

Antibodies to periodontal pathogens and coronary artery calcification in type 1 diabetic and nondiabetic subjects

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Background and Objective: The aim of this study was to examine whether serum immunoglobulin G (IgG) levels to *Porphyromonas gingivalis* and *Actinobacillus actinomycescomitans* are higher in type 1 diabetic patients than in controls and are associated with coronary artery calcification, a measure of atherosclerosis.

Material and Methods: One-hundred and ninety nine type 1 diabetic patients (mean age 38 ± 4 years) and 201 age- and gender-matched nondiabetic subjects had coronary artery calcification, as measured by electron beam computed tomography. Serum IgG levels to *P. gingivalis* W50 and to *A. actinomycescomitans* HK1651 whole cells were measured by enzyme-linked immunosorbent assay.

Results: A similar proportion of diabetic patients (29%) and controls (31%, $p = 0.7$) had elevated serum IgG to periodontal bacteria, defined as being above the median antibody level for both microorganisms. Elevated antibody levels were associated with higher systolic blood pressure ($p = 0.02$) and an increased odds of coronary artery calcification in all subjects combined (odds ratio = 1.7, $p = 0.047$) and in diabetic subjects examined separately (odds ratio = 2.01, $p = 0.027$). Association of serum IgG levels with coronary artery calcification was independent of social class, lipids and antibody levels to other microorganisms, but not systolic blood pressure (odds ratio = 1.4, $p = 0.1$ on adjustment for blood pressure). There was no association between serum IgG level and vascular endothelial function.

Conclusion: Elevated levels of serum IgG to *P. gingivalis* and *A. actinomycescomitans* are associated with coronary artery atherosclerosis. This may reflect a direct role for periodontal infection or a role for the host response to infection in coronary atherosclerosis, particularly in patients with type 1 diabetes.

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Periodontitis is an inflammatory response to bacterial infections of the tissues surrounding the teeth in which

damage to the periodontal ligament and bony attachment apparatus occurs. Gingivitis is inflammation without

irreversible damage to the soft and hard tissues. *Actinobacillus actinomycescomitans* and *Porphyromonas gingivalis*

are considered to be important bacterial pathogens in periodontitis (1,2).

As previously comprehensively reviewed, more than 40 studies have reported on the association between oral conditions (mostly periodontal disease and gingivitis) and cardiovascular disease (3). In general, these studies have explored associations between either the history of periodontal disease or oral examination data with cardiovascular disease (4–7). The results are variable. For example, of 16 analyses from prospective cohort studies, 10 showed evidence of association and six did not. Part of this variability may reflect confounding; periodontal disease and cardiovascular disease share common risk factors, including smoking, socio-economic status (8), elevated total cholesterol, blood pressure and, in some studies, higher body mass index (9). Overt periodontal disease reflects infection, the systemic host response and the local tissue response. An association between periodontal disease and cardiovascular disease might be directly secondary to the periodontal pathology, but might also be secondary to the host response to periodontal pathogens. Although several reviews have suggested that information on periodontal pathogen burden and antibody response would be useful (3,9), few studies have examined the association between antibodies and periodontal microorganisms and cardiovascular disease outcomes (3,10–12). A proposed mechanism by which an association might occur includes induction of vascular endothelial damage (13), but only one study has examined the association of vascular endothelial function with overt periodontal disease and this did not include periodontal pathogen antibody measurements (14). More recently, studies of periodontal treatment have shown an improvement in endothelial function concomitant with nonsignificant reductions in C-reactive protein (15).

Diabetes has been reported to be a risk factor for periodontal disease, although the evidence for this is more consistent for type 2 diabetes than for type 1 diabetes (16,17). If true, then this might contribute to the increased

risk of coronary heart disease in diabetes. However, to our knowledge, no studies have addressed the role of periodontal infection in the four- to eight-fold elevation in coronary heart disease risk in type 1 diabetic patients.

Electron beam computed tomography studies established that coronary artery calcification is a valid measure of coronary atherosclerosis (18) and is increased in type 1 diabetic patients, particularly women, consistent with coronary heart disease event rates (19). The objectives of this study were to examine whether: (i) antibodies to *A. actinomycetemcomitans* and *P. gingivalis* are associated with coronary artery calcification and vascular endothelial function; (ii) antibody levels differ between type 1 diabetic patients and controls; and (iii) periodontal antibody levels partly explain the increased coronary artery calcification in diabetic patients compared with controls.

Material and methods

The subject sampling and main examination methods of this study have been described in detail previously (19). In brief, a random sample of type 1 diabetic men and women aged 30–55 years was taken from the registers of five diabetes clinics. Type 1 diabetes was defined as age of onset ≤ 25 years and insulin treatment within 1 year of diagnosis. A random sample of the general population, stratified to have a similar age and gender distribution to the diabetic patients, was drawn from the lists of two London general medical practices. Subjects were included regardless of any history of heart disease. In all, 199 type 1 diabetic patients (95 women) and 201 nondiabetic men and women (107 women) were examined. University College London Hospitals Ethics Committee approval and fully informed written consent was obtained. Antibody data were available on 194 diabetic subjects and 199 nondiabetic subjects.

Participants completed a standardized questionnaire. Two measures of socio-economic status were used: current social class by own occupation; and educational status (having left

continuous full-time education below age 19 years or not). Social class was defined based on occupation, with subjects grouped into those in manual vs. nonmanual occupations. The average weekly consumption of alcohol units was calculated, and smoking exposure was counted in pack years and categorized as current, ever, or never. Three supine blood pressure recordings were made after 5 min of rest by using an Omron 705c oscillometric device (Omron Healthcare Inc., Bannockburn, IL, USA). The mean of the second and third readings was used. Hypertension was defined as having a systolic blood pressure of ≥ 140 mmHg or a diastolic blood pressure of ≥ 90 mmHg, or being on antihypertensive drugs. Obesity was defined as a body mass index of ≥ 30 kg/m². After an overnight fast, blood samples were taken and total cholesterol, high-density lipoprotein cholesterol and triglyceride concentrations were measured using standard enzymatic colorimetric methods (intra-assay coefficients of variation of 2.6%, 2.6% and 2%, respectively). High-density-lipoprotein cholesterol was measured directly after stabilization of other lipoproteins and low-density-lipoprotein cholesterol was calculated by the Friedewald equation (20). Haemoglobin A1c was measured using a latex-enhanced immunoassay (intra-assay coefficient of variation 1.7%).

Vascular endpoints

An ultrafast computed tomography scanner (IMATRON C-150XL, San Francisco, CA, USA) was used to quantify coronary calcification, as previously described (19). A total score for each artery and for the entire heart was calculated by summing the lesion scores. All scans were scored by the same radiologist who was blinded to the gender and the diabetes status of the subject. Forearm blood flow in response to the endothelium-dependent vasodilator acetylcholine and the endothelium-independent vasodilator glyceryl trinitrate were measured by venous occlusion plethysmography, as previously described (21). Basal nitric oxide-dependent vascular tone was

assessed with the use of L-N^G-monomethyl-L-arginine (a nitric oxide synthase inhibitor). Each infusion was separated by a 10-min saline washout. The order of vasodilators was randomized.

Bacterial culture and enzyme-linked immunosorbent assay of serum antibodies

P. gingivalis strain W50 was grown in an anaerobic work station (Don Whitley, Shipley, UK; N₂/H₂/CO₂, 80 : 10 : 10) in brain–heart infusion medium (Oxoid, Basingstoke, UK) supplemented with 5 mg/L of haemin for 6 d. *A. actinomycetemcomitans* strain HK1651 was cultured in 5% CO₂ for 48 h in brain–heart infusion medium supplemented with 0.05% cysteine and 0.5% yeast extract. Cells were harvested by centrifugation at 10,000 g for 30 min at 4°C. The culture pellets were resuspended and washed in 0.05 M sodium carbonate buffer, pH 9.2, and then resuspended in the same buffer to an optical density at 640 nm of 1.0. The bacteria were heated at 60°C for 45 min to inactivate any proteolytic activity that could influence antibody detection and binding, and were then diluted 1 : 10 in sodium carbonate buffer and dispensed into 96-well plates (Costar, code 2595; Jenkns, East Grinstead, UK) in 100-μL aliquots per well. The plates were then incubated at 37°C for 4 h and at 4°C overnight. Excess bacteria were removed by washing five times in 0.005% Tween 20 in phosphate-buffered saline, pH 7.2, using an automated plate washer (Skatron, Lier, Norway). Plates were allowed to dry and stored at –20°C until needed.

Analysis of the immunoglobulin G (IgG) response to periodontal pathogens was carried out as previously described (22). Two serum samples available in quantity from patients with periodontal disease were selected as the reference serum and the reproducibility control. These were dispensed into small volumes and frozen at –70°C until needed. For each experiment, aliquots were defrosted and the residue was discarded after a single use. Serial dilutions of reference serum, test sera

and controls were made in 1% bovine albumin (Pentex, Serologicals Proteins Inc., Kankakee, IL, USA) in phosphate-buffered saline, pH 7.2. Participants' serum was diluted from 1 : 200 to 1 : 3200 (1 : 200, 1 : 400, 1 : 800, 1 : 1600, 1 : 3200). Reference serum was diluted from 1 : 200 to 1 : 4800 through 10 points (1 : 200, 1 : 300, 1 : 400, 1 : 600, 1 : 800, 1 : 1200, 1 : 1600, 1 : 2400, 1 : 3200, 1 : 4800). Each dilution was added to the plate in duplicate and the plates were incubated for 2 h at 37°C. The plates were washed as before and 200 μL peroxidase-labelled rabbit antihuman IgG (Dako, Ely, UK), diluted 1 : 500 in 1% bovine albumin in phosphate-buffered saline, was added to each well and the plates were incubated for 2 h at 37°C. The plates were again washed five times and the assay was developed using 0.4 mg/mL of *o*-phenylenediamine (Sigma, Gillingham, UK) in citrate-phosphate buffer pH 5.0 with 0.4 μL/mL of hydrogen peroxide added.

For each organism, the optical density of the reference sample was given an arbitrary value of 60 arbitrary units (AU) and the values for each test sample were expressed relative to this. So, for example, a result of 30 AU for antibody to *P. gingivalis* would mean that the optical density for that patient's assay was half that of the reference sample. Plates were read in a VersaMax plate reader (Molecular Devices, Sunnyvale, CA, USA) set at 492 nm and the results were calculated in SOFTMAX.PRO software. This calculates a value for dilutions of the test samples that fall within the range of the standard curve, multiplies by the dilution factor and reports an adjusted mean. The results were inspected to ensure that the dilution curves of the test and reference samples were parallel and only results within the linear portion of the curve were used. If fewer than two dilution points fell within this range, the test dilutions were adjusted accordingly and the assay repeated. From these values a weighted average for the relative antibody level for each test sample was calculated where the smaller the difference in the duplicates, the greater the weight given to the mean for that dilution. A reproducibility

sample was included in every plate, and any plate where that sample gave greater than 10% divergence was repeated. Fourteen blinded duplicate serum samples were also included in the analysis. These showed that the Spearman rank correlation coefficient between repeat pairs of samples was 0.8 for *A. actinomycetemcomitans* and 0.5 for *P. gingivalis*.

Serum IgG to human cytomegalovirus, herpes simplex virus, *Chlamydia pneumoniae* and *Helicobacter pylori* were also determined through the use of standard enzyme-linked immunosorbent assay (ELISA) kits; BioElisa cytomegalovirus-IgG (Biokit SA, ETI-HSV-G ½ kit (DiaSorin Ltd, Wokingham, UK), *C. pneumoniae* (Medac, Wedel, Germany) and *H. pylori* (Meridian Bioscience Inc., Cincinnati, OH, USA), as previously described (23).

Statistical methods

Analyses were carried out using STATA 7 (StataCorp, College Station, TX, US). We examined whether the prevalence of elevated antibodies differed between diabetic and nondiabetic participants using logistic regression, adjusting for age and then other possible confounders. As the distribution of antibody levels was very skewed, and to maximize the specificity of our measure of seropositivity, we defined elevated antibody levels as being above the median for both *A. actinomycetemcomitans* and *P. gingivalis* antibodies. Calcification scores (for the total heart) were positively skewed with a high frequency of zero values. As data transformation would not have normalized this distribution, logistic regression was used to examine the odds of having any calcification (a score of > 0) associated with antibody levels adjusting for covariates. As smoking has been shown to be associated with reduced serum IgG levels (24) and antibody responses to periodontal bacteria (25,26), we examined this association stratified by smoking status (ever/never).

Results

Table 1 shows the distribution of characteristics by diabetes status and

Table 1. Characteristics of diabetic and nondiabetic subjects

	Men			Women			Men and women		
	Nondiabetic (n = 94)	Diabetic (n = 101)	p-value	Nondiabetic (n = 105)	Diabetic (n = 93)	p-value	Nondiabetic (n = 199)	Diabetic (n = 194)	p-value ^a
Age (years)	37.8 (4)	38.0 (4)	0.7	37.9 (4)	37.5 (4)	0.5	38 (0.3)	38 (0.3)	0.8
Systolic BP	124 (12)	129 (12)	0.02	112 (13)	120 (14)	< 0.001	118 (14)	124 (14)	< 0.001
TG mmol/L ^b	1.3 (1.0–1.9)	1.1 (0.9–1.5)	0.04	0.9 (0.7–1.3)	0.9 (0.7–1.2)	0.9	1.1 (0.8–1.5)	1.0 (0.8–1.4)	0.14
LDL-C mmol/L	3.3 (1.1)	3.0 (0.96)	0.03	2.9 (0.83)	2.8 (0.87)	0.3	3.1 (0.96)	2.9 (0.92)	0.03
HDL-C mmol/L	1.6 (0.34)	1.7 (0.34)	0.01	1.8 (0.39)	2.0 (0.49)	0.04	1.7 (0.41)	1.8 (0.46)	0.001
BMI kg/m ²	24.9 (3.4)	25.4 (3.2)	0.3	25.6 (5.6)	25.3 (3.8)	0.6	25.3 (4.7)	25.3 (3.5)	0.9
HbA1c percentage	5.3 (0.4)	8.4 (1.2)	< 0.001	5.3 (0.4)	9.1 (1.7)	< 0.001	5.3 (0.4)	8.9 (1.5)	< 0.001
Diabetes duration (years)	3/4	23 (7.6)	3/4	3/4	24 (7.4)	3/4	3/4	23 (7.5)	3/4
Obese	7 (3)	8 (3)	0.8	21 (4)	8 (3)	0.02	14 (2)	8 (2)	0.08
White ethnicity	91 (3)	97 (2)	0.1	91 (3)	95 (2)	0.4	91 (2)	96 (1)	0.08
Nonmanual social class	68 (5)	82 (4)	0.02	83 (4)	92 (3)	0.07	76 (3)	87 (2)	0.004
Left school < 19 years	63 (5)	45 (5)	0.01	67 (5)	54 (5)	0.1	64 (3)	49 (4)	0.004
With calcification	54 (5)	52 (5)	0.6	22 (4)	48 (5)	< 0.001	37 (3)	50 (4)	0.015
Ever smoked	56 (5)	50 (5)	0.4	49 (5)	45 (5)	0.6	52 (4)	48 (4)	0.4
Above median antibody to PG	59 (5)	3 (5)	0.002	51 (5)	54 (5)	0.7	55 (4)	45 (4)	0.06
Above median antibody to AA	45 (5)	47 (5)	0.9	55 (5)	52 (5)	0.7	50 (4)	50 (4)	0.6
Above median antibody to both	28 (5)	26 (4)	0.7	34 (5)	32 (5)	0.7	31 (3)	29 (3)	0.8

^aThe p-values shown are for the comparison of the characteristic between those with and without diabetes adjusted for age and gender.

^bThe median and 25th and 75th centiles are shown for triglycerides (TG) because the distribution was skewed.

In the rows 'Age' to 'Diabetes duration', the data in the columns headed diabetics and nondiabetics are expressed as mean (standard deviation); in the rows 'Obese' to 'Above median antibody to both', the data in the columns headed diabetics and nondiabetics are expressed as percentage (standard error).

AA, *Actinobacillus actinomycetemcomitans*; BMI, body mass index; BP, blood pressure; HbA1c, haemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; PG, *Porphyromonas gingivalis*.

gender in the subjects studied. As reported previously, diabetic men had a prevalence of calcification similar to that of nondiabetic men, although its severity was significantly greater ($p = 0.03$ Mann–Whitney U -test) (19). Diabetic women had more than twice the prevalence of any calcification than nondiabetic women ($p < 0.001$; Chi-square test). There was no evidence of an elevation in antibodies in diabetic subjects of either sex for either organism. Indeed, diabetic men had significantly lower levels of antibody to *P. gingivalis* than nondiabetic men ($p = 0.002$ adjusted for age).

Table 2 shows the distribution of factors, including potential coronary heart disease risk factors, in those with elevated antibodies (i.e. above median antibody levels to both microorganisms) compared to those without. The only factors that differed between those with and without elevated antibodies were systolic blood pressure and ethnic origin. Those with elevated antibodies

Table 2. Cardiovascular disease risk factors and other characteristics, by periodontal antibody status

Risk factor	Antibodies not elevated (n = 275)	Above median antibodies for both AA and PG (n = 118)
Age	37.7 (3.8)	38.2 (4.4)
Systolic BP	120 (14)	123 (15)*
BMI	25.2 (4.1)	25.6 (4.2)
LDL-cholesterol	3.03 (0.97)	3.0 (0.9)
HDL-cholesterol	1.76 (0.45)	1.79 (0.41)
Triglycerides ^a	1.1 (0.8–1.5)	1.0 (0.8–1.4)
Percentage ever smoking	52 (3)	46 (5)
Percentage nonmanual	82 (2)	78 (4)
Percentage left school before 19 year	56 (3)	56 (5)
Percentage White	96 (1)	87 (3)**

*, $p = 0.016$, **, $p < 0.001$ for the difference in risk factor between those with and without elevated antibodies adjusted for age, gender and diabetes.

^aFor triglycerides the median and 25th and 75th centiles are shown as the distribution was skewed.

In the rows 'Age' to 'Triglycerides', the data are expressed as mean (standard deviation); in the rows 'Percentage ever smoking' to 'Percentage White', the data are expressed as percentage (standard error).

AA, *Actinobacillus actinomycetemcomitans*; BMI, body mass index; BP, blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PG, *Porphyromonas gingivalis*.

had a higher systolic blood pressure (3.3 mmHg higher, $p = 0.016$, adjusted for age and gender and diabetes).

Table 2 shows that the distribution of ethnicity differed significantly between those with and without elevated anti-

Table 3. Odds ratio for coronary artery calcification associated with having above median antibodies to both PG and AA

	Nondiabetic subjects	Diabetic subjects	All subjects adjusted for diabetes
Adjusted for age and gender	1.2 (0.6–2.3)	2.1 (1.1–4.0)*	1.6 (1.0–2.5)*
Adjusted for age, gender and SBP	1.1 (0.6–2.3)	1.7 (0.9–3.3)	1.4 (0.9–2.3)
Adjusted for age, gender, SBP, BMI, lipids, smoking, social class and ethnicity	1.0 (0.7–1.04)	2.0 (0.9–4.6)	1.4 (0.8–2.6)

*, $p < 0.05$.

BMI, body mass index; SBP, systolic blood pressure.

bodies. Indeed, among the small number of subjects of nonwhite ethnicity studied ($n = 25$), the prevalence of elevated antibodies was twice as high (60%) than in whites (28%, $p = 0.002$). The higher systolic blood pressure in those with elevated antibodies was independent of ethnicity (3.1 mmHg higher; $p = 0.025$ on adjustment for ethnicity).

Table 3 shows the association of elevated antibodies with coronary artery calcification, in diabetic and nondiabetic subjects separately, and then among subjects combined but with adjustment for diabetes. Among all subjects combined, those with elevated antibodies were more likely to have coronary artery calcification (odds ratio 1.6, $p = 0.047$). The strength of this association did not differ significantly between diabetic and nondiabetic subjects; however, as shown in Table 3, it was most apparent among diabetic subjects. Adjusting for systolic blood pressure reduced the odds ratio associated with elevated antibodies and it was no longer significant. Adjusting further for other factors (body mass index, lipids, smoking pack years, social class and ethnicity) did not reduce this odds ratio further (Table 3). Adjusting for seropositivity for herpes simplex virus, cytomegalovirus, *C. pneumoniae* and *H. pylori* did not alter the strength of the association.

There was no significant association between above median antibody levels for the two microorganisms and flow response to intra-arterial acetylcholine, glyceryl trinitrate or L-N^G-monomethyl-L-arginine. In a logistic regression model, the relationship of diabetes

to coronary calcification, adjusting for elevated antibody levels, did not alter the odds ratio for calcification associated with diabetes (odds ratio 1.7, 95% confidence interval: 1.1–2.7, $p = 0.01$), consistent with antibody levels not differing by diabetes.

If the sum of antibodies to *P. gingivalis* and *A. actinomycetemcomitans* was used instead as a periodontitis exposure measure, the total calcification score was positively correlated with the total antibody level in diabetic subjects (Spearman's $\rho = 0.19$, $p = 0.007$), but not in nondiabetic subjects ($\rho = 0.01$).

Discussion

The data presented in this report provide evidence of an association between periodontal antibodies and coronary artery calcification, a measure of atherosclerosis burden. We found that, particularly among diabetic subjects, those with elevated periodontal antibodies had significantly more coronary artery calcification and also higher systolic blood pressure. Adjusting for blood pressure attenuated the association between periodontal antibodies and calcification. This is consistent with systolic blood pressure confounding the relationship between the host response to periodontal pathogens and calcification. However, it is also consistent with elevated systolic blood pressure being part of the mechanism by which the periodontal pathogen response might influence atherosclerosis. Although a difference of 3.3 mmHg in systolic blood pressure between those with and without eleva-

ted antibodies may not seem very large clinically, a prolonged difference of this size is associated with about 20% more stroke and 15% more coronary heart disease (27). Importantly, we did not find impaired vascular endothelial function to be an explanation of, or a mediator of, the relationship between antibody level and coronary calcification. To our knowledge only one previous study, in 269 subjects, has examined the relationship between coronary artery calcification and any periodontal related measure. Nakib *et al.* reported that subjects with moderate or severe periodontitis had a higher prevalence of high calcification scores, although the difference was not statistically significant (28).

It is important to emphasize that the associations we are reporting here are with antibody levels and not directly with pathogen burden or overt periodontal disease. The levels of IgG to *P. gingivalis* and to *A. actinomycetemcomitans* have been found to correlate with pathogen burden and the level of periodontal disease in White people and in other ethnic groups (1,2,29,30), and even where they do not correlate with current bacterial load they have been shown to have reasonable sensitivity and specificity for periodontal disease (31,32). IgG levels remain persistently elevated, even when in those with previous periodontal disease (33). Thus, the levels of IgG to *P. gingivalis* and *A. actinomycetemcomitans* have clearly been shown to be sensitive indicators for current or past periodontal disease. Whether they are specific for these two organisms, or cross-react with other organisms, is less clear. However, checkerboard assessments of the levels of IgG to many periodontal pathogens have shown that IgG to *P. gingivalis* and *A. actinomycetemcomitans* were the most useful at discriminating periodontal cases and controls, consistent with such levels having reasonable specificity for periodontal disease (31). With regard to the ELISA used in this study, *P. gingivalis* W50 was chosen as an example of a highly virulent strain of the organism as it is a highly invasive strain in an animal model (34). Griffen *et al.* used analysis of the polymer

phism in the internal spacer region of the 16S ribosomal RNA region to type *P. gingivalis* isolates and showed that the strongest association with disease was with a clonal type resembling strain W50/W83 (35). We chose *A. actinomycetemcomitans* HK1651, which has been described as a member of the high-toxicity JP2 clone of serotype b. A study by Yang *et al.* showed that of the two most commonly occurring serotypes of *A. actinomycetemcomitans*, serotype b is most often isolated from patients with periodontal disease and serotype c is isolated from periodontally healthy subjects (36). Fan *et al.* showed that there is significant cross-reactivity between serotypes of *P. gingivalis* (37), but *A. actinomycetemcomitans* patients infected with other strains may not have had elevated antibody levels. We note that this reduced sensitivity weakens the power of the study to detect an association between *A. actinomycetemcomitans* infection and disease, rather than causing spurious associations. Previously, we have shown significantly higher IgG levels to *P. gingivalis* in patients with periodontal disease when compared to controls, in a case-control study using the ELISA used in the present study (22).

The relationship of IgG levels with periodontal disease is not a direct one (3) and can vary with age, host immune status and level of previous tooth loss (10). Thus, an association between periodontal disease and cardiovascular disease might be directly secondary to periodontal pathogens, but might also be secondary to the host response to periodontal pathogens. Our data are useful because at the very least they demonstrate that failure to mount an immune response to periodontal pathogens is not likely to be the main explanation for the reported relationships between pathogen burden or periodontal disease and cardiovascular disease. As reviewed recently by Beck, all but three studies in this field have focussed on clinical examination or a history of periodontal disease (3). Pussinen *et al.* reported that having a combined antibody response to *A. actinomycetemcomitans* and *P. gingivalis* of more than three standard

deviations above the healthy population mean was more common in men with coronary heart disease than in men without (10). The mean age of subjects studied was 67 years and 19% were edentulous. The association between antibody levels and coronary heart disease was strongest in edentulous subjects. The authors noted that in dentate subjects the number of teeth increased with antibody levels. They postulated that tooth loss was an important negative confounder of the relationship between antibody levels and cardiovascular disease, noting that antibody levels decrease following tooth extraction. Unfortunately, it was not possible to conduct dental examinations in these subjects and so we were not able to examine this issue in our data. However, the lack of data on number of teeth has the effect of reducing the power of our study to detect associations and it would not lead to a spurious association being found. Also, given the much younger age of subjects in the present study, few, if any, would be expected to have been edentulous. In another analysis, the same authors reported a borderline association between antibodies to *A. actinomycetemcomitans* and incident first stroke, and between *P. gingivalis* antibodies and incident and recurrent stroke (11,12). Of note, in some studies the relationships between cardiovascular status and IgA, but not IgG, antibodies to periodontal pathogens have been reported (12,38). We did not measure IgA antibody levels, which are a marker of very recent infection, and it is possible that stronger relationships between calcification and IgA levels exist that we could not examine. Beck *et al.* reported on a principal components analysis of data on clustering of cardiovascular disease status, periodontal status, antibody levels and levels of microorganisms (3). They reported that elevated antibodies appeared to be associated with both more severe periodontal disease and chronic systemic disease. Beck *et al.* noted that periodontitis prevalence was lowest in those with both low microorganism level and low antibody level and that those with a low microorganism but a high antibody level had less periodontitis than those

with a high microorganism and low antibody level.

Another important aspect of this study is that we were able to adjust properly for possible confounders and interacting factors. A meta-analysis of five prospective cohort studies of clinically measured periodontal disease with coronary heart disease reported a combined relative risk for coronary heart disease of 1.2 (95% confidence interval: 1.1–1.38) associated with periodontal disease. Possible residual confounding by socio-economic status was seen as a particular problem (9) and this has been reinforced by recent studies showing that socio-economic status is strongly associated with periodontal disease (8). However, it is very plausible that periodontitis is part of the mechanism by which low socio-economic status leads to coronary heart disease. Our study is useful in this regard, as social class and educational status were not associated with periodontal antibodies and, as such, the association of periodontal antibodies with coronary artery calcification cannot be attributed to confounding by social class. The lack of an association between social class and periodontal antibodies may be because we examined a fairly young, urban-dwelling population in a country where there is easy and cheap access to dental care. Furthermore, most of the participants were of the nonmanual social classes. Smoking is associated with reduced periodontal antibodies despite being known to be associated with an increased prevalence of periodontal disease. This may reflect depression of the specific immune response in smokers (24–26). Accordingly, we adjusted for smoking in the logistic regression model summarized in Table 3. Studies in type 2 diabetic subjects have found that elevated levels of IgG to *P. gingivalis* are associated with carotid stenosis (39). Elevated levels of IgG to *P. gingivalis* have been found to be associated with elevated lipids (40), C-reactive protein and albuminuria (41). In contrast, we did not find any association between antibody levels and lipids in these type 1 diabetic patients. Nor did we find any relationship with C-reactive protein or albuminuria (data not shown).

We did not find any evidence to support the idea that diabetic patients have a greater level of circulating antibodies to periodontal pathogens. However, what was notable in our data was that the relationship between antibody levels and coronary artery calcification was strongest in diabetic patients. It remains possible that diabetic patients have more disease or a greater pathogen burden, but fail to mount an antibody response as high as in nondiabetic patients or that an increased risk is counteracted by increased dental hygiene awareness and clinical care among the diabetic patients in our study. The data on diabetes and periodontal disease is conflicting, with both higher levels of disease than controls (42–44), and lower or similar levels, being reported (16,45). A meta-analysis concluded that overall the evidence did support increased periodontal disease in diabetes (46). As diabetic patients in our study were not found to have elevated antibodies, a higher prevalence of antibodies is not the explanation for differences in calcification between patients and controls. However, our data imply that periodontal disease prevention may be particularly important in diabetic patients, because among diabetic patients a stronger relationship between antibody level and calcification was seen than in the general population.

Methodological considerations

In this study, our measure of periodontal disease was determined by estimation of the relative antibody titres to two common periodontal pathogens. As described above, this is not a direct measure of disease. Furthermore, the assay repeatability was not perfect but the effect of this was to cause an underestimation in the association between calcification and antibodies so that the degree of association reported here is a conservative one.

In conclusion, our data suggest that the relationship between periodontal infection, host response and coronary heart disease and high blood pressure deserves further investigation. As with all cross-sectional studies, our data do

not prove causality. Indeed, given that in many studies periodontal disease is likely to be correlated with other factors, including social class, and is likely to be correlated with other infections, the only way to demonstrate convincingly that it actually causes coronary heart disease is to carry out a randomized controlled trial of the effect of preventing it on coronary heart disease or atherosclerosis. Given the prevalence of periodontal disease in the population, a small relative risk associated with it would be of large public health significance. A randomized trial is justified as there are effective ways of preventing periodontal disease. Our data suggest that such interventions may be particularly useful in diabetic patients as there was a strong association between periodontal antibody level and coronary calcification in this group.

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