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Dietary ratio of n-6 to n-3 polyunsaturated fatty acids and periodontal disease in community-based older Japanese: A 3-year follow-up study

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ABSTRACT

The longitudinal relationship between dietary n-6 to n-3 PUFAs ratio and periodontal disease in 235 Japanese subjects for whom data were available for the years 2003–2006 was investigated. PUFAs intake was assessed at baseline with a brief-type self-administered diet history questionnaire. Full-mouth periodontal status, measured as the clinical attachment level (CAL), was recorded at baseline and once a year for 3 years. The number of teeth with a change in the loss of CAL ≥ 3 mm at any site over a year was calculated as 'periodontal disease events'. Poisson regression analysis was conducted, with dietary n-6 to n-3 PUFAs ratio as the main predictor, to estimate its influence on periodontal disease events.A high dietary n-6 to n-3 PUFAs ratio was significantly associated with greater number of periodontal disease events. The findings suggest the dietary n-6 to n-3 PUFAs ratio is associated with periodontal disease among older Japanese.

1. Introduction

Periodontal disease is defined as an inflammatory condition of the gingival tissues, characterized by loss of attachment of the periodontal ligament and the bony support of the tooth. Periodontal disease is one of the main risk factors for tooth loss in older people [1,2]. Tooth loss has been associated with a sub-optimum intake of some nutrients and changes in food preference [3,4].

Diet plays an important role in terms of optimizing health and disease prevention. Polyunsaturated fatty acids (PUFAs) are of major importance in normal physiological functions linked to membrane integrity and regulatory cell signals [5]. Two groups of PUFAs, n-3 series and n-6 series, have attracted a great deal of attention with regard to health and diseases [6]. Linoleic acid (LA; 18:2n-6) represents the basis of the n-6 family and α-linolenic acid (ALA; 18:3n-3) represents the basis of the n-3 family. Both LA and ALA are metabolized to longer-chain fatty acids of 20 and 22 carbon atoms. LA is metabolized to arachidonic acid (AA; 20:4n-6), and ALA to eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3), increasing the chain length and degree of unsaturation by adding extra double bonds to the carboxyl end of the fatty acid molecule. These two types of PUFA are not interconvertible, are metabolically and functionally distinct, and often have important opposing physiological functions [7,8].

Previous studies have shown that diets rich in n-6 PUFAs create an inflammatory environment that may increase the risk of chronic diseases associated with an inflammatory state [7,8]. On the other hand, higher intakes of n-3 PUFAs appear to reduce the risk of many diseases, including coronary heart disease [9], type 2 diabetes [10], rheumatoid arthritis [11], asthma [12], depression [13], and cancer [14]. Both PUFAs compete for the activity of a fatty acid desaturase and the overabundance of dietary LA gives a quantitative advantage that limits the conversion of ALA to EPA *in vivo* [15]; therefore, it has been suggested that a balanced ratio of these two types of PUFAs may be necessary for the prevention and treatment of chronic diseases [7,8,]. A high n-6 to n-3 PUFA ratio, i.e. n-6 PUFAs consumed in much larger quantities than n-3 PUFAs, is characteristic of modern day diets [16]. In observational and experimental studies, a high ratio of n-6 to n-3 PUFAs has an adverse influence on health [17,18]. It is still debated which explains the PUFA effect on health and diseases more

Abbreviations: PUFA, polyunsaturated fatty acid; LA, linoleic acid; ALA, α-linolenic acid; AA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; BDHQ, brief-type self-administered diet history questionnaire; CAL, clinical attachment level; BMI, body mass index; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; RR, relative risk; CI, confidence intervals; PG, prostaglandin; LT, leukotriene; IL, interleukin; TNF-α, tumor necrosis factor-α

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significantly, the n-6 to n-3 PUFA ratio or the absolute amount of each PUFA [19].

Recently, we demonstrated an inverse independent relationship of dietary DHA intake with periodontal disease progression in a small sample study ($n=36$) [20]; however, information on n-6 PUFA intake was not available in the previous study; therefore, it was not possible to assess both the effects of the dietary n-6 to n-3 PUFA ratio and each intake of n-3 PUFAs and n-6 PUFAs on periodontal disease in one study. So far, there have been no reports in the literature on the relationships of the dietary ratio of n-6 to n-3 PUFAs to changes in the periodontal condition over time.

Investigating the relationship between the dietary n-6 to n-3 PUFA ratio and periodontal disease is important to address the value of the dietary n-6 to n-3 PUFA ratio and to understand the potential role of dietary modification in the prevention and treatment of periodontal disease and the ultimate prevention of tooth loss through periodontal disease. Consequently, the hypothesis of the present study is that a high dietary ratio of n-6 to n-3 PUFAs may contribute to periodontal disease progression. This study aimed to determine if there is a relationship between the dietary n-6 to n-3 PUFA ratio and periodontal disease progression in community-dwelling older Japanese.

2. Materials and methods

2.1. Study population (Fig. 1)

The current investigation was a subset study of the Niigata study over the study period of 2003–2006. The original participants in the Niigata study included 600 randomly selected 70-year-old Japanese individuals who were residents of Niigata city, Japan in 1998. All participants were fully informed of the purpose of the survey and consented to participate. Details of the sampling methodology and selection have been previously published [21].

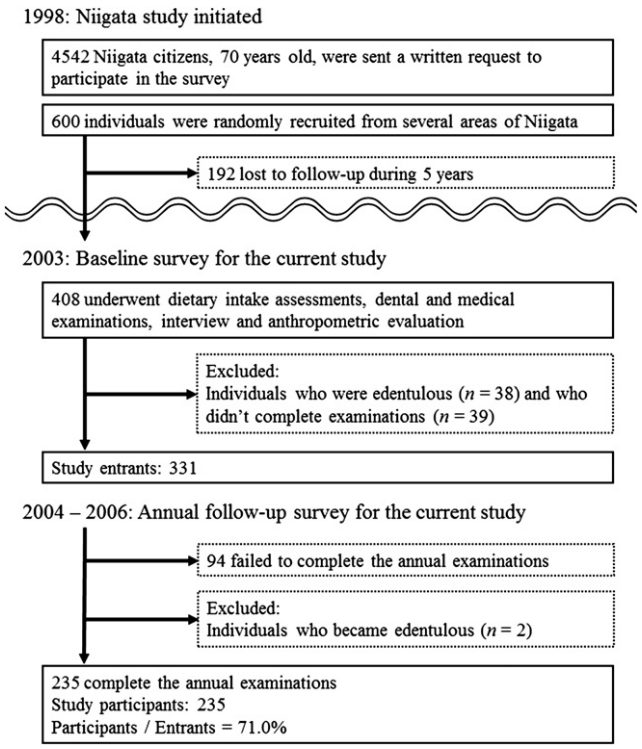

Fig. 1. Flow diagram of the study.

Five years after the Niigata study was initiated, 408 subjects who were 75 years of age in 2003 underwent dietary intake assessments, dental and medical examinations, interview and anthropometric evaluation as part of the baseline assessment for the current study. At baseline, people who were already edentulous ($n=38$) or who did not submit complete data ($n=39$) were excluded, leaving 331 eligible subjects to enter the study. Study entrants underwent annual follow-up examinations including dental assessment. During the study period (2003–2006), 94 failed to complete the annual examinations and 2 became edentulous. Data were therefore analyzed for 235 participants (121 men, 114 women) examined as dentate in 2006. The examination protocol used for this study was reviewed and approved by the Ethics Committee of the Faculty of Dentistry, Niigata University.

2.2. Dietary intake assessment

Dietary habits during the preceding month were assessed with a brief-type self-administered diet history questionnaire (BDHQ) [22]. This validated retrospective method of dietary assessment is based on a the food frequency quesionnaire method [23]. Responses to the BDHQ were checked for completeness and, where necessary, clarified by direct questioning of the subject.

The BDHQ is a questionnaire that inquires about the frequency of consumption of a total of 56 food and beverage items, with specified serving sizes described in terms of the natural portion or the standard weight and volume measurement of servings commonly consumed in general Japanese populations. The BDHQ was developed based on a comprehensive version of a self-administered diet history questionnaire [24]. Estimates of mean daily intake for energy (kcal), total PUFAs, total n-3 PUFAs, ALA, EPA, DHA, total n-6 PUFAs, LA, AA were calculated using an ad hoc computer algorithm for the BDHQ, which was based on the Standard Tables of Food Composition in Japan [25]. Values of dietary PUFA intake were energy-adjusted (i.e., amount per 1000 kcal for PUFA). Information on dietary supplement use was not available to the investigators and therefore PUFA intake from dietary supplements was not incorporated into the analysis.

2.3. Dental examination

Dental examinations were carried out at baseline (2003) and once a year for 3 years. The same methods as the baseline survey were used in follow-up dental examinations (2004–2006). Numbers of teeth present were counted and periodontal conditions were assessed for participants with at least one remaining tooth. The periodontal condition, measured as the clinical attachment level (CAL), was recorded. Teeth were probed at six sites per tooth for all teeth present, and measurements were recorded approximately to the nearest millimeter. A change in the loss of attachment of 3 mm or greater in 1 year at any site was considered as a periodontal disease progression [26]. Teeth with periodontal disease progression were excluded from additional-year assessments. Finally, the cumulative numbers of teeth with periodontal disease progression over 3 years per person were calculated as 'periodontal disease events' [20]. Clinical periodontal parameters were recorded by calibrated examiners as previously reported [21].

2.4. Interview, anthropometric evaluation and blood pressure determination

An interview was conducted to obtain information regarding smoking habits. Participants were classified as non-, previous-, or current-smoker according to their smoking histories. Information about oral hygiene habits, namely, the status of visits to a dentist

(regularly or episodically), was also gathered. Anthropometric evaluation included measurements of weight and height to calculate body mass index (BMI) (weight (kg)/height $(m)^2$). Obesity was defined as a BMI $\geq 25\ kg/m^2$. In addition, blood pressure levels were evaluated. Hypertension was defined according to the Japanese Society of Hypertension criteria as follows: systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg were defined as hypertensive.

2.5. Biochemical examination of blood

Biochemical values, including serum levels of albumin, hemoglobin A1c (HbA1c), total cholesterol, triglycerides, and high-density lipoprotein cholesterol (HDL-C) were evaluated. Hypoalbuminemia was defined as albumin ≤ 4.0 g/dl, and hyperglycemia was defined as HbA1c $\geq 6.0\%$. Lipid abnormalities were classified as follows: hypercholesterolemia, total cholesterol ≥ 220 mg/dl; hypertriglyceridemia, triglycerides ≥ 250 mg/dl; and low HDL-C, HDL-C ≤ 35 mg/dl.

2.6. Statistical analyses

Selected characteristics of study participants and those who withdrew were compared using independent *t*-tests and chi-square tests.

Tests of the hypothesis that those who have a higher dietary ratio of n-6 to n-3 PUFAs may have greater risk for periodontal disease events used bivariable and multivariable Poisson regression models. The major exposure variable was the dietary ratio of PUFAs, i.e. total n-6 to total n-3 PUFAs. Participants were classified by tertiles of the n-6 to n-3 PUFA ratio (lowest, middle, and highest). The multivariable regression model was developed as follows. First, the backward stepwise method was used to develop a candidate model. The significance level for removal from the model was 0.20 and the significance level for addition to the model was 0.10. Then, covariates which were not available in the candidate model by the stepwise method, but were previously recognized as relevant to periodontal disease, were included in the final candidate model. Effect modification by gender, BMI and smoking was evaluated using interaction terms.

Further analyses included the absolute amount of each PUFA (total n-3 and total n-6 PUFAs) as secondary exposure variables to estimate their influence on periodontal disease progression. Dietary n-3 and n-6 PUFA intake was also modeled as tertiles (lowest, middle, and highest).

The level of significance was set at $p < 0.05$. All calculations and statistical analyses were performed using the STATA™ software package (Stata Corp., TX, USA).

3. Results

3.1. Study participant characteristics

Selected characteristics of study participants and withdrawals are listed in Table 1. No significant differences were found between the two groups. Table 2 represents study participants' dietary PUFA ratio and intake.

3.2. Associations between periodontal disease events and PUFA ratio

Table 3 shows the results from Poisson regressions of periodontal disease events. The third column shows crude associations and the fourth column shows adjusted associations, simultaneously taking into account the number of teeth present at baseline, mean CAL at baseline, gender, smoking status, the pattern of visits to a dentist, obesity, hypoalbuminemia, and hyperglycemia as covariates. There were no interactions of dietary PUFAs with gender, BMI and smoking.

Table 1
Selected characteristics of participants and withdrawal.

	Participants ($n=235$)	Withdrawals ($n=96$)	*P*-value†
Dental condition			
No. of teeth at baseline	18.4 ± 8.4	17.5 ± 8.3	0.368
Mean CAL at baseline	2.1 ± 0.5	2.2 ± 0.5	0.214
Periodontal disease events	4.4 ± 3.7	N/A	N/A
Demographic status			
Gender			
Men	121 [51.5]	49 [51.0]	0.941
Women	114 [48.5]	47 [49.0]	
Health behavior and health status			
Smoking status			
Non-smoker	128 [54.5]	49 [51.0]	0.654
Previous smoker	81 [34.5]	33 [34.4]	
Current smoker	26 [11.1]	14 [14.6]	
Visits to dentist			
Regularly	125 [53.2]	49 [51.0]	0.722
Episodically	110 [46.8]	47 [49.0]	
BMI (kg/m^2)	23.1 ± 2.9	22.5 ± 3.0	0.100
Systolic blood pressure (mmHg)	130 ± 16	131 ± 19	0.777
Diastolic blood pressure (mmHg)	71 ± 10	70 ± 9	0.689
Serum albumin (g/dl)	4.1 ± 0.2	4.1 ± 0.3	0.539
HbA1c (%)	5.3 ± 0.7	5.4 ± 0.8	0.130
Serum total cholesterol (mg/dl)	200 ± 32	202 ± 35	0.552
Serum triglycerides (mg/dl)	137 ± 75	136 ± 77	0.911
Serum HDL-C (mg/dl)	60 ± 15	60 ± 18	0.836

Values expressed as average ± standard deviation for continuous variables and frequency [%] for categorical variables.
CAL, clinical attachment level; BMI, body mass index; BP, blood pressure; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol.
Withdrawals, individuals who participated in baseline examination but failed to complete the annual examinations ($n=94$) and persons who became edentulous during the study ($n=2$); periodontal disease events, number of teeth with periodontal disease progression during 3 years.

† *P*-value for the comparison of selected characteristics between participants and withdrawals.

According to the results of bivariable models (third column), a high dietary n-6 to n-3 PUFA ratio was significantly associated with a greater number of periodontal disease events. The crude relative risks (RRs) (95% confidence interval (CI)) for the mean number of periodontal disease events in the lowest, middle, and highest tertiles of the n-6 to n-3 PUFA ratio were 1.00 (reference), 1.20 (1.03–1.40), and 1.26 (1.08–1.47), respectively. This statistically significantly positive dose–response association remained, with slight attenuation, after simultaneously adjusting for possible confounders (fourth column), with multivariable adjusted RRs (95% CI) lowest, middle, and highest tertiles of the dietary n-6 to n-3 PUFA ratio of 1.00, 1.14 (0.97–1.33), and 1.29 (1.10–1.51), respectively (Table 3).

3.3. Associations between periodontal disease events and PUFA intakes

Table 3 also displays the results of Poisson regression models with the intake of each PUFA (total n-3 and total n-6 PUFAs) as secondary exposure variables. The unadjusted model (third column) showed a significant association of n-3 PUFA intake with periodontal disease events. The mean number of periodontal disease events in the highest n-3 PUFAs consumption tertile was approximately 0.8 times smaller (crude RR=0.79, 95%

Table 2
Study participants' dietary PUFA ratio and intake.

	Average ± SD	Minimum	33th Percentile	Median	66th Percentile	Maximum
Energy intakes (kcal)	2207 ± 622	739	1875	2095	2435	4049
PUFA ratio[a]						
Total n-6:total n-3 PUFAs	3.71 ± 0.77	1.91	3.36	3.71	4.02	5.67
PUFA intake[a]						
Total PUFAs (g/kcal)	9.10 ± 1.47	3.96	8.51	9.02	9.66	12.71
Total n-3 PUFAs (g/kcal)	1.98 ± 0.48	0.80	1.77	1.97	2.16	3.57
ALA (g/kcal)	1.20 ± 0.24	0.48	1.10	1.20	1.29	1.92
EPA (g/kcal)	0.24 ± 0.13	0.02	0.17	0.22	0.28	0.69
DHA (g/kcal)	0.38 ± 0.19	0.02	0.29	0.37	0.45	1.06
Total n-6 PUFA (g/kcal)	7.09 ± 1.16	3.15	6.59	7.03	7.46	10.61
LA (g/kcal)	6.92 ± 1.14	3.08	6.39	6.88	7.30	10.47
AA (g/kcal)	0.09 ± 0.03	0.03	0.08	0.09	0.10	0.19

PUFA, polyunsaturated fatty acid; ALA, alpha-linolenic acid (18:3n-3); EPA, eicosapentaenoic acid (20:5 n-3); DHA, docosahexaenoic acid (22:6 n-3); LA, linoleic acid (18:2n-6); AA, arachidonic acid (20:4n-6).

[a] Energy-adjusted (i.e., amount per 1000 kcal of each nutrient).

Table 3
Relationships of PUFA ratio and specific PUFA intake to periodontal disease events.

	PUFA ratio and specific PUFA intake tertiles	RR_{Crude} (95% CI)	RR_{Adj} (95% CI)
PUFA ratio[a]			
Total n-6:total n-3 PUFAs	Lowest (reference)	1.00	1.00
	Middle	1.20 (1.03–1.40)*	1.14 (0.97–1.33)
	Highest	1.26 (1.08–1.47)*	1.29 (1.10–1.51)*
PUFA intake[a]			
Total n-3 PUFA (g/kcal)	Lowest (reference)	1.00	1.00
	Middle	0.92 (0.80–1.06)	0.92 (0.79–1.07)
	Highest	0.79 (0.68–0.92)*	0.88 (0.75–1.03)
Total n-6 PUFA (g/kcal)	Lowest (reference)	1.00	1.00
	Middle	0.99 (0.86–1.15)	1.07 (0.92–1.25)
	Highest	0.96 (0.83–1.11)	1.10 (0.94–1.28)

Periodontal disease events, number of teeth with periodontal disease progression during 3 years.
RR_{Crude}, unadjusted relative risk for the associations between periodontal disease events and PUFA ratio and intake; RR_{Adj}, relative risk for the associations between periodontal disease events and PUFA ratio and intake, simultaneously taking into account the number of teeth present at baseline, mean CAL at baseline, gender, smoking status, the pattern of visits to a dentist, obesity, hypoalbuminemia, and hyperglycemia as covariates; CI, confidence interval.

[a] Energy-adjusted (i.e., amount per 1000 kcal of each nutrient).
* $P < 0.05$.

CI = 0.68–0.92) than the reference group (lowest tertile). This inverse association disappeared in the multivariable model (fourth column), although there was a tendency for the highest n-3 PUFA intake group to have smaller RR for periodontal progression (adjusted RR = 0.88, 95% CI = 0.75–1.03), while there was no statistical association between the number of periodontal disease events and dietary n-6 PUFAs in the bivariable model (third column). Non-significant positive dose–response relationship of n-6 PUFA intake to periodontal disease was observed in the multivariable model (fourth column).

4. Discussion and conclusions

A high dietary n-6 to n-3 PUFA ratio was significantly associated with a greater risk for periodontal disease events. The mean number of periodontal disease events for study participants who consumed the highest tertiles of the n-6 to n-3 PUFA ratio was approximately 1.3 times larger (adjusted RR = 1.29, 95% CI = 1.10–1.51) than the referent group (lowest tertile), after simultaneously adjusting for possible confounders. Although not all n-6 to n-3 PUFA ratio tertile categories had statistically significantly different periodontal events compared with the lowest tertile reference group in the multivariable model, there was a tendency for more periodontal disease events with a higher n-6 to n-3 PUFA ratio. One explanation for the lack of statistical significance of the 3-category specification may be sample size limitations. On the other hand, neither total intake of n-3 PUFAs nor n-6 PUFAs predicted periodontal progression in the multivariable adjusted model. These findings suggest that a balance between total n-6 and n-3 PUFAs, rather than the absolute amount of each PUFA, would be a more significant risk predictor for periodontal progression among older Japanese.

To our knowledge, this is the first longitudinal study to show a relationship between the dietary PUFA ratio and periodontal disease in older people. The average ratio of n-6 to n-3 PUFAs was 3.7. This is slightly lower than the reported average n-6 to n-3 PUFA ratio of 4.0:1 among Japanese [27]. Major changes characterized by increased intake of n-6 PUFAs and an accompanying decrease in intake of n-3 PUFAs has taken place in the diet as a result of nutrition transition and modern agricultural practices [8]. Although, paleolithic nutrition studies show that early hunter-gatherer diets had nearly equal amounts of n-6 to n-3 PUFAs [18], agricultural changes led to an increase in the ratio of n-6 to n-3 PUFAs [16]. Because of the increased amounts of n-6 PUFAs in modern diets (compared with n-3 PUFAs), the

eicosanoid metabolic products from AA, such as prostaglandins (PGs), thromboxanes, and leukotrienes (LTs), are formed in larger quantities than those formed from n-3 PUFAs, such as EPA. Diets with a high ratio of n-6 to n-3 PUFAs may enhance the synthesis of proinflammatory mediators [8].

Periodontal disease is a bacterial infection that results in inflammatory destruction of tissues that support the teeth, including connective tissue and bone [28]. There is evidence that periodontal disease is initiated by dental plaque biofilm, and abnormal inflammatory responses aggravate periodontal tissue destruction. Proinflammatory cytokines, such as interleukin-1 (IL-1), IL-6, and tumor necrosis factor-α (TNF-α), and prostanoids, particularly PGE2 play a key role in the development and progression of periodontal disease [29]. A number of studies have examined the effects of PUFAs in rodent periodontitis models. Two of these studies investigated the effect of therapeutic usage of n-3 PUFAs on periodontitis. They demonstrated reductions in gingival tissue levels of PGE2, PGF2α, LTB4 and platelet activating factor and it was suggested that reduced alveolar bone loss may have been seen with longer periods of n-3 PUFA administration [30].

Many previous studies have provided mechanistic pathways whereby PUFAs might regulate inflammatory mediators, such as eicosanoids and cytokines, which could help explain the relationship with periodontal disease. Studies have shown that differing ratios of n-6 to n-3 PUFAs alter the synthesis of PGs [31], and that high levels of n-6 PUFAs increase PGE2 production [32]. Whereas EPA reduces the level of AA available for metabolism and also competes against AA for metabolism to form metabolites of the 5-series LTs and 3-series PGs, which are considered less biologically active and less inflammatory than 4-series LTs and the 2-series PGs derived from AA, thereby taming the inflammatory reaction [33]. Additionally, a higher intake of n-3 PUFAs leads to blocking the synthesis of proinflammatory cytokines, including IL-1, IL-6, and TNF-α [34]. On the other hand, a high LA intake interferes with the desaturation and elongation of ALA to EPA and DHA [35]. According to Cleland et al. [36] LA inhibits EPA incorporation from dietary fish oil supplements in human subjects. Overall, these findings indicate a potential mechanism of the adverse effects of a PUFA imbalance on periodontal disease. The composition of fatty acids in inflamed gingival tissues is significantly different from that in healthy tissues [37] and a serum fatty acid imbalance was reported in patients suffering from periodontal bone loss [38]. These results lend further support for a potential relationship as discussed above.

In this cohort, a homogenous group restricted to the age of 75 years at baseline examinations was selected to exclude the influence of race and age variation in the results. Among 331 study entrants (170 men and 161 women) 96 did not complete the study because they were unable to participate, became edentulous, or did not provide the data that were necessary during the study period. Individuals who withdrew were excluded from the following statistical analyses. There was no significant difference in dental conditions, demographic, and health status and health behavior between withdrawals and study participants; therefore, it was believed that the participants in this study were representative of the parent study group, which would be the strength of the present study.

There are several limitations to the present study. First, information on participants' serum levels of PUFAs was not available to the investigators and therefore we were unable to fully assess whether the BDHQ record appropriately reflects dietary intake. Further work would be necessary on using serum biochemistry to substantiate our findings. Second, information on nutritional supplement use was not available; therefore, it was not possible to fully assess the total amount of dietary PUFAs, leading to potential misclassification. However, if misclassification of the amount of PUFAs occurred, it would likely be non-differential (i.e. the same for those experiencing and not experiencing periodontal disease events) and therefore bias the results towards the null. Third, the participants were restricted to the age of 75 years. Extending the interpretation of the findings to other age groups is limited because of the narrow age range. Fourth, because other information regarding participants' socioeconomic status (e.g. income and educational status [39]) was not collected, a number of other potentially important confounders could not be included in the analyses. Although the most important confounders were assessed, residual confounding remains a risk. Finally, the current study was an observational cohort study and was insufficient to determine the value of a particular therapeutic approach.

In conclusion, within the limitations of the reported study, an independent relationship between the dietary n-6 to n-3 PUFA ratio and periodontal disease progression was found. Future intervention studies are needed to resolve the question of whether the dietary n-6 to n-3 PUFA ratio or total amount of dietary PUFAs is of more importance to periodontal health. Such studies are important to gain insight into the contribution of nutritional approaches in reducing the risk for the development or progression of periodontal disease.

Conflicts of interest

The authors have no conflicts of interest to report.

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