BMI status in Swedish children and young adults in relation to caries prevalence

<table>
<thead>
<tr>
<th>Age in years</th>
<th>BMI &lt; 25</th>
<th>BMI 25-29.9</th>
<th>BMI ≥ 30</th>
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<td>8</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
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<td>3</td>
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<td>15</td>
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<tr>
<td>20</td>
<td>2</td>
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Effect of post-brushing mouthrinse solutions on salivary fluoride retention
Mystikos, Yoshino, Ramberg, Birkhed

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BMI status in Swedish children and young adults in relation to caries prevalence

Anita Alm1, Helen Isaksson2, Christina Fählæus3, Göran Koch2, Boel Andersson-Gäre3, Mats Nilsson3, Dowen Birkhed4, Lill-Kari Wendt5

Abstract

Overweight and obesity are increasing as health problems at global level. Dental caries and obesity are both multifactorial diseases and are associated with dietary habits.

The aim of the present study was to investigate the relationship between body weight status and caries prevalence in an unselected population followed from pre-school years to young adulthood. The present investigation was designed as a longitudinal analysis of the association between overweight/obesity and dental caries in one population at 3, 6, 15 and 20 years of age.

The result shows that adolescents (15 years) and young adults (20 years) who are overweight/obese had a statistically significantly higher caries prevalence than normal-weight young people. At 6 years of age, the odds (OR) of having caries among obese children are 2.5 times higher than the odds for caries among six-year-old children of normal weight (p=0.04). At 3 years of age, no association between overweight/obesity and caries was found.

To conclude, overweight and obese adolescents and young adults had more caries than normal-weight individuals. The present study emphasises the need for multidisciplinary approaches to change the lifestyle factors causing both overweight/obesity and dental caries.

Key words
Caries, BMI, overweight, obesity, young individuals

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Sammanfattning

Förekomst av övervikt och fetma ökar globalt och är idag ett stort hälsoproblem. Både karies och fetma är multifaktoriella sjukdomar associerade till bland annat kostvågor och andra livsstilsfaktorer. Vissa studier tyder på att det finns ett samband mellan övervikt och kariesförekomst, även om resultaten inte är entydiga.

Syftet med föreliggande studie var att studera sambandet mellan övervikt/fetma och karies i en oselekterad population från förskoleåldern till ung vuxen ålder. Studien var designad som en longitudinell studie där sambandet mellan övervikt/fetma och karies analyserades hos en population vid 3, 6, 15 och 20 års ålder.

Studien visar att ungdomar (15 år) och unga vuxna (20 år) med övervikt/fetma har statistiskt signifikant högre kariesprevalens än de som är normalviktiga. Vid 6 års ålder var oddsren för barn med fetma 2,5 gånger högre att få karies jämfört med 6-åringar med normalvikt (p=0.04). Vid 3 års ålder sågs inget samband mellan övervikt/fetma och karies.

Sammanfattningsvis påvisar studien högre kariesförekomst hos tonåringar och unga vuxna med övervikt/fetma. Studien understryker vikten av ett multidisciplinärt samarbete för att förändra livsstilsfaktorer som orsakar övervikt/fetma och karies.
Introduction
Overweight and obesity are increasing as health problems in many countries around the world, including those in Europe (34, 17). The World Health Organisation (WHO) has compared this marked change in body weight to a “global epidemic disease”. Overweight and obese children/adolescents run an increased risk of developing medical and psychosocial problems compared with individuals of normal weight (21, 30, 34).

Changes in the environment that are promoting a sedentary lifestyle and a high consumption of energy-dense foods and drinks have resulted in a larger number of children becoming obese (6). Studies have also shown a relationship between the consumption of sugar-sweetened drinks and snacks and childhood obesity (14, 18, 35).

Dental caries and obesity are both multifactorial diseases with a complex aetiology and both are associated with dietary habits. A sugar-rich diet, including beverages, is associated with various health problems such as obesity, dental caries and poor diet quality (5, 15). Although the eating pattern among overweight and obese children may represent a risk of dental caries, the documentation is sparse and a systematic review has revealed contradictory results (15).

Recently published studies have also reported conflicting results. Bailleul-Forestier et al. (3), Hilgers et al. (12), Willerhausen et al (31-33) and Modéer et al. (20) found an association between high weight and high caries frequency in children and adolescents, while Macek & Mitola (19), Pinto et al. (22), Kopycka-Kedzierawski et al. (16) and Tramini et al. (24) reported no statistically significant association between weight and caries.

The aim of the present study was therefore to evaluate the association between dental caries and body mass index (BMI) in an unselected population from pre-school years to young adulthood.

Material and methods
The present investigation was designed as a longitudinal analysis of the association between overweight/obesity and dental caries at 3, 6, 15 and 20 years of age. The study is part of a series of surveys of oral health in a cohort of 671 children followed from 1 to 20 years of age and living in the Municipality of Jönköping (1, 2, 26-28). The Ethics Committee at the University of Linköping, Sweden, approved the study. All 671 children who were 1 year of age in 1988 and living within the districts of four of the thirteen child welfare centres in the Municipality of Jönköping were invited to participate. These four districts included the town, suburbs and rural areas and were chosen to reflect the socio-economic levels of the population living in this part of Sweden. During the study period, the children in this study received dental care free of charge up to and including 19 years of age (at Public Dental Service Clinics), as well as regular check-ups of weight and growth as part of regular health care (at the child welfare centre and at school). For the children in the original cohort, data relating to caries and adiposity status were available in 525, 506, 402 children at 3, 6 and 15 years of age respectively and in 491 subjects at 20 years of age.

Dental examination
At 3 and 6 years
The dental examinations at 3 and 6 years of age were conducted by one of the authors (LKW) and have been described in detail elsewhere (27, 28). Caries was registered clinically by visual examination and probing and radiographically if proximal contacts in primary molars made clinical examination impossible. Decayed, extracted and filled surfaces (defs), including initial carious lesions, were registered. Clinically, initial caries was defined as a demineralised surface without cavitation, while manifest caries was defined as a carious lesion with cavitation. Radiographically, initial approximal caries was defined as a radiolucency in the enamel that had not passed the dentino-enamel junction, while manifest caries was defined as a radiolucency extending into the dentine (27).

At 15 years
Information on approximal caries at 15 years of age was obtained from bitewing radiographs which were analysed by one of the authors (AA). The radiographic procedure and dental examinations among the teenagers have been described in detail elsewhere (1). Caries was registered as initial or manifest caries as follows: initial caries (Dia) was defined as “a carious lesion in the enamel that has not reached the dentino-enamel junction or a lesion that reaches or penetrates the dentino-enamel junction but does not appear to extend into the dentine”. Manifest caries (Dma) was defined as “a carious lesion that clearly extends into the dentine”. The total approximal caries prevalence and fillings (Di+mFSa) were used in the analysis, here called DFSa total.
At 20 years
A dental health examination was conducted by one of the authors (HI) as close as possible to the age of 20; about 50% were examined at 20 years of age and 50% at 21 years of age. Caries was registered clinically by visual examination and probing. The clinical examination was supplemented by four posterior bite-wing radiographs. Decayed and filled tooth surfaces (including initial carious lesions) were recorded. Caries was registered clinically on smooth surfaces as follows: 1) initial caries (Di) – a carious lesion on an enamel surface with whitish areas without cavitation; 2) manifest caries (Dm) – a carious lesion with cavitation that extends into the dentine. For the radiographic procedure, please see the definition at 15 years of age. The total caries prevalence and fillings (Di+mFS) were used in the analysis, here called DFS total.

Body adiposity status
At 3, 6 and 15 years
Data relating to the weight and height of the pre-school children were obtained from child welfare records as close as possible to the dental examination at 3 (i.e. 2.5) and 6 (i.e. 5.5) years of age. Data relating to the weight and height of the 2.5-year-old children were acquired between 2.0 and 3.4 years of age (median 2.5 years). Data relating to the 5.5-year-old children were obtained between 4.9 and 6.6 years of age (median 5.5 years). The corresponding data for the 402 15-year-olds were obtained from school health care records when the teenagers were between 13.5 and 16.4 years of age (median 14.7 years). Body adiposity status was determined by calculating BMI (kg/m²) using the international classification system for childhood obesity recommended by the International Obesity Task Force cut-off values (8) (age- and gender-adjusted BMI; here called isoBMI). Information relating to weight and height was not available for 166 teenagers, mainly due to computer failure. As a result, 402 (568 minus 166) teenagers were finally included in the analyses. The children and teenagers were divided into three groups according to this classification system: 1) low-normal weight (isoBMI < 25), 2) overweight (isoBMI 25-29.9) and 3) obesity (isoBMI ≥ 30). In some of the analyses, groups 2 and 3 were combined, here called: overweight/obesity (isoBMI ≥ 25). No classification into underweight exists with isoBMI (see Table 1).

At 20 years
At the dental examination at 20 years of age, anthropometric measurements were made. The body weight (kg) and height (m) of the subjects were determined and the subjects’ BMI (kg/m²) was calculated. Information relating to weight and height was not available for 3 young adults as they did not want to check their weight. As a result, 491 (494 minus 3) young adults were finally included in the analyses. The subjects were divided into four groups as follows: 1) underweight (BMI < 18.5), 2) normal weight (BMI 18.5-24.9), 3) overweight (BMI 25-29.9) and 4) obesity (BMI ≥ 30).

Statistical analysis
The data analysis was generated using SAS/STAT software (version 9 of the SAS System for Windows Copyright © 2002 SAS Institute Inc.), STATISTICA (data analysis software system), version 8.0, StatSoft, Inc. (2007), and SPSS 17.0, SPSS Inc. (2007). In the analyses of variance (ANOVA), Sheffe’s test was used to compensate for multiple comparisons. When two groups were compared in terms of continuous variables, an unpaired t-test was used. Fisher’s exact test was used to analyse differences when the data were categorical. p-values below 0.05 were considered statistically significant.

Results
Body adiposity status at 3, 6, 15 and 20 years
At 3 years of age, 15% were overweight/obese, while the corresponding figure at 6 years of age was 18%. At 15 years of age, 16% were overweight/obese, while the corresponding figure at 20 years of age was 26% (Table 1). At 20 years of age, 5% were underweight and the median BMI for the 27 underweight (BMI < 18.5) individuals was 17.9 kg/m².

Caries prevalence in relation to BMI
At 3 years
Table 2 and Figure 1 show the mean numbers of defs total (including initial carious lesions) and defs

Table 1. Percentage distribution according to isoBMI groups at 3, 6 and 15 years and BMI groups at 20 years of age.

<table>
<thead>
<tr>
<th>Groups</th>
<th>3 years</th>
<th>6 years</th>
<th>15 years</th>
<th>20 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Underweight*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5%</td>
</tr>
<tr>
<td>Normal</td>
<td>86%</td>
<td>82%</td>
<td>84%</td>
<td>69%</td>
</tr>
<tr>
<td>Overweight</td>
<td>12%</td>
<td>14%</td>
<td>12%</td>
<td>19%</td>
</tr>
<tr>
<td>Obese</td>
<td>3%</td>
<td>4%</td>
<td>4%</td>
<td>7%</td>
</tr>
</tbody>
</table>

*At ages 3, 6, and 15, no classification of underweight exists with isoBMI.
BMI AND CARIES PREVALENCE

Table 2. Mean number of decayed, extracted and filled tooth surfaces (defs), in all children and in different isoBMI groups at 3 and 6 years of age (total=initial+manifest).

<table>
<thead>
<tr>
<th>Groups</th>
<th>3 years of age</th>
<th>6 years of age</th>
<th>6 years of age</th>
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<tr>
<td></td>
<td>n</td>
<td>Mean</td>
<td>SD</td>
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<tr>
<td>All</td>
<td>525</td>
<td>1.64</td>
<td>4.88</td>
</tr>
<tr>
<td>isoBMI &lt; 25</td>
<td>449</td>
<td>1.75</td>
<td>5.11</td>
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<td>isoBMI ≥ 25</td>
<td>76</td>
<td>1.01</td>
<td>3.23</td>
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<td>isoBMI 25-29.9</td>
<td>61</td>
<td>1.02</td>
<td>3.16</td>
</tr>
<tr>
<td>isoBMI ≥ 30</td>
<td>15</td>
<td>1.00</td>
<td>3.61</td>
</tr>
</tbody>
</table>

Figure 1. Mean defs total* (3 and 6 years), DFSa total* (15 years) and DFS total* (20 years) distributed according to isoBMI/BMI. For the 20 year olds the underweight group (BMI < 18.5) is excluded. *(initial+manifest)

Table 3. Mean number of decayed and filled approximal tooth surfaces (DFSa) in all children and in different isoBMI groups at 15 years of age (total= initial + manifest).

<table>
<thead>
<tr>
<th>Groups</th>
<th>DFSa total</th>
<th>DFSa manifest</th>
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<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>All (n=402)</td>
<td>3.21</td>
<td>3.95</td>
</tr>
<tr>
<td>isoBMI &lt; 25</td>
<td>2.94</td>
<td>3.62</td>
</tr>
<tr>
<td>isoBMI ≥ 25</td>
<td>4.64</td>
<td>5.15</td>
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<tr>
<td>isoBMI 25-29.9</td>
<td>4.18</td>
<td>5.14</td>
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<tr>
<td>isoBMI ≥ 30</td>
<td>6.29</td>
<td>5.04</td>
</tr>
</tbody>
</table>

p-values obtained using an unpaired two-sample t-test. *denotes p < 0.05

manifest at 3 years of age distributed according to isoBMI (low-normal weight, overweight and obesity). Children who were overweight/obese (isoBMI ≥ 25; n=76) had a lower caries prevalence (defs total) than low-normal weight (isoBMI < 25; n=449), 86% were free from manifest carious lesions and fillings compared with 38% in the obese group (isoBMI ≥ 30; n=15). This difference is statistically significant (Fisher’s exact test, p=0.04 and OR=2.5; 95% CI: 1.0-5.9).

At 15 years
Table 3 and Figure 1 show the mean numbers of approximal initial and manifest carious lesions and fillings at 15 years of age distributed according to isoBMI. Adolescents who were overweight/obese (isoBMI ≥ 25; n=64) had a statistically significantly higher proximal caries prevalence (DFSa total) than low-normal weight individuals (isoBMI < 25; n=338) (p < 0.05). There was a numerical difference in number of manifest carious lesions and fillings (DFSa manifest) between these groups, however not statistically significant. At 15 years of age, the 402 examined children had a mean of 3.21 (±3.95) D_in+mFa and 0.42 (±1.13) D_mFa (Table 3) compared with 3.28 (±4.06) and 0.50 (±1.35) for the 166 teenagers who dropped...
out. These differences were compared with the main group and were found not to be statistically significant. Obese individuals (isoBMI ≥ 30; n=14) had more than twice as many approximal carious lesions and fillings compared with low-normal weight individuals (isoBMI < 25; n=338) (p < 0.05). In the low-normal weight group (isoBMI < 25; n=338), 80% were free from approximal manifest carious lesions and fillings compared with 59% in the obese group (isoBMI ≥ 30; n=14). This difference is statistically significant (Fisher’s exact test, p=0.04 and OR=2.8; 95% CI: 1.0-7.7).

At 20 years

Table 4 and Figure 1 show the mean numbers of initial and manifest carious lesions and fillings at 20 years of age distributed according to BMI. Young overweight/obese adults (BMI ≥ 25; n=124) had a statistically significantly higher caries prevalence (DFS total) than normal-weight individuals (BMI 18.5-24.9: n=340) (p < 0.05). In the normal-weight group (BMI 18.5-24.9), 36% were free from manifest carious lesions and fillings compared with 35% in the obese group (BMI ≥ 30; n=14). This difference is statistically significant (Fisher’s exact test, p=0.01 and OR=3.1; 95% CI: 1.2-7.7). The young adults who were underweight had more manifest carious lesions and fillings (Table 4). This group is small (27 individuals) and there were 2 “outliers” with 23 and 28 carious lesions and fillings respectively. If these two individuals were excluded from the group of underweight subjects, the mean would not differ from the normal-weight group.

Discussion

The main finding in the present study was that overweight/obese adolescents and young adults had more caries than normal-weight young people. At 6 years of age the odds of having caries among obese children are 2.5 times higher than the odds for caries among six-year-old children of normal weight. However, at 3 years of age, there were no statistically significant differences in the caries prevalence between different BMI groups.

Our finding that overweight and obesity are linked to a higher prevalence of dental caries is in agreement with other clinical studies (2, 11, 12, 20). It is possible to speculate about the reason why overweight and obese children/young people have more caries than normal-weight individuals. In a longitudinal study, Ludwig et al. (18) found that the increasing prevalence of obesity in children was linked to the consumption of sugar-sweetened drinks. One reason could therefore be that the frequent consumption of sugar-containing products may result in an increased number of cariogenic micro-organisms. Furthermore, Barkeling et al. (4) showed that the mutans streptococcus count was correlated to BMI and to a higher intake of sweet foods. Another possible explanation could be the novel finding by Modéer et al. (20), who found that obese adolescents exhibit a lower flow rate of stimulated whole saliva compared with normal-weight controls.

In the present study, we found no relationship between dental caries and body weight status at 3 years of age. This is in line with Chen et al. (7) and Hong et al. (13). It is possible to hypothesise about the reason why no association between dental caries and BMI is seen at 3 years of age, although it is seen at a later stage in the same population. One reason could be that dental caries in very young children is often a more rapid process if the children have unsuitable habits, while the increase in body weight is slower. A Swedish study by Garemo et al. (10) concluded that 4-year-old Swedish children eat too much junk food and sucrose (10). Junk food supplied 24% of the energy and 67% had a sucrose intake exceeding Nordic Nutrition Recommendations (NNR). A high sucrose intake could be compensated for by the consumption of less regular food among the youngest children. Consequently, high sucrose could be associated with underweight or normal weight (lower nutritional status). Another explanation of the absence of a correlation between isoBMI and caries at 3 years of age in our study might be that the children identified as overweight/obese at that age are not the same as...
those identified at 6 years of age – i.e. the predictive value of isoBMI at 3 years of age is low. The mechanism for overweight/obesity at the lower age might therefore be different than in older children. Further longitudinal analysis of this issue is called for.

The present study shows that statistically significantly more 6-year-old children were caries free in the normal-weight group compared with children in the obese group. This is supported by Hong et al. (13) who studied the associations between obesity and dental caries in children aged 2-6 years. When caries experience was compared across BMI categories, a statistically significant association between caries and obesity was only found for the 60- to <72-month age group. This is in line with Gardén et al. (9), who found that weight at birth and early preschool age was not predictive of overweight/obesity at 9 years of age, but the weight status at 5 years of age was.

The 15-year-olds who were overweight/obese had a statistically significantly higher proximal caries prevalence (DFSa total) than low-normal weight individuals. However when comparing DFSas manifest between different isoBMI levels, there were no statistically significant differences. This might be explained by the fact that the t-test takes into account that there are unequal variances, and therefore no statistical difference was found. However, among those who are classified as normal weight the mean DFSa manifest is less than half the mean among the overweight/obese children. This difference has to be considered, since it might be of "clinical significance".

There was a difference in dropout rates between the groups, especially at 15 years of age. Weight and height data are missing for 166 of the 15-year-olds, mainly due to computer failure. The differences in caries prevalence (DFSa total+manifest) for the 166 teenagers who dropped out were compared with the main group and were found not to be statistically significant. At 20 years of age 3 out of 494 individuals did not want to check their weight, this number is significant. At 20 years of age 3 out of 494 individuals. However when comparing DFSas manifest between different isoBMI levels, there were no statistically significant differences. This might be explained by the fact that the t-test takes into account that there are unequal variances, and therefore no statistical difference was found. However, among those who are classified as normal weight the mean DFSa manifest is less than half the mean among the overweight/obese children. This difference has to be considered, since it might be of "clinical significance”.

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Both dental caries and obesity are multifactorial diseases related to lifestyle including dietary habits. Several characteristics of today's society are contributing to the “obesogenic environment” in which children and adolescents are to be found. Soft drinks and energy-dense fast food are currently very inexpensive and easily accessible. Changes in dietary patterns and eating habits are expected to be factors related to dental caries and to the increased prevalence of obesity. Preventive measures based on an “up-to-date” approach appear to be essential.

Government-led regulations can help the framework of society to be changed to a “healthier environment”, even though many conflicts of interest can be expected. Long-term population-level strategies, as well as strategies for individuals at risk, are needed in this perspective. The present study emphasizes the need for multi-level approaches. This means that health professionals should work in a multidisciplinary manner with these patients with the aim of establishing a healthy lifestyle including good dietary habits.

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References

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Comparison between high concentration EDTA (24%) and low concentration EDTA (3%) with surfactant upon removal of smear layer after rotary instrumentation: A SEM study

Mohsen Daghustani1, Ahmad Alhammadi1, Khalid Merdad2, Johan Ohlin1, Fredrik Erhardt1, Michael Ahlquist1.

Abstract
This in vitro study compare cleanliness of tooth canal walls regarding smear layer after final treatment with 24% ethylenediaminetetraacetic acid (EDTA) and 3% EDTA with or without surfactant. Sixty extracted teeth, randomly distributed into four groups, were prepared using ProFile® instruments (DENTSPLY, Maillefer, Ballaigues, Switzerland), and subjected to different final irrigation solutions: group A, 24% EDTA; group B, 3% EDTA with surfactant; group C (positive control), 3% EDTA; and group D (negative control), 0.5% sodium hypochlorite. Roots were sectioned, examined and evaluated under scanning electron microscope; microphotographs were taken for the coronal, middle and apical third of each specimen. Statistical analysis showed no difference regarding presence of smear layer between test groups in the coronal and apical sections. They were cleaned in the coronal sections and uncleaned in the apical sections. In the middle section, group B was significantly cleaner (p<0.05) than the other groups. In conclusion, surfactant in combination with EDTA did not improve root canal cleanliness and there is no difference between different EDTA concentrations in removing the smear layer.

Key words
Chelating agents, ethylenediaminetetraacetic acid, irrigation, smear layer, surfactant, endodontics.

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Smear hos rotkanaler efter rensning och behandling med EDTA i hög respektive låg koncentration med eller utan ytspänningsnedsättande tillsats

MOHSEN DAGHUSTANI, AHMAD ALHAMMADI, KHALID MERDAD, JOHAN OHLIN, FREDRIK ERHARDT, MICHAEL AHLQUIST

Sammanfattning

Renheten hos rotkanaler jämfördes in vitro med avseende på smear efter rensning och EDTA-behandling med 24% respektive 3% lösning med eller utan ytspänningsnedsättande medel. Sextio extraherade tänder delades slumpvis in i fyra grupper och preparerades med roterande maskinfilar under spolning med: grupp A, 24% EDTA; grupp B, 3% EDTA med ytspänningsnedsättande medel; Grupp C, 3% EDTA; och Grupp D, 0,5% natriumphoklorit (Dakin’s) lösning. Rötterna klövs och kanalerna delades in i koronal, intermediär, apikal tredjedel. Mängden kvarvarande smear kvantifierades under SEM. Statistisk analys visade ingen skillnad i smearmängd mellan testgrupperna i de koronala och apikala delarna. Kanalerna var rena i den koronala delen men uppvisade mycket smear apikalt. I den mittersta tredjedelen av kanalerna i grupp B, var kanalen signifikant renare (p<0.05) än andra grupper. Det förefaller som varken koncentrationen av EDTA eller ytspänningsnedsättande tillsats påverkar kanalens renhet med avseende på smear.
Introduction
Microorganisms induce inflammatory and pathological changes in dental pulp and periradicular tissues (18). Therefore, the basic objective of endodontic therapy is to eliminate intracanal infection by chemomechanical debridement (5). During canal preparation, dentine chips, organic material and bacterial by-products form a smear layer that adheres to the canal walls (12, 27).

A sodium hypochlorite (NaOCl) solution is considered the most common irrigant in endodontics because of its unique capability to dissolve tissue remnants and its high antimicrobial potency (19). In addition to hypochlorite, the use of a chelating agent, such as ethylenediaminetetraacetic acid (EDTA), has been suggested to clean the root canal system of inorganic components (29). The efficiency of EDTA depends on many factors, such as penetration depth through the entire canal length, duration of application, concentration and volume (4, 29).

In order to increase the effectiveness of the smear layer removal, the irrigant must come in contact with dentine walls, which depends on its wettability, a property correlated to surface tension (22). Reducing surface tension of irrigating solutions by adding a surfactant may improve dentin wettability and increase the flow into narrow root canals (2).

There is no consensus regarding the appropriate EDTA concentration for irrigation, with a various range between 3% up to 24% was recommended (13, 23). These variations in concentration result in different levels of undesirable effects on the dentinal wall; since EDTA has a strong demineralizing effect; it denatures the dentine collagen fibers, which may negatively affect the adherence of root filling materials to the canal wall (13).

The most debated factors affecting smear layer removal are volume of irrigation and contact time. One study suggested that a final rinse with 10 ml of 17% EDTA followed by 10 ml of 5.25% NaOCl was the most effective method (28), while another identified several possible effective methods, ranged from 2 to 30 ml of 15% EDTA as a final rinse used during instrumentation (9). Calt & Serper reported that the irrigation of 10 ml with 17% EDTA for 1 minute was effective in removing the smear layer; however, 10 minutes of application caused excessive peritubular and intertubular dentinal erosion (6). The same authors showed that increasing contact time and concentration of EDTA from 10% to 17% intensified dentin demineralization (25).

The aim of this in vitro study was to compare the removal of smear layer after final treatment with either high concentration EDTA (24%) or 3% EDTA with and without a surfactant.

Materials and Methods
Sample selection
After verbal permission from the patients, 60 extracted, mature, intact, permanent first molars, with less than 30° curved mesial roots and closed apices (24) were selected and stored in 0.5% chlorhexidine (Fresenius Kabi, Uppsala, Sweden). The mesial roots were radiographed from the buccal and mesial aspects to ensure closed apices and similar root canal widths, and then teeth were divided randomly into four groups of 15 each. This sample size was calculated based on a desired power of 80% for the study.

Root canal instrumentation
Working length was determined using a manual K15 file (DENTSPLY, Maillefer, Ballaigues, Switzerland) introduced to a distance of 1 to 2 mm from the root apex and holding the tooth in front of a table lamp.

Teeth were instrumented with rotary Ni-Ti files (ProFile®, DENTSPLY, Maillefer, Ballaigues, Switzerland) according to the manufacturer’s crown-down description, with the final apical preparation shaped with ProFile® (0.04/30) (14).

Canals were irrigated between every instrument change with 2 ml of 0.5% sodium hypochlorite solution (Apoteksbolaget, Uppsala, Sweden) using a 30-gauge irrigation needle (Navitip, Ultradent Products, Inc., South Jordan, Utah, USA) applied 1 to 2 mm from the working length.

Final irrigation sequence
After instrumentation, the teeth were randomly divided into four groups: group A, irrigated with 24% EDTA (Ultradent Products inc, South Jordan, Utah, USA); group B, irrigated with 3% EDTA with surfactant (Dental Therapeutics AB, Nacka, Sweden); group C (positive control), irrigated with 3% EDTA (Tubulicid Plus, Dental Therapeutics AB, Nacka, Sweden); and group D (negative control) irrigated with 0.5% sodium hypochlorite solution (Apoteksbolaget, Uppsala, Sweden). All solutions were applied with a 30-gauge irrigation needle inserted into the canal 1 to 2 mm from the working length, with a total of 2 ml/canal for 40 seconds according to the manufacturer’s instructions (one application of EDTA for 20 seconds, then the canals are dried out with paper points, followed by another 20 seconds application).
Following the final irrigation with EDTA, each canal received a final flush with 2 ml of 0.5% sodium hypochlorite.

**Scanning electron microscope (SEM) evaluation**

The roots were split using a diamond disk (DT-126, PHP, Dentatus, Hägersten, Sweden), a groove was cut along the buccal and lingual surfaces in a longitudinal direction. A plastic instrument was used to complete the separation to avoid canals contaminating.

The specimens were dried, attached firmly to aluminum stubs (Silverpaint, Agar Scientific Ltd., Stansted, Essex, UK), covered with a 20-nm platinum coating (Polaron Instruments Ltd., Hatfield, UK), and evaluated under SEM (ULTRA 55, Carl Zeiss NTS GmbH, Oberkochen, Germany). Microphotographs were taken of every specimen at the coronal, middle, and apical levels, at a magnification of x 800. The canals walls were then quantitatively evaluated for the amount of smear layer by two operators masked to the irrigation sequence after intra- and inter-examiner reliability was evaluated using kappa test. The kappa value based on 20% of the sample was 0.85. The amount of smear layer was determined using a five point scoring system (Table 1). The scores were statistically evaluated using the Kruskal-Wallis ANOVA rank sum test.

<table>
<thead>
<tr>
<th>Table 3. Smear layer scoring</th>
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<tr>
<td>Score</td>
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</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>5</td>
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</tbody>
</table>

**Results**

The smear layer scores in each group are presented in (Figs. 1-3). Representative microphotographs are shown in (Fig. 4). The Kruskal-Wallis test showed that Group D (negative control) resulted significantly with higher scores than the rest of the study groups (A, B, C) (P <0.001) with respect to smear
layer removal at coronal, middle and apical thirds. There is no statistically significant difference in the distribution of scores between the study groups (A, B, C) in the coronal third.

There was a statistically significant difference in scores between the groups in the middle third ($P < 0.05$; with the greatest variation in mean ranks between groups C and B). Pairwise comparisons showed that group C had significantly higher scores ($P < 0.05$) than group B. All study groups failed to clean the smear layer in the apical sections without any statistically significant difference in scores between them.

**Discussion**

In this study, we evaluated the addition of surfactant to our standard irrigant used in the dental school (group C), and to compare them with a high concentration EDTA (24%) upon removal of smear layer.

Analysis of all specimens demonstrated that, the use of NaOCl alone is not effective in removing the smear layer, which is in agreement with Baumgartner et al (4). In this study, the other groups regardless of their irrigation protocol, cleaning was only partially effective. The root canal surfaces of the coronal and middle thirds were relatively clean, permitting the visualization of dentinal tubules orifices. Although in the middle third of group B significant cleaner results were demonstrated when compared to the study groups (A and C), still both these groups showed relatively clean root canal walls in the middle third. The explanation could be that the accessibility and the wide diameter of these canals, when compared to the apical third, enhanced the flow and the effect of the irrigating solution, making a more complete removal of the smear layer possible. These results are in agreement with previous studies that also observed effective cleaning action in the coronal and middle sections of the root canal when different quantities of solutions and duration of irrigation were employed (1, 4, 26).

All the groups in this study were failed to clean root canal walls in the apical third, these result was consistent with other studies showing similar difficulties in removing the smear layer in the apical section (3, 7, 20). In contrast, a similar study was using EDTA for 30 seconds at 3% and 17% concentrations reported good cleaning of the apical third, although they did notice the presence of smear plugs in some of the specimens (13).

Surface tension is defined as the force between molecules that tends to inhibit the spread of a liquid over a surface or limit its ability to penetrate a capillary tube (22). It has been suggested that reduction of surface tension by adding a surfactant to the irrigating solution would improve its efficacy in the narrow apical region of the root canal (2). Our study
showed that the surfactant added in group B did not improve its performance in smear layer removal at the apical third compared to EDTA alone. However, in the middle third, the presence of surfactant in group B enhanced the smear layer removal. Recent studies showed that the reduced surface tension of endodontic chelator solutions did not improve their ability to remove smear layer and they concluded that the addition of a wetting agent is not effective (16, 30), which confirmed our results. An explanation for this may be that root canal walls, after irrigation and preparation, are already wet and do not require any further moisture; in addition to the hydrophilic properties of root dentin (21).

In this study, application time of final EDTA irrigation was 40 seconds in all groups. However, there is a controversy over the most appropriate application EDTA time and its effect in removing the smear layer. One study reported that a few seconds of EDTA administration was sufficient to remove the smear layer (23). In contrast, other studies showed that the smear layer was completely removed after 10% EDTA irrigation for 1 minute (6, 17). Similar findings were also reported when 15% EDTA was applied for 4 minutes (8). EDTA changes pH during demineralization of dentine and its effect is self-limiting due to the buffering effect of dentine, as the increase in pH decreases both the rate of dentine demineralization and the amount of dentine dissolved (11). This action may provide the basis for recommending a short EDTA application time (15).

There is no consensus that the volume of irrigation has the most effect, since some authors suggest that application of 10 ml of EDTA for 1 minute is enough in removing smear layer (6), while others found that the same results can be obtained with 2 ml (15). Crumpton et al demonstrated that 1 ml of EDTA with a contact time of 1 minute was as effective as 10 ml (10).

No matter what the concentration, volume, or application time, there are undesirable consequences of dentinal erosion with EDTA. It was shown that application of 10% EDTA for 1 minute produces a less erosive effect on dentin than 17% EDTA and it has been suggested that to limit erosion, the best method appears to be the use of low concentration EDTA solutions (25). An earlier study reported 3% EDTA had similar results to a 17% solution without excessive dentine erosion (13). In our study no dentinal erosion was noticed in any of the specimens, and this might be explained by the short application time of the irrigants.

It may be concluded that, under the conditions of this study: 1) there was no difference and improvement in smear layer removal when the surface tension of EDTA was lowered with a surfactant except in the middle part of the root; and 2) increasing the concentration of EDTA to 24% did not improve the smear layer removal compared to 3% EDTA. Since the exposure time of EDTA might not be sufficient to influence the removal of the smear layer, further studies are recommended with an increased application time and volume of EDTA in order to identify the most efficacious regimen to remove the smear layer with minimal dentinal erosion.

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Effect of post-brushing mouthrinse solutions on salivary fluoride retention

Chrysostomos Mystikos, Takashi Yoshino, Per Ramberg, Dowen Birkhed

Abstract
Fluoride (F) toothpaste is one of the most effective means of caries prevention. There is also evidence that mouthrinse solutions with antimicrobial agents reduce plaque formation and gingivitis and may be used as adjuncts to daily self-performed oral hygiene for risk patients. The authors hypothesize that using these solutions – without or with just a low F concentration after brushing – will have a "wash-out" effect on F toothpaste. Mouthrinse solutions with more F might be beneficial in this respect.

Two groups of 10 (Series I) and 12 (Series II) healthy subjects were recruited. They brushed for 1 min with toothpastes containing either 1450 or 5000 ppm F. After brushing and spitting out the toothpaste, the participants in Series I rinsed for 30 sec with 10 ml of a variety of products with various F concentrations (0, 100, 226 or 900 ppm F). In Series II, they first rinsed with water after the brushing and directly thereafter with 20 ml of the post-brushing rinsing solution for 30 sec. Saliva samples in both series were collected at different time points up to 1 h and the F concentration was measured.

There was significantly less F in saliva after rinsing with no F or with a low F concentration (100 ppm) compared with just brushing with a F toothpaste. Rinsing with 226 ppm F displayed significantly higher F concentrations in saliva compared with only toothbrushing. Products with a high F concentration (i.e. toothpaste with 5000 ppm F or a mouthrinse solution with 900 ppm F) produced the highest F retention in saliva compared with all other protocols. The quantity of mouthrinse solution (20 vs. 10 ml) did not seem to have any effect on the F retention.

The results from both test series show that a post-brushing rinsing solution without F or with just 100 ppm F exerts a "wash-out" effect on toothbrushing with either 1450 or 5000 ppm F, which may be negative for all patients, especially those with a risk of caries. The general population will benefit more from higher concentrations of F in mouthrinse solutions and, based on the results of the present investigation, 226 ppm F (corresponding to 0.05% NaF) should be the lowest concentration used. Furthermore, caries risk patients are recommended to use a high-F toothpaste (5000 ppm F) or a post-brushing mouthrinse solution with 900 ppm F (corresponding to 0.2% NaF).

Key words
Fluoride, mouthrinse solutions, post-brushing rinsing, toothpastes, toothbrushing, water rinsing
Effekt av munsköljmedel på fluorretention i saliv

CHRYSOSTOMOS MYSTIKOS, TAKASHI YOSHINO, PER RAMBERG, DOWEN BIRKHEJD

Sammanfattning

Flor (F) i tandkräm anses vara en effektiv metod för att motverka karies. Det finns också data som tyder på att munsköljmedel med olika antimikrobiella ämnen reducerar plackbildning och gingivit om de används som complement till daglig munhygien t ex av riskpatienter. Fluorhalten i dessa produkter varierar dock mycket. Målsättningen med denna undersökning var därför att utvärdera dels om sköljvätskor med låg fluorhalt - eller helt utan fluor - minskar fluorretentionen i munhålan efter tandborstning med fluor tandkräm, dels eventuell tilläggseffekt i detta avseende av produkter med en hög fluorhalt.

Två grupper med 10 (Serie I) respektive 12 friska försökspersoner (Serie II) deltog. De fick i båda testserierna borsta tänderna under 1 min med tandkräm innehållande 1450 eller 5000 ppm F. Efter borstningen spottade de ut tandkrämen och i Serie I sköljde de direkt därefter i 30 sec med 10 ml munsköljmedel innehållande olika koncentrationer av F (0, 100, 226 eller 900 ppm). I Serie II sköljde deltagarna först med 10 ml vatten efter borstningen och därefter med 20 ml av munsköljmedlet under 30 sek. Salivprov samlades in vid olika tidpunkter upp till en timma, varefter fluorkoncentrationen bestämdes med hjälp av en jonselektiv elektrod.

Det var signifikant mindre fluor i saliven efter tandborstning följt av ett sköljmedel utan F eller med låg fluorhalt (100 ppm) jämfört med den anbefalade fluorhalten. Sköljning med 226 ppm F (0,05% NaF) resulterade i högre fluorhalt jämfört med den anbefalade fluorhalten. Produkter med hög fluorhalt, dvs. tandkräm med 5000 ppm eller munsköljmedel med 900 ppm, gav de högsta fluorretentionsvärdena i saliven. Mängden sköljvätska (10 eller 20 ml) var ej påverkan vid studien.

Resultaten av båda testserierna visar sålunda att ett munsköljmedel utan F eller med så låg fluorhalt som 100 ppm resulterar i en utspädning av fluor i saliven efter tandborstning med fluor tandkräm. Krävdes att den anbefalade fluorhalten innehållande såväl 1450 som 5000 ppm F. Detta kan anses vara negativt för samtliga människor, speciellt kariesriskpatienter. Det är därför lämpligt ur kariologisk synpunkt att ett munsköljmedel bör innehålla minst 0,05% NaF (226 ppm F). För kariespatienter kan rekommenderas en högre fluorhalt, 0,2% NaF (900 ppm F) i sköljmedel eller 1,1% NaF (5000 ppm F) i tandkräm.
Introduction
There is a great deal of evidence that fluoride (F) toothpaste is one of the most effective means of caries prevention, along with appropriate self-performed oral hygiene. It is well known that F inhibits mineral loss during demineralization and increases mineral uptake during remineralisation (9). A randomized clinical trial involving 2800 ppm F toothpastes revealed 11% higher efficacy against caries than 1500 ppm F (1). More recent studies indicate that F in toothpaste exerts a dose-response effect with respect to caries reduction, at least up to 1500 ppm (10). Even 5000 ppm F toothpaste may be favourable for caries risk patients (6, 11).

Many post-brushing mouthrinse solutions are available on the market. These products are used with the aim of controlling supragingival plaque in order to contribute to the prevention of both caries and gingivitis/periodontitis (7). If no approximal plaque control is practiced by the patient, the chemical support of mechanical plaque removal in the approximal area would be desirable (8). However, mouthrinse solutions do not reach the subgingival area (2), with no significant shifts in oral microbial populations and without affecting the potential pathogens (5, 12).

Mouthrinse solutions have various F concentrations, from 0 to 900 ppm F. Since F is an important ingredient of toothpastes for caries prevention, it is essential that it is not “washed out” by any post-brushing mouthrinse solution. No systematic investigation has been conducted on the effect of these products on F retention in the oral cavity after toothbrushing. However, during the preparation of this manuscript, a study on the effect of rinsing with mouthwashes after brushing with a fluoridated toothpaste on salivary fluoride concentration was published (3).

The aim of the present study was to evaluate the effect of post-brushing rinsing solutions on salivary F retention, when using toothpastes with either 1450 or 5000 ppm F, in combination with mouthrinse solutions containing from 0 to 900 ppm F.

Materials and methods
Study design

Two series were conducted (here called I and II). They were designed as cross-over, randomized, single-blind studies. The subjects were asked not to use any product containing F on the day of the experiment. The products included in Series I and II are presented in Table 1. They are all available on

<p>| Table 1. Test products used in Series I and II |</p>
<table>
<thead>
<tr>
<th>Product (Manufacturer)</th>
<th>Series I</th>
<th>Series II</th>
<th>F concentrations ppm F % NaF</th>
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<tr>
<td>Tootpastes</td>
<td></td>
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<tr>
<td>Pepsodent (Unilever, USA)</td>
<td>+</td>
<td>+</td>
<td>1450 0.32</td>
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<tr>
<td>Duraphat (Colgate, USA)</td>
<td>+</td>
<td>+</td>
<td>5000 1.1</td>
</tr>
<tr>
<td>Mouthrinse solutions</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Listerine Cool Mint (Johnson &amp; Johnson, USA)</td>
<td>+</td>
<td>+</td>
<td>0 0</td>
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<td>Listerine Fluoride (Johnson &amp; Johnson, USA)</td>
<td>+</td>
<td>+</td>
<td>100 0.022</td>
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<tr>
<td>Listerine Fluoride Extra (Experimental)</td>
<td>+</td>
<td>-</td>
<td>226 0.05</td>
</tr>
<tr>
<td>SB12 (Antula, Sweden)</td>
<td>-</td>
<td>+</td>
<td>226 0.05</td>
</tr>
<tr>
<td>Flux (Actavis, Norway)</td>
<td>+</td>
<td>+</td>
<td>900 0.2</td>
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<p>| Table 2 A. Procedures for Series I |</p>
<table>
<thead>
<tr>
<th>Code</th>
<th>Toothpaste (1 min)</th>
<th>Water rinsing (10 ml)</th>
<th>Post-brushing rinsing (10 ml)</th>
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<td>Duraphat</td>
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</tr>
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<td>Duraphat</td>
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<p>| Table 2 B. Procedures for Series II |</p>
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<th>Code</th>
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<td>-</td>
<td>-</td>
<td>Flux</td>
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<tr>
<td>LI</td>
<td>Pepsodent</td>
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<td>Listerine F Extra</td>
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</table>
the Scandinavian market, apart from “Listerine F Extra”, which was prepared in the laboratory by adding NaF to the commercial product “Listerine Fluoride” (which contains 100 ppm F) to produce a final concentration of 226 ppm (corresponding to 0.05% NaF). After this study was completed, a new product has been launched called “Listerine Total Care Tooth Guard” containing 226 ppm F.

On a given day (Day 0), the subjects were asked to follow the brushing-rinsing protocols presented in Tables 2A and 2B. The brushing time was set at 1 min and the amount of toothpaste 1 gram. In Series I (Table 2A), the subjects simply spat out the toothpaste after brushing without any water rinsing afterwards. In Series II (Table 2B), the toothpaste was kept in the mouth and 10 ml of water was taken in and used as slurry rinsing for 10 sec. The subjects were then asked to spit out the slurry. After this, 10 ml (Series I) or 20 ml (Series II) of the post-brushing rinsing solution were taken in and used to rinse carefully for 30 sec (with active movements of the lips and cheeks) and were then spat out. The reason for using different rinsing volumes is that the recommendations by companies varies. An unstimulated saliva sample (≈0.3 ml) was collected before (0 min) and at various time-points after brushing-rinsing (3, 5, 10, 20, 30, 45 and 60 min). The subjects were not allowed to speak, eat or drink during these 60-min test periods.

Subjects
In Series I, 10 subjects, aged 15-59 years old (mean 41 years; 9 females and 1 male), were recruited and, in Series II, 12 subjects, aged 18-60 years (mean age 32 years; 7 females and 5 males). Each volunteer had to fulfil the following four criteria: (i) good general health, (ii) no sign of gingivitis or destructive periodontal disease and no active caries lesions, (iii) a minimum of 24 teeth without extensive restorations, six in each quadrant, and (iv) no orthodontic appliance or any other kind of removable appliance. All subjects received a verbal and written description of the study design and signed an informed consent form. The study was approved by the Ethics Committee at the University of Gothenburg.

Fluoride analysis
The samples were analyzed blind in terms of subjects and methods. 200 µl of the saliva was mixed with 20 µl of TISAB III (Thermo Electron Corp., Waltham, USA). 100 µl of the solution was then placed as a drop on a Petri dish and the F concentration was measured by carefully lowering an ion-specific electrode (model 96-09, Orion Research Inc.) into the fluid. The surface tension of the drop ensured that the liquid enclosed the entire membrane surface of the electrode. In order to calibrate the electrode, three standard solutions were used (0.1, 1 and 10 ppm F). The F concentration in saliva was expressed as parts per million (ppm).

Data analysis
The F concentration was plotted vs. time and the area under the curve (AUC0-60 min) was calculated for each treatment and each individual using a computer program (Kaleida Graph 3.01, Synergy, Software, Reading, PA, USA). Two-way ANOVA was used for both the logarithmic and non-logarithmic values of the AUC0-60 min values because of the non-normal distribution of the data. A p-value of < 0.05 was considered statistically significant.

Results
Series I
Figure 1 shows the mean AUC0-60 min values and standard errors (SE) of salivary F retention from Series I. For statistically significant differences, see Table 3. The highest values were found after brushing with Duraphat toothpaste (5000 ppm F) and using Flux (900 ppm F) as a post-brushing rinsing solution. Duraphat toothpaste alone also produced high AUC values. These three treatments did not differ from a statistical point of view, but they produced significantly higher AUC values than the other nine protocols. Duraphat toothpaste in combination with SB12 (226 ppm F) and Listerine F (100 ppm F) solu-
Salivary Fluoride Retention

Series I vs. II

In general, the salivary F retention in Series II was somewhat lower compared with Series I (in Series II, water rinsing was performed after the tooth-brushing, but not in Series I). However, the rinsing volume (10 ml in Series I and 20 ml in Series II) did not appear to have any impact on the salivary F retention. On the contrary, the 10 ml rinsing solution produced a somewhat higher F retention than 20 ml.

Discussion

It is well known that F toothpaste is one of the most effective means of caries prevention, along with good oral hygiene and good dietary habits. The persistence of F in saliva and plaque, at potentially active concentrations between toothbrushing with F toothpaste, is clearly an important factor. This is even more important in caries risk patients and in periodontal patients with exposed root surfaces. In the present investigation, we studied a number of products, such as toothpastes with 1450 ppm F or 5000 ppm F (the latter recommended for caries risk patients), and three types of post-brushing mouthrinse solutions. These three products were: i) one with anti-bacterial properties (Listerine), ii) one for improving malodour (SB12), and iii) one product enhancing the F concentration for caries risk patients (Flux). Numerous Listerine mouthrinse solutions are sold worldwide and are recommended by the manufacturer to be used immediately after brushing. However, most of them are without fluoride and just a few contains 100 ppm F (one with 226 ppm). Listerine as well as the other two rinsing solutions included in this study (SB12 and Flux), are sold “over the counter” (OCT) in Scandinavia without a prescription. Since a variety of post-brushing mouthrinse solutions are currently available on the market, and are promoted extensively on TV and in the daily press, we wanted to examine their efficacy as post-brushing rinsing products in relation to the well-established evidence for F toothpaste.

Post-brushing mouthrinse solutions with 226 ppm F produced more F in saliva; the level was statistically significantly higher compared with 100 ppm F. The lowest acceptable F concentration of a mouthrinse solution, in order not to reduce the F effect in toothpaste, was therefore found to be 226 ppm (0.05% NaF). Based on these observations, we conclude that the experimental formulation “Listerine F Extra” and SB12 could combine the anti-bacterial effect of Listerine or the anti-malodour effect of SB12, while maintaining, or even improving, the anti-caries properties.
Figure 2. Pair-wise comparison between Duraphat and Pepsodent toothpaste and the effect of various post-brushing rinsing procedures. The inserted bars show the mean AUC0-60 min (n=10; Series I)
The present study indicates that there is no reason in combining a toothpaste with a high F concentration (such as Duraphat) in high caries risk patients and a post-brushing rinsing solution with a low F concentration. Another interesting observation was that, when Flux (containing 0.2% NaF) is used after brushing, the concentration in toothpaste does not have any significant impact on the salivary F retention. In general, Duraphat toothpaste with 5000 ppm F (sold in Sweden as an OCT product without a prescription to individuals more than 16 years old) should be used alone without any post-brushing mouthrinse solution and Flux with 900 ppm should be combined with regular toothpastes with 1450 ppm F.

The present investigation, the subjects did not rinse with water in Series I, while they did in Series II. An examination of the F values presented in the graphs clearly reveals that the saliva F concentration is generally higher in Series I, even though the amount of the post-brushing rinsing solution was half that in Series II. This observation underlines the importance of not using water after brushing, while just spitting it out carefully would be more beneficial when it comes to retaining more F. This has recently been confirmed in a study by Nordström & Birkhed (6).

The present results are in many respects in agreement with the recent study by Duckworth et al. (3). They concluded that “a non-F mouthwash after toothbrushing with a F toothpaste may reduce the anti-caries protection provided with a F toothpaste alone”. They also found that a “226-ppm mouthwash soon after brushing with a 1450 ppm paste may achieve a potential additional anti-caries benefit to that a F toothpaste”. Their study give – in contrast to our study - some benefit of a 100-ppm F rinsing solution. The difference may depend on whether water rinsing or not is used after the brushing.

As a general conclusion, the salivary F concentration after brushing with a F toothpaste is strongly affected by the post-brushing rinsing solution. This means that post-brushing mouthrinse solutions ex-
ert a “wash-out” if the concentration of F is too low. For this reason, solutions with no F or just 100 ppm remove a great deal of the F, which comes from the toothpaste.

Acknowledgements
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Firing temperature accuracy of four dental furnaces

Per Haag¹, Edina Ciber², Tore Dérand²

Abstract
In spite of using recommended firing and displayed temperatures, low-fired dental porcelain more often demonstrates unsatisfactory results after firing than porcelain fired at higher temperatures. It could therefore be anticipated that temperatures shown on the display are incorrect, implying that the furnace does not render correct firing programs for low-fired porcelain. The purpose of this study is to investigate deviations from the real temperature during the firing process and also to illustrate the service and maintenance discipline of furnaces at dental laboratories.

Totally 20 units of four different types of dental furnaces were selected for testing of temperature accuracy with usage of a digital temperature measurement apparatus, Thermo 1. In addition, the staffs at 68 dental laboratories in Sweden were contacted for a telephone interview on furnace brand and on service and maintenance program performed at their laboratories.

None of the 20 different dental furnaces in the study could generate the firing temperatures shown on the display, indicating that the hypothesis was correct. Multimat MCII had the least deviation of temperature compared with display figures. 62 out of 68 invited dental laboratories chose to participate in the interviews and the result was that very few laboratories had a service and maintenance program living up to quality standards.

There is room for improving the precision of dental porcelain furnaces as there are deviations between displayed and read temperatures during the different steps of the firing process.

Key words
Dental furnaces, firing temperature, low-fired dental porcelain

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Bränntemperaturprecision för porslinsugnar

Per Haag, Edina Ciber, Tore, Dérand

Sammanfattning

Trots användande av rekommenderade bränntemperaturer, har det visat sig att lågbränt porslin i högre utsträckning uppvisat otillfredsställande resultat efter bränning i jämförelse med resultatet vid arbete med traditionellt högbränt porslin. Man kan därför anta att de temperaturer som visas på ugnens display inte alltid är korrekta, dvs. ugnarna bränner inte vid en för lågbränt porslin korrekt temperatur. Syftet med denna studie är att studera förekomsten av avvikelse mellan på ugnens display visad, och för porslinet korrekt bränntemperatur, och verklig temperatur.

Ugnar för bränning av dentalporslin är känsliga apparater som fordrar underhåll och service. Därför har vi även undersökt disciplinen avseende underhåll och service. Totalt 20 enheter av fyra olika ugnar valdes ut för att studera temperaturstabilitet med hjälp av en digital temperaturmätare, Therma 1. I tillägg härtill tillfrågades personalen på 68 dentallaboratorier i Sverige via telefonintervjuer om vilken typ av ugn som användes, dess ålder och huruvida ett service- och underhållsprogram var rutin på laboratoriet.

Ingen av de 20 ugnarna som studerades kunde uppvisa en verklig bränntemperatur som stämde överens med vad displayen visade, vilket indikerade att hypotesen var korrekt. Multimat MCII var den ugn som uppvisade minst avvikelse från korrekt bränntemperatur. 62 av 68 tillfrågade dentallaboratorier ställde upp i telefonintervju, från vilken resultatet var att få dentallaboratorier hade ett service- och underhållsprogram som lever upp till kvalitetsstandard.
Introduction:
Fixed all-ceramic and metal-ceramic restorations have been used for about 100 and 50 years respectively in clinical work, and the use of metal cores has widened the application of dental porcelain (26). Porcelain veneering of metal frameworks contains several crucial points like proper mixing of liquid and porcelain powder, proper cleaning of metal surface, and stable humidity of firing object when placing it into the furnace. In the firing process the liquid is vaporized and the gaps between porcelain crystals are closed, which results in a properly sintered product (3). The temperature curve is important for avoiding problems caused by thermal stresses created in the different phases at sintering (7, 9, 11, 15, 21, 25). The breakthrough of modern implantology, successfully using commercially pure titanium, also created an interest in using titanium in bridgeworks covered with ceramics (2, 20). The reaction pattern of titanium to high temperatures made it necessary to develop low-temperature fused porcelain (1), for firing at temperatures around 200°C below the temperatures conventionally adopted for veneering of base metal or noble alloys. As a consequence of initially positive experiences with titanium ceramics, low-temperature fused porcelain has positive properties also at firing of conventional metals and cores, and several concepts of low-fired porcelain have since then been broached (17). The advantages could be described as better control of pigmentation and marginal distortion (10, 21) and also a safer margin to melting temperature of core metals (5). Another aspect is that it is easier to control the coordination of the thermal expansion curve between porcelain and core material at a lower firing temperature (4, 6, 12-14, 16, 18, 22, 25).

The introduction of these porcelains in other situations demands a good control of firing temperature in the furnaces. Failures which have occurred with titanium porcelain might be suspected to depend on inadequate firing temperature control. The aim of this study was therefore to test the hypothesis that there may be a lack of accuracy in firing temperatures of dental furnaces, and that there is a difference between furnaces emanating from both type of furnace and management of maintenance at dental laboratories. Also an investigation of dental laboratories was to be performed in order to gain, by interviews, information on their furnaces and how the control and maintenance of these furnaces was performed.

Material and Methods
The furnaces investigated were selected from different dental laboratories in southern and western Sweden, to enable 5 of each brand to be tested. The furnaces were selected to reflect furnaces used routinely at dental laboratories. Among the laboratories a sample of large and one-man laboratories was selected.

Four brands of dental furnaces were used:
• Multimat® Touch (Dentsply De Trey GmbH, Konstanz, Germany)
• Vacumat 300 (Vita Zahnfabrik, Bad Säckingen, Germany)
• Austromat 3001 (Dekema Dental-Keramiköfen GmbH, Freilassing, Germany)
• Multimat® MC II (Dentsply De Trey GmbH, Konstanz, Germany)

Four thermo wires (Thermocouple type k, batch 8 103 000, Pentronic AB, Gunnebo, Sweden) were brought through the middle of one firing tray (Keystone, 2 7/8” x ½”, item no. 680 050) each. These thermo wires were connected to a digital temperature measurement apparatus (Therma 1, Indikator T/C K, Pentronic, Gunnebo, Sweden). The distance from the top of the wires, where the registration is done, to the firing table was chosen to be 1 cm, in order to make registration from the position where ceramic restorations are placed at a firing sequence. (Fig. 1) In order to optimize the vacuum set in at firing,
the entrance of the thermo wires was made airtight
by aid of a 3 mm thick gasket (AxFlow, Bergman
AXAB AB, Stockholm, Sweden). Three tempera-
ture processes, corresponding to the firing sequences re-
commended by the manufacturer of the different
porcelain brands (Finesse, Dentsply International,
York USA; d-Sign, Ivoclar AG, Schaan, Liechtenstein;
AllCeram, Degudent AG, Hanau, Germany), were
chosen for measurement of all furnaces (Table 1).
Five units each of four different furnace brands were
tested in two firing sequences at each temperature
level, which means that totally 120 firing sequences
were performed.
Registrations were made of the difference between
displayed and temperature measured with thermo
coupling during the holding temperature.

Questionnaire
In addition, the staffs at 62 out 68 invited dental
laboratories in Sweden were contacted for a phone
interview, where the following questions were to be
answered:
• How many furnaces do you have at your labora-
tory?
• Which furnace brands, ages and maintenance
service interval do you have?

Statistics
Kruska-Wallis one way analysis of variance (23) was
used to study the differences between the different
furnaces

Results
Firing temperature measurement with thermocouples
The discrepancy between measured and displayed
temperature at holding temperature, varied between
10.9 °C (1.9%) and 48.4 °C (5.3%) for the different
brands of furnaces, demonstrating that the measu-
red temperatures were always higher than displayed
temperature (Table 2). The best accuracy percenta-
gewise was achieved at the highest firing tempera-
ture and there was a significant difference between
all the furnaces the furnaces at 760º C, 870º C and
920º C (p<0.05).

It could also be seen that the Multimat MCII fur-
naces had the best accuracy in average percentage of
deviation, 1.9 %, compared to the other three fur-
naces, among which Vacumat 300 showed the hig-
hest degree of inaccuracy, 5.2%. Between Multimat®
Touch and Dekema Austromat no difference in ac-
curacy could be documented.

Consequently, the variables for firing temperature
accuracy are both level of firing temperature and
brand of furnace.

Questionnaire
The results were split into three groups: small labo-
ratories in possession of 1-2 furnaces, medium-size
laboratories owning 3-6 furnaces, and large labora-
tories with 7 or more furnaces.

Table 1. Firing schedules for first dentine firing of Finesse,
d-SIGN and AllCeram porcelain, with pre-drying and drying
times eliminated, for Multimat® Touch furnace

<table>
<thead>
<tr>
<th>Porcelain</th>
<th>Firing stage</th>
<th>Finesse</th>
<th>d-SIGN</th>
<th>AllCeram</th>
</tr>
</thead>
<tbody>
<tr>
<td>Start temperature</td>
<td>450 °C</td>
<td>403 °C</td>
<td>575 °C</td>
<td></td>
</tr>
<tr>
<td>Temperature raise</td>
<td>35 °C/60 °C/55 °C</td>
<td>min/ min/ min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Firing temperature</td>
<td>760 °C</td>
<td>870 °C</td>
<td>920 °C</td>
<td></td>
</tr>
<tr>
<td>Vacuum on</td>
<td>50 hPa</td>
<td>50 hPa</td>
<td>50 hPa</td>
<td></td>
</tr>
<tr>
<td>Holding time vacuum</td>
<td>0 min</td>
<td>0 min</td>
<td>1 min</td>
<td></td>
</tr>
<tr>
<td>Firing time</td>
<td>½ min</td>
<td>1 min</td>
<td>2 min</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Mean value (m) and standard deviation (S.D.) of the temperature discrepancies between measured and displayed
temperatures at holding firing temperature for four furnace brands at three different firing programs measured in centigrade (°C)
and also expressed in percentage deviation (%)

<table>
<thead>
<tr>
<th>Furnace Programme for</th>
<th>Finesse (760°C)</th>
<th>d-SIGN (870°C)</th>
<th>AllCeram (920°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>m</td>
<td>S.D.</td>
<td>%</td>
</tr>
<tr>
<td>Multimat Touch</td>
<td>35.0°</td>
<td>12.72</td>
<td>4.9</td>
</tr>
<tr>
<td>Vacumat 300</td>
<td>39.6°</td>
<td>9.19</td>
<td>5.2</td>
</tr>
<tr>
<td>Austromat</td>
<td>36.3°</td>
<td>4.24</td>
<td>4.8</td>
</tr>
<tr>
<td>Multimat MCII</td>
<td>18.5°</td>
<td>12.72</td>
<td>2.4</td>
</tr>
</tbody>
</table>
Totally 62 laboratories (10 small, 42 medium-size and 10 large laboratories) participated in the study, while 6 laboratories did not accept participation, out of 68 invited. Eleven types of dental furnaces, with differing age, the oldest 28 years, were in use at the laboratories. Among the 62 laboratories, the following maintenance was performed:

(a) 9 laboratories have a maintenance program at fixed intervals – six of them once a year and the other three in this group four times/year, two times/year and once every second year, respectively.

(b) 53 laboratories performed maintenance at their furnaces “when needed”, which means when the function declined because of technical errors or visible changes of firing results.

Calibration was performed by the laboratory staff themselves. The interval between calibrations differed among the laboratories, from once a month to once every second year.

Discussion

Thermocouple measuring of firing temperature

The most important part of the firing cycle is the holding temperature, which is the final temperature where the final sintering is performed. This temperature must be held accurately, and that is what is shown by the display.

As a general statement, the temperature given by the displays during the whole firing process deviated from the values measured with the thermo element as illustrated in Fig. 2. According to the given firing schedules the temperature rise should be 35°C/min for Finesse, 60°C/min for d.SIGN, and 55°C/min for AllCeram. The temperatures read from the displays followed, for all furnaces the firing schedules with, however, only a few degrees of deviation. With all furnaces it was shown that the real temperature was lower or at about the same level as the display temperature at the beginning of the firing process, but subsequently rose away from the displayed one, so that during holding the real temperature was higher than the displayed one.

Multimat® MC II showed smallest gap between displayed and real temperature, during temperature rise. The temperature stability during the rising period is important since it influences the thermal expansion curve (8, 16, 25).

The firing process at hold temperature is the most enduring but also the most crucial part of the firing cycle, since the temperature should be held as con-
constant as possible. It has sometimes been claimed that a prolonged firing (holding) time could compensate for incorrect firing temperature, a statement which has been disputed by Cheung & Darvell (10) in a study which showed the difference in effect between a correct temperature and trying to compensate for correct temperature with a longer holding time (10). They showed a variation in display temperature from 0°C to 3°C during firing, while the real temperature showed a divergence. Multimat MCII performed best with an average range of variation of 6.2°C during holding sequence in the firing process. The results could be explained by soft- and hardware design of the different furnaces, reflected in a large variation in pricing and may be also the fact that furnaces are equipped with different types of vacuum pumps functions. This might create a situation where the vacuum level is attained gradually during the firing process, hindering the furnaces from keeping a constant temperature.

Questionnaire
A fixed service program was used by 15% of the interviewed laboratories, which is low not least in view of the large variation in accuracy of temperature illustrated by the thermo element measurements performed in this study. Dental furnaces are sensitive equipment, which undergo high stress in the daily production at a dental laboratory. The development of dental porcelain, in order to render better aesthetics and strength by adding new and more sensitive components, makes it surprising that there is such a variation in maintenance discipline between different laboratories. Since the firing process gives the dental restoration its aesthetic, physical and mechanical properties, the function of dental furnaces used should be controlled following a standard procedure. Since the long-term consequences for patients as well as dentists of uncontrolled firing are crucial, a mandatory maintenance program of dental furnaces should be part of the quality program for dental laboratories. It should be a demand from the dentist when accepting a dental laboratory for dental restorations with porcelain compounds.

Conclusion
The deviation between display and real temperatures during the different steps of a firing process, demonstrated with different furnaces in this study, clearly indicates that there is substantial room for improving precision of dental porcelain firing. There is also a lack of furnace maintenance and since the process of firing porcelain in the end will result in a medical restorative process, standards should be established for temperature stability of furnaces and also for demands on maintenance of furnaces at the dental laboratories.

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Alpha-1-antitrypsin deficiency and periodontitis, a pilot study

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Abstract

The aim of this study was to investigate if periodontal parameters and elastase in gingival crevicular fluid (GCF) are different in alpha-1-antitrypsin deficient (AATD) subjects compared to subjects with normal AAT level.

Thirty subjects were included, 20 of whom with severe AATD, phenotype PiZZ. Ten AATD subjects suffered from chronic obstructive pulmonary disease (COPD, group 1) and 10 were asymptomatic (group 2). Ten control subjects, phenotype PiMM, (group 3) were recruited from a public dental clinic. The examination comprised of sampling of GCF, Gingival Index (GI), Plaque Index (PlI), probing pocket depth (PPD) and radiography. GCF was collected with paper strips (Periopaper®). Plasma AAT concentration was measured by nephelometry and AAT in GCF with ELISA. Elastase activity and protein in GCF were determined by spectrophotometry.

The mean values for GI, PlI, PPD and the radiological measurements did not show any statistically significant differences between the groups. AAT in plasma and GCF demonstrated very low values in groups 1 and 2 with no significant difference between these groups but a statistical difference in comparison with group 3. Elastase in GCF did not show any difference between the three groups.

In conclusion, neither the periodontal parameters nor the elastase in GCF were different in AATD subjects, phenotype PiZZ, when compared to subjects with normal AAT level, phenotype PiMM, in this material.

Key words

Alpha-1-antitrypsin, chronic obstructive pulmonary disease, elastase, gingival crevicular fluid
Alfa-1-antrypsinbrist och parodontit, en pilotstudie

Viveca Wallin-Bengtsson, Eeva Piitulainen, Kristina Hamberg, Christina Lindh, Gunilla Bratthall

Sammanfattning

Målet med studien var att undersöka om parodontala parametrar och elastas i gingivalvätska (GCF) skilde sig hos individer med alfa-1-antrypsin-brist (AAT-brist) jämfört med individer med normal AAT-halt.

I studien ingick 30 individer, varav 20 med allvarlig AAT-brist, fenotyp PiZZ. Tio individer med AAT-brist led av kronisk obstruktiv lungsjukdom (KOL) (grupp 1) och 10 var symptomfria (grupp 2). Tio individer med normal AAT-halt, fenotyp PiMM (grupp 3), utgjorde kontrollgrupp och rekryterades från en allmäntandvårdsklinik. Undersökningen bestod av insamling av GCF, gingivalindex (GI), plackindex (PlI), fickdjupsmätning (PPD) och röntgen. GCF samlades in med hjälp av pappers-strips (Periopaper®). AAT i plasma mättes med nefelometri och AAT i GCF mättes med ELISA. Elastasaktivitet och protem- mängd i GCF bestämdes med spektrofotometri.

Medelvärden för GI, PlI, PPD och röntgenmätningar visade inga statistiskt signifikantha skillnader mellan grupperna. AAT i plasma och GCF visade mycket låga värden i grupp 1 och 2 utan några signifikantha skillnader mellan grupperna men en signifikanth skillnad i jämförelse med grupp 3. Elastas i gingivalvätska visade inga skillnader mellan de tre grupperna.

Sammanfattningsvis visade varken parodontala värden eller elastas i GCF några skillnader hos individer med AAT-brist, fenotyp PiZZ, jämfört med individer med normal AAT-halt, fenotyp PiMM, i detta material.
Introduction
It is important to identify risk patients for periodontitis. Proteases such as elastase are enzymes able to break down the tissue in periodontitis and pulmonary emphysema. Blood plasma and gingival exudate contain antiproteases, such as alpha-1-antitrypsin (AAT) (4, 9, 17, 19). In healthy tissue there is a balance between the proteases and antiproteases. Excess of proteases may lead to tissue destruction and diseases as periodontitis and emphysema.

AAT is mainly synthesized by the liver, excreted in the circulation and can be found in all body liquids (3). The synthesis is coded by a gene located in the chromosome 14(q31-32.2). There are more than 100 different genetic variants of the AAT molecule caused by mutations in the molecule. The Pi (Protease inhibitor) phenotypes reflect the concentration and function of the protein. In the PiMM phenotype the plasma levels and function of AAT are normal. In the PiZZ phenotype plasma concentration of AAT is 1/1600 in Sweden (3, 28).

Peterson & Marsh (21) have shown that certain Pi types appear to be related to increased chronic periodontitis susceptibility. Sandholm et al. (25), however, could not relate the reduced periodontal resistance in juvenile periodontitis subjects by AAT deficiency. All subjects in their study displayed the most common phenotype M, indicating that the AAT levels in the patients’ sera were within normal limits. Fokkema et al. (6) have investigated the periodontal condition in a small group of subjects with severe AAT deficiency (PiZZ) in comparison with healthy control subjects, matched for age, gender and socioeconomic status. The AAT-deficient group had a higher number of sites with probing depths ≥ 5 mm. Another approach was made by Scott et al. (26) who compared the prevalence of the Pi*Z and Pi*S alleles in whole blood from 31 subjects with periodontal disease with periodontally healthy subjects. Periodontal disease was defined as ≥ 5 sites with probing depths of ≥ 5 mm. There was no difference in the proportion of any alpha-1-antitrypsin genotype in these two groups. Because of low prevalence of PiZZ and PiSZ phenotypes, the power to detect differences in Pi phenotypes was low in their study.

The aims of the present investigation were to study if periodontal parameters and elastase in gingival crevicular fluid (GCF) are different in AATD subjects, phenotype PiZZ, compared to subjects with normal AAT level, phenotype PiMM.

Material and methods

Study subjects
The study included 30 subjects each of them with at least 20 teeth. Twenty of the study participants had severe alpha-1-antitrypsin (AAT) deficiency, phenotype PiZZ, and were included in the Swedish national register of AAT deficiency (23).

They were out patients at the Department of Respiratory Medicine at the University Hospital, Malmö (UMAS), Sweden. Ten of the AAT-deficient subjects suffered from chronic obstructive pulmonary disease (COPD) (5 women and 5 men, mean age = 59 years, range 42-74 years), and 10 were asymptomatic and had normal lung function (5 women and 5 men, mean age = 47 years, range 20-65 years). An age and sex matched control group of 10 individuals (6 women and 4 men, mean age = 49 years, range 22-71 years) with phenotype PiMM were recruited from the waiting list of a public dental clinic in Lund, Sweden. Smoking was an exclusion criterion. Patients using inhaled corticosteroids stopped the treatment one week before the participation in the study.

The study was conducted in accordance with the Helsinki Declaration, and approved by the Regional Ethical Review Board. All study participants gave their signed, informed consent.

Clinical examination
Assessment of periodontal status was performed by one observer (VWB) in the following sequences:

1. Gingival crevicular fluid (GCF)
2. Gingival index, GI (15)
3. Plaque index, PI (27)
4. Probing pocket depth (PPD)
5. X-rays

GCF was collected with prefabricated paper strips (Periopaper® GCF strips IDE Interstate, Amityville, N.Y., USA), 6 sites in each patient (representing molars, premolars and incisors in each jaw). The sites to be sampled were isolated with cotton rolls and gently dried with an air syringe. The strips were inserted into the crevice and kept for 30 seconds. Samples contaminated with blood or saliva were discarded. The strips were collected in an Eppendorf® tube and 1000 ul phosphate buffered saline (PBS), pH 7.4, were added. The sample was mixed 4 x 30 seconds and then centrifuged at 8000 rpm for 5 minutes. The supernatant was divided in 100 ul aliquots and stored at -18 °C until analysed.
The fluid volume was determined by weight, the paper strips were weighed before and after insertion into the gingival crevice.

Gingival and plaque index measurements were made at the mesial and distal sites from the buccal side on all teeth. PPD was recorded using a calibrated periodontal probe (UNC-15, Hu-Friedy, Chicago, USA) to the nearest mm (diameter of the probe tip was 0.5 mm, 1 mm increments).

**Intraexaminer reproducibility**

In order to assess the intraexaminer measurement performance, repeated measurements of PPD were conducted in 5 patients with one week between the first and the second registration.

**Radiology**

Panoramic radiography was performed with the Scanora® (Soredex, Helsinki, Finland) multimodal radiography system using the screen/film combination Lanex medium/T-mat G (Eastman Kodak Co., Rochester, N.Y., USA). The Scanora® dental panoramic program 003 was used, and the voltage settings were 66 or 70 kV at 10, 13, 16 or 20 mA and the exposure time was 15, 19 or 23 s. The vertical angulation of the tube was constantly -5°. The films were processed in an automatic processor (Curix HT-33OU, AGFA, Belgium) with a developing time of 2 min.

The marginal bone level was assessed by one independent observer (C.L.). A classification into three different classes due to the amount of marginal bone loss around each remaining tooth was done (10, 12):

- 0 = no loss of supporting bone tissue (healthy)
- 1 = horizontal loss of supporting bone tissue ≥ 1/3 of root length in < 30 % sites (local periodontitis)
- 2 = horizontal loss of supporting bone tissue ≥ 1/3 of the root length in > 30 % sites (general periodontitis)

Periodontal disease was defined as at least one pocket ≥ 5 mm with bleeding and horizontal loss of supporting bone tissue as clarified above. The reproducibility for PD within 1 mm was 88 %.

**Questionnaire**

All study participants answered a questionnaire on respiratory symptoms. The questionnaire was identical to that used in the Swedish AAT deficiency Register. The questionnaire included the following questions: Do you usually bring up phlegm from your chest for at least three consecutive months a year (Yes/No); Are you troubled by shortness of breath when walking 100 m on the level (Yes/No).

**Laboratory methods**

**AAT in plasma**

Venous blood samples were collected when the study participants visited the Department of Respiratory Medicine, Malmo University Hospital for performing pulmonary function tests. Plasma was directly separated by centrifugation and frozen at -180 until use. Plasma AAT concentration was measured by nephelometry. Pi phenotypes were determined by isoelectric focusing at the Department of Clinical Chemistry, University Hospital, Malmo, Sweden (13).

**AAT in GCF**

The concentrations of AAT in GCF were determined with ELISA modified from Persson et al. (18). The samples and standards were prepared to proper dilutions. The wells of the microtiter plates (Nunc Maxisorp, Nunc, Roskilde, Denmark) were coated with standards and samples overnight at 4 °C. After 4 washes with PBS containing 0.05 % Tween, the polyclonal rabbit antibody against AAT (Sigma-Aldrich A-0409) was added. The plates were incubated at 37 °C for 1 hour and washed 4 times before the alkaline phosphatase conjugated polyclonal goat antibody against rabbit IgG (Sigma-Aldrich A-9919) was added. After incubation at 37 °C for 1 hour and another 4 washes, the substrate 4-p-nitrophenyl-phosphate was added. The absorbance at 405 nm was measured after approximately 30 minutes in a spectrophotometer. The samples were compared to a standard curve obtained by dilution of an AAT from human plasma standard (Sigma-Aldrich A-9024) on the same microtiter plate.

**Elastase and protein in GCF**

Elastase activity / concentration in GCF was measured with a low weight substrate S-2484 (L-pyro-glutamyl-L-propyl-Lvaline-nitroaniline, Hemachrome Diagnostica, Malmö, Sweden), which is specific for neutrophil elastase (14). The substrate was added (67 ul 2 mmol) to the 100 ul sample GCF diluted 1:5 x 2 on a microtiter plate and mixed. The mixture was incubated at 37° C and after 5 hours the absorbance at 405 nm was registered in a spectrophotometer. The total protein concentration in GCF was measured with the Bradford protein staining method (2) using Bio-Rad Dye Reagent. GCF samples were analysed undiluted, 2x10 ul on a microtiter plate. Two
hundred ul Bio-Rad Dye Reagent 1:5 were added to the microtiter plates. After 10 minutes the absorbance at 595 nm was measured in a spectrophotometer. The samples were compared to a standard curve obtained by bovine serum albumin.

Pulmonary function tests
All study participants performed dynamic spirometry including measurement of forced expiratory volume in one second (FEV₁) and forced vital capacity (FVC) at the Department of Respiratory Medicine, University Hospital, Malmo. The results of pulmonary function tests (FEV₁ and FVC) are expressed as percentages of predicted values according to the reference material of European Coal and Steel Community (24). The FEV₁/FVC ratios are expressed as a percentage.

COPD was defined as FEV₁ < 80 % of predicted value, and FEV₁/FVC ratio < 70 %.

Statistical Analyses
When comparing all three groups simultaneously the Kruskal-Wallis test was used to test the hypothesis that the groups are equal. When comparing two groups the exact Wilcoxon Rank Sum Test was used to test the hypothesis that the groups are equal. No adjustment for multiple comparisons were made. All p-values were two-sided and a p-value less than 5 % is considered statistically significant.

Results
The demographic data and results of the lung function tests are shown in Table 1. The AAT deficient subjects with symptoms had poorer (lower) lung function than the asymptomatic AAT deficient subjects and the healthy controls. The difference was statistically significant.

The results of the questionnaire are presented in Table 2. Only the AAT-deficient subjects with symptoms reported wheezing and shortness of breath on exertion, typical symptoms of COPD.

Clinical examination
The mean values for GI, PI, and PPD in the different groups are presented in Table 3. No statistical differences between the groups were observed in this pooled material in the overall comparison.

Radiology
The radiological periodontal status of the different groups is presented in Table 4.

There was no statistically significant radiological difference between any of the groups. There was, however, a tendency of a difference between the two AAT groups (p= 0.057).

Gingival crevicular fluid and plasma
The amount of protein, elastase and AAT in GCF in the different groups is shown in Table 5. There was no statistically significant difference in protein and elastase between any of the groups. Nor was there any difference when the two test groups were pooled and compared to the control group.

When AAT/protein in GCF was calculated there was no statistically significant difference between groups 1 and 2 but a significant difference in comparison with the control group (p< 0.01). When the two test groups were pooled and compared to the control group the difference was even more evident (p< 0.001). When AAT was expressed as ng/ul GCF a trend to statistical difference was found between groups 1 and 3 (p=0.063) only.

AAT in plasma expressed as ng/ul did not show statistical difference between groups 1 and 2 but a statistically significant difference for group 1 and 2 compared to the control group (p< 0.001), since only AAT deficient subjects were included in the two test groups (Table 6).

Discussion
In the present study 20 subjects with AATD, 10 of them with lung symptoms, were examined. All subjects belonged to Pi type PiZZ with very low production of AAT as can be seen in table 6. All control subjects belonged to Pi group PiMM. No test or control phenotypes PiMZ were present in our study. PPDs (Table 3) and radiographic bone level (table 4) demonstrated no difference between AATD and control subjects.

In the study by Peterson & Marsh (21) 50 subjects with severe periodontal disease (Ramfjord periodontal Index above 4) and 100 healthy controls (Ramfjord periodontal Index of less than 4) were examined. In their material no PiZZ individuals was found. The experimental group contained a lesser number of PiMM (normal type, 66 %) compared to the control group (87 %). The percentage of PiMZ (heterozygous deficient) was higher in the experimental group (18 %) compared to the control group (5 %). This can be compared to figures from Sweden where the prevalence of PiMZ in the whole population is 4-5 % (28) corresponding to 2 % in St Louis (21).
Table 1. Demographic data and results of the lung function tests. Percentage of the predicted values of Forced expiratory Volume in one second (FEV1) and Forced Vital Capacity (FVC) are shown. The lung function tests were performed 15 minutes after inhaled bronchodilator (1.0 mg Terbutaline)

<table>
<thead>
<tr>
<th>Category</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>AATD COPD</td>
<td>AATD asymptomatic</td>
<td>Healthy controls</td>
<td></td>
</tr>
<tr>
<td>(n=10)</td>
<td>(n=10)</td>
<td>(n=10)</td>
<td></td>
</tr>
<tr>
<td>Age (range)</td>
<td>47 (20-65)</td>
<td>59 (42-74)</td>
<td>49 (22-71)</td>
</tr>
<tr>
<td>Mean (SD) FEV1 (% predicted)</td>
<td>59.9 (15.3)**</td>
<td>94.3 (7.4)</td>
<td>103.4 (22.4)</td>
</tr>
<tr>
<td>Mean (SD) FVC (% predicted)</td>
<td>84.6 (18.1)*</td>
<td>98.8 (17.3)</td>
<td>106.8 (14.6)</td>
</tr>
<tr>
<td>Mean (SD) FEV1/FVC ratio (%)</td>
<td>59 (15)**</td>
<td>79 (6)</td>
<td>80 (8)</td>
</tr>
</tbody>
</table>

AATD = AAT-deficient subjects COPD = Chronic Obstructive Pulmonary Disease **p<0.001 AATD symptomatic vs. asymptomatic and healthy controls *p<0.01 AATD symptomatic vs. asymptomatic and healthy controls

Table 2. Response to questions on respiratory symptoms

<table>
<thead>
<tr>
<th>Question</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Do you usually bring up phlegm from your chest for at least three consecutive months a year / Yes</td>
<td>4</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Does your chest ever sound wheezy or whistle / Yes</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Are you troubled by shortness of breath when walking 100 m on the level / Yes</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 3. Comparison of periodontal variables between AAT-deficient subjects with symptoms (Group 1), asymptomatic AAT-deficient subjects (Group 2) and control subjects (Group 3).

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n = 10)</td>
<td>(n = 10)</td>
<td>(n=10)</td>
</tr>
<tr>
<td>Mean (SD) GI</td>
<td>1.2 (0.2)</td>
<td>1.3 (0.3)</td>
</tr>
<tr>
<td>Mean (SD) PI</td>
<td>1.1 (0.5)</td>
<td>1.0 (0.4)</td>
</tr>
<tr>
<td>Mean (SD) PD</td>
<td>2.7 (0.6)</td>
<td>2.7 (0.4)</td>
</tr>
</tbody>
</table>

GI = gingival index; PI = Plaque index; PD = pocket depth

Table 4. Radiological periodontal status in the in the AAT-deficient subjects with symptoms (Group 1), asymptomatic AAT-deficient subjects (Group 2) and the control subjects (Group 3)

<table>
<thead>
<tr>
<th>Healthy</th>
<th>Local periodontitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>Group 2</td>
</tr>
<tr>
<td>(n = 10)</td>
<td>(n = 10)</td>
</tr>
<tr>
<td>Healthy: No loss of supporting bone</td>
<td>4</td>
</tr>
<tr>
<td>Local periodontitis: Horizontal loss of supporting bone ≥1/3 of the root length in &lt;30 % sites</td>
<td>6</td>
</tr>
</tbody>
</table>

Table 5. The amount of protein, elastase and AAT in GCF in AAT-deficient subjects with symptoms (Group 1), asymptomatic AAT-deficient subjects (Group 2) and control subjects (Group 3)

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n=10)</td>
<td>(n=10)</td>
<td>(n=10)</td>
</tr>
<tr>
<td>Mean (SD) Protein ng/ul</td>
<td>53.2 (37.2)</td>
<td>84.8 (80.5)</td>
</tr>
<tr>
<td>Mean (SD) Elastase: Mabs/protein</td>
<td>38.7 (42.9)</td>
<td>57.6 (62.5)</td>
</tr>
<tr>
<td>Mean (SD) AAT/ul</td>
<td>0.85 (0.64)</td>
<td>0.25 (0.95)</td>
</tr>
<tr>
<td>% AAT/Protein</td>
<td>1.7*</td>
<td>1.8*</td>
</tr>
</tbody>
</table>

*p<0.01 AATD symptomatic vs. healthy controls *p<0.01 AATD asymptomatic vs. healthy controls

Table 6 The amount of AAT in plasma in AAT-deficient subjects with symptoms (Group 1), asymptomatic AAT-deficient subjects (Group 2) and control subjects (Group 3)

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n=10)</td>
<td>(n=10)</td>
<td>(n=10)</td>
</tr>
<tr>
<td>Mean (SD) AAT ng/ul</td>
<td>229.0 (66.7)**</td>
<td>217.0 (76.6)**</td>
</tr>
</tbody>
</table>

**p<0.001 AATD symptomatic vs. health controls *p<0.01 AATD asymptomatic vs. healthy controls
In our study there were no statistically significant differences in clinical parameters between AATD and control subjects. This can be compared to Fokkema et al. (6) who also examined the periodontal condition in subjects with severe AAT deficiency (PiZZ). Ten subjects with AATD were compared with healthy control subjects. No difference with regard to attachment loss was found between the two groups, but the deficiency group demonstrated a higher number of sites with probing depths ≥5 mm. Besides, the study participants were younger (mean age just over 30 years) than in our study (mean ages between 47 and 59 years, Table 1). Smoking habits were not reported by Fokkema, but in the present study all participants had stopped smoking more than one year prior to investigation.

Smoking results in high levels of elastase in the lower respiratory tract because of neutrophil attraction (11). Smoking also inactivates AAT by oxidation. These mechanisms will make smokers at increased risk of developing emphysema (16). In patients with severe periodontitis, Persson et al. (19) showed the total amount of AAT in GCF to be lower in smokers than in non smokers, suggesting that smoking may be one mechanism affecting the inflammatory response in periodontal disease. Persson et al. (20) also showed that 5 weeks after periodontal surgery the concentrations of alpha-1-AT, alpha-2-MG, and MMP-8 in smokers were unaltered. In non-smokers, the levels of alpha-1-AT and alpha-2-MG increased, whereas MMP-8 levels decreased. This may explain the clinical evidence of inferior treatment outcome in smokers.

For GCF, serum-derived AAT is the major elastase inhibitor, but in our study we did not find any statistically significant differences in GCF elastase between AATD subjects and controls. In whole mouth saliva secretory leukocyte protease inhibitor (SLPI) probably reduces the activity. The inflamed gingivae can be an additional source of SLPI in the oral cavity, but here the molecule is apparently cleaved by GCF cysteine proteinases, such as cathepsin B (4). The presence of the protease inhibitor alpha-2-macroglobulin in GCF has also been investigated. In a study by Figueredo & Gustafsson (5) the presence of free elastase in GCF samples from gingivitis and periodontitis subjects was examined. The activity that could be inhibited by AAT was considered as free elastase, and the uninhibited activity as derived from the elastase-A2MG complex. It was concluded that the elevated levels of free elastase in sites showing tissue destruction seemed to be due to a combination of increased release of elastase and an inactivation of AAT.

Ingman et al. (9) studied the presence of neutrophil elastase and AAT in gingival tissue specimens from adult periodontitis (AP) patients and from controls with clinically healthy periodontium. Neutrophil elastase was strongly positive in the connective tissue of the AP patients but only a few elastase-positive cells were present in the healthy gingival tissue. Both in AP and in control gingival specimens, AAT was detected in the connective tissue but the amount was markedly lower in the control specimens.

Reduction of the elastase inhibitor, AAT, may be of pathogenic significance in inflammatory diseases like periodontal disease. AAT can be inactivated by the formation of an AAT-elastase complex and also by cleavage by elastase or other enzymes such as metalloproteinases of host or bacterial origin. Gingival crevicular fluid from periodontal disease patients, AAT is mainly inactivated and the extent of this inactivation is much higher than in inflammatory fluids from other chronic diseases such as rheumatoid arthritis (22).

In the present study the AAT deficient individuals demonstrated a significantly lower level of AAT in GCF than the control subjects. This deficiency did not seem to influence the amount of GCF elastase and clinical parameters, since there was no difference in elastase and clinical parameters between the groups. Other protease inhibitors such as saliva secretory leukocyte protease inhibitor (SLPI) and alpha2-macroglobulin might mask the importance of AAT in this material.

It has been estimated that approximately 35 % of an adult population will develop moderate periodontal disease whereas 10-15 % will develop severe attachment loss (Hugoson et al. 1998, Albandar et al.1999). In a Swedish population only 1/1600 subjects demonstrate the most severe form of AATD (Pi type PiZZ), corresponding to 0.06 %. This means that it is interesting to involve individuals already typed for AATD and see whether more severe forms of periodontal disease can be found in these individuals. If AATD individuals were at high risk some of our 20 subjects might have shown severe periodontal disease. The pathology of periodontal inflammation is complex, however, and a larger study group may be needed to show differences.
Acknowledgements

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References
Outcome of a scheme for specialist orthodontic care, a follow-up study in 31-year-olds

Lennart Lagerström1, Ann-Charlott Fornell1, Arild Stenvik2

Abstract
Changes in the occlusion after orthodontic treatment have in several studies been analyzed by the use of the PAR Index developed by Shaw & Richmond. The use of the PAR Index has been shown by O’Brien & Shaw to be a reliable and reproducible method to evaluate orthodontic treatment results.

The purpose of the study was to examine the long-range orthodontic treatment outcome by following a group of patients into adulthood to the age of 31 years. For the study 115 individuals from a previous randomized study were invited for follow-up examination at age 31 years. Seventy-two individuals, 32 males (44.4%) and 40 females (55.6%) of the original sample attended for clinical examination. Study casts were obtained and questionnaires addressing the patient’s awareness and opinion of the treatment were distributed. In addition twenty-four subjects responded by returning filled-in questionnaires.

The mean change in wPAR scores from start to retention represents a mean relative improvement in occlusion of 78.7%. The mean wPar score improvement from age 19 to 31 years was 11.9%. The relative mean wPar score change dropped to 53.5% at age 31 years. The differences in wPAR recordings between the recorded stages were all statistically significant.

The treatment outcome as expressed by mean wPAR scores at age 31 years was significantly better among individuals treated with extractions compared to those treated without extractions.

The mean wPAR scores of the individuals with retainers at age 31 years were significantly lower when compared to the mean score for those without retainers (unpaired t-test, p=0.020). This clearly indicates the benefit of long-term retention.

The changes in the concern scores from 19 to 31 years of age were small. At age 31 years only 8 of the 96 respondents (8.3%) expressed concern about the treatment outcome.

Key words
Health services research, long-term outcome of orthodontic treatment, patient satisfaction.

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Utfallet av ortodontisk specialistbehandling; en uppföljningsstudie vid 31 års ålder

LENNART LAGERSTRÖM, ANN-CHARLOTT FORNELL, ARILD STENVIK

Sammanfattning

Förändringar i ocklusionen efter ortodontisk behandling har i ett flertal studier blivit utvärderade genom användandet av PAR Index utvecklad av Shaw & Richmond. Användandet av detta Index har av O’Brien & Shaw visats sig vara en tillförlitlig och reproducierbar metod för att utvärdera ett ortodontiskt behandlingsutfall.

Syftet med denna studie var att utvärdera långtidseffekten av det ortodontiska utfallet genom att följa en grupp patienter från början av behandling till 31 års ålder. För studien användes en grupp bestående av 115 patienter från en tidigare randomiserad studie och dessa kallades in för efterkontroll vid 31 års ålder. Sjuttiofem individer, 32 män (44,4%) och 40 kvinnor (55,6%) från den ursprungliga gruppen deltog genom en klinisk undersökning. Studiemodeller togs och enkäter relaterade till patientens uppfattning om behandlingen gavs patienten för ifyllande. Ytterligare 24 patienter deltog i undersökningen genom insändande av enkäter.

Medelförändringen i wPAR score (vägt PAR score) från start av behandling till reten-
rades från 19 till 31 års ålder med 11,9%. Den relativa förändringen i wPAR scores minskade till 53,5% vid 31 års ålder. Skillnaderna i wPAR scores mellan de registrerade stadierna var statistiskt signifikanta.

Behandlingseffekten uttryckt i wPAR score förändringar vid 31 års ålder var signifikant bättre i gruppen som behandlats med extraktioner jämfört dem som behandlats utan extraktioner.

Medelvärdet av wPAR scores vid 31 års ålder hos individer med retainers var signifi-
kant lägre jämfört med dem som inte hade retainers (un-paired t-test, 0,020).

Övriga uppfattningar om behandlingsutfallet.
Introduction
Numerous studies have examined the outcome of orthodontic treatment related to specific treatment methods or specific groups of patients with similar malocclusions. Schemes for provision of orthodontic care provided by specialists have over the last decades been described (18, 25). Outcome of orthodontic care when provided by general practitioners (12) as well as by a combination of general practitioners and specialists (3, 9, 19, 22) have been reported. Additionally, outcome of treatment in university clinics have also been examined (1, 2, 7, 11). In Sweden, outcome of orthodontic services in areas with different schemes for care related to organization and manpower have been reported by Bergstrom & Halling (4, 5). Few reports are available on the outcome of orthodontic treatment on a population level.

Changes in the occlusion in several studies of outcome have been analyzed by the use of the PAR Index developed by Shaw & Richmond (20, 21, 23). The use of the PAR Index has been shown to be a reliable and reproducible method to evaluate orthodontic treatment results (10, 18, 24). The outcome of orthodontic services on a population level evaluated among individuals in their late adolescence (19 year-olds) in the County of Southern Halland (population 125 000), who had been treated according to an established scheme for orthodontic treatment by specialists, has previously been reported (6, 16). The purpose of the present study was to examine the long-range outcome of care by following this same group of patients into adulthood to the age of 31 years. The objectives were to
1) analyze changes in the occlusion from before treatment to 31 years of age,
2) analyze changes in the patients’ attitudes,
3) compare results after extraction and non-extraction treatment,
4) to compare occlusion in individuals with and without retainers at the age of 31 years.

Material and methods
In previous studies (6, 15, 16) a randomized sample of 118 individuals who had undergone orthodontic treatment performed by specialists was analyzed. The parent population in that study was 1554 19 year old residents in the county of Southern Halland, Sweden, of whom 520 (33%) had received orthodontic treatment, and from this sample 171 were drawn at random of whom 118 attended. Sets of study cast representing the occlusion pre-treatment (T1), post-treatment (T2) and at follow-up at age of 19 years

Table 1. Age distribution in subgroups according to whether 115 invited individuals attended (Reg Group), returned questionnaires (Quest Group), or did not respond (DO Group)

<table>
<thead>
<tr>
<th>Age</th>
<th>min</th>
<th>max</th>
<th>mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reg Group (n=72)</td>
<td>T1</td>
<td>10.0</td>
<td>15.4</td>
<td>12.6</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>12.1</td>
<td>17.6</td>
<td>16.6</td>
</tr>
<tr>
<td></td>
<td>T3</td>
<td>18.1</td>
<td>19.7</td>
<td>18.8</td>
</tr>
<tr>
<td></td>
<td>T4</td>
<td>30.0</td>
<td>34.0</td>
<td>31.4</td>
</tr>
<tr>
<td>Quest Group (n=24)</td>
<td>T1</td>
<td>10.4</td>
<td>15.8</td>
<td>12.6</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>12.2</td>
<td>17.5</td>
<td>16.6</td>
</tr>
<tr>
<td></td>
<td>T3</td>
<td>18.3</td>
<td>19.3</td>
<td>18.3</td>
</tr>
<tr>
<td></td>
<td>T4</td>
<td>30.2</td>
<td>33.6</td>
<td>31.0</td>
</tr>
<tr>
<td>DO Group (n=19)</td>
<td>T1</td>
<td>10.3</td>
<td>14.9</td>
<td>12.5</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>12.0</td>
<td>17.2</td>
<td>16.6</td>
</tr>
<tr>
<td></td>
<td>T3</td>
<td>18.4</td>
<td>19.3</td>
<td>18.9</td>
</tr>
<tr>
<td></td>
<td>T4</td>
<td>(not registered)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 1. Flow-sheet illustrating respondents and groups of patients (Reg Group, Quest Group) attending at Stage T4 as well as the Drop outs (DO Group)
For the present study 115 of the same individuals was invited for follow-up records when they were about 31 years old (mean age 31.4, range 30 to 34 years). 3 were excluded due to missing records. At T4 seventy-two individuals, 32 males (44.4%) and 40 females (55.6%) of the original sample attended for clinical examination (Reg Group, n=72). Study casts were obtained and questionnaires addressing the patient’s awareness and opinion of the treatment were distributed. In addition twenty-four individuals responded by returning filled-in questionnaires (Quest Group, n=24). Nineteen individuals did not respond (DO Group, n=19). A "Flow-sheet" indicating the number of individuals in the subgroups of patients who responded (Reg Group, Quest Group) at T4 as well as the subgroup of drop-outs (DO Group) is presented in Fig. 1. The age and gender distribution and the extraction rate in the subgroups are shown in Tables 1 and 2.

Occlusion
The occlusal relations and alignment of teeth were defined by the PAR Index (8, 20). The study models of the 72 subjects were evaluated at all time-points (T1-T4), and unweighed and weighted PAR-scores (wPAR) were recorded. The measurements were made by one calibrated examiner and re-measured after a time interval of more than 3 months. When differences between the first and second recording occurred a second examiner measured the PAR scores and a final score was agreed. Treatment changes were categorized "worse-no different", "improved" or "greatly improved" according to Richmond et al. (21).

Attitudes
The patient’s attitude related to the outcome of the treatment at T4 was measured by a composite measure ("orthodontic concern") used in a previous study (6). This was recorded at T1-T4 in the Reg Group and in the Quest Group.

Statistical procedures
The SPSS (Version 16.0) statistical program has been used in the statistical analyses. The paired t-test was used to calculate the differences in wPAR scores between the time-points T1, T2, T3, and T4. The Kruskal-Wallis test was used in the drop-out analysis to calculate the differences of the wPAR scores at T3 between the three groups (Reg Group, Quest Group and the DO Group) because the treatment outcome at T3 might have been a determining factor for the patient’s willingness to appear for registration at T4. The Wilcoxon-Mann-Whitney rank sum test was used to calculate the differences in wPAR scores at T4 between the individuals treated with or without extractions in the Reg Group. 95% confidence intervals (CI) were calculated when comparison of numerical variables and p-values below 0.05 were required for the differences to be accepted as statistically significant.

Results
Drop-out analysis
There was no difference in the mean wPAR score between the three groups at T3 (Asymp.Sig 0.8, p<0.05). The wPAR scores at T3 in the three groups were also compared in relation to age and gender distribution as well as to distribution of extraction and non-extraction treatment, and no significant differences between the three groups were observed.

Treatment outcome - occlusion
The wPAR scores at T1-T4 are presented in Table 3, and the treatment changes as expressed by wPAR scores are shown in Table 4. The differences in wPAR recordings between T1-T2, T1-T3, T1-T4 and T3-

<table>
<thead>
<tr>
<th>Treatment</th>
<th>males</th>
<th>females</th>
<th>non-extraction</th>
<th>extraction</th>
</tr>
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<tr>
<td>Reg Group</td>
<td>(n=72)</td>
<td>32 (44.4%)</td>
<td>40 (55.6%)</td>
<td>44 (61.1%)</td>
</tr>
<tr>
<td>Quest Group</td>
<td>(n=24)</td>
<td>9 (62.5%)</td>
<td>15 (62.5%)</td>
<td>15 (62.5%)</td>
</tr>
<tr>
<td>DO Group</td>
<td>(n=19)</td>
<td>7 (36.8%)</td>
<td>12 (63.2%)</td>
<td>12 (63.1%)</td>
</tr>
</tbody>
</table>

| Table 2. Distribution males and females and treatment in subgroups (see Table 1) depending on whether treatment involved extractions or not | Table 3. Weighted PAR scores of 72 treated individuals at start of treatment (T1), at retention (T2) and at postretention at age 19 years (T3) and at age 31 years (T4). (mean values, one standard deviation (SD) and total range) |

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-treatment (T1)</td>
<td>20.2</td>
<td>8.6</td>
<td>3 - 43</td>
</tr>
<tr>
<td>Post-treatment (T2)</td>
<td>4.3</td>
<td>4.6</td>
<td>0 - 26</td>
</tr>
<tr>
<td>Follow-up 19yr (T3)</td>
<td>7.0</td>
<td>5.1</td>
<td>0 - 19</td>
</tr>
<tr>
<td>Follow-up 31yr (T4)</td>
<td>9.4</td>
<td>5.3</td>
<td>0 - 19</td>
</tr>
</tbody>
</table>
T4 were all significant. The mean change in wPAR scores from T1 to T2 (20.2 - 4.3) represents a relative improvement in occlusion of 78.7%. The subsequent mean change from T1 to T3 (20.2 - 7.0) represents a drop in relative improvement to 65.3%, and the change from T1 to T4 (20.2 - 9.4) represents a further drop to 53.5%. A mean increase of the wPAR scores from T3 to T4 (7.0 - 9.4) represents an increase of 11.8%. All changes were statistically significant (p< 0.05) (Table 4).

The relative number of individuals in the category “greatly improved” changed from 27.7% post-treatment (T1-T2) to 19.5% at age 19 (T1-T3) and to 8.4% at 31 years-of-age (T1-T4). The category “improved” showed only small changes, while the number of individuals in category “worse-no different” increased from 4.2% to 13.9% and 25.0% during the same intervals (Table 5).

The treatment outcome as expressed by mean wPAR scores at T4 was significantly better among individuals treated with extractions (n= 28, mean wPAR 7.3) compared to those treated without extractions (n=44, mean wPAR 10.8) (p<0.008) (Wilcoxon-Mann-Whitney rank sum test).

22% of the individuals in the Reg Group had fixed bonded retainers in place at T4, five individuals in the upper arch only and nine in the lower arch only and two individuals had bonded retainers in the upper and lower arch. 25% of the individuals in the Quest Group had fixed bonded retainers in place at stage T4, four individuals in the upper arch only, one individual in the lower arch only and one individual had fixed bonded retainers in upper and lower arch. No removable retainers were used in either group.

The mean wPAR scores at T1 of the individuals with and without retainers at T4 showed no statistical differences. However, the mean wPAR score at T4 of the individuals with retainers was significantly lower when compared to the mean score for those without retainers (unpaired t-test, p=0.020). (Table 6).

Attitudes
Attitudes as expressed by the “orthodontic concern” scores (Berset et al.), showed only minor changes from T3 to T4 in the Reg Group. There was no statistical difference between the Reg Group and the Quest Group in the distribution of the concern scores and distribution of males and females. Two of the 72 individuals in the Reg Group and 4 of the 24 individuals in the Quest Group expressed varying degrees concern related to the treatment result at T4. (Table 7).

Of the individuals who returned the questionnaires but who were not able to/not willing to attend the

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Table 4. Differences in weighted PAR scores between start of treatment and post-treatment (T1-T2) and follow-up at ages 19 (T1-T3) and 31 (T1-T4), and the difference between the follow-up stages (T3-T4) in 72 individuals (paired t-test)

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Mean</th>
<th>% change</th>
<th>SD</th>
<th>95% CI</th>
<th>p-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pair 1 par1w - par2w (T1-T2)</td>
<td>15.9</td>
<td>78.7</td>
<td>8.4</td>
<td>14.564 - 18.611</td>
<td>.020</td>
</tr>
<tr>
<td>Pair 2 par1w - par3w (T1-T3)</td>
<td>13.2</td>
<td>65.3</td>
<td>8.7</td>
<td>1.145 - 15.244</td>
<td>.023</td>
</tr>
<tr>
<td>Pair 3 par1w - par4w (T1-T4)</td>
<td>10.8</td>
<td>53.5</td>
<td>8.3</td>
<td>8.887 - 12.789</td>
<td>.002</td>
</tr>
<tr>
<td>Pair 4 par3w - par4w (T3-T4)</td>
<td>-2.4</td>
<td>11.8</td>
<td>4.0</td>
<td>-3.295 - 1.427</td>
<td>.001</td>
</tr>
</tbody>
</table>

Pair 1 T1 vs. T2: p < 0.05, Pair 2 T1 vs. T3: p < 0.05, Pair 3 T1 vs. T4: p < 0.05 Pair4 T3 vs. T4: p < 0.05

Table 5. Changes during treatment (T1–T2), post-treatment at 19 (T1–T3) and 31 years-of-age (T1–T4) according to the distribution of 72 treated individuals in the categories “greatly improved, improved and worse-no different” (Richmond et al., 1992b). Relative frequencies in parentheses

<table>
<thead>
<tr>
<th></th>
<th>T1-T2*</th>
<th>T1-T3</th>
<th>T1-T4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Greatly improved</td>
<td>20 (27.7)</td>
<td>14 (19.5)</td>
<td>6 (8.4)</td>
</tr>
<tr>
<td>Improved</td>
<td>49 (68.1)</td>
<td>48 (66.6)</td>
<td>48 (66.6)</td>
</tr>
<tr>
<td>Worse-no differences</td>
<td>3 (4.2)</td>
<td>10 (13.9)</td>
<td>18 (25.0)</td>
</tr>
</tbody>
</table>

*n=68 as data were missing for 4 individuals

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Table 6. Mean wPAR scores at T1 and T4 for 72 previous orthodontic patients according to whether or not they had retainers at 31 years-of-age

<table>
<thead>
<tr>
<th>Retainers</th>
<th>Mean</th>
<th>Range</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retainers</td>
<td>19.63</td>
<td>3 – 43</td>
<td>11.65</td>
</tr>
<tr>
<td>No retainers</td>
<td>20.41</td>
<td>6 – 38</td>
<td>11.80</td>
</tr>
<tr>
<td>Retainers</td>
<td>5.13</td>
<td>0 – 19</td>
<td>6.02</td>
</tr>
<tr>
<td>No retainers</td>
<td>10.63</td>
<td>0 – 19</td>
<td>7.41</td>
</tr>
</tbody>
</table>

The difference between groups at T1 is non-significant ((un-paired t-test). The difference between groups at T4 is significant (un-paired t-test, p=0.020).
clinical examination at T4, one patient in the Quest Group claimed to have no interest, one stated lack of time as reason, and 22 said that they were living in a distant area and not able to participate.

Discussion
The post-treatment observation period of more than 15 years is extensive compared to most previous studies on orthodontic treatment outcome. Individuals attending and returning questionnaires together represent a response rate of 82.7%. Taking the extended follow-up period into consideration, this response rate is considered satisfactory for the evaluation of orthodontic services.

Twenty-two of the 24 individuals in the Quest Group gave “living in a distant location” as the reason why they did not attend the clinical registration at T4. As practical circumstances were stated as the main cause why they did not attend, there is no reason to believe that they were different from the attendees in terms of treatment and outcome. As the wPAR scores in the DO Group did not differ from the scores of the other subgroups at T3 it is likely to assume that the drop-out did not affect the evaluation of the outcome of services. No significant difference between the groups at T3 with regard to age, gender and distribution of non-extraction/extraction treatment supports this assumption. The significant decrease in relative improvement from 78.7% post-treatment to 53.5% some 15 years later is similar to the findings of Al-Yami et al. (1, 2), who in a ten year post-retention study reported a mean wPAR score improvement of 46%.

The changes in the concern scores between stage T3 and T4 are small and too few for statistical evaluation. At T4 a total of 8 of the 96 respondents (8.3%) expressed some degree of concern about the treatment outcome. This percentage is similar to findings in other reports (1, 8, 12, 13, 14, 18, 25). The general impression from this study based on both the assessment of the occlusion and patients’ attitudes is that the outcome of services provided through this scheme is generally satisfactory. The results are relevant for the discussion of protocols for retention after orthodontic treatment.

Conclusions
Of 115 previously treated individuals who were invited for a new examination when they were 31 years old, 72 attended and 24 returned filled-in questionnaires. No differences were observed between the attendees and drop-outs relative to the observations when they were 19 years old. Mean relative wPAR score improvement post-treatment was 79% and dropped to 65% at 19 and 53% at 31 years of age. Scores for individuals treated with extractions were on average better than those treated without extractions, and for those with retainers still in place compared to those without. Only 8% expressed concern with the treatment result which was related to crowding or spacing of incisors. The outcome of services

Table 7.
Distribution of concern scores in the Reg Group (n=72) and Quest Group (n=24) at stages T1, T3 and T4

<table>
<thead>
<tr>
<th>Concern score</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reg Group at T1</td>
<td>21</td>
<td>28</td>
<td>12</td>
<td>8</td>
<td>3</td>
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<tr>
<td>Reg Group at T3</td>
<td>65</td>
<td>5</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Reg Group at T4</td>
<td>70</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Quest Group at T1</td>
<td>6</td>
<td>9</td>
<td>6</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Quest Group at T3</td>
<td>20</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Quest Group at T4</td>
<td>20</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

scores for those with no retainers which clearly indicate a benefit of long-term retention. The analysis of patient satisfaction (concern scores) shows that the patients who expressed some concern with treatment outcome at T4 had no retainers in place. The complaints were related to relapse of crowding/rotations or spacing of maxillary incisors.

According to the wPAR scores, close to 50% (score 20.2 pre-treatment and score 9.4 at T4) of the initial malocclusion was present 15 years after treatment as a result of post-treatment change and because some malocclusion traits had not been fully corrected during treatment. However, when using the categories of Richmond et al. (21) a positive treatment outcome (“Greatly improved” and “Improved”) was observed in 75% of the individuals.

The scores applied to record patient attitudes were similar to those applied in the previous study (6) to allow for comparisons. The questionnaire contains retrospective and anamnestic questions and this should be born in mind when interpreting the data. However, this approach has been found adequate for the analysis of psychosocial aspects of malocclusion (14).
provided through this scheme for orthodontic care should be considered as satisfactory in a long-term perspective.

Acknowledgement
For the assistance of the personnel at the Departments of Orthodontics, Community and Preventive Dentistry, Halmstad, Sweden. For statistical support from Amir Emanverdi Baigi and administrative support from Anders Holmen, Department of Research, Education and Development, County Halland, Sweden. This study was supported by grants from the Department of Research, Education and Development, County Halland, Sweden.

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E-mail: lennart.lagerstrom@telia.com
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