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Original Article

Subclinical Oral Inflammation is Associated with Reduced Endothelial Function in Young Adults: Evidence from Oral Neutrophil Burden

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ABSTRACT

The periodontium contains an extensive blood supply, and periodontitis is known to trigger harmful structural and functional vascular alterations. In contrast, the influence of minor oral inflammation—levels commonly seen in many otherwise healthy people—on cardiovascular physiology remains uncertain. This preliminary investigation aims to assess how an objectively quantified whole-mouth oral inflammatory load (OIL) relates to vascular function in individuals without known health issues. In this cross-sectional, correlational project, 28 adults aged 18–30 years (16 men, 12 women) with no systemic conditions were enrolled. Oral neutrophil counts, a validated metric for OIL, were obtained from 30-s mouth-rinse samples. Participants also underwent measurements of blood pressure, arterial stiffness via pulse-wave velocity, and endothelial function using brachial artery flow-mediated dilation. Oral neutrophil count was the only factor that significantly explained variation in flow-mediated dilation % ($p = 0.04$; $R^2 = 0.16$; $\beta = -1.05$). Individuals exhibiting OIL values corresponding to $>2.5 \times 10^5$ neutrophils ($n = 8$) demonstrated reduced flow-mediated dilation (6.0 ± 2.3%) compared with participants whose counts reflected gingival health ($<2.5 \times 10^5$ neutrophils: 10.0 ± 5.2%, $p = 0.05$). No meaningful predictors were identified for arterial stiffness. Higher OIL levels were associated with diminished flow-mediated dilation. Compromised flow-mediated dilation is recognized as an early warning sign for later cardiovascular disease—one of the predominant causes of mortality in North America. These findings emphasize the relevance of oral health and suggest that OIL may influence endothelial function.

Keywords: Oral inflammation, Endothelial, Oral neutrophil, Young adults

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Introduction

Inflammation of gingival tissues can be detected before periodontal disease becomes established, and without management, it may progress to full periodontal disease. Globally, periodontal conditions affect up to 90% of adults [1], and periodontitis has been repeatedly linked to cardiovascular outcomes such as myocardial infarction and stroke [2–5]. A commonly proposed explanation involves inflammatory mediators and bacteria accessing systemic circulation through the periodontal vasculature. This contributes to elevated levels of pro-inflammatory cytokines [2, 6], oral pathogens [7], and excessive nitric oxide (NO) production [2, 8], ultimately driving adverse vascular changes. As a result, maintaining good oral hygiene is considered integral to overall health. Our team developed a rapid, non-invasive oral rinse test that quantifies neutrophils to estimate OIL within a brief 30-s rinse [9, 10], enabling assessment of oral inflammation outside dental clinics and facilitating research on oral–systemic links [11, 12].

Most investigations connecting oral conditions to cardiovascular disease have focused on pronounced inflammation associated with periodontitis [6] in older or sedentary populations. Far less is known about whether milder inflammation—routine among younger individuals—affects cardiovascular physiology. Earlier studies often relied on subjective indicators such as self-report instruments [13, 14] or clinician evaluations [3, 15, 16], which introduce variability and incur additional cost and time. In contrast, oral neutrophil quantification offers a rapid, objective

method for grading inflammation [9, 17–19]. Additionally, research involving middle-aged and older adults [3, 6, 7, 15, 16, 20, 21] is complicated by comorbidities and confounders, making it difficult to isolate associations between early periodontal changes and vascular outcomes. Studying young, healthy cohorts reduces these confounding influences.

Evidence indicates that early cardiovascular risks can emerge in youth with inflammatory conditions (e.g., obesity, diabetes) [22, 23]. Thus, if low-grade OIL influences vascular health, it may be detectable even in young adults. Traditional cardiovascular risk factors include blood pressure, lipid profiles, and glucose levels, but more sensitive indicators of arterial function include pulse-wave velocity (PWV) [24] and flow-mediated dilation (FMD) [25].

Therefore, this exploratory study sought to determine whether objectively assessed OIL predicts early vascular dysfunction in healthy young males and females. OIL was quantified from saliva using oral neutrophil counts, an approach known for its speed, non-invasiveness, objectivity, and scalar measurement of gingival inflammation [9, 17]. Vascular endothelial function was measured non-invasively by FMD, and arterial stiffness—reflecting vascular structure—was evaluated using PWV. Our hypothesis was that higher OIL would correspond to poorer vascular function, signaling elevated future risk for cardiovascular disease.

Materials and Methods

Research design

This cross-sectional, correlational investigation collected primary data on OIL and vascular markers during a single visit involving young adults without self-reported periodontal disease. An objective OIL measure served as a predictor of vascular function through linear regression analyses. Ethical approval was granted by the Hamilton Integrated Research Ethics Board (REB project #14145), and the protocol conformed to the Declaration of Helsinki (2013 revision). Raw data can be provided upon request.

Overview of methodology

After written consent was obtained, each participant attended the laboratory having refrained from eating for a minimum of 6 h and having avoided physical activity as well as any beverages or foods containing caffeine or alcohol for at least 8 h before testing. Female participants were scheduled during the early follicular phase of their menstrual cycle (defined as the first 2 days after menstruation begins or during the placebo interval of oral contraceptives). Only oral hormonal contraception was permitted. Following measurements of height and weight, participants performed a 10-s tap-water rinse to clear debris. They then completed a 30-s saline rinse, and the collected samples were stored for subsequent processing. Participants rested in a supine posture for at least 10 min while connected to an electrocardiogram (ECG) for heart rate monitoring. After the rest period, vascular assessments (blood pressure, PWV, and FMD) were conducted while participants remained supine.

Participant characteristics

Men and women aged 18–30 were recruited from the McMaster and Hamilton areas. Recruitment took place through personal referrals, posted advertisements, and social media announcements. To minimize bias, neither recruitment nor communication was performed by dental personnel, and advertisements were not placed in dental clinics. Prior work in older adults showing a 4.5% difference in vascular outcomes related to gingival inflammation [15] suggested a minimum sample of 10. Anticipating milder inflammatory profiles in a young healthy group, we instead enrolled 28 individuals (16 males, 12 females), which provided power to detect a 2.3% difference at 0.8 power. Changes exceeding 1% are regarded as clinically meaningful [26]. All participants were healthy, non-smokers with no known cardiovascular disease or chronic conditions and were not regularly taking medications affecting cardiovascular physiology. Individuals with a history of hypertension or with a BMI above 30 kg/m² were excluded because of their independent effects on vascular outcomes.

Height and weight

Height and weight were recorded to calculate BMI for eligibility screening and risk evaluation. A stadiometer (AnthroFlex) was used to measure stature, and body mass was obtained using a digital scale (Malama).

Saline mouth rinse

Participants avoided food and beverages (other than water) for 6 h before sample collection. They first rinsed with tap water for 10 s, waited 2 min, and then performed a 30-s rinse with 10 ml of sterile saline (0.9% sodium chloride) (Baxter), expelling the solution into a 20-ml collection tube. Samples were fixed to a final concentration of 4% formaldehyde for transport and storage. They were refrigerated at 4 °C for no longer than 2 days before analysis. For neutrophil enumeration, samples were centrifuged, the supernatant was discarded, and the remaining pellet was resuspended in 500 µl of Hank's Balanced Salt Solution. A 250-µl aliquot was stained with 4 µg

Acridine Orange and incubated at room temperature in the dark for 15 min. Samples were mixed again by pipette to ensure consistency and then diluted 10-fold before counting. Neutrophils were manually counted using a hemocytometer under 200× and 400× total magnification [17].

Blood pressure

Brachial artery blood pressure was obtained three times using an automated sphygmomanometer (GE Dynamap V100). Participants remained in the supine position while the cuff was placed on the upper left arm. The monitor automatically cycled once per minute. Mean arterial pressure (MAP) was derived from systolic and diastolic values using the standard formula:

$$\mathrm{MAP} = [\mathrm{SBP} + (2 \times \mathrm{DBP})]/3. \quad (1)$$

Arterial stiffness

Carotid–femoral PWV served as the index of arterial stiffness and is regarded as the reference method [27]. This approach determines how quickly the pressure waveform travels through the thoracic and abdominal aorta. As vessel elasticity diminishes, arterial walls become more rigid, leading to an elevated PWV. For this non-invasive procedure, individuals rested in a supine position and were fitted with a lead 1 ECG and ground electrode. Measurements were conducted in a quiet, temperature-regulated room with low lighting. After a 10-min rest interval, a pressure-sensitive tonometer (Millar Instruments) was positioned at two locations: (1) the carotid artery in the neck and (2) the femoral artery in the inner thigh. Simultaneous pulse-wave recordings enabled determination of transit time, and distance between these anatomical points was used to compute velocity. A correction coefficient of 0.8 was applied to the carotid–femoral path length to adjust for waveform splitting along the carotid and aortic pathways [28]. PWV signals were recorded with Labchart (ADInstruments) and processed with a band-pass filter to identify the foot of each waveform. The interval between pressure-wave arrival at the carotid and femoral sites was used to determine PWV for a single heartbeat. PWV was derived using the following relation:

$$\frac{[0.8(\mathrm{Carotidto\ femoral\ distance:})]}{\mathrm{Pulsewavetransittime}} \quad (2)$$

Values from 20 consecutive cardiac cycles were averaged for each subject.

Endothelial function

While lying supine, participants had a 1-lead ECG recorded. Duplex ultrasound was used to image the brachial artery on the upper right arm, and an uninflated occlusion cuff was placed on the lower right arm [29]. High-resolution B-mode ultrasound videos were obtained with a probe operating at 7.5–12 MHz, using an insonation angle ≤60°. Each assessment began with a baseline imaging period of at least 30 s to capture resting arterial diameter and blood velocity. This was followed by an occlusion phase to induce ischemia. The forearm cuff was rapidly inflated to 200 mmHg using a rapid cuff inflator (E20 Rapid Cuff Inflator and AG101 Air Source; Hokanson, Bellevue, WA). After 5 min, the cuff was deflated to provoke reactive hyperemia. Imaging continued for an additional 3 min to document maximal vasodilation and the hyperemic blood-flow stimulus. The ultrasound protocol therefore captured three segments: baseline, the final 4-min occlusion period (including the minute prior to release), and the post-release phase. To process FMD data, end-diastolic frames were extracted using Sante DICOM Editing software. Arterial diameters were evaluated with automated edge-detection (Arterial Measurement System, Gothenburg, Sweden), with manual correction applied when necessary. Macrovascular endothelial function was quantified as percent dilation relative to baseline. FMD% was computed using:

$$\mathrm{FMD}(\%) = \frac{(\mathrm{ArteryDiameter}_{\mathrm{Peak}} - \mathrm{ArteryDiameter}_{\mathrm{Baseline}})}{\mathrm{Artery\ Diameter}_{\mathrm{Baseline}}} \times 100\% \quad (3)$$

Baseline diameter was obtained from the average of all frames during the baseline recording. For certain individuals with unusable or unstable baseline images, the mean diameter from the minute prior to cuff release was substituted. The peak value represented the maximum taken from a 3-s averaged segment after cuff release.

Blood velocity was assessed using Doppler ultrasound and stored in Labchart (ADInstruments). Shear rate, representing the flow-induced force that drives FMD, was calculated as the shear rate area under the curve for the initial 30 s following cuff release. The following equations were used:

$$\mathrm{MeanBaselineShearRate} = \frac{4(\mathrm{MeanBaselineBV})}{\mathrm{BaselineArteryDiameter}} \quad (4)$$

$$\mathrm{RHShearRate} = \frac{4(\mathrm{meanRHbloodvelocity})}{\mathrm{ArteryDiameter}} \quad (5)$$

$$\text{ShearRateAreaUndertheCurve} = (30\text{smeanRHShearRate} - \text{BaselineShearRate})30\text{sec} \quad (6)$$

Baseline blood velocity for Equation 1 represented a 30-s average at rest, and RH blood velocity for Equation 2 represented a 30-s average immediately after cuff release.

Statistical analyses

Normality was examined with Shapiro–Wilk tests (FMD, PWV). Correlation analyses were conducted between neutrophil count and FMD%, PWV, shear rate, MAP, and BMI to identify variables suitable for multiple linear regression. Because only FMD% showed a significant association with oral neutrophil count, the primary analysis used simple linear regression to predict FMD% from neutrophil levels rather than a multivariable model.

In an exploratory approach, participants were categorized into "high-OIL'' and "low-OIL'' groups using a threshold of 2.5×10^5 neutrophils. This value was chosen by visually examining the data, which showed two distinct groupings above and below 2.5×10^5. A T-test compared these subgroups. The sample size was insufficient for tertile stratification. A separate T-test evaluated sex differences in neutrophil count and FMD%. Statistical significance was defined as $p < 0.05$.

Results and Discussion

Participant information is presented in **Table 1**. None of the measured baseline variables differed meaningfully between males and females.

Table 1. Baseline characteristics.

Anthropometric Variable	Female ($n = 12$)	Male ($n = 16$)	All ($n = 28$)
Age, *y*	22 ± 3	21 ± 1	22 ± 2
Resting HR, *bpm*	62.8 ± 10.5	61.5 ± 7.7	62.1 ± 8.9
Systolic BP, *mmHg*	111.3 ± 9.1	119.6 ± 10.9	116.0 ± 10.8
Diastolic BP, *mmHg*	65.6 ± 7.5	62.7 ± 4.9	64.0 ± 6.2
MAP, *mmHg*	81.5 ± 8.7	82.3 ± 6.0	82.0 ± 7.2
BMI, kg/m^2	23.4 ± 4.0	24.4 ± 2.8	24.0 ± 3.3
FMD, *%*	9.3 ± 4.3	8.5 ± 5.4	8.9 ± 4.9
PWV, *m/s*	7.0 ± 2.3	6.7 ± 1.8	6.8 ± 2.0

HR, Heart Rate; BP, Blood Pressure; MAP, Mean Arterial Pressure; BMI, Body Mass Index; FMD, Flow-Mediated Dilation; PWV, Pulse Wave Velocity. Values shown as mean ± standard deviation.

Oral neutrophils

Oral neutrophil values fell between 1.2×10^4 and 6.5×10^5 cells, with an average count of 2.09×10^5 ($\pm 1.9 \times 10^5$). Most participants exhibited neutrophil levels consistent with mild OIL.

Arterial stiffness

Figure 1 illustrates the association between PWV and oral neutrophil count. No significant link was detected ($p = 0.3$; $R^2 = 0.045$). Using the 2.5×10^5 cutoff to differentiate "high" and "low" OIL did not reveal any additional relationship ($p = 0.1$).

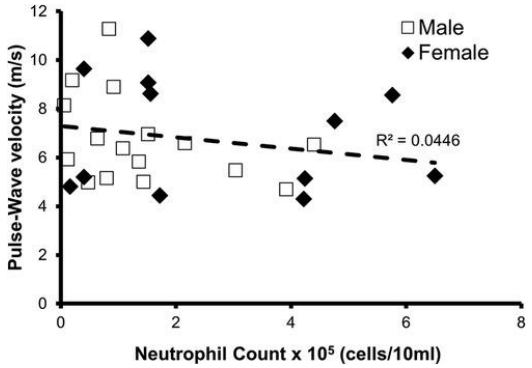


Figure 1. Relationship between oral neutrophil count and pulse-wave velocity (PWV). There was not a significant relationship between neutrophil count and PWV in all participants ($n = 28$, $p = 0.3$, $R^2 = 0.045$).

Endothelial function

Figure 2 shows how FMD% varies with oral neutrophil count. A significant negative association emerged ($p = 0.04$; $R^2 = 0.16$). When categorized at 2.5×10^5, individuals in the high-OIL group ($n = 8$) displayed lower FMD values (6.0 ± 2.3%) than those in the low-OIL group ($n = 20$, 10.0 ± 5.2%, $p = 0.05$) **(Figure 3).**

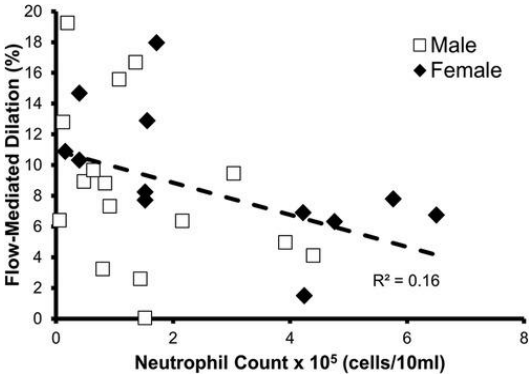


Figure 2. Relationship between oral neutrophil count and flow-mediated dilation % (FMD%). A statistically significant relationship was found to exist between neutrophil count and FMD% ($n = 28$, $p = 0.04$, $R^2 = 0.16$).

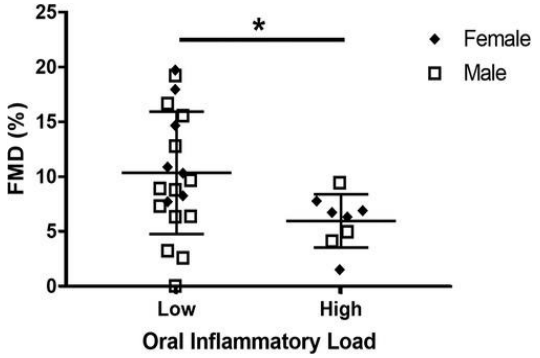
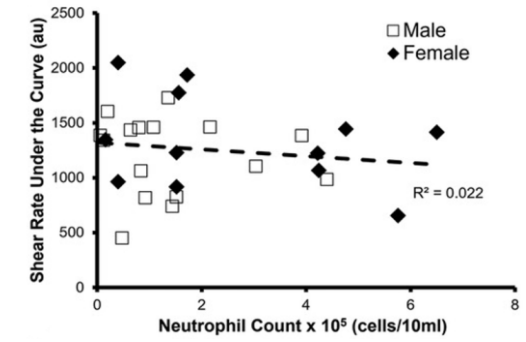
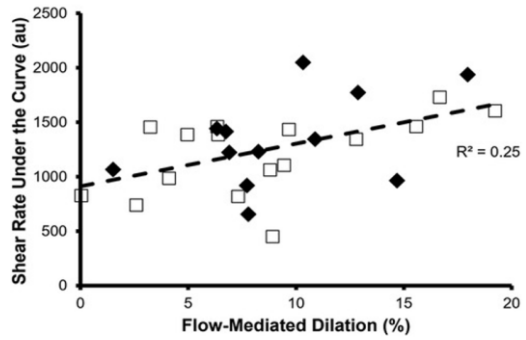


Figure 3. Comparison between high oral inflammatory load (OIL) and FMD% vs. low OIL and FMD%. FMD% was significantly higher in high OIL participants (n = 8) than in low OIL participants (n = 20, p = 0.05).

Shear rate area under the curve showed a significant association with FMD% (p = 0.007; R^2 = 0.25), as displayed in **Figure 4a**. Neutrophil count, however, did not relate to shear rate area under the curve (p = 0.5; R^2 = 0.022) **(Figure 4b)**, indicating that the neutrophil–FMD% link reflects endothelial behavior rather than differences in shear stimulus.



a)



b)

Figure 4. Identifying factors that predict the stimulus for FMD—shear rate area under the curve. (a) There was not a significant relationship between neutrophil count and shear rate under the curve in all participants (n = 28, p = 0.5, R^2 = 0.022). (b) Relationship between flow-mediated dilation % (FMD%) and shear rate under the curve in all participants (n = 28). FMD% significantly predicted shear rate under the curve (p = 0.007, R^2 = 0.25).

A preliminary multi-linear model incorporating MAP, BMI, and oral neutrophils showed that neutrophil count was the only predictor approaching statistical significance (p = 0.06).

Sex differences

Because of limited statistical power, sex-based analyses were inconclusive. In females, the neutrophil–FMD% association reached p = 0.04 (R^2 = 0.36), while in males the trend was weaker (p = 0.2; R^2 = 0.14).

The purpose of this work was to examine whether OIL corresponds with vascular function or arterial properties among young adults free of overt disease. Higher oral neutrophil counts were linked with reduced FMD of the brachial artery, suggesting impaired endothelial responsiveness. Since endothelial dysfunction precedes cardiovascular pathology, the findings imply that even low-level OIL may elevate long-term cardiovascular vulnerability **(Figure 5).** These observations are consistent with a biological pathway connecting OIL to CVD via endothelial mechanisms. Contrary to initial expectations, OIL did not serve as a predictor of arterial stiffness.

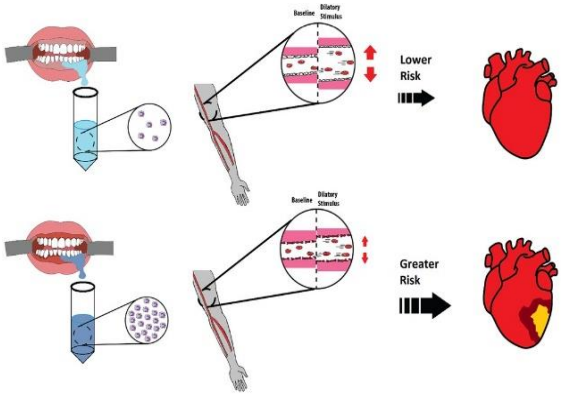


Figure 5. Comparison of healthy and inflamed gingiva and the consequences each may have for later cardiovascular disease (CVD) risk. Healthy gums contain relatively few neutrophils in oral fluid (left top), whereas inflamed gingiva exhibit a markedly higher neutrophil presence (left bottom). Likewise, an intact and responsive endothelium (middle top) demonstrates a larger flow-mediated dilation (%) when exposed to a vasodilatory stimulus, while a compromised endothelium (middle bottom) shows a reduced response. The present study found that greater OIL was linked

with diminished FMD in young, healthy adults, implying a potentially increased likelihood of developing CVD compared to individuals with healthy gingiva.

Mechanistic relationship between oral bacteria and arteries

The junctional epithelium acts as the barrier between the gingival sulcus and the fine vascular network underneath [30]. This tissue is highly permeable and permits metabolic by-products—such as endotoxins, chemotactic molecules, inflammatory mediators, and antigens—from the plaque or biofilm in the sulcus to pass through [31, 32]. Oral inflammation correlates with the amount of plaque-related bacteria within the gingiva [33], and in this study OIL was quantified through oral neutrophil count. These microbial by-products can diffuse into circulation, contributing to elevated systemic inflammatory activity [34]. Circulating inflammatory components may interfere with endothelial integrity, reducing nitric oxide production and altering vascular responsiveness [6, 7, 30, 31]. Such disruptions impair the vessel's capacity to dilate appropriately under shear stress, producing endothelial dysfunction. Over time, this dysfunction is harmful because nitric oxide released by a healthy endothelium protects against atherosclerotic processes and maintains adequate blood flow—even in arteries already affected by disease.

In vessels that function normally, the endothelium detects increased flow via shear stress and triggers the release of vasodilatory substances, especially nitric oxide, which moves into smooth muscle cells to induce dilation. When the endothelium is impaired, the same level of shear stress results in substantially fewer vasodilators being produced, and the artery dilates less [35–37]. In this study, shear rate—a practical surrogate for shear stress—was assessed to determine whether OIL altered the stimulus or the vascular response itself. If shear rate were reduced, FMD would likewise be lower. However, oral neutrophil count did not predict shear rate, showing that the reduction in FMD was not caused by diminished shear stimulus. Although mechanisms were not directly evaluated, the reduced vasodilation may stem from systemic inflammation originating from the diffusion of plaque-derived products into circulation [6, 8, 15, 32, 34].

No association was detected between oral neutrophil count and PWV, an indicator of arterial stiffness, suggesting that structural vascular consequences of OIL had not yet developed. This agrees with prior research conducted in older populations [16, 21] and indicates that OIL exerts minimal influence on arterial structure in younger adults as well.

Strengths and limitations

A large body of research has linked advanced periodontal pathology with altered vascular function [2, 6–8, 20, 21, 32], yet the influence of mild gingival irritation remains unclear [3, 13, 33, 34, 38]. Earlier investigations frequently relied on subjective self-reports [13, 14] or clinical evaluations conducted by multiple examiners [3, 15, 16], which made it difficult to maintain consistent criteria or categorize subtle levels of inflammation. In contrast, the present study used an objective quantification of oral neutrophils—comparable to assessing circulating neutrophils—to determine OIL [9, 17–19]. Notably, endothelial responses were affected even at inflammation levels below the typical clinical threshold for gingivitis [9]. It is plausible that higher, clinically meaningful inflammatory levels would show an even stronger connection to vascular impairment. Another advantage of this work was its focus on young, healthy adults: non-smokers with no CVD history, normal BMI, and without the comorbidities reported in prior studies [3, 13–16, 21]. Although our exclusion criteria for periodontal history relied on participant self-report—which introduces the possibility of recall or reporting bias—the cohort still included many individuals with objectively low OIL, offering a clearer representation of healthy gingival conditions and minimizing common confounders in oral–vascular research.

Because this was a cross-sectional analysis, future investigations should use controlled oral-care interventions paired with a washout design to address unmeasured between-subject differences. Our sample size was smaller than in several earlier studies [3, 13–15], though statistical power was adequate for detecting expected effects, which were indeed observed. Although this study evaluated gingival inflammation levels, subsequent research could include more participants with diagnosed gingivitis (previous study mean 8.8×10^5 neutrophils [9, 17]), confirmed with pocket-depth assessments and/or additional salivary biomarkers. We did not collect standard clinical periodontal measures such as radiographic imaging, probing depth, or bleeding indices, which limits the clinical interpretability of the findings. Nonetheless, the results suggest that even relatively low OIL—levels that might traditionally be viewed as negligible—could still influence markers tied to cardiovascular risk.

Conclusion

Our findings indicate that mild OIL, quantified using oral neutrophil counts, was negatively associated with FMD% in healthy young adults. Reduced FMD is recognized as a predictor of future CVD risk [39]. Consequently, this work supports the idea that oral inflammatory status may play a role in cardiovascular health, even in individuals who otherwise appear to be in good health.

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Conflict of Interest: None

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Ethics Statement: The studies involving human participants were reviewed and approved by Hamilton Integrated Research Ethics Board. The patients/participants provided their written informed consent to participate in this study.

References

1. Nazir M, Al-Ansari A, Al-Khalifa K, Alhareky M, Gaffar B, Almas K. Global prevalence of periodontal disease and lack of its surveillance. Sci World J. 2020;2020:2146160. doi:10.1155/2020/2146160
2. Higashi Y, Goto C, Jitsuiki D, Umemura T, Nishioka K, Hidaka T, et al. Periodontal infection is associated with endothelial dysfunction in healthy subjects and hypertensive patients. Am Heart Assoc. 2008;51(Part 2):446–53. doi:10.1161/ HYPERTENSIONAHA.107.101535
3. Pietropaoli D, Monaco A, D'Aiuto F, Aguilera EM, Ortu E, Giannoni M, et al. Active gingival inflammation is linked to hypertension. J Hypertens. 2020;38(10):2018–27. doi:10.1097/HJH.0000000000002514
4. Sharma JN, Al-Omran A, Parvathy SS. Role of nitric oxide in inflammatory diseases. Inflammopharmacology. 2007;15(6):252–9. doi:10.1007/s10787-007-0013-x
5. Vlachopoulos C, Dima I, Aznaouridis K, Vasiliadou C. Acute systemic inflammation increases arterial stiffness and decreases wave reflections in healthy individuals. Am Heart Assoc. 2005;112:2193–200. doi:10.1161/ CIRCULATIONAHA.105.535435
6. Amar S, Gokce N, Morgan S, Loukideli M, van Dyke TE, Vita JA. Periodontal disease is associated with brachial artery endothelial dysfunction and systemic inflammation. Arterioscler Thromb Vasc Biol. 2003;23(7):1245–9. doi:10.1161/01. ATV.0000078603.90302.4A
7. Elkaïm R, Dahan M, Kocgozlu L, Werner S, Kanter D, Kretz JG, et al. Prevalence of periodontal pathogens in subgingival lesions, atherosclerotic plaques and healthy blood vessels: a preliminary study. J Periodontal Res. 2008;43(2):224–31. doi:10.1111/j.1600-0765.2007.01018.x
8. Batista AC, Silva TA, Chun JH, Lara VS. Periodontal diseases : pathogenesis nitric oxide synthesis and severity of human periodontal disease. Oral Dis. 2002;8:254–60. doi:10.1034/j.1601-0825.2002.02852.x
9. Landzberg M, Doering H, Aboodi GM, Tenenbaum HC, Glogauer M. Quantifying oral inflammatory load: oral neutrophil counts in periodontal health and disease. J Periodontal Res. 2015;50(3):330–6. doi:10.1111/jre.12211
10. Khoury W, Glogauer J, Tenenbaum HC, Glogauer M. Oral inflammatory load: neutrophils as oral health biomarkers. J Periodontal Res. 2020;55(5):594–601. doi:10. 1111/jre.12758
11. Huda S, Doering H, Tenenbaum HC, Whittle W, Sigal MJ, Glogauer M. Oral neutrophil levels: a screening test for oral inflammatory load in pregnancy in a medical setting. J Periodontol. 2015;86(1):72–81. doi:10.1902/jop.2014.140116
12. Moosani A, Sigal MJ, Glogauer M, Lawrence HP, Goldberg M, Tenenbaum HC. Evaluation of periodontal disease and oral inflammatory load in adults with special needs using oral neutrophil quantification. Spec Care Dentist. 2014;34(6):303–12. doi:10.1111/scd.12077
13. Wohlfeil M, Wehner J, Schacher B, Oremek GM, Sauer-Eppel H, Eickholz P. Degree of gingivitis correlates to systemic inflammation parameters. Clin Chim Acta. 2009;401(1-2):105–9. doi:10.1016/j.cca.2008.11.017

14. Ylöstalo PV, Järvelin MR, Laitinen J, Knuuttila ML. Gingivitis, dental caries and tooth loss: risk factors for cardiovascular diseases or indicators of elevated health risks. J Clin Periodontol. 2006;33(2):92–101. doi:10.1111/j.1600-051X.2005. 00875.x
15. Carallo C, Irace C, Tripolino C, De Franceschi MS, Procopio A, Crispino A, et al. Time course analysis of brachial artery flow mediated dilatation in subjects with gingival inflammation. Int Angiol. 2014;33(6):565–72.
16. Hujoel PP, Drangsholt M, Spiekerman C, DeRouen TA. Periodontal disease and coronary heart disease. J Am Med Assoc. 2000;284(11):1406–10. doi:10.1001/jama. 284.11.1406
17. Bender JS, Thang H, Glogauer M. Novel rinse assay for the quantification of oral neutrophils and the monitoring of chronic periodontal disease. J Periodontal Res. 2006;41(3):214–20. doi:10.1111/j.1600-0765.2005.00861.x
18. Fine N, Tasevski N, McCulloch CA, Tenenbaum HC, Glogauer M. The neutrophil: constant defender and first responder. Front Immunol. 2020;11 (September):1–15. doi:10.3389/fimmu.2020.571085
19. Wellappuli NC, Fine N, Lawrence HP, Goldberg M, Tenenbaum HC, Glogauer M. Oral and blood neutrophil activation states during experimental gingivitis. JDR Clin Trans Res. 2018;3(1):65–75. doi:10.1177/2380084417742120
20. Jockel-Schneider Y, Harks I, Haubitz I, Fickl S, Eigenthaler M, Schlagenhauf U, et al. Arterial stiffness and pulse wave reflection are increased in patients suffering from severe periodontitis. PLoS One. 2014;9(8):1–8. doi:10.1371/journalone. 0103449
21. Miyaki K, Masaki K, Naito M, Naito T, Hoshi K, Hara A, et al. Periodontal disease and atherosclerosis from the viewpoint of the relationship between community periodontal index of treatment needs and brachial-ankle pulse wave velocity. BMC Public Health. 2006;6:1–6. doi:10.1186/1471-2458-6-131
22. Slattery DJ, Stuckless TJR, King TJ, Pyke KE. Impaired handgrip exercise- induced brachial artery flow-mediated dilation in young obese males. Appl Physiol Nutr Metab. 2016;537:528–37. doi:10.1139/apnm-2015-0459
23. Bellien J, Costentin A, Dutheil-Maillochaud B, Iacob M, Kuhn J, Thuillez C, et al. Early stage detection of conduit artery endothelial dysfunction in patients with type 1 diabetes. Diab Vasc Dis Res. 2010;7(2):158–66. doi:10.1177/1479164109360470
24. Ueki Y, Miura T, Minamisawa M, Abe N, Nishimura H, Hashizume N, et al. The usefulness of brachial-ankle pulse wave velocity in predicting long-term cardiovascular events in younger patients. Heart Vessels. 2017;32(6):660–7. doi:10.1007/s00380-016- 0919-6
25. Holder SM, Bruno RM, Shkredova DA, Dawson EA, Jones H, Hopkins ND, et al. Reference intervals for brachial artery flow-mediated dilation and the relation with cardiovascular risk factors. Hypertension. 2021;77(5):1469–80. doi:10.1161/ HYPERTENSIONAHA.120.15754
26. Inaba Y, Chen JA, Bergmann SR. Prediction of future cardiovascular outcomes by flow-mediated vasodilatation of brachial artery: a meta-analysis. Int J Cardiovasc Imaging. 2010;26(6):631–40. doi:10.1007/s10554-010-9616-1
27. De Luca M, Iacono O, Valente V, Giardino F, Crisci G, Lettieri M, et al. Can pulse wave velocity (PWV) alone express arterial stiffness? A neglected tool for vascular function assessment. J Basic Clin Physiol Pharmacol. 2022;33(4):373–79. doi:10.1515/jbcpp-2021-0193
28. Huybrechts SAM, Devos DG, Vermeersch SJ, Mahieu D, Achten E, de Backer TLM, et al. Carotid to femoral pulse wave velocity: a comparison of real travelled aortic path lengths determined by MRI and superficial measurements. J Hypertens. 2011;29(8):1577–82. doi:10.1097/HJH.0b013e3283487841
29. Thijssen DHJ, Black MA, Pyke KE, Padilla J, Atkinson G, Harris RA, et al. Assessment of flow-mediated dilation in humans: a methodological and physiological guideline. Am J Physiol Heart Circ Physiol. 2011;300(1):2–12. doi:10.1152/ajpheart.00471.2010
30. Bosshardt DD, Lang NP. Critical reviews in oral biology & medicine the junctional epithelium : from health to disease. J Dent Res. 2005;84(1):9–20. doi:10.1177/154405910508400102
31. Walmsley AD, Walsh TF, Lumley PJ, Burke FJT, Shortall AC, Hayes-Hall R, et al. Restorative dentistry. 2nd ed. Philadelphia: Churchill Livingstone Elsevier; 2007.
32. Beck JD, Elter JR, Heiss G, Couper D, Mauriello SM, Offenbacher S. Relationship of periodontal disease to carotid artery intima-media wall thickness: the atherosclerosis risk in communities (ARIC) study. Arterioscler Thromb Vasc Biol. 2001;21(11):1816–22. doi:10.1161/hq1101.097803

33. Rathee M, Jain P. Gingivitis. Treasure Island (FL): StatPearls Publishing; 2020. pp. 1–6.
34. Eberhard J, Grote K, Luchtefeld M, Heuer W, Schuett H, Divchev D, et al. Experimental gingivitis induces systemic inflammatory markers in young healthy individuals: a single-subject interventional study. PLoS One. 2013;8(2):1–10. doi:10.1371/journal.pone.0055265
35. Hocher B, Schwarz A, Slowinski T, Bachmann S, Pfeilschifter J, Neumayer HH, et al. In-vivo interaction of nitric oxide and endothelin (multiple letters). J Hypertens. 2004;22:111–9. doi:10.1097/01.hjh.0000098151.70956.e6
36. Deanfield JE, Halcox JP, Rabelink TJ. Endothelial function and dysfunction: testing and clinical relevance. Circulation. 2007;115(10):1285–95. doi:10.1161/ CIRCULATIONAHA.106.652859
37. Vogel RA. Measurement of endothelial function by brachial artery flow- mediated vasodilation. Am J Cardiol. 2001;88(SUPPL):31E–4E. doi:10.1016/ S0002-9149(01)01764-7
38. Khader YS, Albashaireh ZSM, Alomari MA. Periodontal diseases and the risk of coronary heart and cerebrovascular diseases: a meta-analysis. J Periodontol. 2004;75(8):1046–53. doi:10.1902/jop.2004.75.8.1046
39. Yeboah J, Folsom AR, Burke GL, Johnson C, Polak JF, Post W, et al. Epidemiology and prevention predictive value of brachial flow-mediated dilation for incident cardiovascular events in a population-based study the multi-ethnic study of atherosclerosis. Circulation. 2009;120(6):502–9. doi:10.1161/CIRCULATIONAHA.109.864801