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ORIGINAL ARTICLE

A randomized controlled clinical study of the effect of daily intake of *Ascophyllum nodosum* alga on calculus, plaque, and gingivitis

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Abstract
Objective The aim of this study is to evaluate, in a randomized controlled cross-over study, the effect of daily intake of the alga *Ascophyllum nodosum* on supragingival calculus, plaque formation, and gingival health over a 6-month period.
Material and methods Sixty-one adults with moderate to heavy calculus formation since their last yearly recall visit participated. In a randomized order over two 6-month periods, they swallowed two capsules daily, comprising a total of 500 mg dried marine alga powder (*Ascophyllum nodosum*, ProDen PlaqueOff®) or two negative control tablets. During the study, the participants maintained their regular oral habits. Their teeth were professionally cleaned at the start of each period and after the 6-month registrations. A wash out period of 1 month separated the two 6-month periods. Supragingival calculus (Volpe Manhold), gingivitis (Löe and Silness), gingival bleeding (Ainamo and Bay), and plaque (Quigley-Hein) were registered at screening and at the end of the two periods. Differences in oral health between the test and control periods were analyzed using a paired *t* test and Wilcoxon signed rank test.
Results Fifty-five participants completed the study. After the alga intake, the mean calculus reduction was 52 % compared to the control ($p<0.0001$). Fifty-two participants showed less calculus formation in the alga group than in the control group. Plaque ($p=0.008$) and gingival bleeding ($p=0.02$) were also significantly less in the alga group. However, no significant difference was found between the groups for gingivitis ($p=0.13$).
Conclusions The alga intake significantly reduced the formation of supragingival calculus and plaque and occurrence of gingival bleeding. The alga has a systemic effect on oral health.
Clinical relevance Daily intake of the alga *Ascophyllum nodosum* as an adjunct to customary oral hygiene showed a major reduction of supragingival calculus formation and reduced plaque formation. In addition, the calculus in the alga group was characterized by a more porous and less solid structure and was easier to remove than the calculus in the control group.

Keywords Alga · *Ascophyllum nodosum* · Calculus · Clinical · Gingivitis · Plaque · Seaweed

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Introduction

Dental plaque is the major etiological factor in the initiation of periodontal disease. Calculus can be defined as calcified dental plaque, composed primarily of calcium phosphate mineral salts deposited between and within remnants of former viable microorganisms [1]. Mature dental calculus organic content resembling bone comprises 70–80 % inorganic salts, of which about two thirds are in crystalline form. The external surface of calculus is covered with non-mineralized plaque and endotoxins [2]. Calculus contributes largely to the chronicity and progression of periodontal disease primarily by being a retention factor for bacterial deposits to form [2–4]. Little research has been conducted on calculus during the last decade. The prevalence and location of calculus are population-specific and affected by the subject's oral hygiene habits and the frequency of professional care, diet, age, and ethnic origin [5, 6]. In a study of adult subjects in Sri Lanka with virtually no oral hygiene or dental care, all participants showed calculus on almost all tooth surfaces [5]. Prevalence figures of the same

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age group from the USA and Norway showed presence of supragingival calculus in 55 % and subgingival or both in 36 % of the subjects [5]. In subjects who practice regular oral hygiene and have access to professional care, supragingival calculus formation tends to be restricted to tooth surfaces adjacent to the salivary ducts [4]. When oral hygiene is poor, calculus may be found throughout the whole dentition where it is associated with gingival recession [6].

Presence of supragingival calculus prevents patients from performing optimal oral hygiene, thereby enhancing plaque formation. Oral hygiene measures are primarily directed at the removal of supragingival plaque and the maintenance of gingival health. Consequently, most studies investigating toothbrushing efficacy focus on removal of plaque and reduction of gingival inflammation; fewer studies have examined formation of calculus, development of stain, and signs of subclinical inflammation [1]. Calculus is traditionally removed mechanically, a time-consuming process involving discomfort for the patient. Many subjects do not visit their dentist or dental hygienist on a regular basis to control calculus formation. Given the widespread presence of calculus, many attempts have been made to prevent the formation of dental plaque and calculus in various ways.

Saliva contains a number of crystal growth inhibitors, such as statherine and proline-rich proteins, whose biological function is to maintain supersaturation with respect to tooth enamel and prevent loss of mineral by dissolution [7]. These proteins contain negatively charged sequences that may adsorb at active sites on the crystallite surfaces and thereby inhibit crystal growth and calculus formation. Pyrophosphate is a salivary component that is able to block adsorption sites available for crystal growth and can also delay the initiation of conversion of dicalcium phosphate hydrate (DCPD), an early component in calculus formation, to hydroxyapatite (HAP). Higher concentrations of pyrophosphates are found in the saliva and plaque of non- or low-formers than in those of heavy calculus formers. Acid and alkaline phosphates in saliva and plaque hydrolyze pyrophosphates thereby favoring calculus formation [8].

These findings provided the impetus to develop products that inhibit or reduce calculus formation by incorporating inhibitors of crystal growth [9]. Several anti-calculus agents have been marketed during the last 30 years as adjunctives to dentifrices and mouth rinses. The most common are soluble pyrophosphate salts, zinc salts such as zinc citrate and zinc chloride, and triclosan in combination with a polyvinylmethyl ether and maleic acid copolymer (PVM MA; Gantrez®), which reduce supragingival calculus formation by delaying plaque calcification [10–13]. Pyrophosphates have been shown to absorb to developing crystallites, inhibiting the mineralization of the amorphous calcium phosphate before it is transformed in the calculus crystalline phase. While calculus-control fluoride dentifrices have been shown to be effective in clinical caries trials, there is no definitive clinical evidence that they are equivalent to regular fluoride dentifrice [14].

During the late-1990s, it was observed that a diet containing a brown marine alga (*Ascophyllum nodosum*) resulted in decreased deposit formation in heavy-calculus-producing patients [15]. A pilot study in beagle dogs confirmed the calculus reducing effect [16]. To date, no in vivo human studies of the effect of the marina alga on oral health, including calculus formation, have been published.

The aim of this study was to evaluate, in a controlled randomized cross-over study, the clinical effect of a daily intake of *Ascophyllum nodosum* alga over a 6-month period on supragingival calculus formation, plaque development, and gingival health in patients showing medium to heavy calculus formation at their annual dental visits. The alga intake was tested intra-individually against a negative control tablet. The hypothesis tested was that there was no difference in effect on oral health conditions between the alga and control intake.

Material and methods

Subjects

In the period October 2011–May 2012, healthy adult patients visiting two public dental health service clinics in the county of Västerbotten (Sweden) were asked to participate in the study. Inclusion criteria were as follows: observed moderate to heavy supragingival calculus formation contiguous to the lingual surfaces of their mandibular anterior teeth formatted since the last dental visit (commonly 1 year before inclusion in the study) and with presence of plaque and gingivitis. The subjects had no clinical signs of periodontal disease or caries and did not wear removable partial dentures, orthodontic appliances, or dental implants. Pregnant women, individuals with poor general health, or using antibiotics, antibacterial, or anti-inflammatory drugs or Waran within a period of 6 months prior to and until the end of the study were excluded. A total of 61 adult subjects, 34 men and 27 women, mean age 53.2 years (range 33–82) agreed to participate. The number of participants was based on a power calculation for the total amount of calculus formation (18 sites) at 80 %, a significance level of 5 %, a longitudinal difference of 3 mm, and a standard deviation of 6 mm, indicating the inclusion of 30 participants. Oral and written information was given to the subjects at the screening visit. All subjects gave their voluntary informed consent prior to start, with the understanding that they could withdraw from the study at any time for any reason. The study was approved by the regional ethical review board at the medical faculty, Umeå University, Sweden (ref. no. 2012-59-31M).

Study design and intakes

The study was designed as a controlled randomized cross-over study, enabling an intra-individual comparison of the unsupervised intake of the following: (1) dried marine alga *Ascophyllum nodosum* (ProDen PlaqueOff™, SDC Swedencare AB, Malmö, Sweden), two 250 mg capsules/day and, as negative control, (2) placebo tablets (Legosan, Kumla, Sweden), two/day.

Each intake period, alga and control, lasted for 6 months [17, 18]. The participants continued to perform their regular mechanical oral hygiene regimes and normal dietary practice throughout the study. The two experimental periods were separated by a 1-month washout period (Fig. 1). The participants were instructed to swallow both the alga capsules and control tablets with water. If adverse events occurred, participants were to directly contact the clinic to report the symptoms.

ProDen PlaqueOff™ is a natural food supplement made from the selected alga *A. nodosum* which contains water (8–14 %), proteins (5–8 %), fat (2–4 %), and polysaccharides (45–60 %) of which fucoidanes, mannitol, and alginate are dominating, as well as vitamins and minerals (Thorlivs analysis, Iceland; Algea AS Norway information). The negative control tablet consisted of 247.5 mg microcrystalline cellulose (E460 Comprecel M120D), 247.5 mg dicalcium phosphate dihydrate (E341), and 5 mg magnesium stearate (E470B) (Legosan). Each subject participated in both groups in a randomized order. Randomization was performed at the start of the study by consecutive numbering using sealed envelopes. The intention of the design was to perform a double-blind study in which the intake alga capsules and negative control tablets were distributed to the participants in similar plastic containers. In addition, the persons performing the clinical examinations were not aware of which treatment each individual participant was subjected to.

Clinical examinations

Clinical examinations were carried out by two trained investigators with long clinical experience. The examinations were performed before the start (screening) and at the end of both 6-month periods (Fig. 1).

Supragingival calculus

Supragingival calculus was scored at the lingual surfaces of the six anterior mandibular teeth using the Volpe-Manhold calculus index (V-M) [19]. Calculus was measured in millimeters with a graduated periodontal probe (Hu-Friedy XP23/127, USA) in three planes of each lingual surface: (1) starting at the tooth cervical site gingival margin, measuring in a mid-facial angle and (2–3) starting interdentally at the gingival margin, measuring in both a mesio-incisal angle and disto-incisal angle. The extent of the deposit was assessed to the nearest 0.5 mm. A zero score denoted the absence of calculus at that site. Prior to grading, the teeth were gently air-dried.

Gingivitis

Gingivitis was determined according to the gingival index (GI, Table 1) [20], followed by the gingival bleeding index (GBI) [21] recorded as presence or absence of bleeding within 10 s upon gentle probing of the orifice of the gingival crevice with a blunt periodontal probe (Hu-Friedy XP23/127).

Dental plaque

The amount of visible plaque was then assessed using the Ouigley-Hein plaque index (QHI, Table 1) modified by Turesky et al. [22, 23] after disclosing the dentition with erythrosine solution. Gingivitis, gingival bleeding, and plaque were measured using the disto-buccal surfaces of two crossed upper and lower quadrant teeth: 16, 14, 11, 31, 34, and 36, which well represents the overall conditions in the dentition when assessing plaque and gingivitis [24, 25]. Teeth with crowns were excluded and replaced by a suitable neighboring tooth. Individual case report and separate forms were used for each index at all examination intervals. Findings were called

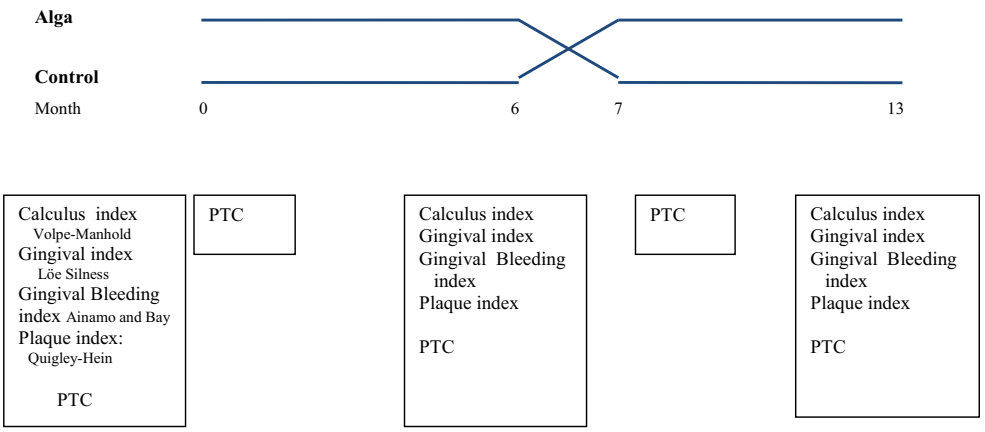

Fig. 1 Study design with analyses at screening and the clinic visits. *PTC* professional tooth cleaning

out to a recording assistant. Calibration sessions for the clinical indices were performed at baseline. The intra-examiner reproducibility kappa value was >0.88. At the start and end of each experimental period and after clinical registrations, each participant underwent a professional tooth cleaning (PTC). Debris, plaque, and calculus were removed using manual- and/or ultrasonic scalers. The tooth surfaces were cleaned with a polishing paste and a rubber cup.

At the end of each 6-month period, the quality and hardness of the removed calculus was described. The participant's compliance was checked; possible adverse events during the period were checked; and the participants were asked to describe their subjective view of the effect of each intake period, with specific emphasis to calculus formation.

Statistical analysis

The data were processed in the SAS statistical program (version 19). Descriptive statistics of the V-M index are presented as group means and standard deviations, while for the gingival index, gingival bleeding index, and plaque index, they are presented as medium and range. Differences in calculus formation at the 6-month examinations between the alga and control groups were tested with Student's paired *t* test. Differences in level of gingivitis and plaque formation were tested with Wilcoxon signed rank test [26]. The null hypothesis was rejected at the 5 % level. Reduction rates were defined as differences in 6-month scores between test and control groups with the control period 6-month scores taken as the individual's normal index score.

Table 1 Scores of the gingival index, gingival bleeding index, and plaque index

Gingival index (GI) according to Löe & Silness [20]

0=Absence of inflammation

1=Mild inflammation; slight chance in color and texture. No gingival bleeding

2=Moderate inflammation; moderate glazing, redness, edema, and hypertrophy. Bleeding upon probing

3=Severe inflammation; marked redness and hypertrophy, a tendency to spontaneous bleeding and ulceration

Gingival bleeding index (GBI) according to Ainamo & Bay [21]

0=No bleeding upon gentle probing

1=Bleeding upon gentle probing

Plaque index OHI according to Quigley-Hein index [22] modified by Turesky et al. [23]

0=No plaque present

1=Separate flecks or discontinuous band of plaque at the cervical margin

2=Thin, continuous band of plaque (up to 1 mm) at the cervical gingival margin

3=Band of plaque wider than 1 mm but covering less than one third of the surface

4=Plaque covering at least one third but less than two thirds of the surface

5=Plaque covering more than two thirds of the surface

Results

Participants

Fifty-five participants, 29 women and 26 men, completed the entire period. The mean age was 58.1 years (range 33–82). At screening, the mean V-M score per tooth was 1.20 (SD 1.24, range 0–7). The mean of the total V-M scores per participant (18 sites) was 24.6 (range 11–58).

Six participants (five females, one male) discontinued their participation in the study for various reasons: moving (one), pregnancy (one), possible interfering medicine (Waran) (one), adverse events (two), and a too-low frequency of intake (one), resulting in a drop out of 9.8 %.

Adverse events

Adverse events were observed in five participants (Table 2). Stomach problems were reported by one participant during the first days of alga intake and by a second participant who complained of stomach pain directly after intake of both alga and control. These two female participants reported a history of stomach problems and discontinued the study. The participant with symptoms in both periods did forget to report her discontinuation of the placebo tablets, during several weeks in the first part of the study, but did report this when she reported symptoms during the second period. Three other participants showed adverse events after intake of the control tablets. Two of these participants discontinued (two females) and one reduced (one female) the intake of the control tablets. All three subjects had previously completed the alga intake period, so it was decided that they could continue the control period but without intake of the control tablets. The adverse events reported were allergic reaction, oral dryness and stomach symptoms, and ill-smelling clothes and skin.

Analysis

The mean and SD values for the V-M index and the median and range values for the other clinical indices at the end of both intake periods for all subjects are presented in Table 3.

Calculus

At 6 months, the mean values of the total V-M scores per participant (18 sites) of each of the participants were 8.97 and 17.02 for the alga and control group, respectively. The mean amount of calculus formed during the 6-month period was

Table 2 Adverse effects observed in alga and control group

Intake	Gender	Adverse events	Discontinuation intake	Discontinuation of study
Alga	Female	Stomach symptoms	Yes	Yes
	Female[a]	Stomach symptoms	Yes	Yes
Control	Female[a]	Stomach symptoms	Yes	Yes
	Female	Allergic reaction	Yes	No
	Female	Oral dryness	Yes	No
	Female	Ill-smelling skin and clothes	Reduced intake	No

[a] Same participant

significantly lower in the alga intake group (−0.45, standard error (SE) 0.04, $p<0.0001$) (Table 3).

The differences were statistically significant for both males (−0.42, SE 0.05) and females (−0.47, SE 0.06) ($p<0.0001$). Fifty-two participants showed less calculus formation after the alga intake period compared to the control period. Two participants showed similar calculus formation after the two periods, and one subject showed a larger calculus formation after the alga intake period. After the alga intake, 64 % of the observed lingual sites showed a score 0, while the number in the control group was 43 %. The individual mean number of registered sites with score 0 was 11.5 (64 %) for the alga and 7.7 (43 %) for the control group, respectively.

When comparing the 6-month scores in the alga group with the 6-month scores in the control group for all participants, the mean calculus reduction, expressed as total V-M scores per participant, was 52.0 % (male 46.3 %, female 58.7 %). Thirty-one of the 52 participants showed >50 % reduction. The total of all registered sites in all subjects (18 sites/subject) [27] showed total V-M scores of 534 for the alga group and 940 for the control group ($p<0.0001$).

In 30 of the participants, it was clinically observed that after the alga intake period, the calculus present was easy to remove and showed a much softer consistency compared to the calculus which had formed after the control intake period. For the other participants, no obvious clinical differences were recorded. Thirty of the participants experienced that they had less calculus formation after the alga intake while the others observed no differences between the two periods.

Gingivitis

The difference in the level of gingivitis between the groups at the end of the 6-month periods was not significant, neither for all participants ($p=0.13$) nor for males ($p=0.42$) and females ($p=0.14$) separately. The total number of sites with GI score 0 was 163 and 154, and the mean number of sites with score 0 was 3.0 in the alga and 2.9 in the control group. When the 6-month alga mean GI scores were compared with the corresponding 6-month control scores, a 0.2 % reduction was observed.

Gingival bleeding

The number of bleeding sites at the 6-month examinations was significantly lower for the alga group ($p=0.024$). The difference was significant when male ($p=0.046$) and not significant when female ($p=0.25$) participants were analyzed separately. A 27.2 % greater reduction was observed in the alga group than in the control group at the 6-month examination. In male subjects, the reduction was 40.2 % and in females 12 %.

Plaque formation

The amount of plaque formed in both groups at the end of the 6-month periods showed significantly less plaque in the alga group for all participants ($p=0.007$) and for males ($p=0.0004$), but not for females ($p=0.62$). The total number of

Table 3 Descriptive values and statistical analysis of differences between the intake groups at 6 months, $N=55$

	Intake group	Mean	SD	*P* value
Calculus index (mm) (Volpe-Manhold)	Alga	0.50	0.45	<0.0001
	Control	0.95	0.54	
		Medium	Range	
Gingival index (Löe and Silness)	Alga	0	0–2	0.13
	Control	1	0–2	
Gingival bleeding (Ainamo and Bay)	Alga	0	0–1	0.024
	Control	1	0–1	
Plaque index (Ouigley-Hein)	Alga	1	0–4	0.008
	Control	2	0–4	

sites with QH index score 0 was 76 and 61, and the mean number of sites per participant with score 0 was 1.40 in the alga and 1.12 in the control group (p=0.0005). When the 6-month scores in the alga group were compared with the corresponding scores in the control group, for all participants, a 7.2 % reduction was observed. In males, the reduction was 19.2 %, and in females, a 6 % increase was observed.

No difference in the amount of calculus, level of gingivitis, and gingival bleeding was found between male and female subjects in the control group. In the alga group, both genders showed lower values for all indices but there was no significant difference between genders. Male subjects showed significant more plaque in the control group than females (p=0.001). In the alga group, the difference was less, but still significant (p=0.024).

Discussion

The present randomized cross-over study assessed the additional effect of daily intake of the alga *A. nodosum* on plaque and calculus formation and gingivitis. The most noticeable result of the present study was the pronounced calculus-inhibiting effect of the alga. Despite the fact that most individuals brush their teeth twice a day and several dentifrices and mouthrinses contain anti-plaque, anti-gingivitis, and/or anti-calculus agents, the prevalence of gingivitis, calculus, and chronic periodontitis remains, although a decreasing trend, relatively high in many populations [28, 29]. Maintaining effective plaque and calculus control is clearly difficult using conventional mechanical cleaning procedures.

The intra-individual comparison design reduces highly variable individual-confounding influences like oral health and oral hygiene habits, diet and salivary quality/quantity, caries experience, socioeconomic status, and genetic differences; these parameters may vary dramatically between subjects and thereby mask the effect of tested chemical agents in a parallel design study [30]. The design requires fewer subjects than a parallel design and has considerable power to detect statistical differences between agents [8, 31]. In almost all clinical evaluations of anti-calculus agents, a parallel group design was used, thereby avoiding the labor intensity and long-term character of cross-over studies. In a recently published systematic review of the effectiveness of anti-calculus dentifrices from 1986 to 1998, 22 of 31 studies included had less than 60 participants despite parallel group design [32]. Only one of these studies reported a sample size calculation. The present study started with 61 participants, and the study duration complied with the 6-month recommendations by ADA and other bodies [8, 17, 18]. However, in Netuveli and Sheiham's systematic review, more than half of the included anti-calculus studies used a 3-month evaluation period [32].

From a dental health behavior perspective, the participants in the present study were characterized by a relatively good standard of dental awareness based on their regular dental attendance and oral hygiene, which often characterize Swedish patients [4]. This was also found in the screening observation of with relatively low plaque and gingivitis levels in the majority of the individuals. The mean of the individual total V-M scores (18 sites) at the screening examinations was 24.6 (range 11–58). This V-M score is higher than in many previously published studies using V-M scoring when evaluating anti-calculus dentifrices. However, in these studies, the pre-test values were based on calculus formation periods of between 1 and 3 months, so participants recruited showed rapid calculus formation [10, 11, 33–37].

Despite marketing of anti-calculus dentifrices, mouth rinses, and chewing gums, sizeable labor-intensive resources are used in traditional mechanical removal of calculus. In addition to dental prophylaxis benefits, traditional scaling has implications to be considered causing, i.e., discomfort for the patients and soft tissue damage, while permanent loss of dentine may be of concern for heavy calculus formers. The resulting bleeding and bacteremia will have implications for patients with for example acquired or congenital valvular defects of the heart [38]. Anti-calculus agents marketed in the last decade include calculus crystal growth inhibitors such as pyrophosphates with or without the PVM/MA copolymer, zinc salts, triclosan combined with zinc citrate, pyrophosphate or PVM/MA, sodium hexametaphosphate, and polyaspartate. In view of the large reduction of calculus after the alga intake period compared to the negative control, it is interesting to compare the present results with the efficacy of other anti-calculus agents.

An extensive electronic search was therefore conducted for clinical studies published 1985–2013, to identify the literature evaluating dentifrices with the most common anti-calculus agents used. PubMed and Google Scholar were searched for relevant published full papers of clinical studies, in English, in which the anti-calculus dentifrices were compared with a negative control or placebo fluoride containing dentifrice. Bibliographies of identified papers were checked for further relevant references. In all included studies, the V-M calculus index was used for measuring supragingival calculus formation at the end of the evaluation period. Studies comparing anti-calculus agents without negative control were excluded. In Table 4, the reported calculus reduction rates are shown. The largest reduction values were observed with the agents 0.2 % triclosan/0.75 % zinc citrate and sodium hexamethaphosphate. However, these reductions were reported in only a few studies. A surprisingly large variation in calculus reduction was observed for the different agents studied. Of 64 dentifrice studies and 90 dentifrice experiments (experiment is used to identify each single experimental dentifrice investigated), only two studies showed

Table 4 Relative calculus reduction percentages observed in clinical studies evaluating the most common marketed anti-calculus agents included in dentifrices compared to a negative control fluoride dentifrice

Anti-calculus agent	Studies	Months	Reduction (%)	Mean reduction (%) (range)
1.3 % Pyrophosphate	Kohut et al. 1989 [39]	3	25	12.9 (2.5–25.0)
	Schiff et al. 1990a [40]	6	2.5	
	Singh et al. 1990 [41]	3	11.2	
3.3 % Pyrophosphate	Gaengler 1993 [42]	3	25.5	29.1 (9.0–45.0)
	Kazmierczak et al. 1990 [43]	6	21.4	
	Kohut et al. 1989 [39]	3	25.0	
	Kurbad et al. 1991 [44]	3	25.0	
	Lobene 1989 [45]	3	38.0	
	Malatt et al. 1985 [46]	2	25.5	
	Rosling and Linde 1987 [47]	6	9.0	
	Rugg-Gun 1988 [48]	6	45.0	
	Rustigo et al. 1986 [49]	3	32.3	
	Schiff 1987 [50]	6	29.4	
	Segreto et al. 1998 [51]	3	28.9	
		6	32.3	
	Zacherell et al. 1985 [52]	6	32.0	
5.0 % Pyrophosphate	Hagiwara et al. 1989 [53]	3	9.0	26.5 (9.0–43.9)
	Petrone et al. 1991 [54]	6	43.9	
1.3 % Pyrophosphate/1.5 % PVM/MA	Bánóczy et al. 1995 [10]	12	54.7	39.1 (20.5–54.7)
	Charles et al. 2001 [37]	4	20.5	
	Cohen et al. 1994 [55]	12	54.4	
	Lobene 1986 [56]	3	44.2	
	Petrone et al. 1991 [54]	6	46.7	
	Rustogi et al. 1991 [57]	6	35.9	
		12	39.8	
	Singh et al. 1990 [41]	3	29.5	
	Triratana et al. 1989 [58]	6	37.1	
	Triratana et al. 1991 [59]	3	35.0	
	Triratana et al. 1991 [60]	6	37.0	
	Triratana et al. 1991 [61]	6	24.1	
		12	40.8	
3.3 % Pyrophosphate/1.5 % PVM/MA	Chikte et al. 1992 [62]	3	20.0	39.0 (14.1–51.3)
	Kohut et al. 1989 [39]	3	38.0	
	Liena et al. 2009 [63]	3	14.1	
	Lobene 1987 [64]	3	50.8	
	Petrone et al. 1991 [54]	6	51.3	
	Rosling and Linde 1987 [47]	6	42.2	
	Schiff 1986a [65]	3	36.5	
		6	46.0	
	Schiff 1987 [66]	6	49.3	
	Schiff et al. 1990a [40]	3	33.0	
	Triratana et al. 1991c [67]	12	37.0	
0.3 % Pyrophosphate/5 % triclosan	Fairbrother et al. 1997 [11]	4	15.0	26.0 (15.0–33.0)
	HCF	4	23.0	
	Lobene 1987a [68]	3	26.0	
	Svatun et al. 1993a [69]	7	33.0	
0.3 % Triclosan/2 % PVM/MA	Allen et al. 2002 [70]	2	28.4	29.7 (4.0–54.6)
	Bánóczy et al. 1995 [10]	12	54.6	
	Fairbrother et al. 1997 [11]	4	4.0	

Table 4 (continued)

Anti-calculus agent	Studies	Months	Reduction (%)	Mean reduction (%) (range)
	HCF	4	8.0	
	Lobene et al. 1990 [71]	3	26.3	
	Lobene et al. 1991 [36]	6	36.3	
	Schiff et al. 1990b [72]	6	23.0	
	Schiff et al. 2008 [34]	3	34.8	
	Sowinski et al. 2002b [73]	2	34.1	
	Svatun et al. 1993a [69]	7	12.0	
	Volpe et al. 1992 [74]	3	35.5	
0.2 % Triclosan/0.5 % zinc citrate or zinc chloride	Fairbrother et al. 1997 [11]	4	13.0	37.1 (13.0–50.0)
	Fairbrother et al. 1997 [11]	4	19.0	
	Stephan et al. 1987 [75]	3	32.4	
	Stephan et al. 1990 [33]	6	50.0	
	Svatun et al. 1990 [76]	7	50.0	
0.2 % Triclosan/0.75 % zinc citrate	Bánóczy et al. 1995 [10]	12	39.2	49.1 (39.2–59.0)
	Svatun et al. 1993b [77]	7	59.0	
0.5 % Zinc citrate	Gaare 1989 [12]	3	34.2	23.7 (9.1–38.0)
	Jowett et al. 2013 [78]	3	13.2	
		6	9.1	
	Lobene 1987b [64]	3	10.7	
	Rustogi et al. 1986 [49]	3	38.0	
	Segreto et al. 1991 [79]	3	13.7	
	Triratana et al. 1991d [61]	6	34.2	
1 % Zinc citrate	Santos et al. 2000 [80]	3	26.4	27.7 (26.4–29.0)
		3	29.0	
2 % Zinc citrate	Kazmierczak et al. 1990 [43]	6	32.3	37.9 (31.9–50.0)
	Kohut et al. 1989 [39]	3	36.0	
	Lobene et al. 1987 [81]	6	39.5	
	Rustogi et al. 1986 [49]	3	50.0	
	Sowinski et al. 1998a [82]	12	31.9	
Na hexamethaphosphate	Hagiwara et al. 1989 [53]	3	30.1	41.3 (38.6–55.0)
	Winston et al. 2007 [83]	3	50.0	
		6	55.0	

HCF heavy calculus formatting subjects

a reduction larger than that found after the alga intake in the present study, while 11 experiments observed a ≥50 % reduction. However, there is a major difference in administration of the anti-calculus agents described above and the alga used in the present study. The alga has an additional systemic effect as an adjunctive to the normal oral hygiene measures, while the anti-calculus agents in dentifrices are based on a local effect, which may vary according to agent, concentration, application time, and frequency used.

Analysis of the clinical anti-calculus literature showed that the majority of the papers were published by or in collaboration with researchers employed by the industry, and many of these articles are published in a small number of commercial journals, not readily accessible to the readers. Adams [38] served that in many of these dentifrice trials, subjects were selected on the basis of high rates of calculus formation during a pre-test screening period.

Blinkhorn et al. emphasized that the absolute differences in the amount of supragingival calculus formed in all of the available studies were modest, and whether they represent a clinical relevance was questionable [84]. Adday and Koltai [85] concluded that presentation of the data as mean values tends to hide the frequency in which the active anti-calculus agent may attain total calculus inhibition at individual sites and teeth in comparison with a placebo group. In this study, the number of calculus-free lingual mandibular anterior tooth sites was 64 % in the alga group and 43 % in the control group. The mean number of sites with score 0 per participant was 11.5 for the alga and 7.7 for the control group. Moreover, at the end of the 6-month study periods, 19 of 55 participants

(35 %) remained almost or totally calculus-free (defined as subjects with ≤5 mm total calculus scores) in the alga group, compared to none in the placebo group.

The present study is the first clinical human study showing a significant reduction of supragingival calculus formation after oral intake of alga compared to a control intake. The hypothesis was therefore rejected. Further research should be performed to investigate the optimal concentration and intake frequency of the alga and its long-term clinical effect on oral health. The mechanism behind the reduction in calculus formation and quality change after intake of the alga is, as yet, unexplained. The explanation may be found in the influence of alga ingredients resulting in crystal growth inhibition, desorbing the pellicle or breakdown of plaque matrix, or controlling the endogenous inhibitors of crystal growth [86]. More than 50 % of *A. nodosum* consists of polysaccharides and proteins, which can be distributed after gastrointestinal enzymatic breakdown or fermentation as monosaccharide, short chain fatty acids, and bioactive peptides to the teeth by saliva and crevicular fluid. These may have a high affinity to tooth surfaces and compete for the same adsorption sites (calcium ions) on hydroxyapatite as the salivary acidic proteins and may thereby block nucleation and crystallization sites [7, 87]. Precipitation and phase transformation of calcium phosphate entities can therefore be inhibited [88], enabling more soluble phases to accumulate [89].

A competitive displacement of the adsorbed proteins may also be able to desorb already adsorbed pellicle proteins as suggested by Rykke et al. [90–92]. Proteins, which inhibit precipitation of calcium phosphates on enamel and control mineralization, contain rigid anionic segments on the molecules, with a high affinity for calcium and apatite surfaces [90, 91]. If present in a soluble form, they may block active sites on the surface or affect the degree of saturation in the liquid phase by binding or complexing calcium [90]. A reduction in supragingival calculus was observed in 95 % of the participants. In the majority of the participants, the remaining calculus observed was also more amorphous and loosely bound, suggesting a disturbed maturation pattern of the calculus, enabling the softer deposits to be removed by normal oral hygiene measures.

The control of supragingival plaque is critical to prevent caries and gingivitis. A significant larger reduction in plaque and bleeding scores was observed after the alga intake period compared to the control period, where the participants used their customary oral hygiene habits. The reduction was more pronounced for men than for women. For male subjects, a 19.2 % plaque reduction was observed. Males harbored significantly more plaque in the control group than females, which is in agreement with earlier findings [92–94]. It was evident that females had more favorable oral hygiene than males, making it more difficult to reduce plaque and gingivitis in women. No difference was seen for the gingivitis scores despite the improved plaque scores. Reductions in plaque unaccompanied by gingivitis reduction have been reported earlier [13, 95].

Addition of various active agents into dentifrices for plaque and gingivitis control has been a field of interest for many years. Dentifrices containing plant extracts, such as sanguinarine; metal salts, i.e., stannous or zinc salts; enzymes; and antimicrobial agents, i.e., triclosan, have been marketed. No or minor anti-plaque effect was shown for dentifrices containing stannous fluoride, triclosan/zinc citrate, and triclosan/soluble pyrophosphate, while a significant effect was observed for triclosan/copolymer containing dentifrices [96].

Pareskevas [97] recently reviewed randomized controlled clinical trials of ≥6 months on the use and effect of agents used for chemical plaque control. A large variation in efficacy was observed between the studies for all reviewed agents. Fourteen studies on triclosan/PVM-MA dentifrices, all parallel, reported reduction rates versus a negative control or placebo between 0 and 35 % (mean 19.7 %), which can be compared with the 7.2 % reduction for the alga group. Mean percentage reductions of gingivitis was 18.5 % (range 1–31.5 %) [93]. Blinkhorn et al. [84] reviewed chemical plaque and gingivitis control via mouth rinses. Only 0.12 % chlorhexidine and essential oils containing mouth rinses were reported to result in an anti-plaque effect. The most consistent anti-gingivitis results were found for the 0.12 % chlorhexidine mouth rinse and moderately consistent results for essential oils. In the review by Pareskevas [97], nine parallel design controlled clinical trials of ≥6 months showed a mean plaque reduction of 30.2 % (range 9.3–56.3 %) and a mean gingivitis reduction of 22.8 % (range 9.4–35.9 %) versus a placebo or negative control. These reviews also showed very large variations in the results for both plaque and gingivitis. The efficacy of anti-plaque, anti-gingivitis, and anti-calculus agents in dentifrices has been studied in short-term evaluations. Evaluations of their long-term effect on oral health are lacking.

Adverse events after use of the alga were reported by two participants: stomach problems during the first days of intake. Both subjects reported afterward that they had a history of stomach problems. Fermentation of the high content of carbohydrates, i.e., soluble fibers of the alga in the gut, with which the participants were not familiar, may explain these symptoms. However, in most cases, the symptoms will disappear with continued intake. No other adverse reactions were observed in the alga intake group. The inclusion of chemical additives, in dentifrices and mouth rinses, has been discussed widely not at least because of their potentially adverse health and environmental effects. Only a few studies reported mucosal reactions after use of mouth rinses with zinc/triclosan and pyrophosphate containing dentifrices probably caused by the increased concentrations of flavorings and detergents [86, 98–100]. A recent systematic review indicated the need to improve reporting adverse events in periodontology [101].

No effect on subgingival calculus has been shown for the various anti-calculus dentifrices and mouth rinses on the market. White [6] expected the efficacy on subgingival calculus to be minimal due to the difficulty for the applied dentifrices and mouth rinses to reach into the periodontal pockets [6]. This may be in contrast to the studied alga, whose systemic distribution via crevicular fluid and saliva may also influence subgingival mineralization, which has to be confirmed in clinical studies.

It can be concluded that a daily intake of the alga *Ascophyllum nodosum* in addition to the participants' customary oral hygiene resulted in a highly significant reduction of calculus formation. In addition, the calculus was less well bonded to the tooth and showed reduced hardness. Plaque formation and gingival bleeding were also significantly reduced. Further research is necessary to evaluate the long-term beneficial effect on oral health.

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Conflict of interest The authors declare that they have no conflict of interest.

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