Fire simulator exposure alters the innate epithelial response and inflammatory status in the airways of firefighters*

Telma Gomes Cordeiro[1], Jônatas Bussador do Amaral[1], Valdir Pavão[2], Ricardo Galesso Cardoso[3], Richard Louis Voegels[4], Pedro Michaluat Pezato[1], Vitoria Paixão[1], Ewin Barbosa de Almeida[1], André Luis Lacerda Bachi[1,5], Rogério Pezato[1,4]

[1] ENT Research Lab, Department of Otorhinolaryngology and Head and Neck Surgery, Universidade Federal de São Paulo, São Paulo, Brazil

[2] Fire Department, Escola Superior de Bombeiros – Polícia Militar do Estado de São Paulo, Franco da Rocha, Brazil

[3] GRAU - Grupo de Resgate da Secretaria Estadual de Saúde de São Paulo. São Paulo, Brazil

[4] Department of Ophthalmology and Otorhinolaryngology, Universidade de São Paulo, São Paulo, Brazil

[5] Post-graduation Program in Health Science, Universidade de Santo Amaro (UNISA), São Paulo, Brazil

Rhinology 59: 3, 267 - 276, 2021
https://doi.org/10.4193/Rhin21.002

***Received for publication:**
January 2, 2021
Accepted: March 31, 2021

Abstract

Background: Firefighters are often exposed to high temperatures and by-products of combustion, which can compromise their health. We aimed to evaluate the effect of fire exposure in fire simulators on the airways of firefighters at different time-points.

Methodology: Thirty-seven male firefighters exposed to fire simulators were evaluated in three phases: pre-exposure, at the end of the first week, and 4 weeks after. Pulmonary function by spirometry, nasal mucociliary clearance; peripheral oxygen saturation, inflammatory markers in the nasal lavage and CC16 in the sputum, nasal obstruction, and quality of life (using the questionnaires NOSE and SNOT-22) were assessed.

Results: Higher levels of IL-8, IL-10, and exhaled carbon monoxide were found more in phase 2 than in phase 1. Higher CC16 levels and lower peripheral oxygen saturation were observed in phase 3 as compared to phase 1. Lower levels of IL-2 and peripheral oxygen saturation were found in phase 3 than in phase 2. Higher nasal mucociliary clearance, as well as the worst quality of life and nasal obstruction, were observed in phases 2 and 3 as compared to phase 1.

Conclusions: The firefighters' exposures to high temperatures and by-products of combustion in the fire simulators elicit an inflammatory process in the airways with impairment in the innate epithelial response of the upper airway lining. Furthermore, changes in O2 transport affected the professionals' quality of life negatively.

Key words: inflammation, airway resistance, spirometry, simulation exercise, firefighters

Introduction

Fire suppression is not only a very dangerous and exhausting activity but it also exposes the firefighters to high temperatures and by-products of combustion; which can impair their health [1, 2].

Fire produces a complex mixture of fumes with airway irritants that can lead to the development of serious respiratory problems. Among the combustion by-products are carbon monoxide (CO), sulfur dioxide (SO_2), nitrogen dioxide (NO_2), small particulate matter 2.5 (PM2.5), and cyanide. Particularly, these by-products hinder oxygen carriage into the bloodstream. Furthermore, smoke inhalation can affect the respiratory tract in different ways and its effects are diagnosed mainly by symptoms assessment and alteration in lung function tests [3-5].

In this regard, it was reported that firefighters presented with an increase in respiratory complaints. These include airway inflammation, pneumonia, rhinosinusitis, allergic processes, airway obstructions, and bronchial hyperresponsiveness [6].

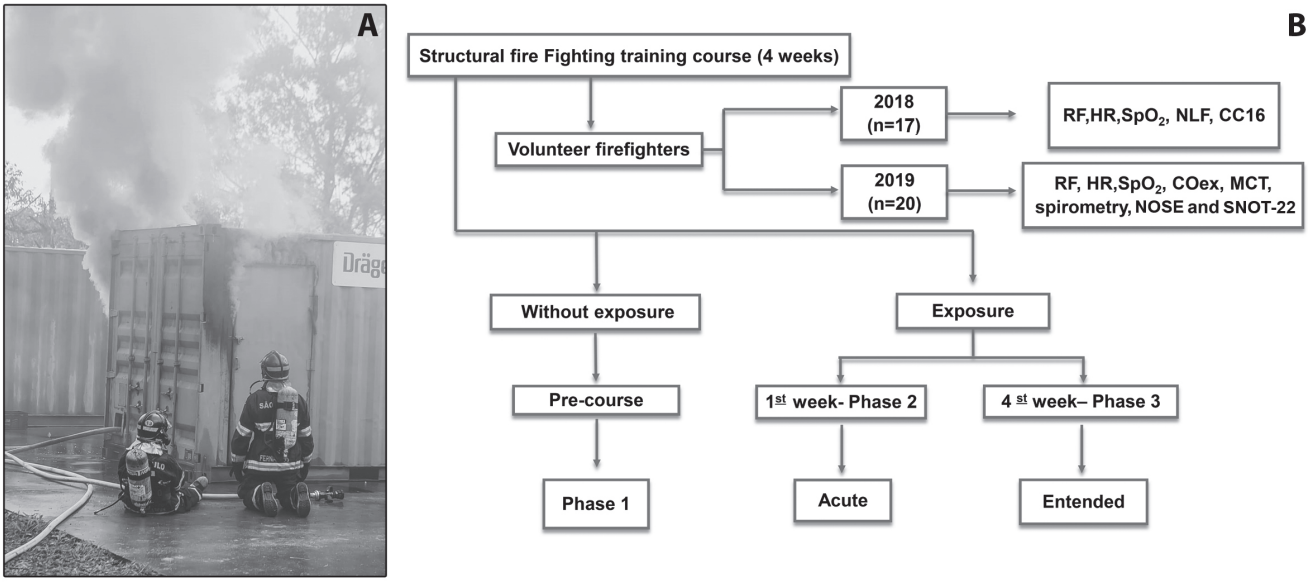

Figure 1. A. Firefighter training using simulators; B. Study flow diagram. RF= Respiratory Frequency, HR= Heart Rate, SpO2= Peripheral Oxygen Saturation, NLF= Nasal Lavage Fluid, CC16= Club Cell Secretory Protein, Coex= Exhaled Carbon Monoxide. MCT= Mucociliary Clearance Time, NOSE= Nasal Obstruction Symptom Evaluation and SNOT-22= SinoNasal Outcome Test 22.

To mitigate the risks imposed by fire on the health of firefighters, the use of self-contained breathing apparatus (SCBA) is mandatory. The use of individual protective equipment (PPE) for thermal insulation is also recommended. However, in situations where the smoke is no longer visible, reduced adherence to the use of these types of equipment is usually observed. Such as during local inspections, or shift changes, and also during rest periods, when high concentrations of combustion by-products are still present at the site. Moreover, there is increased heat that may lead to the continuous generation of some by-products, thereby increasing the susceptibility of their increased cutaneous absorption in firefighters [3].

Beyond the acute risk, the increase in thermal stress can also trigger an increment in the responses of the immune system, which can persist for a long time. It was demonstrated that acute stress originated by repeated work cycles involving intermittent exposure to fires significantly activated the immune system and the maintenance of this activated state can favor the occurrence of adverse effects on the respiratory health of these professionals [7, 8].

Based on this information, training courses are necessary to prepare these professionals for exposure to high temperatures and combustion-derived by-products. Therefore, the purpose of these courses is to offer the firefighters, in a controlled situation, the opportunity to experience the real context of fires. Even in a controlled situation, the firefighters are exposed to high temperatures and, mainly, high concentrations of combustion-derived by-products [9, 10]. There are available data concerning the occurrence of diseases in the airways of firefighters at the end of the course. However, there is a lack of information concerning the effects of repetitive exposure to fire in simulators on the airway mucosa of the firefighters. Therefore, we aimed to evaluate the effect of fire exposure in fire simulators on the airways of firefighters at different time-points.

Materials and methods

This study was carried out by the Department of Otorhinolaryngology and Head and Neck Surgery of the Federal University of São Paulo (UNIFESP). In 2018 (n = 17) and 2019 (n = 20), males volunteer firefighters who were enrolled in the structural firefighting course were invited to participate in the study. Signed informed consent was obtained from all participants. The study was approved by the Local Ethics and Research Committee (number CAAE: 09843919.2.0000.5505). All participants used SCBA and PPE during the practical part of the course. The course was promoted by the Fire Department of São Paulo State of the Superior School of Firefighters in Franco da Rocha. The sample collection took place at the training site immediately after exposure, at an appropriate distance from the simulators, except for the ventilatory tests. The program lasted for four consecutive weeks. Participants were exposed to high temperatures and by-products of combustion in a structural fire simulator that exceeded 600˚C, lasting 20-30 minutes, and repeated 2-3 times a day (Figure 1A). These events took place twice a week in alternating periods. The fuels used for simulation were oriented strand board sheets and pine pallets. The training techniques and fire simulator were the NFPA 1403 standards (National Fire Protection Association) which is the Standard for Live Fire Training

Evolution [11].

The participants were aged between 18-50 years, previously healthy individuals approved by the annual health inspection of the Military Police Corporation of São Paulo State. Exclusion criteria were; 1) smoking, 2) chronic use of corticosteroids, anti-inflammatories, 3) presence of chronic rhinosinusitis using EPOS criteria [12], allergic rhinitis, flu in the last 4 weeks, asthma, chronic bronchitis, 4) women at reproductive age without hormonal contraceptive adherence.

The evaluation of the participants took place in three phases: pre-exposure (phase 1, control), at the end of the first week after exposure (phase 2, acute), 4 weeks after exposure (phase 3, extended) (Figure 1B).

In the first part of the study in 2018 (n=17), anthropometric data, heart rate (HR), respiratory frequency (RF), and SpO_2% (peripheral oxygen saturation) were collected in ambient air, pulmonary sputum, and nasal lavage fluid were assessed. In 2019 (n=20) participants were enrolled in the study. Vital signs were again collected, in addition to exhaled carbon monoxide (COex), mucociliary transport time (MCT), spirometry (FVC, FEV1, FEV1/FVC, FEF 25-75%), and validated nasal obstruction questionnaires, NOSE and SNOT-22 [13-15] (Figure 2).

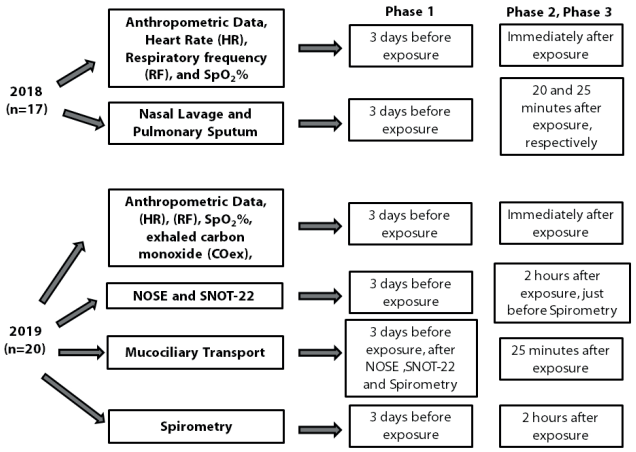

Figure 2: The sequence of tests and timings at the 3 phases.

Collection of estimates of peripheral oxygen saturation, respiratory frequency, and heart rate

Collections of peripheral oxygen saturation (SpO2, in %), respiratory frequency (RF), and heart rate (HR) occurred in three phases during the course. In phases 2 and 3 collections were performed immediately after exposure to the simulator.

In all phases, collections were performed using UT-MD® portable oximeter equipment (Curitiba, PR, Brazil).

Sample collections

Samples of nasal lavage to measure the cytokines and sputum to measure Club cell protein were collected 20 minutes and 25 minutes, respectively, after exposure to the simulator in the morning between 10:00 am and 12:00 pm. Nasal lavage fluid (NLF) samples were collected by introducing 5 ml saline (0.9% NaCl) at room temperature into each nostril with a sterile syringe without a needle. After the introduction, the volunteers were instructed to keep the contents in the nostril, not to perform deep breathing or to swallow for 10 seconds, and then to return the largest amount of the liquid possible into a 50 ml Falcon® tube. The samples were appropriately stored in a thermal box with ice and taken to the laboratory of the Department of Otorhinolaryngology and Head and Neck Surgery at the Federal University of São Paulo (Unifesp). The material was transferred to a graduated polypropylene tube (15 mL Falcon® tube), centrifuged at 3,000 rpm at 4°C for 10 min. No buffer or preservative was added [16]. Subsequently, the supernatant volume was transferred to polypropylene tubes (2 mL Eppendorf® tubes) and stored at 80°C for later use in determining the concentration of cytokines.

Determination of cytokines

The concentration of cytokines (IL-2, IL-8, IL10, IL-12) was measured in the supernatant using an ELISA test (Thermo Fisher Scientific, Invitrogen, Vienna, Austria), following the manufacturer's instructions, initially obtained (pg/mL) in the nasal fluid lavage, and normalized by the total protein concentration according to the Bradford method [17].

Club cell protein dosage (CC16)

The concentration of club cell protein CC16 was determined in the pulmonary sputum of the participants. The collection was carried out spontaneously [18] without induction of 3% hypertonic solution in order to avoid bronchospasm [19] in pre-exposure (phase 1) and acute exposure in phase 3. Participants were instructed not to drink liquids before collection. The samples were analyzed using an ELISA kit according to the manufacturer's instructions (BioVendor Research and Diagnostic Products, Brno, Czech Republic).

Exhaled carbon monoxide

Immediately after exposure in the simulator, COex was measured using a portable monoximeter Bedfont Pico Plus Smokrlyzer® (Bedfont, UK, England). For the measurement, the participant was asked to place the disposable mouthpiece between the teeth with the lips tight and perform a deep inspiration followed by a 20-second apnea. After the established period, the participant was encouraged to exhale the inspired volume slowly and completely [20]. The COex measurement was subsequently recorded on the analyzer display.

Mucociliary transport - Saccharin test

After 25 minutes of exposure, the mucociliary transport time

Table 1. Physical characteristics of participants in 2018 and 2019.

	2018 (n=18)				2019 (n=20)				
	Min	Max	Mean	SD	Min	Max	Mean	SD	p-value
Age (years)	24	49	37.1	7.3	30	49	36.1	5.9	0.65
Height (cm)	167	192	175.8	6.0	166	189	176.5	7.5	0.41
Weight (Kg)	60	100	78.5	9.8	65	100	80.7	10.7	0.66
BMI (Kg/m^2)	20.76	28.76	25.4	5.6	21.5	30.9	22.34	1.79	0.41

BMI = Body Mass Index; Min= minimum; Max= maximum; SD = Standard Deviation. Student's t-test.

was verified using the saccharin test. The individuals were seated with a slight cervical extension (approximately 10°). Gently 25 µg of powdered saccharin was introduced into the edge of the inferior turbinate of the nostril. It was introduced preferably in the right nostril or the nostril without obstruction, without the use of anesthetic, through a plastic straw up to 2 mm in diameter and 5 cm in length; after the procedure, the head was repositioned [21]. At the time of insertion, the stopwatch was activated until the participant reported the sensation of taste. The volunteer was instructed not to inhale deeply, cough, sniff, or sneeze during the test, and not to eat immediately before the test.

Spirometry FEV1, FVC, FEV1/FVC, FEF 25-75%

The spirometry examination was performed using a Mini Spir-Mir® portable flow spirometer (Rome, Italy), properly calibrated, coupled to the computer. The measurements were carried using the guidelines of the Brazilian Society of Spirometry. It was performed two hours after exposure in the simulator with subjects properly rested, cleaned, hydrated. Participants were asked not to take caffeine, and to eat only light snacks after exposure. The following parameters were recorded: FEV1, FVC, FEV1/FVC, FEF 25-75%. Normal values were used according to the Brazilian population. Technical and reproducibility procedures followed the standardization adopted by the Brazilian Consensus on Spirometry and the American Thoracic Society (ATS) [22, 23]. The values were collected in triplicate and the highest value obtained was used. The tests were performed by the same examiner, qualified to perform the exams.

Nasal obstruction questionnaires - NOSE and SNOT-22 quality of life

A validated and standardized questionnaire scale was applied - Nasal Obstruction Symptom Evolution–NOSE [13,15]. Additionally, the SNOT-22 quality of life questionnaire was also used [14]. The questions were asked individually to each participant. SNOT-22 and NOSE were applied before the first exposure and two hours after each exposure phase.

Statistical analysis

The database was built in Excel®, and all cleaning, coding, recoding, and grouping processes were performed in this software. For data analysis, Stata® 12.0 software was used. All the results obtained in this study were initially evaluated the Shapiro–Wilk test in order to verify the adherence to Gaussian distribution. Afterward, the homogeneity of variance was analyzed by the Levene test.

Parametric variables were presented as mean and standard deviation and the Student's t-test was used to verify significant differences for the anthropometric data between volunteer groups in 2018 and in 2019, as well as paired Student's t-test was used in the CC16 analysis. ANOVA test with Tukey posthoc test was used to evaluate the occurrence of significant differences in the spirometric assessment, peripheral oxygen saturation, respiratory frequency, heart rate, and also in the concentration of the cytokines (bars graphics).

Non-parametric variables were presented as a median and interquartile range (box plot graphic) and the Friedman test with the Muller–Dunn posthoc test was used to verify significant differences in the data obtained in the carbon monoxide exhaled, mucociliary clearance time, SNOT-22, and NOSE. The significance level was set to 5% ($p < 0.05$).

Results

Table 1 shows the physical characteristics of the participants. No statistically significant differences were found when volunteers of 2018 and 2019 were compared.

Significant alterations in the respiratory rate, heart rate, and peripheral oxygen saturation after fire simulator exposure

As shown in Table 2, higher levels of RF and HR were found in phases 2 and 3 than in phase 1 in volunteer groups, regardless of the year. Similarly, a higher HF level was observed in phase 2 than in phase 3 both in 2018 and 2019. Interestingly, concerning the HR level, the values of 2018 were higher in phase 2 than in phase 3. Whereas those of 2019 were higher in phase 3 than in phase 2. In addition, concerning peripheral oxygen saturation data, there were a significant reduction in phases 2 and 3 as

Table 2. Description and comparison by phase of the measurements of RF, HR, and SpO_2 (%) - 2018 (n= 17) and 2019 (n=20).

Variables 2018	Phase 1		Phase 2		Phase 3		
	Mean	SD	Mean	SD	Mean	SD	p-value
Respiratory Frequency	20.9	1.8	32.4	4.8	28.3	2.5	<0.01
Heart Rate	70.4	7.9	98.6	8.6	91.8	8.0	<0.01
Peripheral Oxygen Saturation %	97.5	1.2	95.7	1.4	96.6	1.5	<0.01
Variables 2019	**Phase 1**		**Phase 2**		**Phase 3**		
	Mean	SD	Mean	SD	Mean	SD	p-value
Respiratory Frequency	17.6	2.3	35.8	7.6	25.3	4.3	<0.01
Heart Rate	67.8	8.20	121.8	13.0	150.2	19.4	<0.01
Peripheral Oxygen Saturation %	97.1	1.4	95.9	1.9	92	5.3	<0.01

SD= Standard Deviation. ANOVA test with Tukey posthoc test.

Table 3. Comparison of absolutes values and % predicted FVC, FEV1, FEV1/FVC(%), and FEF 25-75 between the phases.

	Phases						
	1		2		3		
	Mean	SD	Mean	SD	Mean	SD	p-value
FVC	5.65	1.05	5.37	5.58	5.49	0.59	0.74
FVC %	104	8	102	7	102	8	0.45
FVE1	4.51	0.60	4.48	0.63	4.48	0.51	0.96
FVE1 %	108	12	107	11	107	7	0.14
FVE/FVC	82.91	4.95	82.39	5.22	83.10	2.85	0.98
FVE1 1 /FVC %	103	7	102	8	102	6	0.89
FEF2575	4.86	1.65	4.80	1.55	4.80	1.68	0.87
FEF2575 %	111	32	109	28	102	28	0.94

FVC=forced vital capacity; FVE1= forced expiratory volume in one second; FVE1/ FVC= ratio forced expiratory volume per second/forced vital capacity; FEF25-75= forced expiratory flow 25-75; SD= standard deviation. ANOVA test with Tukey posthoc test.

compared to phase 1 in 2018. Meanwhile, in 2019 a significant reduction was found only between phases 3 and 1, although the comparison between phases 2 and 1 showed a downward trend (p=0.068).

Pro- and anti-inflammatory cytokines, as well as CC16 levels, are altered in airways after fire simulator exposure

As cited above, the data obtained in the cytokines and CC16 evaluations were normalized by the total protein content found (mg/mL). This presented a similar concentration ($p > 0.05$) between the time-points analyzed (Phase 1 = 16.98 ± 4.38; Phase 2 = 18.85 ± 5.10; and Phase 3 = 16.19 ± 4.28).

Figure 3 shows that the concentration of IL-8 (Figure 3A) and IL-10 (Figure 3B) was higher in phase 2 than in phase 1. Whereas, the IL-2 levels were higher in phase 2 as compared to phases 1 and 3 (Figure 3C). In addition, although the concentrations of IL12p70 (Figure 3D) and IL12p40 (Figure 3E) were unchanged, the ratio of the data obtained was (IL-12p40/IL-12p70, Figure 3F). Thus, there was a significant increase in the ratio in phase 2 compared to phase 1.

Concerning the Club Cell (CC16) analysis in pulmonary sputum (Figure 3G), higher CC16 levels were found in phase 3 than the values observed in phase 1 (p=0.011). The sputum collection procedure for analysis was performed spontaneously [18], without the induction of the hypersaline solution.

Fire simulator exposure did not alter the ventilatory parameters

The ventilatory parameters FVC, FEV1, FEV1/FVC, and FEF 25-75% were assessed by the spirometry exams. No statistically significant differences were found between the phases of the study (Table 3).

Exhaled carbon monoxide and mucociliary transport time are altered after fire simulator exposure

As shown in Figure 4A, the exhaled carbon monoxide concentration found in phases 2 and 3 showed a significant increase as compared to phase 1 (p<0.0001, p=0.0086, respectively).

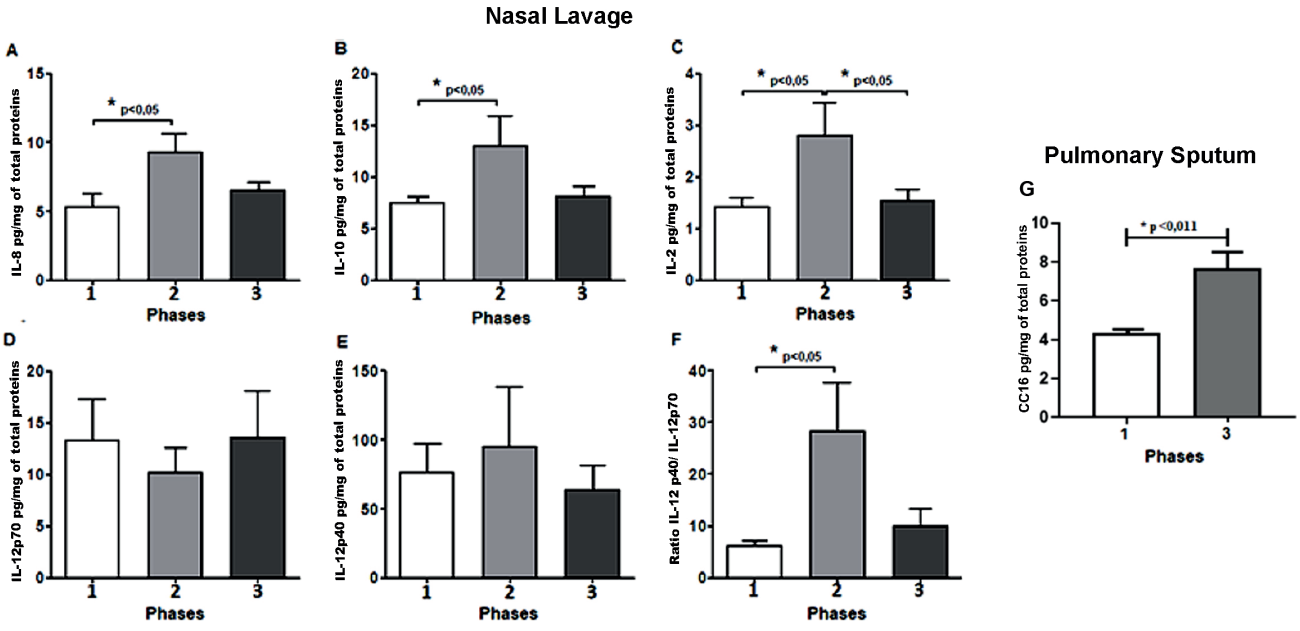

Figure 3. Comparison of cytokine levels of nasal lavage: IL-8 (A), IL-10 (B), IL-2 (C), IL-12p70 (D), IL12 p40 (E), and IL12p40/ILp70 ratio (F) between the phases of the study. Comparison of Club Cell concentrations in pulmonary sputum before and after exposure in the simulators (G).

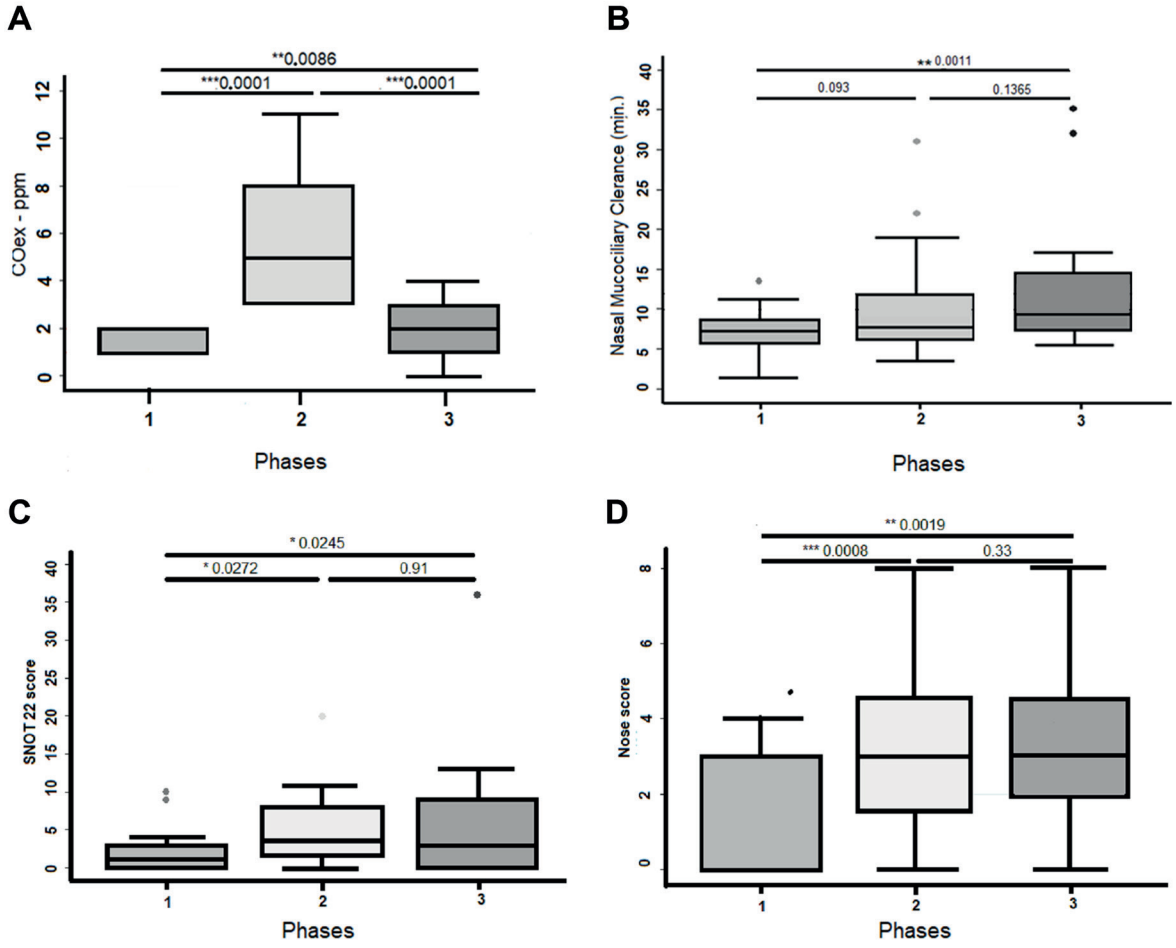

Figure 4. Comparison of the carbon monoxide exhaled (A) and mucociliary clearance time (B) during the phases. Comparison of the questionnaires that demonstrate an increase in scores after exposure to fire simulators and inhalation of combustion by-products – (C) SNOT-22; (D) NOSE.

Table 4. Most significant nasal symptoms - SNOT 22.

Complaints	Phase 1	Phase 2	Phase 3	p-value*
Need to blow nose	4	13	15	0,03
Sneezing	1	13	6	0,11
Running Nose	2	13	25	0,01
Cough	14	23	12	0,85
Post nasal	9	16	14	0,47
Tick nasal discharge	2	8	11	0,14
Decreased sense of smell/taste	4	40	62	<0,01
Nasal obstruction	1	10	34	<0,01

* (Friedman test)

However, lower levels were found in phase 3 as compared to phase 2 ($p<0.0001$).
Concerning the mucociliary transport time (MCT) analysis (Figure 4B), a significant increase in MCT was found in phase 3 compared to the values observed in phase 1 ($p=0.0011$). No other differences were found.

Fire simulator exposure impairs the quality of life and enhances the nasal obstruction symptoms

Figure 4C shows the results concerning the evaluation of the quality of life, which was assessed by the SNOT-22 questionnaire, and a significant increase in their scores was found in phases 2 ($p=0.0272$) and 3 ($p=0.0245$) compared to phase 1. Similarly, the results regarding the evaluation of the nasal obstruction symptoms, which was assessed by the NOSE questionnaire, showed a significant increase in their scores in phases 2 ($p=0.0008$) and 3 ($p=0.0019$) as compared to phase 1 (Figure 4D). Although these results are significant, they were not clinically relevant. Table 4 shows the most significant nasal symptoms - SNOT 22.

Discussion

In this study, our results showed that after the fire exposure in simulators, the firefighters presented with: 1) higher levels of CC16, pro-and anti-inflammatory cytokines, and also an increase in mucociliary transport time in airways; 2) significant increase of exhaled carbon monoxide; 3) an increase in the impairment of the quality of life and nasal obstruction symptoms. Interestingly, although the firefighters did not present alterations in some respiratory parameters (FEV1, FVC, FEV1/FVC, FEF 25-75%), a significant increase in heart rate (HR) and respiratory frequency (RF) were observed after the fire exposure in simulators.
The literature highlighted that the professional firefighters presented with a decrease not only in the peak expiratory flow [24] but also in the scores of pulmonary function test (FVC, FEV1, FEV1/FVC) after fire exposure [25]. However, we did not find any significant alterations in the respiratory parameters. Therefore, our findings permit us to suggest that the correct use of the autonomous respiratory protective equipment putatively minimized the deleterious effect of fire exposure in simulators during the 4 weeks course. Furthermore, it is worth noting that based on the scientific reports, elevations in RF and HR are often associated with exposure to high temperatures and inhalation of fumes. Particularly, it has been reported that repeated heat exposure with thermal impulses can promote physiological adaptations that lead to the improvement in an individual's ability to cope and perform work or exercise in a warm environment. The frequency of adaptations can vary from one to two exposures a day or intermittent exposure [10, 26].
The proposal of occurrence of a "phenomenon of adaptation" in the airways putatively corroborated with our results obtained in the inflammatory markers which were assessed in the nasal lavage. The concomitant elevation of the pro-inflammatory cytokines IL-8 and IL-2 together with the classical anti-inflammatory cytokine IL-10 was observed after fire exposure in a simulator in phase 2. It is of utmost importance to mention that different cell types, such as epithelial, fibroblast, and migrating cells, can produce and release both IL-8 and IL-2 in the mucosa of the airways [27-29]. Higher levels of these cytokines are mainly associated with the manifestation of several disturbances and diseases in the upper and lower airways [30, 31]. In contrast, in order to mitigate the harmful effect of higher levels of these pro-inflammatory cytokines in the airways, it elicited the release of IL-10 at the same time. It is broadly known that IL-10 has anti-inflammatory properties in the airways and can act as a protective factor against inflammation of the mucosa [31, 32]. In addition, it was found that the ratio of IL-12p40/IL-12p70 was increased in phase 2. However, the alterations in the cytokine levels in an isolated way indicate the ability of the upper airways to induce control of inflammation after the fire exposure in simulators. However, it is worth clarifying that, per the literature, cytokine IL-12p70, a heterodimer composed of IL-12p40 and IL-12p35 subunits, is closely associated with a pro-inflammatory action in the airways [33, 34]. Particularly, the subunit IL-12p40, which forms a homodimer known as IL-12p80, can partially antagonize the effects of IL-12p70. In this regard, evidence in the literature showed an increased lung inflammation in IL-12p40-/- mice than in the wild mice. IL-12p40 is also essential for the downregulation of airway hyperresponsiveness in a mouse model of asthma with prolonged antigen exposure [35-37]. Taking together, these results demonstrated that even though an inflammation was elicited by the fire exposure, a system of inflammation control was activated. In addition, it is also paramount to highlight that the observation of a decrease in cytokine concentrations after fire exposure in a simulator in phase 3 to the baseline values (phase 1) can reinforce the idea that adaptations in the airways were achieved during the course period.
Beyond the cytokines evaluation, we also analyzed the mucocili-

ary transport, which is another important characteristic present in the upper airway mucosa. The epithelial layer of the upper airway lining is the first innate barrier that attackers from inhalation encounter. The mucociliary transport is a defense mechanism belonging to this barrier that fights against pathogens and toxins. It is noteworthy to point out that mucociliary transport is a system composed of cilia and a mucus layer, which is susceptible to alterations from physical and climatic agents [38-40].
In this sense, it was reported that agricultural workers who are exposed to burning showed significant alterations in mucociliary transport [41]. This data corroborates our findings in that there was an accumulative increase in mucociliary transport after successive fire exposures, with a significant increase in phase 3. This indicates an adaptive effort to better clear and protect the upper airway.
Although firefighters used autonomous respiratory protection equipment during the training simulations, they removed the equipment for the final inspection and rested when the smoke was no longer evident. This caused them to become susceptible to inhalation of particles suspended in the atmosphere. The effects of smoke exposure is also proven by skin absorption of by-products even when wearing appropriate clothes [9, 42]. This was verified by the significant increase in inflammatory cytokines in the nasal lavage fluid in the acute phase of the study.
In order to improve the understanding of the effect of fire exposure in simulators in the airways, besides cytokines and mucociliary transport evaluations, in the upper airways, we also analyzed the levels of the Club Cell protein-16 (CC16) in the pulmonary sputum. In this regard, higher CC16 levels were found after fire exposure in phase 3. Based on the literature, CC16 is a pneumoprotein secreted by club cells present in the distal airways and the epithelial cells present in the tracheal-bronchial area. It is also associated with surfactants produced in type II alveolar cells [43, 44]. This protein is usually considered as a biomarker of peripheral lung injury since its expression is associated with anti-inflammatory action after exposure to airway irritants [38, 45]. We observed that CC16 was increased in the pulmonary sputum sample obtained in phase 3. This demonstrated that there was fire exposure induced lung inflammation, and also indicated that an alteration in the alveolar-capillary permeability was elicited.
In this sense, the evaluation of exhaled carbon monoxide and peripheral saturation of O_2 was useful in proving that the alveolar-capillary permeability was impaired by fire exposure in simulators. Based on our results, after fire exposure, exhaled carbon monoxide was increased. Whereas, the peripheral saturation of O_2 decreased, signifying a relevant alteration in the transport of oxygen by hemoglobin.
As appealing as these results could be, we believed that evaluations related to the impact of fire exposure in simulators during 4 consecutive weeks in the nasal obstruction symptoms are also very important. The SNOT-22 and NOSE questionnaires showed worsening scores in both phases associated with fire exposure. These alterations were closely associated with our laboratory findings.
The findings obtained in the present study showed that the use of individual respiratory protection materials is useful in many circumstances. We were able to demonstrate that the firefighting carried out by firefighters can elicit a transient inflammatory response in nasal mucosal, an impairment in the innate epithelial response of the upper airway lining, as well as induces an inflammatory process in lower airways that can alter the transport of O_2. Taken together, these can negatively impact the quality of life of these professionals. The NOSE and SNOT-22 questionnaires are developed for assessment for a period longer than one week. Therefore, they were not adapted for this scenario once the subjects did not suffer from sinus conditions. We applied them as an instrument to evaluate the airway symptoms, especially nasal symptoms. They showed significantly higher scores after exposure pointing to a decreased sense of smell/taste and nasal obstruction (Table 4). It is also important to highlight that these results were not clinically relevant.
Our study used firefighters' pre-exposure as a means of comparison and used two different cohorts in different years to be able to extend the measurements. This should be recommended in future studies comparing the regular population (no-firefighters) without previous exposure to smoke and a single cohort to have a more accurate result. Another difficulty encounter in this study was to distinguish the effects of combustion by-products from the effects of heating on the firefighters.

Conclusion

In conclusion, our study indicates that the mechanical alterations in the epithelial barrier in association with the induction of an inflammatory response in airways can lead to disturbances in O_2 transport. Thus, worsening the quality of life and physiological alterations in ventilatory mechanisms. In addition, the importance of further studies to verify these results in the long term is needed.

Acknowledgements

Coordenação de Aperfeiçoamento de Pessoa de Nível Superior (Capes). The study was funded by fellow 88882.43044212019-9.

Authorship contribution

TGC and JBA planning, project execution, data collection, writing article. VP, RLV, RGC, and PMP proofreading and checking of the data. VP and EBA data collection, checking. ALLB guidance, planning, data collection, assistance, article writing. RP guidance, planning, project execution, data collection, assistance, article writing.

Conflict of interest

The authors declare no conflict of interest.

References

1. Burgess JL, Nanson CJ, Bolstad-Johnson DM, Gerkin R, Hysong TA, Lantz RC, et al. Adverse respiratory effects following overhaul in firefighters. J Occup Environ Med 2001;43(5):467-73.
2. Burgess JL, Duncan MD, Hu C, Littau SR, Caseman D, Kurzius-Spencer M, et al. Acute cardiovascular effects of firefighting and active cooling during rehabilitation. J Occup Environ Med. 2012;54(11):1413-20.
3. Fent KW, Toennis C, Sammons D, Robertson S, Bertke S, Calafat AM, et al. Firefighters' and instructors' absorption of PAHs and benzene during training exercises. Int J Hyg Environ Health. 2019;222(7):991-1000.
4. Gianniou N, Giannakopoulou C, Dima E, Kardara M, Katsaounou P, Tsakatikas A, et al. Acute effects of smoke exposure on airway and systemic inflammation in forest firefighters. J Asthma Allergy. 2018;11:81-8.
5. Chia KS, Jeyaratnam J, Chan TB, Lim TK. Airway responsiveness of firefighters after smoke exposure. Br J Ind Med. 1990;47(8):524-7.
6. Miedinger D, Chhajed PN, Stolz D, Gysin C, Wanzenried AB, Schindler C, et al. Respiratory symptoms, atopy and bronchial hyperreactivity in professional firefighters. Eur Respir J. 2007;30(3):538-44.
7. Aránguez E, Ordóñez JM, Serrano J, Aragonés N, Fernández-Patier R, Gandarillas A, et al. [Air pollutants and their monitoring]. Revista espanola de salud publica. 1999;73(2):123-32.
8. Jones L, Burgess JL, Evans H, Lutz EA. Respiratory protection for firefighters--Evaluation of CBRN canisters for use during overhaul II: In mask analyte sampling with integrated dynamic breathing machine. J Occup Environ Hygiene. 2016;13(3):177-84.
9. Van Rooij JG, De Roos JH, Bodelier-Bade MM, Jongeneelen FJ. Absorption of polycyclic aromatic hydrocarbons through human skin: differences between anatomical sites and individuals. J Toxicol Environ Health. 1993;38(4):355-68.
10. Horn GP, Kesler RM, Kerber S, Fent KW, Schroeder TJ, Scott WS, et al. Thermal response to firefighting activities in residential structure fires: impact of job assignment and suppression tactic. Ergonomics. 2018;61(3):404-19.
11. Casey D, Schell S, Kemp M, Kazmierzak B. Live Fire Training: Principles and Practice. 2 ed: Jones & Bartlett Learning; 2018. 260 p.
12. Fokkens WJ, Lund VJ, Hopkins C, Hellings PW, Kern R, Reitsma S, et al. European Position Paper on Rhinosinusitis and Nasal Polyps 2020. Rhinology. 2020;58(Suppl S29):1-464.
13. Stewart MG, Witsell DL, Smith TL, Weaver EM, Yueh B, Hannley MT. Development and validation of the Nasal Obstruction Symptom Evaluation (NOSE) scale. Otolaryngol Head Neck Surg. 2004;130(2):157-63.
14. Kosugi EM, Chen VG, Fonseca VM, Cursino MM, Mendes Neto JA, Gregório LC. Translation, cross-cultural adaptation and validation of SinoNasal Outcome Test (SNOT): 22 to Brazilian Portuguese. Braz J Otorhinolaryngol. 2011;77(5):663-9.
15. Bezerra TF, Padua FG, Pilan RR, Stewart MG, Voegels RL. Cross-cultural adaptation and validation of a quality of life questionnaire: the Nasal Obstruction Symptom Evaluation questionnaire. Rhinology. 2011;49(2):227-31.
16. Belda J, Parameswaran K, Keith PK, Hargreave FE. Repeatability and validity of cell and fluid-phase measurements in nasal fluid: a comparison of two methods of nasal lavage. Clin Exp Allergy. 2001;31(7):1111-5.
17. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry. 1976;72(1):248-54.
18. Out TA, Jansen HM, Lutter R. Methodological aspects in the analysis of spontaneously produced sputum. Monaldi archives for chest disease = Archivio Monaldi per le malattie del torace. 2001;56(6):493-9.
19. Scheicher ME, Terra Filho J, Vianna EO. Sputum induction: review of literature and proposal for a protocol. Sao Paulo medical journal = Revista paulista de medicina. 2003;121(5):213-9.
20. Santos Up, Gannam S, Abe Jm, Esteves Pb, Freitas Filho M, Wakassa Tb, et al. Emprego da determinação de monóxido de carbono no ar exalado para a detecção do consumo de tabaco. Jornal de Pneumologia. 2001;27:231-6.
21. Nakagawa NK, Franchini ML, Driusso P, de Oliveira LR, Saldiva PH, Lorenzi-Filho G. Mucociliary clearance is impaired in acutely ill patients. Chest. 2005;128(4):2772-7.
22. Pereira C. I consenso brasileiro sobre espirometria. J Pneumol. 1996;22(3):105-64
23. Standardization of spirometry--1987 update. Statement of the American Thoracic Society. Am Rev Respir Dis. 1987;136(5):1285-98.
24. Korn GP, Alvarenga EHL, Dall'Oglio GP, Azevedo RR, Chamun W, Cordeiro TG, et al. The Effects in the Upper Airway of Heat and Exposure to Combustion Byproducts of Burning Materials on Larynx of Firefighters. J Voice. 2020 Sep 8;S0892-1997(20)30320-9.
25. Slattery F, Johnston K, Paquet C, Bennett H, Crockett A. Correction to: The long-term rate of change in lung function in urban professional firefighters: a systematic review. BMC Pulm Med. 2019;19(1):86.
26. Bruce-Low SS, Cotterrell D, Jones GE. Effect of wearing personal protective clothing and self-contained breathing apparatus on heart rate, temperature and oxygen consumption during stepping exercise and live fire training exercises. Ergonomics. 2007;50(1):80-98.
27. Moss RB, Nagata H, Goff J, Okubo K, Hausefeld J, Kaliner MA. Constitutive mRNA and immunoreactivity for IL-2 in human nasal mucosa. Clin Exp Allergy. 1997;27(4):406-12.
28. Ohkubo K, Ikeda M, Pawankar R, Gotoh M, Yagi T, Okuda M. Mechanisms of IL-6, IL-8, and GM-CSF release in nasal secretions of allergic patients after nasal challenge. Rhinology. 1998;36(4):156-61.
29. Kim JA, Cho JH, Park IH, Shin JM, Lee SA, Lee HM. Diesel Exhaust Particles Upregulate Interleukins IL-6 and IL-8 in Nasal Fibroblasts. PLoS One. 2016;11(6):e0157058.
30. Vaisberg M, Suguri VM, Gregorio LC, Lopes JD, Bachi AL. Cytokine kinetics in nasal mucosa and sera: new insights in understanding upper-airway disease of marathon runners. Exerc Immunol Rev. 2013;19:49-59.
31. Chen X, Liu J, Zhou J, Wang J, Chen C, Song Y, et al. Urban particulate matter (PM) suppresses airway antibacterial defence. Resp Res. 2018;19(1):5.
32. Zhang S, Huo X, Zhang Y, Huang Y, Zheng X, Xu X. Ambient fine particulate matter inhibits innate airway antimicrobial activity in preschool children in e-waste areas. Environ Int. 2019;123:535-42.
33. Boyaka PN, Lillard JW, Jr., McGhee J. Interleukin 12 and innate molecules for enhanced mucosal immunity. Immunol Res. 1999;20(3):207-17.
34. Bal SM, Bernink JH, Nagasawa M, Groot J, Shikhagaie MM, Golebski K, et al. IL-1β, IL-4 and IL-12 control the fate of group 2 innate lymphoid cells in human airway inflammation in the lungs. Nature Immunol. 2016;17(6):636-45.
35. Cooper AM, Khader SA. IL-12p40: an inherently agonistic cytokine. Trends Immunol. 2007;28(1):33-8.
36. Wang SZ, Bao YX, Rosenberger CL, Tesfaigzi Y, Stark JM, Harrod KS. IL-12p40 and IL-18 modulate inflammatory and immune responses to respiratory syncytial virus infection. J Immunol. 2004;173(6):4040-9.
37. Onari Y, Yokoyama A, Haruta Y, Nakashima T, Iwamoto H, Hattori N, et al. IL-12p40 is essential for the down-regulation of airway hyperresponsiveness in a mouse model of bronchial asthma with prolonged antigen exposure. Clin Exp Allergy. 2009;39(2):290-8.
38. Laucho-Contreras ME, Polverino F, Gupta K, Taylor KL, Kelly E, Pinto-Plata V, et al. Protective role for club cell secretory protein-16 (CC16) in the development of

COPD. Eur Respir J. 2015;45(6):1544-56.
39. Mocellin M, Barrionuevo CE, Pasinato RC, Ferreira RF, Ouriques FL. Nasal Mucociliary Function Study in Children with Adenotonsillar Hypertrophy in the Pre Days After the Adenotonsillectomy. Br J Otorhinolaryngol. 1998;64(3):241-6.
40. Stannard W, O'Callaghan C. Ciliary function and the role of cilia in clearance. J Aerosol Med. 2006;19(1):110-5.
41. Ferreira AD, Ramos EMC, Trevisan IB, Leite MR, Proença M, de Carvalho-Junior LCS, et al. Função pulmonar e depuração muco-ciliar nasal de cortadores de cana-de-açúcar brasileiros expostos à queima de biomassa. Revista Brasileira de Saúde Ocupacional. 2018;43.
42. Banauch GI, Hall C, Weiden M, Cohen HW, Aldrich TK, Christodoulou V, et al. Pulmonary function after exposure to the World Trade Center collapse in the New York City Fire Department. Am J Respir Crit Care Med. 2006;174(3):312-9.
43. Knabe L, Fort A, Chanez P, Bourdin A. Club cells and CC16: another "smoking gun"? (With potential bullets against COPD). Eur Respir J. 2015;45(6):1519-20.
44. Greven F, Krop E, Burger N, Kerstjens H, Heederik D. Serum pneumoproteins in fire-fighters. Biomarkers. 2011;16(4):364-71.
45. Broeckaert F, Clippe A, Knoops B, Hermans C, Bernard A. Clara cell secretory protein (CC16): features as a peripheral lung biomarker. Ann N Y Acad Sci. 2000;923:68-77.

Jônatas Bussador do Amaral
ENT Research Lab.
Department of Otorhinolaryngology--Head and Neck Surgery
Federal University of Sao Paulo.
Rua Pedro de Toledo 781,
1o andar, sala 3
Vila Clementino
Sao Paulo, SP
Brazil CEP 04039-032

Telephone: +55 11 55764848 R:1372
E-mail: jbamaral@unifesp.br