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## Sedentary lifestyle and poor eating habits in childhood: a cohort study

Sedentarismo e práticas alimentares inadequadas na infância: um estudo de coorte

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**Abstract** Worldwide, about 22 million children under five years old are overweight. Environmental factors are the main trigger for this epidemic. The purpose of this study was to evaluate the eating and physical activity habits in a cohort of eight-year-old children in Pelotas, Brazil. Eating habits were assessed based on the Ten Steps to Healthy Eating proposed by the Ministry of Health. To assess the level of physical activity, the physical activity questionnaire for children and adolescents (PAQ-C) was used. Of the 616 interviewed children at 8 years, it was observed that 50.3% were male; 70.3% were white and just over half belonged to economic class C. None of the children were classified as very active and none acceded to a daily consumption of six servings of the cereals, tubers, and roots. The steps that had higher adherence were 8 (do not add salt to ready foods); 4 (consumption of beans, at least 5 times per week) and 1 (have 3 meals and 2 snacks per day), respectively. The high prevalence of physical inactivity and low level of healthy eating habits confirm the importance of strategies to support and encourage the practice of physical activity and healthy eating among youth.

**Key words** Children, Diet, Eating habits, Physical activity

**Resumo** Mundialmente, cerca de 22 milhões de crianças menores de cinco anos têm excesso de peso, sendo que fatores ambientais são os principais desencadeadores da epidemia. O objetivo deste trabalho foi verificar os hábitos alimentares e de atividade física em crianças de oito anos de idade, pertencentes a uma coorte de Pelotas, Brasil. Os hábitos alimentares foram avaliados com base nos Dez Passos para Alimentação Saudável, propostos pelo Ministério da Saúde. Para avaliar o nível de atividade física, utilizou-se o questionário de atividade física para crianças e adolescentes (PAQ-C). Das 616 crianças avaliadas aos 8 anos, observou-se que 50,3% eram do sexo masculino; 70,3% de cor branca e pouco mais de metade pertencia à classe econômica C. Nenhuma das crianças foi classificada como muito ativa ou aderiu ao consumo diário de 6 porções do grupo de cereais, tubérculos e raízes. Os passos de maior adesão foram o 8 (não adicionar sal aos alimentos prontos); o 4 (consumo de feijão, pelo menos, 5 vezes por semana) e o 1 (realização de 3 refeições e 2 lanches por dia), respectivamente. A alta prevalência de inatividade física e o baixo nível de hábitos alimentares saudáveis, confirmam a importância de estratégias para apoiar e incentivar a prática de atividade física e alimentação saudável entre os jovens.

**Palavras-chave** Crianças, Dieta, Hábitos alimentares, Atividade física

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## Introduction

Obesity is a worldwide epidemic and a risk factor in the natural course of several other chronic diseases<sup>1</sup>. With the rise of globalization and its consequences (particularly urbanization, industrialization, and economic development), there are negative changes in the population's lifestyle, including changes in dietary patterns and a decrease in physical activity<sup>2</sup>.

Worldwide, about 22 million children under five years old are overweight and more likely to become obese adults and to suffer from non-communicable chronic diseases (NCDs)<sup>3</sup>. Childhood can be one of the most favorable periods for obesity prevention<sup>4</sup>. However, paradoxically, the management can be more complex than in adult life, since it is related to changes in parents' habits and availability and is inhibited by the lack of knowledge children have on the consequences of this condition<sup>5</sup>.

The etiology of obesity is multifactorial; however, environmental changes comprise the main triggering factors of the epidemic, since they predispose too much consumption of energy associated with lower energy expenditure<sup>6</sup>. Therefore, encouraging the increase of physical activity and the adoption of beneficial dietary habits are the main devices for creating a healthy lifestyle for young people<sup>7</sup>.

Healthy eating should provide water, carbohydrates, proteins, lipids, fiber, vitamins, and minerals, which are essential for preserving health<sup>8</sup>. In terms of physical activity, children and adolescents aged 5-17 years should accumulate at least 60 minutes on a daily basis at a moderate-to-vigorous-intensity level in an activity such as free play, games, sports, transportation, recreation, or planned exercising, in the context of family, school, and community activities<sup>9</sup>. However, national studies indicate unhealthy eating habits such as low consumption of fruits<sup>10</sup> and vegetables<sup>10,11</sup>, in addition to high consumption of candies and fats<sup>10</sup> among children. Added to this scenario is the low level of physical activity found in this age group<sup>12,13</sup>.

The aim of this study was to investigate eating and physical activity habits in eight-year-old children, from a cohort in Pelotas, Rio Grande do Sul, Brazil.

## Methods

This is a prospective cohort study of people born between September 2002 and May 2003 in hospitals in the city of Pelotas (RS), Southern Brazil and followed a random sample of 30% of these, at 30, 90 and 180 days. This study refers to a cross-sectional analysis of data collected in the follow-up that occurred when the children were 8 years of age. These children were visited at home, and trained medical students administered a standardized and pretested questionnaire to mothers or caregivers and children on various aspects of child health, including the frequency, type and intensity of physical activity in the last week and the quantity and quality of the meals in the last month. Details on the cohort methodology are published in Mascarenhas *et al.*<sup>14</sup> and Fonseca *et al.*<sup>15</sup>.

In the present study, the eating and physical activity habits in children aged eight years were evaluated. The assessment of eating habits was based on the Ten Steps to Healthy Eating (*Dez Passos para Alimentação Saudável*), which were developed by the Brazilian Ministry of Health<sup>16</sup>.

To collect data for each of the steps, we formulated questions that helped confirm the frequency of food consumption within one month before the interview. The response options were divided into seven categories: never, 1-2 times a week, 3-6 times a week, once a day, 2 times a day, 3 times a day, and more than 3 times a day. Step 9 (water) was categorized as it follows with answers: "I do not take drink water", "do not take drink water every day", statements referring to 1-2 glasses a day, 3-5 glasses a day, 6-8 glasses a day, and more than 8 glasses a day. Steps 1, 8 and 10 were collected by direct questions whose response options were yes or no.

Individuals who reported having at least three meals and two snacks a day were classified as having acceded to step 1. Children who daily consumed six servings from the group of grains, tubers and roots, joined step 2. Daily consumption of at least three servings of vegetables as part of meals and three or more servings of fruit in desserts and snacks, characterized the membership to step 3. Adhesion to step 4 was considered positive when the individual reported the habit of consuming beans at least five times a week. Individuals who daily consumed 3 portions of milk and dairy products, 1 portion of meat, fish, chicken or eggs and removed fat from meat and skin from poultry were classified as having acceded to step 5. The daily ingestion of no more than

one serving of vegetable oils, olive oil, butter or margarine constituted the membership to step 6. Likewise, step 7 was considered positive when the child consumed sodas, processed juices, sugar cookies, sandwich cookies, and other treats at most once a day. Step 8 was met when the answer to a question about adding salt to prepared food was "no." Adherence to step 9 occurred when water consumption was at least two liters a day, while step 10 adherence was characterized by at least 30 minutes of physical activity every day and the maintenance of appropriate weight.

To calculate the nutritional status, we analyzed anthropometric data (weight and height) by gender and age during the interview, from which body mass index (BMI) was calculated. The subjects were weighed using a portable scale with a 150 kg capacity and accuracy to  $\pm 100$  g, wearing light clothing without shoes. Height were measured using a portable stadiometer. Children with score values  $z \geq -2$  and  $\leq +1$  were considered to have adequate weight; those with the score  $z < -2$  were underweight; those with BMI with score values  $z > +1$  and  $\leq +2$  were overweight, and those with score  $z > +2$  were obese<sup>17,18</sup>.

To assess the level of physical activity, we used the Physical Activity Questionnaire for Children and Adolescents (PAQ-C), developed by Crocker et al.<sup>19</sup>. This survey characterizes the level of physical activity in the seven days preceding its application. It consists of nine questions about the practice of sports, games, and physical activities at school and in free time, including weekends. Each question has a value of one to five, and the final score is obtained by averaging the questions. A score of one is equivalent to a very sedentary lifestyle and five indicates a very active lifestyle. Scores two, three, and four represent the categories sedentary, moderately active, and active, respectively. Participants could be classified as active (score  $\geq 3$ ) or sedentary (score  $< 3$ ). The PAQ -C also includes a question on the average daily time in front of the TV and another on the existence of diseases impeding the normal development of the activities during the previous week, however, these data do not make up the score calculation.

To ascertain the repeatability of the data collected, the field work supervisor repeated a random sample of 10% of the interviews, using a synthesized questionnaire. The sample size was calculated based on a confidence level of 95% and a statistical power of 80%, exposures ranging from 15% to 80%, assuming a Relative Risk (RR)

of 2 and increase of 15% to the initial calculation for losses and confounding factors control. We performed the calculation of the frequencies of the variables, the bivariate analysis between exposure factors and the outcome and between exposure factors and other variables.

The following variables were evaluated: demographic data (gender of the child [male or female], color [white or non-white], and maternal and paternal age in years); socioeconomic data (family income, economic class according to the classification of the Brazilian Association of Research Companies [Associação Brasileira de Empresas de Pesquisa- ABEP]<sup>20</sup>, and mother's education); maternal color (white or non-white); number of children including the one in the study; marital status (living with a partner or not); maternal smoking; maternal overweight (reported by the interviewee); and characteristics of the child (gestational age, birth weight in grams, duration of breastfeeding, and physical activity). Only the associations with  $p < 0.05$  were considered statistically significant.

Epi-Info 6.0 and SPSS (version 21.0) for Windows were used to analyze data. Data were entered twice and checked for consistency. Initially, the frequencies of the variables of interest were obtained to characterize the study sample. Later, bivariate analysis between exposure factors and outcome was done, using chi-squared and linear trend tests.

The research project of perinatal data and visits of one, three, and six months was approved by the Committees of Research and Ethics in Health from Santa Casa de Misericórdia de Pelotas and from Fundação de Apoio Universitário (FAU), and ratified by the Committee of Ethics in Research from Universidade Federal do Rio Grande do Sul and by the Scientific Committee from Universidade Católica de Pelotas. The current research project, with visits to children eight years of age, was approved by the Ethics Committee from Universidade Católica de Pelotas, as this new step was not included in the previous project. Informed consent in writing was obtained from parents or guardians, after they have received detailed and accurate information about the research.

## Results and discussion

In the monitoring conducted at eight years old, it was possible to locate 616 children (63.3%) of the initial sample of 973, losses included 5 refus-

als, 17 deaths, 93 cases of moves to other states or cities, and 242 cases whose addresses could not be found. The sample set of children who were visited was representative of that from the hospital screening.

It was observed that about 10% of children were born preterm, and 8% were underweight at birth (< 2500 g). A little more than half of the sample belonged to economic class C, about 70 % of the children were sedentary and 37.4 % excess weight. The other characteristics are shown in Table 1. More than half of the children were found to be sedentary, and none were found to be very active.

Table 2 shows that frequency of adherence to the Ten Steps to Healthy Eating was low, indicating a possible need to rethink the most effective approach to this population. Vinholes *et al.*<sup>21</sup>, Raphaelli *et al.*<sup>22</sup>, and Couto *et al.*<sup>23</sup>, also exposed low adherence to the steps suggested by the Ministry of Health, in the adult<sup>21</sup> and adolescent<sup>22,23</sup> population of the same city. These results are consistent with the prevalence of overweight in this location<sup>24</sup>. In addition, they confirm the findings of Santos *et al.*<sup>25</sup>, who described the nutritional status of family members in food insecurity in Pelotas/ RS. The authors found high rates of overweight in all age groups, besides height deficit in children aged under five years, implying that food insecurity situation of these families could be related not only to the low availability of food, but also to the poor nutritional value of them.

According to the results, step 1 (having at least three meals and two snacks a day) was mostly adhered by the children (75.2%). This was positive, because although there is no consensus about its benefits, the increase on the frequency of meals may reduce appetite, the loss of nitrogen, and improve lipid oxidation and blood markers such as total cholesterol, LDL-cholesterol, and insulin<sup>26</sup>. In a study with dyslipidemic women, Oliveira and Sichieri<sup>27</sup> suggested that having six daily meals could be a measure of prevention and control of hypercholesterolemia, independent of age, body weight and type of eaten food, fruit, or fiber, once there was a reduction in total and LDL-C serum cholesterol. Furthermore, studies have shown an inverse association between the number of daily meals and BMI<sup>28,29</sup>.

On the other hand, no children adhered to step 2, which is the daily recommended consumption of six servings of the group of cereals, tubers and roots, preferably whole grains. However, the individual analysis of groups of foods of this step showed that 39.1% of children had

**Table 1.** Characteristics of the sample, Pelotas (RS), 2011 (N = 616).

Variable	N	%
Family income*		
≤ 1	85	13.8
1.01-3	308	50.0
3.01-6	144	23.4
> 6	66	10.7
Unknown	13	2.1
Economic class (ABEP) <sup>†</sup>		
A	24	3.9
B	202	32.8
C	325	52.8
D e E	65	10.5
Maternal Age <sup>‡</sup>		
≤ 25	52	8.4
26-35	311	50.5
> 35	249	40.4
Unknown <sup>§</sup>	4	0.6
Maternal Color		
White	456	74.0
Not white	160	26.0
Maternal education		
Illiterate /third- grade	34	5.5
Complete fourth-grade	176	28.6
Complete primary school	133	21.6
Complete secondary school	213	34.6
University	53	8.6
Unknown <sup>  </sup>	7	1.1
Child gender		
Male	316	51.3
Female	300	48.7
Child color		
White	433	70.3
Not White	183	29.7
Physical activity level		
Very Sedentary	103	16.7
Sedentary	336	54.5
Moderately active	160	26.0
Active	17	2.8
Nutricional Status		
Underweight	11	1.8
Normal-weight	375	60.8
Overweight	126	20.5
Obese	104	16.9

\* In minimum wages; <sup>†</sup> Classification according to Brazilian Association of Research Companies (Associação Brasileira de Empresas de Pesquisa), emphasizes people's purchasing power without classifying them into social classes. Economic class A comprises people with the highest purchasing power, and economic class E, those with least purchasing power; <sup>‡</sup> In complete years; <sup>§</sup> Maternal death; <sup>||</sup> Children who do not live with their mother.

eaten the recommended daily servings of cereals, 6.7% ate the portions of tubers and roots, and 2.8% gave priority to whole grains. In the

**Table 2.** Frequency of adherence to the items of the 10 Steps to Healthy Eating of the sample, Pelotas (RS), 2011.

Steps	Items of the 10 Steps to Healthy Eating	N	%
Step 1:	Number of meals: at least three meals and two snacks a day	463	75.2
Step 2:	Daily consumption of six portions of cereal, tuber and roots, preferably whole grains	0	0
Step 3:	Daily consumption at least three portions of vegetables and three portions of fruit	13	2.1
Step 4:	Ingestion of beans: at least five times a week	471	76.5
Step 5:	Daily consumption of 3 portions of milk and dairy products, 1 portion of meat, fish, chicken or eggs and removal of fat from meat and skin from poultry	37	6.0
Step 6:	Have one daily portion, at the most, of vegetable oil, olive oil, butter or margarine	352	57.1
Step 7:	Avoid soda and industrialized juice, cakes, cookies, desserts and sweets. Eat those, at most, once a day	266	43.2
Step 8:	Decrease the amount of salt in food and remove saltshaker from the table	521	84.6
Step 9:	Consumption at least two liters of water a day (six to eight glasses)	54	8.8
Step 10:	Performing regular physical activity and maintaining a healthy weight	101	16.4

studies of Raphaelli et al.<sup>22</sup> and Couto et al.<sup>23</sup>, the total consumption of such foods was 31.5% and 21.0%, respectively. To obtain a healthy diet, 55% to 75% of the total energetic value of the diet (TEV) must come from total carbohydrates (complexes + free or simple sugars). Of these, 45-65% of the supplied energy should be derived from foods rich in complex carbohydrates such as grains, tubers, and roots<sup>8</sup>. Whole foods are rich in fiber, vitamins, minerals, and other nutrients<sup>30</sup>, exerting a protective effect against a number of chronic diseases such as diabetes, obesity, and heart disease<sup>31</sup>. Moreover, Esmailzadeh et al.<sup>32</sup> showed an inverse association between whole grain consumption and metabolic syndrome, corroborating its benefits to health.

The consumption of vegetables and fruits was also low (2.11%). Similar results were found in other studies conducted in Southern Brazil<sup>33,34</sup>, which is worrying because the daily recommendation is for at least three servings of vegetables and three servings of fruits, based on these foods' potential prevention of non-communicable diseases<sup>16</sup>.

Step 4 (consumption of beans at least five times a week) had second-highest adherence (76.5%), confirming previous studies carried out in this city, which also reported good adherence to this step<sup>22,23</sup>. Moreover, this result is higher than the one found by Bortolini et al.<sup>35</sup>, who found daily consumption of this legume by 63.4% of children between 6-59 months of age in Southern Brazil. The percentage found in this study is important, since the Household Budget Surveys (POF)<sup>36</sup> reveal a decrease in annual per capita household consumption of beans, which is an important source of iron, fiber and, associated with rice, of high quality plant protein<sup>8</sup>.

Confirming previous studies, consumption of beans was associated with non-white skin color<sup>37</sup>, lower socioeconomic status<sup>38</sup> and lower maternal education<sup>39</sup> (Table 3).

The fifth step was followed by 6.0% of children, but by analyzing the items separately, 25% consumed three servings of milk and dairy products daily. This is higher than the result found by Filha et al.<sup>40</sup>, who reported adequate intake of these foods by about 7% of the children enrolled in the Public Health System of the city of Aracaju (SE). Regarding the intake of meat, fish, poultry, or eggs, 32.8% of the sample met the recommendation to consume them once a day. In a research study conducted with adolescents in Pelotas (RS), the frequency of daily consumption of red meat and white meat was 43.0% and 9.7%, respectively<sup>41</sup>. In the present study, 80.7% of the respondents reported removing the visible fat from meats. The percentage is higher than the one found in a study with adults, also resident in Pelotas (RS) in which 52.3% reported the consumption of meat with excess fat<sup>42</sup>.

The sixth step had adherence of 57.1% of the sample and as it can be observed in the table 3, being associated with the white race and higher maternal education. Filha et al.<sup>40</sup> found adequate consumption of fats by only 6.2% of the children aged between 24 and 35 months, in Aracaju (SE).

Accession to step 7 was associated with the female gender, white race, socioeconomic status and maternal education (Table 3). Vinholes et al.<sup>21</sup> observed the consumption of foods high in sugar more often than twice a week by 41% of adults in Pelotas (RS), which were positively associated with age and negatively associated with education.



**Table 3.** Frequency of adherence to the 10 Steps to Healthy Eating according to the variables studied. Pelotas (RS), 2011.

Variable	Step 1	Step 2	Step 3	Step 4	Step 5	Step 6	Step 7	Step 8	Step 9	Step 10
Gender [p value*]	0.52	-	0.46	0.11	0.995	0.80	0.02	0.29	0.35	0.12
Male	76.3	-	2.5	79.1	6.0	56.7	38.6	86.1	9.8	18.7
Female	74.0	-	1.7	73.7	6.0	57.7	48.0	83.0	7.7	14.0
Skin color [p value*]	0.13	-	0.29	0.02	0.08	0.001	0.002	0.70	0.20	0.04
White	76.5	-	1.8	74.5	6.8	60.4	46.3	84.3	8.1	14.9
Not White	69.8	-	3.4	84.9	2.5	43.7	30.3	85.7	11.8	22.7
Economic class (ABEP) <sup>α</sup> [p value**]	0.24	-	0.97	0.03	0.98	0.10	0.000	0.30	0.008	0.05
A	75.0	-	0.0	58.3	4.2	62.5	70.8	83.3	8.3	4.2
B	76.7	-	3.0	73.8	5.9	64.4	50.5	87.1	5.5	15.4
C	76.0	-	1.5	78.8	6.5	51.4	37.2	83.7	8.9	16.6
D e E	66.2	-	3.1	80.0	4.6	61.5	40.0	81.5	18.5	23.1
Maternal education [p value**]	0.22	-	0.51	0.000	0.82	0.02	0.000	0.95	0.32	0.12
Illiterate /third- grade	88.2	-	5.9	82.4	2.9	50.0	20.6	85.3	11.8	26.5
Complete fourth-grade	72.7	-	1.7	83.5	5.7	51.7	37.5	83.0	9.7	16.5
Complete primary school	70.7	-	2.3	78.2	9.0	57.9	36.1	86.5	10.5	18.8
Complete secondary school	76.5	-	1.9	70.9	5.2	60.6	49.3	85.5	6.6	15.0
University	79.3	-	1.9	64.2	3.8	66.0	71.7	81.1	9.4	11.3

<sup>α</sup> Classification according to Brazilian Association of Research Companies (Associação Brasileira de Empresas de Pesquisa), emphasizes people's purchasing power without classifying them into social classes. Economic class A comprises people with the highest purchasing power, and economic class E, those with least purchasing power. \* Chi-square test for linear trend. \*\* Chi-square test for heterogeneity.

Step 8 was the one with largest accession in the sample (84.6%), consistent with the result found by Vinholes *et al.*<sup>21</sup>. This finding is important because excessive sodium ingestion is one of the major modifiable risk factors in the genesis of systemic hypertension (SH)<sup>43</sup>. Furthermore, studies show excessive consumption of foods high in sodium by child<sup>44,45</sup>, so that the SH, which previously reached only the elderly, now also included young people<sup>46</sup>.

The item relating to the consumption of at least two liters of water a day had poor accession (8.7%), and was inversely associated with socioeconomic status (Table 3). This percentage is lower than the one found in recent studies<sup>47,48</sup>. Furthermore, the association concerning water ingestion and socioeconomic status, differs from other studies<sup>47,49</sup>.

Analyzing step 10, according to Table 3, there was greater adherence by non-white children and those of lower socioeconomic status. We found that about 60% of the children maintained appropriate weight and 37.4% were excess weight (Table 1). In a study with children and adolescents from Maceió (AL), the authors observed better results when checking 86.2% of normal-weight and 13.8% of excess weight in

the sample<sup>12</sup>. However, when assessing the level of physical activity, the prevalence of physical inactivity was 93.5%, whereas in this study was 71.2%. According to Olds *et al.*<sup>50</sup>, although the rates of childhood overweight and obesity are stabilizing in many countries, they remain high, causing harmful effects on health across the life span. Thus, it becomes necessary to develop interventions that encourage active behaviors such as daily walks, school activities, and programs involving parents and children<sup>51</sup>.

Regarding physical activity, jogging was the most prevalent sport activity both in general population and in the study population (in both genders). Moreover, according to the bivariate analysis, only the gender variable was significantly associated with physical activity, which was found to be more frequent among boys (Table 4). These results confirm previous studies in Pelotas (RS), in which the frequency of physical activity was higher among men<sup>21,52,53</sup>. Corroborating the results found by Hallal *et al.*<sup>54</sup>, in this study soccer and basketball were the sports played significantly more often among boys, while dancing and volleyball had significantly greater adherence among girls.

**Table 4.** Association between physical activity and socio-demographic variables. Pelotas (RS), 2011.

Variable	PR (CI <sub>95%</sub> )	P
Gender		0.03
Male	1.32 (1.03-1.70)	
Female	1.00	
Skin color		0.51
White	1.00	
Not white	1.10 (0.84-1.43)	
Economic class (ABEP) *		0.51
A	1.00	
B	1.87 (0.75 -4.69)	
C	1.66 (0.68-4.14)	
D e E	1.85 (0.70-4.85)	
Maternal education		0.88
Illiterate /third- grade	1.00	
Complete fourth-grade	0.88 (0.51-1.51)	
Complete primary school	0.86 (0.49-1.50)	
Complete secondary school	0.96 (0.57-1.62)	
University	0.76 (0.38-1.49)	

\* Classification according to Brazilian Association of Research Companies (Associação Brasileira de Empresas de Pesquisa), emphasizes people's purchasing power without classifying them into social classes. Economic class A comprises people with the highest purchasing power, and economic class E, those with least purchasing power. PR (IC<sub>95%</sub>): Prevalence Ratio and 95% Confidence Interval.

## Conclusions

Given the increasing rates of childhood obesity and health problems that this condition entails, it is necessary studies to explicate the factors that cause changes in nutritional status in children. Considering this study's findings, it is possible to conclude that adherence to Ministry of Health recommendations was low.

The results confirm the importance of strategies to support and encourage the practice of physical activity and healthy eating among children, which must have intersectional character, comprising health, education and culture policies, and must also integrate the society and family. Only by making children and youth awareness about the importance of healthy habits, the growing obesity epidemic can be reduced, as well as the harms adjacent to this disease, especially the diseases associated with it and, therefore, the burden on health services.

## Collaborations

CC Kaufmann: conception and design of the study, ADB Pretto and GF Dutra: analysis and interpretation of results, drafting and critical revision of intellectual content. EP Albernaz: analysis and interpretation of results and critical review of the content. All authors approved the final version of the manuscript and declare to be responsible for all aspects of the work, ensuring its accuracy and completeness.



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