

# **REVISTA CIÊNCIAS EM SAÚDE**



ISSN 2236-3785

# **ORIGINAL ARTICLE**

# Halitosis in geriatrics: factors related to this oral problem. An observational prospective clinical study

Fernanda Pereira de Caxias<sup>1</sup><sup>®</sup>, Isabela Araguê Catanoze<sup>1</sup><sup>®</sup>, Emily Vivianne Freitas da Silva<sup>1</sup><sup>®</sup>, Júlia Taino Helena<sup>1</sup><sup>®</sup>, Daniela Atili Brandini<sup>2</sup><sup>®</sup>, Daniela Micheline dos Santos<sup>1</sup><sup>®</sup>, Karina Helga Leal Turcio<sup>1</sup><sup>®</sup>, Aimée Maria Guiotti<sup>1,\*</sup><sup>®</sup>

<sup>1</sup>Department of Dental Materials and Prosthodontics, São Paulo State University (UNESP), School of Dentistry, Araçatuba, São Paulo, Brazil; <sup>2</sup>Department of Surgery and Integrated Clinic, São Paulo State University (UNESP), School of Dentistry, Araçatuba, São Paulo, Brazil.

# GENERAL INFORMATION

Received in January 9th, 2020 Accepted in May 14th, 2020

*Keywords* Aged Complete denture Geriatric dentistry Halitosis

# ABSTRACT

**Objective:** To identify the presence and level of halitosis in edentulous individuals with or without prostheses, before and after rehabilitation with complete dentures (CD). Methods: Patients were verbally invited to participate and signed an Informed Consent. Halitosis was analyzed by the volatile sulfur compounds measurement method in the presence and absence of the old prostheses (M1) and 15 to 30 days after the patients received their new prostheses (M2). At M1, the patients answered a questionnaire with clinical data, and the saliva type and presence/absence of tongue coating were assessed. T-test and Spearman correlation test were used, and a p-value < 0,05 was considered statistically significant. Results: Twenty-seven patients (19 women) with an average age of 67 years participated in the study. The mean time of prosthesis usage was 20.2 years. Tong coating was present in 21 patients (77,8%). A good odor level of halitosis was self-claimed in 13 (41,8%) individuals. The odor level of halitosis was significantly lower in M2 compared to M1, either with or without CD and after hygiene (p < 0,001). There was a positive and statistically significant correlation between the level of halitosis and alcoholism, smoking, prostheses hygiene method, and the presence of disease (diabetes). Conclusion: The old complete replacement of the prostheses with new ones was able to improve the odor level in a period of 15 to 30 days after the treatment. The presence of halitosis was correlated with alcoholism, smoking, denture hygiene methods, and the presence of diabetes.

CC BY-NC-SA 4.0 2020 RCSHCI

#### Introduction

Halitosis is the bad odor exhaled by breath and that negatively affects the individuals' life<sup>1-3</sup>. Halitosis might be physiological or pathological. The physiological halitosis is the normal odor level present in almost every individual, whereas the pathological halitosis may have its origin in the oral cavity, the respiratory or gastroesophageal tract, and the bloodstream<sup>1-3</sup>. It can be subjective when a halimeter or another person cannot confirm the patients' complaints<sup>1</sup>. Halitosis may also have an exogenous origin, caused by food, alcohol, and tobacco consumption, and endogenous origin when it is produced by the body itself<sup>1</sup>. Although there are more than 50

\*Correspondence:

Department of Dental Materials and Prosthodontics, São Paulo State University (UNESP). School of Dentistry 1193 José Bonifácio - Vila Mendonça CEP 16015-050 | Araçatuba, São Paulo, Brazil *e-mail:* aimee.m.guiotti@unesp.br

DOI: 10.21876/rcshci.v10i2.930

different causes for halitosis, about 80 to 90% of cases have their origin in the oral cavity  $^4\!\!\!\!$  .

Halitosis originated in the oral cavity is caused by the metabolic products of bacteria that use components present in various sites inside the mouth, mainly the tongue, saliva, periodontal sacks, dentures, and dental restorations<sup>5</sup>. Volatile sulfur compounds (VSC), mainly hydrogen sulfide (H<sub>2</sub>S), methyl mercaptan (CH<sub>3</sub>SH) and dimethyl sulfide [(CH<sub>3</sub>)2S] are the primary molecules related to oral halitosis, originated from short-chain fatty acids and the polyamines produced by anaerobic microorganisms that live on the tongue surface and periodontal sacks<sup>5</sup>.

High levels of VSCs were detected among complete denture users, mainly in elderly people that wear their prostheses during the night<sup>5</sup>. Complete dentures allow more biofilm retention due to the acrylic resin basis that provides a large contact surface, causing the bad odor<sup>5</sup>. Besides the use of dentures, halitosis in older people may be due to several other factors such as burning mouth syndrome, xerostomia, presence of tongue coating <sup>6,7</sup>, educational level and higher saliva viscosity<sup>7</sup>. Halitosis measurement may be done by three methods well accepted by the scientific community: the organoleptic method, utilizing the human olphat<sup>8,9</sup>; the gas chromatography (GC), which uses specific and very reliable equipment<sup>9</sup>; and sulfites measurement<sup>4,8,10</sup>, performed by halimeters (portable measurers of VSCs)<sup>11</sup>.

Thus, this study aimed to identify the presence and level of halitosis in edentulous individuals with or without a prosthesis, before and after rehabilitation with complete dentures, as well as its correlation with alcoholism, smoking, prostheses usage, mouth hygiene methods, type of saliva, presence of tongue coating and systemic diseases. The null hypotheses were that, in elderly people with new complete dentures, the presence and levels of halitosis would not be different with or without prostheses before and after rehabilitation.

# Methods

#### Study design

This study followed an observational prospective design. After approval from the Research Ethical Committee from São Paulo State University (UNESP) (nr. 711.712), patients from the Complete Denture Clinic located at the School of Dentistry of the same University were verbally invited to participate in the study. All recommendations from the Ethics Committee were followed and the study was carried according to the principles of the Declaration of Helsinki. All participants received detailed information about the study and signed a Free Informed Consent.

The patients who attended to the eligibility criteria were subjected to anamnesis and intraoral exams and answered to a clinical questionnaire (Chart 1). As well, they were submitted to halitosis measurements, performed with and without the old prostheses (Moment 1 - M1). Then, 15 to 30 days after the new prosthesis's installation, the measurements were repeated (Moment 2 - M2).

#### Participants

The sample size was calculated from a finite population of 80 patients. The minimum sample required for a 90% confidence interval was 40 subjects, with a margin of error of 9%. As inclusion criteria, the patients had to wear conventional complete dentures, should be subjected to rehabilitation with new prostheses of the same type, and they should be able to answer a printed questionnaire. No systemic condition was considered as exclusion criteria.

*Chart* **1** – Clinical questionnaire applied containing anamnesis and detailed oral exam.

Identification
Name://
ID: Neighborhood: City:
Zip code:
Marital status: Color: Sex:
Nacionality: Hometown: Ocupation:
Anamnesis
1) Smoking habit: ( ) No ( ) Yes Frequency:
2) Alcohol consumption: ( ) No ( ) Yes Frequency:
3) Systemic medicines intake: ( ) No ( ) Yes Which?
4) Systemic disease:
5) Do you have diabetes? ( ) yes ( ) no
6) Do you have any problem in you stomach or intestine ? ( ) no ( ) Yes– Which?
7) Any health problem that you would like to mention?
Clinical data related to complete dentures use
8) Period of use (Years):
9) Complete dentures hygiene method:
10) Oral cavity hygiene method:
11) Presence of tongue coating: ( ) No ( ) Yes
12) Saliva type: ( ) Serous ( ) Mucous
13) How do you think your breath is?
() Very bad () Bad () Indiferent () Good () Very good

#### Anamnesis and intraoral exams

The same examiner performed anamnesis and oral exams. Clinical and demographic data were collected through the questionnaire depicted in Chart 1: age, gender, time of prostheses usage, knowledge of one's breath, alcoholism, smoking, prostheses and mouth hygiene method (self-reported), presence of systemic disease, type of saliva (serous or mucous) and presence of tongue coating.

#### Halitosis measurement

Halitosis was measured at M1 and M2 with and without the prostheses inserted in the mouth. Halitosis measurements were performed using the halimeter HC-212M (Breath Alerth®, TANITA, Japan). This device allowed measurements in six different odor levels (0 – no odor; 1 – very slight odor; 2 – slight odor; 3 – moderate odor; 4 – strong odor; 5 – very strong odor). The device was calibrated before each test, and the same examiner

performed the measurements for three consecutive times and according to the manufacturer's recommendations. When oral odor was detected (levels 1 to 5), patients were asked to and taught how to clean their tongue with a tongue scraper (Higilíngua, Andrade Gomes Ind. e Com. de Artefatos Plásticos Ltda., Americana, SP, Brazil) and water, then this procedure was followed by a new odor measurement.

Statistical Analysis

Levene's test assessed the normality of the data. Odor levels of halitosis were analyzed in both moments of the study by a two-tailed paired t-test. All variables were correlated with the odor level of halitosis by the Spearman's rank correlation test, for both data collection moments, using the IBM SPSS 20.0 (IBM, Armonk, New York, USA) software for  $\alpha = 0.05$ . Data are expressed as mean (± SD).

# Results

Eighty patients were potentially eligible to participate. However, only forty attended to the inclusion criteria. Additionally, 13 patients were dropped out due to the impossibility to perform the second measurements, either because of delay on the prostheses' fabrication, not the use of new prostheses and patients who did not want to keep participating in the study. Thus, 27 patients participated in the study, 19 women and 8 men, ages ranged from 53 to 92 years old (mean 67.0 years). The time of prostheses usage varied from one to 50 years (mean 20.2 years). Data related to knowledge of one's breath, alcoholism, smoking, prostheses and mouth hygiene method, type of saliva, presence of tongue coating and systemic diseases are shown in Table 1. Data related to odor level at M1 are shown in Figure 1, and data obtained at M2 are shown in Figure 2. There was no statistical difference in the odor level between the presence or absence of prostheses (Table 2). There was a statistical difference at the odor level when both Moments 1 and 2 were compared (old prostheses x new prostheses) (Table 3). A decrease of halitosis at M2 could be noted, that is when the patients wore their new prostheses.

Table 4 shows the correlation coefficient between odor level of halitosis and daily habits. There was a positive correlation between alcohol consumption and higher odor level at M1, during the use of the old complete dentures, either before or after tongue hygiene. Therefore, with the new prostheses, there was no correlation between alcohol consumption and odor level. There was positive correlation between the old prosthesis's hygiene method and the odor level. At M2, there was a positive correlation between smoking and the odor level, after hygiene without and with complete dentures. There was no significant correlation between the presence and odor level and the other variables of this study, mouth hygiene method, type of saliva and presence of tongue coating. The correlation coefficient between the odor level of halitosis and the presence of the systemic diseases reported are shown Table 5. There was a positive correlation in between the odor level of halitosis and diabetes mellitus at M1, mainly in the presence of complete dentures.

**Table 1** – Data related to clinical characteristics. The sum regarding the presence of systemic diseases are over N = 27 because some patients reported more than one condition.

Clinical characteristic	Options	n
Knowledge about one's breath	Very good Good Indiferent Bad Very bad	1 13 10 3 0
Alcoholism	Yes No	5 22
Smoking	Yes No	6 21
Prostheses hygiene method	Mechanical method Mechanical method + dentifrice Chemical method	4 15 8
Mouth hygiene method	Mechanical method Mechanical method + dentifrice Washing with water only Washing with mouthwash	15 4 4 4
Saliva type	Serous Mucous Unknown	20 6 1
Tongue coating	Present Abscent	21 6
Systemic diseases	Diabetes Cardiovascular Orthopedic Gastrointestinal Respiratory Other	7 15 4 8 2 12

#### Discussion

The null hypothesis was denied, as a statistical difference was observed between the presence of the old and new prostheses and the odor level of halitosis, as well as a correlation between odor and alcoholism, smoking, prostheses hygiene methods and the presence of diabetes mellitus.

A large number of patients was not aware of the presence of halitosis, which is in agreement with the results of Baran and Nagalci<sup>11</sup>, that also observed that the majority of patients did not know their odor level, despite their unsatisfactory oral condition<sup>11</sup>. Regarding the prosthesis's hygiene method, the mechanical plus dentifrice method was the most used, which shows that patients were not properly instructed, as they were unaware of the need to use the chemical associated with the mechanical method, and having difficulty keeping their dentures clean and disinfected<sup>11</sup>. Another important observation in this study is that 21 patients had tongue coating at M1. It is known that the presence of tongue coating is a determining factor to halitosis and that oral hygiene is capable of reducing the severity of the odor expelled from the oral cavity. Although it was not possible



Figure 1 – Frequency of halitosis at M1, before and after tongue hygiene.



Figure 2 - Frequency of halitosis presence at M2, before and after tongue hygiene.

to correlate this factor to the odor level before and after oral hygiene in this study, the odor levels remained virtually unchanged. Removing the biofilm that covers the tongue decreases the amount of VSCs, reducing the bad odor after cleaning its surface<sup>12,13</sup>. Thus, tongue cleaning is an essential factor in controlling halitosis.

More than 90% of the patients analyzed showed some level of oral odor (from slight to very strong). The prevalence of halitosis in the general population is difficult to obtain since there are different methods of evaluation and variation between the values found in different studies.<sup>14</sup> When comparing the two moments of the study, a decrease in the level of the odor was observed after the placement of the new complete dentures, which shows that the old dentures influenced the odor level. Possibly the acrylic basis functioned as areas of retention of bacterial biofilm, among other factors that cause bad oral odor<sup>15</sup>. Other studies have found a significant relationship

Different stages	Mean (SD)	p-value	
M1			
Without CD	3.67 (1.038)	. 0.00	
With CD	3.67 (1.000)	> 0.99	
After hygiene without CD	3.56 (1.050)	0.405	
After hygiene with CD	3.67 (0.961)	0.185	
Without DC	3.67 (1.038)	0.40 <b>-</b>	
After hygiene without CD	3.56 (1.050)	0.185	
With CD	3.67 (1.000)		
After hygiene with CD	3.67 (0.961)	> 0.99	
,,,			
M2			
Without CD	2.85 (0.770)		
With CD	2.85 (0.770)	> 0.99	
After hygiene without CD	2.74 (0.764)		
After hygiene with CD	2.81 (0.736)	0.327	
	<b>1</b> .01 (0.700)		
Without DC	2.85 (0.770)		
After hygiene without CD	2.74(0.764)	0.185	
inter hygiene without db	2., 1 (0., 01)		
With CD	2.85 (0.770)		
After hygiene with CD	2.81 (0.736)	0.713	
Theor hygicale with up	2.01 (0.750)		

**Table 2** – Paired t test analysis of mean odor level of halitosis at M1 and M2 with and without the old complete dentures (CD), before and after tongue hygiene (N = 27).

*Table 3* – Paired t test analysis of mean odor level of halitosis between both moments (M1 and M2) (N = 27).

Difference between variables	Mean (SD)	p-value
M1 Without CD	3.67 (1.038)	0.002
M2 Without CD	2.85 (0.770)	
M1 With CD	3.67 (1.000)	0.001
M2 With CD	2.85 (0.770)	
M1 After hygiene without CD	3.56 (1.050)	0.001
M2 After hygiene without CD	2.74 (0.764)	
M1After hygiene with CD	3.67 (0.961)	< 0.0001
M2 after hygiene with CD	2.81 (0.736)	
	1.1	

CD, complete dentures. SD, standard deviation.

between the use of prostheses and halitosis, especially if they had been used at night<sup>16</sup>. It is important to observe that in M2, although most patients had a slight odor, there was a significant improvement in the level of odor measured, and no patient had a very strong odor. This might be related to the fact that the new prostheses presented a better level of hygiene and less porosity since they were used for a short period (15 to 30 days). The absence of difference between odor measurements before and after tongue hygiene showed that just one session of cleaning the oral cavity was not enough to reduce the odor, which leads to believe that a process of educating the patient concerning oral and prosthetic hygiene is fundamental, in addition to changing habits and the influence of systemic factors.

Table 4 - Spearman's correlation coefficient between odor level of halitosis and different variables at M1 and M2.

Moment	Variables	Without CD	With CD	Aft. hygiene without CD	Aft. hygiene with CD
M1	Smoking	-0.024	0.078	0.012	0.073
	Alcoholism	0.349	0.483*	0.311	0.402*
	Time of prostheses usage	0.047	0.040	0.053	-0.021
	Prostheses hygiene method	0.607†	0.511†	0.602†	0.552†
	Mouth hygiene method	-0.173	-0.118	-0.558	-0.304
	Presence of tongue coating	0.097	0.193	0.302	0.336
	Type of saliva	0.195	0.314	0.259	0.127
	Knowledge of one's breath	-0.109	-0.188	-0.117	-0.110
M2	Smoking	0.216	0.219	0.468*	0.456*
	Alcoholism	0.040	-0.078	0.311	0.278
	Time of prostheses usage	-0.322	-0.306	-0.027	-0.265
	Prostheses hygiene method	0.119	0.223	0.170	0.249
	Mouth hygiene method	-0.077	0.099	-0.096	-0.122
	Presence of tongue coating	-0.030	-0.040	0.185	0.110
	Type of saliva	-0.127	-0.082	-0.000	-0.254
	Knowledge of one's breath	0.050	-0.048	-0.064	-0.058

\*Correlation is significant at the 0.05 level (2-tailed).

+Correlation is significant at the 0.01 level (2-tailed).

CD: complete dentures.

Moment	Reported systemic diseases	Without	With	Aft. hygiene	Aft. hygiene
		CD	CD	without CD	with CD
M1	Diabetes mellitus	0.458*	0.388*	0.358	0.403*
	Cardiovascular	0.143	0.041	0.159	0.154
	Orthopedic	0.232	0.142	0.182	0.143
	Gastrointestinal	0.022	0.115	0.177	0.132
	Respiratory	-0.364	-0.373	-0.356	-0.385
	Other	0.201	0.120	0.303	0.212
M2	Diabetes mellitus	-0.127	-0.044	-0.148	-0.090
	Cardiovascular	0.075	0.028	-0.090	0.020
	Orthopedic	0.024	0.220	0.624	0.770
	Gastrointestinal	0.210	0,097	0.200	0.279
	Respiratory	-0.224	-0.179	-0.158	-0.225
	Other	0.210	0.259	0.266	0.151

Table 5 – Spearman's correlation coefficient between odor level of halitosis and reported systemic diseases at M1 an	d M2.
--	-------

\*Correlation is significant at the 0.05 level (2-tailed).

CD: complete dentures.

There was a positive correlation between the level of halitosis and alcohol consumption in the presence of the old complete dentures. This fact can also be explained by the presence of porosity and flaws in old prostheses, which can function as niches, absorbing the residues from meals. The correlation of halitosis and the prosthesis's hygiene reinforces the importance of correct orientation for the patient since poorly sanitized prostheses, besides causing halitosis, are also a predisposing factor for stomatitis. Smoking also showed a positive correlation with the level of halitosis, only after tongue hygiene (with or without new prostheses). This may mean that smoking interferes with the detection of VSC by the halimeter, masking the odor of the patient's breathing. However, after oral hygiene, the odor of the smoke was removed, which allowed the real reading of the patients' halitosis.

It is known that diabetes can cause several changes in the oral cavity, such as malfunction of the salivary glands and changes in saliva composition, changes in taste, burning mouth syndrome, a greater tendency to the development of oral infections, delayed healing and formation of the lingual coating.<sup>17</sup> In addition to these oral changes, individuals with diabetes have higher levels of ketone in breathing<sup>18</sup>, and patients with type I diabetes have higher levels of fatty acids<sup>19</sup> and methyl nitrate in their airstream, which produces a specific odor on

# References

- 1. Aydin M, Harvey-Woodworth CN. Halitosis: a new definition and classification. Br Dent J. 2014;217(1):E1. doi: 10.1038/sj.bdj.2014.552
- 2. Cortelli JR, Barbosa MDS, Westphal MA. Halitosis: review of associated factors and therapeutic approach. Braz Oral Res [Internet]. 2008 [cited 2020 May 13];22(Spec Iss 1):44-54. Avaiable from: www.scielo.br/pdf/bor/v22s1/07.pdf
- 3.Gokdogan O, Catli T, lleri F. Halitosis in otorhinolaryngology practice. Iran J Otorhinolaryngol. 2015;27(79):145-53. PMCID: PMC4409959
- 4.vandenBroekAM, FeenstraL, deBaatC.A review ofthe current literature on aetiology and measurement methods of<br/>halitosis.JDent.2007;35(8):627-35.doi:10.1016/j.jdent.2007.04.009
- 5. Wu T, He X, Lu H, Bradshaw DJ, Axe A, LoewyZ, et al. Development of in vitro denture biofilm models for halitosis related bacteria and

breathing<sup>20</sup>. Although in the present study the halimeter was used to measure the presence of VSC, all oral changes and compounds present in breathing may contribute to worse levels of halitosis in patients diagnosed with diabetes.

This study shows that local factors, such as the presence of old prostheses, in addition to systemic and behavioral factors, influence the odor of patients' breathing. It is important to mention as a limitation of the present study the short follow-up period and the lack of control of daily oral hygiene. Future studies with long-term follow-up may help to understand better the role of new prostheses halitosis. The knowledge of concerning the importance of oral hygiene provides conditions for the patient to present better levels of oral odor and, consequently, improvement of quality of life and general well-being.

# Conclusion

The old complete replacement of the prostheses with new ones was able to improve the odor level in the period of 15 to 30 days after the treatment. The presence of halitosis was correlated with alcoholism, smoking, denture hygiene methods and the presence of diabetes.

their application in testing the efficacy of antimicrobial agents. Open Dent J. 2015;9:125-31. doi: 10.2174/1874210601509010125

6. Nalcaci R, Baran I. Oral malodor and removable complete dentures in the elderly. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2008;105(6):e5-9. doi: 10.1016/j.tripleo.2008.02.016

<sup>7.</sup> Ueno M, Takeuchi S, Takehara S, Kawaguchi Y. Saliva viscosity as a potential risk factor for oral malodor. Acta Odontol Scand. 2014;72(8):1005-9. doi: 10.3109/00016357.2014.938115

<sup>8.</sup>Yaegaki K, Coil JM. Examination, classification, and treatment of halitosis; clinical perspectives. J Can Dent Assoc [Internet]. 2000 [cited 2020 May 13];66(5):257-61. Avaiable from: www.cda-adc.ca/jcda/vol-66/issue-5/257.html

<sup>9.</sup> Rosenberg M, Septon I, Eli I, Bar-Ness R, Gelernter I, Brenner S, et al. Halitosis measurement by an industrial sulphide monitor. J Periodontol. 1991;62(8):487-9. doi: 10.1902/jop.1991.62.8.487

10. Shimura M, Yasuno Y, Iwakura M, Shimada Y, Sakai S, Suzuki K, et al. A new monitor with a zinc-oxide thin film semiconductor sensor for the measurement of volatile sulfur compounds in mouth air. J Periodontol. 1996;67(4):396-402. doi: 10.1902/jop.1996.67.4.396

11. Baran I, Nalçaci R. Self-reported denture hygiene habits and oral tissue conditions of complete denture wearers. Arch Gerontol Geriatr. 2009;49(2):237-41. doi: 10.1016/j.archger.2008.08.010

12. Liu XN, Shinada K, Chen XC, Zhang BX, Yaegaki K, Kawaguchi Y. Oral malodor-related parameters in the Chinese general population. J Clin Periodontol. 2006;33(1):31-6. doi: 10.1111/j.1600-051X.2005.00862.x

13. De Boever EH, Loesche WJ. Assessing the contribution of anaerobic microflora of the tongue to oral malodor. J Am Dent Assoc. 1995;126(10):1384-93. doi: 10.14219/jada.archive.1995.0049

14. Rösing CK, Loesche W. Halitosis: an overview of epidemiology, etiology and clinical management Braz Oral Res. 2011;25(5):466-71. doi: 10.1590/S1806-83242011000500015

15. Nalcaci R, Baran I. Oral malodor and removable complete dentures in the elderly. Oral Med Oral Pathol Oral Radiol Endod 2008;105(6):e5-9. doi: 10.1016/j.tripleo.2008.02.016

16. Rosemberg M. Clinical assessment of bad breath: current concepts. J Am Dent Assoc. 1996;127(4):475-82. doi: 10.14219/jada.archive.1996.0239

17. Negrato CA, Tarzia O. Buccal alterations in diabetes mellitus. Diabetol Metab Syndr. 2010;2:3. doi: 10.1186/1758-5996-2-3

18. Tassopoulos CN, Barnett D, Fraser TR. Breath-acetone and bloodsugar measurements in diabetes. Lancet. 1969;1(7609):1282-6. doi: 10.1016/s0140-6736(69)92222-3

19. Galassetti P, Mann S, Tate D, Neill RA, Costa F, Wasserman DH, et al. Effects of antecedent prolonged exercise on subsequent counterregulatory responses to hypoglycemia. Am J Physiol Endocrinol Metab. 2001;280(6):E908-17. doi: 10.1152/ajpendo.2001.280.6.E908

20. Novak BJ, Blake DR, Meinardi S, Rowland FS, Pontello A, Cooper DM, et al. Exhaled methyl nitrate as a noninvasive marker of hyperglycemia in type 1 diabetes. Proc Nat Acad Sci. 2007;104 (40) 15613-18. 10.1073/pnas.0706533104

# The authors declare no conflicts of interest.

# Indications about the contributions of each author:

Conception and design of the study: AMG, DMS, KHLT Analysis and interpretation of data: FPC, DAB, AMG Data collection: FPC, JTH, IAC Writing of the manuscript: FPC, JTH, AMG Critical revision of the article: KHLT, DMS, AMG Final approval of the manuscript: FPC, DAB, JTH, EVFS, KHLT DMS, IAC, AMG Statistical analysis: DAB e EVFS General responsibility for the study: AMG

Funding information: Not applicable.