Influence of Smoking Consumption and Nicotine Dependence Degree in Cardiac Autonomic Modulation

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Abstract

Background: Smoking consumption alters cardiac autonomic function.

Objective: Assess the influence of the intensity of smoking and the nicotine dependence degree in cardiac autonomic modulation evaluated through index of heart rate variability (HRV).

Methods: 83 smokers, of both genders, between 50 and 70 years of age and with normal lung function were divided according to the intensity of smoking consumption (moderate and severe) and the nicotine dependency degree (mild, moderate and severe). The indexes of HRV were analyzed in rest condition, in linear methods in the time domain (TD), the frequency domain (FD) and through the Poincaré plot. For the comparison of smoking consumption, unpaired t test or Mann-Whitney was employed. For the analysis between the nicotine dependency degrees, we used the One-way ANOVA test, followed by Tukey’s post test or Kruskal-Wallis followed by Dunn’s test. The significance level was p < 0,05.

Results: Differences were only found when compared to the different intensities of smoking consumption in the indexes in the FD. LFun (62.89 ± 15.24 vs 75.45 ± 10.28), which corresponds to low frequency spectrum component in normalized units; HFun (37.11 ± 15.24 vs 24.55 ± 10.28), which corresponds to high frequency spectrum component in normalized units and in the LF/HF ratio (2.21 ± 1.47 vs 4.07 ± 2.94). However, in the evaluation of nicotine dependency, significant differences were not observed (p > 0.05).

Conclusion: Only the intensity of smoking consumption had an influence over the cardiac autonomic modulation of the assessed tobacco smokers. Tobacco smokers with severe intensity of smoking consumption presented a lower autonomic modulation than those with moderate intensity. (Arq Bras Cardiol. 2016; 106(6):510-518)

Keywords: Smoking; Tobacco Use / complications; Tobacco Use Disorders.

Introduction

It is known that smoking is considered a serious public health problem with high incidence worldwide. It is estimated that there are 1.3 billion tobacco smokers in the world.¹ Therefore, the consequences of the use of tobacco have, in the last few years,² aroused the attention of researchers. The chronic use of tobacco creates tobacco-related diseases, the most common of which being related to the respiratory system.¹ However, it is clear that smoking has an important extrapulmonary toxicity,³ which could represent serious risk factors for cardiovascular diseases and their respective complications, such as the damage of cardiac autonomic modulation.⁴,⁵

The changes that smoking causes in the cardiac autonomic modulation are thoroughly described in literature⁶,⁷ and can be evaluated through the heart rate variability (HRV),⁸ a non-invasive method, which describes the fluctuations between consecutive heartbeats.⁹ Eryonucu et al.⁸ found that smokers present lower rates of HRV, a result that is similar to those found by Barutcu et al.⁷ when assessing the HRV during controlled breathing exercises and muscle strength tests.

The intensity of smoking consumption, assessed by the number of cigarettes consumed per day, may influence the severity of the alterations observed in the autonomic modulation. Kupari et al.¹⁰ verified that individuals that smoked ten or more cigarettes per day presented greater impairment in cardiac autonomic modulation as compared to those who smoked less. Additionally, the risk of death for smokers increases according to the number of cigarettes smoked per day and the years of smoking.
The intensity of smoking consumption is strongly associated with the level of nicotine dependency, often times seen as the main determinant of the frequent use of cigarettes to avoid withdrawal symptoms.\textsuperscript{11,12} As a consequence of this more intense habit, the damages caused by smoking take bigger proportions.\textsuperscript{12,13}

In spite of its importance, research in pertinent literature did not find studies that addressed the influence of nicotine dependency levels and smoking consumption in cardiac autonomic modulation. This represents a significant gap in the literature, considering that information of this nature could give smokers a more complete orientation on the importance of early cessation of this habit, as well as add elements of the exposed theme to the literature.

In this context, the objective of this study is to evaluate the influence of the intensity of smoking consumption and nicotine dependency degree on cardiac autonomic modulation through the index of HRV.

**Methods**

**Population**

Observational, cross-sectional study, in which 83 smokers were evaluated, determined by sample size calculation, with the LF/HF ratio as its variable. The magnitude of assumed significant difference was 1,8, considering a standard deviation of 1,19, based on a pilot study conducted with 80% beta-risk. The sample size, per evaluated group, resulted in 16 individuals of both genders, between 50 and 70 years of age, with normal lung function evidenced by spirometry. These individuals participated in a cessation program called PROCAT (Program of Anti-Tobacco Orientation and Awareness) of the University of Science and Technology Faculdade de Ciências e Tecnologias – FCT/UNESP, whose objective is the treatment of smokers through cognitive-behavioral and drug therapy.\textsuperscript{13}

This study did not include individuals who used narcotics or medications that influenced cardiac autonomic activity, alcoholics, or individuals with known diseases such as infections, metabolic or cardiorespiratory diseases. The flowchart of study losses is presented in Figure 1.

The volunteers were properly informed of the procedures and objective of this study. After agreement, they signed an informed consent to be part of the possible sample. This research was submitted to the appreciation of the Ethics Committee FCT/UNESP and by approved them (process n° 18/2011). All procedures were in accordance with Resolution 466/2012 of the National Health Council.

**Experimental Protocol**

The protocol was carried out in the morning in order to soften the influences of the circadian rhythm, in a room with a controlled temperature of 23°C and relative air humidity between 50 and 60%. Before the evaluation, the individuals were asked to abstain from smoking, caffeine and physical activities for 12 hours prior to the execution of the protocol. The confirmation of the period of smoking abstinence was done through the uptake of carbon monoxide levels in exhaled air by using the Micro CO monoximeter (Micro Medical Limited, Rochester, England); values of under six parts per million (ppm) were considered to be abstinent.\textsuperscript{14}

![Figure 1 – Flowchart of study losses.](image-url)
On the first day of the protocol, the characterization of the population was initially carried out through interviews with the volunteers to gather personal information, smoking habits (cigarettes per day and years of smoking to calculate packs/year)\(^{15}\) and degree of nicotine dependency. The characterization was concluded with the Fagerström questionnaire, which made it possible to separate the smokers into groups.

To separate the smokers according to their smoking consumption, the rate of packs/year was calculated by dividing the number of cigarettes smoked daily by 20 (number of cigarettes in a pack) and then multiplying that number by the years of smoking.\(^{13}\) Smokers were considered moderate when their smoking habits were between 10 and 20 packs per years and severe when that number surpassed 20 packs/year.\(^{16}\)

Within the same degree of nicotine dependency, however, smokers were divided according to their scores in the Fagerström questionnaire, which consists of six questions that address some of the smoking habits such as the time of the first cigarette of the day, number of cigarettes throughout the day, discomfort for not being able to smoke in places where it is prohibited, satisfaction from smoking, frequency of smoking in the morning and illness occurrences. Each of these alternatives receives a score which allows the rating of three degrees of dependency: mild (0 to 3 points), moderate (4 to 6 points) and severe (7 to 10 points).\(^{17}\)

Still on the first day of the protocol, anthropometric data was measured: weight (digital anthropometric scale W110 H – Welmy) and height (Stadiometer Standard Sanny) to calculate the Body Mass Index (BMI), and finally the lung function was calculated by using a portable spirometer (MIR – Spirobank – Italy) connected to a microcomputer.

Characteristics of individuals and lung function

Table 1 presents the personal, anthropometric and spirometric data of the smokers, separated according to intensity of smoking consumption. The groups were similar in relation to BMI and lung function. Statistically significant differences were found between moderate and severe smokers when the groups were compared by age, cigarettes smoked per day, years of smoking and packs/year.
Table 1 – Characterization of smokers divided according to intensity of smoking consumption in relation to age, BMI, spirometric values and smoking habits, expressed in mean ± standard deviation and median [Interquartile interval 25 – 75%]

| Variables                        | Moderate smokers | Severe smokers | p     |
|---------------------------------|------------------|----------------|-------|
| N                               | 34               | 49             |       |
| **Anthropometry**               |                  |                |       |
| Gender (M/F)                    | (7/27)           | (29/20)        |       |
| Age (years)                     | 52.00 [50.00 – 56.25] | 56.00 [52.00 – 59.00] | 0.0213 |
| BMI (kg/m²)                     | 26.46 ± 4.84     | 26.12 ± 4.72   | 0.7673 |
| **Spirometric values**          |                  |                |       |
| FEV₁ (% Pred)                   | 96.52 [86.00 – 104.30] | 95.07 [90.58 – 99.89] | 0.9112 |
| FVC (% Pred)                    | 99.26 ± 12.47    | 97.81 ± 8.18   | 0.5851 |
| FEV₁/FVC                        | 102.50 [87.14 – 108.80] | 97.08 [91.14 – 103.20] | 0.9560 |
| FEF₂₅-₇₅% (% Pred)              | 91.74 ± 22.93    | 96.97 ± 29.23  | 0.4571 |
| **Smoking consumption history** |                  |                |       |
| Time of smoking (years)         | 28.79 ± 7.85*    | 38.31 ± 7.46   | < 0.0001 |
| Cigarettes/day                  | 29.00 [20.00 – 35.50] | 38.00 [33.00 – 42.50] |       |
| Packs/year                      | 12.82 ± 4.59*    | 22.55 ± 6.77   | < 0.0001 |
|                                | 10.00 [10.00 – 16.25] | 20.00 [20.00 – 20.00] |       |
|                                | 17.05 ± 3.30*    | 42.74 ± 13.34  | < 0.0001 |
|                                | 16.63 [14.75 – 20.00] | 40.00 [30.75 – 50.00] |       |

N: number of volunteers; M: male; F: female; BMI: body mass index; kg: kilogram; - m: meter; FEV₁: forced expiratory volume in the first second; FVC: forced vital capacity; FEV₁/FVC: ratio between FEV₁ and FVC; FEF₂₅-₇₅%: forced expiratory flow between 25 and 75% of FVC; (*) Statistically significant difference in comparison to severe smokers.

Table 2 presents the personal, anthropometric and spirometric data of the smokers, separated according to nicotine dependency. The groups were similar as related to age, BMI and lung function. In the Fagerström questionnaire, according to the score obtained, there was statistically significant difference between the groups only in relation to nicotine dependency.

Indexes of HRV of smokers according to the intensity of smoking consumption and degree of nicotine dependency

Table 3 depicts the indexes of HRV of the smokers, divided according to the intensity of smoking consumption. Statistically significant differences were found in the LF and HF indexes un, LF/HF ratio, and SD1/SD2 ratio.

Table 4 depicts the HRV indexes of the smokers divided into groups, according to the degree of nicotine dependency. No significant differences were found in the analysed indexes.

Qualitative analyses of the Poincaré plot

The qualitative analyses of the Poincaré plot is expressed in figures 2 and 3, which show standard examples of the plot in smokers that presented SD1 and SD2 index values close to the mean, according to the intensity of smoking consumption and the degree of nicotine dependency, respectively.

Discussion

The present study evaluated the influence of smoking consumption and degree of nicotine dependency over cardiac autonomic modulation of smokers by using HRV indexes. The main results showed that smoking consumption alone had influence over the cardiac autonomic modulation of the assessed smokers. In the indexes that describe the HRV in the FD, the LFun index and the LF/HF ratio were increased in severe smokers, as opposed to the HFun index, which was significantly smaller in this group. This characterizes a sympathetic predominance in severe smokers, in comparison...
Table 2 – Characterization of smokers divided according to nicotine dependency in relation to age, BMI, spirometric values and score in the Fagerström questionnaire, expressed in mean ± standard deviation and median [Interquartile interval 25 – 75%]

| Variables                  | Mild smokers | Moderate smokers | Severe smokers | p      |
|----------------------------|--------------|------------------|----------------|--------|
| N                          | 18           | 33               | 32             |        |
| **Anthropometry**          |              |                  |                |        |
| Gender (M / F)             | (5 / 13)     | (15 / 18)        | (16 / 16)      |        |
| Age (years)                | 56.06 ± 5.63 | 55.36 ± 3.75     | 54.41 ± 4.87   | 0.4043 |
| BMI (kg/m²)                | 25.19 ± 5.03 | 26.90 ± 4.94     | 26.22 ± 4.38   | 0.4977 |
| **Spirometric values**     |              |                  |                |        |
| FEV₁ (% Pred)              | 93.21 [83.47 – 104.30] | 94.10 [88.43 – 98.65] | 98.12 [92.42 – 105.80] | 0.1629 |
| FVC (% Pred)               | 98.30 ± 11.98 | 97.11 ± 10.67    | 99.86 ± 8.41   | 0.6472 |
| FEV₁/FVC%                  | 77.08 ± 5.27 | 77.82 ± 6.12     | 79.85 ± 4.99   | 0.2816 |
| FEF₂₅₋₇₅% (% Pred)         | 76.60 [73.75 – 80.60] | 77.20 [73.45 – 82.75] | 79.60 [77.00 – 83.60] | 0.0586 |
| **Smoking Dependency**     |              |                  |                |        |
| Fagerström (SCORE)         | 2.66 ± 0.84† | 5.48 ± 0.61*     | 7.84 ± 1.01    | < 0.0001 |
|                           | 3.00 [3.00 – 3.00] | 6.00 [5.00 – 6.00] | 7.50 [7.00 – 8.75] |        |

N: number of volunteers; M: male; F: female; BMI: body mass index; kg: kilogram; m: meter; FEV₁: forced expiratory volume in the first second; FVC: forced vital capacity; FEV₁/FVC: ratio between FEV₁ and FVC; FEF₂₅₋₇₅: forced expiratory flow between 25 and 75% of FVC. (*) Statistically significant difference in comparison to moderate smokers. (†) Statistically significant difference in comparison to severe smokers. In this study, the studied population is considered between adults and seniors, between 50 and 70 years old, which may justify, in part, the obtained results in the analysed HRV indexes. The results show that the participants in the severe smokers group, separated by the intensity of smoking consumption, were older than the ones in the moderate smokers group. Literature shows that there is an influence of age in the autonomic modulation, that is, the older the individual, the higher the sympathetic action that can be observed; so this factor may have influenced the observed results. 31,32 Age is an important determinant in autonomic modulation, with aging being associated to a progressive cardiac vagal decline as age advances, 31 which may be considered a limitation in the present study. Paschoal et al. 32 found a reduction of the indicative values of parasympathetic activity and an increase in cardiac sympathetic activity, as from the 5th decade of life, in healthy individuals, when compared to younger individuals. Hering et al. 33 showed that the autonomic responses depend on age in smokers as well and may result from alterations in the responses of the adrenal medulla, reduced clearance of norepinephrine and/or inhibition of the process of norepinephrine reabsorption, caused by chronic exposure to smoking.

to moderate smokers. Carcigi et al. 29 found an enlarged LF/HF ratio in smokers with a consumption of over 20 cigarettes/day in comparison to non-smokers. Baructu et al. 7 observed that the length of smoking consumption showed a positive correlation with the LF/HF ratio, which characterizes a smaller vagal modulation and larger sympathetic modulation the longer the length of smoking consumption.

In the quantitative of the Poincaré plot, the SD1/SD2 ratio, which represents the ratio between the long and short-term variations of records of RR intervals, was significantly larger in moderate smokers, who, when compared to severe smokers, had better HRV.

The qualitative Plot analysis did not show differences in the dispersion of RR intervals. However, the analyses of the plot of the different degrees of nicotine dependency showed that mild and moderate smokers present larger RR intervals when compared to severe smokers, but without significant differences. Reduced RR intervals, like the ones found in severe smokers, suggest a higher HR in resting in these individuals, which may be more predisposed to the surging of cardiovascular events. 30 The HR may have a direct effect on the cardiovascular system, because it increases myocardial consumption of oxygen and induces fatigue, in addition to being associated with higher pressure levels. 30
Table 3 – HRV indexes evaluated in the different groups of smokers according to the intensity of smoking consumption expressed in mean ± standard deviation and median [Interquartile interval 25 – 75%]

| Variables | Moderate smokers | Severe smokers | p  |
|-----------|------------------|----------------|----|
| N         | 34               | 49             |    |
| RR (ms)   | 819.40 ± 173.00  | 831.40 ±145.50 | 0.6467 |
| SDNN (ms) | 30.47 ± 12.77    | 31.20 ± 13.79  | 0.5943 |
| RMSSD (ms)| 29.00 [22.75 – 34.25] | 31.00 [20.00 – 41.50] |    |
| LFms²     | 77.82 ± 115.20   | 104.20 ± 138.40 | 0.3617 |
| HFms²     | 39.68 ± 49.93    | 32.69 ± 43.95  | 0.0776 |
| LFun      | 62.89 ± 15.24*   | 75.45 ± 10.28  | < 0.0001 |
| HFun      | 37.11 ± 15.24*   | 24.55 ± 10.28  | < 0.0001 |
| LF/HF     | 2.21 ± 1.47*     | 4.07 ± 2.94    | 0.0002 |
| SD1 (ms)  | 16.99 ± 6.85     | 15.12 ± 7.90   | 0.1473 |
| SD2 (ms)  | 23.50 [14.25 – 48.00] | 16.00 [7.00 – 39.00] |    |
| SD1/SD2   | 0.38 ± 0.13*     | 0.31 ± 0.11    | 0.0204 |

N: number of volunteers; ms: milliseconds; SDNN: Standard Deviation of Normal to Normal intervals; RMSSD: Root Mean Square of Successive Differences; LF: low frequency; un: normalized unit; HF: high frequency; SD1: standard deviation of the instantaneous beat to beat variability; SD2: standard deviation of the long-term continuous R-R intervals; [*] Statistically significant difference in comparison to severe smokers.

The biggest chronicity of smoking was shown to be related to lower vagal activity and higher sympathetic activity, as verified in other studies, which characterize the decrease of HRV indexes in smokers. The reduction of HRV may be associated to health damages, and is a concerning factor associated to the increase in mortality and morbidity in several conditions.

No differences were found in the cardiac autonomic modulation of the evaluated smokers, when comparing different degrees of nicotine dependency. This non-difference may support the evidence that personality traits may be more strongly associated to the dependency than the smoking itself. Some authors are investigating the association between nicotine dependency and psychiatric disturbances such as depression, anxiety, schizophrenia, among others. Such evidence may appear from the assumption that, in the Fagerström questionnaire, only one question addresses the quantity of cigarettes smoked, per day, by the individual, while the others are related to his/her behavior.

As a limitation of the study, the lack of a control group consisting of non-smoking individuals, and of tests to detect asymptomatic heart diseases may be pointed out. These factors could have contributed to a better understanding of the obtained results.

Conclusion

Only the intensity of smoking consumption had influences over cardiac autonomic modulation of the evaluated smokers. Smokers with severe smoking consumption intensity presented worse autonomic modulation than moderate ones.

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Author contributions

Conception and design of the research: Santos APS, Ramos D, Ito JT, Vanderlei LCM, Ramos EMC; Acquisition of data: Santos APS, Oliveira GM, Santos AAS, Freire APCF; Analysis and
Table 4 – Indexes of HRV evaluated in the different groups of smokers according to the degree of nicotine dependency expressed in mean ± standard deviation and median [Interquartile interval 25 – 75%]

| Variables | Mild smokers | Moderate smokers | Severe smokers | p     |
|-----------|--------------|------------------|----------------|-------|
| N         | 18           | 33               | 32             |       |
| RR (ms)   | 844.70 ± 82.51 | 840.5 ± 171.40   | 811.80 ± 179.10 | 0.6632|
| SDNN (ms) | 29.78 ± 11.10 | 32.18 ± 14.16    | 30.69 ± 13.57   | 0.9287|
| RMSSD (ms)| 30.00 [24.75 – 36.00] | 31.00 [22.50 – 40.00] | 29.00 [20.50 – 40.25] |       |
| LFms²     | 21.78 ± 7.44  | 23.68 ± 10.16    | 20.82 ± 12.10   | 0.3369|
| HFms²     | 75.22 ± 56.72 | 108.20 ± 172.90  | 88.72 ± 105.20  |       |
| LFun      | 56.50 [21.75 – 131.00] | 50.00 [24.00 – 101.50] | 45.00 [24.25 – 118.80] | 0.9648|
| HFun      | 28.11 ± 21.93 | 41.12 ± 43.32    | 34.31 ± 56.25   | 0.2748|
| LF/HF     | 22.00 [14.25 – 36.50] | 24.00 [8.50 – 56.00] | 15.50 [8.25 – 36.50] |       |
| SD1 (ms)  | 66.72 ± 11.07 | 68.81 ± 14.61    | 73.72 ± 14.35   |       |
| SD2 (ms)  | 68.35 [60.13 – 75.80] | 71.10 [60.45 – 80.05] | 78.30 [65.53 – 83.00] | 0.0630|
| SD1/SD2   | 33.28 ± 11.07 | 31.19 ± 14.61    | 26.28 ± 14.35   |       |

N: number of volunteers; ms: milliseconds; SDNN: Standard Deviation of Normal to Normal intervals; RMSSD: Root Mean Square of Successive Differences; LF: low frequency; un: normalized unit; HF: high frequency; SD1: standard deviation of the instantaneous beat to beat variability; SD2: standard deviation of the long-term continuous R-R intervals.

Figure 2 – Qualitative analysis of the Poincaré plot in the different intensities of smoking consumption: moderate (individual A – SD1: 16,9 and SD2: 47) and severe (individual B – SD1: 15,2 and SD2: 50,4).
Figure 3 – Qualitative analysis of the Poincaré plot in the different degrees of nicotine dependency: mild (individual A – SD1: 16.9 and SD2: 47), moderate (individual B – SD1: 17.8 and SD2: 52.8) and severe (individual C – SD1: 15.4 and SD2: 46.7).

interpretation of the data: Santos APS, Santos AAS, Freire APCF; Statistical analysis: Santos APS; Obtaining financing: Ramos EMC; Writing of the manuscript: Santos APS; Critical revision of the manuscript for intellectual content: Ramos D, Freire APCF, Ito JT, Fernandes RPP, Vanderlei LCM, Ramos EMC.

Potential Conflict of Interest
No potential conflict of interest relevant to this article was reported.

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References
1. Saleheen D, Zhao W, Rasheed A. Epidemiology and public health policy of tobacco use and cardiovascular disorders in low- and middle-income countries. Arterioscler Thromb Vasc Biol. 2014;34(9):1811-9.
2. Prado GF, Lombardi EM, Morais AM, Martins SR, Santos Ude P Smoking: what has been addressed in Brazilian journals. Arq Bras Cardiol. 2012;99(6):e184-90. Erratum in: Arq Bras Cardiol. 2013;100(5):488.
3. Yanbaeva DG, Dentener MA, Creutzberg EC, Wesseling G, Wouters EF. Systemic effects of smoking. Chest. 2007;131(5):1557-66.
4. Marziano BM, Vanderlei LC, Ramos EM, Ramos D. Smoking implications on cardiac autonomic control. Arq Ciênc Saúde. 2010;17(2):97-101.
5. Middlekauff HR, Park J, Moheiman RS. Adverse effects of cigarette and non-cigarette smoke exposure on the autonomic nervous system: mechanisms and implications for cardiovascular risk. J Am Coll Cardiol. 2014;64(16):1740-50.
6. Eryonucu B, Bilge M, Guler N, Uzun K, Gencer M. Effects of cigarette smoking on the circadian rhythm of heart rate variability. Acta Cardiol. 2000;55(5):301-5.
7. Barutcu I, Emen AM, Kaya D, Turkmen M, Karakaya O, Melek M, et al. Cigarette smoking and heart rate variability: dynamic influence of parasympathetic and sympathetic maneuvers. Ann Noninvasive Electrocardiol. 2005;10(3):324-9.
8. Rajendra Acharya U, Paul Joseph K, Kannathal N, Lim CM, Suri JS. Heart rate variability: a review. Med Bio Eng Comput. 2006;44(12):1031-51.
9. Vanderlei LC, Pastre CM, Hoishi RA, Carvalho TD, Godoy MF. Basic notions of heart rate variability and its clinical applicability. Rev Bras Cir Cardiovasc. 2009;24(2):205-17.
10. Kupari M, Virolainen J, Koskinen P, Tikkanen MJ. Short-term heart rate variability and factors modifying the risk of coronary artery disease in a population sample. Am J Cardiol. 1993;72(12):897-903.
11. Shiffman S, Ferguson SG, Dunbar MS, Scholl SM. Tobacco dependence among intermittent smokers. Nicotine Tob Res. 2012;14(11):1372-81.

12. Park S, Lee JY, Song TM, Cho SJ. Age-associated changes in nicotine dependence. Public Health. 2012;126(6):482-9.

13. Freire AP, Ramos D, Silva BS, David RM, Pestana PR, Fernandes RA, et al. Results of smoking cessation program: analysis of new procedures. CorScienciaesSaúde. 2014;13(3):396-404.

14. Santos UP, Gannam S, Abe JM, Esteves PB, Filho MF, Wakassa TB, et al. Emprego da determinação de monóxido de carbono no ar exalado para a detecção do consumo de tabaco. J Pneumol. 2001;27(5):231-6.

15. Sociedade Brasileira de Pneumologia e Tisiologia. Diretrizes para testes de função pulmonar. J Pneumol. 2002;28(supl. 3):1-221.

16. Nagelmann A, Tonnov Ä, Laks T, Sepper R, Prikk K. Lung dysfunction of chronic smokers with no signs of COPD. COPD. 2011;8(3):189-95.

17. Fagerström K, Russ C, Yu C-R, Yunis C, Foulds J. The Fagerström Test for Nicotine Dependence as a predictor of smoking abstinence a pooled analysis of verencline clinical trial data. Nicotine Tob Res. 2012;14(12):1467-73.

18. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al; ATS/ERS Task Force. Standardization of spirometry. Eur Respir J. 2005;26(2):319-38.

19. Neder JA, Andreoni S, Castelo-filho A, Nery LE. Reference values for lung function tests. I. Static volumes. Braz J Med Biol Res. 1999;32(6):703-17.

20. Gamelin FX, Berthoin S, Bosquet L. Validity of the polar S810 heart rate monitor to measure R-R intervals at rest. Med Sci Sports Exerc. 2006;38(5):807-93.

21. Vanderlei LC, Pastre CM, Azevedo FM, Godoy MF. Comparison of the polar S810i monitor and the ECG for the analysis of heart rate variability in the time and frequency domains. Braz J Med Biol Res. 2008;41(10):854-9.

22. Tarvainen MP, Niskanen JP, Ranta-aho PO, Karjalainen PA. Kubios HR-heart rate variability analysis software. Comput Methods Programs Biomed. 2014;113(1):210-20.

23. Ribeiro JP, Moraes Filho RS. Heart rate variability as a tool for the investigation of the autonomic nervous system. Rev Bras Hipertens. 2005;12(1):14-20.

24. Rassi Jr A. Compreendendo melhor as medidas da análise de variabilidade da frequência cardíaca. J Diag Cardiol. 2001;8ª. ed. [Citado em 2013 nov 15]. Disponível em http://www.cardios.com.br/jornal-01/tese%20completa.htm