Occupational exposure to airborne proteolytic enzymes and lifestyle risk factors for dental erosion—a cross-sectional study

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This study examined the hypothesis that occupational exposure to airborne proteolytic enzymes is associated with dental erosions on the facial surfaces of exposed teeth. Individuals (n = 425) working at a pharmaceutical and biotechnological enterprise (Novozymes A/S) were examined; their mean age was 35 years (range = 18–67 years) and 143 (34%) were women. Two hundred and two of these individuals were newly employed by the company. Occupational exposure was assessed from questionnaire and workplace information. For practical analytical purposes, individuals were categorized as either previously exposed to proteolytic enzymes or not. Information on relevant lifestyle factors and medical history was obtained from a questionnaire. The main effect measure was facial erosion, but lingual erosion indices and the presence of Class V restorations were also considered. The validity of these measures was shown to be very high. Adjusted for potential confounders, there was no association between history of occupational exposure to proteolytic enzymes and prevalent facial or lingual erosion. With respect to prevalence of Class V restorations, the association was significant. The present study did not support directly our primary hypothesis that occupational exposure to airborne proteolytic enzymes is associated with dental erosions on the facial surfaces of exposed teeth. However, the results indicate that exposure to proteolytic enzymes may lead to pronounced tooth substance loss, demanding treatment.

Key words: Dental erosion; industrial dentistry; occupational disease; oral epidemiology; proteolytic enzymes.

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Introduction

A clinically atypical erosive loss of dental enamel was observed on the facial surfaces of maxillary and mandibular incisors and canines of a patient [1] who was referred to the School of Dentistry, University of Copenhagen, for treatment of gingivitis. Information about lifestyle and health parameters gave no ready explanation for the observed substance loss, and a relationship to occupational aetiological factors was suggested. The patient was employed by a large pharmaceutical and biotechnological enterprise (Novozymes A/S), and for a period of more than 8 years had been working in a department producing proteolytic detergent enzymes. Following this primary observation, the remaining 41 employees at the same enzyme-producing division had a dental examination...
within the subsequent few months. Surprisingly, 10 individuals, i.e. almost 25%, showed moderate to severe erosions similar to the erosions first observed in the referred patient. After this observation, protein content and protein activity of saliva from the individuals with erosions were analysed and compared with the values from a control group. No significant differences were found in the two groups. A hypothesis of breakdown of enamel by proteolytic enzymes was raised on the basis of previous findings on the solubility of enamel proteins [2,3]. This hypothesis was further substantiated by preliminary in vitro studies of the effect of proteolytic enzymes on enamel powder, showing that the proteolytic enzymes broke down the enamel proteins.

Non-carious loss of dental hard tissue on non-masticating surfaces is primarily caused by chemical factors generating erosion. Dental erosion is defined as loss of dental hard tissue following a chemical process not involving bacteria [4]. The most frequently involved chemicals are acids, but exposure to proteolytic enzymes and complexing compounds has also been implicated in such enamel decomposition processes [5]. The most well known in vivo exposures are gastro-oesophageal reflux of acid [6], lifestyle, including dietary habits, and acid in the occupational environment [7].

Non-carious loss of dental substance on masticating surfaces is primarily due to mechanical factors, causing attrition and abrasion. The most important abrasive factors are airborne stone dust [8,9] and grinding particles in food [10].

It is important for the understanding of non-carious loss of tooth substance on both masticating and non-masticating surfaces that erosive and abrasive/attritive factors may interact in the pathogenesis of the lesions [11,12].

The present study examined the hypothesis that occupational exposure to airborne proteolytic enzymes is associated with dental erosions on the facial surfaces of exposed teeth. In the analyses, we took into account potential confounders related to lifestyle and occupation. Furthermore, we present the results of explorative analyses tentatively estimating relative weights, i.e. aetiological fractions, of occupational and lifestyle factors for the risk of dental erosions.

Materials and methods

Subjects

Five hundred and seventy-eight individuals were invited to take part in the study. Eighty per cent agreed to participate. Reasons for not participating were: did not want to, or had full dentures. Later, 37 individuals were excluded because: no cast was available; the cast was insufficient; or the presence of crowns, bridges or multiple fillings made it impossible to estimate the degree of dental erosion. There were a total of 425 subjects eligible for study, 74% of those invited. The sample had a mean age of 35 years (range = 18–67 years) and 143 (34%) were women. Of study participants, 223 of the individuals came from five selected departments in the company (Novozymes A/S) and were involved in the production of proteolytic detergent enzymes or insulin, or worked in enzyme analysing laboratories. The remaining 202 study participants had commenced employment within the preceding month.

Questionnaire

Each individual filled in a comprehensive questionnaire giving information on previous and present occupational exposures, relevant lifestyle factors and medical history. Questions were asked about exposure to acids, sugar/flour, solvents, abrasive dust and proteolytic as well as non-proteolytic enzymes. Referring to each type of substance, information was recorded about specific procedures and other exposure circumstances such as mouth pipetting, cleaning, accidents and use of protective masks. Furthermore, oral hygiene routines, including use of toothpaste, were recorded and dietary habits were evaluated by means of questions about consumption of fruit, coarse food, vegetables, sweets, soft drinks, cola, sports drinks, water and tea with lemon, and wine. Subjects were asked if the intake was: (i) seldom or never; (ii) weekly, but not daily; (iii) once or twice daily; or (iv) more frequent. Questions about special fruit or vegetable diets were also included. The questions about medical history dealt with past and present gastric symptoms such as heartburn, acid taste in the mouth, stomach ache, regurgitation and medical treatment with acetylsalicylic acid, vitamin C and antidepressants affecting the salivary flow.

Occupational exposure classification

Exposure was assessed from questionnaire and workplace information, the latter as either self-reported exposure or exposure estimates based on the individual workplace history. For the newly employed individuals, only information on previous jobs and exposures was used. Two occupational physicians (A.I.L., F.G.) classified independently all potentially abrasive or erosive exposures with regard to duration and degree of exposure. For many years, measurements have been carried out by the company’s occupational hygienists, providing information on a large number of chemical and physical exposures, including levels of enzymes and use of protective devices. This information was used to classify individuals on different levels of exposure (B.J.). Two hundred and
of occupational exposure, health and lifestyle of the individuals. The degree of erosion on facial and lingual surfaces was scored with six graduation levels for both facial and lingual surfaces. In order to quantify the erosion, an index value was calculated for both facial and lingual surfaces [15]. Increasing index values represented increasing degrees of erosion as follows.

- No erosion: an index value of 0 indicated that no erosion was observed.
- Slight erosion: index values from 0.1 to 1.5 comprised a spectrum of erosions varying from smoothing of a single tooth to smoothing of all teeth examined, the upper limit being loss of original morphology in up to half of the teeth.
- Moderate erosion: index values covering the interval 1.6–2.5 comprised smoothing of teeth with loss of original morphology and sometimes dentinal exposure, and/or Class V fillings.
- Severe erosion: index values ≥ 2.6 comprised teeth with extensive loss of substance, dentinal exposure and/or Class V restorations.

Classification of erosions

Two dentists (I.B.L., J.W.) performed the final classification of erosions on facial and lingual surfaces of maxillary incisors and canines by consensus decision on the basis of colour slides, casts and data from the clinical records. The classification was blind so that the dentists were unaware of occupational exposure, health and lifestyle of the individuals. The degree of erosion on facial and lingual surfaces was scored with six graduation levels for both facial and lingual surfaces. In order to quantify the erosion, an index value was calculated for both facial and lingual surfaces [15]. Increasing index values represented increasing degrees of erosion as follows.

1. Facial erosion—a facial index value > 1.5 (moderate to severe erosion).
2. Facial erosion likely to have been treated already—presence of one or more facial Class V restorations versus none.
3. Lingual erosion—a lingual erosion index > 0.3 (clinically, a slight erosion with smoothing of 2–3 teeth, defined arbitrarily using the tertile value, 0.4, as a cut-off point) versus less.

In separate analyses, factors that were associated with either facial or lingual loss of tooth substance were investigated, and also those factors that were most ‘predictive’ of the presence of Class V restorations were determined. All factors, except age, were included in a dichotomized form, i.e. as presence or absence of the factor in question, and they are presented as such in Tables 1–3.

Simple bivariate associations between dental characteristics and relevant factors are presented in those tables. Yates corrected \( \chi^2 \) tests were used to test for heterogeneity in the distribution of characteristics of subjects with defined facial or lingual tooth substance loss or presence of Class V restorations. Facial and lingual erosions were subdivided into three groups; a Kruskal–Wallis non-parametric analysis of variance was used to test differences between groups with respect to age and Kendall’s tau B was used to test for trend with respect to various categorical variables. To control simultaneously for the interrelationship of variables, we used multiple logistic regression analyses with the maximum likelihood ratio method and backward elimination of variables. In the analyses of the long-term employed Novozymes group alone, interaction terms were included between proteolytic enzyme exposure history and age. An a priori \( P \)-value of a two-sided test of <0.05 was regarded as a relevant level for rejection of the null hypothesis.

Results

Of the 202 new employees, 21 reported that they had worked with enzymes in previous employment. In the following, they were considered unexposed with respect to proteolytic enzymes. Whether this information was included in the analyses or not had no influence on the results presented.

Characteristics of individuals with different levels of facial tooth substance loss

Table 1 shows characteristics of individuals with different levels of facial tooth substance loss defined as a facial tooth index > 1.5 (moderate to severe erosion). Statistically, the strongest differences between individuals with and without facial loss of tooth substance were: age, exposure to proteolytic enzymes and wine consumption.
Additionally, proportions of individuals with occupational exposure to grinding substances, acids and organic solvents, use of tea with lemon and use of particularly abrasive dentifrice were more frequent among those with pronounced facial tooth substance loss.

**Characteristics of individuals with and without facial Class V restorations**

Table 2 shows characteristics of individuals with and without the presence of Class V restorations.

Statistically, the strongest differences between individuals with and without Class V restorations were age, gender and exposure to proteolytic enzymes and grinding substances. No other factors reached statistical significance. Crude prevalences of one or more Class V restorations on potentially exposed surfaces were 19.3% among those with a history of exposure to proteolytic enzymes (n = 212) and 4.7% among those without (n = 213); the odds ratio with 95% confidence limits adjusted for relevant confounders, including age, was 3.2 (1.3–7.9) (not shown in Table 2).

**Characteristics of individuals with lingual tooth substance loss**

Table 3 shows characteristics of individuals with different levels of lingual tooth substance loss, arbitrarily defined as an index value above the highest tertile, i.e. >0.3. Statistically, the strongest associations were: age, gender and exposure to proteolytic enzymes, grinding substances, acids and organic solvents. No other factors reached statistical significance.

**Multivariable analyses**

Table 4 shows the results of backward logistic regression analyses including all factors from Tables 1–3 associated with each of the outcome measures. Facial tooth substance loss was significantly associated with age and wine consumption. Class V restorations were significantly associated with age and exposure to proteolytic enzymes. Lingual tooth substance loss was significantly associated with age, male sex, a history of exposure to acids and use of lemon tea. Except for age, the odds ratios of significant factors were all >2. A final analysis was performed including the same independent variables and a dependent variable defined as: presence of a facial erosion index > 1.5, one or more Class V restorations, or a lingual erosion index > 0.3 versus the rest. Statistically, this loss of tooth substance, potentially associated with erosion, was significantly associated with age, history of proteolytic enzyme exposure and use of lemon tea (not shown in Table 4).

**Occupation, lifestyle and overall tooth substance loss**

Table 5 shows the crude prevalence of tooth substance loss in individuals with and without identified relevant occupational and lifestyle factors, alone and in combination. Interestingly, individuals with both
occupational and lifestyle risk factors had a 10-fold higher prevalence compared with individuals without any risk factors.

The relative prevalence of tooth substance loss referencing the group without risk factors was estimated in a logistic regression model adjusted for age and gender. An odds ratio of almost 12 was calculated for individuals with occupational as well as lifestyle factors. The odds ratios associated with either occupational or lifestyle factors were almost identical: 7 versus 7.2.

Table 2. Characteristics of individuals with and without Class V restorations [values are mean (SD) or frequency in % (n = 425)]

<table>
<thead>
<tr>
<th>Class V restorations</th>
<th>Present (n = 51)</th>
<th>Absent (n = 374)</th>
<th>P^a</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Occupational exposure</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ever exposed to</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proteolytic enzymes (%)</td>
<td>80.4</td>
<td>45.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Acids (%)</td>
<td>75.0</td>
<td>67.8</td>
<td>0.46</td>
</tr>
<tr>
<td>Grinding substances (%)</td>
<td>70.0</td>
<td>43.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Organic solvents (%)</td>
<td>52.0</td>
<td>45.6</td>
<td>0.49</td>
</tr>
<tr>
<td>Sugar/flour (%)</td>
<td>8.0</td>
<td>8.5</td>
<td>1.0</td>
</tr>
<tr>
<td><strong>Dietary factors</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wine consumption (%)</td>
<td>64.1</td>
<td>58.6</td>
<td>0.63</td>
</tr>
<tr>
<td>Lemon tea (%)</td>
<td>15.4</td>
<td>7.9</td>
<td>0.21</td>
</tr>
<tr>
<td>Fruit intake (%)</td>
<td>73.2</td>
<td>80.2</td>
<td>0.40</td>
</tr>
<tr>
<td>Soda pop intake (%)</td>
<td>74.4</td>
<td>83.4</td>
<td>0.24</td>
</tr>
<tr>
<td><strong>Dental cleaning parameter</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Use of particularly abrasive dentifrice (%)</td>
<td>27.5</td>
<td>19.4</td>
<td>0.32</td>
</tr>
<tr>
<td><strong>Health parameters</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Digestion problems (dyspepsia/habitual vomiting/nausea) (%)</td>
<td>20.0</td>
<td>18.4</td>
<td>0.98</td>
</tr>
<tr>
<td>Use of tablets containing acetylsalicylic acid (%)</td>
<td>17.5</td>
<td>18.6</td>
<td>1.0</td>
</tr>
<tr>
<td><strong>Sociodemographic characteristics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>44.1 (10.6)</td>
<td>33.7 (9.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gender, proportion male (%)</td>
<td>88.2</td>
<td>63.4</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

^aP-value of a Mann–Whitney non-parametric rank sum analysis (for variable age) or Yates corrected \( \chi^2 \) test (for categorical variables).

Table 3. Characteristics of individuals with different levels of lingual tooth substance loss [values are mean (SD) or frequency in %]

<table>
<thead>
<tr>
<th>Lingual tooth substance loss index value</th>
<th>0 (n = 241)</th>
<th>&gt;0–0.3 (n = 61)</th>
<th>≥0.4 (n = 90)</th>
<th>P^a</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Occupational exposure</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ever exposed to</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proteolytic enzymes (%)</td>
<td>37.3</td>
<td>47.5</td>
<td>70.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Acids (%)</td>
<td>62.8</td>
<td>66.7</td>
<td>80.3</td>
<td>0.006</td>
</tr>
<tr>
<td>Grinding substances (%)</td>
<td>35.3</td>
<td>42.4</td>
<td>67.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Organic solvents (%)</td>
<td>40.0</td>
<td>45.8</td>
<td>57.3</td>
<td>0.004</td>
</tr>
<tr>
<td>Sugar/flour (%)</td>
<td>7.2</td>
<td>8.5</td>
<td>7.9</td>
<td>0.39</td>
</tr>
<tr>
<td><strong>Dietary factors</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wine consumption (%)</td>
<td>55.4</td>
<td>58.8</td>
<td>63.4</td>
<td>0.12</td>
</tr>
<tr>
<td>Lemon tea (%)</td>
<td>7.6</td>
<td>2.0</td>
<td>14.1</td>
<td>0.16</td>
</tr>
<tr>
<td>Fruit intake (%)</td>
<td>79.4</td>
<td>74.5</td>
<td>85.9</td>
<td>0.24</td>
</tr>
<tr>
<td>Soda pop intake (%)</td>
<td>85.4</td>
<td>78.4</td>
<td>80.3</td>
<td>0.10</td>
</tr>
<tr>
<td><strong>Dental cleaning parameter</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Use of particularly abrasive dentifrice (%)</td>
<td>19.2</td>
<td>15.4</td>
<td>26.8</td>
<td>0.18</td>
</tr>
<tr>
<td><strong>Health parameters</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Digestion problems (dyspepsia/habitual vomiting/nausea) (%)</td>
<td>17.3</td>
<td>17.3</td>
<td>23.9</td>
<td>0.15</td>
</tr>
<tr>
<td>Use of tablets containing acetylsalicylic acid (%)</td>
<td>18.9</td>
<td>15.7</td>
<td>14.1</td>
<td>0.16</td>
</tr>
<tr>
<td><strong>Sociodemographic characteristics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>31.7 (8.2)</td>
<td>34.4 (9.2)</td>
<td>41.1 (10.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gender, proportion male (%)</td>
<td>58.1</td>
<td>65.6</td>
<td>84.4</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

^aP-value of a Kruskall–Wallis non-parametric analysis of variance (for variable age) or Kendall’s tau B test for trend (for categorical variables).

The table includes 392 individuals from whom a lingual tooth substance loss index was obtained.
Analyses including only long-term employees at Novozymes

Figures 1–3 illustrate the interplay of proteolytic enzyme exposure history and age with facial tooth erosions [using as endpoints the erosion index (Figure 1) and Class V restorations (Figure 2)] and the interplay of proteolytic enzyme exposure history and age with lingual tooth erosion (Figure 3) in the group of long-term employees at

### Table 4. Factors associated with facial tooth substance loss (using the dichotomized erosion index or Class V fillings as markers) and lingual loss (dichotomized lingual erosion index) after multivariable adjustment in a logistic regression model, using backward elimination of variables and the maximum likelihood ratio method (values are presented according to the strength of association with tooth substance loss)

<table>
<thead>
<tr>
<th></th>
<th>Odds ratio with 95% confidence limit</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Facial tooth substance loss (erosion index)</strong></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>1.09 (1.07–1.15)</td>
</tr>
<tr>
<td>Wine consumption</td>
<td>2.7 (1.3–5.5)</td>
</tr>
<tr>
<td><strong>Facial tooth substance loss (Class V fillings)</strong></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>1.10 (1.06–1.15)</td>
</tr>
<tr>
<td>Proteolytic enzyme exposure history</td>
<td>3.2 (1.3–7.9)</td>
</tr>
<tr>
<td><strong>Lingual tooth substance loss (erosion index)</strong></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>1.09 (1.05–1.12)</td>
</tr>
<tr>
<td>Male sex</td>
<td>3.1 (1.5–6.4)</td>
</tr>
<tr>
<td>Acid exposure history</td>
<td>2.4 (1.2–4.7)</td>
</tr>
<tr>
<td>Lemon tea</td>
<td>2.8 (1.1–7.4)</td>
</tr>
</tbody>
</table>

Variables with $P$ values >0.05 after multivariable adjustment were removed from the model. Included in all analyses were: proteolytic enzyme exposure history; grinding substance exposure history; acid exposure history; organic solvent exposure history; sugar/flour exposure history; wine consumption; use of acetylsalicylic acid tablets; digestion problems; soda pop consumption; intake of lemon tea (tea with lemon); use of particularly abrasive dentifrice; age; and gender.

### Table 5. Crude prevalence and adjusted odds ratio of overall tooth substance loss defined as: facial tooth substance loss (facial index $>1.5$ versus index $\leq 1.5$), Class V restorations (presence versus absence) and lingual tooth substance loss (lingual index $>0.3$ versus index $\leq 0.3$), according to occupational and lifestyle factors identified as being associated with substance loss

<table>
<thead>
<tr>
<th>Group</th>
<th>Crude prevalence of overall tooth substance loss (%; n/total)</th>
<th>Odds ratios of overall tooth substance loss referencing Group 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4 (1 of 25)</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>22 (11 of 50)</td>
<td>7.0 (0.8–60.3), $P = 0.08$</td>
</tr>
<tr>
<td>3</td>
<td>27 (21 of 77)</td>
<td>7.2 (0.9–61.3), $P = 0.06$</td>
</tr>
<tr>
<td>4</td>
<td>41 (74 of 180)</td>
<td>11.9 (1.5–93.9), $P = 0.02$</td>
</tr>
</tbody>
</table>

The odds ratios were calculated from coefficients obtained from a logistic regression model also including age and gender using backward elimination of variables and the maximum likelihood ratio method. Based on complete data available from 332 individuals. Definitions: group 1—no occupational, no lifestyle factors; group 2—one or more lifestyle factors, no occupational; group 3—one or more occupational, no lifestyle factors; group 4—one or more occupational and one or more lifestyle factors. Occupational factors were: exposure to proteolytic enzymes, acids and grinding material. Lifestyle factors were: use of lemon tea, particularly abrasive dentifrice and wine consumption.

**Figure 1.** Proportion of individuals with facial tooth substance loss (index $>1.5$) according to proteolytic enzyme exposure and age. Analysis including only long-term employees ($n = 223$).

**Figure 2.** Proportion of individuals with facial Class V restorations according to proteolytic enzyme exposure and age. Analysis including only long-term employees ($n = 223$).

**Figure 3.** Proportion of individuals with lingual erosion (index $>0.3$) according to proteolytic enzyme exposure and age. Analysis including only long-term employees ($n = 191$).
Novozymes. Figure 3 includes the 191 individuals from whom a lingual erosion index was obtainable. Substantial exposure was defined as the characteristic of exposure group 4, comprising individuals with the highest exposure. The associations were tested in logistic regression models, including also an interaction term between the dichotomous exposure variable and age (results were in accordance whether age was included as a categorical or a continuous variable).

Substantial exposure to proteolytic enzymes was associated with a significantly lower prevalence of pronounced facial enamel loss than less exposure or non-exposure (Figures 1 and 2). There was a significant interaction between substantial enzyme exposure and age. Figure 2 shows that among those below the age of 30, 3 out of 19 had Class V restorations in the highly exposed group, compared with 0 out of 21 in the less exposed or unexposed group. In the oldest age group, the prevalence of Class V restorations among those with a history of substantial exposure was only two-thirds that of those less exposed. With lingual erosion as the outcome variable (Figure 3), the prevalence of erosion rose with age, but no difference was seen between the two exposure groups.

Discussion

The results from cross-sectional studies should not be interpreted without taking into consideration the potential influence of three traditional forms of bias associated with measurement, confounding and selection.

Measurement errors or imprecision may be relevant for both exposure and effect variables. Classifying individuals according to different lifetime levels of lifestyle factors and occupational factors is always imprecise. In the present study, the degree of exposure to proteolytic enzymes was complicated by the fact that ~50% of those classified as highly exposed reported use of a mask during working hours, as compared with <10% among others (data not shown). Accordingly, it was an open question as to whether those classified as highly exposed were indeed the group with the more relevant exposure, so for the overall analyses we preferred to use a dichotomous grouping, i.e. study participants either had a history of exposure to proteolytic enzymes or they did not. Still, in analysis of long-term employees only, a clear association was found between exposure level and facial outcomes, erosion and Class V fillings (Figures 1 and 2). In contrast, and in accordance with our a priori hypothesis, no association whatsoever was observed between lingual erosion and exposure level (Figure 3).

Effect variables in the form of erosion indices were established to register, both quantitatively and qualitatively, tooth substance loss on the facial and lingual surfaces of front teeth. As described in detail elsewhere, these indices proved very reliable, with a high inter- and intra-observer correlation [15]. However, the most extensive erosions may escape relevant registration because treatments with large Class V restorations, crowns, bridges and dentures were not included in the erosion index, although these restorations may be due to erosive loss of tooth substance.

To ascertain that associations between exposure and effect were not simply the result of occupational exposure being a proxy for some other underlying risk factor, major potentially confounding erosive or abrasive factors related to lifestyle, health status and occupation were included.

A priori facial erosion was considered the primary effect measure. This was based on the original observation of a clinically atypical erosive loss of dental enamel [1]. It is interesting that the analysis of the entire data, except for age, only revealed wine consumption as significantly associated with facial erosion. However, this association was present only among new employees, despite the fact that similar wine-drinking patterns were reported by both groups (data not shown). A reasonable interpretation is that other factors had affected facial surfaces of long-term employees of Novozymes. In support of this is a surprisingly strong association of proteolytic enzyme exposure with Class V restorations. These restorations may represent erosive lesions which needed treatment and are considered as secondary effect measures. If, on the other hand, a number of these restorations represent treatment of caries, it may have one of three major implications: (i) the strength of an association between exposure to proteolytic enzymes and Class V restorations has been underestimated; (ii) exposure to proteolytic enzymes may facilitate the cariogenic process; and/or (iii) undetected social confounding.

The observed loss of tooth substance was much more pronounced on the facial than on the lingual surfaces of the front teeth, as expected for extrinsic aetiological factors such as those in the working environment. The most advanced lesions were often seen in the gingival part of the teeth, which were frequently restored with Class V restorations. This cervical location of the lesions contrasts with the previously described dental erosions after industrial exposure to acid, characterized by initial inciso-labial loss of tooth substance extending in a gingival direction and, in advanced cases, resulting in shortening of the teeth and an anterior open bite [16–22].

Wine consumption was quite strongly associated with, in particular, erosion of the facial surfaces, as expected for an extrinsic factor. Previous studies have reported on dental erosion in connection with professional wine tasting [23,24] and in patients with chronic alcoholism [25,26]. In these cases of higher intake of alcohol, severe erosions with dentine exposure of the lingual and incisal or occlusal surfaces of the maxillary teeth were observed. In professional wine tasters, this location may be due to the special tasting procedure. The location of erosions in
alcoholics has been ascribed to frequent regurgitation or vomiting. In contrast, the erosions seen on the lingual surfaces in the present study were only slight erosions affecting the enamel and did not show any correlation to wine consumption.

The association between proteolytic enzyme exposure and tooth substance loss is biologically plausible. An increased wear of the incisors of grazing sheep in New Zealand has been associated with dissolution of the organic substance of the teeth by proteolytic enzymes in grass and clover [27]. Proteolytic enzymes may act via both breakdown of the pellicle and decomposition of organic components of the enamel. Decomposition of the organic substance in the pellicle due to exposure to proteolytic enzymes may render the tooth surface more susceptible to physical and chemical damage. A biologically plausible explanation of a direct action of proteolytic enzymes on dental enamel is based on the assumption that dental enamel contains organic material likely to decompose after exposure to proteolytic enzymes. Thus, in vitro studies indicate that proteolytic enzymes are able to dissolve both enamel [3] and dentine [28], and that proteolytic activity is involved in maturation of enamel [29,30]. In vitro studies have also shown that treatment of human enamel with carbamide peroxide decreases the fracture toughness and abrasion resistance of the enamel, which may be due to an alteration of the organic matrix of the enamel [31]. Additionally, the association between exposure to proteolytic enzymes and other potentially erosive or abrasive occupational factors should be borne in mind. It is possible that synergistic effects between enzyme exposure and other factors may have been acting, or that exposure to grinding substances and/or acids may have been even less precise, or relevant, exposure variables. Occupational history provided a strong discrimination between those with and without overall pronounced tooth substance loss; compared with those without any potentially tooth-eroding work exposures, an isolated odds ratio of seven for history of either of the occupational factors was found.

The design of the study made it possible to estimate the relative significance of occupational and lifestyle factors associated with tooth substance loss. Such factors appeared to have an equally strong association with loss of tooth substance, and the presence of both occupational and lifestyle factors was additively associated with the effect.

The present study did not give support to our primary hypothesis that occupational exposure to airborne proteolytic enzymes is associated with facial dental erosions. However, the data presented showed that exposure to proteolytic enzymes was associated with an increased presence of Class V restorations. This indicates that exposure to proteolytic enzymes may lead to more pronounced tooth substance loss, demanding treatment. A significantly lower prevalence of facial dental erosions among those >40 years of age most heavily exposed (Figure 1) was a finding consistent with a higher degree of treated surfaces among those exposed. Also, the above-mentioned observation that wine drinking was not associated with facial erosion among those who had been working with proteolytic enzymes for a long time was consistent with such a hypothesis.

Overall, our results indicate that various occupational and lifestyle factors may be relevant in the pathogenesis of dental erosion. Some of these factors are well established in the pathogenesis of non-curious loss of tooth substance, i.e. acids [17, 20–22], grinding substances [9], lemon tea [32,33], wine consumption [24] and particularly abrasive dentifrice [34]. Since both occupational and lifestyle factors were involved in the observed erosions, further studies are warranted, not only to elucidate further the potential enzymatic degradation of tooth substance, but also to disclose further today’s prevalence of dental erosion in the general population.

References