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Collider bias in the association of periodontitis and carotid intima-media thickness

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Abstract

Objectives: This cross-sectional study tested the presence of collider bias in the relationship between periodontitis and the carotid intima-media thickness (cIMT).

Methods: Data from 480 members of the 1982 Pelotas Birth Cohort, Brazil, were used. Periodontitis at the age of 24 years was determined as the main exposure. cIMT at the age of 30 years was set as the outcome. High-sensitivity C-reactive protein (hsCRP) was considered the mediator (collider). Confounding variables included sex, income, BMI and smoking. The association between cIMT and periodontitis was tested in conventional logistic regression stratified on hsCRP levels, marginal structural modelling and sensitivity analysis for collider stratification bias.

Results: Conventional adjusted logistic regression analysis showed a positive association between periodontitis and cIMT (OR 1.5; 95% CI 1.1; 2.3). Stratified analysis according to the hsCRP levels revealed that the magnitude of the association was even higher among participants with hsCRP ≥ 3 mg/L (OR 2.2, 95% CI 1.1; 4.2) with 36% collider bias probability. No association between periodontitis and cIMT was found among participants with hsCRP < 3 mg/L (OR 1.3; 95% CI 0.8; 1.1). The association was not detected using marginal structural modelling (OR 1.3, 95% CI 0.8; 2.0).

Conclusions: The association between periodontitis and surrogate markers of cardiovascular disease might be induced by collider bias stratification using conventional regression analysis.

KEYWORDS

cardiovascular diseases, carotid intima-media thickness, periodontitis, C-reactive protein, Epidemiology, periodontal medicine

1 | INTRODUCTION

Coronary artery diseases are the most common types of cardiovascular disease (CVD) and the leading cause of death in the world.[1] The increase in carotid intima-media thickness (cIMT) may be a sign of atherosclerosis and a surrogate marker of the risk of CVD.[2] Systemic inflammation participates in endothelial dysfunction and in the establishment of the atherosclerotic plaque.[3]

The United Nations stated CVD as a concern, with a multidisciplinary plan to detect intervenable risk factors.[4] Smoking, excessive body weight, physical inactivity and hyperglycaemia are modifiable risk factors, which contribute to systemic inflammation.[5,6] Periodontitis has been posed to contribute to systemic inflammation.[7]

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It has been hypothesized that periodontopathogens can enter the bloodstream and initiate or increase the development rate of atherosclerotic plaques. Additionally, cytokines from periodontal lesions induce the production of pro-atherogenic molecules, such as C-reactive protein (CRP), which in turn affect the progression rate of atherosclerotic plaques.[8] While a claim exists on the causal association between periodontitis and CVD, the effect of periodontal therapy on prevention of cardiovascular disease seems to be, if any, very limited.[9,10] However, the evidence supporting this causal relationship mainly originates from cross-sectional studies.[11,12] Additionally, studies suffer from relevant methodological flaws, including self-reported assessment of exposure and outcome, lack of proper mediation analysis and presence of residual confounding, especially from smoking and socioeconomic condition.

Another explanation for the associations between periodontitis and CVD may be selection bias.[13] Collider stratification biases have gained attention after the publication of the obesity paradox. Even though obesity has been associated with an increased risk of death in the general population, it would confer a survival advantage in patients with CVD.[14] Collider stratification bias has provided a reasonable explanation for the paradox. A collider is a variable influenced by at least two variables, and, in most cases, it is a mediator in the causal pathway between exposure and outcome. Conditioning on a collider may happen in conventional regression analysis adjustment, sample stratification or participant recruitment. When exploring causal relationships, conditioning on a collider usually induces an association where none exists, as it re-opens a path otherwise blocked at the collider.[15] An example would be restricting participants' inclusion to those who present CVD or systemic inflammation at baseline.

Accordingly, this study assessed the relationship between periodontitis and the cIMT. Additionally, it also investigated whether this association could be explained by collider stratification bias.

2 | METHODS

This report was elaborated according to the STROBE guidelines. All waves of this study were approved by the Ethics Committee of the Federal University of Pelotas, Brazil.

2.1 | The 1982 Pelotas Birth Cohort

In 1982, all live-born infants of the maternity hospitals of Pelotas, Southern Brazil, were identified.[16] Mothers were interviewed, and different information and measures of the infants obtained (n = 5914). Regular interviews, blood sampling and physical examinations were performed since then. Interviews encompassed socioeconomic data, dietary and smoking habits, health-related conditions and others. Physical examinations included height, weight and blood pressure. Cholesterol, CRP and glycated haemoglobin were analysed. Figure 1 illustrates the flow chart of participation along the years.

At 15 years of age in 1997, a representative sample of the cohort members was invited to participate in the oral health study (OHS)-97 (n = 888).[17,18] At 24 years of age (OHS-06), full-mouth examination was carried out and the periodontal condition was assessed using the Community Periodontal Index in six sites per tooth in 720 participants of the OHS-97. The information was recorded at the tooth level, with the worst condition of the six sites being registered. All teeth were examined for the presence of bleeding on probing, calculus and periodontal pocket depth (PPD) ≥ 4 mm. At 31 years of age (OHS-13), the periodontal condition was evaluated in 539 participants of the OHS-97 using full-mouth examination, and the information was recorded for the six sites individually. Data on PPD, gingival margin level, bleeding on probing and calculus were collected.[18] Attachment level was obtained by the sum of the values from PPD and gingival margin level. Oral examination was performed by six trained and calibrated dentists. Calibration was executed by an experienced periodontist as the standard examiner.[18] Practical exercises were performed on 20 participants approximately the same age as enrolled individuals. The lowest intraclass correlation coefficient for PPD and gingival margin level was 0.85.

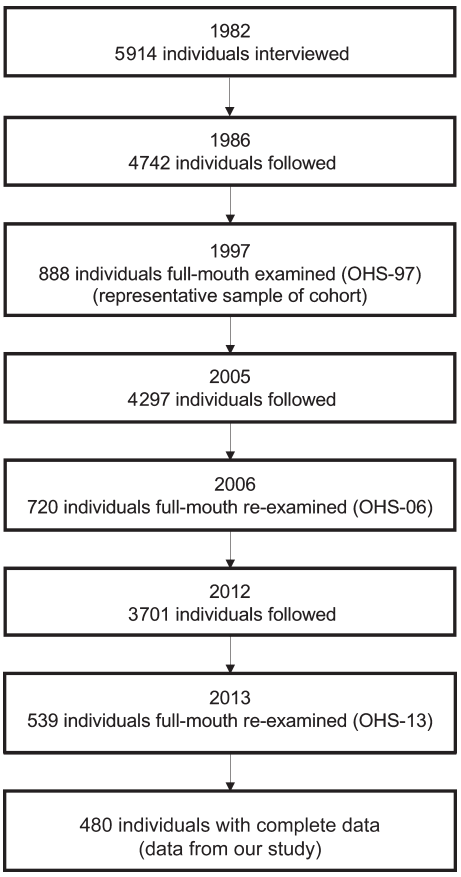

FIGURE 1 Flow chart comprising enrolment and participation in the 1982 cohort until 2013

2.2 | Exposure – periodontitis at 24 years of age

The case definition for periodontitis recommended by the Center for Disease Control and Prevention and the American Academy of Periodontology (CDC/AAP) was chosen.[19] Periodontitis at age 24 was estimated based on data from age 31 using regression models as previously described and consisted of cases of mild, moderate and severe periodontitis.[18] The models were based on a combination of oral, systemic and socioeconomic data using decision tree analysis. Variables in the first two levels were maintained in multivariable logistic regression models. Values for sensitivity, specificity and area under the receiver operating characteristic curve were determined. A sum of sensitivity and specificity above 120% was considered satisfactory in view of the epidemiologic characteristic of our study.[20]

2.3 | Outcome – carotid intima-media thickness at 30 years of age

cIMT was determined in longitudinal planes at the left posterior wall of the left carotid artery. A B-mode imaging ultrasound (Xario, Premium Compact, Toshiba) equipped with 7.5 MHz linear array transducer and 4 cm depth and gain settings optimized to image quality was used (Medical Imaging Applications, MIA-LLC).[21] A section of the carotid artery was imaged proximal to the carotidal bulb in a moving scan for eight seconds. The mean value of 90 frames in a 10-mm-long section was calculated by the software. For analytical purposes, cIMT was dichotomized using the 75th percentile as the cut-off point.[22] Further information can be found elsewhere.[16,23]

2.4 | Mediator (Collider) – high-sensitivity C-reactive protein at 30 years of age

Nonfasting venous blood was collected at the age of 30. High-sensitivity C-reactive protein (hsCRP) was measured using an immunoassay (Immulite, DPC/Siemens). hsCRP variable was dichotomized <3 or ≥3 mg/L to discriminate between individuals at risk of CVD.[24]

2.5 | Confounders

Confounders were determined as variables influencing exposure, outcome and mediator. Sex information was obtained at birth. The following variables were set as time-varying confounders: household income at birth and at age 30 (categorized into tertiles and converted to a dichotomous variable in which the second and third tertiles were grouped), body mass index at ages 23 and 30, and smoking status (current or former smoker; never smoker) at ages 23 and 30. The prevalence of participants at age 30 with fasting plasma glucose levels ≥ 126 mg/dL or glycated haemoglobin ≥ 6.5% was around 2.0%; therefore, diabetes was not included as a confounder. High blood pressure defined as systolic blood pressure of ≥140 mm Hg and/or diastolic blood pressure of ≥90 mm Hg was set as confounder but later removed from the analytical models due to collinearity with the body mass index.

2.6 | Theoretical framework

A directed acyclic graph (Figure 2) was elaborated to illustrate the direct and indirect causal pathways between periodontitis and increase in cIMT.[25]

2.7 | Statistical analysis

Despite data being originated from a birth cohort study, the study may be considered cross-sectional. Initially, we have investigated the association between periodontitis and cIMT conditional on the levels of hsCRP (mediator/collider) using conventional logistic regression. Analyses were adjusted for sex (1982), household income (1982, 2013), body mass index (2006, 2013) and smoking behaviour (2006, 2013). Following the approach of Banack and Kaufman[15] to test for collider stratification bias, we performed conventional logistic regression models stratified on hsCRP levels. Similarly, we used the *episens* macro (STATA 14.2, StataCorp) to carry out bias analysis to understand the magnitude of bias induced by studying a highly selected population drawn from the total population.

Finally, we have estimated the controlled direct effect of periodontitis at age 24 (exposure) on cIMT at the age of 30 (outcome) using marginal structural modelling. Such an approach prevents collider stratification bias, as it estimates the causal effect of time-dependent exposures while handling time-dependent covariates at the population level and differentiates confounders from mediators in the analytical modelling.[15] To test the hypothesis that hsCRP did not mediate the relationship between periodontitis and cIMT, the mediator (hsCRP) was set as 1. The stabilized inverse probability weights (SW) for the exposure and the mediator were estimated accounting for sex (time-unvarying variable), and the time-varying covariates household income, body mass index and smoking behaviour.[26,27] Instead of inverse-probability-to-treatment weights, stabilized

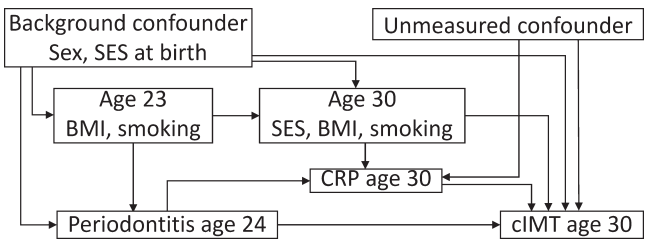

FIGURE 2 Directed acyclic graph representing causal relations between periodontitis and cIMT increase mediated by CRP (mediator/collider). Unmeasured common causes of C-reactive protein (CRP) and carotid intima-media thickness (cIMT) are assumed to play a role in the association (eg genetics, physiology, and behaviours). BMI, body mass index; SES, socioeconomic status

weights were chosen because they preserve the original sample size in the weighted dataset while determining robust confidence intervals. Predicted probabilities for both numerator and denominator were given and then divided to find the stabilized weight.[27] Final stabilized weights were determined by multiplying the weight of the exposure by the weight of the mediator. The model specification was verified by checking the distribution of the final stabilized weight, which required a mean value close to 1 to indicate a proper fit of the marginal structural model.

3 | RESULTS

Complete data were obtained from 480 participants, who provided data for all the assessment, and therefore comprised the final sample for this study. Socioeconomic and demographic characteristics of the sample of this study were similar to the original cohort (Table 1). The prevalence of periodontitis in this population was 36.5% and included mild, moderate and severe cases. The sample characteristics according to the periodontitis status are summarized in Table 2. A comprehensive description of the clinical periodontal data at ages of 24 and 31 is shown in Table A1.

In Table 3 (Model 1), conventional adjusted logistic regression, including the levels of hsCRP, analysis showed a positive association between periodontitis at 24 years of age and cIMT at the age of 30 years (OR 1.5; 95% CI 1.1; 2.3). In Models 2 and 3, respectively, stratified analysis according to the dichotomous hsCRP levels revealed that the magnitude of the association was even higher among participants with hsCRP ≥ 3 mg/L (OR 2.2, 95% CI 1.1; 4.2), whereas among participants with hsCRP < 3 mg/L the association vanished (OR 1.3; 95% CI 0.8; 1.1). Sensitivity analysis revealed 36% bias probability induced by high levels of hsCRP (Model 4, Table 3). Using marginal structural modelling, which considered the levels of hsCRP

TABLE 1 Demographic and socioeconomic characteristics of the original sample at birth in 1982 and of the final sample included in this study. 1982 Pelotas Birth Cohort study, Brazil

	Total sample (1982 at birth) (N = 5914)	Current study (N = 480)
	N (%)	N (%)
Sex		
Male	3037 (51.4)	240 (50.0)
Family income at birth per month		
≤1 BMW	1288 (21.9)	90 (18.8)
1.1-3 BMW	2789 (47.4)	252 (52.5)
>3 BMW	1808 (30.7)	138 (28.8)
Maternal education at birth (in years)		
0-4	1960 (33.2)	155 (32.4)
5-8	2454 (41.5)	215 (44.8)
9+	1493 (25.3)	110 (22.8)
Smoking status		
Nonsmoker	—	372 (77.5)
Smoker	—	108 (22.5)
cIMT		
<75% percentile	—	355 (73.8)
≥75% percentile	—	125 (26.2)
hsCRP		
Low (≤ 3 mg/L)	—	297 (62.8)
High (>3 mg/L)	—	176 (37.2)

Abbreviation: BMW Brazilian Minimum wage.

TABLE 2 Comparison of the sample characteristics regarding the participants with and without periodontitis at age 24 (n = 480). 1982 Pelotas Birth Cohort study, Brazil

	No periodontitis (N = 305)	Any periodontitis (N = 175)	*P*-value
Sex [N (%)]			
Males	140 (45.9)	100 (57.1)	.03
Females	165 (54.1)	75 (42.9)	
Family income at birth in BMW [N (%)]			
≤1	54 (17.5)	38 (21.3)	.865
1.1-3	160 (52.0)	95 (53.4)	
>3	94 (30.5)	45 (25.3)	
Smoking status			
Current/former at age 23 [N (%)]	95 (31.2)	58 (33.1)	.682
Cigarettes/day at age 23 [mean (95% CI)]	14.3 (11.3, 17.3)	13.4 (9.6, 17.1)	.710
Current/former at age 30 [N (%)]	114 (37.5)	73 (42.2)	.416
Cigarettes/day at age 30 [mean (95% CI)]	12.4 (10.3, 14.5)	12.4 (8.7, 16.0)	.743
cIMT [N (%)]			
<75% percentile	227 (75.7)	130 (70.6)	.224
≥75% percentile	73 (24.3)	54 (29.4)	
hsCRP in mg/L [mean (95% CI)]	3.7 (3.2, 4.2)	3.6 (2.9, 4.2)	.552
BMI [mean (95% CI)]			
At age 23 in kg/m^2	23.5 (23.0, 24.0)	23.9 (23.3, 24.5)	.381
At age 30 in kg/m^2	26.6 (15.1, 44.1)	27.4 (17.2, 43.8)	.250

Abbreviations: BMI, Body mass index; BMW, Brazilian minimum wage; hsCRP, High-sensitivity C-reactive protein.

TABLE 3 Association between periodontitis at 24 years of age and cIMT at age 30 comparing results using conventional regression and marginal structural model analyses

	Carotid intima-media thickness				
	Conventional regression			Bias analysis	MSM
	Model 1	Model 2	Model 3	Model 4	Model 5
	All hsCRP values	hsCRP > 3	hsCRP ≤ 3	hsCRP > 3	
Periodontitis OR (95% CI)	1.5 (1.1; 2.3)	2.2 (1.1; 4.2)	1.3 (0.8; 1.1)	1.6 36% bias	1.3 (0.8; 2.0)

Note: Results are given as odds ratio (OR) with respective 95% confidence intervals (95% CI) 1982 Pelotas Birth Cohort study, Brazil. All analyses adjusted for sex (1982), household income (1982, 2013), body mass index (2006, 2013) and smoking behaviour (2006, 2013). In Model 1, levels of hsCRP were also included in the analytical model.

Abbreviations: cIMT, Carotid intima-media thickness; hsCRP, High-sensitivity C-reactive protein; MSM, Marginal structural model.

as a mediator (collider) in the analysis, the association between periodontitis and cIMT was not detected and reached similar values to those observed among participants with low levels of hsCRP (OR 1.3, 95% CI 0.8; 2.0) (Model 5, Table 3).

4 | DISCUSSION

It is biologically plausible to assume a potential relationship between periodontitis and surrogate markers of CVD mediated by systemic inflammation. CRP has been proposed as one of the potential molecules in the causal pathway.[28] We hypothesized that periodontitis leads to an increase in the levels of CRP and that this marker is a predictor of atherosclerosis development. According to the European Federation of Periodontology,[29] there is moderate evidence that periodontal treatment reduces CRP and oxidative stress, and leads to improvements of clinical and biochemical measures of vascular endothelial function. However, the effect of periodontal therapy on clinical endpoints of CVD and biomarkers such as CRP remains inconclusive,[30] as some studies show lack of effect whereas others report positive results.[31-33] Since the majority of the studies present high risk of bias, the findings should be interpreted judiciously. We demonstrated that the association between periodontitis and increased cIMT may result from a statistical phenomenon, known as collider stratification bias.

Collider stratification bias occurs as the result of conditioning the analysis on a common effect of exposure and outcome, that is a mediator. It can lead to spurious associations and even reverse the direction of the association. The directed acyclic graph (Figure 2) explains collider bias in the association between periodontitis and cIMT, given hsCRP as both mediator and collider. As an example, we will consider the genetic variant rs4420638 in the APOE-CI-CII that is associated with both high hsCRP and CVD as the unmeasured confounder.[34] In periodontitis individuals, those with high hsCRP level might have achieved such high levels because they have either periodontitis or the genetic variant. However, among periodontally healthy individuals, more individuals with high hsCRP levels must have high hsCRP because of genetic factors, as periodontitis is not present. Consequently, since high hsCRP is exacerbated by periodontitis, when the effect is estimated within levels of hsCRP, having periodontitis with high hsCRP makes it less likely a participant has a genetic factor, whereas among those with low levels of hsCRP, being periodontally healthy makes it more likely the person has the genetic variant. Therefore, stratification on or adjustment for hsCRP induces an association between periodontitis and the genetic variant that distorts the causal relationship between periodontitis and CVD, because hsCRP is a mediator and conditioning on a mediator may lead to collider bias. In the future, if one can identify a gene variant associated with risk factor for periodontitis, one may use Mendelian randomization in a large sample size to evaluate whether periodontitis may contribute for cIMT increase.

When the association between periodontitis and cIMT was examined using conventional regression conditioned on hsCRP levels, the magnitude of the association was overestimated in 36% among those with high hsCRP levels. As conventional regression modelling does not differentiate between confounders and mediators (or colliders), this analysis risks to distort the association. However, these results differ from those using marginal structural modelling, which, by properly modelling mediators/colliders (hsCRP, in this case), has revealed a null association between periodontitis and CVD. In addition, marginal structural modelling allows the modelling of time-varying exposures and confounders, and can be used to estimate unbiased total and direct effects of periodontitis on cIMT.[35]

It is possible that periodontitis is only a small component of the multiple causes of cIMT increase. Ultimately, periodontitis might not be involved in the causal pathways of cIMT increase. One may speculate that the lack of association was due to a low prevalence of periodontitis cases or low values of cIMT considering the age of participants. However, the prevalence of periodontitis among our cohort members was slightly higher compared to other cohorts in the US and New Zealand,[36,37] and cIMT values were similar to those observed in other studies.[38,39] In order to broaden the implications of our findings, future studies might investigate this relationship in older populations with higher prevalence of periodontitis and values of cIMT.

People at risk of developing periodontitis and CVD are more likely to share risk factors, for example smoking, low physical activity, inadequate diet, low access to health treatment and others. Although these conditions are accounted for in the analysis, statistical adjustment does not rule out residual confounding. The most discussed residual confounder is smoking.[40,41] Usually, studies attempt to capture all dimensions of smoking using self-reported questions on current and past smoking habit, or, on a lower scale, frequency and duration of smoking. This information is converted into a variable with two or three categories: smoker, nonsmoker and/or never smoker. Nevertheless, the detrimental effect of smoking depends on several aspects, which include amount and duration of exposure, chemical composition and more. Moreover, one should consider the social undesirability in reporting smoking. Therefore, despite statistical adjustment, it is challenging to eliminate the residual effect of smoking in the association between periodontitis and systemic diseases. Additionally, if the above-mentioned risk factors have a stronger effect on CVD than periodontitis, they may induce a spurious association where none exists. Finally, collider bias may be observed concomitantly with other sources of bias, such as hawthorn, misclassification, sample size and others, further distorting the existence or magnitude of the association.

Our sample remains representative of the original population,[42,43] and no significant changes in findings could be observed in performing multiple imputations of data.[43] One may speculate whether the absence of the new periodontitis classification[44] should be considered a limitation. However, at data collection, the system had not been released and information needed was not assessed. Instead, our decision to use the classification recommended by the CDC/AAP for epidemiological studies should be instead faced as a strength, given the study design.[19] Among the limitations, we cannot rule out residual confounding, especially due to the low specificity of hsCRP as a marker of systemic inflammation. Future studies should use other relevant biomarkers such as interleukin-6 and VCAMs to investigate the relationship between periodontitis and cIMT. Another drawback relates to the cross-sectional nature of the current study and the lack of longitudinal measures of cIMT and hsCRP, which has precluded an investigation of the baseline status of the participants. However, data from this cohort[45] and population-based studies[46] indicate that the number of risk factors and cardiovascular events is very low among 20-24-year-old adults and considerably greater among 30- to 34-year-old ones.

5 | CONCLUSIONS

The association between periodontitis and surrogate markers of CVD might be induced by collider bias stratification, after conditioning on levels of hsCRP using conventional regression analysis. However, marginal structural modelling revealed a null association between periodontitis and cIMT. Whether the inflammatory periodontal burden requires more time to influence the systemic inflammation to promote cIMT increase remains to be tested. Future studies may consider relevant methodological issues, which include the presence of residual confounding due to smoking and socioeconomic condition, and the use of analytical approaches that account for the particularities of this relationship.

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REFERENCES

1. Roth GA, Johnson C, Abajobir A, et al. Global, regional, and national burden of cardiovascular diseases for 10 causes, 1990 to 2015. *J Am Coll Cardiol*. 2017;70:1-25.
2. Kadota A, Miura K, Okamura T, et al. Carotid intima-media thickness and plaque in apparently healthy japanese individuals with an estimated 10-year absolute risk of cad death according to the japan atherosclerosis society (jas) guidelines 2012: The shiga epidemiological study of subclinical atherosclerosis (sessa). *J Atheroscler Thromb*. 2013;20:755-766.
3. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. *N Engl J Med*. 2005;352:1685-1695.
4. Clark H. Ncds: A challenge to sustainable human development. *Lancet*. 2013;381:510-511.
5. Smolders B, Lemmens R, Thijs V. Lipoprotein (a) and stroke: a meta-analysis of observational studies. *Stroke*. 2007;38:1959-1966.
6. McGill HC Jr, McMahan CA, Gidding SS. Preventing heart disease in the 21st century: implications of the pathobiological determinants of atherosclerosis in youth (pday) study. *Circulation*. 2008;117:1216-1227.
7. Pink C, Kocher T, Meisel P, et al. Longitudinal effects of systemic inflammation markers on periodontitis. *J Clin Periodontol*. 2015;42:988-997.
8. Beck J, Garcia R, Heiss G, Vokonas PS, Offenbacher S. Periodontal disease and cardiovascular disease. *J Periodontol*. 1996;67:1123-1137.
9. Liu W, Cao Y, Dong L, et al. Periodontal therapy for primary or secondary prevention of cardiovascular disease in people with periodontitis. *Cochrane Database Syst Rev*. 2019;12:CD009197.
10. Ramírez JC, Contreras A.Periodontal infection and endothelial dysfunction. 2014. https://clinicaltrials.gov/ct2/show/results/NCT00681564
11. Zeng XT, Leng WD, Lam YY, et al. Periodontal disease and carotid atherosclerosis: a meta-analysis of 17,330 participants. *Int J Cardiol*. 2016;203:1044-1051.

12. Bale BF, Doneen AL, Vigerust DJ. High-risk periodontal pathogens contribute to the pathogenesis of atherosclerosis. *Postgrad Med J*. 2017;93:215-220.
13. Stovitz SD, Banack HR, Kaufman JS. Structural bias in studies of cardiovascular disease: Let's not be fooled by the "obesity paradox". *Can J Cardiol*. 2018;34:540-542.
14. Flegal KM, Kit BK, Orpana H, Graubard BI. Association of all-cause mortality with overweight and obesity using standard body mass index categories: A systematic review and meta-analysis. *JAMA*. 2013;309:71-82.
15. Banack HR, Kaufman JS. The obesity paradox: Understanding the effect of obesity on mortality among individuals with cardiovascular disease. *Prev Med*. 2014;62:96-102.
16. Horta BL, Gigante DP, Goncalves H, et al. Cohort profile update: the 1982 pelotas (brazil) birth cohort study. *Int J Epidemiol*. 2015;44(2):441a-441e.
17. Peres KG, Peres MA, Demarco FF, Tarquinio SB, Horta BL, Gigante DP. Oral health studies in the 1982 pelotas (brazil) birth cohort: methodology and principal results at 15 and 24 years of age. *Cad Saude Publica*. 2011;27:1569-1580.
18. Leite FRM, Peres KG, Do LG, Demarco FF, Peres MAA. Prediction of periodontitis occurrence: Influence of classification and sociodemographic and general health information. *J Periodontol*. 2017;88:731-743.
19. Eke PI, Page RC, Wei L, Thornton-Evans G, Genco RJ. Update of the case definitions for population-based surveillance of periodontitis. *J Periodontol*. 2012;83:1449-1454.
20. Blicher B, Joshipura K, Eke P. Validation of self-reported periodontal disease: A systematic review. *J Dent Res*. 2005;84:881-890.
21. Touboul PJ, Hennerici MG, Meairs S, et al. Mannheim carotid intima-media thickness and plaque consensus (2004–2006-2011). An update on behalf of the advisory board of the 3rd, 4th and 5th watching the risk symposia, at the 13th, 15th and 20th european stroke conferences, mannheim, germany, 2004, brussels, belgium, 2006, and hamburg, germany, 2011. *Cerebrovasc Dis*. 2012;34:290-296.
22. Goff DC Jr, Lloyd-Jones DM, Bennett G, et al. 2013 acc/aha guideline on the assessment of cardiovascular risk: a report of the American College of Cardiology/American Heart Association Task Force on practice guidelines. *J Am Coll Cardiol*. 2014;63:2935-2959.
23. Linhares Rda S, Gigante DP, de Barros FC, Horta BL. Carotid intima-media thickness at age 30, birth weight, accelerated growth during infancy and breastfeeding: a birth cohort study in southern brazil. *PLoS ONE*. 2015;10:e0115166.
24. Whelton SP, Roy P, Astor BC, et al. Elevated high-sensitivity c-reactive protein as a risk marker of the attenuated relationship between serum cholesterol and cardiovascular events at older age. The aric study. *Am J Epidemiol*. 2013;178:1076-1084.
25. Greenland S, Pearl J, Robins JM. Causal diagrams for epidemiologic research. *Epidemiology*. 1999;10:37-48.
26. Robins JM, Hernan MA, Brumback B. Marginal structural models and causal inference in epidemiology. *Epidemiology*. 2000;11:550-560.
27. Peres MA, Nascimento GG, Peres KG, Demarco FF, Menezes AB. Oral health-related behaviours do not mediate the effect of maternal education on adolescents' gingival bleeding: a birth cohort study. *Community Dent Oral Epidemiol*. 2018;46:169-177.
28. Schenkein HA, Loos BG. Inflammatory mechanisms linking periodontal diseases to cardiovascular diseases. *J Clin Periodontol*. 2013;40(Suppl 14):S51-S69.
29. Periodontology EFo. Manifesto: Perio and general health. 2012.
30. Watts D. Scrutinising the link between periodontal disease and systemic conditions: does recent evidence continue to support the european federation of periodontology's 2012 manifesto? *J Int Acad Periodontol*. 2017;19:36-41.
31. D'Aiuto F, Orlandi M, Gunsolley JC. Evidence that periodontal treatment improves biomarkers and cvd outcomes. *J Clin Periodontol*. 2013;40(Suppl 14):S85-105.
32. Deng L, Li C, Li Q, Zhang Y. Zhao H. [Periodontal treatment for cardiovascular risk factors: a systematic review]. *Hua Xi Kou Qiang Yi Xue Za Zhi*. 2013;31:463-467.
33. Li C, Lv Z, Shi Z, et al. Periodontal therapy for the management of cardiovascular disease in patients with chronic periodontitis. *Cochrane Database of Syst Rev*. 2014;CD009197.
34. Elliott P, Chambers JC, Zhang W, et al. Genetic loci associated with c-reactive protein levels and risk of coronary heart disease. *JAMA*. 2009;302:37-48.
35. Banack HR, Kaufman JS. From bad to worse: Collider stratification amplifies confounding bias in the "obesity paradox". *Eur J Epidemiol*. 2015;30:1111-1114.
36. Eke PI, Dye BA, Wei L, et al. Update on prevalence of periodontitis in adults in the United States: Nhanes 2009 to 2012. *J Periodontol*. 2015;86:611-622.
37. Thomson WM, Broadbent JM, Poulton R, Beck JD. Changes in periodontal disease experience from 26 to 32 years of age in a birth cohort. *J Periodontol*. 2006;77:947-954.
38. Magnussen CG, Venn A, Thomson R, et al. The association of pediatric low- and high-density lipoprotein cholesterol dyslipidemia classifications and change in dyslipidemia status with carotid intima-media thickness in adulthood evidence from the cardiovascular risk in Young Finns Study, the Bogalusa Heart Study, and the CDAH (childhood determinants of adult health) study. *J Am Coll Cardiol*. 2009;53:860-869.
39. Juonala M, Viikari JS, Kahonen M, et al. Life-time risk factors and progression of carotid atherosclerosis in young adults: the cardiovascular risk in Young Finns Study. *Eur Heart J*. 2010;31:1745-1751.
40. Hujoel PP, Drangsholt M, Spiekerman C, DeRouen TA. Periodontitis-systemic disease associations in the presence of smoking–causal or coincidental? *Periodontol 2000*. 2002;30(1):51-60.
41. Leite FRM, Nascimento GG, Scheutz F, Lopez R. Effect of smoking on periodontitis: a systematic review and meta-regression. *Am J Prev Med*. 2018;54:831-841.
42. Nascimento GG, Peres MA, Mittinty MN, et al. Diet-induced overweight and obesity and periodontitis risk: an application of the parametric g-formula in the 1982 pelotas birth cohort. *Am J Epidemiol*. 2017;185:442-451.
43. Schuch HS, Nascimento GG, Peres KG, et al. The controlled direct effect of early-life socioeconomic position on periodontitis in a birth cohort. *Am J Epidemiol*. 2019;188:1101-1108.
44. Tonetti MS, Greenwell H, Kornman KS. Staging and grading of periodontitis: framework and proposal of a new classification and case definition. *J Periodontol*. 2018;89(Suppl 1):S159-S172.
45. Andrade RLM, Gigante DP, de Oliveira IO, Horta BL. C-peptide and cardiovascular risk factors among young adults in a southern brazilian cohort. *BMC Endocr Disord*. 2018;18:80.
46. Andersson C, Vasan RS. Epidemiology of cardiovascular disease in young individuals. *Nat Rev Cardiol*. 2018;15:230-240.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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