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# Gingival health status in individuals using different types of toothpaste

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ABSTRACT

*Objectives:* To examine the relationship between the medium term use (> 1 year) of a toothpaste containing natural enzymes and proteins (Zendium™) upon gingival index, plaque index and bleeding index compared to medium term use of toothpastes without antimicrobial/antiinflammatory ingredients.

*Methods:* A total of 305 participants eligible for inclusion were grouped according to their toothpaste use and matched with regard to gender and age (18–30, 31–55 and 56+ years of age). A total of 161 persons were using a toothpaste which contained enzymes and proteins (Zendium™, test group), and 144 persons were using a toothpaste without these ingredients (control group). The amount of dental plaque and the gingival condition were assessed at six sites of each tooth using the modified gingival index (MGI), plaque index (Modified Quigley and Hein plaque index, PI), and bleeding index (BI). Mean values of MGI, PI and BI were compared using analysis of covariance.

*Results:* The test group had significantly less gingival inflammation than the control group (adjusted mean scores (SD); 1.80 (0.65) vs. 2.27 (0.63), $p < 0.0001$), as well as lower levels of plaque (2.03 (0.33) vs. 2.12 (0.33), $p = 0.0168$) and gingival bleeding (0.74 (0.45) vs. 1.08 (0.45), $p < 0.0001$). Females had significantly less gingival inflammation ($p < 0.0001$), plaque ($p = 0.0005$) and bleeding ($p = 0.0118$) than males. Participants aged 18–30 years had significantly higher levels of inflammation and bleeding than the older age groups ($p < 0.001$), and also higher plaque levels compared to participants aged 31–55 years ($p = 0.0069$). Potential confounding factors including oral hygiene practices and consistency of dental visits did not differ between groups.

*Conclusions:* Our findings indicate that medium term use of fluoride toothpaste containing enzymes and proteins (Zendium™) is associated with a better gingival health than the use of other types of fluoride toothpastes without antimicrobial active ingredients.

*Clinical significance:* Medium term (> 1 year) use of toothpaste containing naturally occurring enzymes and proteins (Zendium™) in an unsupervised home setting is associated with better gingival health compared to the unsupervised use of other commercially available toothpastes without antimicrobial/antiinflammatory active ingredients.

## 1. Introduction

The oral cavity harbours a complex microbiota comprised of more than 700 different bacterial species [1,2], and the resident microbiota is critical for maintenance of oral homeostasis [3,4]. On a daily basis, the resident oral microbiota is almost constantly stressed by ecological perturbations such as eating and drinking. Self-performed oral hygiene is a frequent perturbation, and the magnitude of this perturbation is probably influenced by frequency, but is also dependent on choice of toothpaste. In attempts to enhance the natural salivary antimicrobial defence mechanisms, oral health products including toothpastes have been used with different added ingredients. Zendium™ toothpaste contains a triple enzyme system including amyloglucosidase, glucose oxidase and lactoperoxidase that generates the natural antimicrobial agents, hydrogen peroxide and the hypothiocyanite ion. Salivary peroxidases catalyse the oxidation of thiocyanate ($SCN^-$) to hypothiocyanite ($OSCN^-$) via hydrogen peroxide. Peroxidases and thiocyanate are natural constituents of saliva, whereas hydrogen peroxide also

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originates from bacterial metabolism in the oral cavity [5–7]. The salivary proteins, lactoferrin and lysozyme are also added to the toothpaste. Lactoferrin binds iron, whereby the availability of iron as a co-factor in bacterial enzymes is reduced. Lactoferrin thereby acts as a bacteriostatic agent. Lactoferrin also exerts direct bactericidal effect on certain cariogenic bacteria, e.g. *Streptococcus mutans* as well as periodontal pathogens [for review 8]. Lysozyme breaks down peptidoglycan, which is an essential part of the cell wall of the gram-positive bacteria, and thus acts as a bactericidal agent. However, lysozyme also acts in a bacteriostatic manner through agglutination of bacteria inhibiting bacterial adhesion and colonisation [for review [8]].

It has recently been shown that the use of a toothpaste containing enzymes and proteins (Zendium™) can boost the natural salivary defences by increasing the levels of lysozyme and hydrogen peroxide *in vivo* and hypothiocyanite *in vitro* and reduce the growth and viability of oral bacteria in microbiological models [9]. Similarly, the findings of a recent randomised clinical study on the composition of supragingival bacterial biofilms indicate that the use of a toothpaste containing enzymes and proteins can augment natural salivary defences [10]. Specifically, by analysis of supragingival plaque samples collected from 102 subjects it was reported that use of toothpaste containing enzymes and proteins for 14 weeks resulted in a statistically significant increase in 12 gingival health-associated taxa together with a statistically significant decrease in 10 periodontitis-associated taxa [10]. However, clinical recordings on gingival health in long term users of toothpaste containing enzymes and proteins (Zendium™) were not investigated.

To address this question we employed clinical data recorded from a cohort of 305 subjects, which had used the same toothpaste for > 1 year (test group: n = 161 vs. control group: n = 144). Accordingly, the purpose of the present investigation was to test the hypothesis that medium term use (> 1 year) of a toothpaste containing natural enzymes and proteins (Zendium™, test) is associated with a better gingival health in terms of gingival inflammation, plaque levels and gingival bleeding than medium term use of toothpastes without antimicrobial/ antiinflammatory active ingredients (control).

## 2. Materials and methods

### 2.1. Study design and objectives

This was a single blind, with respect to the clinician, monadic study. Screening visits and clinical examinations were performed from May 2016 to October 2016 at the Department of Odontology, Faculty of Health and Medical Sciences, University of Copenhagen. Prior to participation, all subjects were informed about the nature and extent of the study and provided informed consent. The study was conducted in accordance with the Helsinki Declaration, and approved by the Regional Ethical Committee (H-15016471). The purpose of the present investigation was to characterise and compare gingival health status in individuals using different types of toothpaste.

### 2.2. Time line and recruitment strategy

The study time line is detailed in Fig.1. Based on a power calculation from a previous pilot study a total of 240 subjects (120 in each group) were estimated to be required to complete the study. The recruitment strategy is visualised in Table 1, which shows that the study participants were recruited with the intention to ensure a balanced age and gender distribution between the test and control groups. The age groups comprised the following three groups: 18–30 years, 31–55 years and 56 years of age and above.

### 2.3. Pre-screening telephone interview

A total of 10,620 potential study participants were contacted by telephone by the market research agency TNS Gallup A/S and asked to take part in this study. The participants were informed about the purpose of the telephone interview, and subsequently screened using a pre-screening questionnaire concerning basic exclusion criteria including age below 18 years, residence in the Capital Region of Denmark for less than 5 consecutive years, employment in oral health care industry, insufficient or irregular oral health care, wearing partial or full dentures, having oral piercings, and use of mouthwash within the previous 4 weeks. Finally, each potential participant was asked about their toothpaste usage within the last 12 months. Participants who had used any kind of Zendium™ toothpaste continuously over the latest 12 months were eligible for inclusion in the test group. Participants who had used any other toothpaste without antimicrobial/ antiinflammatory ingredients apart from Zendium™ were eligible for inclusion in the control group. A total of 4354 persons refused to participate and a further 5735 persons did not fulfil the inclusion criteria based on the pre-screening questionnaire. Thus, a total of 531 participants were scheduled for the screening visit.

### 2.4. Screening visit

A total of 386 participants attended the appointment for the screening visit, which was performed either by DB or AMLP. At the screening visit the participants provided informed consent and then answered a questionnaire with regards to general health and medication intake. Furthermore, a clinical screening of oral health status, including presence of periodontitis and dental caries was performed. Inclusion criteria for the clinical examination included confirmation of continuous use of specific toothpaste eligible for inclusion in either of the study groups, age above 18 years and willingness to participate in the investigation. Exclusion criteria included periodontitis and/or dental caries requiring treatment, less than 20 natural teeth (excluding third molars), on-going orthodontic treatment, scale and prophylaxis in the month prior to enrolment, type 1 and type 2 diabetes, autoimmune, inflammatory systemic diseases, current antibiotic treatment within 3 months of the screening appointment as well as alcohol and drug abuse. Based on the screening visit a total of 341 subjects were invited to attend the clinical examination. As this study was part of an on-going investigation on oral malodour and the composition of the oral microbiota, participants eligible for the clinical examination were informed to avoid the following: consuming spicy foods or alcohol (24 h prior to their appointment), brushing their teeth (from 11 pm on the evening before their appointment), eating and drinking (2 h prior to their appointment), use of mouthwash or changing the toothpaste used as part of normal oral hygiene, use of make-up, body lotions, perfume or after shave (on the morning of the test visit).

### 2.5. Clinical examination

A total of 305 participants completed the clinical examination, in which gingival inflammation, plaque levels and gingival bleeding were recorded at six sites of each tooth (third molars excluded). Gingival inflammation was scored from 0 to 4 (0: absence of inflammation, 1: localised mild inflammation, 2: generalised mild inflammation, 3: moderate inflammation and 4: severe inflammation) using the Modified Gingival Index (MGI) as previously described [11]. Plaque index (PI) was recorded from 0 to 5 after the disclosure of plaque (0: no plaque, 1: speckles of plaque along the gingival margin, 2: a continuous line of plaque up to 2 mm in depth along the gingival margin, 3: plaque covering up to 1/3 of the assessment area, 4: plaque covering up to 2/3 of the assessment area, 5: plaque covering the whole of the assessment area) by use of the Modified Quigley and Hein index [12]. Gingival bleeding index (BI) was scored from 0 to 2 (0: no bleeding, 1: bleeding within 30 s of probing, 2: spontaneous bleeding) as previously described [13]. All clinical examinations were performed by the same examiner (MD).

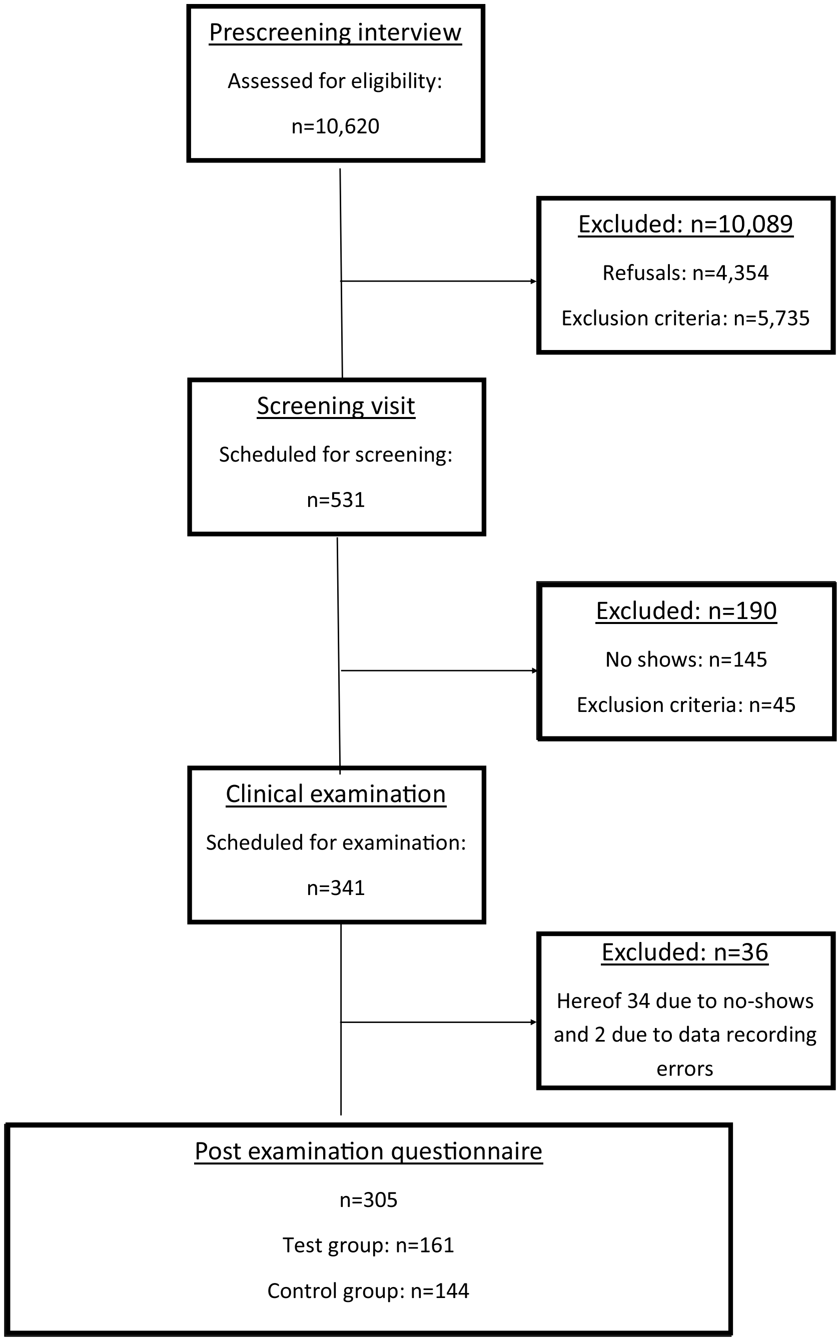


**Fig. 1.** Study timeline.

**Table 1**
Distribution of participants within the test group and control group and stratification by age and gender.

| | Males | | | | | | Females | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Test group | | | Control group | | | Test group | | | Control group | | | |
| Age range | 18-30 | 31-55 | ≥56 | 18-30 | 31-55 | ≥56 | 18-30 | 31-55 | ≥56 | 18-30 | 31-55 | ≥56 | total |
| No. subjects | | | | | | | | | | | | | |
| Expected | 15 | 25 | 30 | 15 | 25 | 30 | 15 | 25 | 30 | 15 | 25 | 30 | 280 |
| Actual | 17 | 29 | 36 | 14 | 27 | 33 | 16 | 29 | 25 | 14 | 29 | 36 | 305 |
| **Difference** | **+2** | **+4** | **+6** | **−1** | **+2** | **+3** | **+1** | **+4** | **−5** | **−1** | **+4** | **+6** | **+25** |

### 2.6. Post examination questionnaire

After completion of the clinical examination each participant underwent a focused questionnaire addressing drinking and eating habits including: smoking habits (daily, occasionally, former or never smoker; type of tobacco used as well as snus, e-cigarettes and nicotine chewing gum), consumption of tea, coffee, soda, soft drink and alcohol (frequency, type, number of beverages, cups and glasses), chewing gum (frequency), candy and snack habits (frequency daily, weekly, monthly, seldom). Furthermore, oral hygiene habits (tooth brushing, manual and/or electric toothbrush, frequency, use of additional oral hygiene products including mouthwash, dental floss, tooth picks, soft picks, interdental brushes; the use of whitening products) and self-perceived oral health status were scored as well.

### 2.7. Statistical analysis

For between group comparisons, an analysis of covariance (ANCOVA) model was conducted for each outcome measure separately. Group was included as a fixed effect, along with gender and age as covariates, and each of their two-way interactions. Brushing frequency and toothbrush type (manual/electric) were also included as fixed effects. The interaction terms were removed if they were not significant based on a significance level of 5%.

## 3. Results

### 3.1. Characteristics of the study population

The distribution of participants within the test group and control group stratified by age and gender is detailed in Table 1. The actual recruitment was slightly different from the intended since a total of 305 participants completed the study compared to a target of 280 participants. Furthermore, the recruitment resulted in a higher number of participants in the test group (n = 161) than in the control group (n = 144).

### 3.2. Differences in gingival health status

Mean levels and standard deviations (SD) of MGI, PI and BI are presented in Figs. 2–4. Adjusted mean levels of gingival inflammation (1.80 (0.65) vs. 2.27 (0.63), $p < 0.0001$), plaque (2.03 (0.33) vs. 2.12 (0.33), $p = 0.0168$) and bleeding (0.74 (0.45) vs. 1.08 (0.45), $p < 0.0001$) were significantly lower in the test group compared to the control group.

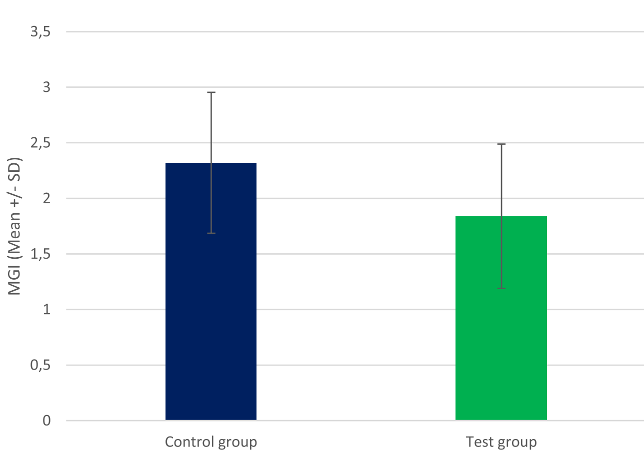


**Fig. 2.** Gingival inflammation. Gingival index expressed as mean (+/-SD) in the test group (green bar) and control group (blue bar).

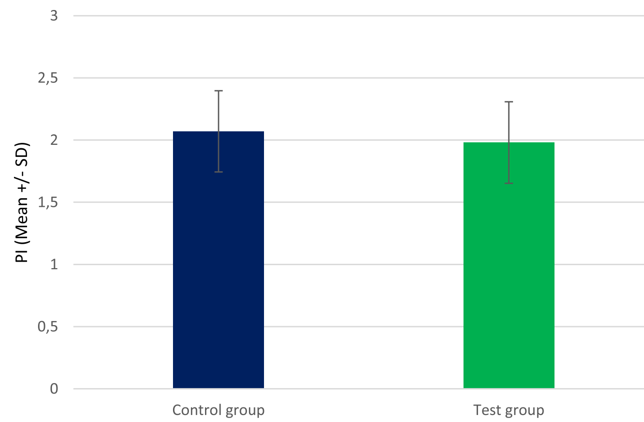


**Fig. 3.** Levels of plaque. Plaque index expressed as mean (+/-SD) in the test group (green bar) and control group (blue bar).

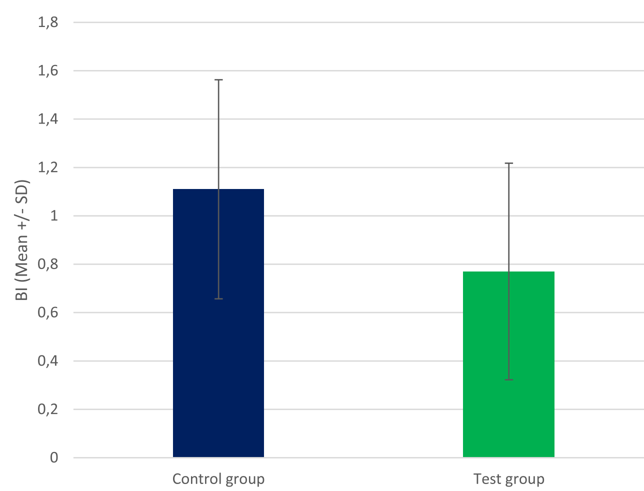


**Fig. 4.** Levels of gingival bleeding. Bleeding index expressed as mean (+/-SD) in the test group (green bar) and control group (blue bar).

### 3.3. Associations between age, gender and gingival health status

Regardless of the toothpaste use, female participants had significantly lower levels of MGI ($p < 0.0001$), PI ($p = 0.0005$) and BI ($p = 0.0118$) than male participants. In addition, study participants aged 18–30 years had significantly higher MGI ($p < 0.0001$) than the older age groups, and significantly higher PI ($p = 0.0069$) and BI ($p = 0.0009$) than participants aged 31–55 years. Finally, participants aged 56 years and above had significantly higher PI ($p = 0.0441$) than participants aged 31–55 years but significantly lower BI ($p = 0.0003$) than participants aged 18–30 years.

#### 3.3.1. Associations between choice of toothpaste, general health and lifestyle

The proportion of daily smokers was 12% in the test group and 15% in the control group, with predominance of cigarette smoking (85%). In the test group and control group, 63% and 47%, respectively, smoked between 3–10 cigarettes daily, whereas fewer subjects in the test group (32%) than in the control group (47%) smoked 11–20 cigarettes daily. An equal number was 'never' smoker (50%). The proportion of former smokers was 30% in the test group vs. 28% in the control group, and the use of nicotine chewing gum 2% vs. 1%. In addition, a comparable proportion of coffee (87% vs. 81%), soft drink (71% vs. 71%), and alcohol consumption (92% vs. 90%) was recorded in the test group and control group ($p > 0.05$). However, the test group tended to drink less soft drinks (67% vs. 81%), drink more tea (83% vs. 72%), to chew less chewing gum (38% vs. 48%) and to eat less candy (88% vs. 94%) than the control group.

Furthermore, the two groups did not differ with regard to overall general health, intake of medication, oral hygiene practices (electric vs. manual toothbrushes, brushing frequency, and use of floss/toothpicks and interdental brushes) and dental attendance. In the test and control group, 68% vs. 69% used manual toothbrush and/or 39% vs. 41% electric toothbrush. In both groups 84% brushed their teeth twice daily and an equal number brushed once or three times or more daily. Eighteen per cent (16% in the test group and 20% in the control group) used toothpicks, dental floss or interdental brush.

## 4. Discussion

The purpose of the present investigation was to test the hypothesis that use of fluoride toothpaste containing naturally occurring enzymes and proteins (Zendium™) for more than a year is associated with a better gingival health than use of toothpastes without antimicrobial/antiinflammatory active ingredients (control). The main finding was that test group who had used Zendium™ had significantly better gingival health status than the control group in terms of gingival inflammation, plaque levels and gingival bleeding.

One way to explain the clinical findings from the present study is that the toothpaste used by the test group contains a triple enzyme system, which includes amyloglucosidase, glucose oxidase and lactoperoxidase. Saliva contains lactoperoxidase, lysozyme and lactoferrin, and salivary levels of these particular enzymes and proteins may be involved in shaping the composition of the resident oral microbiota [14], and therefore potentially influence oral health status [15,16]. One possible explanation, which requires further research, is that use of toothpaste, which contains enzymes and proteins that are naturally present in saliva, may augment salivary defence mechanisms in balancing the oral microbiota. This assumption is supported by data from a randomised clinical trial, which studied the impact of toothpaste use for 14 weeks on the composition of the oral microbiota [10]. Notably, the use of a toothpaste containing enzymes and proteins (Zendium™) induced significant alterations to the supragingival microbial community over time in orally healthy individuals, whereas the control toothpaste did not result in a shift of the supragingival microbial community. Specifically, the use of the test toothpaste with enzymes and proteins induced a significant increase in health-associated bacterial species together with a concomitant decrease in abundance of periodontitis-associated bacterial species [10]. Thus, clinical data from the present study and microbiological data presented in [10] are consistent with each other, and also consistent with the results of a recent controlled clinical trial on gingival health [17].

The supragingival microbiota has been reported to differ between orally healthy individuals with different levels of sugar intake [18], and smoking status seems to influence the composition of the subgingival microbiota in oral health [19] and periodontitis [20], which suggest an impact of diet and lifestyle on the oral microbiota. While it is interesting to know the compositional changes of the microbiota associated with ecological perturbations such as diet, smoking and toothpaste use, such studies provides no information on bacterial phenotypes. Notably, metatranscriptomic analysis has demonstrated that smoking impacts functional signatures of the subgingival microbiota [21] and bacterial metabolic gene expression of saliva is different in patients with periodontitis and dental caries compared to orally healthy persons [22]. Thus in a future study it would be interesting to investigate if long term use of toothpaste with enzymes and proteins (Zendium™) also can be reflected in the metabolic gene expression of the resident microbiota.

In this study, we also found that the women generally had better gingival health status than men, in terms of lower levels of gingival inflammation, plaque and gingival bleeding, which supports the findings of previous studies [23,24]. In addition, participants at the age of 18–30 years had significantly higher levels of gingival inflammation than the participants from the older age groups. Their levels of plaque and gingival bleeding were also higher than those of participants aged 31–55 years, irrespective of the toothpaste use. In Denmark, the government provides free dental care to all children, up to the age of 18 years. From the age of eighteen the young adults need to find a private dentist for regular dental follow-up examination and dental treatment. However, almost 25% of the young adults aged 18–34 years drop out of the dental service system for a period of time, and do not attend a private dentist regularly, mainly due to the costs [23,25]. In this period they are likely to develop dental problems like gingivitis and dental caries, and this may also explain our findings of poorer gingival conditions in the young age group.

In this study, gingival health status was determined by traditional clinical parameters. The continuous development of novel technologies such as metaproteomics and multiplex panels offer new opportunities for investigation of the molecular biological mechanisms underlying these findings. Thus it has been shown that salivary levels of certain immunological markers are associated with periodontitis [26–29] and gingivitis [30–32].

In the present study only participants with good oral health and not requiring treatment for periodontitis or dental caries were included. Thus, the data presented in this study may not be representative of participants with manifest oral disease such as periodontitis or dental caries. Furthermore, no information on socio-economic status was recorded. Oral health status is linked with socioeconomic status [33], and socio-economic status has been reported to impact the composition of the oral microbiota [34]. In this study, the participants in the test group tended to drink less soft drinks and to eat less candy than the control group, which suggest that choice of toothpaste might be associated with consumption and attitude towards health-related consumer choices. Thus, it would be interesting to address these aspects in a future study.

In conclusion, data from the present single-blinded clinical study indicate that long term use of toothpaste containing enzymes and proteins (Zendium™) is associated with better gingival health status than use of other toothpastes. Future studies, which perform simultaneous characterisation and comparison of clinical, microbiological and immunological data in persons using different types of toothpaste, may reveal the mechanisms behind the findings from the present study.

## Conflict of interest statement

The study was financially supported by Unilever Oral Care UK and the Faculty of Health and Medical Sciences, University of Copenhagen.

The Study Coordinator (AMLP), the Principal Investigator (DB) and clinical investigator (MD) are all employed at the University of Copenhagen. JN, MIE and AKG are employees of Unilever.

TNS Gallup DK performed the initial recruitment, and the post-clinical examination interview of study participants regarding habits etc.

The study was performed according to the ICH Harmonised Tripartite Guideline for Good Clinical Practice (CPMP/ICH/135/95) and the Declaration of Helsinki and approved by the Local Committee of Research and Ethics of the Capital Region of Denmark (H-15016471).

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