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Antioxidants and periodontitis in 60–70-year-old men

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Abstract

Objective: The aim was to investigate the association between periodontal health and the serum levels of various antioxidants including carotenoids, retinol and vitamin E in a homogenous group of Western European men.

Materials and Methods: A representative sample of 1258 men aged 60–70 years, drawn from the population of Northern Ireland, was examined between 2001 and 2003. Each participant had six or more teeth, completed a questionnaire and underwent a clinical periodontal examination. Serum lipid-soluble antioxidant levels were measured by high-performance liquid chromatography with diode array detection. Multivariable analysis was carried out using logistic regression with adjustment for possible confounders. Models were constructed using two measures of periodontal status (low- and high-threshold periodontitis) as dependent variables and the fifths of each antioxidant as a predictor variable.

Results: The levels of α- and β-carotene, β-cryptoxanthin and zeaxanthin were highly significantly lower in the men with low-threshold periodontitis ($p < 0.001$). These carotenoids were also significantly lower in high-threshold periodontitis. There were no significant differences in the levels of lutein, lycopene, α- and γ-tocopherol or retinol in relation to periodontitis. In fully adjusted models, there was an inverse relationship between a number of carotenoids (α- and β-carotene and β-cryptoxanthin) and low-threshold periodontitis. β-Carotene and β-cryptoxanthin were the only antioxidants that were associated with an increased risk of high-threshold severe periodontitis. The adjusted odds ratio for high-threshold periodontitis in the lowest fifth relative to the highest fifth of β-cryptoxanthin was 4.02 ($p = 0.003$).

Conclusion: It is concluded that low serum levels of a number of carotenoids, in particular β-cryptoxanthin and β-carotene, were associated with an increased prevalence of periodontitis in this homogenous group of 60–70-year-old Western European men.

Key words: antioxidants; carotenoids; periodontitis

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Periodontitis is an inflammatory condition representing the response of the periodontal tissues to lipopolysaccharide derived from Gram-negative anaerobic bacteria. Inflammation is known to be a protective response that focuses on the removal of the stimuli responsible for damage to the tissues, thereby leading to the restoration of health (Medzhitov 2008). The persistence of the bacterial stimulus results in the inflammatory process becoming chronic in nature. However, this is not associated with progressive damage to the periodontal tissues in all those affected. Some factors associated with progression have been identified. For example smoking is known to be a major environmental risk factor (Tomar & Asma 2000). There may also be regulatory factors, not well characterized as yet, which are protective and limit the progression of periodontal tissue destruction. In this context, antioxidant micronutrients may have an important protective role.

A recent comprehensive review concluded that oxidative stress is at the heart of the periodontal tissue damage that results from host–microbial interactions (Chapple & Matthews 2007). Tissue damage results from dysregulation of chronic inflammation as a consequence of the excessive recruitment

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and activation of phagocytes. Overproduction of cytokines, proteinases and reactive species (RS) further contributes to the chronic nature of the inflammatory lesion (Halliwell & Gutteridge 2007). There is still debate as to whether antioxidant depletion is a cause of disease or a consequence of the tissue damage that accompanies disease progression. Hypothetically, because RS have a role to play, good tissue antioxidant status may then help to reduce tissue injury or damage. It has been shown that reduced dietary intake of vitamin C is associated with an increased risk of periodontitis in participants in the NHANES III study (Nishida et al. 2000). A further study from NHANES III reported that the prevalence of periodontitis was negatively associated with serum levels of antioxidants including vitamin C, bilirubin and various carotenoids (Chapple et al. 2007). There are no published epidemiological studies of antioxidant levels and periodontitis in European populations. We hypothesized that men who had good periodontal health would have higher levels of serum antioxidants than those with periodontal disease. The aim of the current study was to investigate the association between periodontal status in a homogenous group of 60–70-year-old Western European men and the serum levels of various antioxidants including vitamin E and carotenoids.

Materials and Methods

Study population

The study subjects were participants in Prospective Epidemiological Study of Myocardial Infarction (PRIME), which is a cohort study of cardiovascular disease in men in Northern Ireland. The sampling frame was based on industry, the civil service and general medical practices. Between 1991 and 1994, a cohort, representing approximately 5% of 50–60-year-old men from the greater Belfast area, was recruited to match broadly the social class structure of the population in Northern Ireland (Yarnell 1998). Between 2001 and 2003, the surviving men were contacted by post and invited to attend for a review as part of their continuing involvement in the PRIME study. A total of 2010 men were reviewed, and a clinical periodontal examination was completed for 1400 (69.7%) of the men. The remainder of the sample was made up of 363 (18.1%) men who did not have a dental examination because a specialist dental examiner was not available during their visit, 158 (7.9%) who were edentulous and 89 (4.4%) who refused or had a medical condition that precluded periodontal probing. The inclusion criteria for the current study were a valid antioxidant measurement combined with a clinical periodontal examination in men who had six or more teeth. In the sample of 1400 men who had a periodontal examination, there were 38 (2.7%) men who had fewer than six teeth and 104 (7.4%) for whom no measurement of antioxidant levels took place because no blood sample was available for analysis or in $<1\%$ of cases as a result of machine failure. Each subject who met the inclusion criteria completed a questionnaire, which gathered information on their demographic and socioeconomic background and tobacco consumption. Measurements of weight and height were also recorded. Approval for the project was obtained from the Research Ethics Committee of the Faculty of Medicine, Queen's University, Belfast. The aims of the investigation and the nature of the study were fully explained to the subjects, who gave their informed written consent before participation.

All the periodontal examinations were completed by one of four dental hygienists who were calibrated to a ''gold standard'' senior clinical researcher before the study. There were regular monthly meetings to ensure inter- and intra-examiner consistency and reproducibility. Throughout the study, the hygienists maintained the standard set at the outset with κ values of >0.8 at the regular training sessions. In the periodontal examination clinical measurements were made at the mesial, distal, buccal and palatal/lingual aspects of all teeth excluding third molars. Probing pocket depths and clinical attachment level were recorded using Michigan O periodontal probes as described previously (Linden et al. 2007).

Periodontal status

Low-threshold periodontitis was identified by the presence of at least two teeth with non-contiguous inter-proximal sites with $\geqslant 6$ mm loss of attachment and at least one pocket of $\geqslant 5$ mm. High-threshold severe periodontitis was identified when $>15\%$ of all sites measured had loss of attachment $\geqslant 6$ mm and there was at least one site with deep pocketing ($\geqslant 6$ mm).

Antioxidant measurement

Venous blood samples were collected after a 12-h fast and centrifuged within 4 h. The samples were then frozen at -80°C. The serum samples were defrosted and analysed in 2006. The antioxidants should have remained stable with storage at -80°C and the levels of antioxidants recorded in the subsequent analysis should not have been affected (Comstock et al. 1993). Retinol, α-tocopherol, γ-tocopherol, α-carotene, β-carotene, β-cryptoxanthin, zeaxanthin, lutein and lycopene were extracted from the serum, and levels were measured by high-performance liquid chromatography analysis with diode array detection according to Craft et al. (1992). The assay was standardized using serum samples of known concentrations from the National Institute of Standards and Technology, Gaithersburgh, MD, USA.

Potential predictors of poor periodontal status

Body mass index (BMI) was calculated as the body weight/height^2 (kg/m^2). The BMI measured between 2001 and 2003 was categorized using the World Health Organization (2000) classification: normal weight equated to BMI <25 kg/m^2, overweight $\geqslant 25-<30$ kg/m^2 and obese $\geqslant 30$ kg/m^2. Smokers were divided into current, past or never. Diabetes was categorized by self-report of the condition. Socioeconomic status was categorized into high, middle and low using a composite measure of material conditions based on the type of living accommodation (rented or owned/mortgage), number of cars/vans/motorcycles in the household and the number of baths and/or showers and toilets in the home (Wagner et al. 2003).

Statistical analysis

The data for antioxidant levels were not normally distributed and therefore were log transformed before analysis. Results were summarized using the geometric mean and inter-quartile range. Antioxidant levels were compared between men with and without periodontitis using independent-samples *t*-tests and the ratio of geometric means was used to summarize the comparisons. Multivariable analysis was carried out using logistic regression to obtain odds ratios adjusted for possible confounders. Mod-

els were constructed with the periodontitis status (low or high threshold) as the dependent variable. The men were divided into groups using the quintiles of each antioxidant measured. The highest fifth of the distribution of each antioxidant was used as the reference category for the calculation of odds ratios. Confounders included in the analysis were age, smoking, diabetes, socioeconomic status and BMI. A test for trend was used to compare odds ratios across the fifths of antioxidant distribution. To check for possible effect modification interactions between smoking and antioxidants were added in the logistic regression model. To allow for multiple comparisons, the level of significance was set at $p \leqslant 0.01$.

Results

In total, 1258 men with six or more teeth, who had a periodontal examination, and measurements of antioxidant status formed the basis of this investigation (Table 1). The average age of the men studied was 64.2 years (Table 1), with a range from 58.8 to 72.2 years. Only 88 (7%) were aged below 60 years or were 70 years or above. The majority of the men had been exposed to tobacco, with 44% former and 17% current smokers. The mean BMI was 27.4 kg/m^2 and 21% of the men were classified as obese (BMI $\geqslant$ 30 kg/m^2). One quarter of the men (25%) had low-threshold periodontitis while a much lower proportion (8%) had evidence of generalized severe (high-threshold) periodontitis (Table 1).

Periodontitis and antioxidants

The ratios of geometric means show that the levels of α- and β-carotene, β-cryptoxanthin and zeaxanthin were highly significantly ($p < 0.001$) lower in the men with low-threshold periodontitis (Table 2). These carotenoids were also significantly lower in high-threshold periodontitis. There were no significant differences in the levels of lutein, lycopene, α- and γ-tocopherol or retinol in relation to low- or high-threshold periodontitis.

In fully adjusted models, there was an inverse relationship between a number of carotenoids (α- and β-carotene and β-cryptoxanthin) and low-threshold periodontitis (Table 3). Those in the lowest fifth of β-cryptoxanthin had an 83% increased odds of low-threshold periodontitis relative to those in the highest fifth. β-Cryptoxanthin and β-carotene were the only antioxidants that were associated with an increased risk of high-threshold periodontitis. β-Cryptoxanthin yielded an adjusted odds ratio of 4.02 ($p = 0.003$) for the lowest fifth relative to the highest fifth. There was no evidence of a significant interaction between smoking and antioxidants in any of the logistic models.

Discussion

The main finding of this study was that low levels of carotenoids in 60–70-year-old men in Northern Ireland were associated with a significantly increased risk of low-threshold periodontitis. The associations between the carotenoids and low-threshold periodontitis survived adjustment for major confounders that might have affected the periodontal condition. When periodontitis was assessed at two levels, as suggested by Tonetti & Claffey (2005), only β-carotene and β-cryptoxanthin were significantly associated with high-threshold severe periodontitis. Carotenoids are a group of coloured pigments, usually yellow, red or orange, that are widespread in plants. Tissue and plasma levels vary with the diet. There is some evidence for an antioxidant role in animals. In humans, however, they may exert beneficial effects by other mechanisms including regulating cell-to-cell communication or gene expression (Halliwell & Gutteridge 2007). Not all the antioxidants investigated were reduced in men with periodontitis. No associations were found between vitamin E, retinol and periodontitis.

The most striking finding in the current study was the association between β-cryptoxanthin and periodontitis. β-Cryptoxanthin has an anabolic effect on bone metabolism, which is not evident with other carotenoids such as lutein or lycopene (Yamaguchi 2008). β-Cryptoxanthin stimulates bone formation and inhibits bone resorption in a tissue culture model of bone (Yamaguchi & Uchiyama 2004). The mechanism may be through a stimulatory effect on the transcriptional activity in osteoblasts and an increase in the mRNA expression of alkaline phosphatase and Runx2, a master regulator of osteoblast differentiation (Uchiyama & Yamaguchi 2004). β-Cryptoxanthin also suppresses gene

Table 1. Baseline characteristics of the 1258 men studied by whether they exhibited low- or high-threshold periodontitis

	All participants ($n = 1258$)	Low-threshold periodontitis ($n = 320$)	Not low-threshold periodontitis ($n = 938$)	High-threshold periodontitis ($n = 96$)	Not high-threshold periodontitis ($n = 1162$)
Age (years), mean (SD)	64.2 (2.9)	64.6 (2.9)	64.1 (2.9)	64.5 (2.9)	64.2 (2.9)
Teeth, mean (SD)	19.9 (5.6)	18.1 (5.6)	20.5 (5.4)*	15.3 (5.0)	20.3 (5.4)*
BMI, mean (SD)	27.4 (3.5)	27.9 (3.8)	27.2 (3.3)*	28.4 (4.1)	27.3 (3.4)*
Diabetes, *n* (%)	69 (5.5)	28 (8.8)	41 (4.4)*	11 (11.5)	58 (5.0)*
Smoke, *n* (%)					
Never	504 (40.1)	88 (27.5)	416 (44.4)*	27 (28.1)	477 (41.1)*
Past	547 (43.5)	151 (47.2)	396 (42.2)	34 (35.4)	513 (44.1)
Current	207 (16.5)	81 (25.3)	126 (13.4)	35 (36.4)	172 (14.7)
Material conditions, *n* (%)					
High	562 (44.7)	133 (41.6)	429 (45.7)	32 (33.3)	530 (45.6)
Middle	298 (23.7)	67 (20.9)	231 (24.6)	20 (20.8)	278 (23.9)
Low	398 (31.6)	120 (37.5)	278 (29.6)	44 (45.8)	354 (30.5)

*Significant at $p < 0.01$.
BMI, body mass index.

Table 2. Antioxidant levels (μmol/l) by low- or high-threshold periodontitis status

	No periodontitis (low threshold)		Periodontitis (low threshold)		Ratio of geometric means (95%CI)	*p*
	n	geometric mean (IQR)	*n*	geometric mean (IQR)		
α-Carotene	935	0.078 (0.049–0.128)	318	0.062 (0.037–0.103)	0.80 (0.72, 0.89)	<0.001
β-Carotene	933	0.31 (0.21–0.48)	318	0.24 (0.16–0.36)	0.77 (0.70, 0.84)	<0.001
β-Cryptoxanthin	936	0.065 (0.042–0.107)	319	0.051 (0.030–0.086)	0.78 (0.71, 0.85)	<0.001
Zeaxanthin	935	0.035 (0.026–0.047)	319	0.031 (0.023–0.045)	0.90 (0.84, 0.95)	<0.001
Lutein	936	0.17 (0.13–0.23)	318	0.16 (0.12–0.21)	0.94 (0.89, 1.00)	0.035
Lycopene	915	0.37 (0.22–0.67)	311	0.32 (0.18–0.61)	0.88 (0.77, 1.00)	0.054
α-Tocopherol	938	30.7 (26.3–35.7)	320	30.1 (25.1–35.3)	0.98 (0.95, 1.02)	0.34
γ-Tocopherol	937	2.59 (2.06–3.15)	319	2.67 (2.07–3.38)	1.03 (0.98, 1.08)	0.20
Retinol	938	2.09 (1.76–2.50)	320	2.10 (1.74–2.54)	1.01 (0.97, 1.04)	0.75
	No periodontitis (high threshold)		Periodontitis (high threshold)		Ratio of geometric means (95%CI)	*p*
	n	geometric mean (IQR)	*n*	geometric mean (IQR)		
α-Carotene	1157	0.075 (0.047–0.125)	96	0.057 (0.034–0.093)	0.76 (0.64, 0.91)	0.002
β-Carotene	1155	0.29 (0.19–0.46)	96	0.22 (0.15–0.35)	0.75 (0.64, 0.88)	<0.001
β-Cryptoxanthin	1159	0.063 (0.040–0.104)	96	0.043 (0.029–0.072)	0.68 (0.58, 0.79)	<0.001
Zeaxanthin	1158	0.034 (0.025–0.046)	96	0.030 (0.021–0.042)	0.87 (0.79, 0.96)	0.006
Lutein	1158	0.17 (0.13–0.22)	96	0.15 (0.12–0.19)	0.92 (0.84, 1.01)	0.06
Lycopene	1132	0.36 (0.20–0.66)	94	0.32 (0.19–0.57)	0.89 (0.72, 1.10)	0.26
α-Tocopherol	1162	30.6 (26.2–35.6)	96	29.9 (25.2–35.9)	0.98 (0.92, 1.04)	0.43
γ-Tocopherol	1160	2.61 (2.07–3.19)	96	2.63 (2.03–3.44)	1.01 (0.94, 1.08)	0.83
Retinol	1162	2.09 (1.76–2.49)	96	2.09 (1.69–2.63)	1.00 (0.94, 1.06)	0.96

Statistical analysis by *t*-test of log transformed values.

expression of enzymes that are involved in bone resorption in osteoclasts (Uchiyama & Yamaguchi 2006). Translational clinical studies have shown that an increased intake of Satsuma mandarins, which are a rich source of β-cryptoxanthin, leads to changes in circulating markers of bone metabolism such as a decrease in tartrate-resistant acid phosphatase activity and an increase in γ-carboxylated osteocalcin, which suggests stimulation of bone formation and a decrease in bone resorption (Yamaguchi et al. 2005). A recent epidemiological study suggested that a high intake of fruit and vegetables containing β-cryptoxanthin could reduce the risk of osteoporosis (Sugiura et al. 2008). β-Cryptoxanthin, therefore, could be relevant to periodontal destruction, given its potential to inhibit osteoclastic bone resorption and stimulate osteogenesis rather than through its activity as an antioxidant.

There was also a strong association between α-carotene and low-threshold periodontitis and between β-carotene and both low- and high-threshold periodontitis. This may be due to the antioxidant effect of these carotenoids or alternatively due to their role in immune modulation. A 2-year longitudinal study in elderly individuals found that those with low levels of α- and β-carotene and total carotenoids were more likely to have high interleukin-6, an indicator of systemic inflammation, and this was associated with poor health outcomes (Walston et al. 2006). A strong inverse relationship has been demonstrated between β-carotene and systemic markers of inflammation including C-reactive protein (CRP) and white blood cell count (Erlinger et al. 2001). One possibility is that β-carotene has anti-inflammatory properties. High intakes of carotenoid-rich fruit and vegetables have been shown to result in an increase in plasma carotenoid levels and also a decrease in systemic inflammation as indicated by a reduction in CRP (Watzl et al. 2005). Supplementation with β-carotene has been shown to stimulate lymphocyte proliferation in human intervention studies and this may be important because immune cells are particularly sensitive to oxidative stress (Chew & Park 2004).

The findings of the current study broadly agree with those of Chapple et al. (2007), who found an inverse association between carotenoids and periodontitis in the United States using data from NHANES III. The prevalence of low-threshold periodontitis (26%) in the men in PRIME was higher than that of mild periodontitis (14%) in NHANES III, reflecting in part differences in the age profile of the participants in these studies. In addition, NHANES III included both males and females and had participants from the various ethnic groups in the United States. The men in PRIME were a representative sample of 60–70-year-old males in Northern Ireland and were almost exclusively of Western European origin. In the current study, low-threshold periodontitis was relatively common, affecting a quarter of the men, which is broadly similar to a previous work on the population of Northern Ireland (Mullally & Linden 1992). The low prevalence of severe periodontitis, which was identified in only 8% of the men examined, resulted in a reduced power to detect associations. Despite this, it is notable that the associations between both β-cryptoxanthin and β-carotene and high-threshold severe periodontitis were significant.

In general, men in the lowest fifth for each of the antioxidants had a risk factor profile different from those with the highest levels. Men in the current study with the lowest levels of antioxidants had an increased prevalence of smoking, greater cigarette consumption, higher BMI and lower SES, all of which have been associated with an increased risk of periodontitis (Borrell & Papapanou 2005). Previous studies of the men in PRIME have shown an association

Table 3. Multivariable analysis of low- or high-threshold periodontitis by fifths of antioxidant distribution

	Low-threshold periodontitis		High-threshold periodontitis	
	OR (95% CI)	*p* trend	OR (95% CI)	*p* trend
α-Carotene				
1 Low	1.64 (1.07, 2.51)		1.57 (0.80, 3.06)	
2	1.39 (0.91, 2.14)		1.15 (0.57, 2.32)	
3	1.18 (0.76, 1.84)	0.008	0.79 (0.36, 1.72)	0.08
4	1.05 (0.67, 1.64)		0.85 (0.40, 1.84)	
5 High	1.00 (Reference)		1.00 (Reference)	
β-Carotene				
1 Low	1.81 (1.17, 2.82)		1.87 (0.86, 4.06)	
2	1.89 (1.22, 2.92)		2.46 (1.15, 5.28)	
3	1.47 (0.94, 2.29)	<0.001	1.18 (0.51, 2.72)	0.01
4	0.85 (0.53, 1.37)		1.06 (0.45, 2.50)	
5 High	1.00 (Reference)		1.00 (Reference)	
β-Cryptoxanthin				
1 Low	1.83 (1.19, 2.80)		4.02 (1.61, 9.99)	
2	1.51 (0.98, 2.32)		2.90 (1.14, 7.42)	
3	0.98 (0.62, 1.54)	<0.001	2.08 (0.79, 5.51)	0.001
4	1.02 (0.65, 1.61)		2.33 (0.88, 6.12)	
5 High	1.00 (Reference)		1.00 (Reference)	
Zeaxanthin				
1 Low	1.50 (0.99, 2.27)		1.32 (0.69, 2.53)	
2	1.41 (0.93, 2.16)		0.82 (0.41 1.65)	
3	0.96 (0.62, 1.49)	0.02	0.68 (0.33, 1.43)	0.35
4	1.09 (0.71, 1.69)		0.85 (0.41, 1.77)	
5 High	1.00 (Reference)		1.00 (Reference)	
Lutein				
1 Low	1.20 (0.78, 1.83)		1.14 (0.57, 2.29)	
2	0.94 (0.61, 1.45)		0.90 (0.43, 1.87)	
3	1.28 (0.84, 1.95)	0.74	1.43 (0.71, 2.85)	0.56
4	1.15 (0.75, 1.77)		0.72 (0.33, 1.59)	
5 High	1.00 (Reference)		1.00 (Reference)	
Lycopene				
1 Low	1.29 (0.84, 1.97)		1.23 (0.60, 2.49)	
2	1.62 (1.06, 2.46)		1.52 (0.77, 3.03)	
3	0.94 (0.60, 1.46)	0.11	1.00 (0.47, 2.11)	0.45
4	1.23 (0.80, 1.90)		1.25 (0.61, 2.58)	
5 High	1.00 (Reference)		1.00 (Reference)	
α-Tocopherol				
1 Low	1.14 (0.76, 1.70)		0.96 (0.51, 1.81)	
2	0.77 (0.51, 1.17)		0.55 (0.27, 1.13)	
3	0.88 (0.58, 1.33)	0.61	0.87 (0.45, 1.67)	0.68
4	0.80 (0.53, 1.21)		0.74 (0.37, 1.45)	
5 High	1.00 (Reference)		1.00 (Reference)	
γ-Tocopherol				
1 Low	1.04 (0.69, 1.55)		1.11 (0.59, 2.08)	
2	0.73 (0.48, 1.11)		0.64 (0.31, 1.31)	
3	0.87 (0.58, 1.31)	0.82	0.79 (0.41, 1.55)	0.83
4	0.88 (0.58, 1.32)		0.98 (0.52, 1.87)	
5 High	1.00 (Reference)		1.00 (Reference)	
Retinol				
1 Low	1.12 (0.74, 1.69)		1.12 (0.61, 2.08)	
2	1.05 (0.69, 1.59)		0.76 (0.38, 1.49)	
3	1.30 (0.86, 1.96)	0.51	0.81 (0.42, 1.59)	0.97
4	0.97 (0.64, 1.47)		0.68 (0.34, 1.35)	
5 High	1.00 (Reference)		1.00 (Reference)	

Adjusted for age, smoking, diabetes, body mass index and socioeconomic status.
OR, odds ratio.

between obesity and periodontitis (Linden et al. 2007) and so we corrected for this in the multivariate analysis. It is possible that the beneficial effects of antioxidants may have been overshadowed by the harmful effects of smoking. In all multivariate models, smoking was a significant factor associated with periodontitis as has been shown in many previous studies (Tomar & Asma 2000). There is evidence that smoking is related to increased free radical production and antioxidant depletion. Smoking cessation is followed by a marked increase in the plasma antioxidant concentration and an improvement in plasma resistance to an oxidative challenge (Polidori et al. 2003). Smokers have lower circulating levels of antioxidants, which seems to be directly linked to smoking. For example, short-term effects lead to a reduction in the antioxidant micronutrient concentrations after smoking even one cigarette (Alberg 2002). Accordingly, we not only corrected for smoking but also, given the close relationship between smoking and antioxidant levels, we checked the adjusted analyses for possible interactions. No significant interactions were found for any of the antioxidants. Most of the results were no longer significant when a sub analysis was completed on never smokers; however, the small numbers of never smokers with low- or high-threshold periodontitis would have resulted in a reduced power to detect differences. In addition, the association between the carotenoids and low-threshold periodontitis survived adjustment for diabetes and SES. However, it is possible that there are other confounders that were not controlled for in the analysis. Reverse causality cannot be excluded in this cross-sectional study and it is conceivable that the lower levels of antioxidants represent changes in the dietary intake of fruit and vegetables as a result of deterioration in the periodontal condition.

Studies have shown that the total antioxidant capacity is lower in serum from periodontitis subjects (Chapple et al. 2002, Brock et al. 2004). Lipid peroxidation is significantly increased locally in periodontal lesions and this suggests an important role in the pathology of periodontitis (Akalin et al. 2007). Effective periodontal treatment results in a significant decrease in lipid peroxidation (Tsai et al. 2005). Taken together, these studies support the view that oxidative stress and depressed antioxidant function are features of periodontal tissues and fluids in periodontitis subjects. Considerable effort has been targeted at investigating whether interventions using antioxidant supplementation might affect the course of conditions that have been associated with oxidative stress. These trials have been largely unsuccessful (Bjelakovic

et al. 2007). Antioxidant levels may act as biomarkers of ongoing pathological processes related to disease occurrence, and therefore increased intake may have no effect on disease progression (Erlinger et al. 2001). The intervention studies have produced confusing results (Woodside et al. 2005), and it remains unclear at what point antioxidant defences might be most effective. They may be more important in the early stages of disease to prevent its establishment rather than having protective effects in individuals with established disease.

This study was not designed to investigate the possible mechanisms through which the carotenoids may have exerted protective effects on the periodontal tissues. Positive effects on the periodontium may have been through an antioxidant mechanism or alternatively may have resulted from changes in gene expression that affected aspects of connective tissue or bone metabolism or the function of immune or inflammatory cells. It is not possible to state whether low dietary intake or high oxidative stress or a combination of these factors contributed to low antioxidant levels. The men studied were aged 60–70 years and physiological changes during their lifetime such as a reduction in the digestive and absorptive efficiency of the gastrointestinal tract with age could have contributed to impairment of micronutrient status in some cases. The requirements for micronutrients are not reduced with ageing and therefore a sufficient supply of dietary antioxidants remains important (Elmadfa & Meyer 2008).

It is concluded that low serum levels of a number of carotenoids were associated with low-threshold periodontitis in a homogenous group of 60–70-year-old men. A strong association was also evident between low levels of β-carotene and β-cryptoxanthin in particular and severe periodontitis. Low levels of carotenoids may reflect a lifestyle that is inconsistent with periodontal health due to poorer socioeconomic conditions, smoking, obesity and poor nutrition. Nevertheless, it is possible that levels of carotenoids, particularly β-carotene and β-cryptoxanthin, falling below a certain threshold may have independent effects leading to an increased risk of periodontitis. If this is the case, then it raises the possibility that an increased intake of carotenoids may have a protective role against periodontitis. This merits further investigation in controlled trials.

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Clinical Relevance

Scientific rationale for the study: It has been suggested that oxidative stress is involved in the tissue damage that characterizes chronic periodontitis. In this context, epidemiological studies from the United States have shown that a reduced dietary intake or low serum levels of antioxidants are associated with an increased risk of periodontitis.

Principal findings: After adjustment for possible confounders, there was an association between low levels of carotenoids and low-threshold periodontitis in 60–70-year-old men in Northern Ireland. Two of the carotenoids, β-cryptoxanthin and β-carotene, were significantly associated with severe periodontitis.

Practical implications: Dentists should be aware that periodontitis may be associated with low levels of antioxidants. An increased intake of fruit and vegetables can increase antioxidant levels and may also reduce markers of systemic inflammation. Continued focus on improvement in the diet, with an increase in the consumption of foods containing antioxidants, may benefit periodontal health.