Vitamin C analysis and nutritional status of children and adolescents exposed to secondhand smoke

Análise de vitamina C e estado nutricional de crianças e adolescentes expostos ao fumo passivo

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ABSTRACT
The aim of the present study was to analyze ascorbic acid in passive smokers, considering the groups most vulnerable to the exposure of cigarette smoke compounds, i.e., children and adolescents. We quantified of plasma vitamin C levels in passive smokers through blood collection for later analysis. We also recorded nutritional and socioeconomic data in addition to tobacco smoke exposure. There were 33 participants, divided into a group of passive smokers (PS = 16 participants) and a control group (C = 17 participants). Vitamin C levels and reactive oxygen species content were similar in PS and C groups. The secondhand smoke exposure frequency was 5±3.16 hours/day and the biomarker of exposure, carboxyhemoglobin, was an average of 3.69±0.23% in the PS group, slightly above the maximum biological index allowed for non smoking individuals. Food intake of vitamin C was satisfactory in both groups, PS and C. The present study verified that there was no interference from exposure to secondhand smoke on plasma concentrations of ascorbic acid in the individuals studied, because adequate consumption of dietary sources of vitamin C may have helped to maintain satisfactory plasma levels.

Keywords: Passive smoke, Nutritional evaluation, Antioxidant, Free radicals.

RESUMO
O objetivo do presente estudo foi analisar o ácido ascórbico em fumantes passivos, considerando os grupos mais vulneráveis à exposição a compostos de fumaça de cigarro, ou seja, crianças e adolescentes. Foi realizado a quantificação dos níveis plasmáticos de vitamina C em fumantes passivos através da coleta de sangue para análise posterior. Também foram registrados dados nutricionais e socioeconômicos, além da exposição à fumaça do tabaco. Havia 33 participantes, divididos em um grupo de fumantes passivos (FP = 16 participantes) e um grupo controle (C = 17 participantes). Os níveis de vitamina C e o teor de espécies reativas de oxigênio foram semelhantes nos grupos FP e C. A frequência de exposição ao fumo passivo foi de 5 ± 3,16 horas / dia e o biomarcador de exposição, carboxihemoglobina, foi uma média de 3,69 ± 0,23% no grupo FP, um pouco acima do índice biológico máximo permitido para indivíduos não fumantes. A ingestão alimentar de vitamina C foi satisfatória nos dois grupos, FP e C. O presente estudo verificou que não houve interferência da exposição ao fumo passivo nas concentrações plasmáticas de ácido ascórbico nos indivíduos estudados, pois o consumo adequado de fontes alimentares de vitamina C pode ajudaram a manter níveis plasmáticos satisfatórios.

Palavras-chave: Fumo Passivo, Avaliação nutricional, Antioxidantes, Radicais livres.
INTRODUCTION

Data from the National Cancer Institute (INCA) indicate that the Unified Health System (SUS) and Social Security spend about R$ 37 million annually on diseases and deaths caused by passive smoking [1]. According to estimates by the World Health Organization (WHO), 40% of children worldwide are exposed to environmental tobacco smoke (ETS). As this population spends much time indoors, exposure to ETS occurs particularly at home, at school, in daycare settings, in family homes and in cars [2,3,4].

Tobacco is composed of approximately 4720 substances, of which 68 are carcinogens, in addition to a large number of irritants and intoxicants [5,6]. The smoke produced by burning the product contains oxidative and pro-oxidant substances that induce diseases by promoting and propagating increases in oxidative stress (OS). Due to cigarette consumption, the production of reactive oxygen species (ROS) exceeds the antioxidant capacity of the organism, compromising the antioxidant status of the individual [7].

Furthermore, the greater the number of people smoking in a house and the greater the number of cigarettes smoked by these people, the greater the risk of the child for acquiring respiratory diseases and acute infections, in addition to suffering from injuries [8,9]. Depending on the smoking environment and time of exposure, passive smokers may involuntarily inhale the equivalent of ten cigarettes per day [10,11].

Among the major diseases that can be developed by active smokers (cancer, tuberculosis, dental caries, cardiovascular problems, and many others), it has been verified that tobacco users have deficiencies of plasma vitamin C, also known as ascorbic acid [12,13]. This vitamin deficiency can be explained by the fact that ascorbic acid has antioxidant capabilities. In the case of active smokers, this micronutrient has its turnover altered by the compounds present in the cigarette, suggesting greater metabolic consumption; this is primarily because the vitamin acts on the inflammatory response triggered by smoke compounds, in addition to the effects of lower consumption of food sources and reduction in vitamin C bioavailability [14,15].

Studies related to vitamin C and passive smoking are scarce; therefore, in the present study, we analyzed ascorbic acid levels in passive smokers (PS), considering the groups most vulnerable to the exposure to the compounds in cigarette smoke.

MATERIALS AND METHODS

This was a quantitative study; inclusion of the individuals occurred after the signing of the terms of free and informed consent by the parents or relatives and terms of assent by underage
participants. The study was approved by the Research Ethics Committee of the Federal University of the Southern Frontier (UFFS), by means of a Certificate for Ethical Assessment (CAAE), number 48432215.4.0000.5564.

For the case group (PS Group - passive smokers), we included children and adolescents between 2 and 19 years of age exposed to secondhand smoke from the city of Pinhalzinho, located in the western part of the state of Santa Catarina, Brazil. Simultaneously, we enrolled children and adolescents from the same age group who were not exposed to secondhand smoke at home who were attending nurseries and schools in the selected municipality.

The techniques and instruments for data collection included the administration of a socioeconomic questionnaire prepared by the Brazilian Association of Population Studies—ABEP (2015) that estimates the purchasing power of individuals and families. A questionnaire was also applied to analyze the health status of the individuals, including questions regarding the time period of exposure to cigarette smoke, pathological disorders in general, life habits and medication use. The questionnaires were administered directly to the participant, except those under ten years of age.

For the evaluation of nutritional status, anthropometric measurements of weight and height were obtained using a digital scale, with a maximum capacity of 150 kg. The nutritional classification of children and adolescents was performed based on proposed growth curves (W/A), height for age (H/A), and body mass index for age (BMI/A) [16,17]. The evaluation of food consumption was made via administration of a food consumption frequency questionnaire including food sources of antioxidants, validated by Bemvenuti [18] that greater fidelity with respect to quantification of vitamin C.

After the aforementioned evaluations, blood collection was be performed, for determination of plasma vitamin C levels, carboxyhemoglobin (the biomarker of exposure to tobacco), and ROS. We collected a total of 5 ml by venipuncture at the Basic Health Unit of the study municipality. The collection tubes containing EDTA were centrifuged (10 min at 5000 x g) in order to obtain the plasma fraction, that was again centrifuged (10 min at 5000 x g) with equal volumes of 5% trichloroacetic acid.

Determination of plasma vitamin C levels was performed using the technique described by Galley [19] and adapted by Jaques-Silva [20]. This method generates an orange chromogen produced through the reaction of vitamin C with dinitrophenylhydrazine at 37°C that is measured spectrophotometrically at 520 nm. An ascorbic acid curve was used to calculate the vitamin C content, expressed as mg/dl. Carboxyhemoglobin quantification was performed based on the
Malheiro [21] technique, aimed at hemolysis and subsequent spectrophotometer reading at 420 nm and 432 nm. For the analysis of ROS production, the technique of Lebel, Ischiropoulos, and Bondy [22] was followed, based on the oxidation of 2′,7′-dichlorofluorescein diacetate by ROS present in the samples. The final reaction yields a fluorescent compound measured at 488 nm excitation and 525 nm emission. The results were expressed as nmol DCF/ml.

The biochemical analyses, with the exception of ROS, were carried out in the laboratory of the Federal University of the Southern Frontier—UFFS, Realeza Campus, PR, Brazil. The quantification of ROS occurred in the laboratory of the Federal University of Santa Maria—UFSM, RS, Brazil. The data collected were evaluated using Statistica software, version 11.0, and the graphs were generated using GraphPad Prism software version 7.0. For the comparison between the test group (group PS) and the control group (group C) regarding the nutritional evaluation, food consumption, plasma levels of vitamin C and generation of ROS, the Student's T-Test was used for independent samples. In order to verify the correlation between plasma vitamin C levels and nutritional assessment, food intake, secondhand smoke and ROS, a multiple linear regression test was applied. A value of \( p < 0.05 \) was considered statistically significant.

3 RESULTS

There were 33 participants, 16 in the passive smoke group (PS) and 17 in the control group (C). Regarding the age group, both groups had an average age of 15 years of age with standard deviation (SD) of ± 1.66 for the PS group and ± 0.68 for the control group. There were 37.5% (n = 6) males in the PS group and 31.25% (n = 5) males in the control group; correspondingly, females comprised 62.5% (n = 10) and 75% (n = 12), of the PS and C groups, respectively.

Table 1 displays the relevant characteristics for each study group. We observed that whites and urban dwellers predominated. Concerning the number of people living in the household, the PS group had maximum of three persons/residence and group C had maximum variation of four person/residence. Through the administration of the ABEP questionnaire (2015) we classified the level of education of the head of the family, because this data reflects the economic classification. In both groups, incomplete upper/lower secondary education was more common, with 31.25% in the PS group and 47.05% in the C group.

Socioeconomic classification showed a prevalence of 50% for the class of eight-fold minimum wage for the PS group, and 12.5% for the other wage classifications. Group C showed 29.41% for households with eight-fold the minimum wage and eight–ten-fold the minimum wage. Group C households earning two–four-fold minimum wage were 23.55% of all participants, and
households earning more than ten-fold the minimum wage were 11.76%. The average salary of up to two minimum wages was found in a minority of group C (5.88%).

Table 1. Sociodemographic characteristics of children and adolescents exposed and not exposed to secondhand smoke.

| Characteristics                        | Group PS | Group C |
|----------------------------------------|----------|---------|
|                                        | Mean ± SD | Mean ± SD |
| Age                                    | 15.37 ± 1.66 | 15.65 ± 0.68 |
| Gender                                 | n (%)    | n (%)   |
| Male                                   | 6 37.5    | 5 29.41 |
| Female                                 | 10 62.5   | 12 70.58 |
| Ethnicity                              | n (%)    | n (%)   |
| White                                  | 12 75     | 11 64.7 |
| Non-white                              | 4 25      | 6 35.29 |
| Residence Perimeter                    | n (%)    | n (%)   |
| Urban                                  | 14 87.5   | 13 76.47 |
| Rural                                  | 2 12.5    | 4 23.52 |
| N° people residing with subject        | Mean ± SD | Mean ± SD |
|                                        | 3 ± 0.83  | 3 ± 1.31 |
| Head of household’s level of education | n (%)    | n (%)   |
| Illiterate/1° not completed            | 6 62.5    | 1 5.88  |
| 1° completed/2° not completed          | 4 25      | 2 11.76 |
| 2° completed/Middle not completed      | 3 18.75   | 3 17.64 |
| Middle completed/Upper not completed   | 5 31.25   | 8 47.05 |
| Upper completed                        | 3 18.5    | 3 17.64 |
| Socioeconomic class                    | n (%)    | n (%)   |
| Up to 2-fold minimum wage             | 2 12.5    | 1 5.88  |
| 2–4 minimum wages                     | 2 12.5    | 4 23.52 |
| 4–8 minimum wages                     | 8 50      | 5 29.41 |
| 8–10 minimum wages                    | 2 12.5    | 5 29.41 |
| Above 10 minimum wages                | 2 12.5    | 2 11.76 |

According to the main objective of evaluating children and adolescents exposed to PS, Table 2 displays specific characteristics of this group. The biomarker of smoke exposure, carboxyhemoglobin (COHb), was present at an average of 3.69%. The maximum biological index allowed for non-smoking but exposed individuals is 1%–3.5%, a fact that confirms slight exposure to cigarette smoke; however, the level could pose health risks.

Regarding the number of cigarettes consumed per day, the average remained at 9 per day, and the reported maximum reached 20 per day. Regarding the period of time in hours/day in which the PS coexisted with the active smoker, the average was about 5 hours/day, reaching 8 hours/day.

Regarding the frequent smoking environments, the porch or veranda, the interior of the home or the work environment were mentioned, since most respondents justified the smoking habit withdrawn from the home environment in order not to affect the health of their relatives. The most-cited active smokers were uncles and grandparents, classified as ‘other family’. Nevertheless, with
respect to parents, the majority of smokers were male, usually found in the middle echelons of society.

Table 2. Specific characteristics of children and adolescents exposed to secondhand smoke.

| Characteristics                                      | Group PS         |
|------------------------------------------------------|------------------|
| COHb (%)                                             | 3.69 ± 0.23      |
| Nº cigarettes per day                                | 9 ± 5.81         |
| Frequency of smoke exposure (hours/day)              | 5 ± 3.16         |
| Frequent smoking environments                        |                  |
| Veranda/Porch                                        | 9 (56.25)        |
| Interior of the home                                 | 6 (37.5)         |
| Work                                                 | 1 (6.25)         |
| Smoking family member                                |                  |
| Other family                                         | 8 (50)           |
| Father                                               | 6 (37.5)         |
| Mother                                               | 2 (12.5)         |

As a way of assessing the nutritional status of both groups, weight and height data were collected, aiming to calculate and classify BMI for age, as prescribed by the WHO\textsuperscript{16, 17}. The classification result obtained for the control group and the PS group is shown in Figure 1. The PS group was the most eutrophic with 81.25\% (n = 13), and for the other classifications, n = 1 for leanness, overweight and obesity, all of which represented 6.25\%. For group C, eutrophy also prevailed with 88.25\% of the total sample (n = 15), in addition to 5.88\% (n = 1) for both leanness and obesity.

Figure 1. Anthropometric classification of children and adolescents exposed and not exposed to secondhand smoke.

The nutritional assessment also included plasma concentration of vitamin C (mg/dL), daily intake of ascorbic acid (mg/day) and, ROS was quantified in order to analyze the OS exposure to tobacco smoke.
Regarding the evaluation of dietary intake of vitamin C (Figure 2A), the questionnaire identified mean values of 73.23 mg/day for the PS group and 97.94 mg/day for group C, even higher than values recommended for this age group (39–63 mg/day) but within the maximum limit of intake. Among the foods with the highest consumption frequency were pineapple, bergamot/tangerine, orange, strawberry, papaya and lemon juice.

Figure 2. Food consumption of vitamin C (A) and serum vitamin C (B) in children and adolescents.

Based on the biochemical results of plasma vitamin C evaluation (Figure 2B), the control group had a mean ascorbic acid concentration of 1.95 mg/dL, and the PS group had a mean of 1.93 mg/dL. Both groups showed values within the recommended range (0.3–2.0 mg/dL), suggesting that exposure to smoke did not interfere with ascorbic acid levels.
Figure 3. Quantification of EROS in the blood of children and adolescents.

Regarding ROS quantification, no significant difference was found between the two groups. The mean values for the control group were 77.32 nmol DCF/mL and the PS group was 81.51 nmol DCF/mL (Figure 3). This suggests that smoke exposure did not promote an increase in ROS in the PS group.

4 DISCUSSION

We found a predominance of eutrophy in children and adolescents exposed and not exposed to tobacco smoke. This finding was similar to that of a study by Fontoura [23] who evaluated the nutritional status of Brazilian adolescents found eutrophic prevalence in the target population, expressed in 61.9% for females and 72.4% for males, followed by overweight in 33.3% and 13.8%, respectively, and obesity in 4.8% and 10.4%, respectively.

According to a study by Souza [24] carried out with Brazilian children and adolescents, most of the participants were eutrophic, 75.2% for boys and 75.7% for girls. Overweight and obesity were high. Compared to these studies, we observed that their data approximate those of the present study, however, the prevalences of overweight and obesity were lower, a fact that is positive in relation to the anthropometric profile found. The similarity found among the studies nevertheless suggests absence of a correlation between exposure to secondhand smoke and possible nutritional damage including malnutrition that is generally found in active smokers.

Purchasing power is related to the nutritional profile of the consumers, because family income correlates directly with the purchase of foodstuffs. A study by Bigaran [25] showed that better individual purchasing power correlated with greater variety of fruits and vegetables purchased; that is, income played a fundamental role in increasing the variety of products consumed. In the present study, the analysis of the socioeconomic profiles showed that in both groups, the prevalence of earning varied from 4–10-fold minimum wage. This favors direct access to the
purchase of healthier foods, including fruits and vegetables that present relevant values for purchase in the current market.

In relation to the smoke exposure analysis, Abu-Baker, Haddad, and Savage [26] evaluated the carboxyhemoglobin concentration in volunteer active smokers and adult PS; they found an average of 2%–3% of concentration in the PS group, higher than the maximum tolerance limit for toxic symptoms. Thus, in comparison to the present study, the biomarker exposure slightly exceeded the tolerance values, demonstrating that the studied group was exposed to tobacco smoke and was at risk for smoking-related diseases.

Due to the scarcity of studies similar to these, only two studies were close to the objective of the present study that aimed to evaluate the concentration of vitamin C in PS. In a study by Bui [27], the concentration of ascorbic acid in alveolar secretions of adult individuals was evaluated, and researchers did not show a statistical difference of vitamin C in comparison to active and passive smokers; in both groups, the concentrations were adequate relative to the reference values. However, the dietary intake was above the Recommended Dietary Allowance (RDA), with an average consumption of 109 mg/day for the PS group.

According to the objective of the study to relate vitamin C to exposure to secondhand smoke, by assessing the dietary intake of the participants, adequate plasma concentrations may be justified by consumption above the recommended level of the vitamin studied [28].

On the other hand, Tribble, Giuliano, and Fortmann [29], evaluated plasma ascorbic acid concentrations in adult nonsmokers regularly exposed to secondhand smoke and found a tendency to decrease vitamin C for the PS group in comparison to control group not exposed to smoke. However, the average cigarette consumption (20 per day) in their study was a double the value of the present study. In addition, the evaluation of the daily intake of ascorbic acid source foods was also above the recommended level, with a value of 104 mg/day for PS and 142 mg/day for non-smokers.

There is evidence that ROS may be involved in more than 50 diseases, in which case the inhalation of cigarette smoke would cause damage to the respiratory system. Pulmonary diseases associated with ROS include: emphysema, bronchopulmonary dysplasia, pneumoconiosis, chronic kidney disease, asthma and respiratory distress syndrome [30, 31].

Nevertheless, to trigger these pathologies, the production of ROS due to cigarette smoking must exceed the antioxidant capacity of the body, compromising the individual's antioxidant status; according to the results found in the present study, the exposure to cigarette smoke was not reflected in substantial alterations in ROS levels [32,33].
Although we found no significant relationship between plasma vitamin C and smoking exposure through ROS quantification, studies [34, 35, 36, 37, 38], in active smokers have found that smoking causes negative effects on the antioxidant system, because the decrease in concentrations plasma levels of vitamin C have not yet been well established. Smoke could cause less absorption and increased turnover of vitamin C. The increased turnover could be attributed to OS due to cigarette use or exposure to tobacco compounds, thus necessitating a greater amount of vitamin C to counteract free radical formation [39].

Nevertheless, we found that the exposure to tobacco smoke from the daily numbers of smoked cigarettes recorded by the present study did not interfere with the plasma concentration of vitamin C in passive smokers, since the consumption of food sources of such micronutrient were within the recommended values. It is worth noting that the present study is unique with respect to the evaluation of plasma concentration of vitamin C in passive smokers aged between 10 and 19 years, that is, among children and adolescents, thus suggesting that further studies on this subject should performed.

5 CONCLUSION

Furthermore, we recommend that smoking exposure be included in nutritional and anthropometric evaluation studies, because it is a variable that can, biologically, interfere with growth in human infants. Control measures need to be implemented to reduce the prevalence of passive smoking in the population, contributing to healthier lifestyles, especially in the most vulnerable groups such as children and adolescents.

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CONFLICTING INTERESTS STATEMENT

The authors declare that there are no conflicts of interest.
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