Published in final edited form as:
*Cancer Causes Control*. 2013 November ; 24(11): . doi:10.1007/s10552-013-0265-2.

# Lifestyle, dietary factors and antibody levels to oral bacteria in cancer-free participants of a European cohort study

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The authors have no conflicts of interest.

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## Abstract

**Background—**Increasing evidence suggests that oral microbiota play a pivotal role in chronic diseases, in addition to the well-established role in periodontal disease. Moreover, recent studies suggest that oral bacteria may also be involved in carcinogenesis; periodontal disease has been linked several cancers. In this study, we examined whether lifestyle factors have an impact on antibody levels to oral bacteria.

**Methods—**Data on demographic characteristics, lifestyle factors, and medical conditions were obtained at the time of blood sample collection. For the current analysis, we measured antibody levels to 25 oral bacteria in 395 cancer-free individuals using an immunoblot array. Combined total immunglobin G (IgG) levels were obtained by summing concentrations for all oral bacteria measured.

**Results—**IgG antibody levels were substantially lower among current and former smokers (1697 and 1677 ng/mL, respectively) than never smokers (1960 ng/mL; p-trend = 0.01), but did not vary by other factors, including BMI, diabetes, physical activity, or by dietary factors, after adjusting for age, sex, education, country and smoking status. The highest levels of total IgG were found among individuals with low education (2419 ng/mL).

**Conclusions—**Our findings on smoking are consistent with previous studies and support the notion that smokers have a compromised humoral immune response. Moreover, other major factors known to be associated with inflammatory markers, including obesity, were not associated with antibody levels to a large number of oral bacteria.

## Introduction

Within the oral cavity, more than 700 different bacterial species or phylotypes inhabit a complicated ecosystem that remains in dynamic equilibrium[1, 2]. This ecological equilibrium is marked by the stable and diverse composition of the bacterial community and maintains the health of the oral cavity[3, 4]. Disruptions of this balance can promote the growth of opportunistic strains and disrupt the relationship between the microbes and the human host[5]. Increasing evidence indicates that oral microbiota play a pivotal role in chronic diseases; poor oral health (including periodontal disease and tooth loss) has been associated with respiratory disease[6], cardiovascular diseases[7], stroke[8, 9], rheumatoid arthritis[10], diabetes[11, 12], head and neck cancer[12, 13], pancreatic cancer[14, 15], colon cancer[16, 17], and orodigestive cancers[18].

Lifestyle, diet and disease status influence the host in different ways. Studies have consistently shown that smokers have lower immunoglobulin G (IgG) levels to oral bacteria, suggesting that smoking suppresses the humoral immune response[19, 20]. While obesity and diabetes can impact the innate immune response[21, 22], it is less clear whether these conditions can impact the humoral immune response, and specifically the immune response

to oral bacteria. No study has examined the relationship between dietary factors and IgG levels to oral bacteria in blood. A few studies observed that diet can influence bacteria in the mouth suggesting that it may impact the mounted immune response to these bacteria; for example, coffee and wine have strong anti-bacterial and anti-adhesive properties[23], cocoa can reduce bacterial polysaccharide production[24], sugars and acidic foods favor aciduric bacterial species[25], and yogurt may inhibit periodontal pathogens[26]. Furthermore, certain foods are rich in antioxidants and may influence the immune response by lowering levels of oxidative stress, a significant modifier of immune mechanisms. In one study, high levels of systemic oxidative stress were strongly associated with lower total IgG levels, after controlling for a large number of known cardiovascular risk factors[27].

Understanding the association between lifestyle factors and antibodies to oral bacteria may provide new insights into the interaction between the environment and the host immune response to pathogens. We measured antibodies to 25 oral bacteria and examined their association with lifestyle factors, including smoking, physical activity, obesity and diabetes, and dietary factors previously linked to oral bacteria, in a population study of cancer-free individuals.

## Materials and Methods

### Study Population

The European Prospective Investigation into Cancer and Nutrition (EPIC) includes 519,978 participants, mostly aged 35–70 years, who were recruited in 23 centers within 10 European countries (Denmark, France, Germany, Greece, Italy, the Netherlands, Norway, Spain, Sweden, and the United Kingdom) between 1992 and 2000. Detailed descriptions of the study design, population, and baseline data collection of the cohort can be found in a previous report[28]. Each participant provided informed consent. The local ethical review committees approved the EPIC cohort study as well as the current project. For this analysis, we included 395 cancer-free control subjects (i.e., no known medical conditions at the time of blood draw and no major chronic diseases diagnosed during follow-up) who had available antibody measurements for 25 oral bacteria (see previous study[15]). In all but three countries (Denmark, Norway and Sweden), blood samples were collected based on a standardized protocol and aliquoted in plastic straws (plasma, serum, erythrocytes, and buffy coat for DNA). The aliquoted specimens were then stored in a central biorepository (IARC, France) in liquid nitrogen (−196°C). In Sweden, all samples were stored locally in freezers at −80°C and in Denmark in nitrogen vapour (−150°C). Due to small numbers (n=2), samples from Norway were excluded from this analysis.

### Antibody detection

Plasma content of IgG antibodies against a panel of 25 bacterial species/strains selected for a prior study examining the relation between periodontal disease and pancreatic cancer[15]. Measurements were conducted using an immunoblot array as described previously[15, 29]. This method has the advantage of using a very small amount of primary sample (less than 10 μl). Briefly, whole-cell formalin fixed bacteria suspensions and protein A from *Staphylococcus aureus* (control) were used as antigens. Plasma samples (diluted 1:125), and serial dilutions of human IgG immunoglobulin (250, 125, 62.5, 30, 15, and 7.5 ng/ml) were applied to the perpendicular lanes of the immunoblotter. Bound IgG was detected by anti-human IgG antibody. Plasma IgG concentrations were estimated by using the human IgG immunoglobulin reference curve. The lower detection limit of the method was 3.0 ng/ml and interassay correlations were previously reported to be good (percent concordance between 0.67 and 0.84[15]).

### Diet and lifestyle questionnaires

Dietary and lifestyle questionnaires were completed by participants at enrolment. Diet was assessed using country-specific, validated questionnaires designed to capture habitual consumption of food over the preceding year[30]. Extensive quantitative dietary questionnaires with up to 260 food items were used in northern Italy, The Netherlands, Germany, Greece, France and Spain. Semi-quantitative food frequency questionnaires were used in Denmark, Norway, Umeå (Sweden), and Naples (Italy). Combined dietary methods of food records and questionnaires were used in the UK and Malmö (Sweden)[28].

Lifestyle questionnaires included questions on education, occupation, medical history, lifetime history of consumption of tobacco, alcoholic beverages and physical activity[28]. Height and weight were measured directly in most centres at recruitment; self-reported height and weight were obtained in France and at one centre in the United Kingdom. Body mass index (BMI) was calculated as $kg/m^2$. Waist circumference was measured either at the narrowest torso circumference (Italy; Cambridge, United Kingdom; and Utrecht, the Netherlands) or at the midpoint between the lower ribs and iliac crest (Bilthoven, the Netherlands; Potsdam, Germany; Malmö, Sweden; and Oxford, United Kingdom). In Spain, Greece, Denmark, and Heidelberg, Germany, a combination of methods was used, although the majority of participants were measured at the narrowest circumference. Hip circumference was measured at the widest circumference (Italy; Spain; Bilthoven, the Netherlands; Greece; and Malmö, Sweden) or over the buttocks (United Kingdom; Utrecht, the Netherlands; Germany; and Denmark).

### Statistical Analysis

Total IgG level was estimated by summing together the antibody concentration to 25 oral bacteria. A transformation (square root) was conducted to obtain a normal distribution of this variable. We used generalized linear models (GLM) to estimate marginal means (LS Means) and p-values are based on the Type III *F*-test; major demographic, lifestyle and dietary factors were modelled simultaneously to examine the independent contribution of each. We created quartiles for dietary variables and for the waist-to-hip ratio. Mean values were squared to obtain interpretable measures (total IgG in ng/ml). We used a generalized linear model with binomial distribution (PROC GENMOD, LOGIT LINK) to obtain proportions of individuals with elevated antibodies (defined as >200ng/ml; >50 ng/ml; or >7.5 ng/ml, depending on prevalence of values in the higher categories) within different strata of smoking (never, former, current) controlling for age, sex, country of origin and education. This analysis was repeated for education, controlling for smoking status. *P*-values were based on tests for trend (ordered categorical variables modelled as continuous). All statistical analyses were conducted using the Statistical Analysis System (SAS) software package, Version 9.2 (SAS Institute Inc., Cary, North Carolina, USA).

## Results

In this cancer-free population, we observed a statistically significant association between smoking status and total IgG ($p = 0.01$); never smokers had higher total IgG levels than former and current smokers (Table 1) after adjusting for potential confounding variables (i.e., sex, age, country and education). Males had higher antibody level than females ($p=0.02$), and an inverse relationship was observed between education and total IgG levels ($p=0.003$). No statistically significant association was observed for total IgG levels and age, BMI, waist-to-hip ratio, diabetes, hypertension, hyperlipidemia, or physical activity (Table 1). Mean total IgG levels were higher among subjects with low education who were never smokers (2536 ng/ml), compared with subjects with low education who were ever smokers

(2056 ng/ml); the difference between smokers and nonsmokers was similar across educational level (data not shown).

Three of the five oral bacterial pathogens previously associated with periodontal disease were significantly associated with smoking status (*P. gingivalis* ATCC 33277, p = 0.03; *Aggregatibacter actinomycetemcomitans* ATCC 29523, p=0.02; *Tannerella forsythia*, p=0.003; Table 2) after adjusting for potential confounders; antibody levels to these oral pathogens were lower in smokers than in never smokers. For *A. actinomycetemcomitans* ATCC 43718, an inverse association was observed between IgG levels and smoking status, but the association did not reach statistical significance (p=0.17). Half of the antibodies to Gram-negative oral bacterial species measured in this study were associated with smoking status (in addition to the pathogenic strains: *Fusobacterium polymorphum,* p = 0.004; *Prevotella intermedia*, p=0.02; *Prevotella melaninogenica,* p=0.01; *Prevotella nigrescens,* p < 0.001). None of the antibodies to the Gram-positive bacteria were associated with smoking status after adjusting for potential confounders (Table 2).

After adjusting for potential confounders, including smoking, statistically significant associations were observed between education and IgG levels, such that having lower education was associated with higher levels of antibodies to *P. gingivalis* ATCC 33277, *P. intermedia*, *P. melaninogenica*, and *P. nigrescens* (Supplemental Table).

Quartiles of consumption of vegetables, fruits, vitamin C, dairy products, alcohol, and coffee were evaluated in relation to total IgG levels (Table 3); no associations were noted for these dietary factors. Additionally, total IgG levels for the consumption of tea, wine, yogurt, sugar and meats were examined separately, but no associations were observed.

Total IgG levels were not associated with BMI in this population. However, given that *T. forsythia* levels were reported to be significantly higher in subgingival biofilm of obese individuals in a recent study[31], we examined the association between these antibodies and BMI separately. While the association was not statistically significant (p-value = 0.18), overweight subjects had more elevated antibodies to *T. forsythia* (>50 ng/ml; 17.9%) compared to those with BMI<25 kg/m$^2$ (12.8%), adjusting for potential confounders.

## Discussion

In this large cross-sectional analysis of cancer-free individuals living throughout Europe, we observed lower overall antibody levels to 25 oral bacteria in current smokers compared to never smokers, women compared to men, and individuals with higher education compared to lower education. Antibody levels associated with smoking status included antibodies to a number of periodontal pathogens and other Gram-negative bacteria. Antibody levels associated with education were similarly associated with Gram-negative bacteria. No differences in overall antibody levels were noted by age, obesity, diabetes, physical activity, and a number of dietary factors, including fruit and vegetable consumption.

An association between oral bacteria and systemic antibody response is well-established in individuals who have different degrees of periodontal disease; serum antibody levels to oral pathogens, such as *P. gingivalis,* correlate with severity of periodontal disease[32]. However, in the general population, the association between serum antibody levels to oral pathogenic bacteria and periodontal disease status is inconsistent; other factors, such as smoking status, can influence the immune response (i.e., IgG levels)[19, 20]. Additionally, most studies on antibody response to oral bacteria have been performed on small numbers of patients with periodontitis, which limits interpretation to the general population. To date, two large population studies examined the relation between antibody levels to a number of oral bacteria and periodontal disease status. In a study of 8153 individuals (NHANES III), *P.*

*gingivalis* antibodies were the only antibodies (out of 19 species) that were significantly correlated to periodontal disease status (positive associations measured with two different definitions of periodontitis)[19]. In the other study, conducted in the Atherosclerosis Risk in Communities study (ARIC) and including 4,717 individuals, higher total IgG levels to 17 commensal oral bacteria were observed in those with more severe periodontal disease[27].

Smoking has been consistently shown to suppress the humoral immune response[20]. Total IgG levels have been observed to be lower among current smokers[33], and approximately half of IgG levels to specific oral bacteria were also lower in current smokers in a large population based study[19]. In the present study, we also observed that about half of IgG levels to oral bacteria were significantly lower among current smokers.

Dietary intake has been associated with oral bacteria measured directly in saliva and plaques of healthy subjects[23]. Individuals consuming large quantities of coffee, tea or wine, had lower levels of pathogenic bacteria in saliva and plaque than those drinking primarily water[23]; however, the same study made no statistical adjustment for potential confounders, including smoking status. Studies on diet and periodontal disease suggest that whole grains[34] may lower risk of periodontal disease, but overall findings with diet are inconsistent[35]. Dietary factors in our study were not associated with total antibody levels or with antibodies to pathogenic bacteria.

The relation between oral bacteria and obesity was previously examined in one study. Levels of *T. forsythia* measured in subgingival biofilm of subjects with periodontally healthy gums, or exhibiting gingivitis (n=120), were significantly higher among those who were obese ($r=0.43$, $p<0.001$), but other bacteria were not associated with obesity [31]. No association was noted for other pathogenic or commensal bacteria. While we did observe higher levels of antibodies to *T. forsythia* among overweight individuals, compared to normal weight (BMI <25 kg/m$^2$), the association was not statistically significant. Unlike smoking, total antibody IgG levels were not associated with BMI in our population.

Systemic oxidative stress has been shown to be more strongly associated with total oral bacteria antibodies than periodontal disease status, smoking or other demographic and lifestyle factors in a large population study [27]. This finding highlights the need for more studies on modulators of the immune response to bacterial pathogens. Although we did not have any measure of oxidative stress in our study population to examine this question, intake of foods high in antioxidants, including fruits and vegetables, was not related to antibody levels. More studies are also needed to understand factors that impact oxidative stress markers and the humoral immune response.

The major limitation of our study was the lack of data on periodontal disease status. Another limitation was the large differences in antibody levels by country which were not attributable to major lifestyle and medical factors examined. The differences therefore remain unexplained and may have influenced our results. However, the observed association between antibodies for periodontal pathogens and education, and the findings for smoking status, provide some indirect validation of the assays we conducted, given consistency with prior studies.

Our data are consistent with previous studies demonstrating suppressed immune response among smokers. Although obesity and diabetes can modulate the innate immune response, we observed no association with antibodies to oral bacteria in this study; however, as the number of diabetics in this study was low (n=17) more studies will need to confirm this finding. Similarly, consumption of antioxidant rich foods, or foods known to modulate bacteria colonization in the mouth, were not related to plasma levels of oral bacteria antibodies.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Table 1

Total antibody measured to 25 oral bacteria in apparently healthy individuals in the EPIC sub-cohort (n=395) in relation to baseline characteristics and lifestyle factors.

| | Numbers (%) | Total IgG (ng/mL)* | P-values** |
|---|---|---|---|
| **Sex** | | | 0.02 |
| Males | 187 (47) | 1892 | |
| Females | 208 (53) | 1663 | |
| **Age (years)** | | | 0.79 |
| <50 | 53 (13) | 1716 | |
| 50–59 | 166 (42) | 1801 | |
| ≥60 | 176 (45) | 1811 | |
| **Smoking status** | | | 0.01 |
| Never | 174 (44) | 1960 | |
| Former | 134 (34) | 1677 | |
| Current | 87 (22) | 1697 | |
| **BMI (kg/m$^2$)** | | | 0.41 |
| <25 | 171 (43) | 1693 | |
| 25–<30 | 162 (41) | 1809 | |
| ≥30 | 62 (16) | 1827 | |
| **Waist-to-hip ratio** § | | | 0.61 |
| Quartile 1 | 83 (23) | 1863 | |
| Quartile 2 | 97 (27) | 1734 | |
| Quartile 3 | 91 (25) | 1811 | |
| Quartile 4 | 90 (25) | 1688 | |
| **Education** | | | 0.003 |
| None | 17 (4) | 2419 | |
| Primary school completed | 147 (37) | 1662 | |
| Technical/professional school | 104 (26) | 1839 | |
| Secondary school | 54 (14) | 1520 | 18 |
| Higher education | 73 (19) | 1511 | |
| **Country** | | | <0.001 |
| Denmark | 82 (21) | 1020 | |
| France | 15 (4) | 2314 | |
| Germany | 52 (13) | 1803 | |
| Greece | 22 (6) | 1972 | |
| Italy | 38 (10) | 2009 | |
| The Netherlands | 38 (10) | 1548 | |
| Spain | 28 (7) | 2095 | |
| Sweden | 82 (21) | 1529 | |

| | Numbers (%) | Total IgG (ng/mL)* | P-values** |
|---|---|---|---|
| United Kingdom | 38 (10) | 1876 | |
| **Physical activity** # | | | 0.90 |
| Inactive | 106 (30) | 1686 | |
| Moderatively inactive | 123 (34) | 1761 | |
| Moderatively active | 66 (18) | 1781 | |
| Active | 65 (18) | 1784 | |
| **Diabetes** $ | | | 0.20 |
| No | 362 (96) | 1799 | |
| Yes | 17 (4) | 1525 | |
| **Hypertension** $ | | | 0.94 |
| No | 205 (70) | 1829 | |
| Yes | 80 (27) | 1836 | |
| **Hyperlipidemia** $ | | | 0.72 |
| No | 183 (65) | 1823 | |
| Yes | 66 (35) | 1739 | |

*Sum of IgG measured for 25 oral bacteria.

**$p$-values mutually adjusted for other variables in this table (physical activity, waist-to-hip ratio, and diabetes, hypertension and hyperlipidemia not adjusted for to maintain sample size of 395).

§Quartiles: males, <0.90, 0.90 to <0.93, 0.94 to <1.0, and ≥1.0; females, <0.76, 0.76 to <0.81, 0.79 to <0.85, and ≥0.85; 36 with missing data for wait-to-hip ratio.

#Among 360 individuals with data on physical activity.

$Missing data: 16 for diabetes; 110 for hypertension; 146 for hyperlipidemia.

## Table 2

Percent individuals with elevated antibody levels to oral bacteria by smoking status[*].

| Bacteria | % with high IgG level[§] | | | P-values |
|---|---|---|---|---|
| | Never smokers | Past smokers | Current smokers | |
| **Oral periodontal pathogens[**] (Gram negative)** | | | | |
| *Porphyromonas gingivalis* ATCC 33277 | 26.2 | 14.6 | 13.8 | 0.03 |
| *Porphyromonas gingivalis* ATCC 53978[‡] | 38.4 | 30.7 | 36.7 | 0.17 |
| *Aggregatibacter actinomycetemcomitans* ATCC 29523 | 31.9 | 22.3 | 17.2 | 0.02 |
| *Aggregatibacter actinomycetemcomitans* ATCC 43718 | 31.9 | 26.4 | 21.8 | 0.17 |
| *Tannerella forsythia* ATCC 43037[‡] | 27.2 | 11.5 | 12.7 | 0.003 |
| **Oral bacterial species of the human microbiome (Gram negative)** | | | | |
| *Fusobacterium nucleatum* ATCC 25586[‡] | 37.9 | 27.0 | 35.2 | 0.52 |
| *Fusobacterium periodonticum* ATCC 33693[^] | 5.5 | 3.1 | 4.5 | 0.39 |
| *Fusobacterium polymorphum* ATCC 10953[^] | 48.3 | 34.2 | 26.5 | 0.004 |
| *Prevotella intermedia* ATCC 25611[‡] | 60.3 | 40.1 | 46.0 | 0.02 |
| *Prevotella melaninogenica* ATCC 25845[‡] | 73.7 | 62.1 | 57.3 | 0.01 |
| *Prevotella nigrescens* ATCC 33563[‡] | 81.3 | 70.8 | 59.4 | <0.001 |
| *Veillonella atypica* ATCC 17744[‡] | 9.8 | 6.9 | 8.1 | 0.55 |
| *Veillonella parvula* ATCC 10790[‡] | 23.1 | 17.5 | 20.2 | 0.49 |
| **Oral bacterial species of the human microbiome (Gram positive)** | | | | |
| *Bifidobacterium dentium* ATCC 27534[^] | 48.8 | 39.7 | 40.0 | 0.20 |
| *Corynebacterium matruchotii* ATCC 14266[^] | 56.0 | 47.8 | 42.9 | 0.09 |
| *Enterococcus faecalis* ATCC 29212[‡] | 8.7 | 7.0 | 5.7 | 0.34 |
| *Parvimonas micra* ATCC 33270 | 63.2 | 48.1 | 63.5 | 0.69 |
| *Peptostreptococcus anaerobius* ATCC 27337[^] | 7.5 | 5.4 | 4.9 | 0.35 |

| Bacteria | % with high IgG level[§] | | | P-values |
|---|---|---|---|---|
| | Never smokers | Past smokers | Current smokers | |
| *Streptococcus intermedius* ATCC 27335[‡] | 36.5 | 28.5 | 35.4 | 0.68 |
| *Streptococcus mitis* ATCC 49456 | 48.1 | 45.4 | 57.3 | 0.27 |
| *Streptococcus salivarius* ATCC 7073 | 10.9 | 7.7 | 10.2 | 0.73 |

Note: *Captonocytophaga ochracea; Eikenella corrodens; Actinomyces naeslundii; Finegoldia magna are not listed due to insufficient variation after adjusting for country.*

[*] Percentages and p-values for trend are adjusted for sex, age, country, and education.

[**] Oral bacterial species that have been previously associated with periodontal disease.

[§] "High" defined as >200ng/mL, unless otherwise indicated with footnote.

[‡] due to small numbers with antibodies above 200ng/mL, the cutpoint was set at >50 ng/ml

[^] due to small numbers with antibodies above 50ng/mL, the cutpoint was set at >7.5 ng/ml.

## Table 3

Total antibody to 25 oral bacteria in apparently healthy individuals in the EPIC sub-cohort (n=395) in relation to dietary factors*

| | Total IgG (ng/mL) | P-values |
|---|---|---|
| **Vegetable intake** | | 0.61 |
| Quartile 1 (lowest) | 1888 | |
| Quartile 2 | 1748 | |
| Quartile 3 | 1760 | |
| Quartile 4 (highest) | 1836 | |
| **Fruit intake** | | 0.49 |
| Quartile 1 | 1869 | |
| Quartile 2 | 1892 | |
| Quartile 3 | 1760 | |
| Quartile 4 | 1837 | |
| **Dairy intake** | | 0.59 |
| Quartile 1 | 1704 | |
| Quartile 2 | 1794 | |
| Quartile 3 | 1890 | |
| Quartile 4 | 1826 | |
| **Vitamin C** | | 0.50 |
| Quartile 1 | 1840 | |
| Quartile 2 | 1918 | |
| Quartile 3 | 1739 | |
| Quartile 4 | 1884 | |
| **Coffee intake** | | 0.86 |
| Quartile 1 | 1834 | |
| Quartile 2 | 1748 | |
| Quartile 3 | 1783 | |
| Quartile 4 | 1847 | |
| **Alcohol intake** | | 0.93 |
| Quartile 1 | 1820 | |
| Quartile 2 | 1788 | |
| Quartile 3 | 1761 | |
| Quartile 4 | 1734 | |

* Means and p-values for trend are adjusted for sex, age, country, education, BMI and smoking status.