HEALTHY LIFESTYLES FOR HEALTHY GUMS
Towards the implementation of lifestyle modifications to improve oral and general health

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Department of Medical Biotechnologies

PhD Program in Medical Biotechnologies, 36° Cycle

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Towards the implementation of lifestyle modifications

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Introduction

Introduction

Unhealthy lifestyles are at the root of the global burden of NCDs, which account for approximately 41 million deaths (71%) globally each year (WHO, 2021). Unhealthy lifestyle behaviours, such as poor nutrition, physical inactivity, alcohol/tobacco use, poor sleep quality, and high psychological stress, constitute the multiple determinants of "health" together with genetics, environment, and access to medical care, as defined by the U.S. Department of Health and Human Services. Over the past several years, there has been an increased interest in evaluating the benefit of adhering to healthier lifestyle behaviours in the development of morbidity and mortality (Kushner & Sorensen, 2013). In many epidemiological studies, patterns of healthy lifestyle behaviours, characterized by high diet quality (e.g., high adherence to Mediterranean Diet - MD), adequate frequency of physical activity (PA), and not smoking, were associated with a lower risk of Non-Communicable Diseases (NCDs) onset, cardiovascular events, and mortality when compared to subjects with unhealthy lifestyles (Chiuve et al., 2011; Ford et al., 2009; Stringhini et al., 2010). Lately, the importance of effectively managing lifestyle-related risk factors was also linked to the pressing worldwide epidemic of "multimorbidities", i.e., the presence of 2 or more long-term physical or mental disorders, which are becoming a public health concern (Guthrie et al., 2012). From a biological standpoint, the detrimental impact of unhealthy lifestyles on systemic health may be mainly ascribed to the induction of a state of low-grade systemic inflammation (LGSI) and to the overproduction of reactive oxygen species (ROS), leading to oxidative stress (Esposito et al., 2004; Frodermann et al., 2019). Since LGSI has been bi-directionally linked with periodontitis (D'Aiuto et al., 2010; Romandini et al., 2018), the association between lifestyle

behaviours and periodontitis has been analyzed in several epidemiological studies, which frequently reported a higher prevalence as well as a more severe disease phenotype in subjects with unhealthy lifestyle behaviours (Coelho et al., 2020; Karaaslan & Dikilitaş, 2019; Marruganti et al., 2022, 2023; Morales et al., 2022; Romandini et al., 2017). Nonetheless, little is known about the combined effects of lifestyles on periodontitis onset, severity, and treatment outcomes. Therefore, the aim of the present PhD project was to investigate the association between lifestyles, both independently and combined, and periodontal diseases using cross-sectional and longitudinal study designs.

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ADHERENCE TO MEDITERRANEAN DIET, PHYSICAL ACTIVITY LEVEL AND SEVERITY OF PERIODONTITIS. RESULTS FROM A UNIVERSITY-BASED CROSS-SECTIONAL STUDY.

By

Marruganti, C., Traversi, J., Gaeta, C., Cagidiaco, E. F., Parrini, S.,

Discepoli, N., & Grandini, S. (2022).

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Chapter 1. Adherence to Mediterranean diet, physical activity level and severity of periodontitis. Results from a University-based cross-sectional study.

1 Introduction

Periodontitis is defined as a biofilm-mediated noncommunicable chronic inflammatory Disease (NCD) characterized by the progressive destruction of the tooth supporting apparatus. Periodontitis is a highly common chronic inflammatory NCD, with a prevalence of its severe form between 7-11% (Eke et al., 2018); it represents the sixth most prevalent condition worldwide (Eke et al., 2018). Furthermore, periodontitis is associated with a range of systemic diseases, including diabetes (Sanz et al., 2018), cardiovascular disease (CVD) (Sanz, del Castillo, et al., 2020) and adverse pregnancy outcomes (Sanz, del Castillo, et al., 2020). Several factors, such as overweight, smoking, unhealthy diet, and physical inactivity (Reynolds, 2014), are associated with disease occurrence and are shared as risk indicators with other prevalent NCDs, such as type-2 diabetes mellitus (T2DM) and CVD.

Indeed, periodontitis exerts a detrimental impact on both masticatory function and general health, thus resulting in higher dental healthcare costs (Tonetti et al., 2017). Consequently, a lot of research (Allen et al., 2017; Bawadi et al., 2011; Iwasaki et al., 2018; Odorico et al., 2019) focused on the treatment of NCDs through a multifactorial approach targeted at improving various aspects of the patient's lifestyle, such as smoking, diet, or physical activity (PA). In particular, the pioneer Seven Countries study in the 1950s was the first one to highlight the benefic effects of the Mediterranean Diet (MD) (Verschuren et al., 1995); since then, epidemiological evidence flourished regarding the ability of MD to

significantly reduce the risk of developing NCDs such as metabolic syndrome, T2DM, CVD and cancers (Benetou et al., 2008; Sofi et al., 2008). The latest consensus on the Mediterranean pyramid (Bach-Faig et al., 2011) encompassed not only the consumption and serving size of specific food groups, but also other lifestyle dimensions (*i.e.* regular exercise and adequate rest) which, collectively, were framed in the "Mediterranean lifestyle" (ML) (Reynolds, 2014).

While on one hand a lot of studies focused on the effects of each single item of the ML (*e.g.* PA or nutrition) (de Oliveira Ferreira et al., 2019; Diolintzi et al., 2019; Iwasaki et al., 2018), no data are present regarding the impact of the combination of ML components or ML as a whole on oral health. In particular, while there is accumulating evidence (Arouca et al., 2018; Barbaresko et al., 2013; Bonaccio et al., 2017) regarding the anti-inflammatory potential of MD, on the other hand no evidence is present regarding the synergistic/antagonistic impact of adherence to both MD and PA on periodontal health. The hypothesis that we would like to figure out with this current cross-sectional design is that low MD adherence combined with a sedentary behaviour would increase low-grade systemic inflammation, lipid levels and oxidative stress, as well as decrease insulin sensitivity (Beauchamp et al., 2005; Estruch et al., 2006; Tosti et al., 2018); as such, they would contribute to a more severe periodontitis phenotype. Therefore, the aim of the present cross-sectional study was to evaluate the association between MD adherence and PA level with the biometric and inflammatory periodontal parameters of a University-based cohort of individuals.

2 Materials and methods

2.1 *Study design*

The present study is reported according to the Strengthening the Reporting of Observational studies in Epidemiology (STROBE) guidelines for cross-sectional studies (von Elm et al., 2008). The research protocol was approved by the local ethics committee (protocol number: 18993/2021) and received the registration number on Clinicaltrials.gov (NCT04771949).

2.2 *Setting and participants*

All consecutive patients attending the Unit of Periodontology at the University of Siena were screened between January 2021 and August 2021; the inclusion criteria were: i) age between 18 and 70 years old; ii) ability and willingness to give informed consent. The exclusion criteria were: i) pregnancy or lactation; ii) periodontal therapy performed in the last 12 months; iii) administration of antibiotics within the last 6 months; iv) inability to communicate effectively in Italian. Individuals were included in the study after they read and signed the written informed consent, in accordance with the Declaration of Helsinki.

2.3 *Variables*

2.3.1 *Socio-demographic characteristics*

Information regarding patients age, gender, smoking and oral hygiene habits, occupation and education level was registered. Moreover, data regarding the presence of familiarity as well as any comorbidity affecting susceptibility to periodontitis were recorded. The Body

Mass Index (BMI) was computed as weight (kilograms)/height (meters^2). The assessment methods of socio-demographic characteristics are reported in the Supplementary Appendix.

2.3.2 *Dietary assessment*

A validated 15-item questionnaire to measure patients' adherence to MD (QueMD) was administered by two examiners (C.M.,J.T.), following the structured questions and explanations provided by the questionnaire (Gnagnarella et al., 2018). The questionnaire included questions regarding the foods most frequently associated with MD (wholegrain pasta, bread or substitutes; raw or cooked vegetables; all types of fresh fruits; dairies; either red or white wine; olive oil; red meat; fish; dried fruits and pulses), as well as other commonly consumed items (white meat, carbonated beverages or soft drinks; butter, cooking cream or margarine; manufactured sweets or pastries) (Supplementary Table 1). For each component, a standard portion for the Italian population was indicated (SINU, 2019) and participants could choose among 5 consumption frequencies, which differed according to food items. The alternate MD score (aMed) was drawn from the QueMD results in order to evaluate patients' adherence to MD (Gnagnarella et al., 2018); it was calculated by assigning 1 point to participants reporting food consumptions above the Italian National levels (Guidelines for an Italian Healthy Eating, 2003) for each of the following items typical of the MD: wholegrain products (≥1/day), vegetables (≥2/day), fresh fruits (≥2/day), olive oil (≥3/day), wine (1-2 glasses/day for males or 1 glass/day for females), red meat (≤1-3/week), fish (≥2/week), dried fruits (≥2/week), pulses (≥2/day) (Bach-Faig et al., 2011). The sum score ranged between 0

(minimum MD adherence) and 9 (maximum MD adherence); it was dichotomized in order to define cases of low (aMed<5) and high (aMed>4) adherence to MD considering its median in the current study population as the cut-off value.

2.3.3 *Physical activity assessment*

PA was assessed through the validated short version of the International Physical Activity Questionnaire (IPAQ) (Mannocci et al., 2010). It was administered by two examiners (C.M., J.T.), who asked the structured questions and gave the explanations provided by the questionnaire (Mannocci et al., 2010). It consists of 7 items regarding the frequency and amount of time spent doing intense and moderate PA, as well as walking or doing sedentary activities during the last 7 days (Supplementary Table 1). The overall PA level was classified as low, moderate, or high through the IPAQ automatic report (https://theipaq/home).

2.3.4 *Periodontal examination*

All participants received a full periodontal chart by two trained and calibrated examiners (C.M., J.T.) (unweighted *kappa* score of 0.98). Examiners were calibrated by performing a full periodontal chart on two non-study subjects affected by periodontitis; the examiner was considered reproducible if an agreement of at least 95% of Clinical Attachment Level (CAL) (with maximum a 2 mm difference) between two repeated measurements was recorded. Periodontal Probing Depth (PPD), gingival Recession (REC), plaque (O'Leary et al., 1972) and Bleeding on Probing (BoP) (Ainamo & Bay, 1975) were

recorded with a standardized periodontal probe (UNC 15 probe, HuFriedy Group, Chicago, Illinois, United States) six sites per tooth, third molars excluded. Whenever the Cementum Enamel Junction (CEJ) was subgingival, CAL was measured as the difference between PPD and the distance between the free gingival margin and the CEJ. The presence of furcation involvement was recorded according to the classification of Hamp (Hamp et al., 1975); the classification of Miller (Miller, 1950) was used to record tooth mobility. A periodontitis case was defined whenever interdental CAL was detectable at ≥2 non-adjacent teeth, or whenever buccal or oral CAL≥3mm with pocketing (PPD>3mm) was detectable at ≥2 teeth (Tonetti et al., 2018). Periodontitis severity, complexity of management and extent of distribution were assessed with the Staging (Papapanou et al., 2018); the Grading was used to assess the rate of disease progression (Papapanou et al., 2018). Whenever possible, the Grade was assigned with direct evidence of disease progression (*i.e.* longitudinal data of radiographic bone loss or CAL over 5 years); whenever these data were not available, then indirect evidence was used (case presentation or % bone loss/age). As for the Grade modifiers, smoking status was self-reported, while the diagnosis of diabetes was ascertained by checking the patient's medical report; glycohemoglobin levels were considered only when available (Papapanou et al., 2018).

2.4 *Statistical analysis*

2.4.1 *Sample size calculation*

Sample size was calculated considering the prevalence of periodontitis in the reference cohort at 37.3% (Jiao et al., 2020) and its value in the study cohort as 10% higher.

Considering α=0.05 and β=0.80, the computed sample size was of 185 subjects. Given a non-response rate of 20%, the inclusion of 235 participants was planned.

2.4.2 *Descriptive and inferential statistics*

Statistical analysis was performed through an *ad hoc* software (STATA BE, version 17, StataCorp LP, Texas, United States) setting the level of significance at α=0.05. Continuous variables were reported as Mean with 95% Confidence Interval; binomial and categorical data were expressed as number of observations (proportion). After verification of data distribution, Kruskal-Wallis and Fisher's exact tests were used to compare patients' characteristics according to oral health status, MD adherence, PA level and their possible combination (low aMed and low/moderate PA, low aMed and high PA, high aMed and low/moderate PA, and high aMed and high PA).

2.4.3 *Logistic regression models*

Univariate and multivariate logistic regression analyses were performed to compute the association between stage III/IV periodontitis according to PA level, aMed and each component of the aMed score; it was expressed as crude and adjusted odds ratios (ORs). ORs were adjusted for parameters that could affect periodontitis phenotype (*i.e.* age, gender, smoking, and brushing frequency); these parameters were selected according to external knowledge. A multivariate logistic regression model was then built to evaluate the impact of the combination between MD adherence and PA level (independent variable) on the occurrence of stage III/IV periodontitis cases (dependent variable). The best model was chosen

according to the highest value of AUC, and the lowest values of Akaike (AIC) and Bayesian (BIC) information criteria. The predictors included in the final model encompassed: i) age; ii) smoking; iii) familiarity for periodontitis; iv) presence comorbidities. Moreover, the mediating effect of BMI on the impact of the combination between MD adherence and PA level on the presence of stage III-IV periodontitis was investigated.

3 Results

3.1 *Participant characteristics*

A total of 235 participants were included in the present study. The *post-hoc* power analysis for each outcome, *i.e.* association between stage III/IV periodontitis and low MD adherence, low PA level and their combination, respectively, is reported in Supplementary Table 2. All individuals examined for eligibility accepted to participate, were enrolled in the study, and then included in the analysis. Subjects' characteristics overall and by periodontal status are reported in Table 1. The mean age was 53.90 [52.01, 55.79] years, with a proportion of 57.87% females and 25.96% smokers; the mean BMI was 25.49 [24.88, 26.11]. Around 20% of subjects were affected by at least one comorbidity. Moreover, half the participants were affected by stage III/IV periodontitis; significant differences as to age, occupation, education, and familiarity for periodontitis were found across subgroups of periodontitis severity (Table 1). No significant differences were reported as to domiciliary oral hygiene procedures.

Table 1. *Descriptive statistics overall and by oral health status. Values with different superscript letters are different at the 5% level.*

Variable	TOTAL n=235	Healthy-gingivitis n=34	Stage I periodontitis n=27	Stage II periodontitis n=56	Stage III/IV periodontitis n=118	p-value*
Socio-demographic characteristics						
Age	53.90 [52.01, 55.79]	38.81[a] [32.78, 44.85]	48.29[ac] [42.48, 54.09]	53.86[b,c] [50.01, 57.71]	58.49[d] [56.76, 61.34]	.00
BMI	25.49 [24.88, 26.11]	23.79[a] [22.44, 25.12]	24.37[a] [22.73, 26.01]	23.94[a] [22.91, 24.97]	26.98[b] [26.04, 27.91]	.00
Gender, females	136 (57.87%)	20 (58.82%)	17 (62.96%)	38 (67.86%)	61 (51.69%)	.29
Occupation						
Unemployed	42 (17.95%)	9[a] (27.27%)	7[a,b] (25.93%)	3[b] (5.36%)	23[a] (19.49%)	
Employed	133 (56.84%)	20[a] (60.61%)	16[a,b] (59.26%)	39[b] (69.64%)	58[a] (49.15%)	.04
Retired	59 (25.21%)	4[a] (12.12%)	4[a,b] (14.81%)	14[b] (25%)	37[a] (31.36%)	
Education						
Elementary/middle school	69 (29.49%)	6[a] (17.65%)	1[a] (3.70%)	12[a] (21.82%)	50[b] (42.37%)	.00
High school	107 (45.73%)	18[a] (52.94%)	14[a] (51.85%)	29[a] (52.73%)	46[b] (38.98%)	
College or more	58 (24.79%)	10[a] (29.41%)	12[a] (44.44%)	14[a] (25.45%)	23[b] (18.64%)	
Smoking						
Never	107 (45.53%)	22 (64.71%)	15 (55.56%)	26 (46.43%)	44 (37.29%)	.12
Former	67 (28.51%)	5 (14.71%)	4 (14.81%)	19 (33.93%)	39 (33.05%)	
Smoker	61 (25.96%)	7 (20.59%)	8 (29.63%)	11 (19.64%)	35 (29.66%)	
Familiarity for periodontitis, yes	87 (37.02%)	8 (23.53%)	8 (29.63%)	19 (33.93%)	52 (44.07%)	.06
aMed score†	4.31 [4.05, 4.56]	5.35[a] [3.61, 6.09]	6.07[a] [5.55, 6.59]	5.13[a] [4.71, 5.54]	3.5[c] [3.16, 3.84]	.00
High aMed	118 (50.42%)	17[a] (50%)	26[a] (96.30%)	40[b] (71.43%)	35[c] (29.66%)	.00
Low aMed	117 (49.58%)	17[a] (50%)	1[a] (3.70%)	16[b] (28.57%)	83[c] (70.34%)	
PA level						
High PA	67 (28.81%)	10[a] (29.41%)	12[a] (44.44%)	19[a] (33.93%)	26[b] (22.03%)	.04
Low/Moderate PA	168 (71.19%)	24[a] (70.59%)	15[a] (55.56%)	37[a] (66.07%)	92[b] (77.97%)	
Oral health status						
Number of teeth	24.17 [23.47, 24.86]	27.14[a] [25.93, 28.36]	25.33[a] [23.51, 27.16]	24.52[a] [22.97, 26.09]	22.94[b] [21.91, 23.97]	.00
CAL	2.83 [2.72, 2.94]	0.88[a] [0.59, 1.18]	2.18[a] [2.06, 2.30]	2.68[b] [2.52, 2.84]	3.28[c] [3.13, 3.44]	.00
PPD	2.53 [2.45, 2.62]	1.89[a] [1.80, 1.97]	2.17[a] [2.07, 2.27]	2.41[b] [2.28, 2.53]	2.86[c] [2.73, 299]	.00
% PPD≥4mm	9.30 [7.81, 10.79]	0.10[a] [0.01, 0.26]	0.22[b,c] [0.02, 0.40]	7.03[c] [3.57, 9.49]	15.59[d] [13.22, 17.95]	.00
% PPD 5-6mm	7.71 [6.51, 8.90]	0[a] [0, 0]	0.18[b,c] [0.09, 0.39]	6.19[c] [3.03,7.35]	12.83[d] [10.93, 14.72]	.00
% PPD>6mm	4.03 [0.19, 8.24]	0[a] [0, 0]	0[a,b] [0, 0]	9.67[b] [8.19, 27.29]	3.41[c] [2.28, 4.55]	.00
Furcation involvement, yes‡	76 (32.34%)	0[a] (0%)	0[a] (0%)	9[a] (16.07%)	67[b] (56.78%)	.00
Mobility, yes	91 (38.72%)	0[a] (0%)	1[a] (3.70%)	17[a] (30.36%)	73[b] (61.86%)	.00
Number of bleeding pockets§	7.32 [5.98, 8.65]	0[a] [0, 0]	0[b,c] [0, 0]	4.16[c] [2.55, 5.76]	12.26[d] [10.06, 14.46]	.00
FMPS	50.56 [47.45, 53.68]	32.47[a] [27.05, 37.89]	55.66[b] [46.59, 64.74]	50.64[b] [44.41, 56.87]	54.57[b] [50.03, 59.11]	.02
FMBS	28.45 [26.17, 30.74]	14.97[a] [11.29, 18.64]	22.70[a,b] [16.90, 28.50]	28.13[b,c] [23.15, 33.09]	33.81[c] [30.65, 36.97]	.00
Teeth lost for periodontitis, yes	76 (32.34%)	0[a] (0%)	0[a] (0%)	0[a] (0%)	76[b] (64.41%)	.00
Grade¶						
none	34 (14.45%)	34 (100%)	0 (0%)	0 (0%)	0 (0%)	.00
A	21 (8.94%)	0[a] (0%)	12[b] (44.44%)	5[c] (8.93%)	4[a] (3.39%)	
B	111 (47.23%)	0[a] (0%)	11[b] (40.74%)	43[c] (76.78%)	57[d] (48.31%)	
C	69 (29.36%)	0[a] (0%)	4[b] (14.81%)	8[c] (14.29%)	57[d] (48.31%)	
Extent¶						
none	34 (14.45%)	34 (100%)	0 (0%)	0 (0%)	0 (0%)	0.96
Localized	104 (44.25%)	0 (0%)	15 (55.56%)	28 (50%)	61 (51.69%)	
Generalized	97 (41.30%)	0 (0%)	12 (44.44%)	28 (50%)	57 (48.31%)	
Domiciliary plaque control						
Brushing frequency						
Not performed	4 (1.70%)	1 (2.94%)	0 (0%)	0 (0%)	3 (2.54%)	.06
Occasionally	46 (19.57%)	3 (8.82%)	3 (11.11%)	7 (12.50%)	33 (27.97%)	
Every day	185 (78.72%)	30 (88.24%)	24 (88.89%)	49 (87.50%)	82 (69.49%)	

Toothbrush type, powered	122 (51.91%)	15 (44.12%)	14 (51.85%)	30 (53.57%)	63 (53.39%)	.47
Interdental cleaning (IC)						
Not performed	75 (31.91%)	15 (44.12%)	9 (33.33%)	18 (32.14%)	34 (28.81%)	.23
Interdental floss	51 (21.70%)	9 (26.47%)	7 (25.93%)	12 (21.43%)	23 (19.49%)	
Interproximal brushes	109 (45.99%)	10 (29.41%)	11 (40.74%)	26 (46.43%)	61 (51.69%)	
Frequency of IC						
Not performed	75 (31.91%)	14 (41.18%)	9 (33.33%)	17 (30.36%)	35 (29.66%)	.72
Occasionally	48 (20.43%)	6 (17.65%)	4 (14.81%)	9 (16.07%)	29 (24.58%)	
Every day	112 (47.66%)	14 (41.18%)	14 (51.85%)	30 (53.57%)	54 (45.76%)	

Notes. Results of continuous variables are reported as mean [95% Confidence Interval]; results of binary and categorical variables are expressed as number of observations (proportion).

Abbreviations. BMI, body mass index; aMed, alternate Mediterranean diet score; PA, physical activity; CAL, clinical attachment level; PPD, probing depth; FMPS, full-mouth plaque score; FMBS, full-mouth bleeding score; IC, interdental cleaning.

[*] *p*-value of the Kruskal Wallis or Fisher's exact test for patients' characteristics across the four subgroups.

[†] High aMed if aMed>4; Low aMed if aMed<5.

[‡] Class II/III furcation involvement according to the classification of Hamp *et al.* (1975).

[§] Defined as the number of sites with probing depth≥5mm and positive to bleeding on probing.

[¶] According to the 2018 EFP/AAP classification.

3.2 *Outcome data*

3.2.1 *Adherence to Mediterranean Diet and periodontitis*

High adherence to MD was significantly associated to a lower prevalence of stage III/IV periodontitis (29.66%) compared to those with low adherence (70.34%) (Table 2). Other biometric periodontal variables (*i.e.* %PPD>4mm, %PPD 5-6mm, furcation involvement, tooth mobility, number of bleeding pockets, teeth lost for periodontal causes) were reported to be significantly worse in individuals with low MD adherence (Table 2). High aMed

scores as well as the frequent consumption of specific MD foods resulted to be associated with lower odds of periodontitis occurrence as well as stage III/IV periodontitis, even after adjusting for age, gender, smoking and brushing frequency (Supplementary Table 3; Table 3).

3.2.2 *Physical activity and periodontitis*

Around 1/3 of participants reached a high PA level, while the other 2/3 were in the low/moderate PA level category (Table 2). A high PA level was significantly associated with a lower prevalence of stage III/IV periodontitis (22.03%) when compared to low/moderate PA (77.97%). A low/moderate PA level was not associated with periodontitis occurrence (Supplementary Table 3). Conversely, a low/moderate PA level increased the odds of having stage III/IV periodontitis, but not after adjustments (OR=1.65[0.84, 3.28], p=0.42) (Table 3).

Table 2. *Patients' characteristics by adherence to Mediterranean diet (aMed) and physical activity level.*

Variable	aMed			PA level		
	Low (0-4)	High (5-9)	*p*-value*	Low/moderate	High	*p*-value*
	n=117	n=118		n=168	n= 67	
Age	53.63 [50.88, 56.37]	54.17 [51.54, 56.80]	.93	54.52 [52.36, 56.68]	52.39 [48.45, 56.23]	.35
BMI	25.90 [25.02, 26.78]	25.09 [24.22, 25.96]	.11	25.96 [25.19, 26.72]	24.32 [23.33, 25.31]	***.01***
Gender, females	56 (47.86%)	80 (67.80%)	***.00***	105 (62.50%)	31 (46.27%)	***.03***
Occupation						
Unemployed	26 (22.41%)	16 (13.56%)		31 (18.56%)	11 (16.42%)	
Employed	65 (56.03%)	68 (57.63%)	.15	94 (56.29%)	39 (58.21%)	.93
Retired	25 (21.55%)	34 (28.81%)		42 (25.15%)	17 (25.37%)	
Education						

Elementary/middle school	43 (37.07%)	26 (22.03%)		52 (30.95%)	17 (25.76%)	
High school	53 (45.69%)	54 (45.76%)	**.01**	76 (45.24%)	31 (46.97%)	.71
College or more	20 (17.24%)	38 (32.20%)		40 (23.81%)	18 (27.27%)	
Smoking						
Never	47 (40.17%)	60 (50.85%)		70 (41.67%)	37 (55.22%)	
Former	36 (30.77%)	31 (26.27%)	.25	51 (30.36%)	16 (23.88%)	.18
Smoker	34 (29.06%)	27 (22.88%)		47 (27.98%)	14 (20.90%)	
Familiarity for periodontitis, yes	42 (35.90%)	45 (38.14%)	0.79	65 (38.69%)	22 (32.84%)	.46
Oral health status						
Grade[¶]						
none	34 (14.45%)			34 (14.45%)		
A	3 (1.28%)	18 (7.66%)	0.67	10 (4.26%)	11 (4.68%)	0.86

	B	55 (23.41%)	56 (23.83%)		78 (33.19%)	33 (14.05%)	
	C	43 (18.30%)	26 (11.07%)		54 (22.98%)	15 (6.39%)	
Extent¶							
	None	34 (14.45%)			34 (14.45%)		
	Localized	52 (22.13%)	52 (22.13%)	0.67	78 (33.19%)	26 (11.07%)	0.32
	Generalized	48 (20.43%)	49 (20.86%)		66 (28.09%)	31 (13.20%)	
Number of teeth		23.85 [22.81, 24.89]	24.48 [23.55, 25.41]	.48	24.05 [23.24, 24.88]	24.52 [23.09, 25.79]	.48
CAL		2.96 [2.79, 3.14]	2.70 [2.57, 2.83]	.06	2.88 [2.75, 3.02]	2.69 [2.52, 2.86]	.16
PPD		2.62 [2.49, 2.75]	2.44 [2.34, 2.55]	.06	2.56 [2.46, 2.67]	2.45 [2.32, 2.59]	.32
% PPD>4mm		11 [8.74, 13.21]	7.61 [5.69, 9.54]	**.02**	10.21 [8.32, 12.10]	7.01 [4.87, 9.15]	.12
% PPD 5-6mm		9.12 [7.30, 10.95]	6.31 [4.76, 7.84]	**.02**	8.58 [7.04, 10.13]	5.51 [3.94, 7.06]	.09
% PPD>6mm		5.57 [2.81, 13.96]	2.46 [1.37, 3.55]	.18	5 [0.01, 10.90]	1.56 [0.5, 2.64]	.39

Furcation involvement, yes[†]	51 (43.59%)	25 (21.19%)	**.00**	60 (35.71%)	16 (23.88%)	.09
Mobility, yes	55 (47.01%)	36 (30.51%)	**.01**	77 (45.83%)	14 (20.90%)	**.00**
Number of bleeding pockets[‡]	9.18 [6.95, 11.41]	5.46 [4.03, 6.89]	**.03**	7.91 [6.25, 9.58]	5.83 [3.69, 7.97]	.16
FMPS	51.92 [47.45, 56.39]	49.22 [44.82, 53.61]	.48	51.19 [47.27, 55.11]	48.98 [44.08, 53.88]	.61
FMBS	29.78 [26.57, 33.01]	27.14 [23.86, 30.41]	.18	28.69 [25.88, 31.51]	27.85 [23.93, 31.76]	.96
Teeth lost for periodontitis, yes	47 (40.17%)	29 (24.58%)	**.01**	59 (35.12%)	17 (25.37%)	.16
Domiciliary plaque control						
Brushing frequency						
Not performed	3 (2.56%)	1 (0.85%)		4 (2.38%)	0 (0%)	
Occasionally	29 (24.79%)	17 (14.41%)	.06	35 (20.83%)	11 (16.42%)	.39
Every day	85 (72.65%)	100 (84.75%)		129 (76.79%)	56 (83.58%)	
Toothbrush type, powered	62 (52.99%)	60 (50.85%)	.79	90 (53.57%)	32 (47.76%)	.47

Interdental cleaning (IC)						
Not performed	43 (36.75%)	32 (27.12%)		58 (34.52%)	17 (25.37%)	
Interdental floss	29 (24.79%)	22 (18.64%)	.06	36 (21.43%)	15 (22.39%)	.51
Interproximal brushes	45 (38.46%)	64 (54.24%)		74 (44.05%)	35 (42.24%)	
Frequency of IC						
Not performed	44 (37.61%)	31 (26.27%)		58 (34.52%)	17 (25.37%)	
Occasionally	28 (23.93%)	20 (16.95%)	.06	32 (19.05%)	16 (23.88%)	.37
Every day	45 (38.46%)	67 (56.78%)		78 (46.43%)	34 (50.75%)	

Notes. Results of continuous variables are reported as mean [95% Confidence Interval]; results of binary and categorical variables are expressed as number (proportion).

Abbreviations. BMI, body mass index; aMed, alternate Mediterranean diet score; PA, physical activity; CAL, clinical attachment level; PPD, probing depth; FMPS, full-mouth plaque score; FMBS, full-mouth bleeding score; IC, interdental cleaning.

[*] *p*-value of the Mann Whitney or Fisher's exact test for patients' characteristics across the subgroups of diet and physical activity; $p<0.05$

[†] Class II/III furcation involvement according to the classification of Hamp *et al.* (1975).

‡Defined as the number of sites with probing depth≥5mm and positive to bleeding on probing.

¶According to the 2018 EFP/AAP classification.

***Table 3**. Association between aMed and physical activity level with Stage III/IV periodontitis.*

Variable	ORs for Stage III/IV periodontitis							
	Crude ORs	95% CI		*p*-value*	Adjusted† ORs	95% CI		*p*-value*
		Lower	Upper			Lower	Upper	
Low/Moderate PA level	1.83	1.04	3.26	.04	1.65	.84	3.28	.42
Low aMed‡	8.47	4.20	17.09	.00	5.63	3.21	9.84	**.00**
aMed and PA level								
Low aMed,	7.69	3.03	19.47	.01	6.40	2.94	13.91	**.01**

low/moderate PA								
Low aMed, high PA	7.66	1.98	29.95	.00	3.30	1.15	9.50	**.03**
High aMed‡, low/moderate PA	.96	.44	2.20	.97	.85	.32	2.23	.74
High aMed, high PA	REF.							
aMed components								
Wholegrain products	.33	.19	.56	.00	.25	.13	.47	**.00**
Vegetables	.47	.28	.80	.00	.43	.23	.80	**.01**
Fruits	.56	.33	.94	.03	.37	.19	.79	**.00**
Olive oil	.40	.19	.82	.01	.30	.15	.82	**.00**
Wine	.67	.39	1.16	.15	.62	.32	1.19	.15
Red meat and meat products	.67	.37	1.15	.07	.69	.37	1.27	.24

Fish	.36	.21	.63	.00	.38	.20	.71	**.00**
Dried fruits	.33	.19	.58	.00	.35	.15	.59	**.02**
Pulses	.54	.31	.96	.04	.39	.22	.73	**.00**

Abbreviations. ORs, odds ratios; CI, confidence interval; PA, physical activity; aMed, alternate Mediterranean diet score; REF., reference category.

*$p<0.05$

†Adjusted for age, gender, smoking and brushing frequency.

‡ High aMed if aMed>4; Low aMed if aMed<5.

3.2.3 *Combining adherence to Mediterranean Diet and physical activity*

Around 40% of participants belonged to the "low aMed, low/moderate PA" subgroup, while fewer subjects belonged to the other three categories (Table 4). The prevalence of stage III/IV periodontitis was significantly higher in the subgroups "low aMed, low/moderate PA" (73.68%) and "low aMed, high PA" (59.09%), when compared to the "high aMed, low/moderate PA" (30.14%) and the "high aMed, high PA" (28.89%); the periodontal condition was found to be significantly worse in the two former compared to the two latter categories (p=.04). The proportion of subjects who reported having lost teeth for periodontitis almost doubled when shifting from the "high aMed, high PA" (24.44%) to the "low aMed, low/moderate PA" (43.16%) subgroup (p=0.04). Subgroups "low aMed, low/moderate PA" and "low aMed, high PA" resulted in significantly positive adjusted (Table 3) ORs for stage III/IV periodontitis, while only the former was positively associated with periodontitis occurrence (Supplementary Table 3) (OR=1.53[1.04,1.74], p=.01). Moreover, results of the final model are shown in Table 5. The presence of low MD adherence, irrespective of PA level, increased the odds of stage III/IV periodontitis by 9 (low/moderate PA level) and 10 (high PA level) times in both cases (p=.00). Age (OR=1.08[1.05,1.11], p=.00), the presence of smoking habit (OR=2.16[1.02, 4.91], p=.04) and familiarity for periodontitis (OR=2.13[1.08,4.20], p=.029) were significant predictors in this observation. The model resulted statistically significant (p=.00) with a pseudo R^2 of around 30%. Moreover, results from the mediation analysis demonstrated that about 9% of the effect of the combination between MD and PA on stage III-IV periodontitis was mediated by BMI; this mediation was shown to be partial (Sobel test, p-value=.064) (Supplementary Table 4; Supplementary Figure 1).

Table 4. *Patients' characteristics summarized by the combination of adherence to Mediterranean diet (aMed score) and physical activity level. Values with different superscript letters are different at the 5% level.*

Variable	Low aMed, low/moderate PA	Low aMed, high PA	High aMed, low/moderate PA	High aMed, high PA	*p*-value*
	n=95	n=25	n=69	n=46	
Socio-demographic characteristics					
Age	55.26 [52.36, 58.15]	46.58 [39.22, 53.94]	53.56 [50.26, 56.86]	55.15 [50.64, 59.66]	.26
BMI	26.06^{c,d} [25.10, 27.02]	25.22^{a,c,d} [22.95, 27.49]	25.81^{b,c} [24.57, 27.07]	23.88^{a} [22.87, 24.90]	**.04**
Gender, females	50^{c} (52.63%)	6^{b} (27.27%)	55^{a} (75.34%)	25^{a,b,c} (55.56%)	**.00**
Occupation					
Unemployed	22 (23.40%)	4 (18.18%)	9 (12.33%)	7 (15.56%)	.48
Employed	50 (53.19%)	15 (68.18%)	44 (60.27%)	24 (53.33%)	

Retired	22 (23.40%)	3 (13.64%)	20 (27.40%)	14 (31.11%)	
Education					
Elementary/middle school	35 (36.84%)	8 (38.10%)	17 (23.29%)	9 (20%)	
High school	42 (44.21%)	11 (52.38%)	34 (46.58%)	20 (44.44%)	.66
College or more	18 (18.95%)	2 (9.52%)	22 (30.14%)	16 (35.56%)	
Smoking					
Never	37 (38.95%)	10 (45.45%)	33 (45.21%)	27 (60%)	
Former	31 (32.63%)	5 (22.73%)	20 (27.40%)	11 (24.44%)	.69
Smoker	27 (28.42%)	7 (31.82%)	20 (27.40%)	7 (15.56%)	
Familiarity for periodontitis, yes	36 (37.89%)	6 (27.27%)	29 (39.73%)	16 (35.56%)	.46
Oral health status					
Periodontitis[†]					

Healthy-gingivitis	11 (32.35%)[a]	6 (17.65%)[a]	13 (38.24%)[b]	4 (11.76%)[b]	
Stage I	1 (3.70%)[a]	0 (0%)[a]	14 (51.85%)[b]	12 (44.44%)[b]	
Stage II	13 (23.21%)[a]	3 (5.36%)[a]	24 (42.86%)[b]	16 (28.57%)[b]	**.04**
Stage III/IV	70 (73.68%)[a]	13 (59.09%)[a]	22 (30.14%)[b]	13 (28.89%)[b]	
Grade[†]					
none	14 (14.74%)	6 (24%)	12 (17.39%)	2 (4.35%)	
A	3 (3.16%)	0 (0%)	7 (10.15%)	11 (23.91%)	
B	42 (44.21%)	13 (52%)	33 (47.82%)	23 (50%)	.32
C	36 (37.89%)	6 (24%)	17 (24.64%)	10 (21.74%)	
Extent[†]					

none			34 (14.45%)		
Localized	43 (18.30%)	9 (3.84%)	35 (14.89%)	17 (7.23%)	0.12
Generalized	41 (17.45%)	7 (2.98%)	25 (10.65%)	24 (10.21%)	
Number of teeth	23.74 [22.60, 24.89]	24.31 [21.58, 27.04]	24.46 [23.28, 25.64]	24.51 [22.92, 26.09]	.76
CAL (mm)	3.02 [2.82, 3.22]	2.71 [2.31, 3.11]	2.70 [2.52, 2.89]	2.68 [2.50, 2.86]	.07
PPD (mm)	2.64 [2.50, 2.79]	2.52 [2.20, 2.84]	2.46 [2.31, 2.61]	2.42 [2.29, 2.56]	.18
% PPD>4mm	11.64 [9.07, 14.20]	8.22 [3.37, 13.07]	8.35 [5.54, 11.11]	6.42 [4.15, 8.68]	.07
% PPD 5-6mm	9.92 [7,79, 12.05][a]	5.68 [2.65, 8.71][b]	6.84 [4.61, 9.07][b]	5.42 [3.54, 7.30][b]	**.04**
% PPD>6mm	2.42 [1.26, 3.57]	2.63 [0.5, 5.81]	8.36 [5.26, 22]	1.04 [0.45, 1.63]	.43
Furcation involvement, yes‡	43 (45.26%)[a]	8 (36.36%)[a,c,d]	17 (23.29%)[b,c]	8 (17.78%)[b,d]	**.00**
Mobility, yes	50 (52.63%)[a]	5 (22.73%)[b,c]	27 (36.99%)[a,c]	9 (20%)[b,c]	**.00**
Number of bleeding pockets§	9.68 [7.16, 12.20]	7.05 [2.02, 12.07]	5.60 [3.68, 7.52]	5.24 [3.05, 7.43]	.07

FMPS	53.15 [48.12, 58.19]	46.59 [36.43, 56.74]	48.64 [42.34, 54.94]	50.15 [44.52, 55.78]	.59
FMBS	30.48 [26.80, 34.16]	26.77 [20.00, 33.54]	26.39 [21.97, 30.76]	28.37 [23.40, 33.35]	.35
Teeth lost for periodontitis, yes	41 (43.16%)[a]	6 (27.27%)[a,c]	18 (24.66%)[b,c]	11 (24.44%)[c]	**.04**
Domiciliary plaque control					
Brushing frequency					
Not performed	3 (3.16%)	0 (0%)	1 (1.37%)	0 (0%)	
Occasionally	25 (26.32%)	4 (18.18%)	10 (13.70%)	7 (15.56%)	.31
Every day	67 (70.53%)	18 (81.82%)	62 (84.93%)	38 (84.44%)	
Toothbrush type, powered	53 (55.79%)	9 (40.91%)	37 (50.68%)	23 (51.11%)	.63
Interdental cleaning (IC)					

Not performed	36 (37.89%)	7 (31.82%)	22 (30.14%)	10 (22.22%)	
Interdental floss	21 (22.11%)	8 (36.36%)	15 (20.55%)	7 (15.56%)	.16
Interproximal brushes	38 (40%)	7 (31.82%)	36 (49.32%)	28 (62.22%)	
Frequency of IC					
Not performed	37 (38.95%)	7 (31.82%)	21 (28.77%)	10 (22.22%)	
Occasionally	20 (21.05%)	8 (36.36%)	12 (16.44%)	8 (17.78%)	.09
Every day	38 (40%)	7 (31.82%)	40 (54.79%)	27 (60%)	

Note: results of continuous variables are reported as mean [95% Confidence Interval]; results of binary and categorical variables are expressed as number (proportion).

Abbreviations: aMed, alternate Mediterranean diet score; PA, physical activity; CAL, clinical attachment level; PPD, probing depth; FMPS, full-mouth plaque score; FMBS, full-mouth bleeding score; IC, interdental cleaning.

* *p*-value of the Kruskal Wallis or Fisher's exact test for patients' characteristics across the four subgroups; $p<0.05$

† According to the 2018 EFP/AAP classification.

[‡] Class II/III furcation involvement according to the classification of Hamp *et al.* (1975).

[§] Defined as the number of sites with probing depth≥5mm and positive to bleeding on probing.

Table 5. *Multivariate logistic regression analysis for the prediction of Stage III/IV periodontitis by measures of the combination of adherence to Mediterranean diet (aMed score) and physical activity, and socio-demographic characteristics.*

Best model (AUC=0.853, AIC=212.4, BIC=252.5)						
LR chi²	**Prob>chi²**	**Pseudo R²**				
97.61	.00	.2996				
Stage III/IV periodontitis	**OR**	**SE**	**z**	***p*-value***	**95% CI**	
					Lower	**Upper**
High aMed[†], high PA	REF.					

High aMed, low/moderate PA	1.06	.49	.12	.90	.42	2.65
Low aMed†, high PA	9.26	6.28	3.28	**.00**	2.45	35.03
Low aMed, low/moderate PA	10.23	4.88	4.86	**.00**	4.01	26.09
Age	1.08	.01	5.79	**.00**	1.05	1.11
Smoking						
Never	REF.					
Former	1.23	.49	.57	.56	.58	2.69
Smoker	2.16	.90	1.83	**.04**	1.02	4.91
Familiarity for periodontitis	2.13	.74	2.18	**.029**	1.08	4.20
Comorbidities‡	1.59	.72	1.00	.30	.64	3.81
_cons	.00	.00	-6.43	.00	.00	.02

Abbreviations: AUC, Area Under the Curve; AIC, Akaike information criterion; BIC, Bayesian information criterion; LR, likelihood ratio; CI, confidence interval; aMed, alternate Mediterranean diet score; PA, physical activity; REF., reference category. * $p<0.05$

[†] High aMed if aMed>4; Low aMed if aMed<5.

[‡] Presence of at least one comorbidity (*i.e.* diabetes, rheumatoid arthritis, inflammatory bowel diseases, osteoporosis

4 Discussion

4.1 *Summary of findings*

In the current study, the odds of stage III/IV periodontitis were found to be double in subjects with low/moderate PA level compared to those with high PA level and almost 6 times higher in subjects with low MD adherence compared to those with high adherence. The final regression model demonstrated how the combination of poor MD adherence and PA level led to 10-time increased odds of stage III/IV periodontitis. Age, smoking and familiarity for periodontitis resulted as additional predictors for stage III/IV periodontitis. Moreover, the significant mediating effect of BMI accounted for around 9% of the direct effect of the combination between MD and PA on the occurrence of stage III/IV periodontitis.

4.2 *Dietary and physical activity assessments*

This is the first study formulating the hypothesis of a significant association between PA level and MD adherence with periodontitis severity. Overall, high values of physical inactivity were detected in the present cohort (71%), maybe due to the presence of comorbidities in around 20% of the included individuals. High PA levels were associated with lower prevalence of stage III/IV periodontitis, consistently to recent meta-data showing how physically-active patients are 22% less likely to have periodontitis when compared to those physically-inactive (Ferreira et al., 2019).

A linear relationship between decreasing values of aMed scores and the worsening of periodontal indices was also found, with around 70% of those with low MD adherence being affected by stage III/IV periodontitis. Results from

the present study are partially discordant from those of a previous report (Iwasaki et al., 2018) in which no differences in the biometric periodontal indices were found between subjects with high MD adherence compared to those with low adherence. This result may be due to the different target population investigated in both studies: while the former enrolled University students (mean age 20 years), participants from the current report were selected among those coming to a periodontal Unit of a public University Hospital in Italy (mean age 53 years). Consequently, the reported values of periodontitis prevalence widely differ (6.6% *vs* 85%).

Indeed, subjects with low MD adherence and low/moderate PA presented not only worse biometric and inflammatory parameters, but also a more frequent experience of tooth mobility and tooth loss for periodontitis, irrespective of domiciliary oral hygiene measures and plaque accumulation. Therefore, a reciprocal association between such environmental factors (MD and PA) and the periodontium can be speculated. Firstly, the clinical manifestations of severe periodontitis, such as advanced CAL, tooth loss and tooth mobility, hamper masticatory function up to a point that they can lead to dietary changes, characterized by a decrease in fibers, fruits and vegetables intake (Zhu & Hollis, 2014). In turn from a biological standpoint, the consumption of a Western diet, rich in white flour and processed meats, as opposed to MD, induces a state of low-grade inflammation, contributing to the development of many NCDs, including periodontitis (Christ et al., 2019). Conversely, high MD adherence was demonstrated to be inversely related with stage III/IV periodontitis, supposedly due to the synergistic anti-inflammatory potential of the single MD components: wholegrain products demonstrated the strongest protective effect in the current study. In fact, a diet rich in

wholegrains was associated with lower systemic inflammatory markers, such as C-reactive protein, and decreased insulin resistance (Esposito et al., 2004; Jenkins et al., 2000). The improved insulin sensitivity could positively influence periodontal health by lowering the production of glycation end-products, reducing oxidative stress and, therefore, by decreasing cytokines release. From a microbiological standpoint, an increased MD adherence was found to lead to a significant decrease in the salivary concentration of microorganisms such as *Porphyromonas gingivalis*, *Prevotella intermedia* and *Treponema denticola* (Laiola et al., 2020); therefore, the increased consumption of MD components may induce a microbial shift in the saliva, hence contributing to the host defence immunomodulation (Ercolini et al., 2015). On the other hand, low MD adherence and, in particular, a low consumption of wholegrain products, increases the odds of stage III/IV periodontitis by almost 8 times plausibly by decreasing insulin sensitivity and increasing low-grade systemic inflammation (Merchant et al., 2006).

The current study also hypothesized a positive synergistic effect of regular exercise, in conjunction with high MD adherence, on periodontal health. Even though the effects of MD are prominent when compared independently to those of PA, regular physical exercise was previously demonstrated to decrease the concentration of specific pro-inflammatory markers involved in the clinical manifestations of periodontitis (Huck et al., 2019).

4.3 *Final multivariate regression model*

The final regression model highlights the ability of the combination between low MD adherence and low PA to

increase the odds of stage III/IV periodontitis by 10 times; nonetheless, every time MD adherence is below the selected cut-off, the OR increases irrespective of PA level. Patient's lifestyle, in terms of diet and PA, seems to retain higher odds than smoking for stage III/IV periodontitis. It can be speculated that such result may be due to the significant mediating effect exerted by BMI: indeed, low MD adherence and low PA level were previously correlated with higher BMI values (Shai et al., 2008), which in turn can potentially increase the odds for stage III/IV periodontitis (Khan et al., 2018). Such results may be relevant to better understand the etiopathogenesis of periodontitis but may also have critical implications from a therapeutical standpoint: the Step 1 phase (Sanz, Herrera, et al., 2020) of periodontal therapy could be implemented by applying a holistic approach based on common risk factors (Sheiham & Watt, 2000) not only for oral health, but also for other NCDs. Therefore, risk-factor modification should encompass smoking cessation counselling and oral hygiene instructions on one side, but also the promotion of both regular exercise and MD adherence on the other. Thus, this holistic approach based on common risk-factor modification could significantly ameliorate both the treatment efficacy and long-term management of lifestyle-related pathologies, and result in reduced healthcare costs.

4.4 *Strengths and limitations*

This is the first study investigating the association between the combination of MD adherence and PA level and stage III/IV periodontitis. Diet and PA assessments were carried out using reliable and validated tools for the selected sample. In fact, the 15-item questionnaire used to evaluate MD adherence (QueMD) was the first one to

be developed for the Italian population and validated against a Food Frequency Questionnaire (Gnagnarella et al., 2018). Moreover, PA levels were drawn from the results of the short version of the IPAQ, whose reproducibility and validity for the Italian population were demonstrated (Mannocci et al., 2010). Altogether, these factors significantly contribute to the internal validity of the study. The current study presents some limitations. Firstly, the cross-sectional design does not allow for a longitudinal evaluation regarding the cause-effect relationship between the exposure and the outcome, and therefore it can be used only to build a hypothesis. Indeed, this design does not allow also to properly investigate reverse causality (*i.e.* effect of periodontitis on nutrition capability and dietary changes); moreover, no molecular parameters supporting the biological plausibility of such association were registered. Secondly, due to the lack of studies regarding the combined effect of MD and PA on periodontal health, the sample size calculation was instead performed based on the prevalence of periodontitis; therefore, the potential lack of power could explain the absence of association for PA in the multivariate models. Third, the assessments of the exposure (*i.e.* administration of questionnaires) and the outcome (periodontal chart) were performed by the same examiners, who therefore were not blinded during outcome assessment. Furthermore, all participants lived in the urban or suburban areas nearby Siena (Italy), where most adults is Caucasian; hence, any variability in the outcome related to ethnicity may not have been detected. Moreover, given that the study population was selected among patients coming to a public Unit of Periodontology, the risk of selection bias could not be ruled out. Overall, these factors may reduce the generalizability of the study.

5 Conclusion

The present study demonstrated that individuals conducing a lifestyle characterized by low adherence to MD are more than 9 times more likely to have severe manifestations of periodontitis, irrespective of PA level. Further research is needed to elucidate the periodontal response to the implementation of the Step 1 phase of periodontal treatment with nutritional and PA counselling sessions.

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Supplementary information (Chapter 1)

Supplementary Appendix. *Assessment methods of socio-demographic characteristics.*

As socio-demographic characteristics, the following variables were considered: age, Body Mass Index (BMI), gender, occupation, education, smoking, familiarity for periodontitis, presence of comorbidities (diabetes, rheumatoid arthritis, inflammatory bowel diseases and osteoporosis), oral hygiene habits.

- The BMI was calculated as weight (kilograms)/height (meters^2). Weight was measured with a scale in kilograms, and height was measured with a stadiometer in meters.

- Occupation was categorized as unemployed, employed, or retired.

- Educational level was categorized as the highest diploma obtained (primary/middle school, high school, college or more).

- Smoking was self-reported and categorized as smoker, former smoker or never smoked.

- Familiarity for periodontitis was self-reported and categorized as yes/no.

- As for the presence of comorbidities (diabetes, rheumatoid arthritis, inflammatory bowel diseases and osteoporosis), the variable was categorized as: affected, not affected, or having familiarity for the disease. The presence of the specific disease was self-reported but confirmed by checking the patient's medical report. Familiarity for the

specific disease was self-reported.

- Information regarding oral hygiene habits was self-reported. Brushing frequency was categorized as not performed, performed occasionally, every day. Toothbrush type was categorized as powered or manual. The device used for interdental cleaning was categorized as using interproximal brushes, interdental floss or not performed. The frequency of interdental cleaning was categorized as not performed, occasionally or performed every day.

Supplementary Table 1. *Questionnaires regarding adherence to Mediterranean Diet (QueMD) and physical activity (International Physical Activity Questionnaire).*

Adherence to Mediterranean diet (QueMD)

How often do you normally consume a portion of the following foods?

		Daily frequency of consumption				
Food Items	**Reference portions**	**Never or seldom**	**<1 per day**	**1 per day**	**2 per day**	**≥3 per day**
1. Wholegrain pasta or rice	80 g	☐	☐	☐	☐	☐
2. Vegetables, all type (raw and cooked)	200 g 80 g (salad)	☐	☐	☐	☐	☐
3. Fruits, all types fresh and fresh juices	150 g	☐	☐	☐	☐	☐
4. Milk and yoghurt	125 g	☐	☐	☐	☐	☐
		Never or seldom	**<1 per day**	**1-2 per day**	**3-4 per day**	**≥5 per day**
5. Wholegrain bread and substitutes	50 g (1-2 slices)	☐	☐	☐	☐	☐
6. Olive oil to cook and to dress	10 ml (1 spoon)	☐	☐	☐	☐	☐
7. Butter, margarine or cooking cream	10 g (1 spoon)	☐	☐	☐	☐	☐
8. Wine (white and red)	125 ml (1 glass)	☐	☐	☐	☐	☐
		Weekly frequency of consumption				
		Never or seldom	**<1 per week**	**1-3 per week**	**4-6 per week**	**≥7 per week**
9. Red meat (beef, veal, pork), meat products	100 g (raw meat) 50 g (meat products)	☐	☐	☐	☐	☐
10. White meat (chicken, turkey, rabbit)	100 g	☐	☐	☐	☐	☐

11. Carbonated and/or sugar-sweetened beverages	200 ml (1 glass)	☐	☐	☐	☐	☐
12. Manufactured sweets, pastries, biscuits, creams…	100 g	☐	☐	☐	☐	☐
		Never or seldom	**<1 per week**	**1 per week**	**2-3 per week**	**≥4 per week**
13. Fish (fresh or frozen) or sea foods	150 g (fish) 50 g (fish products)	☐	☐	☐	☐	☐
14. Dried fruits (nuts, almonds, hazelnuts)	30 g (1 fist)	☐	☐	☐	☐	☐
15. Pulses (chickpeas, lentils, peas, beans)	50 g (dried) 150 g (canned/raw)	☐	☐	☐	☐	☐

International Physical Activity Questionnaire (IPAQ)

Think about all the ***vigorous activities*** that you did in the last 7 days. Vigorous physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.

1)	During the last 7 days, on how many days did you do vigorous physical activities like heavy lifting, digging, aerobics, or fast bicycling?	☐ Yes … days/week	☐ No Skip to question 3
2)	How much time did you usually spend doing vigorous physical activities on one of those days?	☐ Yes …minutes/day	☐ Not sure/Don't know

Think about all the ***moderate activities*** that you did in the last 7 days. Moderate activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.

3)	During the last 7 days, on how many days did you do moderate physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking.	☐ Yes …days/week	☐ No Skip to question 5

4)	How much time did you usually spend doing moderate physical activities on one of those days?	☐ Yes ... minutes/day	☐ Not sure/Don't know

Think about the time you spent ***walking*** in the last 7 days. This includes at work and at home, walking to travel from place to place, and any other walking that you have done solely for recreation, sport, exercise, or leisure.

5)	During the last 7 days, on how many days did you walk for at least 10 minutes at a time?	☐ Yes ... days/week	☐ No Skip to question 7
6)	How much time did you usually spend walking on one of those days?	☐ Yes ... minutes/day	☐ Not sure/Don't know

The last question is about the time you spent sitting on weekdays during the last 7 days. Include time spent at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading, or sitting or lying down to watch television.

7)	During the last 7 days, how much time did you spend sitting on a week day?	☐ Yes ... minutes/day	☐ Not sure/Don't know

Supplementary Table 2. *Post-hoc power analysis for the association between stage III/IV periodontitis and each evaluated risk indicator.*

Risk indicators for SIII-IV periodontitis	Alfa	Sample Size	Power
Low MD adherence	0.05	235	>90%
Low PA level	0.05	235	76.66%
Low MD adherence and low PA level	0.05	235	>90%

Abbreviations. SIII-IV, Stage III/IV periodontitis; MD, Mediterranean Diet; PA, Physical Activity.

***Supplementary Table 3**. Association between aMed and physical activity level with periodontitis case.*

Variable	ORs for Periodontitis case							
	Crude ORs	95% CI		p-value*	Adjusted† ORs	95% CI		p-value*
		Lower	Upper			Lower	Upper	
Low/Moderate PA level	0.96	0.44	2.15	0.93	1.31	0.51	3.35	0.58
Low aMed‡	1.24	1.01	2.03	0.04	1.57	1.11	2.48	**0.02**
aMed and PA level								
Low aMed, low/moderate PA	1.38	1.02	4.58	0.03	1.53	1.04	5.74	**0.01**
Low aMed,	0.99	0.25	1.44	0.07	1.26	0.61	2.69	0.09

high PA								
High aMed[‡], low/moderate PA	0.35	0.11	1.08	0.09	0.61	0.21	1.78	0.34
High aMed, high PA	REF.							
aMed components								
Wholegrain products	0.55	0.26	1.19	0.13	0.64	0.26	0.96	***0.00***
Vegetables	1.54	0.73	3.23	0.26	0.98	0.81	1.22	0.12
Fruits	1.24	0.59	2.58	0.56	1.00	0.78	1.26	0.45
Olive oil	0.32	0.11	0.75	0.02	0.29	0.09	0.77	***0.01***
Wine	0.59	0.19	1.78	0.35	0.61	0.12	1.67	0.49

Red meat and meat products	1.04	0.48	2.27	0.51	1.44	0.59	3.47	0.65
Fish	0.74	0.36	1.54	0.41	0.83	0.35	1.94	0.66
Dried fruits	0.97	0.47	2.01	0.94	0.95	0.39	2.31	0.91
Pulses	1.09	0.50	2.37	0.82	1.10	0.47	2.54	0.82

Abbreviations. ORs, odds ratios; CI, confidence interval; PA, physical activity; aMed, alternate Mediterranean diet score; REF., reference category.

[*] $p<0.05$

[†]Adjusted for age, gender, smoking and brushing frequency.

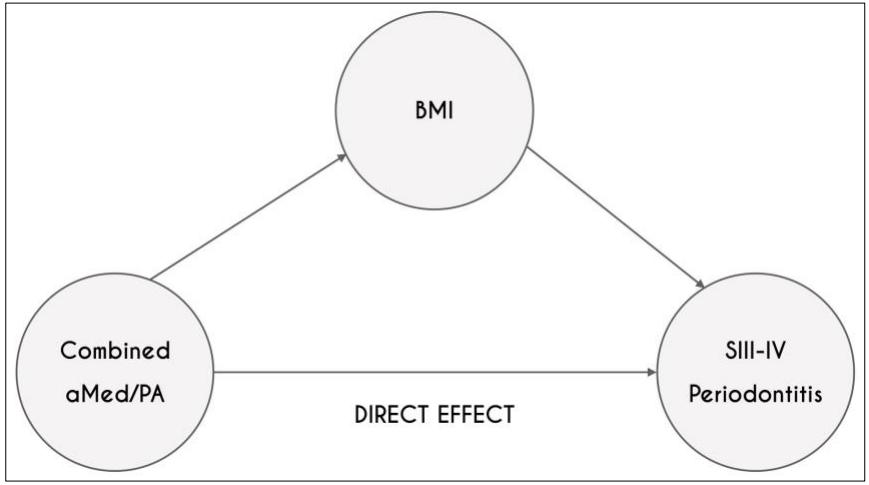
[‡] High aMed if aMed>4; Low aMed if aMed<5.

Supplementary Table 4. *Results of the mediation analysis of BMI on the effect of the combination of diet and physical activity on the presence of Stage III/IV periodontitis.*

Effect	Coefficient	SE	z	*p*-value
BMI <- combined aMed/PA (Step 1)	0.531	0.262	2.03	.042
SIII-IV <- BMI (Step 2)	0.027	0.006	4.56	.000
SIII-IV <- combined aMed/PA (Step 3)	0.156	0.024	6.41	.000
	Results of the Sobel test for the indirect effect			
	Coefficient	**SE**	**z**	***p*-value**
	0.015	0.008	1.854	.064
	Indirect effect/Total effect= .085			

Abbreviations. SE, Standard Error; BMI, Body Mass Index; aMed, adherence to Mediterranean Diet; PA, Physical Activity; SIII-IV, Stage III-IV periodontitis.

Supplementary Figure 1. *Mediating effect of BMI on the impact of combined aMed/PA on stage III/IV periodontitis.*



Abbreviations. BMI, Body Mass Index; aMed, adherence to Mediterranean Diet; PA, Physical Activity; SIII-IV, Stage III-IV periodontitis.

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Data availability statement. The data that support the findings are available upon reasonable request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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LEISURE-TIME AND OCCUPATIONAL PHYSICAL ACTIVITY DEMONSTRATE DIVERGENT ASSOCIATIONS WITH PERIODONTITIS: A POPULATION-BASED STUDY

By

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Chapter 2. LEISURE-TIME AND OCCUPATIONAL PHYSICAL ACTIVITY DEMONSTRATE DIVERGENT ASSOCIATIONS WITH PERIODONTITIS: A POPULATION-BASED STUDY.

1 Introduction

Leisure time physical activity (LTPA) refers to bodily movements performed during free time and not required as part of the essential activities of the daily living. It is performed at subject discretion, and it includes activities such as sports, exercising, and recreational walking. LTPA is regarded as a preventive factor for most chronic non-communicable diseases (NCDs - Katzmarzyk et al., 2022; WHO 2022). Indeed, the LTPA associated dynamic movements and psychological well-being have been linked with an improved cardiometabolic function and a reduced state of low-grade systemic inflammation (LGSI - Hamer et al., 2012; Li et al., 2021). Conversely, LTPA-inactive subjects have shown higher prevalence of cardiovascular diseases (CVDs), type II diabetes mellitus, cancer, depression and, in general, shortened life expectancy (Lee et al., 2012).

Occupational physical activity (OPA) refers instead to bodily movements made as part of the subject's professional tasks (e.g., carrying/lifting heavy loads, digging or construction work, household chores, etc.). OPA often involves long-lasting static load and repetitive working postures, with detrimental consequences on the psycho-physical health and on the LGSI balance. Therefore, contrarily to LTPA, high OPA has shown to increase the incidence of several NCDs (Holtermann et al., 2013, Hallman et al., 2017, Holtermann et al., 2021).

Since LGSI has been bi-directionally linked with periodontitis (Pink et al., 2015; Hajishengallis & Chavakis,

2021; Romandini et al. 2018), the association between physical activity and periodontitis has been analyzed in several epidemiological studies, which however reported conflicting results (Marruganti et al. 2022; Ferreira et al., 2019; Merchant et al., 2003). Nonetheless, these investigations did not differentiate between LTPA and OPA, and this aspect may represent a possible explanation to these contradictory findings. Clarifying the possible preventive role of physical activity on periodontitis would be relevant for oral healthcare providers, since this could represent a target for intervention as part of the Step 1 of periodontal therapy (Ramseier et al., 2020). Therefore, the aim of the present study was to separately investigate the relationship between LTPA/OPA and periodontitis, analyzing a nationally representative sample of the US population.

2 Material and Methods

The present cross-sectional study is reported according to the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) guidelines (Vandenbroucke et al., 2014; von Elm et al., 2007).

2.1 *Study sample: NHANES 2009-2014*

Data for the present study were drawn from the 2009-2014 cycles of the NHANES, a nationwide, stratified, multistage probability survey conducted by the Centers for Disease Control and Prevention (CDC) and designed to be representative of the civilian non-institutionalized US population. Each cycle consists of questionnaires administered in the home of the participants, followed by an examination performed in a designated room at a

mobile examination center (MEC). Detailed information regarding the survey, its contents and sampling methods are provided elsewhere (Dye et al., 2019). All NHANES cycles were ethically approved by the CDC's National Center for Health Statistics (NCHS) Research Ethics Review Board (ERB) and all survey participants provided a written informed consent.

2.2 *Physical activity assessment*

All survey participants aged 2 years or more were eligible for the physical activity assessment, which was based on self-reported answers to the World Health Organization validated "Global Physical Activity Questionnaire" (GPAQ) (National Health and Nutrition Examination Survey, 2009-2010; 2011-2012; 2013-2014; Armstrong & Bull, 2006). The GPAQ has previously shown moderate reliability (Kappa 0.67 to 0.73) and correlation with the International Physical Activity Questionnaire (IPAQ) (Kappa 0.45 to 0.65) (Bull et al., 2009). Briefly, participants were asked to report the frequency and duration of moderate- and vigorous-intensity physical activity during a typical week, discriminating between LTPA and OPA (Hallman et al., 2017; Holtermann et al., 2021). The specific GPAQ questions used to assess LTPA and OPA are reported in the Appendix. Total weekly minutes of LTPA and OPA were calculated as the weekly minutes of moderate-intensity activity plus twice the reported weekly minutes of vigorous-intensity activity (Wiebe et al., 2018). Participants were then categorized, separately for LTPA and OPA, as being inactive (no moderate- or vigorous-intensity activity), insufficiently active (<150 min/week), sufficiently active (150-300 min/week), or highly active (>300 min/week), according to the current guidelines (US Department of Health and

Human Services, 2018; Wiebe et al., 2018). For analytical purposes, only subjects aged at least 30 years were considered (due to the availability of a periodontal examination), and the 4 categories of physical activity were merged to consider participants as either performing high (highly/sufficiently active) or low (insufficiently active/inactive) LTPA and OPA.

2.3 *Periodontitis assessment*

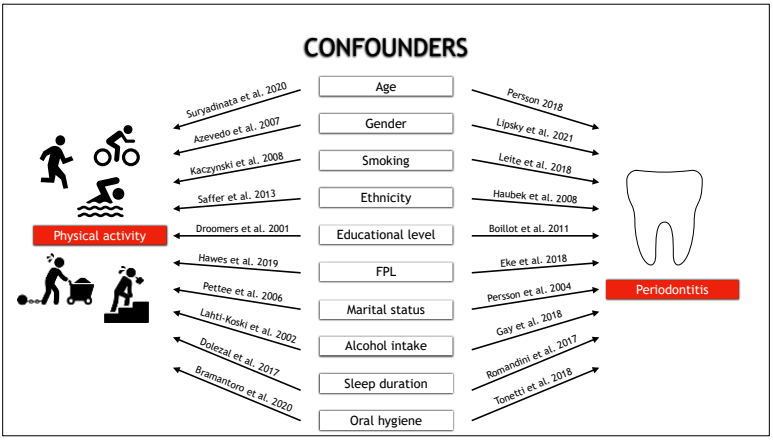
All survey participants aged at least 30 years, presenting at least one tooth (excluding third molars) and not meeting any of the health exclusion criteria (Dye et al., 2014), were eligible for a full-mouth periodontal examination, which was performed by trained dental hygienists (cycle 2009-2010) and general dentists (cycles 2011-2012 and 2013-2014) using a color-coded periodontal probe (PCP2, HuFriedy). Gingival recession and probing pocket depth were measured at 6 sites/tooth (excluding third molars), and clinical attachment level was then calculated. The periodontal examiners were trained and calibrated prior to the beginning of the surveys, and then periodically 2–3 times a year. Further details on the periodontal examination are reported elsewhere (Dye et al., 2019). Only participants with a complete periodontal examination were considered for the present study. Periodontitis was identified applying the AAP/CDC criteria, categorizing its severity as mild, moderate, or severe (Eke et al., 2012).

2.4 *Confounders*

According to the external knowledge, age, gender and pack-year smoking were considered as *a priori*

confounders for both LTPA and OPA, while educational level and family poverty level (FPL) were considered as additional *a priori* confounders for OPA. Putative confounders included: educational level and FPL (only for LTPA), ethnicity, marital status, alcohol intake, sleep duration, and frequency of self-performed interproximal hygiene. The confounders assessment methods, their category boundaries, and the evidence supporting their possible role are reported in the Appendix and in Figure 1.

Figure 1. *Evidence supporting the role of the selected putative confounders.*



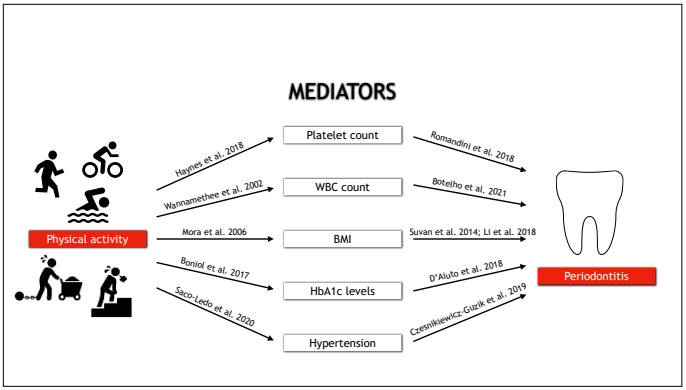
Abbreviations. FPL, family poverty level.

2.5 *Mediators*

Potential mediators encompassed: biomarkers of systemic inflammation (platelet and white blood cells – WBC – counts), body mass index (BMI), diabetes, and

hypertension. The mediators' assessment methods, their category boundaries, and the evidence supporting their possible role are reported in the Appendix and in Figure 2.

Figure 2. *Evidence supporting the role of the selected putative mediators.*



Abbreviations. CVDs, cardiovascular diseases; HbA1c, glycated hemoglobin; WBC, white blood cells count.

2.6 *Statistical analyses*

All statistical analyses were performed using an *ad hoc* statistical software (STATA BE, version 17.1, StataCorp LP, Texas, USA), setting the level of significance at 5%. Continuous variables were described as mean (linearized standard error - SE), while categorical variables were presented as proportions (SE). Prevalence (SE) values of periodontitis/severe periodontitis according to the different combinations of LTPA and OPA were plotted;

simple and multiple logistic regression models were then built to separately evaluate the crude and adjusted estimates of association between the levels of LTPA/OPA (as well as their combinations) and periodontitis/severe periodontitis. For the multiple analyses, age, gender and pack-year smoking (as well as educational level and FPL for models involving OPA) were considered as *a priori* confounders, while the potential role of all the putative confounders was separately tested using the change-in-estimate strategy (Maldonado & Greenland, 1993). Only variables demonstrating a change of 10% or more, compared to the model only adjusted for the *a priori* confounders, were retained in the final model (Appendix). Results from logistic regression models were expressed as Odds Ratios (ORs) with 95% Confidence Intervals (CIs). Moreover, age (<60 / ≥60 years old), gender (male, female), and smoking status (non-smokers, smokers) were considered as *a priori* effect modifiers; accordingly, the results from the respective subgroup analyses were reported. Furthermore, a mediation analysis was performed calculating the percentage (%) of excess odds explained (Han et al., 2016; Romandini et al., 2017). Finally, to explore the possible presence of a dose-response relationship, sensitivity analyses were performed using the 4 different frequencies of LTPA and OPA as exposure (highly/sufficiently/insufficiently active and inactive).

3 Results

The sampling strategy led to the selection of 30,468 subjects; of them, 10,679 participants had a complete periodontal examination and LTPA/OPA data and were then included, representing a weighted population of approximately 431.1 million civilian non-institutionalized

US adults aged 30 years or more.

3.1 *Descriptive statistics*

Table 1 shows the descriptive statistics of the study sample, while Table S1 reports the missing data. The weighted mean age was 50.8 (SE=0.24) years; most of the participants were females (weighted 51.1%) and non-smokers (weighted 82.7%). The weighted prevalence of periodontitis and severe periodontitis was 42.3% and 7.8%, respectively.

With regards to LTPA, the largest proportion of participants were inactive (weighted 46.3%), while the remaining ones were categorized as insufficiently active (17.3%), sufficiently active (14.9%), or highly active (21.4%).

With regards to OPA, although most of the participants were also inactive (weighted 58.5%), a relevant proportion of the study population was considered as highly active (weighted 26.8%); only a minority of the included subjects was finally considered as either sufficiently or insufficiently active (weighted 6.8% and 7.7%, respectively).

Table 1*. Characteristics of the study population.*

Characteristics	N	Weighted N in Millions	Overall	No Periodontitis†	Periodontitis‡
Age (years), mean (SE)	10,679	431.1	50.8 (0.25)	48.1 (0.28)	54.6 (0.34)
Gender, % (SE)					
Males	5,264	210.5	48.9 (0.01)	24.3 (0.01)	24.6 (0.01)

Females	5,415	220.6	51.1 (0.01)	33.5 (0.01)	17.7 (0.01)
Smoking, % (SE)					
Non-smokers	8,672	356.3	82.7 (0.003)	51.2 (0.01)	31.4 (0.01)
Smokers	2,002	74.7	17.3 (0.001)	6.5 (0.003)	10.9 (0.005)
Pack-year smoking, mean (SE)	10,645	430.4	3.3 (0.15)	1.6 (0.11)	5.8 (0.26)
Ethnicity, % (SE)					
Non-Hispanic Black	2,213	45.9	10.7 (0.01)	4.6 (0.004)	6.0 (0.01)
Non-Hispanic white	4,580	295.0	68.4 (0.02)	43.1 (0.02)	25.3 (0.01)
Mexican	1,518	34.8	8.1 (0.01)	3.3 (0.004)	4.8 (0.01)
Other	2,368	55.3	12.8 (0.01)	6.8 (0.005)	6.1 (0.01)
Educational level, % (SE)					
Less than high school	2,496	66.1	15.3 (0.01)	5.4 (0.004)	9.9 (0.01)
High school graduate	2,298	89.7	20.8 (0.01)	9.9 (0.005)	10.9 (0.01)
College degree or more	5,872	275.0	63.8 (0.01)	42.5 (0.02)	21.4 (0.01)
Family Poverty Level, % (SE)					
<100	1,889	48.2	11.2 (0.01)	4.7 (0.003)	7.2 (0.01)
100-199	2,497	76.6	17.8 (0.01)	8.8 (0.005)	10.2 (0.01)

200-399	2,567	115.8	26.9 (0.01)	15.9 (0.01)	12.9 (0.01)
≥400	2,831	161.9	37.6 (0.01)	28.7 (0.01)	11.5 (0.01)
Marital status, % (SE)					
Married	6,226	273.0	63.3 (0.01)	39.4 (0.01)	23.9 (0.01)
Widowed	766	22.9	5.3 (0.002)	2.2 (0.002)	3.1 (0.002)
Divorced	1,352	53.4	12.4 (0.004)	6.5 (0.003)	5.9 (0.004)
Separated	412	11.1	2.6 (0.002)	1.0 (0.001)	1.6 (0.002)
Never married	1,224	44.3	10.3 (0.005)	5.7 (0.004)	4.6 (0.003)
Living with a partner	696	26.3	6.1 (0.004)	2.9 (0.003)	3.1 (0.003)
Alcohol intake, % (SE)					
Below suggested intake	5,660	226.3	56.1 (0.01)	58.0 (0.01)	53.4 (0.01)
Above suggested intake	4,194	177.4	43.9 (0.01)	42.0 (0.01)	46.6 (0.01)
Sleep duration, % (SE)					
<7 hours	4,239	154.9	35.4 (0.01)	19.3 (0.01)	16.7 (0.01)
7-8 hours	2,901	131.4	30.5 (0.01)	19.0 (0.01)	11.5 (0.005)
More than 8 hours	3,527	144.3	33.5 (0.01)	19.4 (0.01)	14.1 (0.007)

Frequency of self-performed interproximal hygiene, % (SE)					
0 days/week	3,393	119.6	27.7 (0.01)	13.0 (0.005)	14.7 (0.01)
1-6 days/week	3,735	169.7	39.4 (0.01)	25.3 (0.01)	14.1 (0.01)
7 days/week	3,551	141.9	32.9 (0.01)	19.5 (0.01)	13.4 (0.01)
Platelet count (1000 cells/uL), mean (SE)	10,315	420.1	237.2 (0.97)	238.0 (0.99)	235.9 (1.55)
WBC count (1000 cells/uL), mean (SE)	10,316	420.1	7.1 (0.04)	6.9 (0.04)	7.3 (0.05)
BMI (kg/m^2), mean (SE)	10,616	429.2	29.2 (0.11)	28.9 (0.13)	29.5 (0.15)
Diabetes, % (SE)					
Yes	1,834	56.2	13.0 (0.004)	5.2 (0.003)	7.8 (0.01)
Hypertension, % (SE)					
Borderline Hypertension	4,249	171.1	39.7 (0.01)	23.9 (0.01)	19.9 (0.01)
Yes	1,155	38.1	8.8 (0.003)	3.9 (0.003)	5.8 (0.004)
LTPA, % (SE)					
Highly active	1,987	92.4	21.4 (0.01)	14.6 (0.01)	6.9 (0.004)
Sufficiently active	1,427	64.2	14.9 (0.01)	9.4 (0.01)	5.5 (0.004)
Insufficiently active	1,731	74.4	17.3 (0.01)	10.8 (0.01)	6.5 (0.004)

Inactive	5,529	199.9	46.3 (0.01)	22.9 (0.01)	23.4 (0.009)
OPA, % (SE)					
Highly active	2,722	115.5	26.8 (0.01)	34.9 (0.01)	23.7 (0.01)
Sufficiently active	630	29.3	6.8 (0.001)	4.7 (0.003)	3.0 (0.002)
Insufficiently active	750	33.3	7.7 (0.002)	3.9 (0.003)	2.8 (0.002)
Inactive	6,561	252.1	58.5 (0.01)	14.2 (0.005)	12.6 (0.01)
Combination of LTPA and OPA, % (SE)					
High LTPA/Low OPA	2,182	97.7	22.7 (0.01)	15.5 (0.01)	7.2 (0.004)
High LTPA/High OPA	1,229	58.9	13.7 (0.01)	8.5 (0.004)	5.2 (0.004)
Low LTPA/Low OPA	5,126	187.7	43.5 (0.01)	24.1 (0.01)	19.5 (0.01)
Low LTPA/High OPA	2,122	85.9	19.9 (0.01)	9.7 (0.004)	10.3 (0.01)

Abbreviations. BMI, Body Mass Index; Kg, kilograms; LTPA, Leisure-Time Physical Activity; m^2, squared meters; OPA, Occupational Physical Activity; SE, Standard Error; WBC, White Blood Cell Count; ul, microliter; %, percentage.

† No periodontitis: N= 5,210; weighted N in millions= 249.1

ǂ Periodontitis: N= 5,469; weighted N in millions = 182.1

3.2 *LTPA/OPA and periodontitis*

Simple and multiple logistic regression analyses for the association between LTPA/OPA and periodontitis are reported in Table 2, while the confounders selection is

reported in Table S2. Multiple logistic regression analyses indicated a statistically significant association between high LTPA and lower odds of suffering from periodontitis (OR=0.81), with similar estimates observed in subgroup analyses by age, gender, and smoking. Conversely, high OPA resulted associated with higher adjusted odds of periodontitis (OR=1.16); an effect modification by smoking, but not by age and gender, was however observed, with stronger estimates in current smokers (OR=1.59).

Sensitivity analyses employing the 4 different frequencies of physical activity as exposure indicated an inverse dose-response relationship between the amount of LTPA and the odds of periodontitis (Tables S3-S4).

***Table 2**. Simple and multiple estimates for the association between physical activity and periodontitis.*

	Periodontitis† OR (95% CI)						
	Overall	Age		Gender		Smoking	
		<60 years	≥60 years	Males	Females	Non-Smokers	Smokers
LTPA							
High LTPA (vs low LTPA)							
Crude	**0.58 (0.52, 0.65)*****	**0.57 (0.50, 0.65)***	0.68 (0.54, 0.85)	**0.52 (0.45, 0.60)***	0.57 (0.50, 0.66)	**0.61 (0.55, 0.69)***	**0.62 (0.47, 0.88)****
Adjusted‡	**0.81 (0.72, 0.92)****	**0.81 (0.70, 0.93)****	**0.70 (0.55, 0.88)****	**0.78 (0.65, 0.93)***	0.85 (0.73, 1.02)	**0.83 (0.72, 0.94)****	0.77 (0.54, 1.09)

				*			
OPA							
High OPA (vs low OPA)							
Crude	**1.26 (1.15, 1.37)****	**1.49 (1.33, 1.68)****	1.03 (0.77, 1.38)	**1.18 (1.05, 1.32)****	1.09 (0.95, 1.27)	1.11 (0.99, 1.25)	**1.52 (1.19, 1.94)***
Adjusted‡	**1.16 (1.04,1.30)*****	**1.15 (1.00,1.32)***	1.12 (0.80, 1.57)	1.14 (0.98, 1.32)	**1.19 (1.01, 1.39)***	1.08 (0.96, 1.22)	**1.59 (1.22,2.07)****
Combination of LTPA and OPA							
High LTPA/ Low OPA							
	Ref	*Ref*	*Ref*	*Ref*	*Ref*	*Ref*	*Ref.*
High LTPA/ High OPA							
Crude	**1.29 (1.07, 1.57)***	**1.54 (1.18, 1.99)****	0.94 (0.56, 1.56)	**1.35 (1.05, 1.72)***	1.06 (0.82, 1.35)	**1.65 (1.01, 2.71)***	1.15 (0.93, 1.40)
Adjusted‡	1.10 (0.90, 1.34)	1.26 (0.97, 1.62)	0.90 (0.51, 1.57)	1.25 (0.97, 1.61)	1.10 (0.86, 1.42)	1.03 (0.83, 1.28)	1.43 (0.80, 2.55)
Low LTPA/ Low OPA							
Crude	**1.74 (1.52, 1.98)****	**1.79 (1.52,2.10)****	**1.39 (1.05, 1.83)***	**2.10 (1.78, 2.48)*****	**1.69 (1.42,2.03)****	**1.68 (1.09, 2.59)***	**1.63 (1.41, 1.88)***
Adjusted‡	**1.35 (1.15, 1.68)***	**1.76 (1.48, 2.09)****	**1.44 (1.08, 1.92)***	**1.53 (1.35, 1.87)***	**1.46 (1.20, 1.77)***	**1.60 (1.33, 1.86)***	1.19 (0.71, 2.00)
Low LTPA/ High OPA							

Crude	**2.28 (1.96, 2.66)****	**2.76 (2.27, 3.35)****	**1.65 (1.15, 2.37)***	**2.29 (1.93, 2.73)****	**2.08 (1.67,2.59)****	**2.64 (1.59, 4.37)****	**1.88 (1.59, 2.23)****
*Adjusted*ǂ	**1.47 (1.26,1.72)*****	**1.48 (1.22,1.80)****	1.40 (0.93, 2.12)	**1.47 (1.19, 1.82)****	**1.46 (1.15,1.86)****	**2.34 (1.13, 4.58)****	**2.04 (1.15, 3.62)***

Abbreviations. CI, Confidence Interval; LTPA, Leisure-Time Physical Activity; OPA, Occupational Physical Activity; OR, Odds Ratio.

ǂ *vs* no periodontitis

ǂ Model adjusted for age, gender, pack-year smoking (as well as educational level and FPL for models involving OPA) and additional confounders identified with the change-in-estimate strategy (reported in Table S2).

*$p<0.05$

**$p<0.01$

***$p<0.001$

3.3 *LTPA/OPA and severe periodontitis*

Simple and multiple logistic regression analyses for the association between LTPA/OPA and severe periodontitis are reported in Table 3; confounders selection is reported in Table S5. As with periodontitis, high LTPA resulted as a protective indicator for severe periodontitis in the adjusted models (OR=0.72), while high OPA was a risk indicator (OR=1.29). These results were consistent in all subgroup analyses, except for smoking: indeed, a stronger estimate of association between OPA and severe periodontitis was present in current smokers (OR=1.69). The same dose-response relationship observed between the amount of LTPA and the odds of periodontitis, was also found in the sensitivity analyses

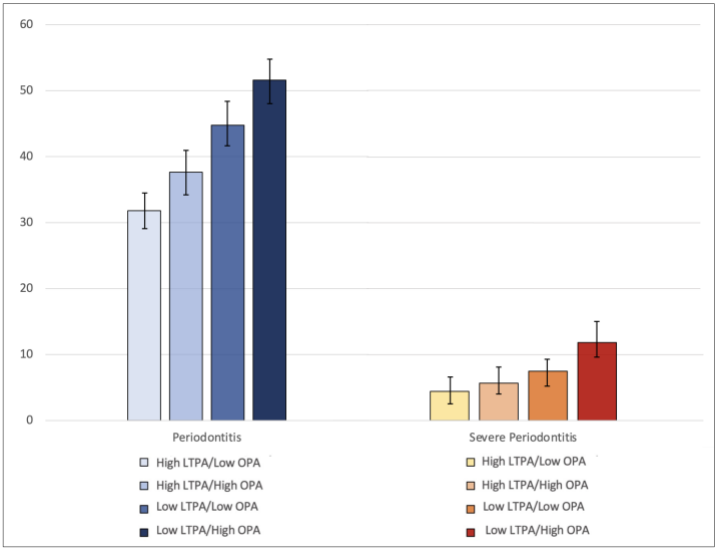
for severe periodontitis (Tables S6 and S7).

3.4 *Combination of LTPA and OPA*

The weighted prevalence of periodontitis and severe periodontitis in subjects performing high LTPA/low OPA was 31.8% and 4.7%, respectively; these proportions doubled in low LTPA/high OPA participants (51.6% and 12.4%, respectively) (Figure 3).

Multiple regression models indicated how the odds of both periodontitis (Table 2) and severe periodontitis (Table 3) gradually increased when shifting from the reference group (high LTPA/low OPA) to the high LTPA/high OPA (OR=1.10 and OR=1.13, respectively) and the low LTPA/low OPA groups (OR=1.35 and OR=1.15, respectively), reaching their peak for the subjects performing low LTPA/high OPA (OR=1.47 and OR=1.66, respectively).

Figure 3. *Weighted prevalence of periodontitis and severe periodontitis with 95% Confidence Intervals in subjects performing different combinations of recreational and occupational physical activity.*



Abbreviations. LTPA, Leisure-Time Physical Activity; OPA, Occupational Physical Activity.

Table 3. *Simple and multiple estimates for the association between physical activity and severe periodontitis.*

	Severe Periodontitis† OR (95% CI)						
	Overall	**Age**		**Gender**		**Smoking**	
		Age<60	**Age≥60**	**Males**	**Females**	**Non-smokers**	**Smokers**
LTPA							
High LTPA (vs low LTPA)							
Crude	**0.53 (0.43, 0.65)****	**0.48 (0.36, 0.64)****	**0.68 (0.49, 0.93)***	**0.49 (0.39, 0.69)***	**0.45 (0.31,0.65)****	**0.63 (0.43, 0.93)***	**0.57 (0.44,0.73)****
Adjusted‡	**0.72 (0.58, 0.90)****	**0.68 (0.52, 0.91)***	0.83 (0.59, 1.15)	**0.72 (0.55, 0.93)****	0.70 (0.47, 1.04)	**0.75 (0.57, 0.97)***	0.75 (0.49, 1.12)
OPA							
High OPA (vs low OPA)							

Crude	**1.49 (1.31, 1.71)***	**1.66 (1.39, 1.99)****	1.31 (0.91, 1.88)	**1.28 (1.04, 1.56)***	1.34 (0.99, 1.80)	**1.27 (1.08, 1.51)***	**1.52 (1.12, 2.06)***
Adjusted‡	**1.29 (1.09,1.53)****	**1.20 (1.00, 1.47)***	1.25 (0.85, 1.85)	1.26 (0.97, 1.62)	**1.41 (1.03, 1.94)***	1.10 (0.89, 1.35)	**1.69 (1.19,2.38)****
Combination of LTPA and OPA							
High LTPA/ Low OPA	*Ref*	*Ref*	*Ref*	*Ref*	*Ref*	*Ref*	*Ref.*
High LTPA/ High OPA							
Crude	1.29 (0.94, 1.77)	**1.47 (1.01, 2.13)***	1.05 (0.54, 2.06)	1.49 (0.99, 2.26)	**0.41 (0.21, 0.78)***	1.86 (0.92, 3.73)	0.95 (0.65, 1.39)
Adjusted‡	1.13 (0.81, 1.57)	0.87 (0.57, 1.33)	1.14 (0.49, 2.65)	1.18 (0.73, 1.91)	**0.44 (0.23, 0.83)***	1.62 (0.76, 3.43)	0.72 (0.47, 1.46)

Low LTPA/ Low OPA							
Crude	**1.74 (1.35, 2.24)****	**1.91 (1.39, 2.64)****	1.35 (0.91, 1.99)	**2.23 (1.61, 3.09)****	1.48 (0.97, 2.25)	**1.85 (1.13, 3.03)****	**1.50 (1.10, 2.04)****
Adjusted‡	1.15 (0.88, 1.50)	1.12 (0.79, 1.62)	1.09 (0.70, 1.71)	**1.28 (1.00, 1.82)***	0.92 (0.56, 1.45)	1.13 (0.81, 1.58)	1.30 (0.74, 2.28)
Low LTPA/ High OPA							
Crude	**2.90 (2.28,3.69)*****	**3.45 (2.48, 4.80)****	**2.07 (1.26, 3.39)****	**2.71 (1.99, 3.71)****	**2.80 (1.74, 4.50)****	**2.82 (1.61, 4.94)****	**2.26 (1.68, 3.06)****
Adjusted‡	**1.66 (1.29,2.15)*****	**1.73 (1.19, 2.51)****	1.57 (0.83, 2.98)	**1.67 (1.14, 2.44)****	**1.77 (1.05, 2.98)***	**1.43 (1.03, 1.99)****	**2.29 (1.22, 4.29)***

Abbreviations. CI, Confidence Interval; LTPA, Leisure-Time Physical Activity; OR, Odds Ratio; OPA, occupational Physical Activity.

†*vs* no/mild/moderate periodontitis

‡Model adjusted for age, gender, pack-year smoking (as well as educational level and FPL for models involving OPA) and additional

confounders identified with the change-in-estimate strategy (reported in Table S5).

*$p<0.05$

**$p<0.01$

***$p<0.001$

3.5 *Mediation analysis*

Table 4 shows a series of ORs after further adjusting the final models for the putative mediators. The association between high LTPA and periodontitis resulted partially mediated by biomarkers of systemic inflammation (platelet count and WBC), BMI and diabetes. Their presence in the final model attenuated the estimate of association by 21.1%. With regards to severe periodontitis, in addition to the same variables, also hypertension showed a mediator effect (10.7%). Conversely, the magnitude of the association between high OPA and periodontitis/severe periodontitis was not attenuated by any of the putative mediators considered.

Table 4*. Mediation analysis for the association between LTPA/OPA and both periodontitis and severe periodontitis.*

	High LTPA		High OPA	
	OR (95% CI)	**% excess odds explained***	**OR (95% CI)**	**% excess odds explained***
Periodontitis				
Base model (B)	0.81 (0.72, 0.92)	-	1.16 (1.04, 1.30)	-
B + Platelet count	0.82 (0.73, 0.93)	5.3%	1.17 (1.04, 1.32)	0.0%
B + WBC count	0.83 (0.74, 0.94)	10.5%	1.18 (1.05, 1.32)	0.0%
B + BMI	0.83 (0.74, 0.94)	10.5%	1.16 (1.04, 1.30)	0.0%
B + Diabetes	0.83 (0.74,	10.5%	1.18 (1.05,	0.0%

	0.93)		1.32)	
B + Hypertension	0.81 (0.70, 0.93)	0.0%	1.17 (1.04, 1.31)	0.0%
B + Platelet count + WBC count	0.83 (0.74, 0.94)	10.5%	1.18 (1.05, 1.32)	0.0%
B + BMI + diabetes	0.85 (0.75, 0.95)	21.1%	1.18 (1.05, 1.32)	0.0%
B + Platelet count + WBC count + BMI + diabetes	0.85 (0.76, 0.96)	21.1%	1.19 (1.06, 1.34)	0.0%
Severe Periodontitis				
Base model (B)	0.72 (0.58, 0.90)	-	1.29 (1.09, 1.53)	-
B + Platelet count	0.73 (0.58, 0.91)	3.6%	1.31 (1.10, 1.56)	0.0%
B + WBC count	0.74 (0.59, 0.93)	7.1%	1.32 (1.11, 1.57)	0.0%
B + BMI	0.73 (0.58, 0.91)	3.6%	1.29 (1.09, 1.53)	0.0%
B + Diabetes	0.73 (0.58, 0.91)	3.6%	1.31 (1.10, 1.56)	0.0%
B + Hypertension	0.75 (0.61, 0.92)	10.7%	1.32 (1.09, 1.59)	0.0%
B + platelet count + WBC count	0.74 (0.59, 0.93)	7.1%	1.32 (1.11, 1.57)	0.0%
B + BMI + diabetes + hypertension	0.75 (0.61, 0.93)	10.7%	1.33 (1.10, 1.60)	0.0%
B + platelet count + WBC count + BMI + diabetes + hypertension	0.76 (0.62, 0.95)	14.3%	1.35 (1.12, 1.64)	0.0%

Abbreviations. BMI, Body Mass Index; CI, Confidence Interval; LTPA, Leisure-Time Physical Activity; OR, Odds Ratio; OPA, Occupational Physical Activity; WBC; White Blood Cell Count.

The Base Model (B) corresponds to the adjusted model reported in Table 2.

Different potential mediators have been added separately to the base model.

* Negative values were considered as 0.00% excess odds explained.

4 Discussion

The findings from the present population-based study indicated that LTPA represents a protective indicator, while OPA represents a risk indicator, of both periodontitis and severe periodontitis. The association of LTPA with periodontitis/severe periodontitis demonstrated a dose-response relationship, while the association of OPA with periodontitis/severe periodontitis resulted more pronounced in current smokers. Moreover, the associations of LTPA and OPA with periodontitis/severe periodontitis were cumulative, with the highest odds observed in subjects performing both low LTPA and high OPA. Mediation analyses suggested that the association between LTPA and periodontitis/severe periodontitis may be partially mediated by biomarkers of systemic inflammation, BMI, and comorbidities (*i.e.,* diabetes and hypertension).

Previous epidemiological studies reported an association between high levels of physical activity and periodontitis (Bawadi et al., 2011; Al-Zahrani et al., 2005; Han et al., 2019). Specifically, a cross-sectional study also using NHANES data, but employing a partial periodontal examination protocol (NHANES III), noted that subjects with normal weight, engaged in regular levels of bodily movement, and with a better diet quality were 40% less likely to have periodontitis compared to individuals presenting none of these healthy lifestyles (Al-Zahrani et

al., 2005). However, other studies have failed to report a significant association between physical activity and periodontitis (Sakki et al., 1995; Sanders et al., 2009). These conflicting results may be explained by the use of different case definitions of periodontitis and by the lack of a clear distinction in the quality of the exposure, since previous studies did not differentiate between LTPA and OPA. Notably, the present study showed an inverse tendency of association when LTPA and OPA are analyzed separately, with the highest odds for periodontitis found in subjects performing low LTPA and high OPA. This 'physical activity paradox' has already been described for the incidence of major cardiovascular events (Holtermann et al., 2021), and therefore it may be present also for periodontitis.

The mechanisms explaining the association between physical activity and NCDs, including periodontitis, are still not clearly understood; however, the modulation of LGSI is regarded as one of the main potential pathways involved (Gleeson et al., 2011). Indeed, high LTPA has been previously associated with a reduction in the levels of both systemic and local (i.e., within the gingival crevicular fluid) inflammatory biomarkers (Kasapis & Thompson, 2005; Rombaldi et al., 2015; Sanders et al., 2009). The results from the present study further underline a possible relevance of the systemic metabolic/inflammatory axis, since platelet count, WBC, BMI, and diabetes partially attenuated the observed estimates of association between LTPA and periodontitis within the mediation analyses.

On the other hand, a high occupational workload may contribute to physical deterioration, leading to chronic stimulation of the hypothalamic–pituitary–adrenal axis

and resulting in the release of high levels of cortisol, pro-inflammatory cytokines, and oxidative stress markers (Tatzber et al., 2022). These blueprints of LGSI have been significantly associated with the incidence and progression of periodontitis (Baima et al., 2022; D'Aiuto et al., 2004). However, OPA is also related to heavy labors which, in turn, are associated with socio-economic disadvantage, a well-established risk indicator for periodontitis and other NCDs (Borrell et al., 2006). While the current analyses involving OPA as exposure were adjusted for socioeconomic status, relevant details about specific job type and duration were not available in NHANES and, hence, they could not be considered as additional confounders. The present study, therefore, cannot exclude the possibility that the identification of OPA as a risk indicator for periodontitis is spurious.

The results from this study should be regarded with caution due the above-mentioned risk of residual confounding and due to its cross-sectional design , which prevents the evaluation of causality and limits the value of the reported mediation analyses. Moreover, a risk of information bias cannot be ruled out, due to the self-reported assessment of physical activity and to the use of PCP2 probes for the periodontal examination. Finally, despite the statistical significance, the lower limit of some OPA-related 95% CIs was only marginally higher than 1.0, so such associations – if present - may potentially be of limited clinical relevance. Nonetheless, the present study has the novelty to differentiate between LTPA and OPA, and the divergent direction of association found with periodontitis may explain the previously reported conflicting results. Finally, the use of data from a complete periodontal examination protocol minimized

the risk of information bias, and the employed sampling procedures allow the generalizability of the present findings to the whole non-institutionalized US population.

5 Conclusions

Leisure-time and occupational physical activity demonstrated divergent associations with periodontitis. Both dose-response and cumulative-type associations were identified, while systemic inflammation, BMI and co-morbidities may partially explain these relationships. Longitudinal cohort studies are needed to rule out the risk of residual confounding and to verify the temporality of these associations. Furthermore, randomized clinical trials should be carried out to investigate the potential impact of physical activity counseling both on primary prevention of periodontitis and as part of the Step 1 of periodontal therapy.

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Supplementary information (Chapter 2)

Supplementary Appendix.

Specific GPAQ questions used to assess LTPA

Leisure-time physical activity (LTPA) was assessed through the following specific GPAQ questions:
(1) "In a typical week, do you do any vigorous-intensity sports, fitness, or recreational activities that cause larger increases in breathing or heart rate like running or basketball for at least 10 minutes continuously [excluding work and transportation activities]?"

(2) "In a typical week, on how many days do you do vigorous-intensity sports, fitness, or recreational activities?"
(3) "How much time do you spend doing vigorous-intensity sports, fitness, or recreational activities on a typical day?"
(4) "In a typical week, do you do any moderate-intensity sports, fitness, or recreational activities that cause a small increase in breathing or heart rate such as brisk walking, bicycling, swimming, or golf for at least 10 minutes continuously?"
(5) "In a typical week, on how many days do you do moderate-intensity sports, fitness, or recreational activities?"
(6) "How much time do you spend doing moderate-intensity sports, fitness, or recreational activities on a typical day?"

Specific GPAQ questions used to assess OPA
Occupational physical activity (OPA) was assessed through the following specific GPAQ questions:
(1) "Does your work involve vigorous-intensity activity

that causes large increases in breathing or heart rate like carrying or lifting heavy loads, digging or construction work for at least 10 minutes continuously?"
(2) "In a typical week, on how many days do you do vigorous-intensity activities as part of your work?"
(3) "How much time do you spend doing vigorous-intensity activities at work on a typical day?"
(4) "Does your work involve moderate-intensity activity that causes small increases in breathing or heart rate such as brisk walking or carrying light loads for at least 10 minutes continuously?"
(5) "In a typical week, on how many days do you do moderate-intensity activities as part of your work?"
(6) "How much time do you spend doing moderate-intensity activities at work on a typical day?""

Assessment methods of putative confounders, mediators and effect modifiers.
As a priori/putative confounders, the following variables were considered: age, gender, pack-year smoking, ethnicity, educational level, Family Poverty Level (FPL), marital status, alcohol intake, sleep duration, and frequency of self-performed interproximal hygiene.
- Age (RIDAGEYR; self-reported) was measured in years and considered as a continuous variable.
- Gender (RIAGENDR; self-reported) was categorized as: males and females.
- Pack-year smoking (self-reported) was calculated as the number of packs/day multiplied by the number of years smoked and it was considered as a continuous variable (Berube et al., 2021). The number of packs/day was calculated by dividing the average number of cigarettes/day during the past 30 days (SMD650) by 20; the number of years smoked was calculated as the difference between the interview age (RIDAGEYR) and the age started smoking regularly (SMD030).

- Ethnicity (RIDRETH1; self-reported) was categorized as: Mexican American, Non-Hispanic White, Non-Hispanic Black.
- Educational level (DMDEDUC2; self-reported) was categorized as: less than high school, high school, college or more.
- FPL (INDFMPIR; self-reported) was categorized as; <100 %, 100-199 %, 200-399 %, ≥400 %. This variable represents the ratio of family income to poverty. It was calculated by dividing the family (or individual) income by the poverty guidelines specific to the survey year. The value was not computed if the respondent only reported income as < $20,000 or ≥ $20,000 or if the income data was missing. If family income was reported as a more detailed category, the midpoint of the range was used to compute the ratio. Values at or above 5.00 were coded as 5.00 or more because of disclosure concerns.
- Marital status (DMDMARTL; self-reported) was categorized as: married, widowed, divorced, separated, never married, living with partner.
- Alcohol intake (ALQ130, ALQ101; self-reported) was identified considering the suggested alcohol intake as threshold (ALQ130; 14 g/d or 1 standard alcoholic drink /d for women, and 28 g/d or 2 standard alcoholic drinks /d for men). Participants were categorized as being below (if they had not drunk at least 12 alcohol drinks/1 year - ALQ101, or if they had drunk at least 12 alcohol drinks/1 year but they were below the suggested intake) or above (if they had drunk at least 12 alcohol drinks/1 year and they were above the suggested intake) the suggested alcohol intake (Ricci et al. 2020).
- Sleep duration (SLD010H; self-reported) was categorized as: less than 7 hours, 7-8 hours, more than 8 hours.

- Frequency of self-performed interproximal hygiene (OHQ870; self-reported) was categorized as follows: 0 days/week, 1-6 days/week, 7 days/week.

As putative mediators, the following variables were considered: platelet count, white blood cell count (WBC), body mass index (BMI), diabetes status, hypertension.

- Platelet count (LBXPLTSI) was measured as 1000 cells/ul and considered a continuous variable.
- WBC count (LBXWBCSI) was measured as 1000 cells/ul and considered a continuous variable.
- BMI (BMXBMI) was calculated with the formula: weight/height^2. Weight was measured with a digital scale in kilograms, while height was measured with a portal stadiometer in meters.
- Diabetes status was categorized as: no diabetes (glycated hemoglobin (HbA1c; LBXGH; measured) <6.5%, and fasting plasma glucose (FPG; LBXGLU; measurred) <126 mg/dL, and no self-reported diagnosis of diabetes - DIQ010) and diabetes (HbA1c- ≥6.5%, or FPG ≥126 mg/dL, or self-reported diagnosis of diabetes).
- Hypertension status was categorized as: normal (measured Systolic Blood Pressure (SBP) < 120 mmHg and measured Diastolic Blood Pressure (DBP) < 80mmHg), borderline hypertension (120≤SBP<140 mmHg or 80≤DBP<90 mmHg), hypertension (average of SBP≥140mmHg or DBP≥90mmHg). The final value of SBP and DBP was obtained by calculating the average of three readings for each parameter (SBP: BPXSY1, BPXSY2, and BPXSY3; DBP: BPXDI1, BPXDI2, and BPXDI3).

Table S1. *Amount of missing data in the study population (N=10,679).*

Variables	Collected data, N (%)	Missing data, N (%)
Age	10,679 (100.0)	0 (0.0)
Gender	10,679 (100.0)	0 (0.0)
Smoking	10,674 (99.9)	5 (0.1)
Ethnicity	10,679 (100.0)	0 (0.0)
Educational level	10,666 (99.9)	13 (0.1)
Family Poverty Level	9,784 (91.6)	895 (8.4)
Marital status	10,679 (100.0)	0 (0.0)
Alcohol intake	9,854 (92.2)	829 (7.8)
Sleep duration	10,663 (99.8)	16 (0.2)
Frequency of self-performed interproximal hygiene	10,675 (99.9)	4 (0.1)
Platelet count	10,315 (96.6)	364 (3.4)
WBC count	10,316 (96.6)	363 (3.4)
Body Mass Index	10,616 (99.4)	63 (0.6)
Diabetes	10,679 (100.0)	0 (0.0)
Hypertension	9,591 (89.8)	1,088 (10.2)
Periodontitis	10,679 (100.0)	0 (0.0)

LTPA	10,674 (99.9)	5 (0.1)
OPA	10,663 (99.9)	16 (0.1)

Abbreviations. LTPA, Leisure-Time Physical Activity; N, number; OPA, Occupational Physical Activity; WBC, white blood cell count; %, percentage.

Table S2. *Confounders selection for the association between LTPA/OPA and periodontitis.*

PA assessment	Ethnicity OR (95% CI)	Educational level OR (95% CI)	Family Poverty level OR (95% CI)	Marital Status OR (95% CI)	Alcohol Intake OR (95% CI)	Sleep duration OR (95% CI)	Freqeuncy of self.performed interproximal hygiene OR (95% CI)
LTPA							
High LTPA (vs low LTPA), Overall (*a priori* OR=0.63, 95% CI 0.56, 0.70) Change if: OR<0.575 or OR>0.703	0.63 (0.56, 0.70)	***0.74*** ***(0.66, 0.83)***	***0.76*** ***(0.67, 0.85)***	0.63 (0.57, 0.70)	0.64 (0.57, 0.70)	0.63 (0.56, 0.70)	0.65 (0.58, 0.73)
High LTPA (vs low LTPA), Age<60 years (*a priori* OR=0.60, 95% CI 0.53, 0.68) Change if: OR<0.540 or OR>0.660	0.61 (0.53, 0.69)	***0.73*** ***(0.64, 0.83)***	***0.74*** ***(0.65, 0.86)***	0.61 (0.53, 0.69)	0.61 (0.53, 0.70)	0.61 (0.53, 0.69)	0.63 (0.55, 0.71)
High LTPA (vs low LTPA),	0.70 (0.56,	0.77	0.78 (0.60,	0.70 (0.55,	0.68 (0.28,	0.74 (0.55,	0.73

Age≥60 years (*a priori* OR=0.70, 95% CI 0.55, 0.88) Change if: OR<0.640 or OR>0.782	0.89)	(0.61, 0.99)	1.01)	0.89)	0.98)	0.99)	(0.57, 0.93)
High LTPA (vs low LTPA), Males (*a priori* OR=0.61, 95% CI 0.52, 0.71) Change if: OR<0.549 or OR>0.671	0.62 (0.53, 0.72)	***0.73*** ***(0.63, 0.85)***	***0.71*** ***(0.60, 0.85)***	0.61 (0.52, 0.71)	0.63 (0.53, 0.75)	***0.69*** ***(0.54, 0.88)***	0.64 (0.55, 0.74)
High LTPA (vs low LTPA), Females (*a priori* OR=0.64, 95% CI 0.55, 0.74) Change if: OR<0.576 or OR>0.704	0.65 (0.56, 0.78)	***0.76*** ***(0.65, 0.87)***	***0.80*** ***(0.69, 0.93)***	0.67 (0.58, 0.77)	***0.65*** ***(0.49, 0.99)***	0.65 (0.56, 0.75)	0.67 (0.57, 0.78)
High LTPA (vs low LTPA), Non-smokers (*a priori* OR=0.62, 95% CI 0.55, 0.69) Change if: OR<0.561 or OR>0.686	0.63 (0.56, 0.71)	***0.75*** ***(0.67, 0.84)***	***0.76*** ***(0.67, 0.86)***	0.63 (0.56, 0.70)	0.62 (0.53, 0.69)	0.62 (0.55, 0.70)	0.64 (0.72, 0.57)
High LTPA (vs low LTPA), Smokers (*a priori* OR=0.77, 95% CI	0.77 (0.55, 1.10)	0.82 (0.58, 1.15)	0.80 (0.56, 1.14)	0.77 (0.55, 1.09)	0.76 (0.38, 1.02)	0.78 (0.56, 1.10)	0.81 (0.58, 1.19)

0.54, 1.09) Change if: OR<0.693 or OR>0.847							
OPA							
High OPA (vs low OPA), Overall (*a priori* OR=1.16, 95% CI 1.04, 1.30) Change if: OR<1.044 or OR>1.276	1.17 (1.05, 1.31)	-	-	1.16 (1.04, 1.31)	1.19 (1.08, 1.30)	1.15 (1.03, 1.29)	1.17 (1.04, 1.31)
High OPA (vs low OPA), Age<60 years (*a priori* OR=1.15, 95% CI 0.99, 1.32) Change if: OR<1.035 or OR>1.265	1.16 (1.01, 1.33)	-	-	1.14 (0.99, 1.31)	1.23 (1.09, 1.38)	1.13 (0.99, 1.30)	1.15 (1.00, 1.32)
High OPA (vs low OPA), Age≥60 years (*a priori* OR=1.12, 95% CI 0.80, 1.57) Change if: OR<1.008 or OR>1.232	1.13 (0.80, 1.58)	-	-	1.14 (0.81, 1.60)	1.06 (0.78, 1.42)	1.11 (0.78, 1.56)	1.12 (0.80, 1.58)
High OPA (vs low OPA), Males (*a priori* OR=1.14, 95% CI 0.98, 1.32)	1.15 (0.98, 1.33)	-	-	1.15 (0.99, 1.34)	1.21 (1.05, 1.39)	1.12 (0.96, 1.30)	1.14 (0.98, 1.32)

Change if: OR<1.026 or OR>1.276							
High OPA (vs low OPA), Females (*a priori* OR=1.19, 95% CI 1.01, 1.39) Change if: OR<1.071 or OR>1.309	1.20 (1.02, 1.40)	-	-	1.17 (0.99, 1.38)	1.14 (0.97, 1.35)	1.18 (1.00, 1.38)	1.20 (1.02, 1.40)
High OPA (vs low OPA), Non-smokers (*a priori* OR=1.08, 95% CI 0.96, 1.22) Change if: OR<0.972 or OR>1.188	1.09 (0.97, 1.24)	-	-	1.08 (0.95, 1.23)	1.12 (0.99, 1.26)	1.07 (0.95, 1.21)	1.09 (0.96, 1.23)
High OPA (vs low OPA), Smokers (*a priori* OR=1.59, 95% CI 1.22, 2.07) Change if: OR<1.431 or OR>1.749	1.59 (1.21, 2.07)	-	-	1.60 (1.23, 2.08)	1.57 (1.22, 2.03)	1.55 (1.19, 2.01)	1.64 (1.25, 2.15)
Combination of LTPA and OPA							
High LTPA/ High OPA (vs High LTPA/ Low OPA), Overall (*a priori* OR=1.10, 95% CI 0.90, 1.34) Change if: OR<0.990 or	1.11 (0.90, 1.36)	-	-	1.10 (0.90, 1.35)	1.18 (0.98, 1.41)	1.09 (0.89, 1.33)	1.10 (0.89, 1.35)

OR>1.210							
High LTPA/ High OPA (vs High LTPA/ Low OPA), Age<60 years (*a priori* OR=1.14, 95% CI 0.86, 1.51) Change if: OR<1.026 or OR>1.254	1.14 (0.86, 1.52)	-	-	1.13 (0.85, 1.50)	***1.27*** ***(0.98, 1.64)***	1.12 (0.85, 1.48)	1.14 (0.86, 1.51)
High LTPA/ High OPA (vs High LTPA/ Low OPA), Age≥60 years (*a priori* OR=0.90, 95% CI 0.51, 1.57) Change if: OR<0.810 or OR>0.990	0.90 (0.52, 1.58)	-	-	0.91 (0.52, 1.60)	0.93 (0.55, 1.54)	0.89 (0.51, 1.57)	0.90 (0.51, 1.58)
High LTPA/ High OPA (vs High LTPA/ Low OPA), Males (*a priori* OR=1.10, 95% CI 0.84, 1.43) Change if: OR<0.990 or OR>1.210	1.11 (0.85, 1.45)	-	-	1.12 (0.86, 1.46)	***1.25*** ***(0.98, 1.61)***	1.09 (0.83, 1.42)	1.10 (0.84, 1.44)
High LTPA/ High OPA (vs High LTPA/ Low OPA), Females (*a priori* OR=1.10, 95% CI 0.86, 1.42) Change if:	1.11 (0.86, 1.43)	-	-	1.08 (0.84, 1.39)	1.04 (0.80, 1.36)	1.09 (0.85, 1.42)	1.11 (0.86, 1.43)

OR<0.990 or OR>1.210							
High LT PA/ High OPA (vs High LTPA/ Low OPA), Non-smokers (*a priori* OR=1.03, 95% CI 0.83, 1.29) Change if: OR<0.927 or OR>1.133	1.05 (0.84, 1.30)	-	-	1.05 (0.84, 1.30)	1.12 (0.92, 1.38)	1.03 (0.83, 1.28)	1.03 (0.83, 1.28)
High LTPA/ High OPA (vs High LTPA/ Low OPA), Smokers (*a priori* OR=1.43, 95% CI 0.80, 2.55) Change if: OR<1.287 or OR>1.573	1.43 (0.80, 2.55)	-	-	1.40 (0.78, 2.52)	1.45 (0.86, 2.48)	1.33 (0.74, 2.41)	1.54 (0.85, 2.78)
Low LTPA/ Low OPA (vs High LTPA/ Low OPA), Overall (*a priori* OR=1.19, 95% CI 1.04, 1.37) Change if: OR<1.071 or OR>1.309	1.19 (1.04, 1.37)	-	-	1.19 (1.04, 1.73)	***1.35*** ***(1.15, 1.68)***	1.19 (1.33, 1.36)	1.17 (1.01, 1.35)
Low LTPA/ Low OPA (vs High LTPA/ Low OPA), Age<60 years (*a priori* OR=1.32, 95% CI 1.10, 1.58)	1.32 (1.10, 1.58)	-	-	1.32 (1.10, 1.58)	***1.76*** ***(1.48, 2.09)***	1.30 (1.09, 1.56)	1.30 (1.09, 1.56)

Change if: OR<1.188 or OR>1.452							
Low LTPA/ Low OPA (vs High LTPA/ Low OPA), Age≥60 years (*a priori* OR=1.16, 95% CI 0.85, 1.57) Change if: OR<1.044 or OR>1.276	1.16 (0.86, 1.58)	-	-	1.16 (0.86, 1.58)	***1.44*** ***(1.08, 1.92)***	1.17 (0.86, 1.58)	1.14 (0.83, 1.56)
Low LTPA/ Low OPA (vs High LTPA/ Low OPA), Males (*a priori* OR=1.25, 95% CI 1.04, 1.51) Change if: OR<1.125 or OR>1.375	1.25 (1.03, 1.50)	-	-	1.27 (1.05, 1.53)	***1.53*** ***(1.15, 1.87)***	1.26 (1.04, 1.52)	1.23 (1.02, 1.48)
Low LTPA/ Low OPA (vs High LTPA/ Low OPA), Females (*a priori* OR=1.15, 95% CI 0.96, 1.38) Change if: OR<1.035 or OR>1.265	1.15 (0.96, 1.39)	-	-	1.13 (0.94, 1.36)	***1.46*** ***(1.20, 1.77)***	1.13 (0.94, 1.36)	1.13 (0.94, 1.36)
Low LTPA/ Low OPA (vs High LTPA/ Low OPA), Non-smokers (*a priori* OR=1.18, 95% CI	1.18 (1.01, 1.38)	-	-	1.19 (1.02, 1.38)	***1.60*** ***(1.38, 1.86)***	1.18 (1.01, 1.37)	1.16 (0.99, 1.36)

1.01, 1.37) Change if: OR<1.062 or OR>1.298							
Low LTPA/ Low OPA (vs High LTPA/ Low OPA), Smokers (*a priori* OR=1.19, 95% CI 0.71, 2.00) Change if: OR<1.071 or OR>1.309	1.19 (0.71, 2.00)	-	-	1.18 (0.70, 1.98)	1.29 (0.81, 2.06)	1.13 (0.67, 1.91)	1.16 (0.69, 1.94)
Low LTPA/ High OPA (vs High LTPA/ Low OPA), Overall (*a priori* OR=1.47, 95% CI 1.26, 1.72) Change if: OR<1.323 or OR>1.617	1.48 (1.27, 1.74)	-	-	1.47 (1.25, 1.72)	1.53 (1.47, 2.04)	1.45 (1.24, 1.69)	1.45 (1.24, 1.70)
Low LTPA/ High OPA (vs High LTPA/ Low OPA), Age<60 years (*a priori* OR=1.48, 95% CI 1.22, 1.80) Change if: OR<1.332 or OR>1.628	1.49 (1.23, 1.81)	-	-	1.48 (1.22, 1.79)	1.61 (1.32, 2.04)	1.45 (1.20, 1.77)	1.47 (1.21, 1.79)
Low LTPA/ High OPA (vs High LTPA/ Low OPA), Age≥60 years (*a priori*	1.42 (0.94, 2.14)	-	-	1.42 (0.94, 2.14)	1.53 (0.95, 2.28)	1.41 (0.93, 2.13)	1.39 (0.92, 2.10)

OR=1.40, 95% CI 0.93, 2.12) Change if: OR<1.260 or OR>1.540							
Low LTPA/ High OPA (vs High LTPA/ Low OPA), Males (*a priori* OR=1.47, 95% CI 1.19, 1.82) Change if: OR<1.323 or OR>1.617	1.48 (1.19, 1.83)	-	-	1.50 (1.21, 1.85)	1.59 (1.12, 2.02)	1.45 (1.17, 1.80)	1.44 (1.16, 1.78)
Low LTPA/ High OPA (vs High LTPA/ Low OPA), Females (*a priori* OR=1.46, 95% CI 1.15, 1.86) Change if: OR<1.314 or OR>1.606	1.48 (1.17, 1.88)	-	-	1.42 (1.11, 1.82)	1.60 (1.14, 2.13)	1.43 (1.13, 1.82)	1.45 (1.14, 1.84)
Low LTPA/ High OPA (vs High LTPA/ Low OPA), Non-smokers (*a priori* OR=1.34, 95% CI 1.14, 1.58) Change if: OR<1.206 or OR>1.474	1.35 (1.15, 1.59)	-	-	1.33 (1.13, 1.57)	1.47 (1.15, 1.95)	1.32 (1.12, 1.56)	1.33 (1.12, 1.57)
Low LTPA/ High OPA (vs High LTPA/ Low OPA), Smokers (*a priori*	2.03 (1.15, 3.61)	-	-	2.04 (1.15, 3.62)	2.22 (1.32, 3.74)	1.91 (1.08, 3.38)	1.99 (1.10, 3.59)

OR=2.04, 95% CI
1.15, 3.62)

Change if:
OR<1.836 or
OR>2.244

Each putative confounder was tested separately and added to a model adjusted for the *a priori* confounders. For LTPA, the *a priori* confounders included age, gender and pack-year smoking. For OPA and the combination of LTPA and OPA, the *a priori* confounders included age, gender, pack-year smoking, educational level, and Family Poverty Level (FPL). A change in the adjusted OR for a putative confounder of 10% or more, compared to the *a priori* OR, has been considered as indication that confounding for that covariate could be present.

Abbreviations. CI, Confidence Interval; LTPA, Leisure-Time Physical Activity; OPA, Occupational Physical Activity; OR, Odds Ratio.

***Table S3**. Confounders selection for the sensitivity analyses for the association between LTPA/OPA (4 frequencies) and periodontitis.*

Physical Activity assessment	Ethnicity OR (95% CI)	Educational level OR (95% CI)	Family Poverty level OR (95% CI)	Marital Status OR (95% CI)	Alcohol Intake OR (95% CI)	Sleep duration OR (95% CI)	Freqeuncy of self-performed interproximal hygiene OR (95% CI)
LTPA							
Highly active (vs inactive), Overall (*a priori* OR=0.51, 95% CI 0.44, 0.59) Change if: OR<0.459 or OR>0.561	0.52 (0.46, 0.62)	***0.63*** ***(0.54, 0.72)***	***0.64*** ***(0.55, 0.76)***	0.51 (0.44, 0.60)	0.52 (0.42, 0.66)	0.51 (0.44, 0.59)	0.54 (0.47, 0.63)
Highly active (vs inactive), Age<60 years (*a priori* OR=0.49, 95% CI 0.41, 0.57) Change if: OR<0.441 or	0.50 (0.42, 0.60)	***0.61*** ***(0.52, 0.75)***	***0.62*** ***(0.52, 0.76)***	0.48 (0.42, 0.58)	0.49 (0.39, 0.60)	0.49 (0.41, 0.58)	0.51 (0.44, 0.63)

OR>0.539							
Highly active (vs inactive), Age≥60 years (*a priori* OR=0.59, 95% CI 0.40, 0.86) Change if: OR< 0.531 or OR>0.649	0.59 (0.41, 0.86)	***0.67*** ***(0.45, 0.99)***	***0.71*** ***(0.48, 1.05)***	0.58 (0.44, 0.86)	0.59 (0.38, 0.91)	0.58 (0.40, 0.85)	0.64 (0.42, 0.94)
Highly active (vs inactive), Males (*a priori* OR=0.53, 95% CI 0.44, 0.63) Change if: OR<0.477 or OR>0.583	0.53 (0.44, 0.64)	***0.61*** ***(0.55, 0.80)***	***0.64*** ***(0.53, 0.80)***	0.51 (0.43, 0.62)	0.55 (0.45, 0.72)	0.52 (0.44, 0.63)	0.56 (0.49, 0.68)
Highly active (vs inactive), Females (*a priori* OR=0.48, 95% CI 0.40, 0.59) Change if: OR<0.432 or OR>0.528	0.49 (0.60, 0.41)	***0.57*** ***(0.47, 0.70)***	***0.64*** ***(0.78, 0.52)***	0.49 (0.40, 0.60)	0.49 (0.38, 0.68)	0.49 (0.40, 0.59)	0.51 (0.42, 0.63)
Highly active (vs inactive), Non-smokers (*a priori* OR=0.50, 95% CI 0.41, 0.60) Change if:	0.52 (0.43, 0.61)	***0.63*** ***(0.53, 0.75)***	***0.65*** ***(0.54, 0.79)***	0.50 (0.42, 0.60)	0.50 (0.41, 0.66)	0.50 (0.42, 0.60)	0.53 (0.44, 0.64)

OR<0.457 or OR>0.555							
Highly active (vs inactive), Smokers (*a priori* OR=0.68, 95% CI 0.44, 1.03) Change if: OR<0.612 or OR>0.748	0.68 (0.44, 1.03)	0.72 (0.47, 1.11)	0.68 (0.44, 1.01)	0.66 (0.44, 1.00)	0.67 (0.43, 1.02)	0.68 (0.44, 1.03)	0.74 (0.48, 1.14)
Sufficiently active (vs inactive), Overall (*a priori* OR=0.77, 95% CI 0.65, 0.92) Change if: OR<0.693 or OR>0.847	0.78 (0.65, 0.93)	0.81 (0.68, 0.97)	0.84 (0.68, 1.03)	0.76 (0.64, 0.90)	0.79 (0.64, 0.98)	0.77 (0.64, 0.92)	0.78 (0.65, 0.93)
Sufficiently active (vs inactive), Age<60 years (*a priori* OR=0.78, 95% CI 0.64, 0.96) Change if: OR<0.702 or OR>0.858	0.79 (0.97, 0.65)	0.82 (0.66, 1.01)	0.88 (0.70, 1.09)	0.78 (0.63, 0.95)	0.81 (0.63, 1.04)	0.79 (0.64, 0.97)	0.79 (0.64, 0.98)
Sufficiently active (vs inactive), Age≥60 years (*a priori* OR=0.74,	0.74 (0.44, 1.20)	0.78 (0.46, 1.28)	0.75 (0.43, 1.28)	0.71 (0.45, 1.15)	0.72 (0.41, 1.22)	0.72 (0.45, 1.15)	0.74 (0.46, 1.19)

95% CI 0.45, 1.19) Change if: OR<0.666 or OR>0.814							
Sufficiently active (vs inactive), Males (*a priori* OR=0.80, 95% CI 0.62, 1.02) Change if: OR<0.720 or OR>0.880	0.81 (0.63, 1.04)	0.83 (0.63, 1.08)	0.85 (0.63, 1.15)	0.78 (0.61, 1.01)	0.81 (0.58, 1.11)	0.79 (0.62, 1.02)	0.81 (0.62, 1.04)
Sufficiently active (vs inactive), Females (*a priori* OR=0.74, 95% CI 0.60, 0.92) Change if: OR<0.666 or OR>0.814	0.74 (0.59, 0.92)	0.80 (0.64, 1.01)	0.83 (0.65, 1.04)	0.73 (0.58, 0.91)	0.74 (0.57, 1.01)	0.74 (0.60, 0.92)	0.72 (0.58, 0.90)
Sufficiently active (vs inactive), Non-smokers (*a priori* OR=0.72, 95% CI 0.58, 0.88) Change if: OR<0.648 or OR>0.792	0.72 (0.59, 0.90)	0.78 (0.61, 0.97)	***0.80*** ***(0.64, 1.01)***	0.71 (0.57, 0.87)	0.72 (0.56, 0.95)	0.71 (0.56, 0.87)	0.72 (0.58, 0.89)
Sufficiently active (vs inactive),	1.27 (0.72,	***1.15***	***1.11*** ***(0.60,***	1.23 (0.70,	1.35 (0.73,	1.27 (0.74,	1.28

Smokers	2.17)	***(0.67, 2.08)***	***2.08)***	2.13)	2.46)	2.13)	(0.74, 2.22)
(*a priori* OR=1.26, 95% CI 0.71, 2.22)							
Change if: OR<1.134 or OR>1.386							
Insufficiently active (vs inactive), Overall							
(*a priori* OR=0.84, 95% CI 0.67, 1.04)	0.85 (0.71, 1.05)	0.85 (0.68, 1.08)	0.88 (0.71, 1.11)	0.79 (0.63, 0.99)	0.84 (0.64, 1.10)	0.83 (0.68, 1.02)	0.84 (0.68, 1.05)
Change if: OR<0.756 or OR>0.924							
Insufficiently active (vs inactive), Age<60 years							
(*a priori* OR=0.85, 95% CI 0.65, 1.11)	0.86 (0.65, 1.14)	0.87 (0.68, 1.15)	0.88 (0.66, 1.18)	0.80 (0.61, 1.05)	0.85 (0.62, 1.22)	0.84 (0.65, 1.10)	0.86 (0.67, 1.12)
Change if: OR<0.765 or OR>0.935							
Insufficiently active (vs inactive), Age≥60 years							
(*a priori* OR=0.81, 95% CI 0.50, 1.32)	0.81 (0.49, 1.30)	0.82 (0.51, 1.33)	0.85 (0.52, 1.37)	0.78 (0.48, 1.25)	0.80 (0.61, 1.40)	0.80 (0.50, 1.29)	0.81 (0.50, 1.31)
Change if: OR<0.729 or							

OR>0.891							
Insufficiently active (vs inactive), Males (*a priori* OR=0.93, 95% CI 0.68, 1.25) Change if: OR<0.837 or OR>1.023	0.93 (0.68, 1.27)	0.95 (0.70, 1.33)	0.96 (0.69, 1.35)	0.88 (0.65, 1.20)	0.93 (0.65, 1.32)	0.93 (0.68, 1.27)	0.93 (0.68, 1.27)
Insufficiently active (vs inactive), Females (*a priori* OR=0.73, 95% CI 0.56, 0.97) Change if: OR<0.657 or OR>0.803	0.74 (0.56, 0.98)	0.74 (0.56, 0.98)	0.76 (0.58, 1.00)	0.68 (0.53, 0.91)	0.74 (0.57, 0.95)	0.70 (0.53, 0.92)	0.74 (0.56, 0.98)
Insufficiently active (vs inactive), Non-smokers (*a priori* OR=0.81, 95% CI 0.64, 1.02) Change if: OR<0.739 or OR>0.903	0.82 (0.64, 1.04)	0.83 (0.64, 1.06)	0.85 (0.67, 1.10)	0.77 (0.60, 0.98)	0.79 (0.58, 1.06)	0.80 (0.63, 1.02)	0.81 (0.64, 1.03)
Insufficiently active (vs inactive), Smokers (*a priori* OR=1.09, 95% CI 0.60,	0.89 (0.55, 1.66)	1.10 (0.61, 1.96)	1.00 (0.63, 1.85)	1.02 (0.56, 1.70)	1.18 (0.69, 1.99)	1.04 (0.56, 1.70)	1.09 (0.54, 1.58)

1.96) Change if: OR<0.981 or OR>1.199							
OPA							
Highly active (vs inactive), Overall (*a priori* OR=1.15, 95% CI 1.01, 1.30) Change if: OR<1.035 or OR>1.265	1.16 (1.02, 1.33)	-	-	1.16 (1.02, 1.32)	1.18 (1.07, 1.31)	1.13 (0.99, 1.29)	1.15 (1.01, 1.31)
Highly active (vs inactive), Age<60 years (*a priori* OR=1.18, 95% CI 1.02, 1.36) Change if: OR<1.062 or OR>1.298	1.19 (1.03, 1.38)	-	-	1.18 (1.03, 1.37)	1.27 (1.13, 1.44)	1.16 (1.00, 1.34)	1.18 (1.02, 1.37)
Highly active (vs inactive), Age≥60 years (*a priori* OR=0.99, 95% CI 0.69, 1.43) Change if: OR<0.891 or OR>1.089	1.00 (0.70, 1.44)	-	-	1.01 (0.70, 1.46)	0.92 (0.66, 1.29)	0.98 (0.67, 1.42)	0.99 (0.69, 1.44)
Highly active (vs inactive), Males (*a priori* OR=1.14, 95%	1.15 (0.98, 1.35)	-	-	1.16 (0.99, 1.36)	1.20 (1.05, 1.38)	1.12 (0.96, 1.32)	1.14 (0.97, 1.33)

CI 0.98, 1.34) Change if: OR<1.026 or OR>1.254							
Highly active (vs inactive), Females (*a priori* OR=1.14, 95% CI 0.95, 1.37) Change if: OR<1.026 or OR>1.254	1.15 (0.97, 1.37)	-	-	1.14 (0.95, 1.36)	1.12 (0.93, 1.35)	1.12 (0.94, 1.35)	1.15 (0.96, 1.37)
Highly active (vs inactive), Non-smokers (*a priori* OR=1.05, 95% CI 0.92, 1.20) Change if: OR<0.945 or OR>1.155	1.06 (0.93, 1.22)	-	-	1.06 (0.93, 1.21)	1.11 (0.98, 1.25)	1.04 (0.91, 1.19)	1.05 (0.92, 1.20)
Highly active (vs inactive), Smokers (*a priori* OR=1.67, 95% CI 1.20, 2.31) Change if: OR<1.503 or OR>1.837	1.66 (1.20, 2.29)	-	-	1.68 (1.21, 2.32)	1.65 (1.24, 2.18)	1.61 (1.16, 2.23)	1.72 (1.23, 2.39)
Sufficiently active (vs inactive), Overall (*a priori* OR=1.14, 95% CI 0.88,	1.15 (0.89, 1.48)	-	-	1.11 (0.86, 1.45)	1.06 (0.83, 1.36)	1.13 (0.87, 1.47)	1.05 (0.88, 1.49)

1.48) Change if: OR<1.026 or OR>1.254							
Sufficiently active (vs inactive), Age<60 years (*a priori* OR=1.00, 95% CI 0.70, 1.42) Change if: OR<0.99 or OR>1.01	1.00 (0.70, 1.42)	-	-	0.96 (0.67, 1.37)	**0.95** **(0.69, 1.31)**	0.99 (0.70, 1.42)	1.00 (0.71, 1.43)
Sufficiently active (vs inactive), Age≥60 years (*a priori* OR=1.52, 95% CI 0.94, 2.48) Change if: OR<1.368 or OR>1.672	1.55 (0.96, 2.51)	-	-	1.54 (0.95, 2.50)	1.40 (0.89, 2.21)	1.52 (0.93, 2.48)	1.54 (0.94, 2.51)
Sufficiently active (vs inactive), Males (*a priori* OR=0.98, 95% CI 0.69, 1.41) Change if: OR<0.882 or OR>1.078	0.99 (0.69, 1.42)	-	-	1.00 (0.70, 1.43)	0.96 (0.67, 1.37)	0.98 (0.68, 1.41)	0.99 (0.69, 1.41)
Sufficiently active (vs inactive),	1.29 (0.86,	-	-	1.20 (0.80,	1.17 (0.80,	1.27 (0.85,	1.30

Females	1.93)			1.82)	1.70)	1.91)	(0.86, 1.94)
(*a priori* OR=1.27, 95% CI 0.85, 1.90) Change if: OR<1.143 or OR>1.397							
Sufficiently active (vs inactive), Non-smokers	1.20			1.16	1.13	1.18	1.19
(*a priori* OR=1.19, 95% CI 0.91, 1.54) Change if: OR<1.071 or OR>1.309	(0.93, 1.55)	-	-	(0.89, 1.49)	(0.87, 1.47)	(0.91, 1.54)	(0.92, 1.55)
Sufficiently active (vs inactive), Smokers							
(*a priori* OR=0.90, 95% CI 0.46, 1.77) Change if: OR<0.810 or OR>0.990	0.90 (0.46, 1.77)	-	-	0.91 (0.46, 1.79)	0.82 (0.43, 1.55)	0.90 (0.45, 1.81)	0.93 (0.46, 1.88)
Insufficiently active (vs inactive), Overall							
(*a priori* OR=0.97, 95% CI 0.75, 1.24) Change if: OR<0.873 or OR>1.067	0.98 (0.76, 1.26)	-	-	0.98 (0.76, 1.26)	0.88 (0.70, 1.09)	0.97 (0.76, 1.24)	0.97 (0.75, 1.24)

Insufficiently active (vs inactive), Age<60 years (*a priori* OR=1.06, 95% CI 0.78, 1.43) Change if: OR<0.954 or OR>1.166	1.07 (0.79, 1.45)	-	-	1.08 (0.80, 1.46)	0.96 (0.73, 1.25)	1.06 (0.78, 1.44)	1.05 (0.78, 1.43)
Insufficiently active (vs inactive), Age≥60 years (*a priori* OR=0.81, 95% CI 0.54, 1.21) Change if: OR<0.729 or OR>0.891	0.82 (0.55, 1.24)	-	-	0.82 (0.55, 1.23)	0.76 (0.52, 1.10)	0.81 (0.54, 1.22)	0.81 (0.54, 1.22)
Insufficiently active (vs inactive), Males (*a priori* OR=0.93, 95% CI 0.68, 1.28) Change if: OR<0.837 or OR>1.023	0.94 (0.69, 1.29)	-	-	0.95 (0.69, 1.32)	0.87 (0.61, 1.09)	0.94 (0.69, 1.29)	0.92 (0.68, 1.26)
Insufficiently active (vs inactive), Females (*a priori* OR=0.98, 95% CI 0.71,	0.99 (0.71, 1.38)	-	-	0.98 (0.71, 1.35)	0.94 (0.71, 1.24)	0.98 (0.70, 1.35)	0.99 (0.71, 1.38)

1.35) Change if: OR<0.882 or OR>1.078							
Insufficiently active (vs inactive), Non-smokers (*a priori* OR=1.04, 95% CI 0.78, 1.38) Change if: OR<0.936 or OR>1.144	1.05 (0.79, 1.40)	-	-	1.05 (0.79, 1.40)	0.95 (0.73, 1.22)	1.04 (0.78, 1.38)	1.03 (0.78, 1.38)
Insufficiently active (vs inactive), Smokers (*a priori* OR=0.62, 95% CI 0.36, 1.08) Change if: OR<0.558 or OR>0.682	0.63 (0.36, 1.09)	-	-	0.63 (0.37, 1.08)	0.57 (0.34, 0.95)	0.62 (0.35, 1.11)	0.63 (0.37, 1.08)

Each putative confounder was tested separately and added to a model adjusted for the *a priori* confounders. For LTPA, the *a priori* confounders included age, gender and pack-year smoking. For OPA, the *a priori* confounders included age, gender, pack-year smoking, educational level, and Family Poverty Level (FPL). A change in the adjusted OR for a putative confounder of 10% or more, compared to the *a priori* OR, has been considered as indication that confounding for that covariate could be present.

Abbreviations. CI, Confidence Interval; LTPA, Leisure-Time Physical Acitivty; OPA, Occupational Physical Activity; OR, Odds Ratio.

Table S4. *Sensitivity analysis for the association between LTPA/OPA (4 frequencies) and periodontitis.*

	Periodontitis† OR (95% CI)						
		Age		Gender		Smoking	
PA	**Overall**	**Age<60**	**Age≥60**	**Males**	**Females**	**Non-smokers**	**Smokers**
LTPA							
Inactive	*Ref*	*Ref*	*Ref*	*Ref*	*Ref*	*Ref*	*Ref*
Insufficiently active							
Crude	0.81 (0.66, 1.01)	0.86 (0.67, 1.10)	0.85 (0.54, 1.33)	0.84 (0.62, 1.12)	***0.66 (0.50, 0.85)****	***0.60 (0.72, 0.84)****	***0.77 (0.61, 0.97)****
Adjusted‡	0.84 (0.67, 1.04)	0.85 (0.65, 1.11)	0.81 (0.50, 1.32)	0.93 (0.68, 1.25)	***0.73 (0.56, 0.97)****	0.81 (0.64, 1.03)	0.80 (0.64, 1.03)
Sufficiently active							
Crude	***0.79 (0.67, 0.93)****	0.83 (0.67, 1.02)	0.80 (0.51, 1.28)	***0.71 (0.57, 0.89)****	***0.70 (0.56, 0.87)****	1.27 (0.78, 2.08)	***0.75 (0.61, 0.92)****
Adjusted‡	***0.77 (0.65, 0.92)*****	***0.78 (0.64, 0.96)****	0.74 (0.45, 1.19)	0.80 (0.62, 1.02)	***0.74 (0.60, 0.92)*****	1.04 (0.57, 1.92)	0.80 (0.64, 1.01)
Highly active							
Crude	***0.46 (0.40, 0.53)*****	***0.49 (0.39,0.53)*****	***0.58 (0.42, 0.82)*****	***0.42 (0.51, 0.36)*****	***0.40 (2.05, 3.00)*****	***0.60 (0.42, 0.85)*****	***0.49 (0.42, 0.58)*****

Adjusted‡	***0.70 (0.60, 0.83)******	***0.69 (0.57,0.83)******	0.75 (0.50, 1.11)	***0.72 (0.60, 0.88)****	***0.68 (0.51, 0.92)****	***0.50 (0.42, 0.60)******	0.69 (0.45, 1.08)
OPA							
Inactive	*Ref*	*Ref*	*Ref*	*Ref*	*Ref*	*Ref*	*Ref*
Insufficiently active							
Crude	0.94 (0.77, 1.15)	0.99 (0.77, 1.28)	0.76 (0.54, 1.07)	0.82 (0.63, 1.06)	1.01 (0.77, 1.32)	0.98 (0.77, 1.26)	0.74 (0.48, 1.15)
Adjusted‡	0.97 (0.75, 1.24)	1.06 (0.78, 1.43)	0.81 (0.54, 1.21)	0.93 (0.68, 1.28)	0.98 (0.71, 1.35)	1.04 (0.78, 1.38)	0.62 (0.36, 1.08)
Sufficiently active							
Crude	1.04 (0.84, 1.29)	1.03 (0.76, 1.39)	1.31 (0.85, 2.03)	0.96 (0.70, 1.33)	1.07 (0.76, 1.49)	1.09 (0.86, 1.38)	0.80 (0.43, 1.49)
Adjusted‡	1.14 (0.88, 1.48)	0.81 (0.51, 1.36)	1.52 (0.94, 2.48)	0.98 (0.69, 1.41)	1.27 (0.85, 1.90)	1.19 (0.91, 1.54)	0.90 (0.46, 1.77)
Highly active							
Crude	***1.31 (1.19, 1.43)******	***1.63 (1.45, 1.83)******	0.91 (0.66, 1.26)	***1.19 (1.06, 1.32)****	1.11 (0.94, 1.30)	1.12 (0.99, 1.25)	***1.64 (1.26,2.13)******
Adjusted‡	***1.15 (1.00, 1.31)****	***1.18 (1.02,1.36)****	0.99 (0.69, 1.43)	1.14 (0.98, 1.34)	1.14 (0.95, 1.36)	1.05 (0.92, 1.20)	***1.67 (1.20,2.31)****

Abbreviations. CI, Confidence Interval; LTPA, Leisure-Time Physical Activity; OR, Odds Ratio;OPA, Occupational Physical Activity.

† *vs* no periodontitis

‡ Model adjusted for age, gender, pack-year smoking (as well as educational level and FPL for models involving OPA) and additional confounders identified with the change-in-estimate strategy (reported in Table S3).

* $p<0.05$

** $p<0.01$

*** $p<0.001$

Table S5. *Confounders selection for the association between LTPA/OPA and severe periodontitis.*

PA assessment	Ethnicity OR (95% CI)	Educational level OR (95% CI)	Family Poverty level OR (95% CI)	Marital Status OR (95% CI)	Alcohol Intake OR (95% CI)	Sleep duration OR (95% CI)	Freqeuncy of self-performed interproximal hygiene OR (95% CI)
LTPA							
High LTPA (vs low LTPA), Overall (*a priori* OR=0.56, 95% CI 0.45, 0.70) Change if: OR<0.504 or OR>0.616	0.56 (0.46, 0.70)	***0.68*** ***(0.55, 0.85)***	***0.66*** ***(0.53, 0.82)***	0.57 (0.46, 0.71)	0.60 (0.52, 1.77)	0.57 (0.47, 0.71)	0.61 (0.50, 0.77)
High LTPA (vs low LTPA), Age<60 years (*a priori* OR=0.53, 95% CI 0.41, 0.75) Change if: OR<0.477	0.53 (0.40, 0.71)	***0.66*** ***(0.48, 0.87)***	***0.62*** ***(0.47, 0.82)***	0.54 (0.41, 0.72)	0.57 (0.40, 0.89)	0.53 (0.39, 0.70)	0.57 (0.42, 0.77)

or OR>0.583							
High LTPA (vs low LTPA), Age≥60 years (*a priori* OR=0.65, 95% CI 0.47, 0.89) Change if: OR<0.585 or OR>0.715	0.65 (0.47, 0.90)	***0.77*** ***(0.56, 1.05)***	***0.76*** ***(0.56, 1.05)***	0.65 (0.47, 0.90)	0.67 (0.47, 0.98)	0.65 (0.47, 0.90)	0.71 (0.51, 0.99)
High LTPA (vs low LTPA), Males (*a priori* OR=0.58, 95% CI 0.46, 0.77) Change if: OR<0.522 or OR>0.638	0.58 (0.44, 0.74)	***0.70*** ***(0.56, 0.91)***	***0.65*** ***(0.50, 0.84)***	0.58 (0.44, 0.75)	0.62 (0.50, 0.77)	0.57 (0.45, 0.74)	0.62 (0.49, 0.80)
High LTPA (vs low LTPA), Females (*a priori* OR=0.51, 95% CI 0.36, 0.75) Change if: OR<0.459 or OR>0.561	0.51 (0.34, 0.75)	***0.62*** ***(0.42, 0.91)***	***0.65*** ***(0.44, 0.96)***	0.53 (0.36, 0.77)	0.52 (0.31, 0.88)	0.53 (0.36, 0.79)	0.56 (0.39, 0.83)
High LTPA (vs low LTPA), Non-	0.55 (0.43,	***0.69*** ***(0.54, 0.90)***	***0.67*** ***(0.52,***	0.55 (0.42,	0.54 (0.42,	0.55 (0.43,	0.59 (0.46, 0.77)

smokers (*a priori* OR=0.55, 95% CI 0.43, 0.70) Change if: OR<0.497 or OR>0.607	0.71)		***0.86)***	0.72)	0.69)	0.70)	
High LTPA (vs low LTPA), Smokers (*a priori* OR=0.75, 95% CI 0.51, 1.11) Change if: OR<0.684 or OR>0.835	0.75 (0.51, 1.11)	0.80 (0.54, 1.19)	0.75 (0.52, 1.09)	0.74 (0.51, 1.10)	0.78 (0.55, 1.12)	0.75 (0.50, 1.11)	0.78 (0.53, 1.16)
OPA							
High OPA (vs low OPA), Overall (*a priori* OR=1.29, 95% CI 1.11, 1.50) Change if: OR<1.161 or OR>1.419	1.29 (1.09, 1.52)	-	-	1.30 (1.10, 1.55)	1.30 (1.12, 1.51)	1.28 (1.08, 1.52)	1.31 (1.11, 1.55)
High OPA (vs low OPA), Age<60 years (*a priori* OR=1.20, 95% CI 1.00,	1.20 (0.98, 1.47)	-	-	1.20 (0.98, 1.47)	1.29 (1.10, 1.52)	1.20 (0.98, 1.47)	1.22 (1.00, 1.48)

1.47) Change if: OR<1.080 or OR>1.320							
High OPA (vs low OPA), Age≥60 years (*a priori* OR=1.25, 95% CI 0.85, 1.85) Change if: OR<1.125 or OR>1.375	1.25 (0.85, 1.85)	-	-	1.27 (0.85, 1.90)	1.19 (0.78, 1.80)	1.24 (0.84, 1.84)	1.26 (0.85, 1.87)
High OPA (vs low OPA), Males (*a priori* OR=1.26, 95% CI 0.97, 1.62) Change if: OR<1.134 or OR>1.394	1.26 (0.97, 1.62)	-	-	1.27 (0.98, 1.65)	1.29 (1.02, 1.62)	1.25 (0.97, 1.63)	1.27 (0.98, 1.63)
High OPA (vs low OPA), Females (*a priori* OR=1.41, 95% CI 1.03, 1.94) Change if: OR<1.269 or OR>1.551	1.40 (1.01, 1.92)	-	-	1.41 (1.03, 1.94)	1.33 (0.97, 1.83)	1.39 (1.01, 1.92)	1.44 (1.04, 2.00)
High OPA (vs low OPA), Non-	1.10 (0.89,	-	-	1.10 (0.89,	1.16 (0.89,	1.09 (0.88,	1.11

smokers (*a priori* OR=1.10, 95% CI 0.89, 1.35) Change if: OR<0.990 or OR>1.210	1.35)			1.36)	1.51)	1.35)	(0.90, 1.37)
High OPA (vs low OPA), Smokers (*a priori* OR=1.69, 95% CI 1.19, 2.38) Change if: OR<1.521 or OR>1.859	1.67 (1.19, 2.35)	-	-	1.73 (1.22, 2.45)	1.69 (1.23, 2.34)	1.68 (1.19, 2.38)	1.69 (1.19, 2.40)
Combination of LTPA and OPA							
High LTPA/ High OPA (vs High LTPA/ Low OPA), Overall (*a priori* OR=0.96, 95% CI 0.68, 1.35) Change if: OR<0.864 or OR>1.056	0.96 (0.68, 1.35)	-	-	0.96 (0.68, 1.36)	***1.13*** ***(0.81, 1.57)***	0.95 (0.67, 1.35)	0.97 (0.69, 1.37)
High LTPA/ High OPA (vs High LTPA/ Low OPA), Age<60 years	***1.08*** ***(0.74, 1.57)***	-	-	***1.07*** ***(0.73, 1.56)***	***1.13*** ***(0.78, 1.65)***	***1.08*** ***(0.74, 1.57)***	***1.09*** ***(0.75, 1.59)***

(*a priori* OR=0.94, 95% CI 0.63, 1.41) Change if: OR<0.846 or OR>1.034							
High LTPA/ High OPA (vs High LTPA/ Low OPA), Age≥60 years (*a priori* OR=0.94, 95% CI 0.46, 1.92) Change if: OR<0.846 or OR>1.034	1.02 (0.51, 2.01)	-	-	***1.04*** ***(0.52, 2.09)***	0.93 (0.55, 1.54)	1.03 (0.52, 2.04)	1.02 (0.52, 2.03)
High LTPA/High OPA (vs High LTPA/ Low OPA), Males (*a priori* OR=1.16, 95% CI 0.74, 1.82) Change if: OR<1.044 or OR>1.276	***1.34*** ***(0.87, 2.08)***	-	-	***1.35*** ***(0.87, 2.09)***	***1.39*** ***(0.90, 2.16)***	***1.39*** ***(0.87, 2.08)***	***1.35*** ***(0.87, 2.08)***
High LTPA/ High OPA (vs High LTPA/ Low OPA), Females (*a priori* OR=0.44,	0.43 (0.23, 0.81)	-	-	0.42 (0.22, 0.79)	0.45 (0.22, 0.84)	0.43 (0.23, 0.80)	0.44 (0.24, 0.82)

95% CI 0.23, 0.83) Change if: OR<0.396 or OR>0.484							
High LTPA/ High OPA (vs High LTPA/ Low OPA), Non-smokers (*a priori* OR=0.72, 95% CI 0.47, 1.09) Change if: OR<0.648 or OR>0.792	0.72 (0.47, 1.09)	-	-	0.72 (0.48, 1.10)	0.78 (0.38, 1.33)	0.72 (0.47, 1.08)	0.72 (0.47, 1.09)
High LTPA/ High OPA (vs High LTPA/ Low OPA), Smokers (*a priori* OR=1.62, 95% CI 0.76, 3.43) Change if: OR<1.458 or OR>1.782	1.61 (0.76, 3.40)	-	-	1.60 (0.75, 3.40)	1.69 (0.84, 3.39)	1.61 (0.76, 3.43)	1.61 (0.74, 3.50)
Low LTPA/ Low OPA (vs High LTPA/ Low OPA), Overall (*a priori* OR=1.16, 95% CI	1.16 (0.89, 1.52)	-	-	1.16 (0.89, 1.52)	***1.53*** ***(1.19, 1.97)***	1.16 (0.89, 1.52)	1.13 (0.86, 1.49)

0.89, 1.52) Change if: OR<1.044 or OR>1.276							
Low LTPA/ Low OPA (vs High LTPA/ Low OPA), Age<60 years (*a priori* OR=1.38, 95% CI 0.97, 1.95) Change if: OR<1.242 or OR>1.518	***1.52*** ***(1.09, 2.14)***	-	-	1.51 (1.08, 2.11)	***1.78*** ***(1.28, 2.46)***	***1.52*** ***(1.08, 2.13)***	1.47 (1.04, 2.08)
Low LTPA/ Low OPA (vs High LTPA/ Low OPA), Age≥60 years (*a priori* OR=1.03, 95% CI 0.68, 1.56) Change if: OR<0.927 or OR>1.133	***1.17*** ***(0.78, 1.76)***	-	-	***1.18*** ***(0.79, 1.78)***	***1.43*** ***(0.93, 2.19)***	***1.19*** ***(0.79, 1.78)***	1.12 (0.74, 1.70)
Low LTPA/ Low OPA (vs High LTPA/ Low OPA), Males (*a priori* OR=1.32, 95% CI 0.93, 1.87) Change if:	***1.46*** ***(1.03, 2.06)***	-	-	***1.47*** ***(1.05, 2.06)***	***1.68*** ***(1.21, 2.33)***	***1.47*** ***(1.04, 2.08)***	1.41 (0.99, 2.01)

OR<1.188 or OR>1.452							
Low LTPA/ Low OPA (vs High LTPA/ Low OPA), Females (*a priori* OR=0.96, 95% CI 0.61, 1.50) Change if: OR<0.864 or OR>1.056	***1.10*** ***(0.72, 1.70)***	-	-	1.05 (0.69, 1.62)	1.03 (0.63, 1.85)	***1.08*** ***(0.70, 1.65)***	1.05 (0.68, 1.62)
Low LTPA/ Low OPA (vs High LTPA/ Low OPA), Non-smokers (*a priori* OR=1.07, 95% CI 0.78, 1.46) Change if: OR<0.963 or OR>1.177	1.07 (0.78, 1.46)	-	-	1.08 (0.79, 1.47)	***1.42*** ***(0.99, 2.03)***	1.07 (0.78, 1.46)	1.03 (0.74, 1.42)
Low LTPA/ Low OPA (vs High LTPA/ Low OPA), Smokers (*a priori* OR=1.30, 95% CI 0.74, 2.28) Change if: OR<1.170 or OR>1.430	1.30 (0.74, 2.29)	-	-	1.28 (0.73, 2.26)	1.33 (0.78, 2.25)	1.28 (0.73, 2.24)	1.30 (0.74, 2.28)

Low LTPA/ High OPA (vs High LTPA/ Low OPA), Overall (*a priori* OR=1.73, 95% CI 1.32, 2.25) Change if: OR<1.557 or OR>1.903	1.72 (1.32, 2.24)	-	-	1.74 (1.33, 2.27)	***2.18*** ***(1.73, 2.76)***	1.71 (1.30, 2.23)	1.69 (1.29, 2.21)
Low LTPA/ High OPA (vs High LTPA/ Low OPA), Age<60 years (*a priori* OR=1.75, 95% CI 1.19, 2.55) Change if: OR<1.575 or OR>1.925	1.91 (1.34, 2.70)	-	-	1.92 (1.36, 2.71)	***2.40*** ***(1.72, 3.36)***	1.91 (1.34, 2.71)	1.86 (1.30, 2.66)
Low LTPA/ High OPA (vs High LTPA/ Low OPA), Age≥60 years (*a priori* OR=1.58, 95% CI 0.89, 2.81) Change if: OR<1.422 or OR>1.738	1.65 (0.95, 2.86)	-	-	1.70 (0.97, 2.98)	***1.86*** ***(1.03, 3.36)***	1.62 (0.93, 2.83)	1.59 (0.90, 2.81)

Low LTPA/ High OPA (vs High LTPA/ Low OPA), Males (*a priori* OR=1.75, 95% CI 1.19, 2.57) Change if: OR<1.575 or OR>1.925	1.84 (1.33, 2.64)	-	-	1.91 (1.36, 2.69)	**2.15** **(1.56, 2.98)**	1.88 (1.32, 2.66)	1.82 (1.29, 2.57)
Low LTPA/ High OPA (vs High LTPA/ Low OPA), Females (*a priori* OR=1.84, 95% CI 1.14, 2.96) Change if: OR<1.656 or OR>2.024	1.98 (1.23, 3.18)	-	-	1.94 (1.22, 3.11)	**2.39** **(1.43, 3.99)**	1.91 (1.19, 3.07)	1.94 (1.20, 3.11)
Low LTPA/ High OPA (vs High LTPA/ Low OPA), Non-smokers (*a priori* OR=1.43, 95% CI 1.04, 1.98) Change if: OR<1.287 or OR>1.573	1.43 (1.03, 1.98)	-	-	1.44 (1.04, 1.99)	**1.95** **(1.32, 2.95)**	1.42 (1.03, 1.96)	1.39 (1.00, 1.95)
Low LTPA/ High OPA (vs High	2.27	-	-	2.35	2.36	2.25	2.29

LTPA/ Low OPA), Smokers (*a priori* OR=2.29, 95% CI 1.22, 4.30) Change if: OR<2.061 or OR>2.519	(1.22, 4.24)		(1.24, 4.46)	(1.36, 4.10)	(1.20, 4.24)	(1.22, 4.29)

Each putative confounder was tested separately and added to a model adjusted for the *a priori* confounders. For LTPA, the *a priori* confounders included age, gender and pack-year smoking. For OPA and the combination of LTPA and OPA, the *a priori* confounders included age, gender, pack-year smoking, educational level, and Family Poverty Level (FPL). A change in the adjusted OR for a putative confounder of 10% or more, compared to the *a priori* OR, has been considered as indication that confounding for that covariate could be present.

Abbreviations. CI, Confidence Interval; LTPA, Leisure-Time Physical Activity; OR, Odds Ratio; OPA, Occupational Physical Activity.

Table S6. *Confounders selection for the sensitivity analyses for the association between LTPA/OPA (4 frequencies) and severe periodontitis.*

Physical Activity assessment	Ethnicity OR (95% CI)	Educational level OR (95% CI)	Family Poverty level OR (95% CI)	Marital Status OR (95% CI)	Alcohol Intake OR (95% CI)	Sleep duration OR (95% CI)	Freqeuncy of self-performed interproximal hygiene OR (95% CI)
LTPA							
Highly active (vs inactive), Overall (*a priori* OR=0.51, 95% CI 0.39, 0.73) Change if: OR<0.459 or OR>0.561	0.51 (0.38, 0.70)	***0.64*** ***(0.54, 0.96)***	***0.63*** ***(0.47, 0.84)***	0.51 (0.37, 0.70)	0.56 (0.42, 0.78)	0.52 (0.38, 0.69)	0.56 (0.41, 0.78)
Highly active (vs inactive), Age<60 years (*a priori* OR=0.46, 95% CI 0.32, 0.67) Change if: OR<0.414 or OR>0.506	0.46 (0.31, 0.67)	***0.59*** ***(0.41, 0.85)***	***0.55*** ***(0.39, 0.79)***	0.47 (0.33, 0.67)	0.50 (0.35, 0.75)	0.47 (0.32, 0.67)	***0.51*** ***(0.35, 0.74)***

Highly active (vs inactive), Age≥60 years (*a priori* OR=0.68, 95% CI 0.43, 1.06) Change if: OR<0.612 or OR>0.748	0.68 (0.42, 1.07)	***0.82*** ***(0.53, 1.28)***	***0.88*** ***(0.56, 1.39)***	0.68 (0.43, 1.08)	0.72 (0.41, 1.25)	0.68 (0.43, 1.05)	***0.76*** ***(0.48, 1.23)***
Highly active (vs inactive), Males (*a priori* OR=0.55, 95% CI 0.40, 0.75) Change if: OR<0.495 or OR>0.605	0.55 (0.40, 0.78)	***0.68*** ***(0.49, 1.05)***	***0.66*** ***(0.47, 0.92)***	0.54 (0.39, 0.75)	0.61 (0.46, 0.83)	0.55 (0.40, 0.74)	***0.69*** ***(0.43, 0.83)***
Highly active (vs inactive), Females (*a priori* OR=0.38, 95% CI 0.22, 0.66) Change if: OR<0.342 or OR>0.418	0.38 (0.20, 0.39)	***0.46*** ***(0.27, 0.81)***	***0.48*** ***(0.24, 0.89)***	0.39 (0.21, 0.71)	***0.43*** ***(0.18, 1.02)***	0.39 (0.23, 0.68)	***0.43*** ***(0.25, 0.76)***
Highly active (vs inactive), Non-smokers (*a priori* OR=0.48, 95% CI 0.33, 0.69) Change if: OR<0.43 or OR>0.53	0.49 (0.33, 0.71)	***0.64*** ***(0.43, 0.94)***	***0.63*** ***(0.43, 0.91)***	0.48 (0.33, 0.69)	0.48 (0.32, 0.72)	0.48 (0.33, 0.68)	***0.54*** ***(0.36, 0.79)***
Highly active (vs inactive), Smokers (*a priori*	0.79 (0.51,	0.85 (0.53, 1.34)	0.81 (0.54,	0.79 (0.55,	0.85 (0.70,	0.81 (0.52,	0.83 (0.53, 1.33)

OR=0.79, 95% CI 0.50, 1.27) Change if: OR<0.711 or OR>0.869	1.25)		1.20)	1.30)	0.53)	1.27)	
Sufficiently active (vs inactive), Overall (*a priori* OR=0.88, 95% CI 0.64, 1.22) Change if: OR<0.792 or OR>0.968	0.88 (0.78, 1.52)	0.92 (0.67, 1.27)	0.92 (0.64, 1.32)	0.87 (0.63, 1.20)	0.93 (0.67, 1.42)	0.88 (0.64, 1.22)	0.91 (0.65, 1.27)
Sufficiently active (vs inactive), Age<60 years (*a priori* OR=0.96, 95% CI 0.64, 1.46) Change if: OR<0.864 or OR>1.056	0.96 (0.62, 1.48)	1.00 (0.65, 1.53)	0.97 (0.61, 1.52)	0.96 (0.64, 1.45)	***1.08*** ***(0.70, 1.91)***	0.96 (0.63, 1.45)	0.97 (0.65, 1.51)
Sufficiently active (vs inactive), Age≥60 years (*a priori* OR=0.76, 95% CI 0.44, 1.33) Change if: OR<0.694 or OR>0.848	0.76 (0.44, 1.33)	0.81 (0.47, 1.43)	0.84 (0.48, 1.47)	0.75 (0.42, 1.31)	0.73 (0.34, 1.51)	0.76 (0.44, 1.33)	0.76 (0.44, 1.32)
Sufficiently active (vs inactive), Males (*a priori* OR=0.88, 95%	0.88 (0.59, 1.28)	0.91 (0.63, 1.32)	0.88 (0.59, 1.31)	0.87 (0.62, 1.25)	0.94 (0.63, 1.43)	0.88 (0.62, 1.27)	0.91 (0.63, 1.30)

CI 0.61, 1.25) Change if: OR<0.792 or OR>0.968							
Sufficiently active (vs inactive), Females (*a priori* OR=0.81, 95% CI 0.43, 1.56) Change if: OR<0.729 or OR>0.891	0.81 (0.40, 1.59)	0.88 (0.43, 1.75)	***0.98*** ***(0.54, 1.91)***	0.83 (0.42, 1.64)	0.88 (0.57, 1.18)	0.81 (0.42, 1.56)	0.82 (0.43, 1.59)
Sufficiently active (vs inactive), Non-smokers (*a priori* OR=0.73, 95% CI 0.92, 2.04) Change if: OR<0.663 or OR>0.811	0.74 (0.49, 1.10)	0.81 (0.53, 1.22)	0.81 (0.51, 1.27)	0.72 (0.49, 1.08)	0.74 (0.44, 1.23)	0.73 (0.49, 1.09)	0.76 (0.50, 1.16)
Sufficiently active (vs inactive), Smokers (*a priori* OR=1.69, 95% CI 0.79, 1.99) Change if: OR<1.521 or OR>1.859	1.66 (0.89, 3.13)	1.61 (0.72, 1.89)	1.61 (0.72, 2.94)	1.69 (0.85, 3.08)	1.79 (0.92, 3.91)	1.69 (0.91, 3.13)	1.66 (0.92, 3.12)
Insufficiently active (vs inactive), Overall (*a priori* OR=1.11, 95% CI	1.11 (0.76, 1.60)	1.15 (0.79, 1.69)	1.19 (0.81, 1.75)	1.08 (0.75, 1.57)	1.19 (0.77, 1.95)	1.10 (0.77, 1.59)	1.12 (0.78, 1.61)

0.78, 1.59) Change if: OR<0.999 or OR>1.221							
Insufficiently active (vs inactive), Age<60 years (*a priori* OR=1.10, 95% CI 0.72, 1.69) Change if: OR<0.989 or OR>1.210	1.09 (0.66, 1.69)	1.15 (0.74, 1.80)	1.12 (0.72, 1.76)	1.04 (0.68, 1.59)	***1.22*** ***(0.82, 2.21)***	1.09 (0.72, 1.69)	1.11 (0.72, 1.72)
Insufficiently active (vs inactive), Age≥60 years (*a priori* OR=1.11, 95% CI 0.64, 1.91) Change if: OR<0.999 or OR>1.221	1.09 (0.60, 1.90)	1.12 (0.64, 1.999)	***1.28*** ***(0.73, 2.18)***	1.11 (0.64, 1.92)	***1.10*** ***(0.51, 2.62)***	1.12 (0.63, 2.00)	1.14 (0.65, 1.96)
Insufficiently active (vs inactive), Males (*a priori* OR=1.23, 95% CI 0.83, 1.81) Change if: OR<1.107 or OR>1.353	1.23 (0.84, 1.78)	1.28 (0.83, 1.92)	1.33 (0.88, 2.04)	1.19 (0.79, 1.75)	1.29 (0.75, 2.12)	1.23 (0.84, 1.82)	1.23 (0.84, 1.82)
Insufficiently active (vs inactive), Females (*a priori* OR=0.78, 95% CI 0.37, 1.64)	0.78 (0.37, 2.64)	0.76 (0.36, 1.64)	0.81 (0.32, 1.83)	0.76 (0.36, 1.61)	***0.88*** ***(0.55, 1.39)***	0.74 (0.35, 1.56)	0.79 (0.37, 1.64)

Change if: OR<0.702 or OR>0.858							
Insufficiently active (vs inactive), Non-smokers (*a priori* OR=0.98, 95% CI 0.64, 1.49) Change if: OR<0.891 or OR>1.089	0.98 (0.64, 1.52)	1.01 (0.66, 1.59)	1.05 (0.67, 1.64)	0.94 (0.62, 1.45)	1.00 (0.54, 1.75)	0.98 (0.63, 1.49)	1.01 (0.66, 1.82)
Insufficiently active (vs inactive), Smokers (*a priori* OR=1.64, 95% CI 0.82, 1.43) Change if: OR<1.476 or OR>1.804	1.69 (0.93, 3.13)	1.67 (0.84, 3.22)	1.72 (0.94, 3.23)	1.64 (0.85, 3.13)	1.72 (0.70, 3.97)	1.67 (0.89, 3.13)	1.61 (0.82, 3.17)
OPA							
Highly active (vs inactive), Overall (*a priori* OR=1.25, 95% CI 1.03, 1.51) Change if: OR<1.125 or OR>1.375	1.25 (1.03, 1.51)	-	-	1.26 (1.04, 1.53)	1.28 (1.09, 1.50)	1.24 (1.02, 1.51)	1.26 (1.05, 1.53)
Highly active (vs inactive), Age<60 years (*a priori* OR=1.16, 95% CI 0.95, 1.42) Change if:	1.16 (0.95, 1.41)	-	-	1.17 (0.96, 1.42)	1.28 (1.02, 1.63)	1.15 (0.94, 1.41)	1.18 (0.97, 1.43)

OR<1.044 or OR>1.276							
Highly active (vs inactive), Age≥60 years (*a priori* OR=1.20, 95% CI 0.74, 1.96) Change if: OR<1.080 or OR>1.320	1.20 (0.74, 1.95)	-	-	1.22 (0.74, 2.02)	1.18 (0.73, 1.89)	1.20 (0.74, 1.95)	1.21 (0.74, 1.97)
Highly active (vs inactive), Males (*a priori* OR=1.17, 95% CI 0.88, 1.56) Change if: OR<1.053 or OR>1.287	1.17 (0.88, 1.56)	-	-	1.19 (0.89, 1.59)	1.20 (0.94, 1.54)	1.17 (0.87, 1.56)	1.18 (0.89, 1.57)
Highly active (vs inactive), Females (*a priori* OR=1.54, 95% CI 1.11, 2.15) Change if: OR<1.386 or OR>1.694	1.53 (1.09, 2.14)	-	-	1.54 (1.11, 2.15)	1.52 (1.09, 2.11)	1.49 (1.07, 2.07)	1.57 (1.11, 2.21)
Highly active (vs inactive), Non-smokers (*a priori* OR=1.01, 95% CI 0.78, 1.30) Change if: OR<0.909 or OR>1.111	1.01 (0.78, 1.30)	-	-	1.01 (0.79, 1.31)	1.11 (0.89, 1.38)	1.00 (0.78, 1.29)	1.02 (0.79, 1.31)

Highly active (vs inactive), Smokers (*a priori* OR=1.69, 95% CI 1.18, 2.42) Change if: OR<1.521 or OR>1.859	1.68 (1.18, 2.40)	-	-	1.73 (1.20, 2.47)	1.72 (1.23, 2.39)	1.69 (1.18, 2.42)	1.68 (1.17, 2.42)
Sufficiently active (vs inactive), Overall (*a priori* OR=1.31, 95% CI 0.88, 1.93) Change if: OR<1.179 or OR>1.441	1.31 (0.88, 1.93)	-	-	1.31 (0.89, 1.95)	***1.13*** ***(0.79, 1.63)***	1.30 (0.88, 1.93)	1.32 (0.90, 1.95)
Sufficiently active (vs inactive), Age<60 years (*a priori* OR=1.38, 95% CI 0.89, 2.16) Change if: OR<1.242 or OR>1.518	1.38 (0.88, 2.16)	-	-	1.38 (0.88, 2.18)	1.29 (0.88, 1.87)	1.39 (0.88, 2.17)	1.40 (0.89, 2.18)
Sufficiently active (vs inactive), Age≥60 years (*a priori* OR=0.97, 95% CI 0.47, 1.99) Change if: OR<0.873 or OR>1.067	0.97 (0.47, 2.01)	-	-	0.98 (0.48, 2.01)	***0.78*** ***(0.37, 1.63)***	0.97 (0.47, 1.99)	0.98 (0.47, 2.01)
Sufficiently active (vs	1.33	-	-	1.34	1.21	1.32	1.33

inactive), Males (*a priori* OR=1.33, 95% CI 0.84, 2.08) Change if: OR<1.197 or OR>1.463	(0.85, 2.08)			(0.84, 2.12)	(0.81, 1.81)	(0.84, 2.07)	(0.85, 1.09)
Sufficiently active (vs inactive), Females (*a priori* OR=1.19, 95% CI 0.58, 2.42) Change if: OR<1.071 or OR>1.309	1.17 (0.58, 2.39)	-	-	1.19 (0.59, 2.43)	***1.01*** ***(0.45, 1.93)***	1.19 (0.58, 2.44)	1.24 (0.62, 2.49)
Sufficiently active (vs inactive), Non-smokers (*a priori* OR=1.26, 95% CI 0.78, 2.06) Change if: OR<1.134 or OR>1.386	1.27 (0.78, 2.06)	-	-	1.27 (0.78, 2.06)	***1.11*** ***(0.71, 1.73)***	1.26 (0.76, 2.06)	1.29 (0.79, 2.12)
Sufficiently active (vs inactive), Smokers (*a priori* OR=1.44, 95% CI 0.67, 3.07) Change if: OR<1.296 or OR>1.584	1.42 (0.67, 2.99)	-	-	1.50 (0.69, 3.29)	1.33 (0.61, 2.93)	1.44 (0.67, 3.07)	1.43 (0.67, 3.08)
Insufficiently active (vs inactive),	0.75 (0.49,	-	-	0.75 (0.49,	***0.65*** ***(0.44,***	0.74 (0.48,	0.75

Overall (*a priori* OR=0.75, 95% CI 0.48, 1.15) Change if: OR<0.675 or OR>0.825	1.15)			1.16)	***0.95)***	1.15)	(0.49, 1.15)
Insufficiently active (vs inactive), Age<60 years (*a priori* OR=1.04, 95% CI 0.59, 1.83) Change if: OR<0.936 or OR>1.144	1.04 (0.59, 1.84)	-	-	1.06 (0.60, 1.87)	***0.83*** ***(0.49, 1.42)***	1.03 (0.58, 1.83)	1.03 (0.58, 1.83)
Insufficiently active (vs inactive), Age≥60 years (*a priori* OR=0.39, 95% CI 0.31, 0.85) Change if: OR<0.351 or OR>0.429	0.39 (0.20, 0.76)	-	-	0.40 (0.21, 0.76)	0.40 (0.22, 0.72)	0.39 (0.21, 0.75)	0.40 (0.21, 0.77)
Insufficiently active (vs inactive), Males (*a priori* OR=0.53, 95% CI 0.32, 0.88) Change if: OR<0.477 or OR>0.583	0.54 (0.32, 0.88)	-	-	0.55 (0.33, 0.91)	*0.48* *(0.29, 0.74)*	0.54 (0.33, 0.89)	0.53 (0.32, 0.88)
Insufficiently active (vs inactive), Females	1.08 (0.51,	-	-	1.09 (0.51,	1.04 (0.50,	1.06 (0.49,	1.12 (0.53, 2.38)

(*a priori* OR=1.09, 95% CI 0.51, 2.31) Change if: OR<0.981 or OR>1.199	2.31)			2.31)	2.15)	2.29)	
Insufficiently active (vs inactive), Non-smokers (*a priori* OR=0.69, 95% CI 0.39, 1.20) Change if: OR<0.621 or OR>0.759	0.69 (0.39, 1.21)	-	-	0.69 (0.39, 1.21)	0.60 (0.36, 1.01)	0.69 (0.30, 1.21)	0.69 (0.39, 1.21)
Insufficiently active (vs inactive), Smokers (*a priori* OR=0.85, 95% CI 0.43, 1.69) Change if: OR<0.765 or OR>0.935	0.84 (0.42 1.68)	-	-	0.88 (0.45, 1.71)	0.77 (0.40, 1.47)	0.84 (0.42, 1.69)	0.84 (0.41, 1.71)

Each putative confounder was tested separately and added to a model adjusted for the *a priori* confounders. For LTPA, the *a priori* confounders included age, gender and pack-year smoking. For OPA, the *a priori* confounders included age, gender, pack-year smoking, educational level, and Family Poverty Level (FPL). A change in the adjusted OR for a putative confounder of 10% or more, compared to the *a priori* OR, has been considered as indication that confounding for that covariate could be present.

Abbreviations. CI, Confidence Interval; LTPA, Leisure-Time Physical Activity; OR, Odds Ratio; OPA, Occupational Physical Activity.

Table S7. *Sensitivity analysis for the association between LTPA/OPA (4 frequencies) and severe periodontitis.*

		Severe Periodontitis† OR (95% CI)					
		Age		Gender		Smoking	
PA	**Overall**	**Age<60**	**Age≥60**	**Males**	**Females**	**Non-smokers**	**Smokers**
LTPA							
Inactive	*Ref*	*Ref*	*Ref*	*Ref*	*Ref*	*Ref*	*Ref*
Insufficiently active							
Crude	***0.47 (0.36, 0.63)******	***0.41 (0.29, 0.58)****	0.70 (0.45, 1.09)	***0.45 (0.33, 0.60)******	***0.33 (0.19, 0.57)******	0.69 (0.44, 1.10)	***0.50 (0.35, 0.72)****
Adjusted‡	1.11 (0.78, 1.59)	1.22 (0.82, 2.21)	1.25 (0.71, 2.22)	1.23 (0.83, 1.81)	0.88 (0.55, 1.39)	0.98 (0.64, 1.49)	1.64 (0.82, 1.43)
Sufficiently active							
Crude	0.93 (0.69, 1.27)	1.02 (0.71, 1.45)	0.86 (0.53, 1.41)	0.79 (0.56, 1.10)	0.81 (0.42, 1.54)	0.80 (0.54, 1.20)	1.61 (0.88, 2.94)
Adjusted‡	0.88 (0.64, 1.22)	0.96 (0.63, 1.45)	0.76 (0.44, 1.33)	0.88 (0.64, 1.27)	0.90 (0.59, 1.33)	0.73 (0.49, 1.09)	1.69 (0.79, 1.99)
Highly active							
Crude	1.12 (0.79, 1.59)	1.14 (0.72, 2.04)	1.22 (0.73, 2.04)	1.10 (0.75, 1.61)	0.76 (0.36, 1.61)	2.22 (0.44, 1.10)	0.97 (0.64, 1.49)

*Adjusted*ǂ	***0.69 (0.51, 0.93)****	***0.65 (0.45, 0.92)****	1.00 (0.68, 1.69)	0.73 (0.52, 1.02)	***0.49 (0.25, 0.92)*****	0.74 (0.52,1.10)	0.79 (0.50, 1.26)
OPA							
Inactive	*Ref*	*Ref*	*Ref*	*Ref*	*Ref*	*Ref*	*Ref*
Insufficiently active							
Crude	0.68 (0.46, 0.99)	0.89 (0.56, 1.44)	0.38 (0.21, 0.69)	0.46 (0.29, 0.71)	1.13 (0.58, 2.18)	0.65 (0.40, 1.04)	0.79 (0.44, 1.43)
*Adjusted*ǂ	***0.65 (0.44, 0.95)****	0.83 (0.49, 1.42)	***0.39 (0.31, 0.85)****	***0.53 (0.32, 0.88)****	1.09 (0.51, 2.31)	0.69 (0.39, 1.20)	0.85 (0.43, 1.69)
Sufficiently active							
Crude	1.13 (0.80, 1.58)	1.30 (0.93, 1.82)	0.87 (0.43, 1.78)	1.14 (0.76, 1.71)	0.95 (0.49, 1.81)	1.15 (0.74, 1.77)	1.06 (0.51, 2.19)
*Adjusted*ǂ	1.48 (0.92, 2.40)	1.38 (0.89, 2.16)	0.78 (0.37, 1.63)	1.33 (0.84, 2.08)	1.01 (0.45, 1.93)	1.11 (0.71, 1.73)	1.44 (0.67, 3.07)
Highly active							
Crude	***1.52 (1.31, 1.76)*****	***1.73 (1.42,2.09)*****	1.31 (0.85, 2.00)	1.19 (0.95, 1.49)	***1.51 (1.09, 2.10)*****	***1.24 (1.03, 1.50)****	***1.56 (1.13, 2.14)*****
*Adjusted*ǂ	***1.25 (1.03, 1.52)****	1.16 (0.95, 1.42)	1.20 (0.74, 1.96)	1.17 (0.88, 1.56)	***1.54 (1.11, 2.15)****	1.01 (0.78, 1.30)	***1.69 (1.18, 2.42)*****

Abbreviations. CI, Confidence Interval; LTPA, Leisure-Time Physical Activity; OR, Odds Ratio; OPA, Occupational Physical Activity.

ǂ vs no periodontitis

ǂ Model adjusted for age, gender, pack-year smoking (as well as educational level and FPL for models involving OPA) and additional confounders identified with the change-in-estimate strategy (reported in Table S6).

*$p<0.05$

**$p<0.01$

***$p<0.001$

References of Supplementary Information (Chapter 2)

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MULTIPLICATIVE EFFECT OF STRESS AND POOR SLEEP QUALITY ON PERIODONTITIS: A UNIVERSITY-BASED CROSS-SECTIONAL STUDY.

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Chapter 3

MULTIPLICATIVE EFFECT OF STRESS AND POOR SLEEP QUALITY ON PERIODONTITIS: A UNIVERSITY-BASED CROSS-SECTIONAL STUDY.

1 Introduction

Stress and lack of adequate rest are among the major issues of the modern lifestyle (Sabbah et al., 2018). In particular, perceived stress refers to a condition described by the afflicted subject as threatening or uncontrollable (Mondo et al., 2021); in 2020, it affected around 20% of Americans (Chambers & Anderson, 2020). Stress has a negative impact on general health, both as a consequence of direct biological mechanisms (e.g., immune system impairment) or indirect consequent behavioral changes (e.g., reduced physical activity, comfort eating, alcohol intake, smoking) (Krueger & Chang, 2008). With regards to rest, epidemiological studies indicated how a similar proportion of the American population suffers from chronic sleep disorders (Peppard et al., 2013). Similarly to stress, poor sleep quality has a negative effect on systemic health, which has been explained through direct mechanisms involving systemic inflammation, oxidative stress and immune system impairment, and indirect mechanisms related to the associated compensatory behaviors (Mullington et al., 2010).

Being inflammation and oxidative stress key components of periodontitis pathogenesis (Baima et al., 2022; Romandini et al., 2018), previous epidemiological studies have proposed high levels of perceived stress and poor sleep quality as modifiable risk indicators for periodontitis (Coelho et al., 2020; Karaaslan & Dikilitaş, 2019). However, studies from the medical field already highlighted that high stress and poor sleep quality exert a multiplicative

effect on the mortality risk for cardiovascular disease (CVD) compared to subjects with low stress and adequate sleep quality (Li et al., 2021). The rationale behind such multiplicative systemic effect may reside on the reciprocal association found between stress and sleep quality (Hall, 2015): the sleep-induced recovery process helps the human body attenuate stress-related acute load reactions (Akerstedt & Nilsson, 2003), resulting in a fine balance between the two factors. Indeed, whenever stress levels are chronically high and there is an incomplete recovery due to poor sleep quality, chronic allostatic load reactions and the disrupted immune/inflammatory responses are additionally triggered (Nakata, 2012). However, studies analyzing the possible multiplicative effect of high perceived stress and poor sleep quality on the periodontium are still lacking. This information would be clinically relevant, being stress and sleep quality modifiable in nature, and thus potential targets of interventions in the context of the Step 1 of periodontal therapy (Sanz et al., 2020; Marruganti et al., 2023a).

Therefore, the aim of the present cross-sectional study was to evaluate the multiplicative effect of stress and poor sleep quality on periodontitis prevalence and severity in a University-based cohort of individuals.

2 Materials and methods

2.1 *Study design*

The present study is reported according to the Strengthening the Reporting of Observational studies in Epidemiology (STROBE) guidelines for cross-sectional studies (von Elm et al., 2008). The research protocol was

approved by the local ethics committee (protocol number: 18993/2021) and it was registered on Clinicaltrials.gov (NCT04771949).

2.2 *Setting and participants*

All consecutive patients attending the Dentistry Department at the University Hospital of Siena were screened between January 2021 and August 2021. The following inclusion criteria were applied: i) age between 18 and 70 years old; ii) presence of at least 2 remaining teeth; iii) ability and willingness to give informed consent. The exclusion criteria were: i) current pregnancy or lactation; ii) periodontal therapy performed in the previous 12 months; iii) administration of antibiotics within the previous 6 months; iv) inability to effectively communicate in Italian. Individuals were enrolled in the study after they read and signed the written informed consent, in accordance with the Declaration of Helsinki.

2.3 *Assessment of study variables*

2.3.1 *Clinical periodontal parameters*

Details on the assessment of study variables are reported in a separate publication on the same study sample (Marruganti et al., 2022). Briefly, all included participants received a full-mouth periodontal examination by two previously calibrated examiners. Probing Pocket Depth (PPD), gingival recession (REC), plaque (O'Leary et al., 1972) and bleeding on probing (BoP) (Ainamo & Bay, 1975) were recorded with a standardized periodontal probe (UNC 15 probe, HuFriedy Group, Chicago, Illinois, United States) at six sites per tooth, third molars

excluded. Whenever the Cementum Enamel Junction was located subgingivally, negative values were recorded for REC. Clinical attachment levels (CAL) were computed as the sum between PPD and REC values. Furthermore, mean PPD and mean CAL, as well as the proportion (%) of sites with PPD≥4mm and PPD≥6mm were calculated from PPD/CAL measurements. The presence of furcation involvement was recorded according to the classification of Hamp (Hamp et al., 1975), while mobility was measured according to the Miller classes (Miller & Boenheim, 1938).

Examiners' calibration was performed for both PPD and REC on two non-study subjects suffering from periodontitis, and it was considered satisfactory only when an agreement in at least 95% of measurements (with a maximum of 2 mm difference) was recorded between the two examiners. Intra-class correlation coefficients (ICCs) were computed using site-level measurements and not considering sites clustering at teeth/participant-level. Inter-examiner agreement resulted in ICC=0.98 (p<0.001) for PPD and in ICC=0.96 (p<0.001) for REC. For the first examiner, intra-examiner agreement resulted in ICC=0.89 (p=0.002) for PPD and in ICC=0.92 (p=0.001) for REC; for the second one, intra-examiner agreement resulted in ICC=0.94 (p<0.001) for PPD and in ICC=0.98 (p<0.001) for REC.

2.3.2 *Periodontitis case definitions*

Periodontitis was diagnosed using both the EFP/AAP (Papapanou et al., 2018) and the CDC/AAP criteria (Eke et al., 2012a; Page & Eke, 2007). According to EFP/AAP criteria, periodontitis cases were identified whenever interdental CAL was detectable at ≥2 non-adjacent teeth, or whenever buccal or oral CAL≥3mm with pocketing

(PPD>3mm) was detectable at ≥2 teeth (Tonetti et al., 2018). Cases were classified according to their stage and extent. Periodontitis stage was identified using severity (i.e., interdental CAL at site of greatest loss, number of missing teeth due to periodontitis) and complexity factors (i.e., sites with PPD≥6mm, furcation involvement, number of opposing pairs). The number of missing teeth due to periodontitis was drawn from clinical records whenever available, otherwise it was self-reported by the patient; the number of opposing pairs was clinically assessed. For each stage, extent was described as either localized (<30% of teeth involved) or generalized. According to the CDC/AAP criteria, periodontitis was categorized as being either mild, moderate, or severe. Severe periodontitis was defined as having ≥2 interproximal sites with CAL≥6mm and ≥1 interproximal site with PPD≥5mm. Moderate periodontitis was defined as having ≥2 interproximal sites with CAL≥4mm and ≥2 interproximal sites with PPD≥5mm, while mild periodontitis was defined as the presence of ≥2 interproximal sites with CAL≥3mm and ≥2 interproximal sites with PPD≥4mm (Eke et al., 2012; Page & Eke, 2007).

2.3.3 *Perceived stress*

The Italian version of a validated 10-items questionnaire to measure patients' level of perceived stress (IPSS-10) was administered by the clinical examiners, who asked structured questions and gave the explanations provided by the questionnaire itself (Cohen et al., 1983). The instrument had 10 questions with response options ranging between 0 (never) and 4 (very often). All questions were negatively stated, except for 4 questions that were positively stated (items 4, 5, 7 and 8). The sum scores were calculated after reversing the positive items'

scores and then summing up all scores. Total scores ranged between 0 and 40; the higher the score, the higher the level of perceived stress. Finally, the IPSS-10 score was categorized according to the guidelines in order to stratify participants into two subgroups: moderate/high (IPSS-10>13) and low perceived stress (IPSS-10≤13) (Biswas et al., 2019; State of New Hampshire Employee Assistance Program, 1983).

2.3.4 *Sleep quality*

Sleep quality was assessed using the validated Italian version of Pittsburgh Sleep Quality Index (PSQI) questionnaire (Mondo et al., 2021). The PSQI was administered by the clinical examiners, who asked structured questions and gave the explanations provided by the questionnaire. The questionnaire included 7 domains (subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medications, daytime dysfunction), each one assessed with a 0 to 3 scoring system; the final scores ranged between 0 and 21, with higher values indicating worse sleep quality. Participants with a total score of 5 or higher were classified as having "poor sleep quality", vice versa for those with lower scores (classified as having "good sleep quality") (Buysse et al., 1988).

2.3.5 *Covariates*

Self-reported socio-demographic characteristics, including age, sex, weight, height, smoking and oral hygiene habits, occupation, and education level, were registered. Moreover, self-reported information regarding the presence of any comorbidity possibly

affecting susceptibility to periodontitis was collected (i.e., diabetes, rheumatoid arthritis, osteoporosis, cardiovascular and inflammatory bowel diseases); in case participants self-reported one of the mentioned comorbidities, the medical reports were checked for verification. The Body Mass Index (BMI) was computed as weight (kilograms)/height (meters^2). The detailed assessment methods of socio-demographic characteristics are reported in the Appendix.

2.4 *Sample size calculation*

The sample size calculation was based on the null hypothesis that the prevalence of periodontitis in the present sample was the same as previously reported (37.3%) (Jiao et al., 2020). For a 10% threshold in prevalence difference (with α=0.05), a sample of 185 participants would have resulted in 80% power to reject the null hypothesis. Due to the broader scope of the present work, this number was inflated of 20%; therefore, the inclusion of 235 participants was planned.

2.5 *Statistical analyses*

Statistical analyses were performed through an *ad hoc* software (StataCorp. 2021; Stata Statistical Software: Release 17. College Station, TX: StataCorp LLC), *a priori* setting the level of significance at 5%. Continuous variables were reported as Mean and Standard Deviation (SD); categorical data were expressed as number of observations (percentage-%).

After verification of data distribution, the unpaired Student's t test and the Chi^2 test were used to compare

patients' characteristics according to the categories of perceived stress and sleep quality.

Simple and multiple ordinal logistic regression analyses were then performed to study the association between perceived stress/sleep quality (binary variables) and their interaction with periodontitis (EFP/AAP and CDC/AAP criteria). Simple and multiple logistic regression analyses were also performed using perceived stress/sleep quality (continuous variables) as exposure. Furthermore, after assumptions verification, multiple linear regression models were built to evaluate the association between perceived stress and sleep quality with additional periodontal variables, *i.e.* mean CAL, and mean PPD. The multiple models were adjusted for age (Bernabe et al., 2020; Hublin et al., 2018; Osmanovic-Thunström et al., 2015), body mass index (Harding et al., 2014; Jepsen et al., 2020; Madrid-Valero et al., 2017), sex (Michelson et al., 2022), smoking (Cohrs et al., 2014), education (Eke et al., 2016; Zhang et al., 2020), toothbrushing frequency (Zimmermann et al., 2015), and comorbidities (binary variable defined as the presence of at least one comorbidity among those mentioned above), *a priori* selected according previous evidence of their association with the exposure, the outcome, or both (VanderWeele, 2019). Results from regression analyses were expressed as odds ratios (ORs) or beta coefficient (β) with 95% CI.

3 Results

3.1 *Participant characteristics*

A total of 235 participants were included in the present study. Table 1 provides the descriptive statistics of the study population. The mean age was 53.9 years and most

of the included participants were females (57.9%) and never smokers (45.5%). The prevalence of periodontitis according to the EFP/AAP classification was 85.5%, while the prevalence according to the CDC/AAP classification was 64.7%. Around 52% of subjects were in the moderate/high-stress subgroup and 48% were in the poor sleep quality subgroup.

Table 1. *Patients' characteristics by perceived stress and sleep quality level.*

Variable	Overall	Perceived Stress			Sleep Quality		
		Moderate/High	Low	*p*-value*	Poor	Good	*p*-value*
	n=235	n=122	n=113		n=113	n= 122	
Age, years	53.9±14.7	55.8±12.9	51.8±16.2	0.07	57.5±12.6	50.5±15.7	***<0.001***
BMI, kg/m^2	25.5±4.8	25.3±4.2	25.7±5.4	0.93	25.9±5.1	25.1±4.5	0.22
Sex, females	136 (57.9)	73 (59.8)	63 (55.8)	0.31	72 (63.7)	64 (52.5)	0.08
Occupation							
Unemployed	42 (17.9)	25 (20.7)	17 (15.1)		18 (15.9)	24 (19.8)	
Employed	133 (56.8)	64 (52.9)	69 (61.1)	0.41	61 (53.9)	72 (59.5)	0.24
Retired	59 (25.2)	32 (26.5)	27 (23.9)		34 (30.1)	25 (20.7)	
Education							

Elementary/middle school	69 (29.5)	44 (36.1)	25 (22.3)		36 (31.9)	33 (27.3)	
High school	107 (45.7)	55 (45.1)	52 (46.4)	**0.02**	53 (46.9)	54 (44.6)	0.45
College or more	58 (24.8)	23 (18.8)	35 (31.3)		24 (21.3)	34 (28.1)	
Smoking							
Never	107 (45.5)	53 (43.4)	54 (47.8)		51 (45.1)	56 (45.9)	
Former	67 (28.5)	37 (30.3)	30 (26.5)	0.77	32 (28.3)	35 (28.7)	0.99
Smoker	61 (25.9)	32 (26.2)	29 (25.7)		30 (26.6)	31 (25.4)	
Familiarity for periodontitis, yes	87 (37.1)	53 (43.4)	34 (30.1)	**0.02**	43 (38.1)	44 (36.1)	0.79
Comorbidities[††], yes	43 (18.3)	21 (17.2)	22 (19.5)	0.69	27 (23.9)	16 (13.1)	0.09
Oral health status							
Periodontitis Staging[†] (EFP/AAP)							

Healthy/Gingivitis	34 (14.5)	7 (5.7)	27 (23.9)		8 (7.1)	26 (21.3)	
Stage 1	27 (11.5)	13 (10.7)	14 (12.4)		9 (7.9)	18 (14.8)	**0.03**
Stage 2	56 (23.8)	27 (22.1)	29 (25.7)	**<0.001**	25 (22.1)	31 (25.4)	
Stage 3	94 (40.0)	58 (47.5)	36 (31.9)		56 (49.6)	38 (31.2)	
Stage 4	24 (10.2)	17 (13.9)	7 (6.2)		15 (13.3)	9 (7.4)	
Periodontitis ǂ (CDC/AAP)							
No Periodontitis	83 (35.3)	21 (17.2)	62 (54.9)		26 (23.0)	57 (46.7)	
Mild	29 (12.3)	20 (16.4)	10 (8.8)	**<0.001**	16 (14.2)	13 (10.7)	**<0.001**
Moderate	70 (29.8)	57 (46.7)	13 (11.5)		41 (36.3)	29 (23.8)	
Severe	53 (22.6)	15 (12.3)	38 (33.6)		30 (26.6)	23 (18.9)	
Number of teeth	24.2±5.4	23.7±5.3	24.7±5.6	0.06	23.7±5.3	24.6±5.5	0.08
Mean CAL, mm	2.8±1.1	2.9±1.1	2.4±1.1	**<0.001**	2.9±1.0	2.4±1.1	**<0.001**

Mean PPD, mm	2.5±0.7	2.6±0.7	2.4±0.6	***0.02***	2.6±0.7	2.4±0.6	***0.03***
% PPD≥4mm	9.3±11.6	11.0±12.7	7.4±9.9	***<0.001***	11.6±12.8	7.2±9.9	***<0.001***
% PPD≥6mm	5.8±9.4	6.4±4.8	3.2±3.9	***0.001***	7.5±4.2	3.6±3.0	***<0.001***
Furcation involvement, yes [§]	76 (32.3%)	42 (34.4%)	34 (30.1%)	0.28	46 (40.7%)	30 (24.6%)	***0.01***
Mobility, yes	91 (38.7%)	53 (43.4%)	38 (33.6%)	***0.04***	57 (50.4%)	34 (27.9%)	***<0.001***
Number of bleeding pockets [¶]	7.3±10.4	8.9±12.1	5.5±7.8	***<0.001***	9.1±10.9	5.7±9.5	***<0.001***
FMPS	50.6±24.3	51.3±23.9	49.8±24.6	0.69	50.6±24.8	50.5±23.8	0.98
FMBS	28.5±17.8	30.1±15.6	26.7±19.8	***0.02***	29.7±17.9	27.3±17.7	0.25
Teeth lost for periodontitis, yes	76 (32.3)	51 (41.8)	25 (22.1)	***<0.001***	49 (43.4)	27 (22.1)	***<0.001***
Domiciliary plaque control							
Brushing frequency							

Not performed	4 (1.7)	2 (1.6)	2 (1.8)		1 (0.9)	3 (2.5)	
Occasionally	46 (19.6)	28 (22.9)	18 (15.9)	0.37	27 (23.9)	19 (15.6)	0.22
Every day	185 (78.7)	92 (75.4)	93 (82.3)		85 (75.2)	100 (81.9)	
Toothbrush type, powered	122 (51.9)	60 (49.2)	62 (54.9)	0.23	55 (48.7)	67 (54.9)	0.20
Interdental cleaning (IC)							
Not performed	75 (31.9)	41 (33.6)	35 (30.9)		37 (32.7)	38 (31.9)	
Interdental floss	51 (21.7)	27 (22.1)	24 (21.2)	0.78	25 (22.1)	26 (21.3)	0.98
Interproximal brushes	109 (45.9)	54 (44.3)	54 (47.8)		51 (45.1)	57 (46.7)	
Frequency of IC							
Not performed	75 (31.9)	42 (34.4)	33 (29.2)	0.56	37 (32.7)	38 (31.2)	0.99
Occasionally	48 (20.4)	26 (21.3)	22 (19.5)		23 (20.4)	25 (20.5)	

Every day	112 (47.7)	54 (44.3)	58 (51.3)	53 (46.9)	59 (48.4)

Note. Data are presented as mean ± standard deviation or numbers (percentages). Comparisons across subgroups of stress/sleep were performed using the unpaired Student's t test for continuous variables, and the Chi2 test for categorical variables.

Abbreviations: BMI, body mass index; CAL, clinical attachment level; PPD, probing depth; FMPS, full-mouth plaque score; FMBS, full-mouth bleeding score; IC, interdental cleaning; EFP, European Federation of Periodontology; AAP, American Academy of Periodontology.

* $p<0.05$ is considered statistically significant.

†According to the 2018 EFP/AAP classification.

‡According to the CDC/AAP classification.

§Class II/III furcation involvement according to the classification of Hamp *et al.* (1975).

¶Defined as the number of sites with probing depth≥5mm and positive to bleeding on probing.

†† Defined as the presence of at least one comorbidity among diabetes, rheumatoid arthritis, osteoporosis, cardiovascular and inflammatory bowel diseases.

3.2 *Perceived stress and periodontitis*

Participants with moderate/high perceived stress presented a significantly higher periodontitis severity (staging) (p=0.00). Other periodontal parameters (*i.e.* mean CAL and mean PPD, tooth mobility, number of bleeding pockets, FMBS, teeth lost for periodontal causes) resulted significantly worse in subjects with high compared to low perceived stress. Conversely, FMPS as well as domiciliary plaque control habits were comparable across subgroups of perceived stress (p=0.56) (Table 1).

Ordinal logistic regression analyses indicated how moderate/high perceived stress was significantly associated with stage I and III periodontitis with increasing estimates of association from stage I to stage III (Table 2), while the estimates were attenuated when considering the CDC/AAP criteria. Moreover, an increase in the PSS (continuous) was associated with significantly higher odds of severe periodontitis (CDC/AAP; OR=2.4) (Table S1). Furthermore, simple and multiple linear regression analyses demonstrated a significant association between moderate/high perceived stress and other measures of periodontitis, *i.e.* mean CAL and mean PPD (Tables 3).

Table 2*. Effect estimates from ordinal logistic regression models for the association between perceived stress, sleep quality, and their interaction with periodontitis (EFP/AAP classification).*

	Stage I†	Stage II†	Stage III/IV†
	OR (95% CI)	OR (95% CI)	OR (95% CI)

Exposure variables	*Crude*	*Adjusted*‡	*Crude*	*Adjusted*‡	*Crude*	*Adjusted*‡
PSQI (continuous)	3.2 (0.9, 7.2)	2.5 (0.7, 5.1)	2.9 (0.9, 6.2)	2.3 (0.8, 5.3)	5.5 (0.9, 9.7)	2.5 (0.2, 5.2)
PSS (continuous)	3.6 (0.9, 7.9)	2.2 (0.4, 5.0)	2.3 (0.4, 5.9)	2.1 (0.7, 6.2)	3.2 (0.9, 6.1)	2.9 (0.6, 4.9)
Poor sleep quality (vs. good sleep quality)	1.4 (0.5, 4.0)	1.2 (0.4, 3.8)	2.0 (0.8, 4.9)	1.7 (0.7, 4.4)	***4.0 (1.8, 8.7)****	***3.0 (1.2, 7.4)****
Moderate/High perceived stress (vs. low perceived stress)	2.3 (0.8, 6.4)	***2.2 (1.0, 8.8)****	2.3 (0.9, 5.7)	***2.6 (1.1, 6.6)****	***4.6 (2.1, 10.1)****	***5.4 (2.2, 13.5)****
Interaction between sleep quality and perceived stress						
Poor sleep quality (vs. good sleep quality)	2.4 (0.7, 9.0)	3.2 (0.7, 15.0)	1.3 (0.4, 3.3)	1.2 (0.3, 4.9)	***3.4 (1.3, 9.0)****	***2.5 (1.1, 8.2)****
Moderate/High perceived stress (vs. low perceived stress)	3.5 (0.7, 12.0)	***5.5 (1.1, 26.3)****	1.4 (0.4, 4.5)	2.0 (0.9, 7.7)	***3.9 (1.4, 10.9)****	***4.0 (1.0, 15.6)****
Moderate/High stress # poor sleep quality§	3.7 (0.5, 12.9)	1.0 (0.1, 7.1)	***2.7 (1.1, 17.9)****	2.7 (0.7, 9.9)	***6.4 (2.1, 19.1)****	***5.8 (1.6, 21.3)****

Abbreviations. CI, Confidence Interval; OR, Odds Ratio; PSQI, Pittsburgh Sleep Quality Index; PSS, Perceived Stress Scale.

* $p<0.05$ is considered statistically significant.

† vs. healthy or gingivitis.

‡ Adjusted for age, sex, smoking, body mass index, education, brushing frequency, and comorbidities (defined as the presence of at least one comorbidity among diabetes, rheumatoid arthritis, osteoporosis,

cardiovascular and inflammatory bowel diseases).

§ The interaction term equaled 1 whenever the participant had moderate/high stress and poor sleep quality, otherwise it equaled 0 (i.e., reference category).

***Table 3**. Effect estimates from linear regression models for the association between perceived stress, sleep quality, and their interaction with mean CAL and mean PPD.*

	Mean CAL, mm		**Mean PPD, mm**	
	β (95% CI)		**β (95% CI)**	
Exposure variables	***Crude***	***Adjusted†***	***Crude***	***Adjusted†***
PSQI (continuous)	***0.7 (0.3, 1.1)****	***0.4 (0.1, 0.8)****	***0.9 (0.3, 1.6)****	***0.2 (0.02, 1.0)****
PSS (continuous)	***0.7 (0.4, 1.2)****	***0.5 (0.2, 1.9)****	***0.4 (0.04, 1.2)****	0.2 (-0.1, 1.3)
Poor sleep quality (vs. good sleep quality)	***0.1 (0.04, 0.2)****	***0.3 (0.05, 0.6)****	***0.1 (0.02, 0.2)****	0.1 (-0.03, 0.3)
Moderate/High perceived stress (vs. low perceived stress)	***0.1 (0.06, 0.2)****	***0.4 (0.1, 0.6)****	***0.1 (0.03, 0.2)****	***0.2 (0.004, 0.3)****
Interaction between sleep quality and perceived stress				
Poor sleep quality (vs. good sleep quality)	***0.1 (0.01, 0.3)****	***0.2 (0.001, 0.5)****	0.2 (0.06, 0.4)	0.1 (-0.1, 0.4)
Moderate/High perceived stress (vs. low perceived stress)	0.1 (-0.003, 0.2)	***0.3 (0.01, 0.6)****	***0.2 (0.03, 0.4)****	***0.2 (0.001, 0.4)****
Moderate/High stress # poor sleep quality§	***0.7 (0.4, 0.9)****	***0.5 (0.2, 0.7)****	***0.2 (0.04, 0.4)****	0.2 (-0.02, 0.3)

Abbreviations. CAL, Clinical Attachment Level; CI, Confidence Interval; PPD, Probing Pocket Depth; PSQI, Pittsburgh Sleep Quality Index; PSS, Perceived Stress Scale.

* $p<0.05$ is considered statistically significant.

† Adjusted for age, sex, smoking, body mass index, education, brushing frequency, and comorbidities (defined as the presence of at least one comorbidity among diabetes, rheumatoid arthritis, osteoporosis, cardiovascular and inflammatory bowel diseases).

§ The interaction term equaled 1 whenever the participant had moderate/high stress and poor sleep quality, otherwise it equaled 0 (i.e., reference category).

3.3 *Sleep quality and periodontitis*

Poor sleep quality presented a significantly higher periodontitis severity (staging) (p=0.03). Other periodontal parameters (*i.e.* mean PPD, %PPD≥4mm, %PPD≥6mm, furcation involvement, tooth mobility, number of bleeding pockets, teeth lost for periodontal causes) resulted significantly worse in subjects with poor compared to good sleep quality. Conversely, domiciliary plaque control habits were comparable across subgroups of sleep quality (p=0.99) (Table 1). Ordinal logistic regression analyses indicated how poor sleep quality was significantly associated with Stage III/IV periodontitis (EFP/AAP; OR=3.0), as well as with severe periodontitis (CDC/AAP; OR=2.5). When considering PSQI (continuous) as exposure, only a non-statistically significant tendency in the same direction was noted (Tables 2-S1). Moreover, multiple linear regression analyses indicated a significant association between poor sleep quality and mean CAL, but not mean PPD (Table 3).

3.4 *Interaction between perceived stress and sleep quality*

When considering the interaction between perceived stress and sleep quality, ordinal logistic regressions

indicated how the subgroup with combined "moderate/high stress, poor sleep quality" (OR=5.8) resulted associated with Stage III/IV periodontitis (EFP/AAP) with around a double magnitude than either poor sleep quality or moderate/high perceived stress alone (Table 2); these results remained consistent also for severe periodontitis (CDC/AAP) (Table S1). Multiple linear regression analyses indicated a similar trend of association also with other additional periodontal parameters, i.e., mean CAL and mean PPD (Tables 3).

4 Discussion

The present cross-sectional study indicated that moderate/high perceived stress and poor sleep quality are associated with severe forms of periodontitis (defined with both the EFP/AAP and the CDC/AAP criteria). The estimates of association were found to be multiplicative for subjects reporting moderate/high perceived stress and poor sleep quality, irrespective of the disease classification employed. Furthermore, moderate/high perceived stress and poor sleep quality also showed a linear relationship with additional periodontal parameters, such as mean CAL and mean PPD. Plaque control was comparable across stressed/non-stressed subjects and among those reporting poor/good sleep quality, suggesting that these associations are not due to this factor.

Results of the current investigation on the association between perceived stress and periodontitis are consistent with those obtained by a previous report conducted on 621 Brazilian individuals (Coelho et al.,

2020), where a significant association was also found. Nonetheless, the magnitude of association resulted weaker than the one found in the present study, possibly due to the different periodontitis case definitions employed (Eke et al., 2012; Page & Eke, 2007; Papapanou et al., 2018). Indeed, the present study underlined similar, but more attenuated results for the CDC/AAP compared to the EFP/AAP criteria, consistently with those findings reporting discrepancies in the identification of periodontitis cases between the two case definitions (Morales et al., 2021). The association between stress and periodontitis may be boiled down to mainly two pathways: a direct biological negative impact on the immune and endocrine system, and behavioral adaptive changes (including smoking) (Sabbah et al., 2018). As for the direct biological impact, stress triggers an increase in neuroendocrine hormones, such as glucocorticoids and catecholamines which, in turn, exert suppressive effects on the immune system, by reducing lymphocyte proliferation, antibody production and natural killer cell activity (Webster Marketon & Glaser, 2008). Consequently, subjects with chronic stress tend to be more prone to infections.

With regards to sleep quality, results from the present research are consistent with those from a previous study, which reported a significant association between staging and grading of periodontitis and PSQI (Karaaslan & Dikilitaş, 2019). A proper comparison of the magnitude of association with the present study could not be performed, due to the differences in the outcome assessment methods and in the employed thresholds for the categorization of variables. Substantial evidence supports the biological plausibility of the association between poor sleep quality and periodontitis, and this

relationship seems to be bi-directional (Dowd et al., 2011; Lee et al., 2014; Nakada et al., 2015; Romandini et al., 2017). Indeed, poor sleep quality was found to be associated with higher markers of systemic inflammation which may influence the development of periodontitis (Besedovsky et al., 2019; Pink et al., 2015); in turn, the presence of a state of systemic inflammation, which can be triggered by periodontitis (Hajishengallis & Chavakis, 2021), may negatively impact on sleep quality.

The combination of moderate/high perceived stress and poor sleep quality was associated with a higher prevalence of both periodontitis and severe periodontitis, and with worse periodontal parameters, irrespective of socio-demographic characteristics and plaque control. Therefore, the results from the current study suggest the presence of a multiplicative effect of poor sleep quality and perceived stress on periodontitis occurrence and severity, similarly to what was observed for CVD (Li et al., 2021). To the best of the authors' knowledge, no previous study evaluated the combined effect of perceived stress and sleep quality on periodontal health. On the grounds of these considerations, it can be hypothesized that the pro-inflammatory systemic action of stress and poor sleep quality and the suppressive action on the immune functions of moderate/high perceived stress may exert a multiplicative effect on the periodontium (Besedovsky et al., 2019; Liu et al., 2017), which may be conducive to an increased susceptibility to periodontitis onset and severity. This information may be relevant for clinicians since stress and sleep quality may potential targets of interventions in the context of the Step 1 of periodontal therapy (Sanz et al., 2020), and for researchers since they may represent potential confounders to be taken into

account in well-established associations between periodontitis and systemic diseases (Botelho et al., 2022; Baima et al., 2022; Romandini et al., 2021; Czesnikiewicz-Guzik et al., 2019; D'Aiuto et al., 2018; Marruganti et al., 2023b; Antonoglou et al., 2023).

Some limitations are worth mentioning when interpreting these findings, including the cross-sectional study design, the risk of information bias for periodontitis (*e.g.,* due to the lack of radiographic information in some non-periodontitis or incipient periodontitis cases) and stress/sleep quality (i.e., self-reported) assessments. Furthermore, the risk of residual confounding cannot be ruled out, also due to the lack of a detailed consideration of the medical conditions that may have affected the periodontal status of the participants as well as the estimates of association between the exposure and the outcome. In addition, all the included participants came from urban or suburban areas in the South-east part of a region in Centre Italy (Tuscany), where most adults are Caucasian; hence, any possible variability in the results related to ethnicity could not be investigated. Moreover, given that the study population was selected among patients coming to a public University Hospital, the risk of selection bias could not be excluded. Overall, these two factors may also reduce the generalizability of the study findings. Finally, the reduced sample size resulted in wide confidence intervals for the multiplicative association of stress and sleep quality with periodontitis, which lead to insecurity towards the central estimates and - consequently - on the reported concept of a multiplicative effect. Nonetheless, to the best of the authors knowledge, this represents the first study investigating the multiplicative effect of perceived stress and sleep quality on periodontal health. Assessments of

perceived stress and sleep quality were performed using reliable and validated tools for the selected sample, whose validity and reproducibility for the Italian population were previously demonstrated (Curcio et al., 2013; Mondo et al., 2021).

5 Conclusion

The present study indicated a multiplicative association of perceived stress and sleep quality with periodontitis. Specifically, individuals whose lifestyle is characterized by both moderate/high perceived stress and poor sleep quality have from 5- to 6-time increased odds of suffering from severe forms of periodontitis.

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Supplementary information (Chapter 3)

Supplementary Appendix. Assessment methods of socio-demographic characteristics.

As socio-demographic characteristics, the following variables were considered: age, Body Mass Index (BMI), sex, occupation, education, smoking, familiarity for periodontitis, oral hygiene habits.

- Weight was measured with a calibrated digital scale in kilograms, and height was measured with a stadiometer in meters. Afterwards, BMI was calculated using an electronic calculator as weight (kilograms)/height (meters^2).
- Occupation was categorized as unemployed, employed, or retired.
- Educational level was categorized as the highest diploma obtained (primary/middle school, high school, college or more).
- Smoking was self-reported and categorized as smoker, former smoker or never smoked.
- Familiarity for periodontitis was self-reported and categorized as yes/no.
- As for the presence of comorbidities (diabetes, rheumatoid arthritis, osteoporosis, cardiovascular and inflammatory bowel diseases), this variable was categorized as yes/no. In case the presence of a specific disease was self-reported, medical reports were checked for verification.
- Information regarding oral hygiene habits was self-reported. Brushing frequency was categorized as not performed, performed occasionally, every day. Toothbrush type was categorized as powered or manual. The device used for interdental cleaning was categorized as using interproximal brushes, interdental floss or not performed. The frequency of interdental cleaning was

categorized as not performed, occasionally or performed every day.

Table S1. *Effect estimates from ordinal logistic regression models for the association between perceived stress, sleep quality, and their combination with periodontitis (CDC/AAP classification).*

	Mild periodontitis†		**Moderate periodontitis†**		**Severe Periodontitis†**	
	OR (95% CI)		**OR (95% CI)**		**OR (95% CI)**	
Exposure variables	***Crude***	***Adjusted‡***	***Crude***	***Adjusted‡***	***Crude***	***Adjusted‡***
PSQI (continuous)	0.9 (0.3, 2.4)	0.7 (0.2, 3.0)	0.9 (0.5, 4.0)	1.1 (0.3, 4.5)	1.9 (0.2, 9.3)	1.3 (0.5, 8.7)
PSS (continuous)	1.6 (0.9, 3.3)	1.4 (0.6, 3.0)	2.0 (0.8, 4.6)	1.9 (0.7, 4.2)	**3.2 (1.2, 6.0)***	**2.4 (1.1, 4.9)***
Poor sleep quality (vs. good sleep quality)	**2.7 (1.1, 6.4)***	1.9 (0.8, 4.9)	**2.9 (1.4, 5.8)****	1.9 (0.9, 4.4)	**3.1 (1.6, 6.0)***	**2.5 (1.2, 4.4)***
Moderate/High perceived stress (vs. low perceived stress)	1.4 (0.3, 2.9)	1.0 (0.3, 2.5)	1.2 (0.5, 2.5)	1.1 (0.5, 2.8)	**12.9 (5.9, 18.2)***	**12.7 (5.4, 21.6)***
Interaction between sleep quality and perceived stress						
Poor sleep quality (vs. good sleep quality)	1.7 (0.7, 3.9)	1.7 (0.4, 4.2)	**3.1 (1.0, 10.5)***	**5.2 (1.2, 21.9)***	**2.4 (1.0, 5.6)***	**1.9 (1.0, 4.4)***
Moderate/High perceived stress (vs.	0.5 (0.4, 1.7)	0.9 (0.4, 2.9)	**6.9 (2.9, 21.2)***	**3.3 (1.2, 7.2)***	**5.2 (2.0, 9.1)***	**2.8 (1.2, 5.0)***

low perceived stress)

Moderate/High stress # poor sleep quality§	1.1 (0.6, 3.9)	1.3 (0.3, 3.0)	0.6 (0.1, 3.1)	1.3 (0.2, 4.0)	***3.4 (1.1, 6.2)****	***4.9 (1.1, 8.9)****

Abbreviations. CI, Confidence Interval; OR, Odds Ratio; PSQI, Pittsburgh Sleep Quality Index; PSS, Perceived Stress Scale; REF, reference category.

* $p<0.05$ is considered statistically significant.

† vs. healthy or gingivitis.

‡ Adjusted for age, sex, smoking, body mass index, education, brushing frequency, and comorbidities (defined as the presence of at least one comorbidity among diabetes, rheumatoid arthritis, osteoporosis, cardiovascular and inflammatory bowel diseases).

§ The interaction term equaled 1 whenever the participant had moderate/high stress and poor sleep quality, otherwise it equaled 0 (i.e., reference category).

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HEALTHY LIFESTYLES AS PROTECTIVE INDICATORS FOR PERIODONTAL DISEASES.

RESULTS FROM TWO LARGE-SCALE SURVEYS.

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Manuscript in preparation.

Chapter 4

HEALTHY LIFESTYLES AS PROTECTIVE INDICATORS FOR PERIODONTAL DISEASES. RESULTS FROM TWO LARGE-SCALE SURVEYS.

1 Introduction

Unhealthy lifestyle behaviours (e.g., smoking, poor nutrition, physical inactivity) are at the root of the global burden of non-communicable diseases (NCDs) and multimorbidities (Marrero et al., et al., 2012). Over the past two decades, several studies have highlighted the detrimental impact of some of these behaviours on the onset and progression of periodontitis (Iwasaki et al., 2021; Karaaslan & Dikilitaş, 2019; Marruganti et al., 2022). Recent pieces of evidence have however focused on the combination of healthy/unhealthy lifestyles as key determinant in the development of future morbidity and mortality (Kushner & Sorensen, 2013). The importance of investigating the whole lifestyle (instead of considering each factor separately) relies on the close interconnection among behaviours and on the ability of one behaviour to trigger other adaptive changes (Sabbah et al., 2018), hence hampering an accurate evaluation of the impact of each separate component on overall health. Evidence on the association between combinations of lifestyles and periodontal diseases is limited. The aim of the present study was therefore to ascertain whether healthy lifestyles are associated with periodontal diseases in two large-scale surveys (the National Health and Nutrition Examination Survey – NHANES in the US and the UK Biobank - UKB).

2 Materials and methods

The cross-sectional studies investigated are reported

according to the STROBE guidelines (Vandenbroucke et al., 2007).

Study samples

Data for the present study were drawn from two large-scale epidemiological surveys:

-NHANES (2009-2014 cycles): a nationwide, stratified, multistage probability survey representative of the civilian non-institutionalized US population. Detailed information about the survey design is provided elsewhere (Dye et al., 2019);

-UKB (2007-2010): a nationwide epidemiological study carried out between 2006 and 2010 in 22 assessment centres throughout the UK. Detailed information about the study design is provided elsewhere (Sudlow et al., 2015).

Lifestyles assessment

In both surveys, participants were included in the analyses if a complete assessment for all the following healthy lifestyle behaviours was available:

-Non-smoking (vs. smoking) (NHANES/UKB);

-Alcohol below the suggested intake (vs. above), corresponding to 2 alcoholic drinks/day for men and 1 alcoholic drink/day for women (NHANES/UKB);

-High leisure-time physical activity (LTPA; vs. low), if the participant was in the top third of the total leisure-time physical activity level (Zhang et al., 2021) (NHANES/UKB);

-High diet quality (vs. low), if the participant was in the top two quintiles of the Healthy Eating Index (HEI) for

NHANES, or if the participant had an ideal intake of at least 5 out of the 10 dietary components considered in UKB;

-Ideal sleep duration (vs. short/long), if the participant reported sleeping 7-9 hours and was <65 years, or 7-9 hours and was ≥65 years (Hirshkowitz et al., 2015) (NHANES/UKB).

A healthy lifestyle score was then constructed considering the reported number of healthy lifestyle behaviours. The score ranged between 0 and 5, with the highest value indicating the healthiest overall lifestyle. Detailed information about lifestyles assessment methods are provided in the Supplementary Information.

Periodontal diseases assessment

In the NHANES, all survey participants aged at least 30 years, presenting at least one tooth (excluding third molars) were eligible for a full-mouth periodontal examination performed by trained and calibrated examiners (Dye et al., 2014, 2019). Periodontitis was identified applying the AAP/CDC criteria (Eke et al., 2012).

In the UKB, participants reported the presence of any periodontal diseases through a questionnaire. Bleeding gums and painful gums were used as surrogates for mild to moderate periodontal diseases, while self-reported loose teeth were indicative of severe periodontal diseases (Larvin et al., 2021). In cases of multiple responses, the most severe indicator was used as the primary surrogate for periodontal diseases.

Statistical analyses

All analyses will be performed setting the level of significance at 5% (STATA BE, version 17.1, StataCorp LP, Texas, USA). For NHANES data, analyses for complex samples were used. Simple and multiple linear/logistic regression analyses were performed to assess the crude and adjusted estimates of association between the healthy lifestyle score and periodontal diseases. Mediation analysis was performed to test the potential mediating effect of biomarkers of systemic inflammation.

3 Results

The selection process led to the inclusion of 9,854 participants for the NHANES and 111,679 participants for the UKB with complete lifestyles and periodontal assessments (Tables 1-2). The amount of missing observations for each variable are shown in Table S1. The prevalence of periodontal diseases was evaluated according to categories of healthy lifestyle scores (Figure 1).

Table 1. *Characteristics of the study population in the NHANES cohort.*

Variables	Overall	Healthy lifestyle score		
		0-1	2-3	4-5
Age (years), mean (SE)	50.9 (0.3)	47.2 (0.4)	51.1 (0.3)	53.2 (0.5)
Gender, % (SE)				
Male	48.9 (0.005)	55.4 (0.01)	49.5 (0.008)	47.3 (0.01)

Female	51.2 (0.005)	44.7 (0.01)	50.5 (0.008)	52.7 (0.01)
Ethnicity, % (SE)				
Non-Hispanic Black	10.7 (0.009)	14.7 (0.02)	11.4 (0.01)	6.2 (0.006)
Non-Hispanic white	68.4 (0.02)	66.4 (0.02)	67.1 (0.02)	75.7 (0.01)
Mexican	8.1 (0.01)	8.5 (0.01)	9.2 (0.01)	5.3 (0.008)
Other	12.8 (0.009)	10.3 (0.01)	12.4 (0.009)	12.8 (0.009)
Family Poverty Level, % (SE)				
<100	11.9 (0.007)	20.9 (0.02)	11.5 (0.007)	6.3 (0.007)
100-199	19.0 (0.008)	25.6 (0.01)	19.5 (0.008)	13.8 (0.01)
200-399	28.8 (0.01)	31.2 (0.01)	30.3 (0.01)	25.6 (0.02)
≥400	40.2 (0.02)	22.2 (0.02)	38.7 (0.02)	54.3 (0.02)
Acculturation score, mean (SE)	2.7 (0.02)	2.8 (0.02)	2.7 (0.02)	2.7 (0.03)
Education, % (SE)				
< High school	15.3 (0.009)	23.7 (0.01)	15.9 (0.009)	7.7 (0.008)
High school graduate	20.8 (0.008)	29.6 (0.02)	22.9 (0.01)	12.8 (0.008)
College degree or more	63.8 (0.01)	46.7 (0.02)	61.1 (0.01)	79.6 (0.01)
Frequency of interproximal hygiene, % (SE)				
0 days/week	27.7 (0.007)	41.7 (0.01)	28.4 (0.008)	18.4 (0.009)

1-6 days/week	39.4 (0.007)	34.6 (0.01)	39.9 (0.009)	41.5 (0.01)
7 days/week	32.9 (0.006)	23.7 (0.01)	31.7 (0.009)	40.1 (0.01)
Comorbidity score, mean (SE)	0.9 (0.01)	0.9 (0.03)	0.9 (0.02)	0.7 (0.02)
BMI, mean (SE)	29.1 (0.1)	29.8 (0.3)	29.8 (0.1)	27.6 (0.2)
Smoking, % (SE)				
Yes	17.4 (0.005)	66.7 (0.01)	11.8 (0.006)	1.0 (0.003)
No	82.6 (0.005)	33.3 (0.01)	88.2 (0.006)	98.9 (0.003)
Alcohol intake, % (SE)				
Below suggested intake	56.1 (0.008)	10.9 (0.009)	52.2 (0.009)	86.9 (0.009)
Above suggested intake	43.9 (0.008)	89.1 (0.009)	47.8 (0.009)	13.2 (0.009)
LTPA, % (SE)				
Low LTPA	62.5 (0.009)	92.5 (0.009)	71.9 (0.008)	28.1 (0.01)
High LTPA	37.5 (0.009)	7.5 (0.009)	28.0 (0.008)	71.9 (0.01)
HEI score, % (SE)				
Unhealthy diet	57.1 (0.009)	93.7 (0.008)	69.5 (0.009)	19.8 (0.01)
Healthy diet	42.9 (0.009)	6.4 (0.008)	30.5 (0.009)	80.2 (0.01)
Sleep duration, % (SE)				

Short/long	39.4 (0.007)	79.4 (0.01)	43.2 (0.009)	11.3 (0.007)
Proper	60.6 (0.007)	20.6 (0.01)	56.8 (0.009)	88.8 (0.007)
Periodontitis, % (SE)				
Mild	4.3 (0.004)	4.3 (0.006)	4.9 (0.005)	3.3 (0.004)
Moderate	30.1 (0.01)	39.5 (0.02)	29.8 (0.01)	25.7 (0.02)
Severe	7.8 (0.005)	15.9 (0.01)	7.5 (0.005)	4.1 (0.005)
WBC (1000 cells/uL), mean (SE)	7.1 (0.04)	7.9 (0.09)	7.1 (0.05)	6.6 (0.05)
C-reactive protein (mg/dL), mean (SE)	0.4 (0.02)	0.5 (0.08)	0.4 (0.02)	0.3 (0.02)
Segmented neutrophils number (1000 cells/uL), mean (SE)	4.2 (0.03)	4.8 (0.07)	4.2 (0.04)	3.9 (0.04)

Abbreviations. BMI, body mass index; HEI, healthy eating index; LTPA, leisure-time physical activity; SE, standard error; uL, microliters; WBC, white blood cell count; %, percentage.

Table 2. *Characteristics of the study population in the UK Biobank cohort.*

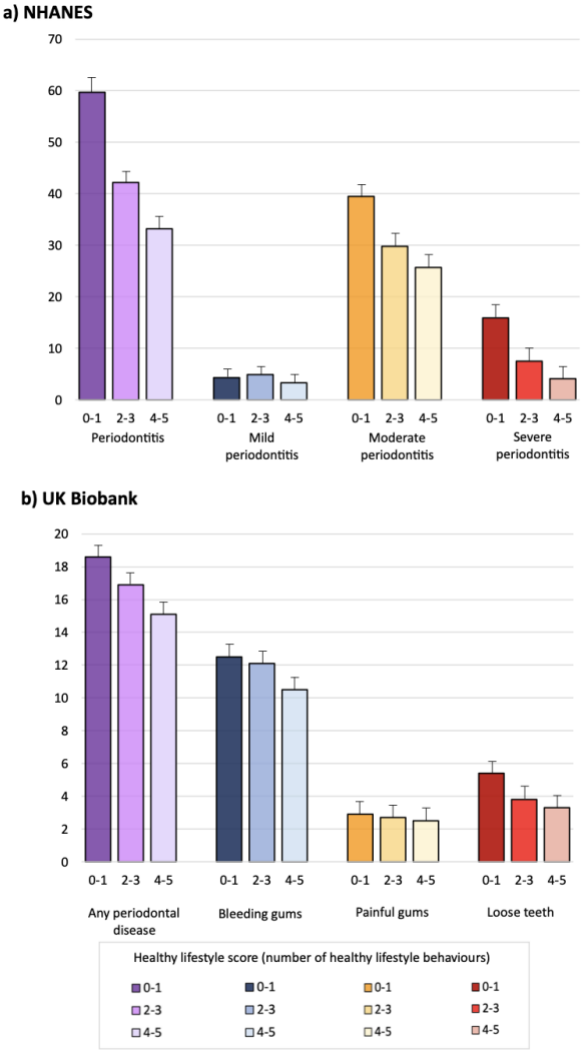
Variables	Overall	Healthy lifestyle score		
		0-1	2-3	4-5
Age (years), mean (SD)	55.7 (7.9)	54.5 (7.6)	55.3 (7.9)	56.7 (7.9)
Gender, n (%)				
Male	45,922 (41.1)	3,734 (53.6)	28,568 (44.2)	13,620 (34.0)
Female	65,757 (58.9)	3,228 (46.4)	36,112 (55.8)	26,417 (66.0)
Ethnicity, n (%)				
White	104,521 (93.8)	6,784 (97.7)	61,649 (95.4)	36,188 (90.5)
Mixed	592 (0.5)	51 (0.7)	341 (0.5)	200 (0.5)
Asian/Asian British	3,585 (3.3)	47 (0.7)	1,307 (2.1)	2,231 (5.7)
Black/Black British	1,628 (1.5)	39 (0.6)	818 (1.3)	771 (1.9)
Other	1,019 (0.9)	19 (0.3)	479 (0.8)	521 (1.3)
Total household income, n (%)				
Less than £18,000	16,774 (17.0)	840 (12.9)	9,065 (15.7)	6,869 (20.1)
£18,000 to £30,999	22,846 (23.2)	1,364 (21.1)	12,805 (22.1)	8,677 (25.4)
£31,000 to £51,999	27,158 (27.5)	1,913 (29.6)	16,164 (27.9)	9,081 (26.5)
£52,000 to £100,000	24,432 (24.8)	1,810 (27.9)	15,161 (26.2)	7,461 (21.8)

Greater than £100,000	7,413 (7.5)	540 (8.3)	4,728 (8.2)	2,145 (6.3)
Acculturation score, mean (SD)	2.9 (0.4)	2.9 (0.3)	2.9 (0.4)	2.8 (0.5)
Education, n (%)				
< High school	10,884 (9.8)	526 (7.6)	5,725 (8.9)	4,633 (11.7)
High school graduate	51,122 (46.1)	3,491 (50.3)	29,857 (46.4)	17,774 (44.8)
College degree or more	48,942 (44.1)	2,927 (42.1)	28,745 (44.7)	17,270 (43.5)
Comorbidity score, mean (SD)	0.8 (0.9)	0.7 (0.6)	0.6 (0.7)	0.7 (0.6)
BMI, mean (SD)	26.8 (4.7)	27.2 (4.5)	26.9 (4.7)	26.5 (4.7)
Smoking, n (%)				
Yes	22,822 (20.4)	5,114 (73.5)	16,463 (25.5)	1,245 (3.1)
No	89,857 (79.6)	1,848 (26.5)	48,217 (74.5)	38,792 (96.9)
Alcohol intake, n (%)				
Below suggested intake	73,030 (65.5)	717 (10.3)	34,375 (53.2)	37,938 (94.8)
Above suggested intake	38,649 (34.6)	6,245 (89.7)	30,305 (46.8)	2,099 (5.2)
LTPA, n (%)				
Low LTPA	28,916 (25.9)	5,258 (75.5)	21,917 (33.8)	1,741 (4.3)
High LTPA	82,763 (74.2)	1,704 (24.5)	42,763 (66.2)	38,296 (95.7)

Diet quality, n (%)				
Unhealthy diet	91,741 (82.1)	6,843 (98.3)	59,558 (92.1)	25,340 (63.3)
Healthy diet	19,938 (17.9)	119 (1.7)	5,122 (7.9)	14,697 (36.7)
Sleep duration, n (%)				
Short/long	32,381 (29.0)	5,108 (73.4)	24,063 (37.3)	3,210 (8.1)
Proper	79,298 (70.9)	1,854 (26.6)	40,617 (62.7)	36,827 (91.9)
WBC (1000 cells/uL), mean (SD)	6.7 (2.2)	7.0 (1.9)	6.7 (1.9)	6.7 (2.7)
C-reactive protein (mg/dL), mean (SD)	2.3 (4.1)	2.5 (4.1)	2.4 (4.1)	2.3 (4.0)
Segmented neutrophils number (1000 cells/uL), mean (SD)	4.1 (1.4)	4.3 (1.4)	4.1 (1.3)	4.1 (1.4)

Abbreviations. BMI, body mass index; LTPA, leisure-time physical activity; SE, standard error; uL, microliters; WBC, white blood cell count; %, percentage.

Figure 1. *Prevalence of periodontitis according to categories of healthy lifestyle score (error bars indicate 95% Confidence Interval).*



In the NHANES, simple and multiple regression analyses confirmed that the presence of 2-3 healthy behaviours (vs. 0-1) was associated with lower odds of periodontitis (OR=0.5) and severe periodontitis (OR=0.5). The same direction of association was confirmed when considering linear surrogate measures of periodontal severity. In the UKB, simple and multiple regression analyses identified the presence of 2-3 healthy behaviours (vs. 0-1) was associated with lower odds of bleeding gums (OR=0.8), painful gums (OR=0.8), and loose teeth (OR=0.6). This protective association increased when considering 4-5 healthy behaviours (vs. 0-1) as exposure in both the NHANES (periodontitis: OR=0.3; severe periodontitis: OR=0.1) and the UKB (bleeding gums: OR=0.9; loose teeth: OR=0.6), hence indicating the presence of a dose-response relationship between the number of healthy behaviours and periodontal diseases (Table 3). Results were consistent also in the sensitivity analyses by gender and age, in systemically healthy subjects, and by subgroups of BMI (Tables S2-S3, S9-S16). Additional supplementary analyses showed the same protective effect when considering each healthy lifestyle independently as exposure (i.e., no smoking, alcohol below the suggested intake, high LTPA, high diet quality, and ideal sleep duration) (Tables S4-S8).

Table 3. *Association between categories of healthy lifestyle score and periodontal diseases.*

	Healthy lifestyle score as exposure - OR/MD (95% CI)			
Outcomes	**2-3 healthy lifestyles (vs. 0-1 healthy lifestyles)**		**4-5 healthy lifestyles (vs. 0-1 healthy lifestyles)**	
	Crude	***Adjusted***	***Crude***	***Adjusted***
NHANES				
Periodontitis	***0.5 (0.4, 0.6)****	***0.5 (0.4, 0.6)****	***0.3 (0.2, 0.4)****	***0.3 (0.2, 0.4)****
Periodontitis severity				
Mild periodontitis	***0.5 (0.3, 0.6)****	0.8 (0.5, 1.1)	0.5 (0.3, 0.6)	***0.6 (0.4, 0.9)****
Moderate periodontitis	***0.4 (0.3, 0.5)****	***0.6 (0.4, 0.9)****	0.4 (0.3, 0.5)	***0.3 (0.3, 0.4)****
Severe periodontitis	***0.2 (0.1, 0.2)****	***0.5 (0.3, 0.8)***	***0.1 (0.1, 0.2)****	***0.1 (0.01, 0.2)****
PPD				
% sites PPD ≥ 4 mm	***-2.0 (-2.6, -1.5)****	***-1.3 (-1.9, -0.8)****	***-3.1 (-3.7, -2.6)****	***-1.9 (-2.5, -1.4)****
% sites PPD ≥ 5 mm	***-0.7 (-0.9, -0.4)****	***-0.4 (-0.7, -0.2)***	***-1.0 (-1.3, -0.7)****	***-0.6 (-0.9, -0.4)****
% sites PPD ≥ 6 mm	***-0.2 (-0.4, -0.09)***	***-0.1 (-0.3, -0.02)****	***-0.3 (-0.5, -0.2)****	***-0.2 (-0.3, -0.09)***

CAL				
% sites CAL ≥ 3 mm	***-6.6 (-7.6, -5.6)*** ***	***-5.9 (-6.9, -4.9)*** ***	***-9.6 (-10.7, -8.5)*** ***	***-8.0 (-9.3, -6.8)*** ***
% sites CAL ≥ 4 mm	***-4.5 (-5.3, -3.7)*** ***	***-4.1 (-4.9, -3.3)*** ***	***-6.4 (-7.2, -5.5)*** ***	***-5.5 (-6.4, -4.5)*** ***
% sites CAL ≥ 5 mm	***-2.6 (-3.1, -2.0)*** ***	***-2.3 (-2.9, -1.7)*** ***	***-3.7 (-4.4, -3.1)*** ***	***-3.1 (-3.8, -2.5)*** ***
% sites CAL ≥ 6 mm	***-1.5 (-1.9, -1.1)*** ***	***-1.3 (-1.8, -0.9)*** ***	***-2.3 (-2.7, -1.8)*** ***	***-1.8 (-2.3, -1.4)*** ***
UK Biobank				
Bleeding gums (vs. healthy)	0.9 (0.8, 1.0)	0.9 (0.8, 1.0)	***0.8 (0.7, 0.9)*** ***	***0.8 (0.7, 0.9)*** ***
Painful gums (vs. healthy)	0.9 (0.7, 1.1)	0.9 (0.7, 1.0)	0.9 (0.8, 1.1)	***0.7 (0.6, 0.9)*** ***
Loose teeth (vs. healthy)	***0.7 (0.6, 0.8)*** ***	***0.6 (0.5, 0.7)*** ***	***0.6 (0.5, 0.7)*** ***	***0.5 (0.4, 0.6)*** ***
Any periodontal disease (vs. healthy)	***0.8 (0.8, 0.9)*** **	***0.8 (0.7, 0.9)*** ***	***0.8 (0.7, 0.9)*** ***	***0.8 (0.7, 0.9)*** ***

Abbreviations. CAL, clinical attachment level; CI, confidence interval; MD, difference in means; mm, millimeter; OR, odds ratio; PPD, probing pocket depth; %, percentage. The following confounders were tested using univariate analysis in order to obtain the adjusted estimates: age, gender, ethnicity, income, acculturation score, education, body mass index, frequency of self-performed oral hygiene (NHANES), last dental visit (NHANES), number of comorbidities. * p<0.05; ** p<0.01; *** p<0.001.

Further, mediation analyses highlighted how the association between the presence of ≥ 2 healthy behaviours and periodontal diseases was partially and mildly explained by markers of systemic inflammation, with a proportion of mediated effect ranging between 2% and 6% for NHANES, and between 1% and 14% for UKB (Table 4).

Table 4. *Mediation analyses for the association between HL score > 1 and periodontitis/severe periodontitis.*

	WBC count			CRP			Neutrophils count		
	Effect estimate	**SE**	**p-value**	**Effect estimate**	**SE**	**p-value**	**Effect estimate**	**SE**	**p-value**
NHANES									
Periodontitis									
a (exposure -> mediator)	-0.232	0.078	***<0.001*** ***	-0.172	0.04	***<0.001*** ***	-0.411	0.030	***<0.001*** ***
b (mediator -> outcome)	0.010	0.003	***0.001*** **	0.027	0.02	0.118	0.010	0.002	***<0.001*** ***
c (total effect)	-0.224	0.012	***0.001*** **	-0.183	0.03	***0.001*** **	-0.110	0.003	***0.001*** **
c' (direct effect)	-0.211	0.02	***<0.001*** ***	-0.178	0.03	***<0.001*** ***	-0.105	0.008	***<0.001*** ***
ab (mediated effect)	-0.012	0.004	***0.001*** **	-0.005	0.003	0.142	-0.004	0.001	***<0.001*** ***

ab/c (percentage mediated) = %	5.5	-	-	2.5	-	-	4.0	-	-
Severe periodontitis									
a (exposure -> mediator)	-0.554	0.504	***<0.001******	-0.063	0.024	***0.001*****	-0.375	0.042	***<0.001******
b (mediator -> outcome)	0.012	0.002	***<0.001******	0.043	0.014	***0.001*****	0.013	0.002	***<0.001******
c (total effect)	-0.107	0.002	***<0.001******	-0.110	0.002	***<0.001******	-0.106	0.001	***<0.001******
c' (direct effect)	-0.100	0.007	***<0.001******	-0.107	0.014	***<0.001******	-0.102	0.008	***<0.001******
ab (mediated effect)	-0.006	0.001	***<0.001******	-0.003	0.001	***0.043****	-0.005	0.001	***<0.001******
ab/c (percentage mediated) = %	6.0	-	-	2.0	-	-	5.0	-	-
UK Biobank									
Bleeding gums									

a (exposure -> mediator)	-0.127	0.010	***<0.001*****	-0.086	0.022	***0.001****	-0.077	0.007	***<0.001*****
b (mediator -> outcome)	0.003	0.002	***<0.001*****	0.001	0.0002	***<0.001*****	0.003	0.001	***<0.001*****
c (total effect)	-0.014	0.001	***0.001****	-0.013	0.0001	***0.001****	-0.014	0.002	***<0.001*****
c' (direct effect)	-0.013	0.002	***<0.001*****	-0.014	0.002	***<0.001*****	-0.013	0.004	***<0.001*****
ab (mediated effect)	-0.001	0.001	***<0.001*****	-0.001	0.0001	***<0.001*****	-0.001	0.0001	***<0.001*****
ab/c (percentage mediated) = %	3.0	-	-	1.0	-	-	2.0	-	-
Loose teeth									
a (exposure -> mediator)	-0.151	0.010	***<0.001*****	-0.092	0.023	***<0.001*****	-0.093	0.008	***<0.001*****
b (mediator -> outcome)	0.006	0.0003	***<0.001*****	0.002	0.0002	***<0.001*****	0.007	0.0004	***<0.001*****
c (total effect)	0.007	0.001	***<0.001*****	0.003	0.001	***<0.001*****	0.002	0.0002	***<0.001*****

c' (direct effect)	-0.006	0.003	0.096	-0.007	0.002	***0.010****	-0.006	0.002	***<0.001******
ab (mediated effect)	-0.001	0.0001	***<0.001******	-0.001	0.001	***<0.001******	-0.001	0.0001	***<0.001******
ab/c (percentage mediated) = %	14.0	-	-	2.0	-	-	10.0	-	-

Abbreviations. CI, confidence interval; CRP, C-reactive protein; HL, healthy lifestyle; SE, standard error; WBC, white blood cell count. * p<0.05; **p<0.01; ***p<0.001.

4 Discussion

The findings from these analyses indicate that the presence of at least 2 healthy lifestyle behaviours could be a protective indicator for any periodontal diseases. Indeed the association between healthy lifestyles and periodontal diseases exhibited a dose-response relationship, with a higher number of healthy behaviours being associated with lower odds of periodontal diseases. Some evidence of a potential mediation of this association based on the host inflammatory status (systemic inflammation as assessed by leucocyte counts) was also noted.

Despite the limited evidence available on the association between healthy lifestyles in their entirety and oral health, the results obtained in the current study are consistent with those achieved in previous prospective investigations (Iwasaki et al., 2018; Marruganti et al., 2023). Iwasaki and coworkers (2018) demonstrated a protective effect of the combined adherence to multiple healthy lifestyles (non-smoking, physical activity, healthy body weight, and high diet quality) when assessing the onset of periodontitis and tooth loss over a period of 6 years in a population of older Japanese adults. In addition, another prospective investigation highlighted the possible impact of lifestyle overall indicators on the response to the treatment of periodontitis; participants presenting with either unhealthy diet, physical inactivity, high stress, and poor sleep quality struggled to achieve the expected clinical endpoints after 3 months of delivery treatment of periodontitis (Marruganti et al., 2023).

The biological mechanisms underpinning the protective effect of the combination of healthy lifestyles on the periodontium are still not clearly understood. The available evidence regarding the local and systemic

repercussions of each individual lifestyle factor points towards shared mechanisms of modulation of inflammation including oxidative stress production (Gleeson et al., 2011; Pitchika et al., 2017) and their impact on the risk of periodontitis (D'Aiuto et al., 2010; Pink et al., 2015). A bi-directional link between periodontitis and systemic inflammation is corroborated by the present study showing a statistically significant mediation of inflammatory biomarkers on the prevalence of periodontal diseases.

These results are in line with the current evidence focusing on lifestyle as a combination of behaviours instead of considering each of them separately. Indeed, several observational studies demonstrated that a combination of healthy lifestyles is associated with lower mortality, incident cardiovascular disease and diabetes (Abdullah Said et al., 2018; Zhang et al., 2021).

As lifestyles behaviours/factors are closely intertwined, it could be easily hypothesized that individuals with patterns of unhealthy behaviours are less likely to follow domiciliary oral hygiene instructions and vice versa (Sakki & Sakki, 1995; Sabbah et al., 2018). Given the recent confirmation of the importance of oral health for achieving good general health, promotion of healthy lifestyle could be revisited to also include better oral health behaviours. Indeed, a more comprehensive approach in promotion of better lifestyles could help reducing the burden of NCDs and multimorbidities.

It is noteworthy to mention that these analyses present some limitations including the risk of residual confounding and the cross-sectional design of the data obtained which limit our ability to assess causality of the associations found as well as the validity of the mediation analysis. Moreover, the risk of information bias cannot be

excluded because of the self-reported assessment of lifestyles in both populations, and of the periodontal status in the UKB. This is however the first comprehensive and robust analysis of two representative and large surveys in assessing a potential beneficial impact of healthy lifestyle behaviours on the periodontium.

5 Conclusion

Healthy lifestyle behaviours are associated with lower prevalence of periodontal diseases in both populations in a dose-response manner, and they are partially mediated by systemic inflammation. Further studies should investigate the effects of promoting healthy lifestyles to improve both oral and general health.

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Supplementary Information (Chapter 4)

Supplementary Appendix. Lifestyle and confounders assessment methods for the NHANES and the UKB databases.

LIFESTYLES ASSESSMENT METHODS

NHANES

- Cigarette smoking (self-reported)*: non-smokers/smokers* - subjects who reported currently smoking every day or some days - SMQ040 - and those who had smoked at least 100 cigarettes during life - SMQ020 - were considered as current smokers;
- Alcohol intake (ALQ130, ALQ101; self-reported): identified considering the suggested alcohol intake as threshold (ALQ130; 14 g/d or 1 standard alcoholic drink /d for women, and 28 g/d or 2 standard alcoholic drinks /d for men). Participants will be categorized as being *below* (if they had not drunk at least 12 alcohol drinks/1 year - ALQ101, or if they had drunk at least 12 alcohol drinks/1 year but they were below the suggested intake) or *above* (if they had drunk at least 12 alcohol drinks/1 year and they were above the suggested intake) the suggested alcohol intake (Ricci et al., 2020);
- Physical activity: moderate-to-vigorous intensity leisure-time physical activity (MVPA) will be assessed using the Global Physical Activity Questionnaire (GPAQ) (PAQ650; PAQ655; PAD660; PAQ665; PAQ670; PAD675).

Metabolic Equivalent Times (MET min/week) will be calculated for each participant by adding the time spent on each activity weighted by its metabolic equivalent

score. The assumed MET scores for MVPA are 4.0 for moderate intensity PA, and 8.0 for vigorous intensity PA (Janssen et al., 2013). According to previous studies, participants will be categorized as being *active (vs. inactive)* if they are in the top third of the total leisure-time physical activity level (in terms of MET min/week) (Abdullah Said et al., 2018; Janssen et al., 2013; Y. Zhang et al., 2021);

- Diet quality: the dietary intake data will be obtained from NHANES two 24-h recall interviews, which were conducted by the trained interviewers based on the automated multiple-pass method. The first interview was arranged face-to-face in the Mobile Examination Center (MEC) and the second was carried out via phone 3–10 days later. The energy and nutrients for each food or beverage intake were calculated using Food and Nutrient Database for Dietary Studies (FNDDS) and the food groups were determined by Food Patterns Equivalence Database (FPED) from the US Department of Agriculture (USDA). The diet quality will be estimated using the Healthy Eating Index (HEI-2015) which was recommended by USDA to assess the adherence to the dietary guidelines of 2015–2020 DGA. The HEI-2015 contains 13 components (food groups or nutrients), including 9 adequacy components (total vegetables, greens and beans, total fruits, whole fruits, whole grains, dairy, total protein foods, seafood and plant proteins and fatty acids) and 4 moderation components (sodium, refined grains, saturated fats and added sugars). These 13 components will be expressed as amounts per 1000 kcal except for fatty acids (expressed as a ratio of unsaturated to saturated fats), saturated

fats (expressed as % energy) and added sugars (expressed as % energy). These components will be scored separately and incorporated to a total score with the maximum possible score being 100. For each participant, the final dietary intake will be estimated using the mean value of the two 24 recall data. The variables collected during the first (https://wwwn.cdc.gov/Nchs/Nhanes/2009-2010/DR1TOT_F.htm) and second (https://wwwn.cdc.gov/Nchs/Nhanes/2009-2010/DR2TOT_F.htm) day of dietary interview will be used to compute the HEI2015. For each cycle, the FPED will be downloaded from the USDA website (https://www.ars.usda.gov/northeast-area/beltsville-md-bhnrc/beltsville-human-nutrition-research-center/food-surveys-research-group/docs/fped-databases/). Participants will be defined as having a *healthy diet* if they are in the top 2 quintiles of the HEI-2015; participants will be defined as having an *unhealthy diet* if they are in the bottom three quintiles of the HEI-2015.

- Sleep duration: According to the National Sleep Foundation guidelines, participants will be categorized as having a *proper sleep duration* (SLD010H) if they are adults (30-64 years) with 7-9 hours of sleep duration, or if they are elderlies (≥65 years) with 7-8 hours of sleep duration, otherwise they will be categorized as having an excessively *short/long sleep duration* (Hirshkowitz et al., 2015).

UKB

- Cigarette smoking (22506, 2644; self-reported): *non-smokers/smokers-* subjects who reported currently smoking on most or all days, or occasionally - 22506 - and those who had smoked at least 100 cigarettes during life - 2644 - were considered as current smokers (Zhang et al., 2021);
- Alcohol intake (1558; self-reported): Participants were asked about the frequency of drinking alcohol (1558), i.e., (almost) daily, three or four times a week, once or twice a week, one to three times a month, special occasions only, never, and prefer not to answer. Participants will be then categorized as drinking more or less than the suggested intake (Ricci et al., 2020);
- Physical activity (104900, 104910, 104920, 11005, 11006, 884, 894, 904, 914, 3637, 3647; self-reported): Consistently with the US NHANES, the level of leisure-time physical activity in the UKB will be assessed. Three kinds of activities will be considered: walking for pleasure (assigned 3.3 MET equivalents), strenuous sports (assigned 8 MET equivalents), and other exercises (i.e., swimming, cycling, keep fit, bowling) (assigned 4 MET equivalents). The frequency and duration for each time will be asked for each participant. Participants could choose one of the following frequency options, i.e., once in the last 4 weeks, 2-3 times in the last 4 weeks, once a week, 2-3 times a week, 4-5 times a week, every day, do not know, and prefer not to answer. Options including a range would be substituted by the midpoint of the range, e.g., we assigned 2.5 times a week for the option "2-3 times a week". The duration for each time will be also obtained by some options, i.e., less than 15 minutes,

between 15 and 30 minutes, between 30 minutes and 1 hour, between 1 and 1.5 hours, between 1.5 and 2 hours, between 2 and 3 hours, over 3 hours, do not know, and prefer not to answer. Each option would be substituted by the midpoint of the range, and those who chose over 3 hours will be substituted by 3 hours as recommended by other studies. According to previous studies, participants will be categorized as being active (vs. inactive) if they are in the top third of the total leisure-time physical activity level (in terms of MET min/week), otherwise they will be defined as inactive (Abdullah Said et al., 2018; Janssen et al., 2013; Zhang et al., 2021).

- Diet quality: According to a previous UK Biobank study, dietary quality will be evaluated as ideal or poor. Ideal diet will be defined as having an adequate intake of at least half of the following dietary components: increased consumption of fruits, vegetables, whole grains, (shell)fish, dairy products, and vegetable oils; and reduced or no consumption of refined grains, (un)processed meats and sugar-sweetened beverages. Fruit intakes were evaluated according to daily consumptions of fresh fruit (pieces) and dried fruit (pieces). Vegetable intakes were evaluated according to daily consumptions of cooked vegetables (tablespoons) and salad/raw vegetables (tablespoons). Wholegrain intakes were evaluated according to weekly consumptions of bread (slices, if the participant consumed whole meal/whole grain bread) and cereal (bowls, if the participant consumed bran, oat, or Muesli). (Shell)fish were evaluated according to the frequency of eating oily fish and non-oily fish. Dairy intakes were evaluated

according to the frequency of cheese consumption and whether the participant consumed milk. Vegetable oils were evaluated according to the weekly consumption of bread (slices) if the participants consumed flora pro-active/benecol, soft margarine, olive oil based, polyunsaturated/sunflower oil based, or other low/reduced fat spread. Refined grain intakes were evaluated according to weekly consumptions of bread (slices, if the participant consumed white, brown, or other bread) and cereal (bowls, if the participant consumed biscuit or others). Processed meat consumptions were evaluated according to the frequency of consumption and whether the participants did not eat it anymore (according to a question about age when last ate meat). Unprocessed meat consumptions were evaluated according to the frequency of consumption of poultry, beef, lamb/mutton, and pork and whether the participants did not eat meat anymore (according to a question about age when last ate meat). Sugar-sweetened beverage intakes were evaluated by a question "Which of the following do you NEVER eat?" Those who chose sugar or foods/drinks containing sugar were regarded as never drinking sugar-sweetened beverages. The diet components, the intake goal, the field IDs, and the amount per serving were reported in a previous publication (Zhang et al., 2021). Participants will be categorized as having a *healthy diet* if they have the ideal intake of 5 or more dietary components, otherwise they will be considered as having an *unhealthy diet*.

- Sleep duration: According to the National Sleep Foundation guidelines, participants will be

categorized as having a *proper sleep duration* (1160) if they are adults (30-64 years) with 7-9 hours sleep duration, or if they are elderlies (≥65 years) with 7-8 hours sleep duration, otherwise they will be categorized as having an excessively *short/long sleep duration* (Hirshkowitz et al., 2015).

CONFOUNDERS ASSESSMENT

NHANES

The following set of confounders will be tested:

- Age (RIDAGEYR; *self-reported*);

- Sex (RIAGENDR; *self-reported; male/female*);

- Ethnicity (RIDRETH1/RIDRETH3; *Mexican American, non-Hispanic white, non-Hispanic black, other race*);

- Family Poverty Level (FPL) (INDFMPIR; self-reported; *<100%, 100-199%, 200-399%, ≥400%*);

- Acculturation score: A 3-point acculturation score will be constructed according to the country of birth (DMDBORN2/ DMDBORN4) and length of time in the US (DMDYRSUS). A 3-point score will be assigned to the country of birth and length of time in the US, i.e., 3 points for US-born, 2 points for foreign-born and lived in the US ≥20 years, 1 point for foreign-born and lived in the US 10 to 19 years, and 0 points for foreign-born and lived in the US <10 years. The higher the score, the more acculturated the participants (Y. B. o. Zhang et al., 2021);

- Educational level (DMDEDUC2*; self-reported; <high school, high school, >high school*);

- Body Mass Index (BMI; BMXBMI; *the following formula will be used "weight/height^2"; weight was measured with a digital scale in kilograms, while height was measured with a portal stadiometer in meters*);

- Frequency of self-performed interproximal oral hygiene (OHQ870; *0 days/week, 1-6 days/week, 7 days/week*);

- Last dental visit (OHQ030; *more or less than 6 months*)

- Comorbidity score: a 4-point score will be built to account for the number of comorbidities. The following comorbidities will be identified: i) diabetes (*no diabetes* - glycated hemoglobin (HbA1c; LBXGH) <6.5%, and serum glucose at 2 hours following a 75 g glucose load (OGTT; LBXGLT) <200 mg/dL, and fasting plasma glucose (FPG; LBXGLU) <126 mg/dL, and no self-reported diagnosis of diabetes - DIQ010, and *diabetes* - HbA1c- ≥6.5%, or OGTT ≥200 mg/dL, or FPG ≥126 mg/dL, or self-reported diagnosis of diabetes); ii) hypertension (*no hypertension* - measured Systolic Blood Pressure (SBP) < 120 mmHg and measured Diastolic Blood Pressure (DBP) < 80mmHg, *borderline hypertension* - 120≤SBP<140 mmHg or 80≤DBP<90 mmHg, *hypertension* - average of SBP≥140mmHg or DBP≥90mmHg); iii) cardiovascular or cerebrovascular diseases (*yes* - if participant reported history of congestive heart failure - MCQ160b, or coronary heart disease - MCQ160c, or angina/angina pectoris - MCQ160d, or heart attack - MCQ160e, or stroke - MCQ160f, and *no* if otherwise); iv) depressive symptoms (DPQ010/ DPQ020/ DPQ030/ DPQ040/ DPQ050/ DPQ060/ DPQ070/ DPQ080 / DPQ090; assessed with the Patient Health Questionnaire - PHP-9; participants had depressive symptoms whenever PHP-9 ≥ 10) (Manea et al., 2012).

UKB

The following set of confounders will be tested:

- Age (21003; *self-reported*), defined as the "Age when attended the assessment center";

- Sex (31; *self-reported; male/female*);

- Ethnicity (21000; *white, mixed, Asian/Asian British, Black/Black British*);

- Total household income before tax (738; self-reported; "*less than £18 000*"/"*£18 000 to 30 999*"/ "*£31 000 to 51 999*"/"*£52 000 to 100 000*"/"*greater than £100 000*").

- Acculturation score: A 3-point acculturation score will be constructed according to the country of birth (1647; *UK/elsewhere*) and length of time in the UK (3659; "*Year immigrated to the UK*"). A 3-point score will be assigned to the country of birth and length of time in the UK, i.e., 3 points for UK-born, 2 points for foreign-born and lived in the UK ≥20 years, 1 point for foreign-born and lived in the UK 10 to 19 years, and 0 points for foreign-born and lived in the UK <10 years. The higher the score, the more acculturated the participants (Y. B. o. Zhang et al., 2021);

- Education attainment (6138; "*College or university degree*"/"*A levels/AS levels or equivalent*"/"*O levels/GCSEs or equivalent*"/"*CSEs or equivalent*"/"*NVQ or HND or HNC or equivalent*"/"*Other professional qualifications*"/"*None of the above*" *(equivalent to less than high school diploma)*/"*Prefer not to answer*);

- Body Mass Index (BMI; 21001; *the following formula will be used "weight/height^2"; weight was measured with a digital scale in kilograms, while height was measured with a portal stadiometer in meters*);

- Comorbidity score: a 4-point score will be built to

account for the number of comorbidities. The following comorbidities will be identified: i) diabetes (2443; *no diabetes* - glycated hemoglobin (HbA1c; LBXGH) <6.5%, and serum glucose at 2 hours following a 75 g glucose load (OGTT; LBXGLT) <200 mg/dL, and fasting plasma glucose (FPG; LBXGLU) <126 mg/dL, and no self-reported diagnosis of diabetes - DIQ010, and *diabetes* - HbA1c- ≥6.5%, or OGTT ≥200 mg/dL, or FPG ≥126 mg/dL, or self-reported diagnosis of diabetes); ii) hypertension (*no hypertension* - measured Systolic Blood Pressure (SBP) < 120 mmHg and measured Diastolic Blood Pressure (DBP) < 80mmHg, *borderline hypertension* - 120≤SBP<140 mmHg or 80≤DBP<90 mmHg, *hypertension* - average of SBP≥140mmHg or DBP≥90mmHg); iii) cardiovascular or cerebrovascular diseases (131059/131375/131377; *yes/no)*.; iv) bipolar/major depression status (20126).

Each confounder was tested for its association with the outcome using simple regression analysis. Whenever a confounder was associated both with the exposure and the outcome, it was selected to be included in the multiple models.

Table S1. *Missing values for each variable.*

Variables	NHANES		UK Biobank	
	Collected values – N (%)	Missing values – N (%)	Collected values – N (%)	Missing values – N (%)
Healthy lifestyle score	9,854 (100.0)	0 (0.0)	111,679 (100.0)	0 (0.0)
Periodontitis	9,854 (100.0)	0 (0.0)	111,679 (100.0)	0 (0.0)
Age	9,854 (100.0)	0 (0.0)	111,679 (100.0)	0 (0.0)
Gender	9,854 (100.0)	0 (0.0)	111,679 (100.0)	0 (0.0)
Ethnicity	9,854 (100.0)	0 (0.0)	111,679 (100.0)	0 (0.0)
Family Poverty Level/Total household income	9,076 (92.1)	778 (7.9)	98,623 (88.3)	13,056 (21.7)

Acculturation score	9,732 (98.8)	122 (1.2)	111,543 (99.9)	136 (0.1)
Education	9,842 (99.9)	12 (0.1)	1110,948 (99.3)	731 (0.7)
BMI	9,800 (99.5)	54 (0.5)	111,200 (99.6)	479 (0.4)
Frequency of interproximal hygiene	9,851 (99.07)	3 (0.03)	-	-
Comorbidity score	8,960 (89.3)	894 (9.7)	111,679 (100.0)	0 (0.0)
Smoking	9,854 (100.0)	0 (0.0)	111,679 (100.0)	0 (0.0)
Alcohol intake	9,854 (100.0)	0 (0.0)	111,679 (100.0)	0 (0.0)
LTPA	9,854 (100.0)	0 (0.0)	111,679 (100.0)	0 (0.0)
HEI/Diet quality	9,854 (100.0)	0 (0.0)	111,679 (100.0)	0 (0.0)
Sleep duration	9,854 (100.0)	0 (0.0)	111,679 (100.0)	0 (0.0)

WBC (1000 cells/uL)	9,602 (97.4)	252 (2.6)	107,366 (96.6)	4,313 (3.4)
C-reactive protein (mg/dL)	3,344 (97.1)	99 (2.9)	105,253 (94.2)	6,426 (5.8)
Segmented neutrophils number (1000 cells/uL)	9,584 (100.0)	0 (0.0)	107,140 (95.9)	4,539 (4.1)

Abbreviations. BMI, body mass index; HEI, healthy eating index; LTPA, leisure-time physical activity; uL, microliters; WBC, white blood cell count; %, percentage.

Table S2. *Association between categories of healthy lifestyle score (2-3 vs. 0-1 healthy lifestyles) and periodontitis by subgroups of age and sex.*

2-3 healthy lifestyles (vs. 0-1 healthy lifestyles) as exposure – OR/MD (95% CI)								
Outcomes	**Age**				**Sex**			
	< 60 years		**≥ 60 years**		**Males**		**Females**	
	Crude	***Adjusted***	***Crude***	***Adjusted***	***Crude***	***Adjusted***	***Crude***	***Adjusted***
NHANES								
Periodontitis	***0.4 (0.4, 0.5)****	***0.5 (0.4, 0.6)****	***0.5 (0.3, 0.7)****	***0.5 (0.3, 0.7)****	***0.5 (0.4, 0.6)****	***0.5 (0.4, 0.7)****	***0.5 (0.4, 0.6)****	***0.5 (0.4, 0.6)****
Periodontitis severity								
Mild periodontitis	***0.4 (0.3, 0.6)****	***0.6 (0.4, 0.9)****	1.7 (0.4, 7.4)	1.3 (0.3, 6.0)	***0.4 (0.2, 0.7)****	0.6 (0.3, 1.0)	***0.5 (0.3, 0.9)****	0.6 (0.4, 1.0)

Moderate periodontitis	**0.3 (0.2, 0.3)*** **	**0.4 (0.3, 0.5)*** **	**0.4 (0.3, 0.7)** **	**0.4 (0.2, 0.8)** **	**0.4 (0.3, 0.5)*** **	**0.4 (0.3, 0.5)*** **	**0.5 (0.4, 0.6)*** **	**0.4 (0.3, 0.6)*** **
Severe periodontitis	**0.1 (0.08, 0.2)*** **	**0.2 (0.1, 0.3)*** **	**0.1 (0.08, 0.2)*** **	**0.2 (0.1, 0.3)*** **	**0.2 (0.1, 0.2)*** **	**0.2 (0.1, 0.3)*** **	**0.1 (0.09, 0.2)*** **	**0.1 (0.08, 0.2)*** **
PPD								
% sites PPD ≥ 4 mm	**-1.9 (-2.6, -1.4)*** **	**-1.1 (-1.7, -0.5)** **	**-2.3 (-3.7, -0.9)** **	**-1.7 (-3.0, -0.4)** **	**-2.1 (-2.9, -1.4)*** **	**-1.1 (-1.9, -0.2)** **	**-1.7 (-2.5, -0.9)*** **	**-1.3 (-2.1, -0.4)** **
% sites PPD ≥ 5 mm	**-0.6 (-0.9, -0.3)*** **	**-0.3 (-0.6, -0.05)** **	**-0.8 (-1.4, -0.1)** **	-0.6 (-1.1, 0.03)	**-0.7 (-1.1, -0.3)** **	**-0.3 (-0.8, 0.1)**	**-0.5 (-0.9, -0.2)** **	**-0.4 (-0.8, -0.08)** **
% sites PPD ≥ 6 mm	**-0.2 (-0.4, -0.06)** **	-0.1 (-0.3, 0.04)	**-0.2 (-0.4, -0.02)** **	-0.1 (-0.3, 0.08)	**-0.2 (-0.4, -0.02)** **	-0.08 (-0.3, 0.1)	**-0.2 (-0.3, -0.05)** **	**-0.1 (-0.3, -0.08)** **
CAL								

% sites CAL ≥ 3 mm	**-7.0 (-8.2, -5.8)*** **	**-5.2 (-6.3, -3.9)*** **	**-9.5 (-12.8, -6.3)*** **	**-7.9 (-10.7, -5.2)*** **	**-7.4 (-8.9, -5.8)*** **	**-6.1 (-7.7, -4.4)*** **	**-5.1 (-6.5, -3.6)*** **	**-4.8 (-6.3, -3.2)*** **
% sites CAL ≥ 4 mm	**-4.6 (-5.5, -3.6)*** **	**-3.6 (-4.6, -2.6)*** **	**-7.2 (-9.6, -4.9)*** **	**-5.9 (-7.9, -3.8)*** **	**-4.9 (-6.1, -3.8)*** **	**-4.1 (-5.5, -2.7)*** **	**-3.4 (-4.6, -2.3)*** **	**-3.3 (-4.5, -2.1)*** **
% sites CAL ≥ 5 mm	**-2.6 (-3.3, -1.9)*** **	**-2.0 (-2.8, -1.3)*** **	**-4.2 (-6.0, -2.5)*** **	**-3.2 (-4.8, -1.7)*** **	**-2.9 (-3.9, -1.9)*** **	**-2.3 (-3.3, -1.2)*** **	**-1.9 (-2.5, -1.2)*** **	**-1.8 (-2.6, -1.1)*** **
% sites CAL ≥ 6 mm	**-1.5 (-2.0, -1.1)*** **	**-1.1 (-1.7, -0.6)*** **	**-2.5 (-3.9, -1.2)** **	**-1.8 (-3.1, -0.6)** **	**-1.9 (-2.6, -1.1)*** **	**-1.4 (-2.2, -0.6)** **	**0.9 (-1.4, -0.6)*** **	**-0.9 (-1.4, -0.5)*** **
UK Biobank								
Bleeding gums (vs. healthy)	0.9 (0.8, 1.1)	1.1 (0.9, 1.2)	1.0 (0.9, 1.1)	**0.9 (0.8, 0.99)***	1.0 (0.9, 1.1)	1.0 (0.8, 1.2)	1.0 (0.9, 1.1)	**0.9 (0.8, 0.99)***
Painful gums (vs. healthy)	0.9 (0.7, 1.1)	1.3 (0.9, 1.8)	0.9 (0.7, 1.2)	0.9 (0.7, 1.1)	0.9 (0.7, 1.2)	1.2 (0.8, 1.7)	0.9 (0.7, 1.2)	0.9 (0.7, 1.1)

Loose teeth (vs. healthy)	***0.7*** ***(0.6, 0.8)*** ***	***0.6*** ***(0.5, 0.8)*** ***	0.7 (0.8, 1.0)	***0.6*** ***(0.5, 0.7)*** ***	***0.7*** ***(0.6, 0.8)*** ***	***0.6*** ***(0.5, 0.8)*** ***	***0.7*** ***(0.6, 0.9)*** ***	***0.6*** ***(0.5, 0.7)*** ***
Any periodontal disease (vs. healthy)	***0.9*** ***(0.8, 0.99)*** ***	0.9 (0.8, 1.1)	0.9 (0.8, 1.0)	***0.8*** ***(0.7, 0.9)*** ***	***0.9*** ***(0.8, 0.9)*** *	0.9 (0.8, 1.1)	0.9 (0.8, 1.0)	***0.8*** ***(0.7, 0.9)*** ***

Abbreviations. CAL, clinical attachment level; CI, confidence interval; MD, difference in means; mm, millimeter; OR, odds ratio; PPD, probing pocket depth; %, percentage. * p<0.05; ** p<0.01; *** p<0.001.

Table S3. *Association between categories of healthy lifestyle score (4-5 vs. 0-1 healthy lifestyles) and periodontitis by subgroups of age and sex.*

4-5 healthy lifestyles (vs. 0-1 healthy lifestyles) as exposure – OR/MD (95% CI)								
Outcomes	**Age**				**Sex**			
	< 60 years		**≥ 60 years**		**Males**		**Females**	
	Crude	***Adjusted***	***Crude***	***Adjusted***	***Crude***	***Adjusted***	***Crude***	***Adjusted***
NHANES								
Periodontitis	***0.2 (0.1, 0.3)****	***0.4 (0.3, 0.5)****	***0.3 (0.2, 0.5)****	***0.4 (0.2, 0.6)****	***0.3 (0.2, 0.4)****	***0.4 (0.3, 0.5)****	***0.4 (0.3, 0.5)****	***0.4 (0.3, 0.5)****
Periodontitis severity								
Mild periodontitis	***0.4 (0.3, 0.6)****	***0.6 (0.4, 0.9)****	1.7 (0.4, 7.4)	1.1 (0.2, 5.1)	***0.4 (0.2, 0.7)****	0.5 (0.3, 1.1)	***0.5 (0.3, 0.9)****	0.7 (0.3, 1.1)

Moderate periodontitis	0.3 (0.2, 0.3)***	0.4 (0.3, 0.5)***	0.4 (0.3, 0.7)**	0.3 (0.2, 0.9)**	0.4 (0.3, 0.5)***	0.3 (0.2, 0.6)**	0.5 (0.4, 0.6)***	0.5 (0.3, 0.7)**
Severe periodontitis	0.1 (0.09, 0.2)***	0.2 (0.1, 0.3)***	0.1 (0.08, 0.2)***	0.1 (0.1, 0.4)***	0.2 (0.1, 0.2)***	0.1 (0.09, 0.4)***	0.1 (0.09, 0.2)***	0.2 (0.1, 0.3)***
PPD								
% sites PPD ≥ 4 mm	-3.2 (-3.9, -2.6)***	-1.6 (-2.2, -1.0)***	-3.0 (-4.5, -1.5)***	-1.9 (-3.3, -0.6)**	-3.7 (-4.4, -2.9)***	-1.7 (-2.6, -0.7)**	-2.3 (-3.0, -1.6)***	-1.6 (-2.3, -0.8)***
% sites PPD ≥ 5 mm	-1.1 (-1.4, -0.8)***	-0.6 (-0.8, -0.3)***	-0.9 (-1.6, -0.3)**	-0.5 (-1.1, 0.1)	-1.2 (-1.6, -0.8)***	-0.5 (-0.9, -0.04)*	-0.7 (-1.1, -0.4)***	-0.5 (-0.9, -0.2)**
% sites PPD ≥ 6 mm	-0.4 (-0.6, -0.2)***	-0.2 (-0.3, -0.07)**	-0.2 (-0.4, 0.009)	0.03 (-0.2, 0.3)	-0.4 (-0.6, -0.2)***	-0.1 (-0.3, 0.06)	-0.3 (-0.4, -0.1)**	-0.2 (-0.3, -0.04)*
CAL								

% sites CAL ≥ 3 mm	-10.3 (-1..7, -8.8)***	-6.4 (-7.9, -4.9)***	-13.3 (-16.9, -9.8)***	-9.3 (-12.2, -6.4)***	-11.1 (-12.6, -9.6)***	-7.9 (-9.8, -5.9)***	-7.1 (-8.5, -5.7)***	-5.7 (-7.1, -4.4)***
% sites CAL ≥ 4 mm	-6.4 (-7.4, -5.3)***	-4.2 (-5.3, -3.1)***	-10.3 (-12.9, -7.7)***	-6.9 (-9.1, -4.7)***	-7.6 (-8.7, -6.4)***	-5.4 (-6.9, -3.9)***	-4.6 (-5.7, -3.5)***	-3.8 (-4.9, -2.7)***
% sites CAL ≥ 5 mm	-3.6 (-4.3, -2.9)***	-2.4 (-3.1, -1.7)***	-6.3 (-8.1, -2.5)***	-3.9 (-5.3, -2.4)***	-4.7 (-5.6, -3.8)***	-3.2 (-4.3, -2.0)***	-2.4 (-3.1, -1.8)***	-2.0 (-2.8, -1.3)***
% sites CAL ≥ 6 mm	-2.2 (-2.6, -1.7)***	-1.4 (-1.9, -0.9)***	-3.7 (-5.2, -2.2)***	-2.1 (-3.3, -0.9)**	-2.9 (-3.7, -2.3)***	-1.9 (-2.8, -1.1)***	-1.3 (-1.7, -0.9)***	-1.0 (-1.5, -0.6)***
UK Biobank								
Bleeding gums (vs. healthy)	0.8 (0.7, 0.9)***	0.9 (0.8, 1.1)	0.8 (0.7, 0.9)**	0.7 (0.6, 0.8)***	0.8 (0.7, 0.9)**	0.9 (0.7, 1.0)	0.8 (0.7, 0.9)**	0.8 (0.7, 0.8)***
Painful gums (vs. healthy)	0.8	1.4	0.9	0.8	0.7 (0.5, 0.9)	1.2	0.9	0.7

	(0.7, 0.9) *	*(0.9, 1.9)*	(0.7, 1.2)	*(0.6, 0.9)* *	*	(0.8, 1.7)	(0.7, 1.2)	*(0.6, 0.9)* **
Loose teeth (vs. healthy)	*0.6* *(0.5, 0.7)* ***	*0.6* *(0.5, 0.7)* ***	*0.7* *(0.6, 0.8)* ***	*0.6* *(0.5, 0.7)* ***	*0.5* *(0.4, 0.6)* ***	*0.5* *(0.4, 0.7)* ***	*0.7* *(0.6, 0.8)* ***	*0.4* *(0.3, 0.5)* ***
Any periodontal disease (vs. healthy)	*0.8* *(0.7, 0.9)* ***	*0.9* *(0.8, 0.99)* *	*0.8* *(0.7, 0.9)* ***	*0.7* *(0.6, 0.8)* ***	*0.7* *(0.6, 0.8)* ***	*0.8* *(0.7, 0.9)* *	*0.8* *(0.7, 0.9)* ***	*0.7* *(0.6, 0.8)* ***

Abbreviations. CAL, clinical attachment level; CI, confidence interval; MD, difference in means; mm, millimeter; OR, odds ratio; PPD, probing pocket depth; %, percentage. * p<0.05; ** p<0.01; *** p<0.001.

***Table S4**. Association between smoking and periodontitis overall and by subgroups of age and sex.*

Outcomes	Non-smoking (vs. smoking) as exposure – OR/MD (95% CI)									
	Overall		Age				Sex			
			< 60 years		≥ 60 years		Males		Females	
	Crude	*Adjusted*	*Crude*	*Adjusted*	*Crude*	*Adjusted*	*Crude*	*Adjusted*	*Crude*	*Adjusted*
NHANES										
Periodontitis	**0.4 (0.3,0.4)*****	**0.4 (0.3, 0.4)*****	**0.3 (0.2, 0.4)*****	**0.4 (0.3, 0.5)*****	**0.3 (0.2, 0.4)*****	**0.3 (0.2, 0.4)*****	**0.3 (0.2, 0.4)*****	**0.3 (0.3, 0.4)*****	**0.4 (0.3, 0.5)*****	**0.4 (0.3, 0.5)*****
Periodontitis severity										
Mild periodontitis	**0.6 (0.5, 0.9)****	**0.8 (0.6, 1.2)**	**0.6 (0.4, 0.9)****	0.8 (0.6, 1.2)	1.4 (0.4, 4.9)	1.3 (0.4, 3.1)	0.7 (0.5, 1.1)	0.9 (0.6, 1.6)	**0.6 (0.3, 0.9)***	0.7 (0.4, 1.1)
Moderate periodontitis	**0.4 (0.4,0.5)*****	**0.4 (0.3, 0.4)*****	**0.3 (0.3,**	**0.4 (0.3,**	**0.3 (0.2,**	**0.3 (0.2,**	**0.4 (0.3,**	**0.3 (0.2,**	**0.5 (0.4,**	**0.4 (0.3,**

			0.4)***	0.5)***	0.5)***	0.5)***	0.5)***	0.4)***	0.6)***	0.5)***
Severe periodontitis	0.2 (0.2,0.3)***	0.2 (0.1, 0.3)***	0.2 (0.1, 0.2)***	0.2 (0.1, 0.3)***	0.1 (0.08, 0.2)***	0.2 (0.1, 0.3)***	0.2 (0.1, 0.3)***	0.2 (0.1, 0.3)***	0.2 (0.1, 0.3)***	0.2 (0.1, 0.3)***
PPD										
% sites PPD ≥ 4 mm	-3.1 (-3.6,-2.5)***	-2.2 (-2.8, -1.5)***	-2.9 (-3.6, -2.4)***	-2.0 (-2.7, -1.3)***	-3.7 (-5.4, -1.9)***	-2.9 (-4.6, -1.2)**	-3.7 (-4.4, -2.9)***	-2.5 (-3.4, -1.7)***	-2.1 (-2.8, -1.4)***	-1.6 (-2.4, -0.8)***
% sites PPD ≥ 5 mm	-0.9 (-1.1,-0.6)***	-0.6 (-0.9, -0.3)***	-0.9 (-1.2, -0.6)***	-0.6 (-0.9, -0.3)***	-0.9 (-1.7, -0.2)*	-0.7 (-1.4, 0.04)	-1.1 (-1.4, -0.8)***	-0.7 (-1.1, -0.3)***	-0.6 (-0.9, -0.3)***	-0.5 (-0.8, -0.2)**
% sites PPD ≥ 6 mm	-0.3 (-0.4, -0.2)***	-0.2 (-0.3, -0.05)**	-0.3 (-0.4, -0.2)***	-0.2 (-0.3, -0.03)*	-0.2 (-0.5, 0.002)	-0.08 (-0.3, 0.2)	-0.3 (-0.5, -0.2)***	-0.2 (-0.4, -0.01)*	-0.1 (-0.3, 0.05)**	-0.1 (-0.2, 0.002)
CAL										
% sites CAL ≥ 3	-9.3	-8.2	-9.7	-7.3	-14.3	-12.5	-11.0	-9.9	-6.6	-6.1

mm	(-10.2, -8.4)***	(-9.3, -7.2)***	(-10.7, -8.7)***	(-8.5, -6.2)***	(-17.8, -10.9)***	(-15.6, -9.4)***	(-12.3, -9.7)***	(-11.4, -8.3)***	(-7.9, -5.3)***	(-7.6, -4.7)***
% sites CAL ≥ 4 mm	-6.3 (-7.2, -5.5)***	-5.7 (-6.7, -4.8)***	-6.3 (-7.1, -5.5)***	-5.0 (-5.9, -4.2)***	-10.9 (-14.1, -7.7)***	-9.3 (-12.3, -6.4)***	-7.9 (-9.2, -6.7)***	-7.3 (-8.7, -5.8)***	-1.9 (-5.1, -2.9)***	-3.8 (-4.9, -2.6)***
% sites CAL ≥ 5 mm	-3.8 (-4.4, -3.2)***	-3.4 (-4.1, -2.7)***	-3.7 (-4.4, -3.1)***	-3.0 (-3.7, -2.4)***	-6.8 (-9.3, -4.4)***	-5.6 (-7.9, -3.3)***	-5.1 (-6.1, -4.0)***	-4.6 (-5.9, -3.3)***	-2.0 (-2.7, -1.4)***	-1.9 (-2.7, -1.3)***
% sites CAL ≥ 6 mm	-2.3 (-2.8, -1.9)***	-2.1 (-2.6, -1.6)***	-2.2 (-2.6, -1.8)***	-1.8 (-2.2, -1.3)***	-4.5 (-6.5, -2.5)***	-3.7 (-5.6, -1.8)***	-3.3 (-4.2, -2.5)***	-2.9 (-3.9, -1.9)***	-1.0 (-1.4, -0.7)***	-0.9 (-1.4, -0.6)***
UK Biobank										
Bleeding gums (vs. healthy)	1.2 (1.1, 1.3)***	1.1 (1.1, 1.2)***	1.2 (1.1, 1.2)***	1.1 (1.0, 1.2)***	1.3 (1.1, 1.4)***	1.2 (1.0, 1.3)**	1.2 (1.1, 1.3)***	1.1 (1.0, 1.2)**	1.2 (1.1, 1.3)***	1.1 (1.0, 1.2)**
Painful gums (vs. healthy)	0.9 (0.9, 1.1)	0.9 (0.8, 1.0)	0.9 (0.7, 0.9)*	0.9 (0.7, 0.9)*	1.1 (0.9, 1.3)	1.1 (0.9, 1.3)	0.8 (0.7, 0.9)**	0.8 (0.7, 0.9)*	1.0 (0.9, 1.1)	0.9 (0.8, 1.1)

Loose teeth (vs. healthy)	***0.5*** ***(0.4, 0.6)*** ***	***0.5*** ***(0.4, 0.6)*** ***	***0.4*** ***(0.3, 0.5)*** ***	***0.4*** ***(0.3, 0.5)*** ***	***0.6*** ***(0.5, 0.7)*** ***	***0.6*** ***(0.5, 0.7)*** ***	***0.5*** ***(0.4, 0.6)*** ***	***0.5*** ***(0.4, 0.6)*** ***	***0.5*** ***(0.4, 0.6)*** ***	***0.5*** ***(0.4, 0.5)*** ***
Any periodontal disease (vs. healthy)	***0.8*** ***(0.7, 0.9)*** ***	***0.9*** ***(0.8, 0.9)*** **	***0.9*** ***(0.8, 0.9)*** **	***0.9*** ***(0.8, 0.9)*** **	0.9 (0.8, 1.1)	0.9 (0.8, 1.1)	***0.9*** ***(0.8, 0.9)*** ***	***0.9*** ***(0.8, 0.9)*** ***	0.9 (0.9, 1.0)	***0.9*** ***(0.8, 0.9)*** **

Abbreviations. CAL, clinical attachment level; CI, confidence interval; MD, difference in means; mm, millimeter; OR, odds ratio; PPD, probing pocket depth; %, percentage. * p<0.05; ** p<0.01; *** p<0.001.

***Table S5**. Association between alcohol drinking and periodontitis overall and by subgroups of age and sex.*

Outcomes	Alcohol below the suggested intake (vs. above the suggested intake) as exposure – OR/MD (95% CI)									
	Overall		Age				Sex			
			< 60 years		≥ 60 years		Males		Females	
	Crude	*Adjusted*	*Crude*	*Adjusted*	*Crude*	*Adjusted*	*Crude*	*Adjusted*	*Crude*	*Adjusted*
NHANES										
Periodontitis	***0.8 (0.7, 0.9)****	***0.7 (0.6, 0.8)*****	***0.7 (0.6, 0.8)*****	***0.7 (0.6, 0.8)*****	0.8 (0.7, 1.1)	0.8 (0.7, 1.1)	***0.6 (0.5, 0.7)*****	***0.7 (0.6, 0.8)*****	1.1 (0.9, 1.3)	0.9 (0.7, 1.0)
Periodontitis severity										
Mild periodontitis	0.9 (0.7, 1.1)	0.9 (0.8, 1.2)	0.9 (0.7, 1.1)	0.9 (0.7, 1.2)	1.3 (0.7, 2.5)	1.3 (0.7, 2.5)	0.8 (0.6, 1.1)	0.9 (0.6, 1.3)	1.0 (0.7, 1.5)	1.0 (0.7, 1.5)
Moderate periodontitis	0.9 (0.7, 1.0)	***0.7 (0.6,***	***0.7 (0.6,***	***0.7 (0.6,***	0.9 (0.7, 1.1)	0.9 (0.7, 1.1)	***0.7 (0.6,***	***0.6 (0.5, 0.7)***	1.2 (0.9,	0.9 (0.7, 1.1)

		0.9)***	0.8)***	0.9)**			0.8)***	***	1.4)	
Severe periodontitis	0.6 (0.5, 0.8)***	0.6 (0.4, 0.7)***	0.6 (0.4, 0.7)***	0.6 (0.4, 0.8)***	0.6 (0.4, 0.8)***	0.7 (0.4, 0.9)*	0.5 (0.4, 0.6)***	0.5 (0.4, 0.6)***	0.9 (0.7, 1.4)	0.8 (0.6, 1.2)
PPD										
% sites PPD ≥ 4 mm	-1.0 (-1.4, -0.7)***	-0.7 (-1.0, -0.3)***	-1.0 (-1.4, 0.7)***	-0.7 (-1.2, -0.3)**	-0.9 (-1.5, -0.4)**	-0.6 (-1.2, -0.04)*	-1.6 (-2.1, -1.1)***	-0.9 (-1.5, -0.3)**	-0.4 (-0.7, -0.08)*	-0.3 (-0.6, -0.04)*
% sites PPD ≥ 5 mm	-0.4 (-0.5, -0.2)***	-0.3 (-0.4, -0.1)**	-0.4 (-0.6, -0.2)***	-0.3 (-0.5, -0.1)**	-0.3 (-0.5, -0.07)**	-0.1 (-0.3, 0.08)	-0.6 (-0.8, -0.3)***	-0.3 (-0.6, -0.07)*	-0.1 (-0.3, 0.03)	-0.1 (-0.3, 0.03)
% sites PPD ≥ 6 mm	-0.1 (-0.2, -0.05)	-0.1 (-0.2, -0.03)**	-0.2 (-0.1, -0.06)**	-0.1 (-0.2, -0.04)**	-0.06 (-0.2, 0.03)	0.03 (-0.06, 0.1)	-0.2 (-0.3, -0.08)**	-0.2 (-0.3, -0.02)*	-0.04 (-0.1, 0.03)	-0.05 (-0.1, 0.03)
CAL										
% sites CAL ≥ 3	-2.2	-2.6	-3.0	-2.5	-3.7	-2.5	-4.2	-3.8	0.03	-1.1

mm	**(-3.1, 1.4)** ***	**(-3.4, -1.8)** ***	**(-3.9, -2.1)** ***	**(-3.4, -1.5)** ***	**(-6.2, -1.2)** **	**(-4.7, -0.3)** *	**(-5.5, -2.9)** ***	**(-5.0, -2.5)** ***	(-0.9, 0.9)	**(-1.8, -0.3)** **
% sites CAL ≥ 4 mm	**-1.6** **(-2.3, -1.0)** ***	**-1.9** **(-2.4, -1.3)** ***	**-2.0** **(-2.7, -1.4)** ***	**-1.7** **(-2.4, -1.1)** ***	**-2.9** **(-4.6, 1.1)** **	**-1.9** **(-3.5, -0.2)** *	**-2.8** **(-3.8, -1.9)** ***	**-2.5** **(-3.6, -1.5)** ***	-0.3 (-0.9, 0.4)	**-0.9** **(-1.4, -0.3)** **
% sites CAL ≥ 5 mm	**-1.0** **(-1.5, -0.6)** ***	**-1.1** **(-1.6, -0.7)** ***	**-1.2** **(-1.7, -0.8)** ***	**-1.1** **(-1.5, -0.6)** ***	**-1.9** **(-3.0, -0.7)** **	-1.1 (-2.2, 0.04)	**-1.8** **(-2.5, -1.1)** ***	**-1.6** **(-2.5, -0.8)** ***	-0.2 (-0.5, 0.2)	**-0.4** **(-0.8, -0.1)** *
% sites CAL ≥ 6 mm	**-0.7** **(-1.0, -0.4)** ***	**-0.8** **(-1.1, -0.4)** ***	**-0.8** **(-1.1, -0.5)** ***	**-0.7** **(-0.9, -0.4)** ***	**-1.3** **(-2.1, -0.4)** **	-0.8 (-1.6, 0.1)	-1.3 (-1.8, -0.7) ***	-1.1 (-1.8, -0.5)	-0.08 (-0.3, 0.1)	**-0.2** **(-0.4, -0.01)** *
UK Biobank										
Bleeding gums (vs. healthy)	**0.9** **(0.8, 0.9)** ***	**0.9** **(0.8, 0.9)** ***	**0.9** **(0.8, 0.9)** *	**0.9** **(0.8, 0.9)** ***	**0.9** **(0.8, 0.9)** *	**0.8** **(0.7, 0.9)** ***	**0.8** **(0.7, 0.9)** ***	**0.8** **(0.8, 0.9)** ***	**0.7** **(0.7, 0.8)** ***	**0.8** **(0.8, 0.9)** ***
Painful gums (vs. healthy)	**1.4** **(1.2, 1.5)** ***	1.0 (0.9, 1.1)	**1.4** **(1.2, 1.5)** ***	1.0 (0.9, 1.1)	**1.5** **(1.3, 1.8)** ***	1.1 (0.9, 1.4)	**1.4** **(1.2, 1.6)** ***	1.1 (0.9, 1.3)	**1.2** **(1.0, 1.3)** **	0.9 (0.8, 1.1)

Loose teeth (vs. healthy)	***1.3 (1.2, 1.4)*** ***	0.9 (0.8, 1.0)	***1.5 (1.4, 1.7)*** ***	1.0 (0.9, 1.1)	1.0 (0.9, 1.1)	0.9 (0.7, 1.0)	***1.4 (1.3, 1.6)*** ***	0.9 (0.8, 1.1)	***1.3 (1.1, 1.4)*** ***	0.9 (0.8, 1.1)
Any periodontal disease (vs. healthy)	***1.0 (0.9, 1.1)***	***0.9 (0.8, 0.9)*** **	***1.1 (1.0, 1.2)*** *	***0.9 (0.8, 0.9)*** *	1.0 (0.9, 1.1)	0.9 (0.8, 1.0)	1.0 (0.9, 1.1)	***0.9 (0.8, 0.9)*** **	***0.9 (0.8, 0.9)*** ***	***0.8 (0.8, 0.9)*** ***

Abbreviations. CAL, clinical attachment level; CI, confidence interval; MD, difference in means; mm, millimeter; OR, odds ratio; PPD, probing pocket depth; %, percentage. * p<0.05; ** p<0.01; *** p<0.001.

Table S6. *Association between leisure-time physical activity and periodontitis overall and by subgroups of age and sex.*

Outcomes	High LTPA (vs. low LTPA) as exposure – OR/MD (95% CI)									
	Overall		Age				Sex			
			< 60 years		≥ 60 years		Males		Females	
	Crude	*Adjusted*	*Crude*	*Adjusted*	*Crude*	*Adjusted*	*Crude*	*Adjusted*	*Crude*	*Adjusted*
NHANES										
Periodontitis	*0.6 (0.5, 0.6)****	*0.7 (0.6, 0.8)****	*0.6 (0.5, 0.7)****	*0.7 (0.6, 0.8)****	*0.7 (0.5, 0.8)***	*0.7 (0.6, 0.9)**	*0.5 (0.4, 0.6)****	*0.7 (0.6, 0.8)****	*0.6 (0.5, 0.7)****	*0.8 (0.7, 0.9)***
Periodontitis severity										

Mild periodontitis	**0.6 (0.5, 0.8)****	0.8 (0.6, 1.0)	**0.6 (0.5, 0.8)****	0.8 (0.6, 1.1)	0.7 (0.4, 1.2)	0.7 (0.4, 1.3)	**0.5 (0.3, 0.7)*****	**0.6 (0.4, 0.9)***	0.7 (0.5, 1.1)	1.0 (0.7, 1.5)
Moderate periodontitis	**0.6 (0.5, 0.7)*****	**0.8 (0.7, 0.9)****	**0.6 (0.5, 0.7)*****	**0.8 (0.7, 0.9)***	**0.7 (0.5, 0.9)****	0.8 (0.6, 1.0)	**0.6 (0.5, 0.7)*****	**0.7 (0.6, 0.9)****	**0.6 (0.5, 0.7)*****	**0.8 (0.7, 0.9)****
Severe periodontitis	**0.4 (0.3, 0.5)*****	**0.6 (0.5, 0.8)*****	**0.4 (0.3, 0.5)*****	**0.6 (0.4, 0.8)****	**0.6 (0.4, 0.8)****	0.8 (0.6, 1.1)	**0.4 (0.3, 0.5)*****	**0.5 (0.4, 0.7)*****	**0.4 (0.3, 0.6)*****	0.6 (0.4, 0.9)
PPD										
% sites PPD ≥ 4 mm	**-1.2 (-1.5, -0.9)*****	**-0.8 (-1.0, -0.5)*****	**-1.4 (-1.8, 1.1)*****	**-0.9 (-1.3, -0.6)*****	**-0.5 (-0.9, -0.03)***	-0.1 (-0.6, 0.4)	**-1.8 (-2.2, -1.3)*****	**-1.1 (-1.6, -0.7)*****	**-0.9 (-1.2, -0.6)*****	**-0.4 (-0.7, -0.09)***
% sites PPD ≥ 5 mm	**-0.4 (-0.5, -0.2)*****	**-0.2 (-0.4, -0.1)*****	**-0.5 (-0.6, -0.3)*****	**-0.3 (-0.5, -0.2)*****	-0.1 (-0.4, 0.08)	0.003 (-0.2, 0.3)	**-0.6 (-0.8, -0.4)*****	**-0.4 (-0.6, -0.2)*****	**-0.2 (-0.4, -0.08)****	-0.09 (-0.2, 0.06)

% sites PPD ≥ 6 mm	**-0.1 (-0.2, -0.04)****	-0.06 (-0.1, 0.005)	-0.2 (-0.2, -0.07)***	-0.09 (-0.2, -0.02)*	0.02 (-0.1, 0.1)	0.08 (-0.06, 0.2)	**-0.2 (-0.3, -0.1)*****	**-0.1 (-0.2, -0.02)***	-0.06 (-0.1, 0.01)	0.001 (-0.07, 0.07)
CAL										
% sites CAL ≥ 3 mm	**-4.2 (-4.8, -3.6)*****	**-2.0 (-2.6, -1.4)*****	**-3.8 (-4.6, -3.1)*****	**-1.9 (-2.8, -1.2)*****	**-4.1 (-5.6, -2.6)*****	**-2.0 (-3.4, -0.6)****	**-5.6 (-6.7, -4.7)*****	**-2.8 (-3.8, -1.8)*****	**-3.6 (-4.5, -2.7)*****	**-1.3 (-2.1, -0.5)****
% sites CAL ≥ 4 mm	**-2.7 (-3.2, -2.2)*****	**-1.3 (-1.7, -0.9)*****	**-2.3 (-2.8, -1.8)*****	**-1.2 (-1.7, -0.8)*****	**-3.1 (-4.3, -1.9)*****	**-1.4 (-2.5, -0.3)***	**-3.7 (-4.5, -2.9)*****	**-1.8 (-2.5, -1.1)*****	**-2.1 (-2.7, -1.6)*****	**-0.8 (-1.3, -0.2)****
% sites CAL ≥ 5 mm	**-1.5 (-1.8, -1.2)*****	**-0.7 (-0.9, -0.4)*****	**-1.2 (-1.6, -0.9)*****	**-0.7 (-1.0, -0.3)*****	**-1.8 (-2.6, -0.9)*****	-0.6 (-1.4, 0.09)	**-2.2 (-2.7, -1.6)*****	**-0.9 (-1.5, -0.5)*****	**-1.1 (-1.4, -0.7)*****	**-0.4 (-0.7, -0.03)***
% sites CAL ≥ 6 mm	**-0.8 (-1.1, -0.6)*****	**-0.4 (-0.6, -0.2)*****	**-0.7 (-0.9, -0.5)*****	**-0.4 (-0.6, -0.2)****	**-0.9 (-1.6, -0.4)****	-0.2 (-0.8, 0.3)	**-1.3 (-1.6, -0.9)*****	**-0.5 (-0.9, -0.2)****	**-0.6 (-0.8, -0.4)*****	**-0.2 (-0.4, -0.01)***

UK Biobank										
Bleeding gums (vs. healthy)	**0.9 (0.8, 0.9)*** **	**0.9 (0.8, 0.9)*** **	**0.8 (0.8, 0.9)*** **	**0.9 (0.8, 0.9)*** **	**0.9 (0.8, 0.9)** **	**0.9 (0.8, 0.9)* **	**0.9 (0.8, 0.9)* **	**0.9 (0.8, 0.9)* **	**0.9 (0.8, 0.9)*** **	**0.9 (0.7, 0.9)*** **
Painful gums (vs. healthy)	1.0 (0.9, 1.1)	0.9 (0.8, 1.0)	0.9 (0.8, 1.1)	0.9 (0.8, 1.0)	1.2 (0.9, 1.3)	0.9 (0.8, 1.2)	0.9 (0.8, 1.2)	0.8 (0.7, 1.0)	1.1 (0.9, 1.2)	0.9 (0.8, 1.1)
Loose teeth (vs. healthy)	**1.2 (1.1, 1.3)*** **	1.0 (0.9, 1.1)	**1.3 (1.1, 1.4)*** **	1.0 (0.9, 1.2)	1.1 (0.9, 1.2)	0.9 (0.8, 1.1)	**1.2 (1.0, 1.3)* **	1.0 (0.9, 1.1)	**1.2 (1.1, 1.3)** **	1.0 (0.9, 1.1)
Any periodontal disease (vs. healthy)	**0.9 (0.8, 0.9)** **	**0.9 (0.8, 0.9)*** **	0.9 (0.9, 1.0)	**0.9 (0.8, 0.9)*** **	0.9 (0.9, 1.0)	**0.9 (0.8, 0.9)* **	0.9 (0.8, 1.0)	**0.9 (0.8, 0.9)* **	**0.9 (0.8, 0.9)** **	**0.9 (0.8, 0.9)*** **

Abbreviations. CAL, clinical attachment level; CI, confidence interval; LTPA, leisure-time physical activity; MD, difference in means; mm, millimeter; OR, odds ratio; PPD, probing pocket depth; %, percentage. * p<0.05; ** p<0.01; *** p<0.001.

***Table S7**. Association between diet quality and periodontitis overall and by subgroups of age and sex.*

Outcomes	Healthy diet (vs. unhealthy diet) as exposure – OR/MD (95% CI)									
	Overall		Age				Sex			
			< 60 years		≥ 60 years		Males		Females	
	Crude	*Adjusted*	*Crude*	*Adjusted*	*Crude*	*Adjusted*	*Crude*	*Adjusted*	*Crude*	*Adjusted*
NHANES										
Periodontitis	***0.8 (0.7, 0.9)****	***0.8 (0.7, 0.9)****	***0.7 (0.6, 0.8)****	***0.8 (0.7, 0.9)****	***0.8 (0.7, 0.9)****	0.8 (0.7, 1.0)	0.9 (0.8, 1.0)	0.9 (0.7, 1.0)	***0.8 (0.7, 0.9)****	***0.7 (0.6, 0.9)****
Periodontitis severity										
Mild periodontitis	***0.7 (0.6, 0.8)****	***0.8 (0.6, 0.9)****	***0.7 (0.6, 0.9)****	0.9 (0.7, 1.1)	0.6 (0.4, 1.1)	***0.5 (0.3, 0.9)****	***0.6 (0.4, 0.9)****	0.7 (0.5, 1.2)	0.8 (0.6, 1.2)	0.9 (0.6, 1.3)

Moderate periodontitis	**0.9 (0.8, 0.9)****	**0.8 (0.7, 0.9)****	**0.7 (0.6, 0.8)*****	**0.8 (0.7, 0.9)****	0.9 (0.7, 1.0)	0.9 (0.7, 1.1)	0.9 (0.8, 1.1)	0.9 (0.8, 1.1)	**0.8 (0.7, 0.9)****	**0.8 (0.7, 0.9)****
Severe periodontitis	**0.6 (0.5, 0.8)*****	**0.7 (0.6, 0.9)****	**0.6 (0.5, 0.8)*****	**0.7 (0.6, 0.9)***	**0.6 (0.4, 0.8)*****	**0.7 (0.5, 0.9)***	0.8 (0.7, 1.0)	0.8 (0.6, 1.0)	**0.5 (0.3, 0.6)*****	**0.5 (0.3, 0.7)*****
PPD										
% sites PPD ≥ 4 mm	**-0.8 (-1.1, -0.5)*****	**-0.3 (-0.7, -0.01)***	**-0.9 (-1.2, -0.6)*****	**-0.4 (-0.8, -0.01)***	**-0.6 (-1.0, -0.1)***	-0.2 (-0.6, 0.2)	**-0.7 (-1.2, -0.2)****	-0.2 (-0.7, 0.4)	**-0.7 (-0.9, -0.4)*****	**-0.5 (-0.8, -0.2)****
% sites PPD ≥ 5 mm	**-0.3 (-0.4, -0.1)*****	-0.1 (-0.2, 0.03)	**-0.31 (-0.5, -0.1)****	-0.1 (-0.3, 0.03)	-0.2 (-0.4, 0.001)	-0.05 (-0.2, 0.1)	-0.2 (-0.5, 0.01)	-0.04 (-0.3, 0.2)	**-0.3 (-0.4, -0.1)*****	**-0.2 (-0.3, -0.06)****
% sites PPD ≥ 6 mm	**-0.09 (-0.2, -0.04)****	-0.04 (-0.1, 0.02)	**-0.1 (-0.2, -0.03)****	-0.06 (-0.1, 0.02)	-0.05 (-0.2, 0.06)	0.03 (-0.08, 0.1)	-0.06 (-0.2, 0.05)	-0.01 (-0.1, 0.1)	**-0.1 (-0.2, -0.04)****	**-0.08 (-0.1, -0.02)****
CAL										

% sites CAL ≥ 3 mm	**-1.6 (-2.4, -0.9)*** **	**-1.3 (-2.0, -0.5)** **	**-2.2 (-2.9, -1.4)*** **	**-1.2 (-1.9, -0.5)** **	**-2.5 (-4.3, -0.8)** **	-1.3 (-2.8, 0.2)	-0.8 (-1.9, 0.3)	-0.9 (-2.1, 0.1)	**-1.8 (-2.6, -0.9)*** **	**-1.7 (-2.4, -0.9)*** **
% sites CAL ≥ 4 mm	**-1.0 (-1.5, -0.5)*** **	**-0.8 (-1.3, -0.3)** **	**-1.1 (-1.6, -0.6)*** **	**-0.6 (-1.1, -0.1)* **	**-2.1 (-3.5, -0.7)** **	-1.0 (-2.1, 0.09)	-0.6 (1.4, 0.2)	-0.7 (-1.5, 0.09)	**-0.9 (-1.4, -0.5)*** **	**-0.9 (-1.4, -0.4)*** **
% sites CAL ≥ 5 mm	**-0.6 (-0.9, -0.3)** **	**-0.5 (-0.8, -0.1)** **	**-0.6 (-1.0, -0.2)** **	-0.4 (-0.7, 0.03)	**-1.4 (-2.4, -0.4)** **	-0.6 (-1.4, 0.3)	-0.5 (1.1, 0.2)	-0.5 (-1.1, 0.2)	**-0.5 (-0.8, -0.3)*** **	**-0.5 (-0.8, -0.2)** **
% sites CAL ≥ 6 mm	**-0.4 (-0.7, -0.1)** **	**-0.3 (-0.5, -0.02)* **	**-0.4 (-0.7, -0.1)** **	-0.2 (-0.5, 0.03)	**-0.8 (-1.5, -0.02)* **	-0.2 (-0.9, 0.4)	-0.3 (-0.8, 0.1)	-0.3 (-0.7, 0.2)	**-0.3 (-0.5, -0.1)** **	**-0.3 (-0.5, -0.1)** **
UK Biobank										
Bleeding gums (vs. healthy)	***0.8 (0.7, 0.9)****	***0.8 (0.8, 0.9)****	***0.8 (0.7, 0.9)****	***0.8 (0.7, 0.9)****	***0.9 (0.8, 0.9)****	0.9 (0.8, 1.0)	***0.8 (0.7, 0.9)****	***0.8 (0.7, 0.9)****	***0.8 (0.7, 0.9)****	***0.9 (0.8, 0.9)****
Painful gums (vs.	1.0 (0.9,	0.9	1.0 (0.9,	0.9	1.1 (0.9,	1.0	0.9 (0.8,	0.9	1.0 (0.9,	0.9

healthy)	1.2)	(0.8, 1.0)	1.1)	(0.8, 1.0)	1.3)	(0.9, 1.2)	1.2)	(0.7, 1.1)	1.2)	(0.8, 1.1)
Loose teeth (vs. healthy)	1.1 (0.9, 1.2)	0.9 (0.8, 1.1)	1.1 (0.9, 1.3)	0.9 (0.8, 1.1)	1.0 (0.9, 1.2)	0.9 (0.8, 1.1)	1.1 (0.9, 1.3)	1.0 (0.9, 1.2)	1.1 (0.9, 1.2)	1.1 (0.9, 1.2)
Any periodontal disease (vs. healthy)	***0.9 (0.8, 0.9)*** ***	***0.9 (0.8, 0.9)*** ***	***0.9 (0.8, 0.9)*** ***	***0.9 (0.8, 0.9)*** ***	0.9 (0.9, 1.1)	0.9 (0.8, 1.0)	***0.9 (0.8, 0.9)*** **	***0.9 (0.8, 0.9)*** ***	***0.9 (0.8, 0.9)*** ***	***0.9 (0.8, 0.9)*** ***

Abbreviations. CAL, clinical attachment level; CI, confidence interval; MD, difference in means; mm, millimeter; OR, odds ratio; PPD, probing pocket depth; %, percentage.

* p<0.05; ** p<0.01; *** p<0.001.

Table S8. *Association between sleep duration and periodontitis overall and by subgroups of age and sex.*

Outcomes	Proper sleep duration (vs. short/long sleep duration) as exposure – OR/MD (95% CI)									
	Overall		Age				Sex			
			< 60 years		≥ 60 years		Males		Females	
	Crude	*Adjusted*	*Crude*	*Adjusted*	*Crude*	*Adjusted*	*Crude*	*Adjusted*	*Crude*	*Adjusted*
NHANES										
Periodontitis	***0.7 (0.6, 0.8)****	***0.8 (0.7, 0.8)****	***0.7 (0.6, 0.7)****	***0.7 (0.6, 0.8)****	***0.8 (0.6, 0.9)****	***0.8 (0.7, 0.9)****	***0.8 (0.7, 0.9)****	***0.7 (0.6, 0.9)****	***0.7 (0.6, 0.8)****	***0.8 (0.7, 0.9)****
Periodontitis severity										
Mild periodontitis	***0.7 (0.6, 0.9)****	0.8 (0.6, 1.0)	***0.7 (0.6, 0.9)****	0.8 (0.6, 1.0)	0.8 (0.4, 1.7)	0.8 (0.4, 1.6)	0.8 (0.6, 1.2)	0.9 (0.6, 1.2)	***0.6 (0.4, 0.9)****	***0.7 (0.5, 0.9)****

Moderate periodontitis	**0.7 (0.7, 0.8)*** **	**0.8 (0.7, 0.9)*** **	**0.7 (0.6, 0.8)*** **	**0.7 (0.6, 0.9)*** **	**0.8 (0.6, 0.9)***	0.8 (0.7, 1.1)	**0.8 (0.6, 0.9)** **	**0.7 (0.6, 0.9)** **	**0.7 (0.6, 0.9)*** **	0.9 (0.7, 1.0)
Severe periodontitis	**0.6 (0.5, 0.7)*** **	**0.7 (0.6, 0.8)*** **	**0.6 (0.5, 0.7)*** **	**0.7 (0.6, 0.9)** **	**0.6 (0.5, 0.8)** **	**0.7 (0.5, 0.9)** **	**0.8 (0.6, 0.9)***	**0.7 (0.6, 0.9)***	**0.4 (0.3, 0.5)*** **	**0.5 (0.4, 0.6)*** **
PPD										
% sites PPD ≥ 4 mm	**-0.5 (-0.8, -0.3)*** **	-0.2 (-0.4, 0.09)	**-0.5 (-0.8, -0.2)** **	-0.09 (-0.4, 0.2)	**-0.5 (-0.9, -0.1)** **	-0.4 (-0.8, 0.03)	-0.2 (-0.6, 0.2)	0.03 (-0.4, 0.5)	**-0.7 (-0.9, -0.4)*** **	**-0.5 (-0.8, -0.2)** **
% sites PPD ≥ 5 mm	**-0.2 (-0.3, -0.07)** **	-0.07 (-0.2, 0.06)	**-0.2 (-0.3, -0.03)***	-0.05 (-0.2, 0.1)	-0.1 (-0.3, 0.05)	-0.08 (-0.3, 0.1)	-0.08 (-0.3, 0.1)	0.02 (-0.2, 0.2)	**-0.2 (-0.4, -0.1)** **	**-0.2 (-0.3, -0.03)***
% sites PPD ≥ 6 mm	**-0.09 (-0.2, -0.02)***	-0.04 (-0.1, 0.03)	**-0.1 (-0.2, -0.02)***	-0.05 (-0.1, 0.04)	-0.04 (-0.2, 0.06)	-0.02 (-0.1, 0.1)	-0.06 (-0.2, 0.06)	-0.03 (-0.2, 0.1)	**-0.09 (-0.2, -0.04)** **	**-0.08 (-0.1, -0.01)***
CAL										

% sites CAL ≥ 3 mm	**-2.3 (-3.1, -1.5)*** **	**-1.3 (-2.1, -0.6)** **	**-2.4 (-3.2, -1.6)*** **	**-1.3 (-2.0, -0.5)** **	**-2.6 (-3.9, -1.3)*** **	**-1.6 (-2.8, -0.4)** **	**-1.9 (-3.1, -0.7)** **	**-1.8 (-3.1, -0.5)** **	**-2.4 (-3.2, -1.6)*** **	**-1.2 (-2.0, -0.4)** **
% sites CAL ≥ 4 mm	**-1.4 (-1.9, -0.8)*** **	**-0.8 (-1.3, -0.2)** **	**-1.4 (-1.9, -0.8)*** **	**-0.7 (-1.3, -0.2)** *	**-1.8 (-2.9, -0.7)** **	-1.1 (-2.2, 0.05)	**-1.1 (-2.1, -0.2)** *	-1.0 (-2.0, 0.01)	**-1.5 (-2.1, -0.9)*** **	**-0.8 (-1.4, -0.3)** **
% sites CAL ≥ 5 mm	**-0.8 (-1.2, -0.4)*** **	**-0.4 (-0.8, -0.05)** *	**-0.8 (-1.2, -0.4)*** **	**-0.4 (-0.8, -0.06)** *	**-1.0 (-1.9, -0.1)** *	-0.6 (-1.5, 0.3)	-0.7 (-1.4, 0.05)	-0.6 (-1.3, 0.1)	**-0.8 (-1.2, -0.5)*** **	**-0.5 (-0.9, -0.1)** *
% sites CAL ≥ 6 mm	**-0.4 (-0.7, -0.2)** **	-0.2 (-0.5, 0.03)	**-0.4 (-0.7, -0.2)** **	-0.2 (-0.5, 0.08)	-0.5 (-1.1, 0.1)	-0.3 (-0.9, 0.3)	-0.3 (-0.7, 0.1)	-0.2 (-0.7, 0.2)	**-0.5 (-0.7, -0.2)*** **	**-0.3 (-0.6, -0.07)** *
UK Biobank										
Bleeding gums (vs. healthy)	**0.9 (0.8, 0.9)*** **	**0.9 (0.8, 0.9)*** **	**0.8 (0.8, 0.9)*** **	**0.9 (0.8, 0.9)*** **	**0.9 (0.8, 0.9)** **	**0.9 (0.8, 0.9)** *	**0.8 (0.8, 0.9)*** **	**0.8 (0.7, 0.9)*** **	**0.9 (0.8, 0.9)*** **	**0.9 (0.8, 0.9)*** **

Painful gums (vs. healthy)	0.6 (0.5, 0.7)***	0.7 (0.6, 0.7)***	0.6 (0.5, 0.7)***	0.6 (0.5, 0.7)***	0.7 (0.6, 0.8)***	0.8 (0.7, 0.9)**	0.6 (0.5, 0.7)***	0.6 (0.6, 0.8)***	0.6 (0.5, 0.7)***	0.7 (0.6, 0.8)***
Loose teeth (vs. healthy)	0.6 (0.6, 0.7)***	0.8 (0.7, 0.8)***	0.6 (0.5, 0.7)***	0.7 (0.6, 0.8)***	0.7 (0.6, 0.9)***	0.8 (0.7, 0.9)*	0.7 (0.6, 0.7)***	0.8 (0.7, 0.9)***	0.6 (0.5, 0.7)***	0.7 (0.7, 0.8)***
Any periodontal disease (vs. healthy)	0.7 (0.6, 0.8)***	0.8 (0.7, 0.9)***	0.7 (0.6, 0.8)***	0.8 (0.7, 0.8)***	0.8 (0.7, 0.9)***	0.9 (0.8, 0.9)***	0.7 (0.6, 0.8)***	0.8 (0.7, 0.9)***	0.8 (0.7, 0.8)***	0.8 (0.7, 0.9)***

Abbreviations. CAL, clinical attachment level; CI, confidence interval; MD, difference in means; mm, millimeter; OR, odds ratio; PPD, probing pocket depth; %, percentage.* p<0.05; ** p<0.01; *** p<0.001.

Table S9. *Association between categories of healthy lifestyle score (2-3 vs. 0-1 healthy lifestyles) and periodontitis in systemically healthy individuals only (sensitivity analyses) overall and by subgroups of age and sex.*

2-3 healthy lifestyles (vs. 0-1 healthy lifestyles) as exposure – OR/MD (95% CI)										
Outcomes	**Overall**		**Age**				**Sex**			
			< 60 years		**≥ 60 years**		**Males**		**Females**	
	Crude	***Adjusted***	***Crude***	***Adjusted***	***Crude***	***Adjusted***	***Crude***	***Adjusted***	***Crude***	***Adjusted***
NHANES										
Periodontitis	***0.5 (0.4, 0.7)*** ***	***0.5 (0.3, 0.7)*** ***	***0.5 (0.4, 0.6)*** ***	***0.5 (0.3, 0.7)*** ***	***0.4 (0.3, 0.6)*** **	***0.5 (0.3, 0.6)*** ***	***0.5 (0.4, 0.8)*** ***	***0.5 (0.3, 0.8)*** **	***0.5 (0.3, 0.7)*** ***	***0.4 (0.3, 0.7)*** **
Periodontitis severity										
Mild periodontitis	***0.3 (0.2,***	0.5	***0.3 (0.2,***	***0.5 (0.2, 0.9)***	NE	NE	0.5 (0.2,	0.7	***0.2 (0.09,***	0.3 (0.09,

	0.6)***	(0.2, 1.0)	**0.5)*****	*			1.1)	(0.3, 1.2)	**0.6)** ***	1.0)
Moderate periodontitis	**0.4** **(0.3, 0.5)*****	**0.4** **(0.3, 0.6)** ***	**0.3** **(0.2, 0.4)*****	**0.4** **(0.3, 0.7)** **	**0.3** **(0.2, 0.6)*****	**0.4** **(0.3, 0.8)** ***	**0.3** **(0.2, 0.6)*****	**0.4** **(0.3, 0.6)** ***	**0.4** **(0.3, 0.6)** ***	**0.5** **(0.3, 0.8)** *
Severe periodontitis	**0.1** **(0.08, 0.3)*****	**0.2** **(0.08, 0.4)** ***	**0.1** **(0.07, 0.2)*****	**0.2** **(0.07, 0.5)** **	**0.1** **(0.03, 0.2)****	**0.1** **(0.09, 0.3)** ***	**0.1** **(0.05, 0.2)** ***	**0.1** **(0.03, 0.5)*****	**0.3** **(0.09, 0.7)** **	**0.2** **(0.06, 0.9)** *
PPD										
% sites PPD ≥ 4 mm	**-1.8** **(-2.5, -1.1)*****	**-1.3** **(-1.9, -0.6)** ***	**-1.9** **(-2.6, -1.1)*****	**-1.4** **(-2.2, -0.6)** **	-0.4 (-0.5, 1.0)	-0.1 (-0.3, 0.2)	**-2.1** **(-3.4, -0.9)** **	**-1.6** **(-2.9, -0.3)** *	**-1.2** **(-2.2, -0.3)** *	-0.9 (-1.9, 0.04)
% sites PPD ≥ 5 mm	**-0.5** **(-0.8, -0.1)** *	-0.3 (-0.7, 0.04)	**-0.5** **(-0.9, -0.09)** *	-0.4 (-0.8, 0.04)	0.2 (-0.1, 0.2)	0.2 (-0.2, 0.4)	-0.7 (-1.3, 0.03)	-0.5 (-1.2, 0.2)	-0.2 (-0.5, 0.09)	-0.1 (-0.5, 0.2)
% sites PPD ≥ 6 mm	-0.1 (-0.4, 0.08)	-0.09 (-0.3, 0.1)	-0.1 (-0.4, 0.09)	-0.09 (-0.4, 0.2)	0.05 (-0.1, 0.3)	0.06 (-0.2, 0.3)	-0.2 (-0.6, 0.2)	-0.1 (-0.6, 0.3)	-0.02 (-0.1, 0.09)	-0.003 (-0.1, 0.1)

CAL										
% sites CAL ≥ 3 mm	**-5.8 (-8.7, -2.9)*** **	**-5.5 (-7.9, -3.0)*** **	**-5.8 (-8.4, -3.2)*** **	**-5.1 (-7.6, -2.7)*** **	**-11.1 (-16.2, -3.1)*** **	**-9.4 (-13.8, -2.9)*** **	**-7.1 (-10.9, -3.2)** **	**-7.1 (-11.1, -3.1)** **	**-4.0 (-6.6, -1.5)** **	**-3.9 (-6.1, -1.6)** **
% sites CAL ≥ 4 mm	**-3.9 (-6.1, -1.9)*** **	**-3.9 (-5.9, -1.9)*** **	**-4.0 (-6.1, -1.9)*** **	**-3.7 (-5.8, -1.6)*** **	**-6.4 (-9.1, -1.3)*** **	**-5.9 (-8.7, -1.9)*** **	**-5.1 (-7.8, -2.4)*** **	**-5.1 (-8.1, 2.1)** **	**-2.6 (-4.6, -0.6)* **	**-2.6 (-4.4, -0.7)** **
% sites CAL ≥ 5 mm	**-2.3 (-3.7, -0.9)** **	**-2.2 (-3.6, -0.9)** **	**-2.4 (-3.9, -0.9)** **	**-2.3 (-3.8, -0.7)** **	**-2.4 (-3.3, -1.0)** **	**-2.2 (-3.5, -1.2)** **	**-3.3 (-5.3, -1.3)** **	**-3.3 (-5.4, -1.1)** **	**-1.2 (-2.1, -0.2)* **	**-1.1 (-2.0, -0.3)* **
% sites CAL ≥ 6 mm	**-1.4 (-2.5, -0.4)** **	**-1.4 (-2.5, -0.3)* **	**-1.5 (-2.6, -0.4)* **	**-1.4 (-2.6, -0.3)* **	**-1.0 (-2.1, -0.3)* **	**-0.9 (-2.0, -0.4)** **	**-2.3 (-3.9, -0.7)** **	**-2.2 (-3.9, -0.5)* **	-0.5 (-0.9, 0.02)	**-0.5 (-0.9, -0.05)* **
UK Biobank										
Bleeding gums (vs. healthy)	0.9 (0.8, 1.1)	0.9 (0.8, 1.1)	1.0 (0.9, 1.1)	0.9 (0.9, 1.1)	0.9 (0.7, 1.1)	0.8 (0.6, 1.1)	1.0 (0.8, 1.2)	0.9 (0.8, 1.2)	0.9 (0.8, 1.0)	0.9 (0.8, 1.0)

Painful gums (vs. healthy)	0.8 (0.7, 1.0)	***0.8*** ***(0.6, 0.9)*** *	***0.8*** ***(0.6, 0.9)*** *	***0.7*** ***(0.5, 0.9)*** **	1.5 (0.7, 2.9)	1.4 (0.7, 2.8)	0.8 (0.5, 1.1)	0.7 (0.5, 1.0)	0.8 (0.6, 1.1)	0.8 (0.6, 1.1)
Loose teeth (vs. healthy)	***0.6*** ***(0.5, 0.8)*** ***	***0.6*** ***(0.5, 0.7)*** ***	***0.6*** ***(0.5, 0.8)*** ***	***0.6*** ***(0.5, 0.7)*** ***	***0.7*** ***(0.5, 0.9)*** *	***0.6*** ***(0.4, 0.9)*** *	0.7 (0.6, 1.0)	***0.7*** ***(0.5, 0.9)*** *	***0.6*** ***(0.4, 0.7)*** ***	***0.5*** ***(0.4, 0.7)*** ***
Any periodontal disease (vs. healthy)	***0.9*** ***(0.8, 0.9)*** **	***0.9*** ***(0.8, 0.9)*** **	***0.9*** ***(0.8, 0.9)*** *	***0.9*** ***(0.7, 0.9)*** *	0.9 (0.7, 1.1)	0.8 (0.7, 1.1)	0.9 (0.7, 1.1)	0.9 (0.8, 1.0)	***0.8*** ***(0.7, 0.9)*** **	***0.8*** ***(0.7, 0.9)*** **

Abbreviations. CAL, clinical attachment level; CI, confidence interval; MD, difference in means; mm, millimeter; OR, odds ratio; PPD, probing pocket depth; %, percentage.* p<0.05; ** p<0.01; *** p<0.001.

Table S10. *Association between categories of healthy lifestyle score (4-5 vs. 0-1 healthy lifestyles) and periodontitis in systemically healthy individuals only (sensitivity analyses) overall and by subgroups of age and sex.*

4-5 healthy lifestyles (vs. 0-1 healthy lifestyles) as exposure – OR/MD (95% CI)										
Outcomes	**Overall**		**Age**				**Sex**			
			< 60 years		**≥ 60 years**		**Males**		**Females**	
	Crude	***Adjusted***	***Crude***	***Adjusted***	***Crude***	***Adjusted***	***Crude***	***Adjusted***	***Crude***	***Adjusted***
NHANES										
Periodontitis	***0.3 (0.2, 0.4)*** ***	***0.3 (0.2, 0.5)*** ***	***0.3 (0.2, 0.3)*** ***	***0.3 (0.2, 0.5)*** ***	***0.3 (0.1, 0.5)*** **	***0.4 (0.2, 0.6)*** **	***0.3 (0.2, 0.4)*** ***	***0.3 (0.2, 0.5)*** ***	***0.4 (0.2, 0.5)*** ***	***0.4 (0.2, 0.6)*** ***
Periodontitis severity										
Mild periodontitis	***0.5 (0.3,***	***0.5 (0.4,***	***0.3 (0.2,***	***0.5 (0.3, 0.7)***	NE	NE	0.5 (0.2,	***0.5 (0.3, 0.7)***	***0.2 (0.09,***	0.7

	0.6)***	**0.7)*****	**0.5)*****	***			1.1)	**	**0.6)****	(0.4, 1.1)
Moderate periodontitis	**0.4** **(0.3, 0.5)*****	**0.4** **(0.3, 0.5)*****	**0.3** **(0.2, 0.4)*****	**0.3** **(0.2, 0.4)*****	**0.3** **(0.1, 0.5)*****	**0.4** **(0.3, 0.8)****	**0.3** **(0.2, 0.6)*****	**0.4** **(0.3, 0.5)*****	**0.4** **(0.3, 0.6)*****	**0.5** **(0.4, 0.6)*****
Severe periodontitis	**0.2** **(0.1, 0.2)*****	**0.2** **(0.1, 0.2)*****	**0.1** **(0.07, 0.2)*****	**0.2** **(0.1, 0.2)*****	**0.1** **(0.05, 0.3)*****	**0.2** **(0.09, 0.3)*****	**0.1** **(0.05, 0.2)*****	**0.2** **(0.1, 0.3)*****	**0.3** **(0.09, 0.7)****	**0.2** **(0.1, 0.3)*****
PPD										
% sites PPD ≥ 4 mm	**-2.8** **(-3.4, -2.1)*****	**-1.7** **(-2.4, -1.0)*****	**-2.9** **(-3.6, -2.2)*****	**-1.8** **(-2.6, -1.1)*****	**-1.0** **(-2.9, -0.1)****	-0.6 (-1.2, 0.2)	**-3.6** **(-4.8, -2.3)*****	**-2.1** **(-3.3, -0.8)****	**-1.8** **(-2.7, -0.9)*****	**-1.3** **(-2.2, -0.3)***
% sites PPD ≥ 5 mm	**-0.8** **(-1.1, -0.4)*****	**-0.5** **(-0.8, -0.1)****	**-0.8** **(-1.2, -0.4)*****	**-0.5** **(-0.9, -0.1)***	-0.08 (-0.9, 1.0)	-0.04 (-0.2, 0.9)	**-1.1** **(-1.7, -0.4)****	-0.6 (-1.3, 0.05)	**-0.4** **(-0.7, -0.1)****	-0.3 (-0.6, 0.02)
% sites PPD ≥ 6 mm	**-0.3** **(-0.5, -0.07)****	-0.2 (-0.4, 0.04)	**-0.3** **(-0.5, -0.07)***	-0.2 (-0.4, 0.06)	-0.02 (-0.1, 0.3)	-0.02 (-0.1, 0.4)	**-0.4** **(-0.8, -0.03)***	-0.2 (-0.6, 0.2)	**-0.09** **(-0.2, -0.003)***	-0.06 (-0.2, 0.04)

CAL

% sites CAL ≥ 3 mm	-8.4 (-11.2, -5.6)***	-7.2 (-9.9, -4.4)***	-8.5 (-11.1, -5.9)***	-6.5 (-9.2, -3.8)***	-14.7 (-18.1, -6.9)**	-12.6 (-21.1, -5.3)***	-10.5 (-14.6, -6.4)***	-9.0 (-13.7, -4.3)***	-5.8 (-8.3, -3.3)***	-5.3 (-7.8, -2.9)***
% sites CAL ≥ 4 mm	-5.5 (-7.5, -3.4)***	-4.9 (-6.9, -2.8)***	-5.4 (-7.5, -3.4)***	-4.4 (-6.6, -2.3)***	-9.1 (-10.8, -4.2)***	-8.1 (-10.1, -3.9)***	-7.0 (-9.7, -4.3)***	-6.2 (-9.5, -2.9)***	-3.7 (-5.6, -1.7)***	-3.5 (-5.2, -1.7)***
% sites CAL ≥ 5 mm	-3.1 (-4.4, -1.7)***	-2.7 (-4.1, -1.3)***	-3.1 (-4.5, -1.7)***	-2.6 (-4.1, -1.1)**	-3.8 (-4.1, -1.9)***	-3.3 (-3.9, -1.3)***	-4.3 (-6.2, -2.4)***	-3.8 (-6.0, -1.6)**	-1.7 (-2.6, -0.7)**	-1.6 (-2.5, -0.7)**
% sites CAL ≥ 6 mm	-1.8 (-2.8, -0.8)**	-1.6 (-2.7, -0.6)**	-1.9 (-2.9, -0.8)**	-1.6 (-2.7, -0.5)**	-1.7 (-2.8, -0.7)**	-1.5 (-2.5, -0.4)**	-2.9 (-4.4, -1.3)**	-2.5 (-4.2, -0.7)**	-0.7 (-1.2, -0.3)**	-0.7 (-1.2, -0.3)**
UK Biobank										
Bleeding gums (vs. healthy)	0.8 (0.7, 0.9)**	0.8 (0.7, 0.9)**	0.9 (0.7, 0.9)*	0.8 (0.7, 0.9)**	0.8 (0.6, 1.1)	0.8 (0.6, 1.0)	0.8 (0.7, 1.0)	0.8 (0.7, 1.0)	0.7 (0.6, 0.9)***	0.8 (0.7, 0.9)**

Painful gums (vs. healthy)	**0.8 (0.6, 0.9)***	**0.6 (0.5, 0.8)*****	**0.6 (0.5, 0.8)****	**0.5 (0.4, 0.7)*****	1.6 (0.8, 3.2)	1.4 (0.7, 2.9)	0.7 (0.5, 1.1)	0.6 (0.4, 0.8)	**0.7 (0.5, 0.9)***	**0.6 (0.5, 0.9)****
Loose teeth (vs. healthy)	**0.6 (0.5, 0.7)*****	**0.5 (0.4, 0.6)*****	**0.6 (0.4, 0.7)*****	**0.5 (0.4, 0.6)*****	**0.6 (0.4, 0.8)****	**0.5 (0.4, 0.8)****	**0.7 (0.5, 0.9)***	**0.5 (0.4, 0.8)*****	**0.5 (0.4, 0.7)*****	**0.4 (0.3, 0.6)*****
Any periodontal disease (vs. healthy)	**0.8 (0.7, 0.8)*****	**0.7 (0.7, 0.8)*****	**0.8 (0.7, 0.9)*****	**0.7 (0.6, 0.8)*****	**0.6 (0.4, 0.8)****	**0.8 (0.6, 1.0)**	**0.8 (0.7, 0.9)****	**0.7 (0.6, 0.9)*****	**0.7 (0.6, 0.8)*****	**0.7 (0.6, 0.8)*****

Abbreviations. CAL, clinical attachment level; CI, confidence interval; MD, difference in means; mm, millimeter; OR, odds ratio; PPD, probing pocket depth; %, percentage. * p<0.05; ** p<0.01; *** p<0.001.

Table S11. *Association between categories of healthy lifestyle score (2-3 vs. 0-1 healthy lifestyles) and periodontitis across subgroups of BMI (sensitivity analyses).*

2-3 healthy lifestyles (vs. 0-1 healthy lifestyles) as exposure – OR/MD (95% CI)								
Outcomes	**Underweight**		**Normal weight**		**Overweight**		**Obese**	
	Crude	***Adjusted***	***Crude***	***Adjusted***	***Crude***	***Crude***	***Adjusted***	***Crude***
NHANES								
Periodontitis	0.2 (NE)	0.2 (NE)	***0.3 (0.3, 0.4)*** ***	***0.3 (0.2, 0.5)*** ***	***0.5 (0.4, 0.7)*** ***	***0.5 (0.3, 0.7)*** ***	***0.6 (0.5, 0.7)*** ***	***0.5 (0.4, 0.7)*** ***
Periodontitis severity								
Mild periodontitis	NE	NE	***0.3 (0.1, 0.8)****	***0.3 (0.1, 0.7)****	0.7 (0.4, 1.2)	***0.9 (0.4, 2.0)***	***0.4 (0.2, 0.7)****	***0.4 (0.2, 0.8)****
Moderate periodontitis	0.02 (NE)	0.05 (NE)	***0.4***	***0.1***	***0.5***	***0.4***	***0.4***	***0.3***

			(0.3, 0.5) ***	**(0.07, 0.2)** ***	**(0.4, 0.7)** ***	**(0.3, 0.7)** ***	**(0.3, 0.6)** ***	**(0.2, 0.5)** ***
Severe periodontitis	0.004 (NE)	0.003 (NE)	**0.1** **(0.04, 0.1)** ***	**0.04** **(0.02, 0.09)** ***	**0.1** **(0.09, 0.2)** ***	**0.09** **(0.05, 0.2)** ***	**0.4** **(0.2, 0.6)** ***	**0.3** **(0.2, 0.6)** ***
PPD								
% sites PPD ≥ 4 mm	-6.4 (NE)	-6.9 (NE)	**-3.1** **(-4.2, -1.9)** ***	**-2.1** **(-3.2, -0.9)** **	**-2.4** **(-3.2, -1.6)** ***	**-2.1** **(-2.9, -1.1)** ***	**-1.0** **(-1.9, -0.09)** **	-0.7 (-1.6, 0.3)
% sites PPD ≥ 5 mm	-1.9 (NE)	-2.3 (NE)	**-0.9** **(-1.4, -0.4)** **	**-0.6** **(-1.0, -0.09)** *	**-1.0** **(-1.5, -0.5)** ***	**-0.9** **(-1.4, -0.4)** **	-0.2 (-0.7, 0.2)	-0.2 (-0.6, 0.3)
% sites PPD ≥ 6 mm	-0.9 (NE)	-0.4 (NE)	**-0.3** **(-0.6, -0.04)** *	-0.1 (-0.3, 0.09)	**-0.4** **(-0.6, -0.1)** **	**-0.3** **(-0.6, -0.09)** **	-0.03 (-0.2, 0.2)	-0.01 (-0.2, 0.2)
CAL								
% sites CAL ≥ 3 mm	-23.3 (NE)	-15.2 (NE)	**-10.4** **(-13.0, -7.7)**	**-9.1** **(-12.1, -6.2)**	**-6.7** **(-8.7, -4.6)**	**-6.9**	**-3.9** **(-5.6, -2.2)**	**-3.9**

			***	***	***	(-8.9, -4.9) ***	***	(-5.5, -2.4) ***
% sites CAL ≥ 4 mm	-23.9 (NE)	-21.2 (NE)	-6.9 (-8.8, -4.2) ***	-5.7 (-7.9, -3.6) ***	-4.7 (-6.2, -3.2) ***	-5.1 (-6.5, -3.6) ***	-2.2 (-3.4, -1.1) ***	-2.3 (-3.5, -1.1) ***
% sites CAL ≥ 5 mm	-17.8 (NE)	-13.9 (NE)	-3.9 (-5.2, -2.7) ***	-3.2 (-4.5, -1.9) ***	-2.9 (-3.9, -1.9) ***	-3.2 (-4.2, -2.2) ***	-1.0 (-1.8, -0.3) **	-1.0 (-1.8, -0.2) *
% sites CAL ≥ 6 mm	-14.6 (NE)	-6.1 (NE)	-2.0 (-2.9, -1.2) ***	-1.5 (-2.4, -0.5) **	-1.8 (-2.4, -1.1) ***	-1.8 (-2.4, -1.1) ***	-0.7 (-1.2, -0.1) *	-0.6 (-1.2, -0.008) *
UK Biobank								
Bleeding gums (vs. healthy)	0.9 (0.8, 1.1)	0.9 (0.8, 1.1)	0.7 (0.2, 2.1)	0.5 (0.2, 1.6)	0.9 (0.8, 1.0)	0.9 (0.8, 1.0)	1.1 (0.9, 1.3)	1.1 (0.9, 1.3)
Painful gums (vs. healthy)	0.9 (0.7, 1.3)	0.9 (0.7, 1.3)	1.1 (0.1, 8.9)	1.1 (0.1, 9.1)	0.9 (0.7, 1.1)	0.9 (0.7, 1.1)	1.1 (0.8, 1.6)	0.9 (0.7, 1.4)
Loose teeth (vs. healthy)	**0.5**	**0.5**	0.4	0.3	**0.6**	**0.6**	1.2	1.0

	(0.4, 0.6) ***	***(0.4, 0.6)*** ***	(0.1, 1.4)	(0.06, 1.3)	***(0.5, 0.7)*** ***	***(0.5, 0.7)*** ***	(0.9, 1.5)	(0.8, 1.3)
Any periodontal disease (vs. healthy)	***0.8*** ***(0.7, 0.9)*** **	***0.8*** ***(0.7, 0.9)*** **	0.6 (0.3, 1.5)	0.5 (0.2, 1.2)	***0.8*** ***(0.7, 0.9)*** ***	***0.8*** ***(0.7, 0.9)*** ***	1.1 (0.9, 1.3)	1.1 (0.9, 1.2)

Abbreviations. CAL, clinical attachment level; CI, confidence interval; MD, difference in means; mm, millimeter; NE, not estimable; OR, odds ratio; PPD, probing pocket depth; %, percentage.

* p<0.05; ** p<0.01; *** p<0.001.

Table S12. *Association between categories of healthy lifestyle score (4-5 vs. 0-1 healthy lifestyles) and periodontitis across subgroups of BMI (sensitivity analyses).*

4-5 healthy lifestyles (vs. 0-1 healthy lifestyles) as exposure – OR/MD (95% CI)								
Outcomes	**Underweight**		**Normal weight**		**Overweight**		**Obese**	
	Crude	***Adjusted***	***Crude***	***Adjusted***	***Crude***	***Adjusted***	***Crude***	***Adjusted***
NHANES								
Periodontitis	0.01 (NE)	0.01 (NE)	***0.2 (0.1, 0.3)*** ***	***0.2 (0.1, 0.3)*** ***	***0.4 (0.3, 0.5)*** ***	***0.4 (0.3, 0.5)*** ***	***0.4 (0.3, 0.6)*** ***	***0.4 (0.3, 0.5)*** ***
Periodontitis severity								
Mild periodontitis	***0.5 (0.3, 0.6)*** ***	***0.7 (0.5, 0.9)*** *	***0.5 (0.3, 0.6)*** ***	***0.5 (0.3, 0.8)*** **	***0.5 (0.3, 0.6)*** ***	***0.5 (0.3, 0.8)*** ***	***0.5 (0.3, 0.6)*** ***	***0.5 (0.3, 0.8)*** **

Moderate periodontitis	**0.4 (0.3, 0.5)** **	**0.6 (0.2, 0.8)** **	**0.4 (0.3, 0.5)** ***	**0.3 (0.2, 0.4)** ***	**0.4 (0.3, 0.5)** ***	**0.3 (0.2, 0.4)** ***	**0.4 (0.3, 0.5)** ***	**0.3 (0.2, 0.4)** ***
Severe periodontitis	**0.2 (0.1, 0.2)** **	**0.3 (0.1, 0.4)** ***	**0.2 (0.1, 0.2)** ***	**0.1 (0.08, 0.2)** ***	**0.2 (0.1, 0.2)** ***	**0.1 (0.08, 0.2)** ***	**0.2 (0.1, 0.2)** ***	**0.1 (0.09, 0.2)** ***
PPD								
% sites PPD ≥ 4 mm	-8.2 (NE)	-6.5 (NE)	**-4.5 (-5.6, -3.3)** ***	**-2.9 (-4.1, -1.7)** ***	**-3.2 (-3.9, -2.5)** ***	**-2.5 (-3.3, -1.7)** ***	**-1.9 (-2.7, -1.1)** ***	**-1.4 (-2.3, -0.5)** **
% sites PPD ≥ 5 mm	-2.4 (NE)	-3.1 (NE)	**-1.4 (-1.9, -0.9)** ***	**-0.8 (-1.3, -0.4)** **	**-1.2 (-1.6, -0.8)** ***	**-1.0 (-1.5, -0.5)** ***	**-0.5 (-0.9, -0.1)** *	-0.4 (-0.9, 0.07)
% sites PPD ≥ 6 mm	-1.2 (NE)	-0.5 (NE)	**-0.5 (-0.7, -0.2)** **	-0.2 (-0.4, 0.02)	**-0.4 (-0.6, -0.2)** ***	**-0.3 (-0.6, -0.1)** **	-0.1 (-0.3, 0.07)	-0.09 (-0.3, 0.2)
CAL								

% sites CAL ≥ 3 mm	-35.2 (NE)	-23.1 (NE)	**-14.8 (-17.6, -11.9)** ***	**-12.4 (-15.7, -9.1)** ***	**-8.8 (-10.9, -6.7)** ***	**-8.7 (-10.9, -6.6)** ***	**-5.9 (-7.4, -4.4)** ***	**-5.9 (-7.6, -4.1)** ***
% sites CAL ≥ 4 mm	-30.9 (NE)	-21.6 (NE)	**-10.1 (-12.1, -8.0)** ***	**-8.2 (-10.6, -5.7)** ***	**-6.2 (-7.7, -4.7)** ***	**-6.3 (-7.8, -4.8)** ***	**-3.3 (-4.5, -2.2)** ***	**-3.1 (-4.5, -1.7)** ***
% sites CAL ≥ 5 mm	-21.5 (NE)	-19.3 (NE)	**-5.9 (-7.3, -4.6)** ***	**-4.7 (-6.4, -3.1)** ***	**-3.8 (-4.8, -2.8)** ***	**-3.9 (-4.9, -2.9)** ***	**-1.7 (-2.5, -0.9)** ***	**-1.6 (-2.5, -0.6)** **
% sites CAL ≥ 6 mm	-16.9 (NE)	-11.1 (NE)	**-3.3 (-4.3, -2.4)** ***	**-2.5 (-3.6, -1.4)** ***	**-2.3 (-2.9, -1.6)** ***	**-2.2 (-2.9, -1.5)** ***	**-1.1 (-1.6, -0.6)** ***	**-0.9 (-1.6, -0.4)** **
UK Biobank								
Bleeding gums (vs. healthy)	**0.8 (0.7, 0.9)** **	**0.8 (0.7, 0.9)** *	0.4 (0.1, 1.2)	**0.3 (0.08, 0.9)** *	**0.8 (0.7, 0.9)** ***	**0.8 (0.7, 0.9)** **	0.9 (0.7, 1.1)	0.9 (0.8, 1.1)
Painful gums (vs. healthy)	0.9	0.9	0.9	0.9	0.8	**0.6**	1.1	0.8

	(0.7, 1.2)	(0.7, 1.3)	(0.1, 7.1)	(0.1, 7.4)	(0.6, 1.1)	***(0.5, 0.8)*** **	(0.8, 1.6)	(0.6, 1.2)
Loose teeth (vs. healthy)	***0.4 (0.4, 0.5)*** ***	***0.4 (0.3, 0.5)*** ***	***0.1 (0.03, 0.6)*** *	***0.1 (0.03, 0.7)*** *	***0.6 (0.5, 0.7)*** ***	***0.5 (0.4, 0.6)*** ***	1.1 (0.9, 1.5)	0.8 (0.6, 1.1)
Any periodontal disease (vs. healthy)	***0.7 (0.6, 0.8)*** ***	***0.7 (0.6, 0.8)*** ***	***0.4 (0.1, 0.9)*** *	***0.3 (0.1, 0.8)*** *	***0.8 (0.7, 0.8)*** ***	***0.7 (0.6, 0.8)*** ***	0.9 (0.8, 1.1)	***0.9 (0.8, 1.0)***

Abbreviations. CAL, clinical attachment level; CI, confidence interval; MD, difference in means; mm, millimeter; NE, not estimable; OR, odds ratio; PPD, probing pocket depth; %, percentage.

* p<0.05; ** p<0.01; *** p<0.001.

Table S13. *Association between categories of healthy lifestyle score (2-3 vs. 0-1 healthy lifestyles) and periodontitis according to last dental visit and frequency of interproximal brushing (sensitivity analyses) in the NHANES cohort.*

2-3 healthy lifestyles (vs. 0-1 healthy lifestyles) as exposure – OR/MD (95% CI)										
Outcomes	**Last dental visit**				**Frequency of interproximal brushing**					
	6 months or less		**More than 6 months**		**0 days/week**		**1-6 days/week**		**7 days/week**	
	Crude	***Adjusted***	***Crude***	***Adjusted***	***Crude***	***Adjusted***	***Crude***	***Adjusted***	***Crude***	***Adjusted***
NHANES										
Periodontitis	***0.4 (0.3, 0.5)*** ***	***0.4 (0.3, 0.6)*** ***	***0.5 (0.4, 0.7)*** ***	***0.4 (0.3, 0.6)*** ***	***0.5 (0.4, 0.6)*** ***	***0.4 (0.3, 0.5)*** ***	***0.6 (0.5, 0.7)*** ***	***0.6 (0.4, 0.8)*** ***	***0.5 (0.4, 0.7)*** ***	***0.5 (0.3, 0.6)*** ***
Periodontitis severity										
Mild	0.4	0.6	1.2	1.2	**0.3**	**0.2**	0.7	0.6	0.5	0.5

periodontitis	(0.09, 1.4)	(0.2, 2.1)	(0.4, 3.4)	(0.4, 3.7)	**(0.2, 0.6)** ***	**(0.1, 0.5)** ***	(0.4, 1.2)	(0.3, 1.1)	(0.2, 1.0)	(0.2, 1.1)
Moderate periodontitis	**0.4** **(0.3, 0.6)** ***	**0.3** **(0.2, 0.5)** ***	**0.3** **(0.2, 0.5)** ***	**0.2** **(0.1, 0.4)** ***	**0.3** **(0.2, 0.4)** ***	**0.1** **(0.01, 0.2)** ***	**0.4** **(0.3, 0.6)** ***	**03** **(0.2, 0.4)** ***	**0.5** **(0.4, 0.7)** ***	**0.3** **(0.2, 0.5)** ***
Severe periodontitis	**0.2** **(0.1, 0.3)** ***	**0.1** **(0.05, 0.2)** ***	**0.2** **(0.09, 0.3)** ***	**0.09** **(0.05, 0.2)** ***	**0.2** **(0.1, 0.3)** ***	**0.07** **(0.04, 0.1)** ***	**0.2** **(0.1, 0.3)** ***	**0.1** **(0.06, 0.2)** ***	**0.2** **(0.1, 0.3)** ***	**0.1** **(0.05, 0.2)** ***
PPD										
% sites PPD ≥ 4 mm	**-1.3** **(-1.8, -0.8)** ***	**-0.9** **(-1.6, -0.4)** **	**-1.3** **(-2.0, -0.6)** **	**-1.3** **(-2.2, -0.5)** ***	**-2.2** **(-3.4, -1.1)** ***	**-1.9** **(-3.1, -0.7)** **	**-1.3** **(-1.9, -0.6)** ***	**-0.9** **(-1.7, -0.2)** *	**-1.9** **(-2.7, -1.1)** ***	**-1.6** **(-2.5, -0.8)** ***
% sites PPD ≥ 5 mm	**-0.3** **(-0.5, -0.08)** **	**-0.2** **(-0.4, -0.009)** *	**-0.4** **(-0.7, -0.06)** *	**-0.4** **(-0.8, -0.02)** ***	**-0.8** **(-1.3, -0.2)** *	**-0.6** **(-1.2, -0.04)** *	**-0.4** **(-0.7, -0.09)** *	**-0.3** **(-0.7, -0.003)** *	**-0.6** **(-0.9, -0.2)** **	**-0.5** **(-0.9, -0.09)** *
% sites PPD ≥ 6 mm	-0.05 (-0.2, 0.05)	-0.03 (-0.1, 0.09)	-0.04 (-0.2, 0.1)	-0.04 (-0.2, -0.1)	-0.3 (-0.6, 0.02)	-0.2 (-0.5, 0.09)	-0.06 (-0.2, 0.06)	-0.01 (-0.1, 0.1)	**-0.2** **(-0.5, -0.001)** *	-0.2 (-0.5, 0.04)

CAL

% sites CAL ≥ 3 mm	-5.5 (-7.5, -3.5) ***	-4.9 (-7.2, -2.8) ***	-6.1 (-8.0, -4.1) ***	-6.9 (-9.1, -4.8) ***	-8.1 (-10.3, -6.0) ***	-9.2 (-11.5, -6.9) ***	-3.6 (-5.3, -1.9) ***	-3.8 (-5.8, -1.9) ***	-5.7 (-8.1, -3.4) ***	-6.2 (-8.4, -3.9) ***
% sites CAL ≥ 4 mm	-3.3 (-4.5, -1.9) ***	-2.9 (-4.3, -1.4) ***	-3.9 (-5.4, -2.4) ***	-4.3 (-6.0, -2.6) ***	-5.6 (-7.4, -3.9) ***	-6.3 (-8.3, -4.3) ***	-2.0 (-3.2, -0.9) **	-2.1 (-3.3, -0.8) **	-4.1 (-5.9, -2.2) ***	-4.2 (-6.2, -2.3) ***
% sites CAL ≥ 5 mm	-1.5 (-2.2, -0.7) ***	-1.2 (-2.0, -0.5) **	-2.1 (-3.2, -0.9) **	-2.4 (-3.7, -1.1) **	-3.4 (-4.7, -2.2) ***	-3.9 (-5.4, -2.5) ***	-0.9 (-1.5, -0.2) *	-0.8 (-1.6, -0.04) *	-2.3 (-3.6, -1.0) **	-2.4 (-3.8, -0.9) **
% sites CAL ≥ 6 mm	-0.6 (-0.9, -0.2) **	-0.5 (-0.8, -0.07) *	-1.2 (-1.9, -0.4) **	-1.3 (-2.2, -0.5) ***	-2.2 (-3.0, -1.4) ***	-2.6 (-3.5, -1.6) ***	-0.4 (-0.9, 0.05)	-0.3 (-0.8, 0.3)	-1.2 (-2.0, -0.4) **	-1.2 (-2.1, -0.3) *

Abbreviations. CAL, clinical attachment level; CI, confidence interval; MD, difference in means; mm, millimeter; NE, not estimable; OR, odds ratio; PPD, probing pocket depth; %, percentage.

* p<0.05; ** p<0.01; *** p<0.001.

Table S14. *Association between categories of healthy lifestyle score (4-5 vs. 0-1 healthy lifestyles) and periodontitis according to last dental visit and frequency of interproximal brushing (sensitivity analyses) in the NHANES cohort.*

4-5 healthy lifestyles (vs. 0-1 healthy lifestyles) as exposure – OR/MD (95% CI)										
Outcomes	**Last dental visit**				**Frequency of interproximal brushing**					
	6 months or less		**More than 6 months**		**0 days/week**		**1-6 days/week**		**7 days/week**	
	Crude	***Adjusted***	***Crude***	***Adjusted***	***Crude***	***Adjusted***	***Crude***	***Adjusted***	***Crude***	***Adjusted***
NHANES										
Periodontitis	**0.4 (0.3, 0.5)*** **	**0.4 (0.2, 0.6)*** **	**0.3 (0.2, 0.5)*** **	**0.2 (0.2, 0.4)*** **	**0.3 (0.2, 0.3)*** **	**0.2 (0.1, 0.2)*** **	**0.4 (0.3, 0.5)*** **	**0.4 (0.3, 0.6)*** **	**0.4 (0.3, 0.6)*** **	**0.4 (0.3, 0.5)*** **
Periodontitis severity										
Mild periodontitis	0.3 (0.09,	0.5	1.0 (0.5,	1.2	**0.2 (0.1,**	**0.2 (0.1, 0.5)**	0.5	0.5	**0.4 (0.2,**	0.4

	1.2)	(0.2, 2.1)	2.9)	(0.4, 3.9)	**0.4)** ***	***	(0.3, 1.1)	(0.2, 1.0)	**0.9)** *	(0.1, 1.0)
Moderate periodontitis	**0.3** **(0.2, 0.6)** ***	**0.2** **(0.1, 0.4)** ***	**0.3** **(0.2, 0.5)** ***	**0.2** **(0.1, 0.5)** ***	**0.2** **(0.1, 0.3)** ***	**0.1** **(0.09, 0.2)** ***	**0.4** **(0.2, 0.6)** ***	**0.2** **(0.1, 0.5)** ***	**0.4** **(0.2, 0.7)** **	**0.2** **(0.1, 0.5)** ***
Severe periodontitis	**0.1** **(0.03, 0.2)** ***	**0.09** **(0.02, 0.2)** ***	**0.1** **(0.04, 0.3)** ***	**0.09** **(0.04, 0.2)** ***	**0.1** **(0.03, 0.2)** ***	**0.07** **(0.04, 0.1)** ***	**0.1** **(0.04, 0.2)** ***	**0.1** **(0.02, 0.2)** ***	**0.1** **(0.06, 0.2)** ***	**0.1** **(0.02, 0.3)** ***
PPD										
% sites PPD ≥ 4 mm	**-1.7** **(-2.2, -1.2)** ***	**-1.3** **(-1.9, -0.8)** ***	**-2.7** **(-3.5, -1.8)** ***	**-2.5** **(-3.4, -1.6)** ***	**-3.2** **(-4.3, -2.0)** ***	**-2.4** **(-3.6, -1.2)** **	**-2.3** **(-2.9, -1.6)** ***	**-1.7** **(-2.4, -0.9)** ***	**-2.7** **(-3.5, -1.9)** ***	**-2.2** **(-3.1, -1.3)** ***
% sites PPD ≥ 5 mm	**-0.4** **(-0.6, -0.2)** ***	**-0.3** **(-0.5, -0.1)** **	**-0.9** **(-1.2, -0.6)** ***	**-0.8** **(-1.2, -0.4)** ***	**-0.9** **(-1.5, -0.4)** **	-0.5 (-1.1, 0.05)	**-0.7** **(-1.1, -0.4)** ***	**-0.6** **(-0.9, -0.2)** **	**-0.9** **(-1.3, -0.5)** ***	**-0.7** **(-1.1, -0.3)** **
% sites PPD ≥ 6 mm	-0.07 (-0.2, 0.02)	-0.04 (-0.2, 0.06)	**-0.2** **(-0.4, -0.05)** *	**-0.2** **(-0.4, -0.02)** *	**-0.3** **(-0.6, -0.02)** *	-0.1 (-0.4, 0.2)	**-0.2** **(-0.3, -0.06)** **	-0.09 (-0.2, 0.01)	**-0.3** **(-0.6, -0.09)** **	**-0.3** **(-0.6, -0.03)** *

CAL

% sites CAL ≥ 3 mm	-6.5 (-8.7, -4.3) ***	-6.0 (-8.6, -3.5) ***	-10.6 (-12.9, -8.2) ***	-10.6 (-13.4, -7.8) ***	-11.1 (-13.5, -8.7) ***	-11.9 (-14.9, -8.9) ***	-6.7 (-8.5, -4.9) ***	-6.1 (-8.3, -3.9) ***	-7.5 (-9.8, -5.3) ***	-7.8 (-10.1, -5.4) ***
% sites CAL ≥ 4 mm	-4.0 (-5.3, -2.7) ***	-3.5 (-5.1, -1.9) ***	-6.7 (-8.4, -4.9) ***	-6.4 (-8.5, -4.2) ***	-7.5 (-9.4, -5.5) ***	-7.7 (-10.2, -5.2) ***	-3.7 (-4.8, -2.6) ***	-3.3 (-4.5, -2.0) ***	-5.5 (-7.4, -3.5) ***	-5.5 (-7.6, -3.4) ***
% sites CAL ≥ 5 mm	-1.9 (-2.6, -1.3) ***	-1.7 (-2.4, -0.9) ***	-3.8 (-4.9, -2.6) ***	-3.6 (-5.2, -2.1) ***	-4.5 (-6.0, -3.1) ***	-4.8 (-6.6, -2.9) ***	-1.9 (-2.5, -1.2) ***	-1.6 (-2.3, -0.9) ***	-3.2 (-4.6, -1.8) ***	-3.2 (-4.8, -1.7) ***
% sites CAL ≥ 6 mm	-0.9 (-1.3, -0.5) ***	-0.7 (-1.1, -0.3) **	-2.2 (-2.9, -1.5) ***	-2.1 (-3.0, -1.2) ***	-2.9 (-3.9, -2.1) ***	-3.2 (-4.4, -1.9) ***	-0.9 (-1.4, -0.6) ***	-0.7 (-1.2, -0.3) **	-1.7 (-2.6, -0.8) ***	-1.7 (-2.7, -0.7) **

Abbreviations. CAL, clinical attachment level; CI, confidence interval; MD, difference in means; mm, millimeter; NE, not estimable; OR, odds ratio; PPD, probing pocket depth; %, percentage.

* p<0.05; ** p<0.01; *** p<0.001.

Table S15. *Crude association between lifestyles and last dental visit, frequency of interproximal brushing, and categories of BMI.*

	OR (95% CI)				
	Non-smoking (vs. smoking)	**Low alcohol consumption** (vs. high alcohol consumption)	**High LTPA** (vs. low LTPA)	**Healthy diet** (vs. unhealthy diet)	**Proper sleep duration** (vs. short/long sleep duration)
NHANES					
Last dental visit					
6 months or less	REF.	REF.	REF.	REF.	REF.
More than 6 months	**0.4 (0.3, 0.5)** ***	**0.6 (0.5, 0.7)** ***	**0.6 (0.5, 0.7)** ***	**0.7 (0.6, 0.8)** ***	**0.7 (0.6, 0.8)** ***
Frequency of interproximal brushing					
0 days/week	REF.	REF.	REF.	REF.	REF.
1-6 days/week	**1.6 (1.4, 1.9)** ***	**1.3 (1.1, 1.5)** **	**1.9 (1.8, 2.2)** ***	**1.5 (1.3, 1.7)** ***	**1.3 (1.1, 1.4)** ***
7 days/week	**1.9 (1.6, 2.2)** ***	**1.6 (1.4, 1.9)** ***	**2.0 (1.8, 2.3)** ***	**1.8 (1.6, 2.1)** ***	**1.3 (1.1, 1.4)** ***
BMI					
Normal weight	REF.	REF.	REF.	REF.	REF.
Underweight	**0.4 (0.2, 0.7)** **	0.9 (0.6, 1.5)	**0.5 (0.3, 0.9)** *	1.2 (0.7, 1.9)	0.8 (0.5, 1.2)
Overweight	**1.4 (1.2, 1.6)** ***	0.9 (0.8, 1.1)	**0.8 (0.7,**	**0.7 (0.6,**	0.9 (0.8, 1.0)

			0.9) **	0.8) ***	
Obese	1.3 (1.1, 1.6) **	0.8 (0.7, 0.9) *	0.5 (0.4, 0.6) ***	0.5 (0.4, 0.6) ***	0.7 (0.6, 0.8) ***
UK Biobank					
BMI					
Normal weight	REF.	REF.	REF.	REF.	REF.
Underweight	0.8 (0.7, 0.9) *	2.3 (1.9, 2.8) ***	1.0 (0.9, 1.2)	1.5 (1.2, 1.8) ***	0.7 (0.6, 0.8) ***
Overweight	0.9 (0.8, 0.9) ***	0.8 (0.7, 0.8) ***	1.0 (0.9, 1.1)	0.8 (0.7, 0.9) ***	0.8 (0.7, 0.9) ***
Obese	0.9 (0.8, 0.9) ***	1.1 (1.0, 1.2) **	1.1 (1.0, 1.2) **	0.9 (0.8, 0.9) *	0.6 (0.5, 0.7) ***

Abbreviations. CAL, clinical attachment level; CI, confidence interval; MD, difference in means; mm, millimeter; OR, odds ratio; PPD, probing pocket depth; REF, reference category; %, percentage.

* p<0.05; ** p<0.01; *** p<0.001.

Table S16. *Correlation between markers of systemic inflammation and periodontitis in both cohorts.*

		WBC	CRP	Neutrophils count
NHANES				
Periodontitis				
	R/F value	5.01	50.01	188.85
	p-value	***0.025****	***<0.001******	***<0.001******
% sites PPD ≥ 4 mm				
	R/F value	0.068	0.097	0.082
	p-value	***<0.001******	***<0.001******	***<0.001******
% sites PPD ≥ 5 mm				
	R/F value	0.035	0.078	0.047
	p-value	***<0.001******	***<0.001******	***<0.001******
% sites PPD ≥ 6 mm				
	R/F value	0.020	0.077	0.028
	p-value	***0.005****	***<0.001******	***<0.001******
% sites CAL ≥ 3 mm				
	R/F value	0.062	0.111	0.099
	p-value	***<0.001******	***<0.001******	***<0.001******
% sites CAL ≥ 4 mm				
	R/F value	0.059	0.094	0.086

	p-value	**<0.001*****	**<0.001*****	**<0.001*****
% sites CAL ≥ 5 mm				
	R/F value	0.049	0.087	0.069
	p-value	**<0.001*****	**<0.001*****	**<0.001*****
% sites CAL ≥ 6 mm				
	R/F value	0.036	0.085	0.051
	p-value	**<0.001*****	**<0.001*****	**<0.001*****
UK Biobank				
Bleeding gums				
	F value	87.85	47.68	71.77
	p-value	**<0.001*****	**<0.001*****	**<0.001*****
Painful gums				
	F value	114.79	63.96	54.51
	p-value	**<0.001*****	**<0.001*****	**<0.001*****
Loose teeth				
	F value	214.39	72.27	148.77
	p-value	**<0.001*****	**<0.001*****	**<0.001*****
Any periodontal disease (vs. healthy)				
	F value	114.09	83.14	208.87
	p-value	**<0.001*****	**<0.001*****	**<0.001*****

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Data availability statement. The data that support the findings of this study are available upon reasonable request from the corresponding author.

Authors contribution. CM contributed to study conception, to study design, to data analysis, and to manuscript drafting. SL contributed to data analysis, data interpretation, and to manuscript drafting. HS contributed to data analysis, data interpretation, and to manuscript drafting. JS contributed to study design, data interpretation, and critically revised the manuscript. FD contributed to study conception, to study design, to data analysis, and to manuscript drafting.

HEALTHY LIFESTYLES ARE ASSOCIATED WITH A BETTER RESPONSE TO PERIODONTAL THERAPY: A PROSPECTIVE COHORT STUDY.

By

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Chapter 5

HEALTHY LIFESTYLES ARE ASSOCIATED WITH A BETTER RESPONSE TO PERIODONTAL THERAPY: A PROSPECTIVE COHORT STUDY.

1 Introduction

Unhealthy lifestyle behaviours are at the root of the global burden of noncommunicable diseases (NCDs), which account for about 63% of all deaths (Marrero et al., 2012). Unhealthy lifestyle behaviours include poor nutrition, physical inactivity, poor sleep quality, high stress, and tobacco/alcohol use (Kushner & Sorensen, 2013). Each of these lifestyle behaviours constitute the multiple determinants of "health" as defined by the US Department of Health and Human Services (US Department of Health and Human Services). Over the past several years, there has been an increased interest in evaluating the benefit of adhering to healthier lifestyle behaviours in the development of morbidity and mortality (Kushner & Sorensen, 2013). In many epidemiological studies, patterns of healthy lifestyle behaviours, characterized by high diet quality (e.g., high adherence to Mediterranean Diet - MD), adequate frequency of physical activity (PA), and not smoking, were associated with a lower risk of NCDs onset, cardiovascular events, and mortality when compared to subjects with unhealthy lifestyles (Chiuve et al., 2011; Ford et al., 2009; Stringhini et al., 2010).

The detrimental impact of unhealthy lifestyles on systemic health may be mainly ascribed to the induction of a state of low-grade systemic inflammation (LGSI) and to the overproduction of reactive oxygen species (ROS), leading to oxidative stress (Esposito et al., 2004; Frodermann et al., 2019). Since LGSI has been bi-directionally linked with periodontitis (D'Aiuto et al.,

2010a; Romandini et al., 2018), the association between lifestyle behaviours and periodontitis has been analyzed in several epidemiological studies, which frequently reported a higher prevalence as well as a more severe disease phenotype in subjects with unhealthy lifestyle behaviours (Coelho et al., 2020; Karaaslan & Dikilitaş, 2019; Marruganti et al., 2022, 2023; Morales et al., 2022; Romandini et al., 2017). On these premises, unhealthy lifestyles may also negatively influence the efficacy of the Steps 1 and 2 of periodontal therapy through the molecular pathways of LGSI imbalance and ROS overproduction. Although the detrimental impact of each single unhealthy lifestyle behavior on periodontal treatment outcomes has been investigated in previous longitudinal studies (Bartha et al., 2022; Leite et al., 2018; Marruganti et al., 2022; Woelber et al., 2019; Bakri et al., 2013; Chang et al., 2021; Costa et al., 2020; Dommisch et al., 2018), little is known regarding their combined effect on periodontal treatment outcomes. Moreover, while on one hand the latest treatment guidelines recommend smoking cessation interventions as part of Step 1, it is still unclear whether other lifestyle interventions, such as dietary counselling and increase in physical activity, may provide an added benefit to periodontal therapy as evidence is still scarce (Sanz et al., 2020). Therefore, the aim of the current prospective cohort study was to investigate the association between lifestyle behaviours and the efficacy of the Steps 1/2 of periodontal therapy.

2 Materials and methods

The present study is reported according to the Strengthening the Reporting of Observational studies in Epidemiology (STROBE) guidelines for cohort studies (von Elm et al., 2008). The research protocol was

approved by the local Ethical Review Board (ERB; protocol number: 19037/2021) and was registered on Clinicaltrials.gov before commencing recruitment (NCT04769622).

2.1 *Participants*

Participants were selected among those identified in the baseline study (Marruganti et al., 2022, under review) according to the following inclusion criteria: i) presence of untreated Stage II/III periodontitis, either localized or generalized (Tonetti et al., 2018), with at least one site with Probing Pocket Depth (PPD) ≥ 4 mm; ii) presence of at least 20 remaining teeth; iii) age between 18 and 70 years old; iv) ability and willingness to give informed consent. The exclusion criteria were: i) current pregnancy or lactation; ii) periodontal therapy received during the previous 12 months; iii) antibiotics intake within the previous 6 months; iv) inability to effectively communicate in Italian. Participants were recruited between February 2021 and August 2021 and were enrolled in the study after they read and signed a written informed consent, in accordance with the Declaration of Helsinki.

2.2 *Assessment of study variables*

2.2.1 *Lifestyle behaviours*

Lifestyle behaviours were assessed by interviewers at BL by asking structured questions and giving the explanations provided by each questionnaire. In >90% of the cases, the interviewers were trained undergraduate students not involved in study clinical examination

procedures; in <10% of the cases, the interviews were made by the same clinical examiners (CM, CG) due to COVID-19 restrictions. The following four validated questionnaires were administered: i) adherence to Mediterranean Diet questionnaire (QueMD) (Gnagnarella et al., 2018); ii) International Physical Activity Questionnaire (IPAQ) (Mannocci et al., 2010); iii) Perceived Stress Score (PSS) questionnaire (Cohen et al., 1983); iv) Pittsburgh Sleep Quality Index (PSQI) questionnaire (Mondo et al., 2021). According to the sum scores obtained in each questionnaire, participants were categorized as having either high vs. low adherence to MD, moderate/high vs. low PA level, low vs. moderate/high PSS, and good vs. poor sleep quality. Details regarding the thresholds selected for all categories are provided in the Appendix. In addition, smoking status and frequency of alcohol consumption were also assessed, and thus participants were further categorized as being non-smokers vs. smokers, and consuming alcohol below vs. above the suggested intake. Further details regarding the structure of the questionnaires and their sum scores assessment methods are reported in the Appendix 1.

2.2.2 *Clinical periodontal variables*

A full-mouth periodontal examination was performed at BL and 3 months (3M) after completing the Steps 1/2 of periodontal therapy by two previously calibrated examiners (C.M., C.G.). The clinical examiners were different from the interviewers in >90% the cases, and they were therefore blinded with respect to lifestyles assessment in most cases. Examiners' calibration was performed for both Probing Pocket Depth (PPD) and recession (REC) on two non-study subjects suffering from

periodontitis, and it was considered satisfactory only when an agreement in least 95% of measurements (with a maximum of 2 mm difference) was recorded between the two examiners (C.M., C.G.). Inter-examiner agreement resulted in ICC=0.98 (p<0.001) for PPD and in ICC=0.96 (p<0.001) for REC. For the first examiner, intra-examiner agreement resulted in ICC=0.89 (p=0.002) for PPD and in ICC=0.92 (p=0.001) for REC; for the second one, intra-examiner agreement resulted in ICC=0.94 (p<0.001) for PPD and in ICC=0.98 (p<0.001) for REC. PPD, recession (REC), plaque (O'Leary et al., 1972) and bleeding on probing (BoP) (Ainamo & Bay, 1975) were recorded with a standardized periodontal probe (UNC 15 probe, HuFriedy Group, Chicago, Illinois, United States) at six sites per tooth, third molars excluded. Clinical attachment levels (CAL) were computed. Tooth mobility was measured according to the Miller index (Miller & Boenheim, 1938).

2.2.3 *Other variables*

Socio-demographic characteristics (age, gender, Body Mass Index - BMI, education) and diabetes status were collected. Household disposable income was approximated from the participant's post code. Their detailed assessment methods are reported in the Appendix 1.

2.3 *Periodontal therapy*

The included participants received Steps 1/2 of periodontal therapy by two therapists (C.M., C.G.). Briefly, Step 1 included oral hygiene instructions (OHIs) and motivation, as well as supragingival Professional

Mechanical Plaque Removal (PMPR) performed with ultrasonic instruments (Cavitron® Select SPS, Dentsply Sirona, Rome, Italy) (Step 1). Moreover, smokers received simple counseling in the form of advice to limit or possibly quit smoking (Sanz et al., 2020). No interventions aimed at modifying the other lifestyles behaviours were implemented. Step 2 of periodontal therapy included quadrant-wise subgingival instrumentation performed with both ultrasonic and hand instruments (Gracey curetters, HuFriedy, Chicago, USA) under local anesthesia (Articaine 4% with epinephrine 1:100.000) (Sanz et al., 2020). One month after the completion of the Steps 1/2, participants received a reinforcement of OHIs and motivation.

2.4 *Study outcomes*

The primary outcome of this study was the presence of a composite measure of "endpoint of therapy" defined at patient-level as the absence of sites with residual PPD ≥ 4mm with BoP, and of sites with PPD ≥ 6mm. Additional patient-level outcomes encompassed: FMPS, FMBS, % sites PPD ≥ 5mm, % PPD ≥ 6mm, and number of teeth with mobility ≥ 1. Moreover, site-level PPD, REC, CAL changes (3M - BL), and BoP at 3 months were also considered.

2.6 *Statistical analyses*

Analyses were performed using a statistical software (STATA BE, version 17, StataCorp LP, Texas, United States), *a priori* setting the level of significance at 5%. Sample size calculation has been performed for the baseline study (Marruganti et al., 2022); no specific a priori power analysis has been performed for this follow-

up examination, since all the patients identified in the baseline study fulfilling the inclusion criteria have been included in the present analysis. Continuous variables were described as Mean and Standard Deviation (SD), while the categorical ones were expressed as number of observations (percentage-%).

After verification of data distribution, the response to Steps 1/2 of periodontal therapy was evaluated comparing 3M and BL values through the paired Student's t-test. At patient-level (unilevel analyses), simple and multiple linear regression analyses were performed to investigate the association between each lifestyle behaviour (binary variables) and continuous periodontal outcomes at 3M (i.e., FMPS, FMBS, % PPD ≥ 5mm, % PPD ≥ 6mm, number of teeth with mobility ≥ 1), while simple and multiple logistic regression analyses were used to investigate the association between each lifestyle behaviour (binary variables) and the endpoint of therapy (binary outcome). At site-level (multilevel analyses), simple and multiple linear regression analyses were performed to investigate the association between each lifestyle behaviour (binary variables) and the change from baseline (3M-BL) of continuous periodontal outcomes (i.e., PPD change, PPD change for sites with PPD≥6mm, REC change, and CAL change), while simple and multiple logistic regression analyses were used to investigate the association between each lifestyle behaviour (binary variables) and the presence of BoP at 3 months (binary outcome). Whenever possible, sensitivity analyses using the lifestyle behaviours as continuous variables were also performed. In addition, the binary variable "unhealthy lifestyles" (which equaled 1 whenever each participant had: low adherence to MD *and* low PA level *and* high perceived stress *and* poor sleep quality, otherwise it equaled 0) was built and was used as a further exposure variable in the simple/multiple

regression models. Multiple regression models were adjusted as follows: i) Model 1: estimates adjusted for the value of the outcome at baseline (e.g., outcome "PPD change" adjusted for the PPD value at BL); ii) Model 2: Model 1 + BMI + diabetes + household disposable income + 3M FMPS (for patient-level analyses) or plaque at site-level (for site-level analyses); iii) Model 3: Model 2 + other lifestyle behaviours (except for the exposure(s)). Confounders were selected due to the previous knowledge of association with either the exposure, the outcome, or both (Hsu et al., 2019; Suvan et al., 2020; Tomasi et al., 2022; VanderWeele, 2019). For each multiple model, multicollinearity was tested using Pearson's correlation coefficient and Variance Inflation Factors (VIF) values; correlation coefficients ≥0.80 or VIF ≥5 indicated the presence of multicollinearity and therefore the variable with the highest correlation coefficient/VIF was eventually dropped out from the model. Results from regressions analyses were expressed as odds ratios (ORs) or difference in means (MD) with 95% Confidence Interval (CI).

3 Results

From the initially selected 235 participants, a total of 120 subjects was included in the present study (Figure S1). Most of the participants were males (58.3%) and had a mean age of 58.8 years. Fifty-five (55.0%) participants had low MD adherence, 42.5% performed low PA, 34.2% had moderate/high PSS, 39.2% had poor sleep quality, 35.8% were smokers, and 41.7% consumed alcohol above the suggested intake (Table 1). At 3M, one subject was lost to follow up due to non-study related reasons (n=119).

Table 1. *Characteristics of the study population.*

Variables	Overall (N=120)
Age (years), mean (±SD)	58.8 (±11.2)
Gender, females, N (%)	38 (31.7)
Education, N (%)	
Elementary/middle school	42 (35.0)
High school	61 (50.8)
College or more	17 (14.2)
Household disposable income, mean (±SD)	18,031.02 (±3,415.68)
BMI (kg/m^2), mean (±SD)	27.2 (±4.4)
Diabetes, yes, N (%)	15 (12.5)
Low aMed, N (%)	66 (55.0)
Low PA, N (%)	51 (42.5)
Moderate/high PSS, N (%)	41 (34.2)
Poor sleep quality, N (%)	47 (39.2)
Smoking, N (%)	
Non-smokers	77 (64.2)
Smokers	43 (35.8)
Alcohol use, N (%)	
Below the suggested intake	70 (58.33)
Above the suggested intake	50 (41.67)
aMed, mean (±SD)	4.37 (±1.66)

MET-min/week (PA), mean (±SD)	2,703.55 (±3,840.47)
PSS, mean (±SD)	11.41 (±6.66)
PSQI, mean (±SD)	4.93 (±3.00)

Abbreviations. aMed, alternate Mediterranean diet score; BMI, Body Mass Index; MET-min/week, metabolic equivalent of task minutes per week; N, number; PA, Physical Activity; PSS, Perceived Stress Score; PSQI, Pittsburgh Sleep Quality Index; SD, Standard Deviation; %, percentage.

Overall, 76 (63.9%) participants achieved the endpoint of therapy at 3M after Steps 1/2. Steps 1/2 led to a significant reduction of FMPS and FMBS, as well as a significant reduction in the % of sites with PPD ≥ 5mm and PPD ≥ 6 mm ($p<0.001$). The mean number of teeth with mobility almost halved from BL to 3M (Table 2).

***Table 2**. Periodontal variables at baseline and 3-month follow up (patient- and site-level).*

Variables	BL (N=120)	3M (N=119)	3M-BL
Patient-level			
FMPS, %, mean (±SD)	73.15 (±18.78)	18.69 (±14.67)	***-54.46 (±16.13)****
FMBS, %, mean (±SD)	37.73 (±20.37)	13.73 (±10.65)	***-24.00 (±17.30)****
% PPD ≥ 5 mm, mean (±SD)	10.60 (±9.97)	3.99 (±5.23)	***-6.61 (±8.49)****
% PPD ≥ 6 mm, mean (±SD)	4.03 (±6.50)	1.64 (±3.05)	***-2.39 (±4.73)****
n teeth with mobility ≥ 1, mean (±SD)	1.96 (±3.16)	1.00 (±2.91)	***-0.96 (±3.79)****
% sites with PPD ≥ 4 mm	21.41 (±14.23)	4.70 (±5.91)	***-16.71 (±7.02)****

with BoP, and PPD ≥ 6 mm			
Endpoint of therapy†, yes, N (%)	-	76 (63.33)	-
Site-level			
PPD, mm, mean (±SD)	2.59 (±1.37)	2.34 (±1.15)	***-0.25 (±1.28)*** **
PPD (sites with PPD≥6mm), mm, mean (±SD)	6.42 (±0.90)	3.19 (±1.57)	***-3.23 (±1.81)*** *
REC, mm, mean (±SD)	0.10 (±0.61)	0.18 (±0.73)	***0.08 (±0.71)*** **
CAL, mm, mean (±SD)	2.70 (±1.49)	2.53 (±1.34)	***-0.17 (±1.46)*** *

Abbreviations. BL, baseline; FMBS, Full Mouth Bleeding Score; FMPS, Full Mouth Plaque Score; N, number; PPD, Probing Pocket Depth; SD, Standard Deviation; 3M, 3 months; %, percentage.

* $p<0.05$

** $p<0.01$

*** $p<0.001$

† Participants with no sites with PPD ≥ 4 mm with BoP, and PPD ≥ 6 mm.

Subjects with poor sleep quality (OR=0.13; 95% CI: 0.03, 0.47; $p<0.01$), smokers (OR=0.18; 95% CI: 0.06, 0.52; $p<0.05$), and excessive alcohol use (OR=0.21; 95% CI: 0.07, 0.63; $p<0.01$) demonstrated a reduced rate of endpoint of therapy in the fully adjusted model (Model 3), while subjects with low aMed (OR=0.51; 95% CI: 0.18, 1.46; $p>0.05$), low PA (OR=0.52; 95% CI: 0.18, 1.49; $p>0.05$), and moderate/high PSS (OR=0.65; 95% CI: 0.18, 2.38; $p>0.05$) showed the same tendency, even though not statistically significant. Furthermore, smokers showed a higher proportion of residual PPD ≥ 5 mm (Table 3).

Table 3. *Simple and multiple linear/logistic regression analyses for the association between lifestyle behaviours and periodontal outcomes at 3M (patient-level).*

Low aMed – MD/OR (95% CI)				
Outcomes	**Crude**	**Model 1†**	**Model 2‡**	**Model 3§**
FMPS¶, %	0.79 (-5.31, 6.89)	0.37 (-4.81, 5.54)	0.30 (-5.52, 5.00)	-0.23 (-5.72, 5.20)
FMBS, %	2.09 (-1.80, 5.97)	1.47 (-1.86, 4.80)	1.59 (-1.42, 4.66)	1.03 (-2.10, 4.21)
% PPD ≥ 5 mm	3.60 (-0.08, 5.69)	2.62 (-0.04, 5.85)	2.11 (-0.03, 4.24)	1.37 (-0.02, 3.03)
% PPD ≥ 6 mm,	0.10 (-0.30, 1.91)	0.10 (-0.27, 1.44)	0.60 (-0.46, 1.28)	0.56 (-0.34, 1.44)
n teeth with mobility ≥ 1, mean (±SD)	-0.68 (-1.75, 0.38)	-0.66 (-1.73, 0.41)	-0.67 (-1.79, 0.44)	-0.60 (-1.76, 0.56)
Endpoint of therapy¶, yes, N (%)	0.77 (0.36, 1.63)	0.83 (0.39, 1.79)	0.92 (0.39, 2.11)	0.50 (0.18, 1.47)
Low PA – MD/OR (95% CI)				
Outcomes	**Crude**	**Model 1†**	**Model 2‡**	**Model 3§**
FMPS¶, %, mean (±SD)	5.59 (-0.47, 11.64)	2.82 (-2.41, 8.06)	2.80 (-2.43, 8.04)	1.37 (-4.44, 7.18)
FMBS, %, mean (±SD)	3.35 (-0.52, 7.23)	2.02 (-1.34, 5.38)	1.10 (-2.09, 4.21)	1.87 (-1.52, 5.25)
% PPD ≥ 5 mm, mean (±SD)	1.57 (-0.33, 3.47)	0.73 (-0.92, 2.39)	0.69 (-0.99, 2.37)	0.42 (-1.38, 2.22)
% PPD ≥ 6 mm, mean (±SD)	0.83 (-0.29, 0.02)	0.13 (-0.16, 1.11)	0.34 (-0.41, 0.99)	-0.30 (-1.03, 0.64)
n teeth with mobility ≥ 1, mean (±SD)	0.66 (-0.41, 1.74)	0.49 (-0.60, 1.59)	0.56 (-0.60, 1.69)	0.67 (-0.59, 1.92)
Endpoint of therapy¶,	0.62 (0.29,	0.68 (0.32,	0.75 (0.33,	0.54 (0.19, 1.49)

yes, N (%)	1.31)	1.46)	1.71)	
Moderate/High PSS – MD/OR (95% CI)				
Outcomes	**Crude**	**Model 1†**	**Model 2‡**	**Model 3§**
FMPS‖, %, mean (±SD)	2.89 (-3.53, 9.29)	4.91 (-0.49, 10.30)	4.18 (-1.38, 9.72)	5.14 (-1.30, 11.58)
FMBS, %, mean (±SD)	-2.49 (-6.58, 1.59)	-2.23 (-5.72, 1.26)	-1.81 (-5.09, 1.47)	-0.99 (-4.65, 2.66)
% PPD ≥ 5 mm, mean (±SD)	0.42 (-1.60, 2.44)	0.19 (-1.53, 1.92)	0.04 (-1.73, 1.80)	0.74 (-1.24, 2.71)
% PPD ≥ 6 mm, mean (±SD)	0.15 (-1.02, 1.33)	0.08 (-0.82, 0.99)	0.03 (-0.87, 0.93)	0.11 (-0.95, 1.13)
n teeth with mobility ≥ 1, mean (±SD)	-0.04 (-1.17, 1.10)	-0.18 (-1.34, 0.97)	-0.29 (-1.49, 0.92)	0.05 (-1.35, 1.44)
Endpoint of therapy‖, yes, N (%)	1.96 (0.86, 4.46)	0.48 (0.21, 1.11)	***0.34 (0.13, 0.92)****	0.65 (0.18, 2.38)
Poor sleep quality – MD/OR (95% CI)				
Outcomes	**Crude**	**Model 1†**	**Model 2‡**	**Model 3§**
FMPS‖, %, mean (±SD)	-0.37 (-6.58, 5.85)	0.99 (-4.28, 6.26)	0.25 (-5.11, 5.59)	-2.29 (-8.29, 3.70)
FMBS, %, mean (±SD)	***4.20 (0.30, 8.10)****	1.92 (-0.52, 5.38)	1.85 (-0.49, 4.11)	2.01 (-0.37, 4.92)
% PPD ≥ 5 mm, mean (±SD)	1.41 (-0.52, 3.35)	0.78 (-0.89, 2.44)	1.05 (-0.67, 2.72)	1.58 (-0.31, 3.50)
% PPD ≥ 6 mm, mean (±SD)	-0.53 (-1.67, 0.61)	0.32 (-0.58, 1.21)	0.46 (-0.41, 1.37)	0.51 (-0.51, 1.53)
n teeth with mobility ≥ 1, mean (±SD)	-0.39 (-1.48, 0.70)	-0.48 (-1.58, 0.62)	-0.55 (-1.69, 0.60)	-0.64 (-1.99, 0.69)
Endpoint of therapy‖, yes, N (%)	***0.31 (0.12, 0.59)****	***0.27 (0.11, 0.65)****	***0.22 (0.08, 0.59)****	***0.13 (0.04, 0.47)****
Smoking – MD/OR (95% CI)				

Outcomes	Crude	Model 1†	Model 2‡	Model 3§
FMPS‖, %, mean (±SD)	2.47 (-3.87, 8.81)	1.60 (-3.77, 6.98)	1.50 (-3.88, 6.87)	1.85 (-3.77, 7.47)
FMBS, %, mean (±SD)	-2.19 (-6.24, 1.85)	-0.50 (-4.01, 3.01)	-0.58 (-3.84, 2.72)	-0.42 (-3.76, 2.93)
% PPD ≥ 5 mm, mean (±SD)	***2.89 (0.97, 4.82)*** **	***2.06 (0.38, 3.74)*** *	***2.07 (0.38, 3.76)*** *	***2.26 (0.47, 4.02)*** *
% PPD ≥ 6 mm, mean (±SD)	***1.19 (0.05, 2.34)*** *	0.79 (-0.10, 1.68)	0.70 (-0.17, 1.57)	0.87 (-0.06, 1.77)
n teeth with mobility ≥ 1, mean (±SD)	0.48 (-0.63, 1.58)	0.51 (-0.61, 1.62)	0.51 (-0.64, 1.65)	0.48 (-0.75, 1.68)
Endpoint of therapy¶, yes, N (%)	***0.33 (0.15, 0.71)*** **	***0.35 (0.16, 0.77)*** **	***0.33 (0.14, 0.74)*** **	***0.18 (0.06, 0.52)*** *
Alcohol use (above suggested intake) – MD/OR (95% CI)				
Outcomes	**Crude**	**Model 1†**	**Model 2‡**	**Model 3§**
FMPS‖, %, mean (±SD)	-2.12 (-8.26, 4.02)	-3.70 (-8.89, 1.48)	-3.34 (-8.67, 1.97)	-3.84 (-9.37, 1.72)
FMBS, %, mean (±SD)	***5.74 (1.95, 9.54)*** **	***3.44 (1.23, 5.66)*** **	***2.74 (1.08, 5.80)*** *	1.06 (-0.66, 4.65)
% PPD ≥ 5 mm, mean (±SD)	1.10 (-0.83, 3.01)	0.77 (-0.87, 2.42)	0.65 (-1.07, 2.34)	1.08 (-0.64, 2.81)
% PPD ≥ 6 mm, mean (±SD)	-0.80 (-1.88, 0.36)	-0.21 (-1.09, 0.65)	0.10 (-1.01, 0.76)	0.44 (-0.70, 1.12)
n teeth with mobility ≥ 1, mean (±SD)	-0.84 (-1.92, 0.23)	-0.64 (-1.74, 0.47)	-0.78 (-1.93, 0.40)	-0.73 (-1.94, 0.50)
Endpoint of therapy¶, yes, N (%)	0.52 (0.24, 1.12)	0.53 (0.24, 1.18)	***0.39 (0.16, 0.95)*** *	***0.21 (0.07, 0.63)*** **

Abbreviations. aMed, Alternate Mediterranean Diet Score; BL, baseline; FMBS, Full Mouth Bleeding Score; FMPS, Full Mouth Plaque Score; N, number; PA, Physical Activity; PPD, Probing Pocket Depth; PSS, Perceived Stress Score; SD, Standard Deviation; 3M, 3 months; %, percentage.

* $p<0.05$

** $p<0.01$

† Model 1: Adjusted for its baseline value.

‡ Model 2: Model 1 + BMI + diabetes + household disposable income + 3M FMPS.

§ Model 3: Model 2 + the remaining five lifestyle behaviours (except for the main exposure)

†† Participants with no sites with PPD ≥ 4 mm with BoP, and PPD ≥ 6 mm.

‡‡ Multiple models unadjusted for 3M FMPS.

At site-level, low aMed and smoking showed a negative association with PPD, REC, and CAL changes, while moderate/high PSS and excessive alcohol use only with CAL and PPD (only considering sites with PPD ≥ 6 mm at baseline), respectively. Moreover, low PA, poor sleep quality, and excessive alcohol use resulted associated with higher odds of BoP-positive sites at 3 months (Table 4). These trends of association were confirmed also by the sensitivity analyses performed using the exposures - lifestyle behaviours - as continuous variables (Tables S1-S2).

Table 4. *Simple and multiple linear/logistic regression analyses for the association between lifestyle behaviours and the changes in periodontal outcomes (3M - BL) (site-level).*

	Low aMed – MD/OR (95% CI)			
Outcomes	**Crude**	**Model 1†**	**Model 2‡**	**Model 3§**
PPD change, mm	0.17 (-0.005, 0.34)	***0.17 (0.01, 0.34)****	***0.21 (0.05, 0.37)****	***0.17 (0.02, 0.33)****

PPD change (sites with PPD≥6mm), mm	0.16 (-0.37, 0.68)	0.25 (-0.25, 0.76)	0.33 (-0.17, 0.82)	0.35 (-0.10, 0.82)
REC change, mm	0.02 (-0.06, 0.09)	0.02 (-0.03, 0.07)	***0.04 (0.01, 0.07)****	***0.04 (0.02, 0.08)****
CAL change, mm	0.15 (-0.05, 0.35)	0.15 (-0.06, 0.35)	0.17 (-0.01, 0.34)	***0.17 (0.02, 0.34)****
BoP, yes	***1.10 (1.01, 1.20)****	1.08 (0.99, 1.18)	1.04 (0.96, 1.20)	1.02 (0.92, 1.10)
Low PA – MD/OR (95% CI)				
Outcomes	**Crude**	**Model 1†**	**Model 2‡**	**Model 3§**
PPD change, mm	0.11 (-0.06, 0.28)	***0.24 (0.08, 0.40)****	***0.23 (0.08, 0.39)****	0.16 (-0.01, 0.34)
PPD change (sites with PPD≥6mm), mm	0.23 (-0.29, 0.75)	0.12 (-0.38, 0.63)	0.17 (-0.30, 0.65)	0.16 (-0.32, 0.69)
REC change, mm	-0.02 (-0.10, 0.06)	-0.01 (-0.10, 0.08)	0.01 (-0.08, 0.12)	0.06 (-0.06, 0.15)
CAL change, mm	0.09 (-0.11, 0.29)	***0.23 (0.02, 0.44)****	***0.22 (0.03, 0.42)****	0.21 (-0.11, 0.41)
BoP, yes	***1.33 (1.22, 1.45)*****	***1.24 (1.13, 1.36)*****	***1.21 (1.11, 1.43)****	***1.19 (1.13, 1.40)****
Moderate/High PSS – MD/OR (95% CI)				
Outcomes	**Crude**	**Model 1†**	**Model 2‡**	**Model 3§**
PPD change, mm	***0.26 (0.09, 0.44)****	***0.22 (0.05, 0.39)****	***0.19 (0.02, 0.36)****	0.16 (-0.03, 0.35)
PPD change (sites with PPD≥6mm), mm	0.17 (-0.39, 0.73)	0.12 (-0.42, 0.67)	0.03 (-0.51, 0.58)	0.02 (-0.47, 0.50)
REC change, mm	***0.09 (0.006, 0.17)****	***0.10 (0.01, 0.19)****	***0.08 (0.01, 0.17)****	0.06 (-0.05, 0.21)
CAL change, mm	***0.37 (0.17, 0.57)*****	***0.33 (0.12, 0.54)****	***0.28 (0.07, 0.49)****	***0.22 (0.01, 0.45)****

BoP, yes	0.89 (0.78, 1.03)	0.90 (0.82, 1.06)	0.94 (0.84, 1.07)	0.98 (0.89, 1.16)††
Poor sleep quality – MD/OR (95% CI)				
Outcomes	**Crude**	**Model 1†**	**Model 2‡**	**Model 3§**
PPD change, mm	***0.28 (0.12, 0.46)*****	***0.23 (0.06, 0.29)****	0.12 (-0.07, 0.26)	0.05 (-0.12, 0.24)
PPD change (sites with PPD≥6mm), mm	0.12 (-0.45, 0.69)	0.09 (-0.59, 0.52)	0.10 (-0.62, 0.43)	0.08 (-0.46, 0.61)
REC change, mm	***0.08 (0.003, 0.17)****	***0.09 (0.002, 0.18)****	0.08 (-0.005, 0.17)	0.09 (-0.02, 0.19)
CAL change, mm	***0.37 (0.18, 0.56)*****	***0.21 (0.007, 0.42)****	0.21 (-0.02, 0.39)	0.16 (-0.09, 0.38)
BoP, yes	***1.43 (1.30, 1.56)*****	***1.31 (1.19, 1.45)****	***1.37 (1.23, 1.52)****	***1.34 (1.19, 1.49)****
Smoking – MD/OR (95% CI)				
Outcomes	**Crude**	**Model 1†**	**Model 2‡**	**Model 3§**
PPD change, mm	0.08 (-0.09, 0.24)	0.15 (-0.02, 0.32)	***0.21 (0.03, 0.43)****	***0.28 (0.02, 0.46)****
PPD change (sites with PPD≥6mm), mm	***0.64 (0.12, 1.15)****	***0.53 (0.03, 1.03)****	***0.80 (0.31, 1.29)****	***0.85 (0.39, 1.31)*****
REC change, mm	0.03 (-0.05, 0.11)	0.04 (-0.05, 0.13)	***0.04 (0.01, 0.14)****	***0.06 (0.01, 0.16)****
CAL change, mm	0.12 (-0.09, 0.33)	0.20 (-0.01, 0.42)	***0.18 (0.02, 0.38)****	***0.17 (0.03, 0.38)****
BoP, yes	***0.84 (0.76, 0.92)*****	***0.89 (0.81, 0.98)****	0.90 (0.83, 1.04)	0.91 (0.79, 1.02)
Alcohol use (above suggested intake) – MD/OR (95% CI)				
Outcomes	**Crude**	**Model 1†**	**Model 2‡**	**Model 3§**
PPD change, mm	0.09 (-0.08, 0.27)	0.08 (-0.09, 0.25)	0.08 (-0.10, 0.23)	0.12 (-0.03, 0.29)

PPD change (sites with PPD≥6mm), mm	0.39 (-0.12, 0.91)	***0.62 (0.13, 1.10)****	0.44 (-0.08, 0.96)	***0.73 (0.23, 1.22)*****
REC change, mm	0.05 (-0.03, 0.13)	0.02 (-0.07, 0.11)	0.04 (-0.06, 0.15)	0.05 (-0.04, 0.14)
CAL change, mm	0.14 (-0.06, 0.34)	0.10 (-0.11, 0.31)	0.12 (-0.12, 0.33)	0.11 (-0.09, 0.32)
BoP, yes	***1.43 (1.31, 1.57)******	***1.47 (1.34, 1.61)******	***1.37 (1.25, 1.51)*****	***1.31 (1.19, 1.45)*****

Abbreviations. aMed, Alternate Mediterranean Diet Score; BoP, Bleeding on Probing; BL, baseline; CAL, Clinical Attachment Level; CI, Confidence Interval; MD, Mean Difference; mm, millimeters; OR, Odds Ratio; PA, Physical Activity; PPD, Probing Pocket Depth; PSS, Perceived Stress Score; REC, recession; SD, Standard Deviation; 3M, 3 months.

* $p<0.05$

** $p<0.01$

*** $p<0.001$

† Model 1: Adjusted for its baseline value.

‡ Model 2: Model 1 + BMI + diabetes + household disposable income + Plaque at site-level

§ Model 3: Model 2 + the remaining five lifestyle behaviours (except for the main exposure)

†† Smoking was dropped out from the model due to multicollinearity.

Subjects with a combination of unhealthy newly-explored lifestyles (low aMed *and* low PA *and* moderate/high PSS *and* poor sleep quality) showed a reduced probability of reaching the endpoint of therapy (OR=0.85; 95% CI: 0.32, 0.99; $p<0.05$), even after adjusting for smoking and alcohol. Those subjects also showed a higher proportion of residual PPD ≥ 6 mm, and a reduced site-level impact of the Steps 1/2 of periodontal therapy in PPD, REC, and CAL changes (Table 5).

Table 5. *Simple and multiple linear/logistic regression analyses for the relationship between the combination of unhealthy lifestyles and the response to periodontal treatment at 3M (site- and patient-level).*

Unhealthy lifestyles† - MD/OR (95% CI)				
Variables	**Crude**	**Model 1ǂ**	**Model 2§**	**Model 3¶**
Patient-level				
FMPS, %, mean (±SD)	4.80 (-4.59, 14.19)	6.06 (-1.85, 13.97)	5.53 (-2.43, 13.50)	5.06 (-2.97, 13.12)
FMBS, %, mean (±SD)	0.15 (-5.89, 6.17)	1.54 (-3.62, 6.70)	1.39 (-3.43, 6.20)	1.81 (-3.11, 6.69)
% PPD ≥ 5 mm, mean (±SD)	***3.16 (0.25, 6.06)****	2.18 (-0.32, 4.69)	2.13 (-0.41, 4.66)	1.78 (-0.72, 4.24)
% PPD ≥ 6 mm, mean (±SD)	***2.09 (0.4, 3.78)****	***1.68 (0.39, 2.98)****	***1.59 (0.32, 2.86)****	***1.51 (0.23, 2.80)****
n teeth with mobility ≥ 1, mean (±SD)	0.97 (-0.67, 2.61)	0.96 (-0.67, 2.59)	0.93 (-0.74, 2.59)	0.79 (-0.89, 2.45)
Endpoint of therapy‡‡, yes, N (%)	0.75 (0.24, 2.31)	0.89 (0.47, 1.28)	***0.81 (0.31, 0.99)****	***0.85 (0.32, 0.99)****
Site-level¶				
PPD change, mm, mean (±SD)	***0.59 (0.35, 0.85)*****	***0.56 (0.32, 0.80)*****	***0.54 (0.31, 0.78)*****	***0.52 (0.30, 0.82)*****
PPD change (sites with PPD≥6mm), mm, mean (±SD)	0.41 (-0.37, 1.19)	0.50 (-0.25, 1.25)	0.56 (-0.18, 1.23)	0.27 (-0.43, 0.93)
REC change, mm, mean (±SD)	0.07 (-0.05,	***0.19 (0.05, 0.33)****	***0.17 (0.04, 0.30)****	***0.17 (0.03, 0.30)****

0.19)

CAL change, mm, mean (±SD)	***0.67 (0.37, 0.96)*****	***0.77 (0.47, 1.07)*****	***0.74 (0.45, 1.02)*****	***0.73 (0.40, 1.02)*****
BoP, yes	1.11 (0.97, 1.28)	1.07 (0.93, 1.24)	***1.17 (1.10, 1.34)****	***1.10 (1.01, 1.27)****

Abbreviations. BL, baseline; BMI, Body Mass Index; BoP, Bleeding on Probing; CAL, Clinical Attachment Level; CI, Confidence Interval; MD, Mean Difference; mm, millimeters; PPD, Probing Pocket Depth; REC, recession; SD, Standard Deviation; 3M, 3 months.

* $p<0.05$

** $p<0.01$

*** $p<0.001$

† Binary variable "unhealthy lifestyles", which equaled 1 whenever each participant had: low adherence to Mediterranean Diet AND low physical activity level AND high perceived stress AND poor sleep quality; otherwise it equaled 0.

‡ Model 1: Adjusted for its baseline value.

§ Model 2: Model 1 + BMI + diabetes + household disposable income + 3M FMPS for patient-level analyses and Plaque at the site for site-level analyses

¶ Model 3: Model 2 + smoking + alcohol.

4 Discussion

The results from the present study indicated a significant association between lifestyle behaviours and the clinical outcomes after Steps 1/2 of periodontal therapy. Specifically, poor sleep quality, smoking, and alcohol use above the suggested intake were associated with significantly lower rates of endpoint of therapy; the same tendency was also observed for low MD adherence, low PA level, and high perceived stress, even if not reaching statistical significance. Moreover, subjects reporting a

combination of low MD adherence, low PA level, high perceived stress, and poor sleep quality showed reduced rates of endpoint of therapy and a higher proportion of residual PPD ≥ 6 mm.

In the current investigation, Steps 1/2 led to a reduction in the proportion of sites with PPD≥5mm of around 60%, which is consistent with previous meta-analytical data (Suvan, Leira, et al., 2020). Nonetheless, the proportion of residual pockets achieved in the current study varied widely across subgroups of lifestyle behaviours. These differences were consistent and significant also after adjusting for baseline values, patient's compliance (plaque at 3M), systemic conditions/diseases (i.e., BMI and diabetes), as well as the other tested lifestyle behaviours, that could have otherwise influenced the subject's response to Steps 1/2 (Hsu et al., 2019; Suvan, Leira, et al., 2020). Specifically, the magnitude of the association of poor sleep quality and excessive alcohol use was similar or even greater than the one noted for smoking, which is considered as the major behavioural risk factor for periodontitis onset, severity, and response to treatment (Chang et al., 2021; Labriola et al., 2005; Leite et al., 2018). The association between the investigated lifestyle behaviours and the clinical response after Steps 1/2 of periodontal therapy resulted independent from possible confounders, including plaque control. Hence, a direct biological effect of lifestyle behaviours on the periodontium is suggested. These findings may be relevant for clinicians since those behaviours may represent potential targets of interventions in the context of the Step 1 of periodontal therapy (Sanz et al., 2020), and for researchers since they may represent potential confounders to be considered when analyzing risk factors of periodontitis or its association with

systemic diseases (Botelho et al., 2022; Baima et al., 2022; Romandini et al., 2020, 2021; Antonoglou et al., 2023; Marruganti et al., 2023b, 2023c; D'Aiuto et al., 2018).

At the local level, previous investigations indicated that participants under high psychosocial stress exhibited lower PPD and CAL changes 6 months after Steps 1/2 of periodontal therapy (Bakri et al., 2013). Consistently, the administration of supplemental micronutrients (e.g., vitamin C, D, E, calcium, magnesium, etc.) as well as specific dietary patterns (e.g., anti-inflammatory or Mediterranean diet) were shown to improve periodontal clinical measures (Bartha et al., 2022; Dommisch et al., 2018; Woelber et al., 2019). At the systemic level, low MD adherence, low PA, high stress, and poor sleep quality were shown to lead to an increased LGSI state through a variety of molecular pathways (Besedovsky et al., 2019; Esposito et al., 2004; Frodermann et al., 2019; Sabbah et al., 2018). In particular, a low MD adherence dietary pattern is usually characterized by the frequent consumption of pro-inflammatory foods, such as white flour and processed meats, which also contribute to increasing oxidative stress levels (Christ et al., 2019). Similar molecular pathways leading to an imbalance of LGSI and an overproduction of ROS were also involved in the association between low PA (or sedentary lifestyle), poor sleep quality and periodontitis, respectively (Besedovsky et al., 2019; Frodermann et al., 2019). In addition to these mechanisms, high stress was also found to exert an immunosuppressive action on the immune system (e.g., by reducing lymphocyte proliferation and antibody production) and to trigger other behavioural adaptive changes (e.g., alcohol/drug use, increased smoking, etc.) (Sabbah et al., 2018). Since it has been previously shown that these molecular pathways can

negatively influence the periodontium and render the subjects more prone to periodontitis onset and rapid progression (Baima et al., 2021; D'Aiuto et al., 2010; Pink et al., 2015), it can be hypothesized that the same mechanisms can reduce the efficacy of Steps 1/2, as observed in the current investigation.

The biological mechanisms underpinning the detrimental impact of smoking and alcohol on the periodontium are well established. Indeed, several investigations have suggested that smoking is related to a specific pattern of periodontal pathogens colonization and to high levels of pathogenic species even in shallow sites, while, from an immunoinflammatory standpoint, smoking was shown to increase oxidative stress and to trigger cytokine overproduction through the modification of intracellular signaling mechanisms (Labriola et al., 2005; Nociti et al., 2014). Similarly, the possible mechanisms linking excessive alcohol consumption to periodontitis may be mainly ascribed to cytokines overproduction as well as to the impairment of the immune host defense (i.e., alteration of neutrophils, macrophages, and T-cells functioning) (Barr et al., 2016; Szabo & Saha, 2015). From a clinical standpoint, results from the current study are consistent with previous longitudinal investigations showing a detrimental impact of smoking and excessive alcohol consumption on periodontal treatment outcomes (Chang et al., 2021; Costa et al., 2020; Ryder et al., 2018).

The present study also indicated how subjects reporting a combination of low MD adherence, low PA, high stress, and poor sleep quality had a poorer response to periodontal therapy. These results are in line with the

current medical literature focusing on lifestyle behaviours not as separate, independent entities, but instead as a closely intertwined pattern. Indeed, several prospective cohort studies highlighted how a combination of unhealthy lifestyle factors (e.g., diet quality, physical activity level, smoking, stress levels, etc.) was significantly associated with a shorter life expectancy free from multiple chronic diseases, such as diabetes, cardiovascular diseases, and cancer (Nyberg et al., 2018; O'Doherty et al., 2016; Stenholm et al., 2016). In addition, it can also be hypothesized that subjects undertaking a pattern of unhealthy lifestyle behaviours may be less prone to brush regularly, and thus be unmotivated to maintain oral health (Sakki, 1995; Sabbah et al., 2018). Therefore, the promotion of a comprehensive healthy lifestyle, acting jointly on all the major lifestyle-related risk factors, would crucially help reduce the burden of NCDs, including periodontitis (Li et al., 2020).

When interpreting the present findings, some limitations should be considered. They include the limited external validity (due to the inclusion of a non-representative sample), the risk of residual confounding, the inability to attribute causation (due to the observational study design), and the risk of information bias since lifestyle behaviours were assessed through questionnaires which, although validated, represent self-reported information. Moreover, a risk of information bias also related to periodontal variables assessment cannot be excluded since, despite calibrated, the examiners were not blinded when evaluating a little proportion (<10%) of the included patients. Additionally, because of the potential for type I error due to multiple comparisons, findings for analyses of secondary end points should be interpreted as exploratory. Furthermore, no specific sample size

calculation has been performed for this follow-up examination and the study size may have resulted limited to verify the statistical significance of MD adherence, PA, and stress estimates of association with the endpoint of periodontal therapy. Moreover, the smoking cessation advice intervention, made due to ethical reasons as part of the Step 1 of periodontal therapy, may potentially have reduced the magnitude of association between that behaviour and the reported outcomes. Similarly, lifestyles were monitored only at baseline, so it is not possible to exclude that lifestyle changes may have occurred during the study period. Finally, the validity of the present findings only applies for a short follow-up period (3 months). Overall, the described limitations may have influenced the results in both directions (i.e., lower or higher associations between lifestyles behaviours and periodontal therapy outcomes).

5 Conclusion

The present prospective cohort study showed that unhealthy lifestyle behaviours are associated with a worse response to the Steps 1 and 2 of periodontal therapy. Although randomized clinical trials are warranted to verify whether the modification of those lifestyle behaviours as part of the Step 1 of periodontal therapy may have a significant impact on the clinical outcomes, these results may lay the groundwork for the implementation of lifestyle interventions as part of the Step 1 and 2 of periodontal therapy.

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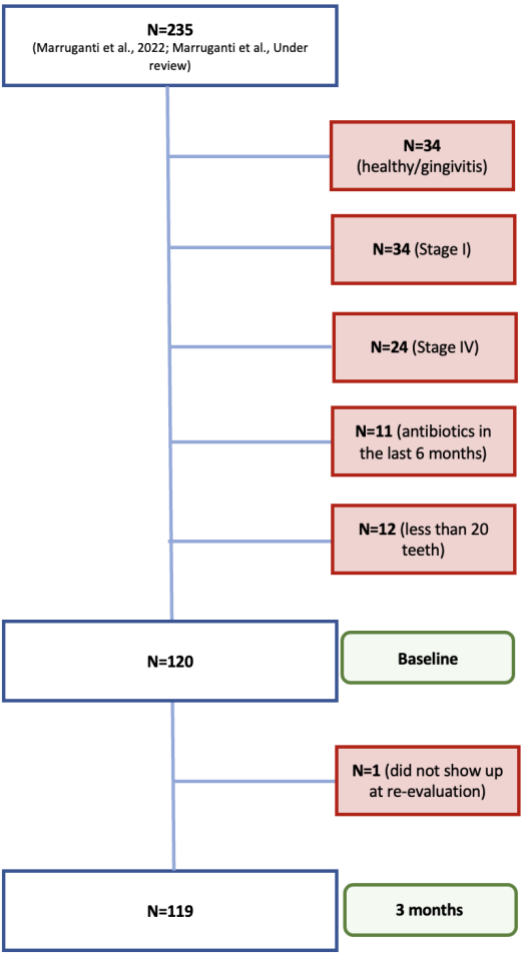
Supplementary information (Chapter 5)

Supplementary Figure 1. *Flow chart for participants' inclusion.*



Appendix 1. *Assessment methods of patients' characteristics and lifestyle behaviours.*

Socio-demographic characteristics

As socio-demographic characteristics, the following variables were identified and measured as follows:
- Age was self-reported and measured in years.
- Gender was self-reported and dichotomized as male/female.
- Education was self-reported and categorized as primary/middle school, high school, and college or more.
- Average disposable income was approximated from the patient's post code using the data publicly available on the website of the Italian finance government (https://www1.finanze.gov.it/finanze/analisi_stat/public/index.php?search_class[0]=cCOMUNE&opendata=yes).
- BMI was calculated using an electronic calculator as weight (kilograms)/height (meters^2). Weight was measured with a calibrated digital scale in kilograms, and height was measured with a stadiometer in meters.
- Diabetes status was self-reported; in case of self-reported presence of diabetes, medical reports were checked for verification.

Lifestyle behaviours

Lifestyle behaviours were assessed using a set of validated questionnaires. Details on the questionnaires and the sum scores are reported below.

Adherence to Mediterranean Diet

A validated 15-item questionnaire to measure patients'

adherence to Mediterranean Diet (MD; QueMD) was administered by two examiners, following the structured questions and explanations provided by the questionnaire (Gnagnarella et al., 2018). The questionnaire included questions regarding the foods most frequently associated with MD (wholegrain pasta, bread, or substitutes; raw or cooked vegetables; all types of fresh fruits; dairies; either red or white wine; olive oil; red meat; fish; dried fruits and pulses), as well as other commonly consumed items (white meat, carbonated beverages, or soft drinks; butter, cooking cream or margarine; manufactured sweets or pastries). For each component, a standard portion for the Italian population was indicated (Italian Society of Nutrition, 2019) and participants could choose among 5 consumption frequencies, which differed according to food items. The alternate MD score (aMed) was drawn from the QueMD results in order to evaluate patients' adherence to MD (Gnagnarella et al., 2018); it was calculated by assigning 1 point to participants reporting food consumptions above the Italian National levels (National Research Institute for Food and Nutrition, 2003) for each of the following items typical of the MD: wholegrain products (≥1/day), vegetables (≥2/day), fresh fruits (≥2/day), olive oil (≥3/day), wine (1-2 glasses/day for males or 1 glass/day for females), red meat (≤1-3/week), fish (≥2/week), dried fruits (≥2/week), pulses (≥2/day) (Bach-Faig et al., 2011). The sum score ranged between 0 (minimum MD adherence) and 9 (maximum MD adherence); it was dichotomized to define cases of low (aMed<5) and high (aMed>4) adherence to MD considering its median in the current study population as the cut-off value, as previously performed (Marruganti et al., 2022).

Physical activity level

Physical Activity (PA) was assessed through the validated short version of the International Physical Activity Questionnaire (IPAQ) (Mannocci et al., 2010). It was administered by two examiners, who asked the structured questions and gave the explanations provided by the questionnaire (Mannocci et al., 2010). It consists of 7 items regarding the frequency and amount of time spent doing intense and moderate PA, as well as walking or doing sedentary activities during the last 7 days. The overall PA level was classified as low, moderate, or high through the IPAQ automatic report (https://theipaq/home). Participants were further categorized as performing either moderate/high vs. low PA level, as previously performed (Marruganti et al., 2022).

Perceived stress

The Italian version of a validated 10-items questionnaire to measure patients' level of perceived stress (PSS-10) was administered by the examiners, who asked structured questions and gave the explanations provided by the questionnaire itself (Cohen et al., 1983). The instrument had 10 questions with response options ranging between 0 (never) and 4 (very often). All questions were negatively stated, except for 4 questions that were positively stated (items 4, 5, 7 and 8). The sum scores were calculated after reversing the positive items' scores and then summing up all scores. Total scores ranged between 0 and 40; the higher the score, the higher the level of perceived stress. Finally, the PSS-10 score was categorized according to the guidelines to stratify participants into two subgroups: moderate/high (PSS-10>13) and low perceived stress (PSS-10≤13) (Biswas et al., 2019; State of New Hampshire Employee Assistance

Program, 1983).

Sleep quality

Sleep quality was assessed using the validated Italian version of Pittsburgh Sleep Quality Index (PSQI) questionnaire (Mondo et al., 2021). The PSQI was administered by the examiners, who asked structured questions and gave the explanations provided by the questionnaire. The questionnaire included 7 domains (subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medications, daytime dysfunction), each one assessed with a 0 to 3 scoring system; the final scores ranged between 0 and 21, with higher values indicating worse sleep quality. Participants with a total score of 5 or higher were classified as having "poor sleep quality", vice versa for those with lower scores (classified as having "good sleep quality") (Buysse et al., 1988).

Smoking/Alcohol use

Smoking was self-reported and participants were then categorized as smoker vs. non-smokers. Alcohol consumption was identified considering the suggested alcohol intake as threshold (1 standard alcoholic drink/d for women, and 2 standard alcoholic drinks/d for men); participants were then categorized as consuming alcohol above vs. below the suggested intake (Ricci et al., 2020).

Table S1. *Simple and multiple linear/logistic regression analyses for the association between lifestyle behaviours (continuous variables) and periodontal outcomes at 3M (patient-level).*

aMed – MD/OR (95% CI)				
Outcomes	**Crude**	**Model 1†**	**Model 2‡**	**Model 3§**
FMPS‖, %	-1.31 (-3.12, 0.52)	-0.42 (-1.90, 1.21)	-0.63 (-2.21, 1.03)	-0.91 (-2.56, 0.64)
FMBS, %	0.51 (-0.73, 1.66)	0.74 (-0.30, 1.74)	0.72 (-0.33, 1.75)	0.93 (-0.04, 1.93)
% PPD ≥ 5 mm	0.54 (-2.21, 1.20)	0.3 (-1.72, 1.24)	0.53 (-1.92, 0.95)	0.34 (-1.82, 1.10)
% PPD ≥ 6 mm	0.12 (-0.40, 0.31)	0.04 (-0.33, 0.35)	0.02 (-0.32, 0.39)	0.02 (-0.31, 0.34)
n teeth with mobility ≥ 1, mean (±SD)	-0.42 (-0.71, 0.04)	-0.37 (-0.61, 0.01)	-0.33 (-0.60, 0.12)	-0.30 (-0.62, 0.05)
Endpoint of therapy‖, yes, N (%)	1.10 (0.91, 1.42)	-	1.11 (0.94, 1.45)	1.13 (0.90, 1.56)
MET-min/week (PA) – MD/OR (95% CI)				
Outcomes	**Crude**	**Model 1†**	**Model 2‡**	**Model 3§**
FMPS‖, %, mean (±SD)	0.001 (-0.002, 0.002)	0.001 (-0.001, 0.002)	0.001 (-0.002, 0.002)	0.0009 (-0.002, 0.003)
FMBS, %, mean (±SD)	0.0001 (-0.0006, 0.0004)	0.00009 (-0.0005, 0.0003)	0.0009 (-0.0005, 0.003)	0.0003 (-0.0007, 0.002)
% PPD ≥ 5 mm, mean (±SD)	0.0004 (-0.001, 0.003)	0.0002 (-0.0009, 0.003)	0.0002 (-0.0008, 0.003)	0.004 (-0.0009, 0.009)
% PPD ≥ 6 mm, mean (±SD)	0.0001 (-0.0002, 0.0008)	0.00003 (-0.0001, 0.00009)	0.00005 (-0.0002, 0.0001)	-0.00006 (-0.0002, 0.0007)
n teeth with mobility	0.0001 (-	0.0001 (-	0.0001 (-	0.0009 (-0.0002,

≥ 1, mean (±SD)	0.0002, 0.0003)	0.0003,0.0006)	0.0003, 0.0007)	0.005)
Endpoint of therapy††, yes, N (%)	1.03 (0.92, 1.15)	-	1.02 (0.84, 1.16)	1.02 (0.99, 1.12)
PSS – MD/OR (95% CI)				
Outcomes	**Crude**	**Model 1†**	**Model 2‡**	**Model 3§**
FMPS‡‡, %, mean (±SD)	0.32 (-0.21, 0.80)	0.32 (-0.93, 0.76)	0.22 (-0.23, 0.61)	0.35 (-0.12, 0.79)
FMBS, %, mean (±SD)	-0.12 (-0.44, 0.13)	-0.25 (-0.42, 0.90)	-0.26 (-0.42, 0.15)	-0.13 (-0.41, 0.19)
% PPD ≥ 5 mm, mean (±SD)	0.24 (-0.21, 0.75)	0.23 (-0.16, 0.61)	0.22 (-0.25, 0.51)	0.29 (-0.23, 0.59)
% PPD ≥ 6 mm, mean (±SD)	0.03 (-0.06, 0.12)	0.03 (-0.04, 0.11)	0.05 (-0.03, 0.12)	0.04 (-0.03, 0.12)
n teeth with mobility ≥ 1, mean (±SD)	-0.001 (-0.1, 0.08)	-0.07 (-0.2, 0.07)	-0.13 (-0.22, 0.07)	0.0008 (-0.09, 0.09)
Endpoint of therapy††, yes, N (%)	1.02 (0.91, 1.10)	-	***0.89 (0.71, 0.95)****	***0.83 (0.62, 0.95)****
PSQI – MD/OR (95% CI)				
Outcomes	**Crude**	**Model 1†**	**Model 2‡**	**Model 3§**
FMPS‡‡, %, mean (±SD)	-0.43 (-0.62, 0.29)	-0.22 (-0.68, 0.12)	-0.34 (-0.62, 0.19)	-0.42 (-0.93, 0.89)
FMBS, %, mean (±SD)	0.61 (-0.01, 0.93)	0.45 (-0.22, 0.93)	0.39 (-0.22, 0.94)	0.31 (-0.25, 0.89)
% PPD ≥ 5 mm, mean (±SD)	0.34 (-1.21, 0.65)	0.63 (-0.41, 1.25)	0.52 (-0.34, 1.39)	0.77 (-0.07, 1.52)
% PPD ≥ 6 mm, mean (±SD)	-0.23 (-0.41, 0.03)	0.14 (-0.06, 0.36)	0.13 (-0.05, 0.38)	0.08 (-0.22, 0.09)
n teeth with mobility ≥ 1, mean (±SD)	-0.03 (-0.25, 0.16)	-0.05 (-0.22, 0.14)	-0.06 (-0.21, 0.17)	-0.02 (-0.23, 0.27)
Endpoint of	***0.82 (0.76,***	-	***0.82 (0.63,***	***0.84 (0.72, 0.95)****

therapy‡‡, yes, N (%)	**0.92)****	**0.95)****

Abbreviations. aMed, Alternate Mediterranean Diet Score; BL, baseline; FMBS, Full Mouth Bleeding Score; FMPS, Full Mouth Plaque Score; N, number; PA, Physical Activity; PPD, Probing Pocket Depth; PSS, Perceived Stress Score; SD, Standard Deviation; 3M, 3 months; %, percentage.

*$p<0.05$

**$p<0.01$

† Model 1: Adjusted for its baseline value.

‡ Model 2: Model 1 + BMI + diabetes + household disposable income + 3M FMPS.

§ Model 3: Model 2 + the remaining five lifestyle behaviours (except for the main exposure)

‡‡ Participants with no sites with PPD ≥ 4 mm with BoP, and PPD ≥ 6 mm.

Multiple models unadjusted for 3M FMPS.

Table S2. *Simple and multiple linear/logistic regression analyses for the association between lifestyle behaviours (continuous variables) and the changes in periodontal outcomes (3M - BL) (site-level).*

aMed – MD/OR (95% CI)				
Outcomes	**Crude**	**Model 1†**	**Model 2‡**	**Model 3§**
PPD change, mm	0.01 (-0.13, 0.05)	0.04 (-0.01, 0.16)	0.12 (-0.003, 0.21)	0.03 (-0.13, 0.01)
PPD change (sites with PPD≥6mm), mm	0.12 (-0.15, 0.34)	0.12 (-0.05, 0.34)	0.12 (-0.17, 0.32)	0.13 (-0.05, 0.36)
REC change, mm	0.001 (-0.02, 0.25)	0.003 (-0.02, 0.03)	0.002 (-0.02, 0.03)	0.01 (-0.02, 0.03)
CAL change, mm	0.01 (-0.07, 0.17)	0.03 (-0.12, 0.07)	0.04 (-0.02, 0.13)	0.03 (-0.03, 0.09)
BoP, yes	***0.96 (0.94, 0.98)******	0.99 (0.95, 1.02)	***0.96 (0.93, 0.99)****	0.99 (0.97, 1.2)
MET-min/week (PA) – MD/OR (95% CI)				
Outcomes	**Crude**	**Model 1†**	**Model 2‡**	**Model 3§**
PPD change, mm	0.0002 (-0.0004, 0.001)	0.0002 (-0.0004, 0.002)	***0.006 (0.001, 0.008)****	***0.0003 (0.0005, 0.008)****
PPD change (sites with PPD≥6mm), mm	0.002 (-0.0004, 0.004)	0.0002 (-0.0004, 0.001)	0.0004 (-0.00002, 0.001)	0.0005 (-0.0001, 0.001)
REC change, mm	0.002 (-0.01, 0.35)	0.006 (-0.01, 0.04)	0.003 (-0.02, 0.05)	0.001 (-0.02, 0.02)
CAL change, mm	0.00001 (-0.00004, 0.0001)	0.00003 (-0.00005, 0.0002)	***0.00002 (0.0004, 0.00004)****	***0.00003 (0.00001, 0.0001)****
BoP, yes	***1.01 (1.00, 1.02)****	***1.02 (1.00, 1.04)****	1.01 (0.97, 1.11)	1.02 (0.96, 1.12)

PSS – MD/OR (95% CI)				
Outcomes	**Crude**	**Model 1†**	**Model 2‡**	**Model 3§**
PPD change, mm	0.008 (-0.006, 0.02)	0.008 (-0.004, 0.02)	***0.008 (0.004, 0.02)****	0.008 (-0.003, 0.02)
PPD change (sites with PPD≥6mm), mm	-0.04 (-0.08, 0.005)	-0.03 (-0.08, 0.009)	0.05 (-0.01, 0.14)	0.05 -(0.01, 0.09)
REC change, mm	***0.007 (0.001, 0.01)****	***0.009 (0.002, 0.02)****	0.006 (-0.0009, 0.01)	0.006 (-0.0009, 0.01)
CAL change, mm	***0.02 (0.001, 0.03)****	***0.02 (0.004, 0.03)****	0.02 (-0.0001, 0.03)	***0.02 (0.001, 0.03)****
BoP, yes	1.01 (0.99, 1.02)	0.99 (0.98, 1.04)	1.00 (0.99, 1.03)	1.01 (0.99, 1.02)
PSQI – MD/OR (95% CI)				
Outcomes	**Crude**	**Model 1†**	**Model 2‡**	**Model 3§**
PPD change, mm	***0.04 (0.01, 0.07)****	0.02 (-0.006, 0.05)	0.02 (-0.008, 0.05)	0.03 (-0.003, 0.05)
PPD change (sites with PPD≥6mm), mm	0.03 (-0.14, 0.06)	0.05 (-0.17, 0.06)	0.05 (-0.13, 0.08)	0.03 (-0.18, 0.05)
REC change, mm	0.01 (-0.0006, 0.02)	***0.02 (0.004, 0.03)****	*0.02 (0.002, 0.03)*	*0.01 (0.0004, 0.03)*
CAL change, mm	***0.05 (0.02, 0.08)****	***0.04 (0.006, 0.07)****	***0.04 (0.003, 0.07)****	*0.04 (0.009, 0.07)*
BoP, yes	***1.02 (1.00, 1.03)****	***1.04 (1.00, 1.06)****	***1.05 (1.01, 1.09)****	1.02 (0.99, 1.02)

Abbreviations. aMed, Alternate Mediterranean Diet Score; BoP, Bleeding on Probing; BL, baseline; CAL, Clinical Attachment Level; CI, Confidence Interval; MD, Mean Difference; mm, millimeters; OR, Odds Ratio; PA, Physical Activity; PPD, Probing Pocket Depth; PSS, Perceived Stress Score; REC, recession; SD, Standard Deviation; 3M, 3 months.

* $p<0.05$

** $p<0.01$

*** $p<0.001$

† Model 1: Adjusted for its baseline value.

‡ Model 2: Model 1 + BMI + diabetes + household disposable income + Plaque at site-level

§ Model 3: Model 2 + the remaining five lifestyle behaviours (except for the main exposure)

†† Smoking was dropped out from the model due to multicollinearity.

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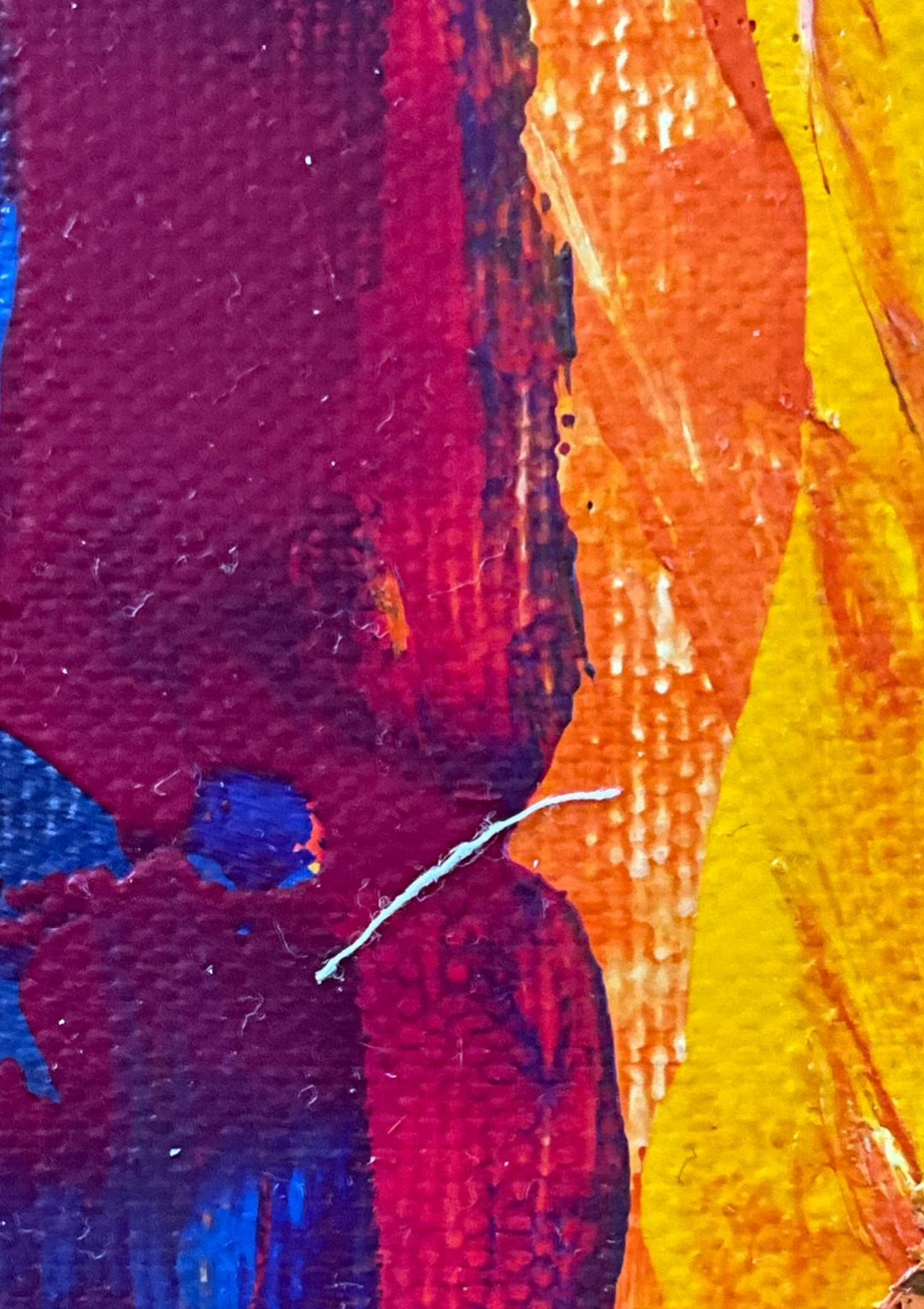
Data availability statement. The data that support the findings of this study are available upon reasonable request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Authors contribution. CM contributed to study conception, to study design, to data analysis, to data collection and to manuscript drafting. MR contributed to data analysis, to data interpretation, and to manuscript drafting. CG contributed to data interpretation and to manuscript drafting. EFC, ND, SP, FG contributed to data interpretation, and critically revised the manuscript. SG contributed to study conception, to study design, and to manuscript drafting.



Discussion

Discussion

Results from the present PhD project show a positive association between lifestyles and periodontitis. Chapter 1 highlighted that physical inactivity was associated with a 2-fold increase in the odds of having Stage III/IV periodontitis when compared to physically active subjects; the odds increased by 6 times in subjects with low adherence to Mediterranean Diet (vs. high adherence). Such associations were shown to be multiplicative, with the odds of Stage III/IV periodontitis skyrocketing up to 10 times when considering the combination between physical inactivity and low adherence to Mediterranean Diet. In Chapter 1, it could be noted that the effect of diet was of a higher magnitude than that of physical activity. It can be hypothesized that the lower effect of physical activity noted in Chapter 1 may be due to the lack of distinction between leisure-time and occupational physical activity, whose divergent effects on the periodontium have been investigated in Chapter 2. Indeed, Chapter 2 indicated that leisure-time physical activity represents a protective indicator, while occupational physical activity represents a risk indicator for periodontitis. The divergent effects of the two types of physical activity were also found to have a cumulative effect. Consistently, Chapter 3 investigated the impact of perceived stress and sleep quality on periodontitis prevalence and severity, and highlighted that the combination of the two lifestyles led to 5 to 6 times increased odds of suffering from severe forms of periodontitis. The cumulative effect of lifestyles has also been demonstrated by the results reported in Chapter 4, where the combination of healthy lifestyles has been identified as a protective indicator against periodontitis; in addition, a dose-response relationship between the number of reported healthy lifestyles and the lower odds of periodontitis was also recognised. Finally, Chapter 5

provided longitudinal evidence of the association between unhealthy lifestyles, either individually or combined, and response to periodontal treatment. Specifically, poor sleep quality, smoking, and alcohol use above the suggested intake were associated with significantly lower rates of endpoint of therapy; the same tendency was also observed for low adherence to Mediterranean Diet, low physical activity level, and high perceived stress, even if not reaching statistical significance. The odds of reaching the endpoint of therapy 3 months after Steps 1/2 of periodontal therapy further decreased in subjects reporting a combination of unhealthy lifestyles, namely low adherence to Mediterranean Diet, low physical activity level, high perceived stress, and poor sleep quality.

Despite the limited evidence available on the association between healthy lifestyles in their entirety and oral health, the results obtained in the current PhD project are consistent with those achieved in a previous prospective investigation (Iwasaki et al., 2018). Iwasaki and coworkers (2018) demonstrated a protective effect of the combined adherence to multiple healthy lifestyles (non-smoking, physical activity, healthy body weight, and high diet quality) when assessing the onset of periodontitis and tooth loss over a period of 6 years in a population of older Japanese adults.

The biological mechanisms underpinning the protective effect of the combination of healthy lifestyles on the periodontium still need further investigation. The available evidence regarding the local and systemic repercussions of each individual lifestyle factor points towards shared mechanisms of modulation of inflammation including oxidative stress production (Gleeson et al., 2011; Pitchika et al., 2017) and their impact on the risk of periodontitis (D'Aiuto et al., 2010; Pink et al.,

2015). A bi-directional link between periodontitis and systemic inflammation is corroborated by Chapters 2 and 4 showing a statistically significant mediation of inflammatory biomarkers on the prevalence of periodontal diseases.

The results obtained are consistent with the current evidence focusing on lifestyle as a combination of factors instead of considering each behaviour as independent. Indeed, in the medical literature several observational studies demonstrated that a combination of healthy lifestyles is associated with lower mortality, incident cardiovascular disease and diabetes (Abdullah Said et al., 2018; Zhang et al., 2021). As lifestyles behaviours/factors are closely intertwined, it could be speculated that individuals with patterns of unhealthy behaviours are less likely to follow domiciliary oral hygiene instructions and vice versa (Sakki & Sakki, 1995; Sabbah et al., 2018). Given the recent confirmation of the importance of oral health for achieving good general health, promotion of healthy lifestyle could be revisited to also include better oral health behaviours. Indeed, a more comprehensive approach in the promotion of better lifestyles could help reducing the burden of NCDs and multimorbidities.

It is noteworthy to mention some limitations when interpreting these findings. Chapters 1-4 rely on cross-sectional study designs, which pose the risk of residual confounding and the impossibility to assess causality; as such, the mediation analyses performed in Chapters 1,2 and 4 may be of limited validity. Moreover, the risk of information bias cannot be excluded because of the self-reported assessment of lifestyles throughout. Nonetheless, the studies presented embody the first pieces of evidence on the association between combined lifestyles and periodontitis onset, severity (Chapters 1-4), and response to periodontal therapy (Chapter 5).

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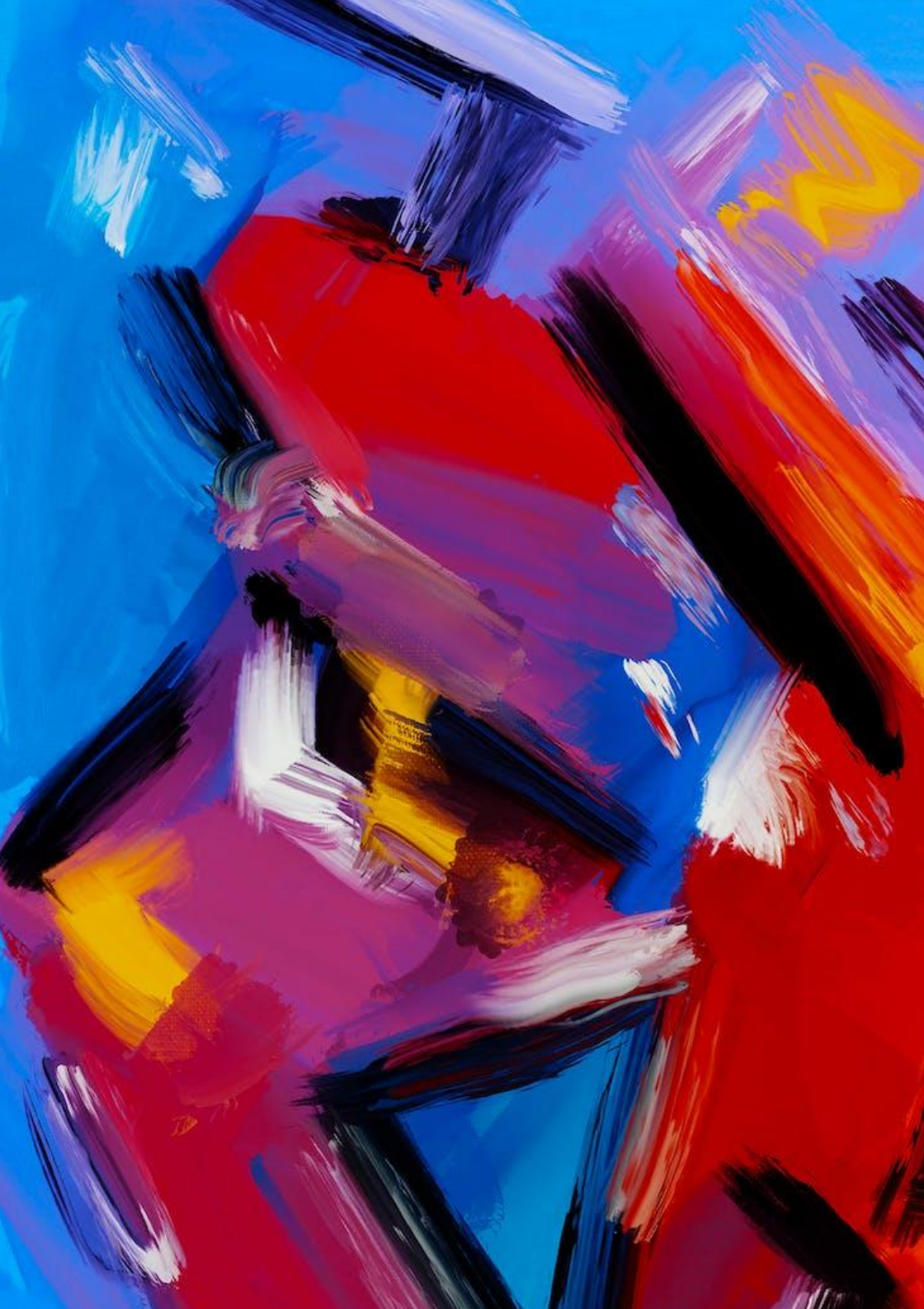

Conclusions

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Future directions

CONCLUSIONS

- Low adherence to Mediterranean Diet, physical inactivity, high perceived stress, and poor sleep quality were identified as risk indicators for periodontitis onset and severity (Chapters 1-5).
- Low adherence to Mediterranean Diet and physical inactivity were found to have a multiplicative effect, leading to 9-time increased odds of being affected by severe forms of periodontitis (Chapter 1).
- With regards to physical activity, leisure-time and occupational physical activity demonstrated divergent associations with periodontitis, with the former identified as a protective and the latter as a risk indicator for the disease (Chapter 2).
- The same multiplicative effect highlighted for diet and physical activity was identified for high perceived stress and poor sleep quality, with the combination of the two unhealthy behaviours leading to a 5- to 6-fold increase in the odds of having severe forms of periodontitis (Chapter 3).
- Healthy lifestyle behaviours demonstrated a positive cumulative effect on the periodontium, meaning that the higher the number of healthy lifestyles, the lower the prevalence of periodontitis in a dose-response fashion (Chapter 4).
- Consistently, the combination of unhealthy lifestyles was associated with poorer periodontal treatment outcomes at 3 months, in terms of lower odds of achieving the endpoint of therapy and a higher number of residual pockets (Chapter 5).

FUTURE DIRECTIONS

- Long-term longitudinal studies should be performed to evaluate the impact of lifestyle behaviours on the risk of developing periodontitis over time.
- Randomized controlled trials to verify whether the modification of those lifestyle behaviours as part of the Step 1 of periodontal therapy may have a significant impact on the clinical outcomes, and to verify whether those improvements in oral health are also accompanied by improvements in general health.
- These findings underline the pivotal role of oral healthcare providers during the screening, the detection, and the lifestyle modification counselling to prevent or slow down the progression of periodontitis, as well as of other associated NCDs. Hence, policy makers should foster the implementation of an interdisciplinary approach to tackle NCDs which should include a joint approach among clinicians to successfully achieve lifestyle modifications.

Curriculum Vitae

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List of Publications

CURRICULUM VITAE

EDUCATION/QUALIFICATIONS

2022-2023

Affiliate PhD Candidate in Periodontology

Periodontology Unit, UCL Eastman Dental Institute, London, UK

2021-2022

Research Fellow in Periodontology

Unit of Periodontology, University of Pisa, Pisa, Italy

2021

Research Intern

Zahnmedizinische Kliniken der Universität Bern (ZMK), Bern, Switzerland

2020-2023

Doctor of Philosophy in Medical Biotechnologies (PhD Candidate)

University of Siena, Siena, Italy

2020

Annual Course in Periodontology and Implant Dentistry

Italian Society of Periodontology and Implantology (SIdP), Florence, Italy

2014-2020

Doctor of Dental Medicine (DMD)

University of Florence, Florence, Italy (Inter-university with the University of Siena)

2009-2014

Scientific Bachelor of Science

Liceo Scientifico Galileo Galilei, Siena, Italy

DENTISTRY ROLL REGISTRATIONS

2023-

General Dental Council, UK (number: 306551)

2020-

Ordine dei Medici Chirurghi e degli Odontoiatri di Siena, Italy (number: 0444)

PROFESSIONAL SOCIETIES MEMBERSHIP

2023-

British Society of Periodontology and Implant Dentistry (BSP), London, UK

2023-

Italian Society of Dermatology and Sexually Transmitted Diseases (SIDeMaST), Milan, Italy

2022-

International Association for Dental Research (IADR), Alexandria, USA

2020-

Italian Dental Association (ANDI), Rome, Italy

2019-

Italian Society of Periodontology and Implantology (SIdP), Florence, Italy

AWARDS

June 2023

Finalist Hatton Award for the Best Senior Clinical Research, International Association for Dental Research (IADR), with the presentation entitled: "*The physical activity paradox and periodontitis: a population-based study.*", by **Marruganti C**, Baima G, Grandini S, Sanz M, Graziani F, Aimetti M, Sanz M, Romandini M.

September 2022

CED-IADR Robert Frank Award for the best SENIOR - Clinical Research – CED-IADR (International Academy for Dental Research), with the presentation entitled: entitled "*The physical activity paradox and periodontitis: a population-based study.*", by **Marruganti C**, Baima G, Grandini S, Sanz M, Graziani F, Aimetti M, Sanz M, Romandini M.

April 2022

Prize for the best poster, session of Periodontology – Collegio dei Docenti di Discipline Odontostomatologiche (CDUO), with the presentation entitled: "*Acute-phase response following quadrant SRP vs full-mouth SRP in patients with comorbid type 2 diabetes. A randomized clinical trial*", by **Marruganti C**, Pioli MC, Gennai S, Graziani F.

April 2021

Prize for the best poster, session of Endodontics – Collegio dei Docenti di Discipline Odontostomatologiche (CDUO), with the presentation entitled: "*Caries and apical periodontitis in patients with Inflammatory Bowel Disease. A systematic review and meta-analysis*", by **Marruganti C**, Discepoli N, Gaeta C, Franciosi G, Ferrari M, Grandini S.

October 2020

H.M. Goldman Prize for the best Basic Research – Italian Society of Periodontology and Implantology (SIdP), with the presentation entitled: "*Clinical, radiographic and molecular analysis of the relationship between a restorative class II margin and the periodontal tissues: a pre-post cohort study*", by **Marruganti C**, Discepoli N, Santoro F, Mirra R, Pozzi G, Ferrari M, Grandini S.

INVITED TALKS

June 2023

"*The Physical activity paradox and periodontitis: a population-based study*", International Association for Dental Research (IADR), Bogotà, Colombia

September 2022

"*Risk factors and protective indicators of peri-implant diseases among psoriatic subjects. A cross-sectional study*.", European Prosthodontic Association (EPA), Siena, Italy.

September 2022

"*The Physical activity paradox and periodontitis: a population-based study*", International Association for Dental Research (IADR), Marseille, France

June 2022

"*Local and systemic risk factors for periodontal diseases*", Italian Association of Dentistry (AIO), Foggia, Italy

June 2022

"*Periodontal diseases: definition and relationship with skin diseases*", Italian Society of Dermatology and Sexually Transmitted Diseases (SIDeMaST), Milan, Italy

February 2022

"*Local and systemic risk factors for periodontal diseases*", Italian Dental Association (ANDI), Florence, Italy

February 2022

"*E. faecalis as a risk factor for post-treatment apical periodontitis*", Italian Academy of Endodontics (AIE), Bologna, Italy

UNIVERSITY TEACHING

2020-

"*Inviolability of the Supracrestal Tissue Attachment*", MSc Restorative Dentistry and Endodontics, University of Siena, Siena, Italy

2020-

"*Periodontal tissue reactions after the placement of iuxta/subgingival margins of restorations*", MSc Advanced Endodontics, University of Siena, Siena, Italy

EDITORIAL COMMITMENTS

2022-

Editorial Board Member, Journal of Osseointegration – Section of Periodontics

REVIEWER (AD HOC) IN SCIENTIFIC JOURNALS

2022-

International Journal of Oral Implantology

2022-

BMC Oral Health

2022-

Oral Diseases

2021-

Journal of Clinical Periodontology

LIST OF PUBLICATIONS

Gliga A, Imre M, Grandini S, **Marruganti C**, et al. The Limitations of Periapical X-ray Assessment in Endodontic Diagnosis-A Systematic Review. J Clin Med. 2023;12(14):4647. Published 2023 Jul 12. doi:10.3390/jcm12144647

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