilation was exceeded, epithelial damage was found. Our study also highlights the complexity of the

heat and water transfers in the upper and lower airways. To improve our estimate of these transfers, a more refined geometric description of the airways is now necessary.

Finally, this study is the first step towards the development of innovative training programs, based on the level of ventilation, for both athletes and people with respiratory pathologies. The objective will be to determine the consequences on the epithelium of chronically exceeding the threshold of bronchial dehydration. This could help to prevent maladaptation to exercise training or rehabilitation, such as exercise-induced bronchoconstriction.

Disclosure of interest

No conflicts of interest, financial or otherwise, are declared by the authors.

Ethics approval

For data on healthy subjects, the exercise protocol had been previously obtained by The University of Brighton Ethics Committee (25).

Code availability

Custom code written in Mathematica® 12. Algorithms and equations are described in text or have been previously published.

Author contributions

CK, VB and BM designed the study; CK, BH and BM designed the computation algorithms and models; CK performed the computations; VB and GB interpreted and processed the experimental data; AB, FD, JD and VB collected and analyzed the experimental data; CK and VB wrote the initial manuscript. All the authors participated in proofreading and correcting the manuscript.

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570 tracheobronchial tree. *Appl Pathol*. 4(1-2): 24-32, 1986.
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572

573 **Table 1 Characteristics of the participants.**

574 Data are expressed as mean±SEM. VE during continuous exercise was calculated during the
575 last 27^{th} minutes. *p<0.05 and **p < 0.001 between groups A and B. The effect is considered
576 weak if d-value is below 0.2, moderate if between 0.2 and 0.8 and strong if greater than 0.8.

577

578 **Table 2** Inputs and outputs of the computational model, depending on the minute ventilation
579 $\dot{V}_E$, for a room temperature T_{room} = 19°C and a room relative humidity RH_{room} = 55%. **I**: input
580 data from Haverkamp et al. (23) are compiled as described in Annex I. **II**: from the input data,
581 the values of the tracheal air temperature T_T depending on $\dot{V}_E$ are derived, based on a correlation
582 from McFadden et al. data (24) (see Annex I). **III**: All the input parameters allow for deriving
583 the tracheal relative humidity RH_T, based on our phenomenological model of the upper airways,
584 as described in Annex II. **IV**: Finally, the values of the evaporation in the conducting airways
585 are numerically computed (see Annex IV). Here are only presented the values of the
586 evaporation flux J_{evap} in the trachea, as compared with Fig. 1 where J_{evap} is plotted for all
587 conducting airways. The table is established for FRC =3 l, with f_b = breathing frequency, V_T =
588 tidal volume, t_i = inspiration time, t_e = expiration time. $t_{tot} = t_i + t_e$ is the respiratory cycle time.

589

590

Figures

Fig. 1 Modelling of the evaporation flux in the bronchial tree of each of the 16 male participants**.**

Each graph represents a participant (n=16 participants, n=16 pictures). Participants' graph are ranked according to their $\dot{V}_E$, from the smallest at the top left to the largest at the bottom right. Data from the participants in group B are shown in the first 5 graphs on the top left. FRC: functional residual capacity; $\dot{V}_E$: minute ventilation during 30-min exercise.

The dehydration threshold of 0.32 to 0.35 x 10^{-3} $\mu l.mm^{-2}.s^{-1}$ (10) is represented by a horizontal grey zone. The first generation represents the trachea.

Fig. 2 Mean evaporation flux per surface unit in the bronchial tree ($J_{evap,i}$) for functional residual capacity of a) 3 l and b) 3.5 l. $J_{evap,i}$ was determined at the level of the trachea (1^{st} generation) up to the last generation of the conducting airways (17^{th} generation). The horizontal shaded area delimits the limit at which the water supply is insufficient to cover losses (between 0.32 and 0.35 x 10^{-3} $\mu l.mm^{-2}.s^{-1}$) (10). The first generation is the trachea. The values correspond to an ambient temperature of 19 °C and a relative humidity of 55%.

Fig 3. Correlation between the change in cc16/cr (Δcc16/cr) and mean ventilation ($\dot{V}_E$) during exercise in the 16 male participants.

cc16: club cell protein; cr: creatinine. The correlation coefficient is a Pearson coefficient (r=0.69, p=0.003, n=16). The linear regression equation is y=0.0112x-0.6103.

Fig. 4 Maximum change in cc16/cr ratio according to the ventilation status in 16 healthy male participants, after a 30-min continuous exercise at 70% of their maximal work rate.

613 cc16: Club cell protein; cr: creatinine. The median [Q1-Q3] was of 106.9% [42.7-136.6%] in
614 group A and of 15.8% [0-25.7%] in group B. The middle line in the box is the median and
615 upper and lower line of the boxes are respectively Q3 and Q1. The mean is represented by a
616 cross inside the box. The top and bottom bars represent maximum and minimum values,
617 excluding extreme points. The single point is an extreme value.

618 Groups: Participants exceeding the threshold of 0.35 x 10^{-3} $\mu l.mm^{-2}.s^{-1}$ for bronchial
619 dehydration (group A "above", n=11) and those who did not (group B "below", n=5).

620 A Mann-Whitney rank sum test was used to compare delta cc16/cr ratio between groups.

621

622 **Fig. 5** Individual values of cc16/cr from baseline to post-exercise in both groups.

623 Baseline values are post-warm-up values. Groups: Participants exceeding the value of 0.35 x
624 10^{-3} $\mu l.mm^{-2}.s^{-1}$ for bronchial dehydration (group A "above", n=11) and those who did not
625 (group B "below", n=5).

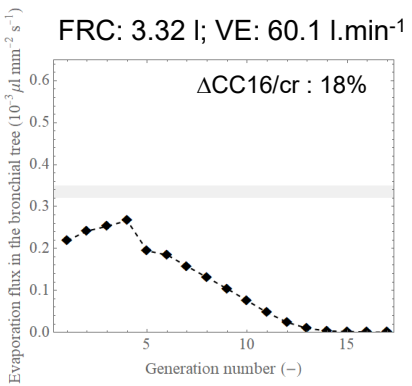
626 A two-way ANOVA for repeated measures was done with factors being time (pre- and post-
627 exercise) and group (A *vs* B) (Interaction Time × Group : F=12.74, p=0.003). The Holm-
628 Sidak post-hoc test for multiple comparisons was applied to localize any difference (see p-
629 values on the graph).

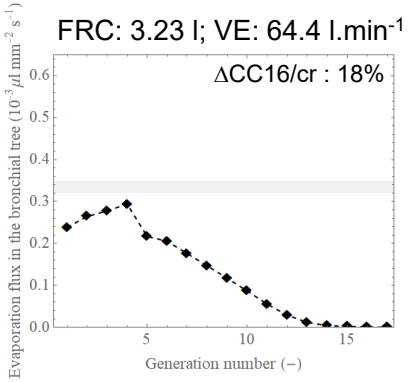
630

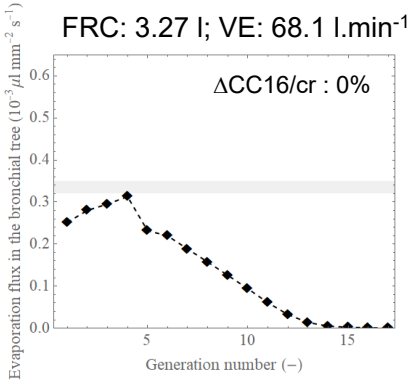
631

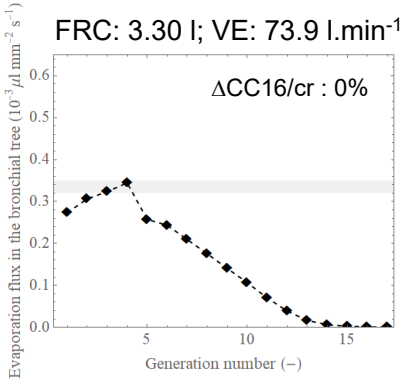
632

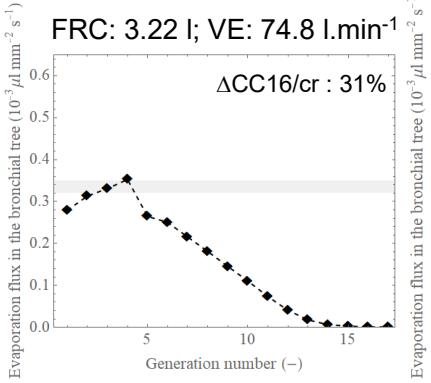
Figure 1

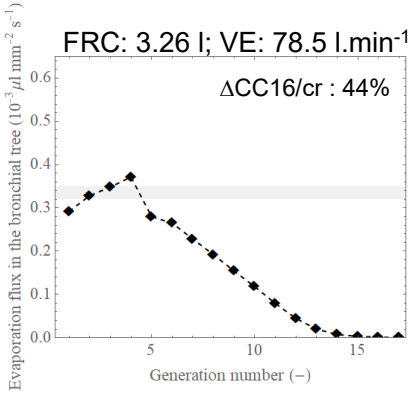

FRC: 3.32 l; VE: 60.1 l.min-1
ΔCC16/cr : 18%
Evaporation flux in the bronchial tree (10-3 µl mm-2 s-1)
Generation number (–)

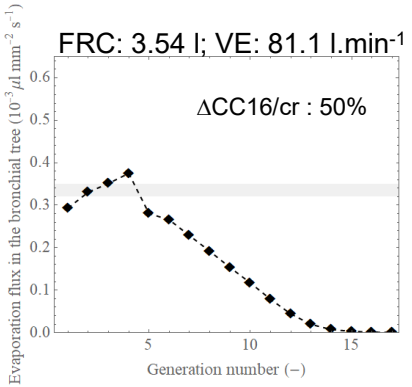

FRC: 3.23 l; VE: 64.4 l.min-1
ΔCC16/cr : 18%
Evaporation flux in the bronchial tree (10-3 µl mm-2 s-1)
Generation number (–)

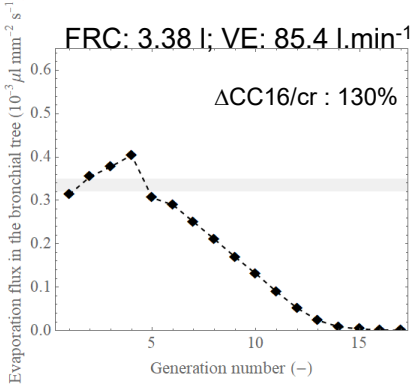

FRC: 3.27 l; VE: 68.1 l.min-1
ΔCC16/cr : 0%
Evaporation flux in the bronchial tree (10-3 µl mm-2 s-1)
Generation number (–)

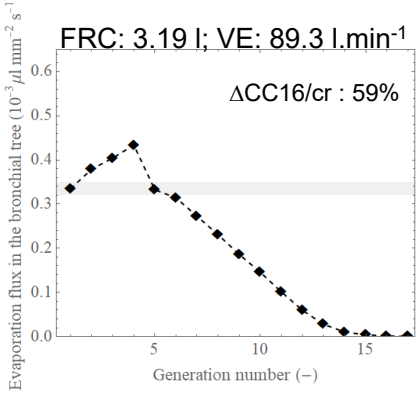

FRC: 3.30 l; VE: 73.9 l.min-1
ΔCC16/cr : 0%
Evaporation flux in the bronchial tree (10-3 µl mm-2 s-1)
Generation number (–)

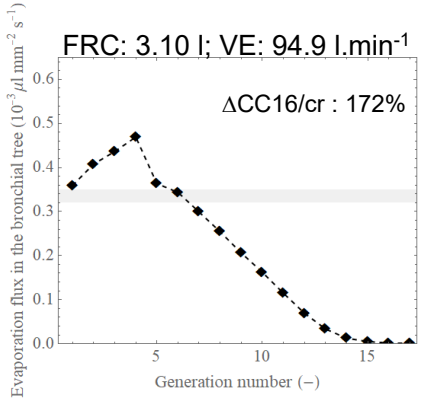

FRC: 3.22 l; VE: 74.8 l.min-1
ΔCC16/cr : 31%
Evaporation flux in the bronchial tree (10-3 µl mm-2 s-1)
Generation number (–)

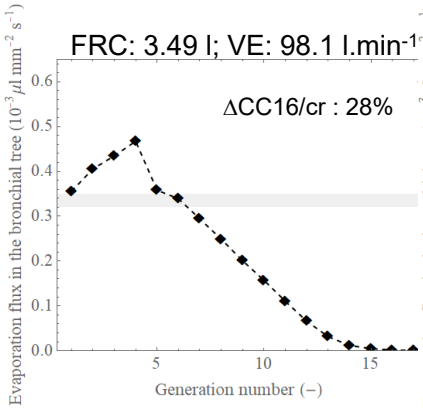

FRC: 3.26 l; VE: 78.5 l.min-1
ΔCC16/cr : 44%
Evaporation flux in the bronchial tree (10-3 µl mm-2 s-1)
Generation number (–)

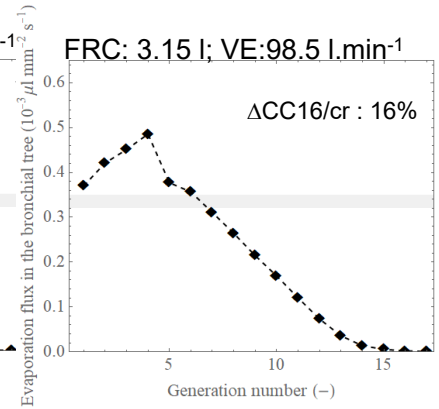

FRC: 3.54 l; VE: 81.1 l.min-1
ΔCC16/cr : 50%
Evaporation flux in the bronchial tree (10-3 µl mm-2 s-1)
Generation number (–)

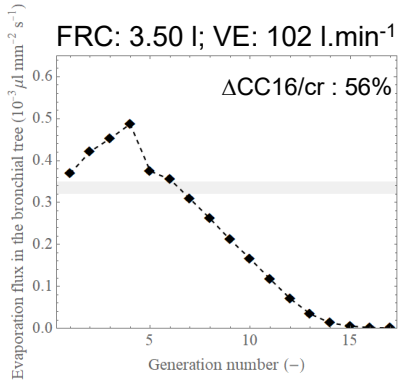

FRC: 3.38 l; VE: 85.4 l.min-1
ΔCC16/cr : 130%
Evaporation flux in the bronchial tree (10-3 µl mm-2 s-1)
Generation number (–)

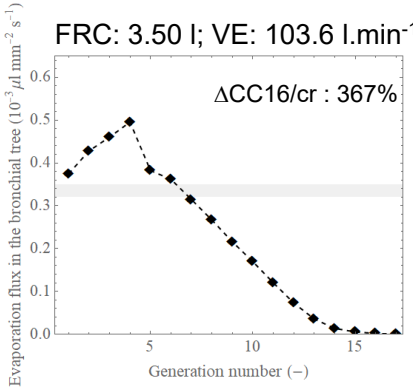

FRC: 3.19 l; VE: 89.3 l.min-1
ΔCC16/cr : 59%
Evaporation flux in the bronchial tree (10-3 µl mm-2 s-1)
Generation number (–)

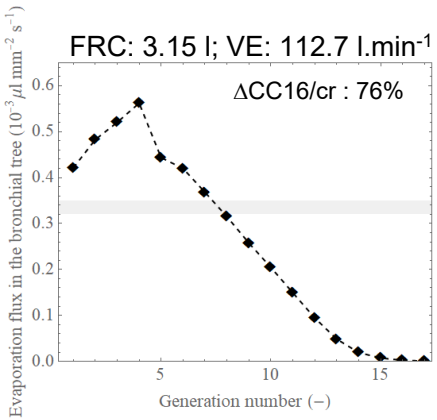

FRC: 3.10 l; VE: 94.9 l.min-1
ΔCC16/cr : 172%
Evaporation flux in the bronchial tree (10-3 µl mm-2 s-1)
Generation number (–)


FRC: 3.49 l; VE: 98.1 l.min-1
ΔCC16/cr : 28%
Evaporation flux in the bronchial tree (10-3 µl mm-2 s-1)
Generation number (–)


FRC: 3.15 l; VE:98.5 l.min-1
ΔCC16/cr : 16%
Evaporation flux in the bronchial tree (10-3 µl mm-2 s-1)
Generation number (–)


FRC: 3.50 l; VE: 102 l.min-1
ΔCC16/cr : 56%
Evaporation flux in the bronchial tree (10-3 µl mm-2 s-1)
Generation number (–)


FRC: 3.50 l; VE: 103.6 l.min-1
ΔCC16/cr : 367%
Evaporation flux in the bronchial tree (10-3 µl mm-2 s-1)
Generation number (–)


FRC: 3.15 l; VE: 112.7 l.min-1
ΔCC16/cr : 76%
Evaporation flux in the bronchial tree (10-3 µl mm-2 s-1)
Generation number (–)

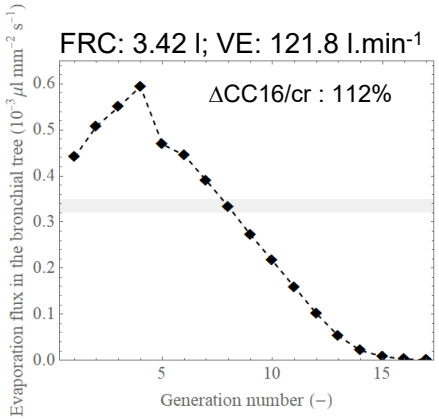
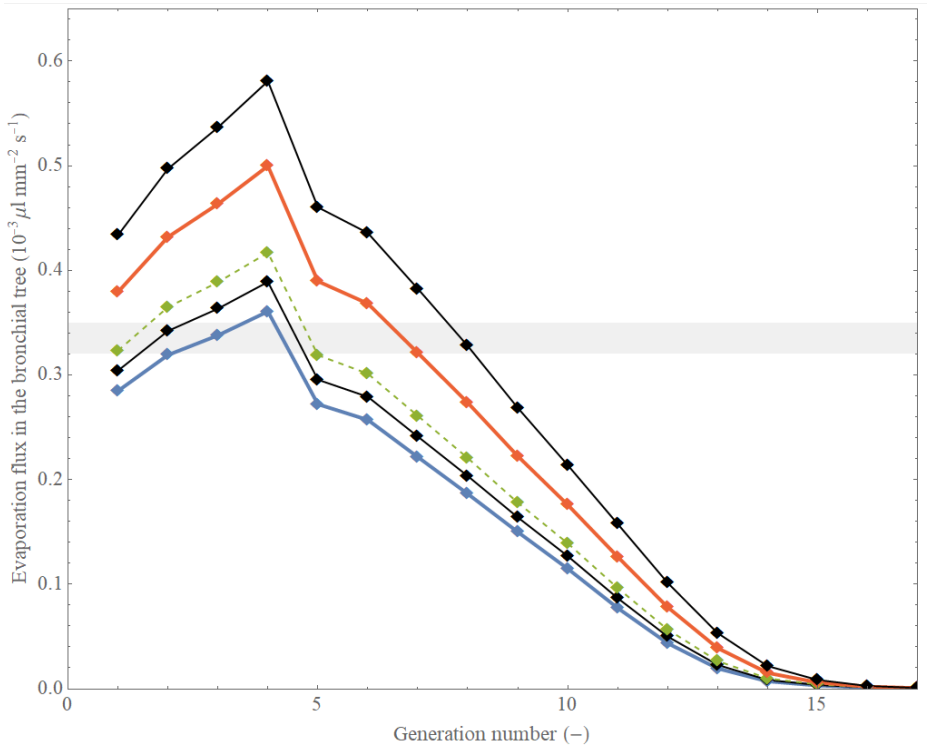
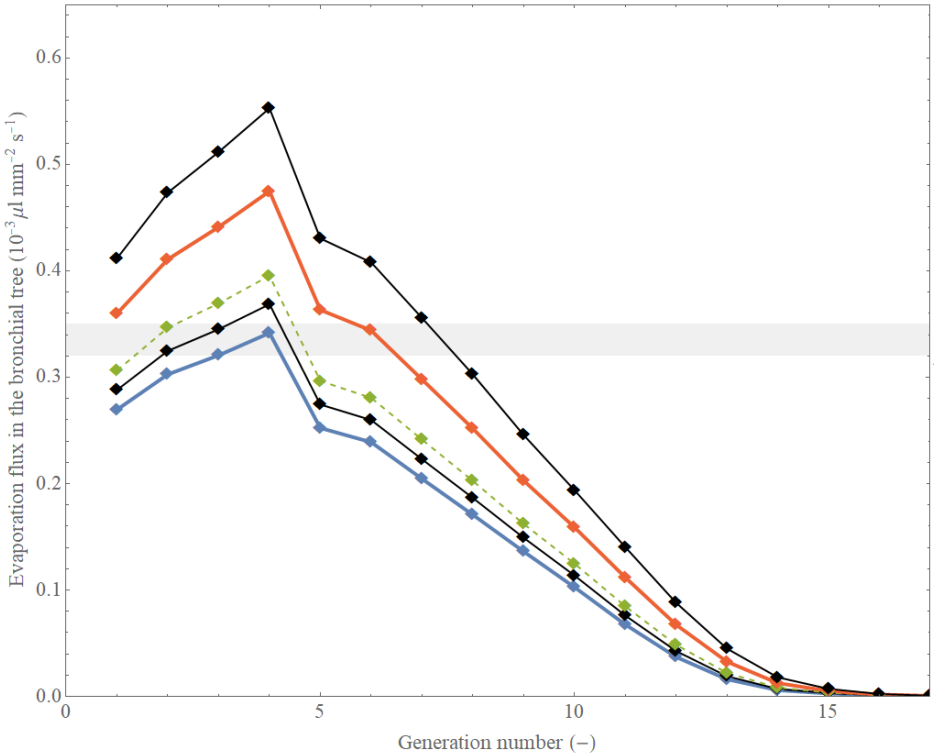

FRC: 3.42 l; VE: 121.8 l.min-1
ΔCC16/cr : 112%
Evaporation flux in the bronchial tree (10-3 µl mm-2 s-1)
Generation number (–)

Figure 2

a) FRC = 3 l

b) FRC = 3.5 l





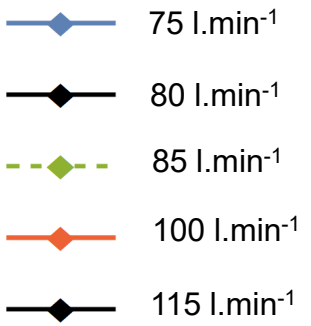


Figure 3

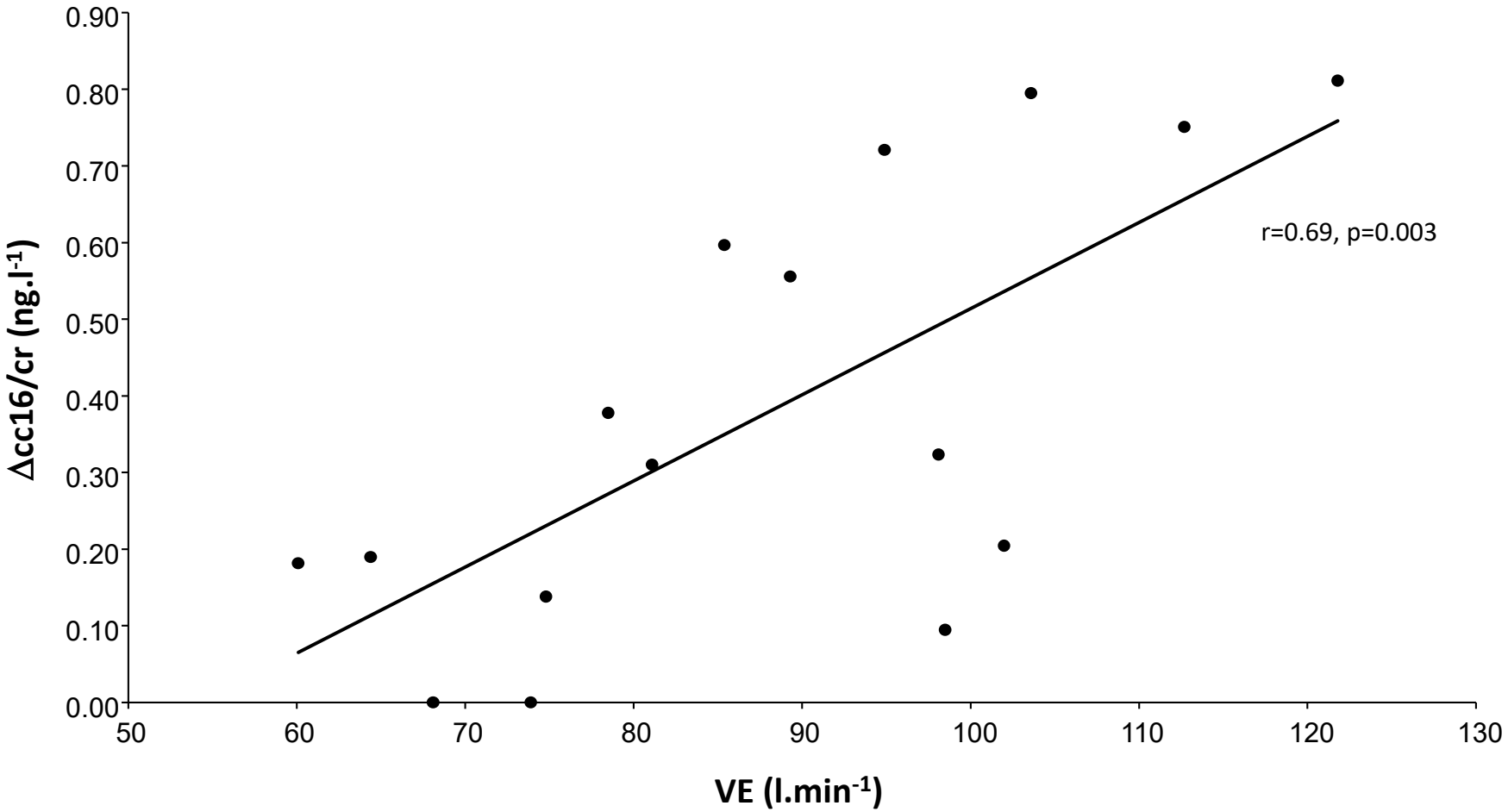

0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
Δcc16/cr (ng.l-1)
r=0.69, p=0.003
50
60
70
80
90
100
110
120
130
VE (l.min-1)

Figure 4

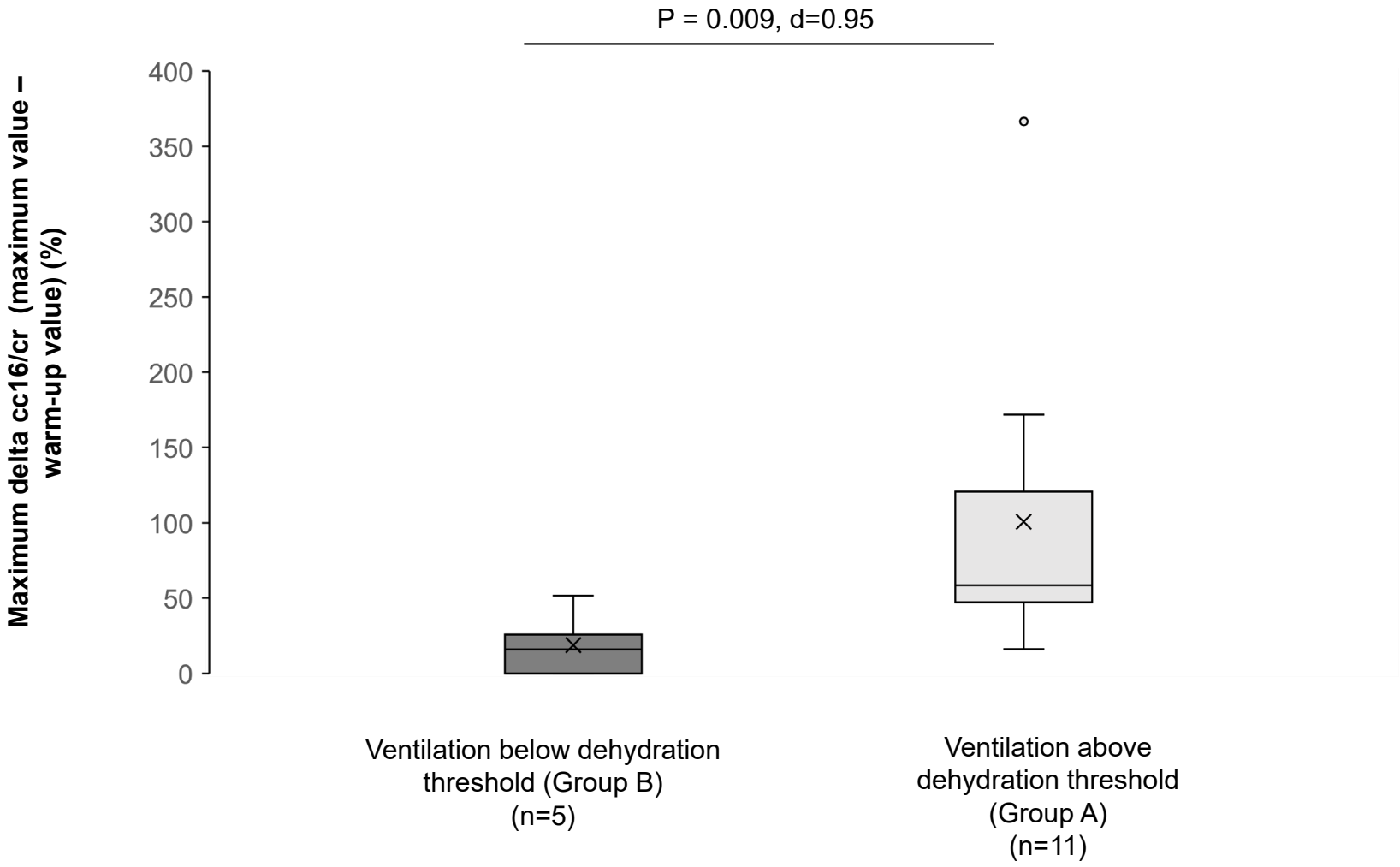

P = 0.009, d=0.95
Maximum delta cc16/cr (maximum value – warm-up value) (%)
400
350
300
250
200
150
100
50
0
Ventilation below dehydration threshold (Group B) (n=5)
Ventilation above dehydration threshold (Group A) (n=11)

Figure 5

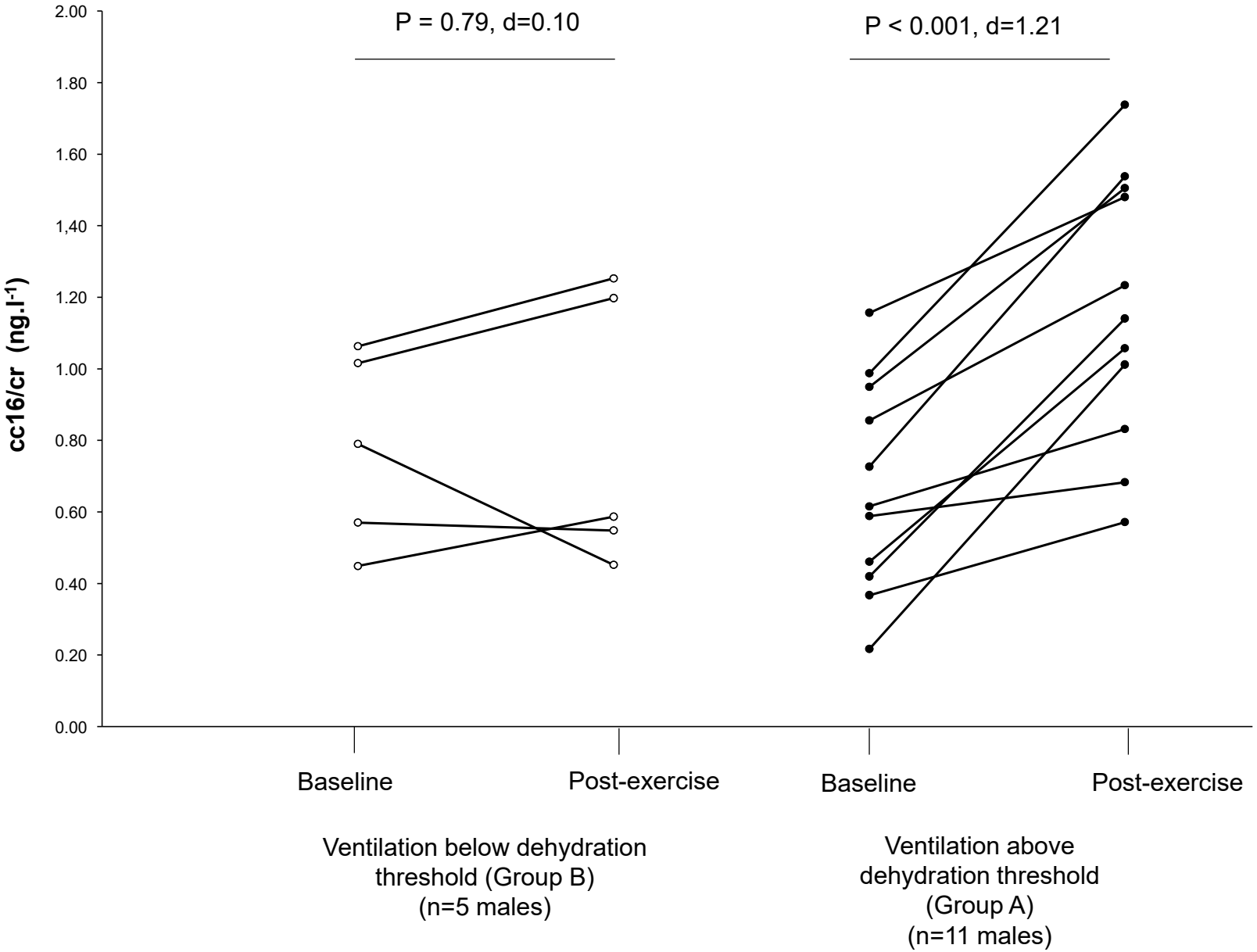

P = 0.79, d=0.10
P < 0.001, d=1.21
cc16/cr (ng.l-1)
2.00
1.80
1.60
1,40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
Baseline
Post-exercise
Baseline
Post-exercise
Ventilation below dehydration threshold (Group B) (n=5 males)
Ventilation above dehydration threshold (Group A) (n=11 males)

Table 1

Participants	All (n=16)	Group A (n=11)	Group B (n=5)	Effet-size
Baseline				
Age (years)	24±1	24±2	23±2	0.09
Mass (kg)	74.2±2.5	74.1±3.4	74.3±4.0	0.03
Height (cm)	180±2	181±3	177±2	0.56
FEV_1 (l)	4.61±0.19	4.72±0.23	4.37±0.39	0.48
FEV_1 (% predicted)	104±3	104±3	104±5	0.02
FVC (l)	5.54±0.119	5.58±0.26	5.45±0.31	0.16
FVC (% predicted)	105±3	105±4	106±6	0.12
Incremental exercise test				
PPO (watts)	280±9	278±12	284±16	0.15
VO_2max ($ml.kg^{-1}.min^{-1}$)	45.4±1.9	46.6±2.4	42.8±3.4	0.51
VE max ($l.min^{-1}$)	145.1±5.6	150.6±6.1	133.2±11.6	0.81
VE max (% MVVt)	92±4	92±3	91±14	0.05
30-min continuous exercise				
HR (bpm)	167±3	172±3	156±6*	1.27
HR (% max theoretical HR)	85±2	88±1	80±3*	1.28
VE (l/min)	88.0±4.6	96.9±4.2	68.3±3.1**	1.62
VE (% MVVt)	55±3	60±3	46±6*	1.09

Table 2 :

Inputs							Outputs	
I						II	III	IV
$\dot{V}_E$	f_b	V_T	$t_i + t_e$	t_i/t_{tot}	t_i	T_T	RH_T	J_{evap}
$l.min^{-1}$	min^{-1}	l	s	–	s	°C	%	10^{-3} µl.$mm^{-2}.s^{-1}$
75	32	2.37	1.89	0.46	0.87	29.9	0.46	0.285
80	33.1	2.417	1.81	0.46	0.837	28.9	0.45	0.304
85	34.5	2.46	1.74	0.47	0.81	29.5	0.43	0.323
100	39	2.58	1.55	0.48	0.74	29.1	0.40	0.379
115	43	2.68	1.40	0.49	0.69	28.6	0.38	0.434