

# Influence of a submaximal and maximal aerobic exercise session on mucociliary clearance and autonomic function in smokers

*Influência de uma sessão de exercício aeróbico submáximo e máximo sobre o transporte mucociliar e função autonômica em tabagistas*

*Influencia de una sesión de ejercicio aeróbico submáximo y máximo sobre el transporte mucociliar y la función autonómica en fumadores*

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**ABSTRACT** | The aim of this study was to evaluate and to correlate the behavior of mucociliary clearance and the autonomic nervous system of smokers after submaximal and maximal aerobic exercise sessions. We evaluated 25 smokers and 15 nonsmokers aged between 30 and 50 years. Both groups were submitted to the saccharin transit time (STT) test and heart rate variability (HRV) before and after a submaximal (six-minute walk test) and maximal (cardiopulmonary test) exercise. Paired t-test or Wilcoxon were used for intragroup analysis and the unpaired t-test or Mann-Whitney for intergroup analysis. The correlation was performed using Pearson or Spearman coefficients ( $p < 0.05$ ). Saccharine transit time reduced significantly after submaximal and maximal exercises in both groups. After the submaximal exercise, both groups presented significant reduction of the RR interval and increased heart rate (HR). In the nonsmoker group there were significant reductions in the RMSSD, HFms<sup>2</sup> and SD1 indexes. After maximal exercise, both groups showed significant reductions in SDNN, RMSSD, RR, LF and HF interval, in ms<sup>2</sup> and normalized units,

SD1 and SD2, in addition to the increase in HR, LFun, and LF/HF ratio. STT positively correlated with LFms<sup>2</sup> ( $r = 0.520$ ,  $p = 0.008$ ) after the maximal exercise for the smoker group. We concluded, that regardless of the intensity of aerobic exercise, mucociliary clearance increases in smokers, but this alteration seems to be influenced by the autonomic nervous system only during maximum exercise.

**Keywords** | Mucociliary Clearance; Autonomic Nervous System; Smoking; Exercise.

**RESUMO** | O objetivo do estudo foi avaliar e correlacionar o comportamento da depuração mucociliar e do sistema nervoso autônomo de fumantes após sessões de exercício aeróbico submáximo e máximo. Foram avaliados 25 fumantes e 15 não fumantes, entre 30 e 50 anos. Ambos os grupos foram submetidos ao teste do tempo de trânsito de sacarina (TTS) e variabilidade da frequência cardíaca (VFC) antes e após uma sessão de exercício submáximo (teste de caminhada de seis minutos) e máximo (teste de

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exercício cardiopulmonar). Teste t pareado ou Wilcoxon foi utilizado para análise intragrupos e o teste t não pareado ou Mann-Whitney para a análise intergrupos. A correlação foi realizada utilizando os coeficientes de Pearson ou Spearman ( $p < 0,05$ ). Houve redução significativa do TTS após exercícios submáximo e máximo em ambos os grupos. Após o exercício submáximo, ambos grupos apresentaram redução significativa do intervalo RR e aumento da FC em comparação ao repouso, no grupo de não fumantes houve reduções significativas nos índices RMSSD, HFms<sup>2</sup> e SD1. Após o exercício máximo, ambos grupos apresentaram reduções significativas no SDNN, RMSSD, intervalo RR, LF e HF, em ms<sup>2</sup> e un, SD1 e SD2, além do aumento da FC, LFun e da razão LF/HF. Houve correlação positiva entre TTS e LFms<sup>2</sup> ( $r = 0,520$ ,  $p = 0,008$ ) após o exercício máximo para o grupo de fumantes. Conclui-se que independentemente da intensidade do exercício aeróbio, houve um aumento na depuração mucociliar em fumantes, mas essa alteração parece ser influenciada pelo sistema nervoso autônomo apenas frente o exercício máximo.

**Descritores** | Depuração Mucociliar; Sistema Nervoso Autônomo; Tabagismo; Exercício.

**RESUMEN** | El objetivo de este estudio fue evaluar y correlacionar el comportamiento de la depuración mucociliar y del sistema nervioso autónomo de fumadores después de sesiones de ejercicio aeróbico submáximo y máximo. Se evaluaron a 25 fumadores y a

15 no fumadores de entre 30 y 50 años de edad. Ambos grupos se sometieron a la prueba de tiempo de tránsito de sacarina (TTS) y la variabilidad de la frecuencia cardíaca (VFC) antes y después de una sesión de ejercicio submáximo (prueba de caminata de seis minutos) y de ejercicio máximo (prueba de esfuerzo cardiopulmonar). Para el análisis intragrupo se utilizó la prueba t pareada o Wilcoxon, y para el análisis intergrupar, la prueba t no pareada o Mann-Whitney. Para realizar la correlación se utilizaron los coeficientes de Pearson o Spearman ( $p < 0,05$ ). Hubo una reducción significativa en TTS después de ejercicios submáximo y máximo en ambos grupos. Después del ejercicio submáximo, ambos grupos mostraron una reducción significativa en el intervalo RR y un aumento en la FC en comparación con el reposo; en el grupo de no fumadores hubo reducciones significativas en los índices RMSSD, HFms<sup>2</sup> y SD1. Después del ejercicio máximo, ambos grupos mostraron reducciones significativas en SDNN, RMSSD, intervalo RR, LF y HF, en ms<sup>2</sup> y un, SD1 y SD2, además de un aumento de FC, LFun y la relación LF/HF. Hubo una correlación positiva entre TTS y LFms<sup>2</sup> ( $r = 0,520$ ,  $p = 0,008$ ) después del ejercicio máximo para el grupo de fumadores. Se concluye que, de manera independiente a la intensidad del ejercicio aeróbico, hubo un aumento de la depuración mucociliar en los fumadores, pero este cambio parece haber sido influido por el sistema nervioso autónomo solamente en el ejercicio máximo.

**Palabras clave** | Depuración Mucociliar; Sistema Nervoso Autônomo; Tabagismo; Ejercicio.

## INTRODUCTION

Smoking can promote several alterations in the body, including those in autonomic modulation<sup>1</sup> and mucociliary clearance (MC)<sup>2,3</sup>. In autonomic modulation, sympathetic activation and vagal removal occur<sup>4</sup>; and due to changes in the respiratory mucosa, mucus composition, structure and ciliary function<sup>2,5,6</sup> smokers present increased MC time, which may be directly associated with the smoking load<sup>6</sup>. However, after smoking cessation this condition can be reversed<sup>7</sup>.

Physical exercise can also accelerate MC time<sup>8</sup>, suggesting that pulmonary hyperventilation and sympathetic stimulation<sup>9</sup> after exercise accelerates the ciliary beat, facilitating the elimination of particles that are harmful to the respiratory system, increasing its defense action<sup>10,11</sup>. In smokers, the effects of submaximal and maximal aerobic exercise on mucociliary clearance are still unclear, as well as the influence of the autonomic nervous system in different exercise intensities.

Considering that smoking alters both MC and autonomic modulation, and that physical exercise can accelerate MC (which, at least in parts, is related to changes in the autonomic nervous system). It is important for clinical practice to assess whether this also occurs in smokers in different intensities of physical exercise, since it is a therapeutic resource that can be used in the clinical treatment of smokers. Therefore, this study aimed to evaluate the alterations and relationships of nasal MC behavior and autonomic modulation of smokers after submaximal and maximal aerobic exercise stimulus.

## METHODOLOGY

This is a prospective and cross-sectional study, conducted with 69 participants aged between 30 and 50 years, allocated to a smokers group ( $n = 44$ ) and a nonsmokers group (control,  $n = 25$ ). Of these, 29 lost to follow-up, 14 for no-show and 15 for other reasons. Thus,

the final sample included 40 participants, 25 smokers and 15 non-smokers (Figure 1).

Inclusion criteria were physically inactive individuals (who did not perform regular physical activity for 20 continuous minutes, three times a week), with normal pulmonary function confirmed by spirometry and absence of known osteopathies, cardiorespiratory, metabolic, and neuromuscular diseases. For the smoking group, individuals

who smoked at least 20 cigarettes per day for one year or more were included. Exclusion criteria were non-understanding or non-collaboration of the volunteer in relation to the procedures or methods, absence in one of the exercise protocols, recent respiratory infections, nasal septum deviation, history of surgery or nasal trauma and alcohol consumption, or any adverse health condition that could interfere with exercise performance or autonomic modulation.

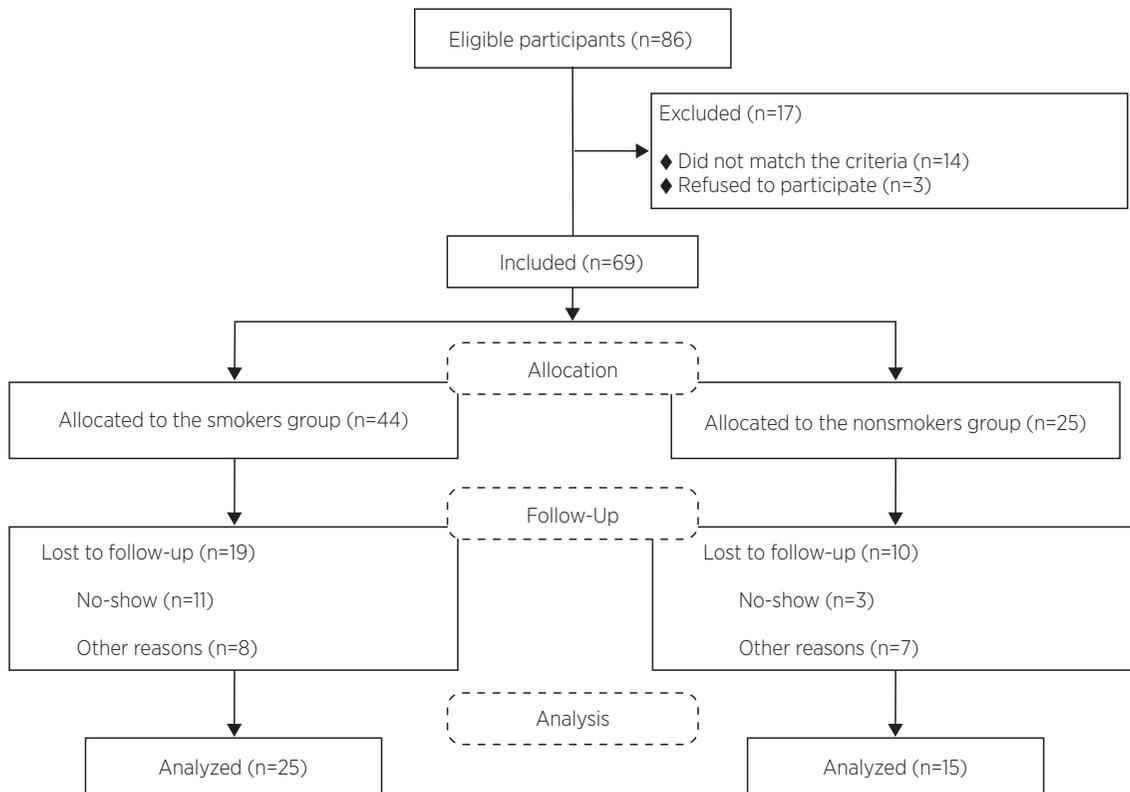


Figure 1. Flowchart of the study

This study was approved by the Research Ethics Committee (Protocol No. 223,033). The participants were informed about the objectives and procedures of the study before signing the consent form.

The protocol was performed on three non-consecutive days. All evaluations happened in the morning, in an environment with controlled temperature ( $22.93 \pm 1.32^\circ\text{C}$ ) and relative air humidity ( $53.96 \pm 3.82\%$ ). The individuals were instructed to have light meals 2 hours before the evaluations and to abstain from alcohol, caffeine, cigarettes, and vigorous physical activity for 12 hours before the evaluations.

On the first day of the protocol, all individuals were submitted to an initial evaluation, which included interviews for collection of personal data, anthropometric measurement, general health status

(comorbidities and history of surgery and nasal trauma), smoking history, and level of nicotine dependence assessed through the *Fagerström* test<sup>12</sup>. The measurement of exhaled carbon monoxide ( $\text{CO}_{\text{ex}}$ , cutoff point of 10 ppm<sup>13</sup>) was evaluated using the standard technique with the carbon monoxide analyzer (Micro CO, Micro Medical Ltd. apparatus, Rochester, Kent, UK)<sup>14</sup>, and the evaluation of pulmonary function was performed by spirometry ( $\text{VEF}_1/\text{FVC} > 70\%$  – Spirobank 3.6, Medical International Research, Rome, Italy) according to the standards of the American Thoracic Society and European Respiratory Society<sup>15</sup>, using reference values for the Brazilian population<sup>16</sup>.

The other two days of protocol had a minimum interval of 48 hours between them. On the first day, the participants took a six-minute walk test (6MWT),

which was the submaximal physical exercise according to the guide of the American Thoracic Society<sup>17</sup>. On the second day, the participants made the maximal physical exercise, which was a progressive effort test on a treadmill<sup>18</sup> with a gas analyzer (VO2000, Medical Graphics, USA). It was considered maximal exercise when at least two of the following criteria were met: HR reached >90% of maximum HR (220 – age); subjective perception of effort<sup>19</sup> above 17; eventual plateau in the oxygen consumption chart (VO<sub>2</sub> l/min) in the face of increased effort; and ratio of respiratory equivalents carbon dioxide and oxygen consumption (QR) >1,<sup>19-21</sup>.

Before performing the exercise protocols, the individuals remained seated at rest for 20 minutes to stabilize cardiorespiratory variables and to measure heart rate variability with the Polar S810i heart rate variability meter (Polar Electro, Kempele, Finland)<sup>22,23</sup>. Then, the COex was collected and the saccharin transit time (STT) test<sup>2,3,7,8,24,25</sup> was performed.

The exercise protocols were initiated after the first perception of a sweet taste. To avoid the influence of respiratory rate on MC, the evaluations were repeated ten minutes after the end of both exercises, thus, COex, STT test, and HRV were collected again. The heart rate variability was measured until the perception of the taste of saccharin.

Heart rate variability was recorded at rest, in sitting position for 20 minutes before and after exercise protocols, until the perception of a sweet taste during STT test. To analyze the indexes of heart rate variability (HRV) in each protocol, 256 RR intervals (interval oscillations between consecutive heartbeats) were selected in the most stable phase – during initial rest and during the final evaluation of STT, that is, 256 heartbeats prior to the exact moment the individuals reported having felt the taste of saccharine.

The selected section was submitted to digital filtering (Polar Precision Performance SW software, version 4.01.029) complemented with manual filtering, and only series with more than 95% of sinus beats were included in the study. Kubios software (Biosignal and Medical Image Analysis Group, Department of Physics, University of Kuopio, Kuopio, Finland)<sup>26</sup> was used to calculate HRV indexes: RR intervals; Root Mean Square of the Successive Differences (RMSSD, expressed in ms) between the adjacent normal RR intervals; and standard deviation of the mean (SDNN, expressed in ms) of the normal RR intervals. In the frequency domain, we analyzed the spectral components of low (LF: 0.04 - 0.15 Hz) and high frequency (HF: 0.15 – 0.40 Hz), in normalized units (un) and in milliseconds squared (ms<sup>2</sup>), and the ratio between these components (LF/HF)<sup>22,27,28</sup>. This analysis used the Fast Fourier Transform algorithm<sup>29</sup>.

The Poincaré plot was used to calculate the following indexes: SD1 (standard deviation of the instantaneous beat-to-beat variability); SD2 (standard deviation of the long-term variability of continuous RR intervals)<sup>22,27,29</sup>.

The statistical program SPSS 22.0 was used for analysis. We verified data normality using the Shapiro-Wilk test. For intragroup comparison, the paired t-test or Wilcoxon test was used, according to the normality of the data. For analysis between the groups, the unpaired t-test was used for data with normal distribution or Mann-Whitney test for those with non-normal distribution. Correlation analyses were performed using Pearson or Spearman coefficients according to data normality. The significance level was p<0.05 in all tests.

**RESULTS**

Table 1 shows the characteristics of the two evaluated groups.

Table 1. Characterization of the sample according to sex, age, anthropometric measurements, spirometric indexes, physical capacity, and smoking history

Variables	Smoking Group (n=25)	Control Group (n=15)	p
Sex F/M	11/14	9/6	0,3276
Age (years)	43.0 (35.0 – 46.0)	46.0 (32.0 – 49.0)	0.211‡
BMI (kg/m <sup>2</sup> )	27.0 (24.2 – 30.7)	26.2 (22.7 – 29.9)	0.419 <sup>+</sup>
FEV <sub>1</sub> /FVC (predicted %)	85.8 (82.2 – 90.5)	85.8 (82.2 – 90.5)	0.679‡
FVC(predicted %)	97.0 (87.5 – 107.5)	92.0 (90.0 – 107.0)	0.638 <sup>+</sup>
FEV <sub>1</sub> (predicted %)	93.0 (81.5 – 104.0)	97.0 (91.0 – 108.0)	0.206 <sup>+</sup>
D6MWT (%)	92.0 (85.0 – 100.0)	102.8 (100.2 – 90.5)	<0.0001‡*
VO <sub>2</sub> max. (ml/kg/min)	36.0 (26.0 – 48.0)	47.0 (41.0 – 58.0)	0.008* <sup>+</sup>

(continues)

Table 2. Continuation

Variables	Smoking Group (n=25)	Control Group (n=15)	p
Cigarettes/day	20.0 (20.0 – 32.5)	---	
Years-Pack	30.0 (17.5 – 39.0)	---	
<i>Fagerström</i>			
Moderate (%)	32	---	
High (%)	48	---	
Very High (%)	20	---	

n: number of individuals; F/M: female/male; kg: kilograms; BMI: body mass index; FEV1: forced expiratory volume in the first second; FVC: forced vital capacity; D6MWT: distance covered in the six-minute walk test; VO<sub>2</sub> max: maximum oxygen consumption; ml/kg/min: millilitres per kilogram per minute; Moderate smoker: consumption of 15 to 24 cigarettes per day; Heavy smoker: consumption of 25 or more cigarettes per day.

Data expressed as median (interquartile range 25% and 75%).

†: Student t-test for independent samples.

‡: Mann-Whitney test.

δ: Chi-square test.

\*Statistically significant difference (p<0.05)

In the intragroup analysis, in submaximal and maximal exercise, both groups showed a reduction in the saccharine transit time (STT) post-exercise. In the HRV indexes, the smoking group showed a significant reduction in the RR interval and a significant increase in HR compared with the pre-exercise moment; while the control group presented significantly lower

values of RMSSD, RR interval, HFms<sup>2</sup> and SD1 and a significant increase in HR in the post-exercise period, compared with the pre-exercise. Regarding maximal exercise, both groups showed a significant reduction in the SDNN, RMSSD, RR interval, LFms<sup>2</sup>, HFms<sup>2</sup>, HFun, SD1 and SD2 indexes; HR, LFun and LF/HF ratio increased (Table 2).

Table 2. Data of monoximetry, MC and variability of the heart rate frequency of the groups before and after submaximal and maximal exercise.

Variables	Smoking Group (n=25)			Control Group (n=15)		
	Resting	Post-exercise	P	Resting	Post-exercise	P
<b>Submaximal Exercise</b>						
COex (ppm)	7.0 (5.0 – 9.0)	7.0 (5.0 – 9.0)	0.100†	2.0 (1.0 – 2.0)	1.0 (0.0 – 2.0)	0.075‡
STT (min.)	14.7 (9.1 – 18.4)	7.3 (5.9 – 9.6)	0.002‡*	11.0 (7.8 – 13.2)	7.7 (5.7 – 9.0)	0.023‡*
HR (bpm)	71.0 (64.5 – 79.0)	80.0 (67.5 – 87.5)	0.002‡*	71.0 (68.0 – 76.0)	82.0 (75.0 – 88.0)	<0.0001‡*
SDNN (ms)	30.5 (24.0 – 37.1)	28.7 (24.9 – 36.4)	0.514‡	29.4 (21.4 – 32.2)	20.3 (15.6 – 29.2)	0.083‡
RMSSD (ms)	25.2 (17.8 – 29.7)	21.0 (17.2 – 29.3)	0.268‡	21.0 (16.5 – 23.5)	11.5 (10.2 – 21.0)	0.031‡*
RR (ms)	844.0 (767.0 – 902.0)	779.0 (724.5 – 870.5)	0.037‡*	827.0 (742.0 – 881.0)	757.0 (688.0 – 838.0)	<0.0001‡*
LF (ms <sup>2</sup> )	509.0 (326.0 – 840.0)	569.0 (248.5 – 897.5)	0.619†	502.0 (314.0 – 685.0)	369.0 (157.0 – 511.0)	0.496†
HF (ms <sup>2</sup> )	208.0 (114.0 – 300.5)	199.0 (92.5 – 302.5)	0.989†	159.0 (85.0 – 270.0)	104.0 (38.0 – 156.0)	0.029‡*
LF (un)	70.5 (58.2 – 82.0)	73.8 (65.3 – 85.5)	0.339†	73.9 (61.4 – 82.3)	82.2 (66.9 – 86.1)	0.105‡
HF (un)	24.8 (18.0 – 41.6)	27.6 (15.7 – 37.7)	0.469‡	26.1 (17.7 – 38.6)	17.8 (13.8 – 33.1)	0.104‡
LF/HF (ms <sup>2</sup> )	2.4 (1.4 – 4.6)	2.8 (1.9 – 5.9)	0.581†	2.8 (1.6 – 4.7)	4.6 (2.0 – 6.2)	0.179‡
SD1 (ms)	17.8 (12.6 – 21.0)	14.9 (12.2 – 20.8)	0.269‡	14.8 (11.7 – 16.6)	8.2 (7.2 – 14.9)	0.031‡*
SD2 (ms)	38.5 (31.3 – 48.2)	38.3 (32.3 – 47.6)	0.577‡	37.4 (28.0 – 42.8)	27.8 (21.2 – 38.8)	0.106‡
<b>Maximal Exercise</b>						
COex (ppm)	7.0 (4.5 – 11.0)	6.0 (4.0 – 10.0)	0.075‡	1.0 (0.0 – 2.0)	0.0 (0.0 – 2.0)	0.034‡*
STT (min.)	12.2 (9.1 – 18.7)	8.1 (6.0 – 11.0)	0.006‡*	8.7 (6.9 – 13.7)	5.9 (5.0 – 7.5)	0.017‡*
HR (bpm)	74.0 (68.0 – 78.5)	96.0 (84.5 – 103.0)	<0.0001‡*	76.0 (72.0 – 84.0)	102.0 (98.0 – 106.0)	<0.0001‡*
SDNN (ms)	27.0 (22.7 – 36.4)	16.9 (12.4 – 23.2)	<0.0001‡*	31.0 (24.5 – 34.7)	13.5 (10.7 – 18.4)	<0.0001‡*
RMSSD (ms)	24.1 (18.0 – 29.6)	10.1 (6.0 – 16.1)	<0.0001‡*	20.5 (15.5 – 23.3)	6.6 (6.0 – 9.9)	0.001‡*
RR (ms)	820.0 (762.5 – 887.0)	663.0 (589.5 – 725.0)	<0.0001‡*	779.0 (745.0 – 857.0)	613.0 (593.0 – 642.0)	<0.0001‡*
LF (ms <sup>2</sup> )	412.0 (279.5 – 599.0)	187.0 (119.0 – 323.0)	0.002‡*	488.0 (289.0 – 746.0)	149.0 (61.0 – 260.0)	0.003‡*

(continues)

Variables	Smoking Group (n=25)			Control Group (n=15)		
	Resting	Post-exercise	P	Resting	Post-exercise	P
HF (ms <sup>2</sup> )	159.0 (114.0 – 319.0)	31.0 (12.0 – 108.0)	<0.0001 <sup>†*</sup>	198.0 (136.0 – 277.0)	14.0 (8.0 – 40.0)	0.001 <sup>†*</sup>
LF (un)	71.0 (60.0 – 80.5)	86.5 (81.6 – 90.2)	<0.0001 <sup>†*</sup>	69.4 (61.2 – 77.2)	85.8 (79.3 – 93.3)	0.001 <sup>†*</sup>
HF (un)	29.0 (19.5 – 40.0)	13.5 (9.7 – 18.3)	<0.0001 <sup>†*</sup>	30.6 (22.8 – 38.8)	14.2 (6.7 – 20.7)	0.001 <sup>†*</sup>
LF/HF (ms <sup>2</sup> )	2.5 (1.5 – 4.1)	6.4 (4.5 – 9.3)	<0.0001 <sup>†*</sup>	2.3 (1.6 – 3.4)	6.1 (3.8 – 14.0)	0.004 <sup>†*</sup>
SD1 (ms)	16.6 (12.7 – 20.0)	7.2 (4.2 – 11.4)	<0.0001 <sup>†*</sup>	14.5 (11.0 – 16.5)	4.7 (4.2 – 7.0)	0.001 <sup>†*</sup>
SD2 (ms)	35.0 (29.4 – 46.3)	22.6 (16.7 – 31.1)	<0.0001 <sup>†*</sup>	41.3 (31.9 – 44.8)	18.5 (14.7 – 25.3)	<0.0001 <sup>†*</sup>

COex: exhaled carbon monoxide; ppm: parts per million; STT: saccharin transit time; min: minutes; HR: heart rate; bpm: beats per minute; SDNN: standard deviation of the mean of all normal RR intervals, expressed in milliseconds; RMSSD: root mean square of the successive differences between the adjacent normal RR intervals in a time interval, expressed in milliseconds; LF: low frequency; HF: high frequency; ms: milliseconds; un: normalized units; SD1: standard deviation of instantaneous beat-to-beat variability; SD2: standard deviation of long-term variability. Data expressed as median (interquartile range 25% and 75%).

<sup>†</sup>: Student *t*-test for paired samples.

<sup>‡</sup>: Wilcoxon test;

\*Statistically significant difference (p<0.05)

Table 3 presents the delta analyses of intragroup and between groups data. The smoking group showed a significant decrease of SDNN, RMSSD, RR interval, LFsms<sup>2</sup>, HFms<sup>2</sup> and un, SD1 and SD2 indexes during the maximal exercise when compared with submaximal,

and an increase in HR and LF/HF indexes. The control group also showed a reduction in SDNN, RMSSD, RR interval, HFms<sup>2</sup> and un, SD1 and SD2 and HR, LFun, and LF/HF increased during maximal exercise, when compared with submaximal.

Table 3. Magnitude of response of the evaluated groups according to the submaximal and maximal exercise deltas in monoximetry, mucociliary clearance and heart rate variability indexes. Data expressed in mean and standard deviation

	Submaximal Exercise			Maximal Exercise			Smoking Group	Control Group
	Smoking Group	Control Group	P	Smoking Group	Control Group	P	P (Submaximal vs. Maximal)	P (Submaximal vs. Maximal)
COex (ppm)	-0.6±1.6	-0.7±1.4	1 <sup>†</sup>	-0.4±1.6	-0.6±1.3	0.244 <sup>†</sup>	0.527 <sup>§</sup>	0.154 <sup>§</sup>
STT (min)	-5.8±8.7	-2.1±3.5	0.067 <sup>‡</sup>	-4.9±8.1	-4.3±6.2	0.806 <sup>‡</sup>	0.847 <sup>§</sup>	0.777 <sup>§</sup>
HR (bpm)	6.3±9.1	22.2±15.7	0.303	9.1±6.6	23.3±11.3	0.813	<0.0001 <sup>§*</sup>	<0.0001 <sup>§*</sup>
SDNN (ms)	-1.1±8.3	-5.4±11.2	0.173 <sup>‡</sup>	-11.2±8.8	-14.3±9.8	0.380 <sup>‡</sup>	<0.0001 <sup>§*</sup>	0.033 <sup>§*</sup>
RMSSD (ms)	-1.5±6.7	-5.0±8.0	0.152 <sup>‡</sup>	-21.9±42.6	-13.0±8.5	0.600 <sup>†</sup>	<0.0001 <sup>§*</sup>	0.008 <sup>†*</sup>
RR (ms)	-28.8±70.1	-56.6±47.7	0.125 <sup>†</sup>	-156.9±90.0	-169.0±39.5	0.563 <sup>‡</sup>	<0.0001 <sup>§*</sup>	<0.0001 <sup>§*</sup>
LF (ms <sup>2</sup> )	27.4±409.8	-80.0±440.8	0.440 <sup>‡</sup>	-251.6±382.4	-309.5±308.2	0.543 <sup>†</sup>	0.026 <sup>§*</sup>	0.124 <sup>§</sup>
HF (ms <sup>2</sup> )	-14.7±162.5	-85.6±151.0	0.106 <sup>‡</sup>	-152.2±175.5	-187.6±154.7	0.507 <sup>†</sup>	<0.006 <sup>§*</sup>	0.027 <sup>§*</sup>
LF (un)	23.0±98.5	7.4±16.6	0.581 <sup>†</sup>	15.5±12.8	16.4±8.9	0.790 <sup>‡</sup>	0.104 <sup>§</sup>	0.033 <sup>§*</sup>
HF (un)	-2.9±19.6	-7.4±16.6	0.458 <sup>‡</sup>	-15.4±12.7	-16.4±8.8	0.796 <sup>‡</sup>	0.018 <sup>§*</sup>	0.033 <sup>§*</sup>
LF/HF (ms <sup>2</sup> )	2.5±11.7	1.2±3.3	0.489 <sup>†</sup>	5.7±6.8	8.9±10.1	0.292 <sup>†</sup>	0.014 <sup>§*</sup>	0.004 <sup>§*</sup>
SD1 (ms)	-1.1±4.8	-3.5±5.7	0.154 <sup>‡</sup>	-9.7±6.2	-9.2±6.0	0.800 <sup>‡</sup>	<0.0001 <sup>§*</sup>	0.008 <sup>†*</sup>
SD2 (ms)	-1.3±11.2	-6.7±15.1	0.198 <sup>‡</sup>	-13.0±11.6	-18.1±12.8	0.202 <sup>‡</sup>	<0.0001 <sup>§*</sup>	0.040 <sup>§*</sup>

COex: exhaled carbon monoxide; ppm: parts per million; STT: saccharin transit time; min: minutes; HR: heart rate; bpm: beats per minute; SDNN: standard deviation of the mean of all normal RR intervals, expressed in milliseconds; RMSSD: root mean square of the successive differences between the adjacent normal RR intervals in a time interval, expressed in milliseconds; LF: low frequency; HF: high frequency; ms: milliseconds; un: normalized units; SD1: standard deviation of instantaneous beat-to-beat variability; SD2: standard deviation of long-term variability.

<sup>†</sup>: Mann Whitney Test

<sup>‡</sup>: Student *t*-test for independent samples.

<sup>§</sup>: Wilcoxon Test

<sup>§</sup>: Student *t*-test for paired samples.

\*Statistically significant difference (p<0.05).

Saccharine transit time correlated positively with the LFsms<sup>2</sup> index (r= 0.520; p=0.008) in the maximal exercise for the smoking group. The control group presented a negative correlation between STT and the SDNN

post-exercise indexes (r= -0.833; p<0.0001), RMSSD (r= -0.614; p=0.15), HFms<sup>2</sup> (r= -0.645; p=0.009), SD1 (r= -0.623, p=0.013) and SD2 (r= -0.832; p<0.0001) after maximal exercise.

## DISCUSSION

The results of the study showed acceleration of nasal mucociliary clearance in smokers and non-smokers after a submaximal and maximal exercise. Heart rate variability in the submaximal exercise, in both groups, showed a reduced RR interval and increased HR. In the non-smoking group, reductions in RMSSD, HFms<sup>2</sup> and SD1 indexes were also observed and the LFun indexes and LF/HF ratio increased. The magnitude of response between the groups in this stimulus was not different. In the maximum exercise, however, both groups presented alterations in HRV indexes, characterized by decreased parasympathetic modulation and increased HR, LFun and LF/HF ratio. In addition, a positive correlation was observed between STT and the LFms<sup>2</sup> index in the smoking group and a negative correlation between STT and the SDNN, RMSSD, HFms<sup>2</sup>, SD1 and SD2 indexes in the non-smoking group after maximal exercise.

The acceleration of MC in both groups after exercise corroborates with other studies<sup>10,11,24,30</sup>. Ramos et al.<sup>24</sup> compared the behavior of MC in smokers in three stimuli (isolated moderate aerobic exercise – 60 to 70% VO<sub>2</sub> max –, moderate aerobic exercise combined with smoking and smoking) and verified that all stimuli accelerated MC compared to rest. Other authors also found acceleration of MC in exercise, but in healthy individuals<sup>10,11</sup>.

The accelerated mucociliary response to physical exercise seems to be influenced by the autonomic nervous system (ANS), because pulmonary hyperventilation stimulates the central receptors (chemoreceptors) that, in turn, stimulate the autonomic activity of the sympathetic branch and, consequently, increase catecholamine levels (epinephrine and norepinephrine) that accelerate the ciliary beat<sup>10,31</sup>. At the same time, this hyperventilation promotes cortical irradiation on the bulbar region that results in progressive vagal removal<sup>32</sup>, potentiating sympathetic action on the ciliary beat and reducing saccharine transit time<sup>10</sup>.

After submaximal and maximal exercise, both smokers and non-smokers presented reduction of RR intervals (ms) and increased HR, indicating that the heart rate of these individuals remains high in the analyzed post-exercise period. In the submaximal exercise, only the control group showed a significant decrease in the RMSSD, HFms<sup>2</sup> and SD1 indexes in the post-exercise period (compared with rest), suggesting that these individuals had not yet had recovered from parasympathetic modulation after

exercise. This behavior was not observed in the smoking group, which, except for the RR intervals, did not present significant differences in heart rate variability in the same condition, indicating that these individuals were recovered from the autonomic point of view. These results may be related to the lower intensity performed in the 6MWT by smokers, because in participants could choose their own pace, unlike the maximal test, in which a speed and inclination are imposed until maximum effort. Thus, the recovery of cardiac autonomic modulation is faster in the submaximal work with lower intensity, which may have happened before the end of the nasal MCC evaluation in this group.

In the maximum exercise, both groups presented autonomic responses that indicate that they had not yet recovered. During high intensity exercises, recovery is slower because the changes induced in exercise are more intense<sup>33,34</sup>, justifying the presented responses and allowing the evaluation of their influence on nasal mucociliary clearance.

In addition, a positive correlation was observed between the STT and the LFms<sup>2</sup> index in the smoking group and a negative correlation between STT and SDNN, RMSSD, HFms<sup>2</sup>, SD1 and SD2 indexes in the non-smoking group after maximal exercise. Such alterations demonstrate the influence of the sympathetic and parasympathetic branches of the ANS on the ciliary beat after maximal exercise in both groups. This influence was not observed in submaximal exercise, because this type of exercise has less effect on autonomic modulation, so the increase in STT may have been in parts caused by mechanical effects of pulmonary hyperventilation<sup>14</sup>. Santos et al.<sup>30</sup> found similar results, not associating autonomic modulation and mucociliary function in smokers.

As a limitation of the study, we can highlight the lack of evaluation of MC at different moments after exercise, which could help the better understanding of the action of autonomic modulation and pulmonary hyperventilation on the nasal ciliary beat. Another limitation was the absence of an electrocardiogram that would ensure that the individuals evaluated did not present arrhythmias. However, all necessary care was taken when measuring the heart rate variability that originated the RR interval series: exclusion of series with more than 5% error, filtering and visual inspection of the series, which made them more reliable. So, even if the individuals presented some arrhythmia, it did not influence the results.

The present study has important clinical relevance because it suggests that the intensity of aerobic exercise

influences the response of nasal MC in smokers and nonsmokers, increasing the efficiency of mucociliary function in the post-exercise period, which leads to a more efficient pulmonary defense mechanism against respiratory infections and harmful agents to the respiratory tract. Besides, the results also showed that the intensity of the exercise is related to the participation of the ANS.

## CONCLUSION

We concluded that regardless of the intensity of aerobic exercise, there is an increase in nasal MC in smokers. However, this alteration seems to be influenced by the autonomic system only in maximum exercise.

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