

The systemic effect of a food additive on dental plaque and calculus

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Short title: A food additive and dental deposits

Abstract

A food additive containing the brown algae *Ascophyllum nodosum* SW1313 was used in an eight-week clinical trial with the purpose to explore its possible effect on reducing supragingival plaque and calculus. 105 subjects were randomly assigned to one of three groups, each containing 35 subjects at baseline. Two groups received tablets of either a low (125mg) or a higher (250mg) concentration of the algae. A third group (control) received placebo tablets. Significant reduction in plaque levels compared to control were observed after eight weeks in both the low concentration (49%) and the high concentration (66%) algae groups ($P = 0.002$). Significant reductions in supragingival calculus levels were observed after four weeks ($P = 0.037$) improving after eight weeks ($P = 0.000$). The effect appeared to be dose-dependent. It is concluded that a food additive containing brown algae reduces the amounts of supragingival plaque and calculus in humans.

Key words

Dental calculus, dental plaque, food additive

Introduction

Several attempts have been made to prevent the formation of dental plaque and calculus by topically applied chemotherapeutic agents, e g., by incorporating antiseptics in dentifrices and mouthwash. There is currently, however, limited knowledge about the possibility of interfering with plaque and calculus formation using agents via the systemic route.

Naturally, it would be preferable to prevent the formation of plaque and calculus, instead of removing them once they are established.

One of the authors of the present study had observed that a patient who normally formed a lot of calculus had none a year after he had retired and moved from Sweden to another country. On examination, his oral hygiene regime had not changed, and neither, as far as could be ascertained, the quality of his dental care. His diet, however, had changed quite significantly. He had started eating a salad containing a particular fresh seaweed:

Ascophyllum nodosum (AN). Pilot tests with tablets made from dried AN confirmed in other patients that calculus appeared to reduce, and, interestingly, the tablets were swallowed whole – neither chewed nor sucked (Mattson B & Wikner S, unpublished data). The results of these simple experiments suggested that the observed dissolution of calculus may have been caused by some salivary factor that was systemically influenced by the AN tablets.

Ascophyllum nodosum is a brown seaweed which is confined to the North Atlantic basin.

AN is used widely in the production of alginic acid and carrageenan; polysaccharides commonly used as food additives for thickening such as ice cream and other dairy products.

It is also used as a feed additive in cattle and as a soil conditioner and fertilizer.

AN contains a large number of nutrients (Table 1) but several of them in concentrations (1) which seem to be too low to have any significant effect on human metabolism e g the protein and most vitamins and minerals.

Nutrients that prevail in the highest concentrations are alginic acid, phenol, fucoidan, mannitol, laminaran and sulphated fucoidan. Also the concentrations of vitamin D and iodine are high considering recommended daily allowances (RDA). AN also contains several minerals and trace elements which are vital to enzymes or hormones e.g. magnesium, manganese, zinc, cobalt, chromium and selenium. Those may be important to human metabolism in spite of low concentrations.

Plaque and dental calculus are widespread in adults. A study in 9689 Americans, 30 years of age and older, reported supragingival calculus in 91,8 per cent (2). A British study reported visible plaque in 72 per cent of adults and calculus deposits in 73 per cent (3). It is agreed that calculus does not exert a detrimental effect on the gingiva but facilitates plaque retention. Dental calculus is removed at the dental office in order to facilitate oral hygiene. For a long time dental calculus was defined as calcified deposits adhering to the teeth. It was not until 1969 that Schröder (4) provided the definition which is regarded appropriate to day: mineralised dental plaque that is permeated with crystals of various calcium phosphates. Calculus consists initially of an organic matrix which becomes calcified when certain conditions prevail. These include a sufficiently high pH and a supersaturated saliva with respect to ions which participate in the calcification process e.g. calcium, phosphate and carbonate. Precipitation does not occur below pH 7 but may be heavy at higher, alkaline, values (5).

Calculus contains 83 per cent inorganic salts and the most common are calcium phosphate (75%) calcium carbonate (3%) and magnesium phosphate (4%).

Others are calcium oxalate and ammonium magnesium phosphate (6).

Apatite is the most abundant structure followed in order by brushite, whitlockite, octacalcium phosphate, monetite and calcite (7). A high number of other minerals (8) have been identified as well; sodium, potassium, aluminium, iron, copper, silicon, nickel zinc just

to mention a few. The composition of dental calculus may differ in persons from different geographical locations and from the same area and also within the deposit (7). No one has yet provided a theory of calculus formation that satisfactorily explains all the observed variations in calculus formation in human subjects. E g no theory has explained the presence of calculus in some persons having an excellent oral hygiene and its absence in others having a poor oral hygiene.

Besides mechanical elimination of plaque and calculus several chemical compounds have been used for topical application. E g mouthwashes containing triclosan and sodium lauryl sulphate produced a significant reduction in plaque formation (9). Topical treatment of dog's teeth with 8-hydroxyquinoline sulphate also retarded the formation of plaque (10). Other effective compounds against plaque are chlorhexidine and the essential oils in Listerine (11, 12). Polyphenols e g tannins are abundant in most herbs, bushes and trees and also in tea, coffee and wine. They are astringent and antiseptic and may prevent plaque formation. E g polyphenols in cacao beans have antiplaque activity in vitro and in vivo (13). Phenolic compounds in hop may inhibit growth of oral streptococci in vitro (14). Also cranberry has been suggested for plaque inhibition due to ability to interfere with aggregation and adhesion of *S mutans* (15).

Topically applied compounds have also been suggested for prevention of calculus formation e g zinc (16-18), silica (19) and pyrophosphate (5, 20, 21). Also structural analogs of pyrophosphate e g bisphosphonates have been used for topical oral application in order to inhibit calculus formation (22). Hexametaphosphate used as a feed additive retards the growth of calculus on previously cleaned teeth in squirrel monkeys. However, no effect was observed on existing calculus that was left in situ (23). There are no reports on the systemic effect of the compounds accounted for above.

Several studies have reported influence on the deposit formation rate by altering the composition and consistency of the diet (24). However there is little evidence that the formation of calculus was systemically influenced in those studies. One problem is to distinguish between the topical effect during consumption and the systemic effect from nutrients absorbed from the intestine and then transported to saliva by the blood system. Similarly, due to the study design, the pilot tests (mentioned above) conducted prior to the present study do not prove that diet, rather than other factors, caused the apparent calculus reduction.

An Australian study indicates that diet might play a role for calculus formation. It reported that dental calculus scores were significantly higher in domestic cats than in feral cats (25). The assumption that AN may inhibit calculus formation, when used systemically, is contradictory to the opinion that dental calculus is merely a calcification product of bacterial plaque (26). That view is supported by the observation that calculus may form in the absence of bacterial plaque (27-29).

The purpose of this clinical study was to investigate the systemic effects of tablets made from AN on plaque and calculus formation in persons who had existing supragingival calculus at baseline.

Material and methods

150 subjects were recruited after an advertisement was placed in a local paper in Stockholm, Sweden. After a primary screening, 105 subjects were admitted after meeting the inclusion criterion of visible deposits of supragingival calculus. Persons with advance periodontal disease were excluded. Participants were randomly assigned to one of three groups, each containing 35 subjects at baseline.

The clinical examination comprised the assessments of plaque and calculus on three tooth surfaces per person. The assessment of plaque was based on the lingual surface of 26, and the buccal surfaces of 11 and 31. Supragingival calculus was assessed on the buccal, lingual and lingual surfaces, respectively, of the same teeth. The extension of plaque as well as calculus was assessed using Green and Vermillion's Oral Health Index Short Form (30). In this system score 0 indicated absence of plaque or calculus, score 1 that one third or less of the gingival part of the tooth surface was covered, score 2 that two thirds were covered, and score 3 that more than two thirds were covered. Plaque and calculus scores, respectively, were summarised across surfaces and given per subject. A preselected tooth, when missing, was replaced by 27, 41 and 21, respectively.

The food supplement selected was the brown algae _ *Ascophyllum nodosum* SW1313.

Tablets (ProDen PlaqueOff™) containing dried and pulverised AN were used in this trial, and in order to minimise any perioral absorption the tablets were wax-coated.

Tablets of two different concentrations of dried algae were tested: 125mg and 250mg. Two groups received tablets of either the low or the high concentration and a third group (control) received placebo tablets. Participants were instructed to take two tablets a day for four weeks when a second dental examination was made. The subjects were clearly instructed that the tablets should be swallowed whole.

Following the four-week examination, participants were instructed to take three tablets a day for a further four weeks. Eight weeks after baseline a final dental examination was conducted. Subsequent to the final examination, any remaining plaque and calculus were removed. The trial was performed double blind. Participants were asked to maintain their normal oral hygiene regimes during the course of the trial.

Statistics

The distributions of individuals with reference to plaque and calculus were normal at baseline. Differences between groups were tested by means of 1-factor ANOVA and, for comparisons over time, repeated measures ANOVA. Statistical significance was accepted at $P < 0.05$.

Results

14 persons dropped out during the course of the trial, leaving a total of 89 participants (85%), 47 women and 42 men, to be accounted for. The mean age was 45 years (range 23-68 years) and there were no significant differences between groups. Adverse events were rare. One person complained about abdominal pain.

The mean levels of plaque and calculus at baseline and during the course of the trial are presented in Tables 2 and 3, respectively. There were no statistically significant differences between groups at baseline. After four weeks, slight reductions of plaque were observed in all groups. There were, however, no significant differences between groups. Further reductions were observed at eight weeks in both the low concentration alga (LA) and the high concentration alga (HA) groups but not in the placebo group (Table 2). The reductions compared to baseline were 49% and 66% in LA and HA groups, respectively, compared to just 20% in the placebo group. The differences between groups were statistically significant ($P = 0.002$). Post hoc comparisons testing revealed a significant difference between HA and placebo groups (Scheffé $P = 0.002$) but not between LA and placebo groups or LA and HA groups.

The changes in plaque levels over time are illustrated in Figure 1. Repeated measures ANOVA disclosed a significant plaque reduction effect over time associated with substance use. Although differences between groups regarding change were small during the first 4 week period, there were significant differences between LA and placebo groups and between

HA and placebo groups during the second 4 week period ($P = 0.038$ and $P = 0.015$, respectively). For the total duration of the trial, the differences in plaque reduction between LA and placebo groups as well as between HA and placebo groups were statistically significant ($P = 0.015$ and $P = 0.001$, respectively).

There were no statistically significant differences with reference to calculus extension between groups at baseline. After four weeks, however, a statistically significant reduction associated with the test substance was observed ($P = 0.037$). Further reductions were observed after eight weeks in LA and HA groups but not in the placebo group. The differences between groups were statistically significant ($P = 0.000$). Post hoc comparisons testing suggested significant differences between HA and placebo groups as well as between LA and placebo groups (Scheffé $P = 0.000$ and $P = 0.004$, respectively). The difference between LA and HA groups was almost significant ($P = 0.080$). The changes in calculus levels over time are illustrated in Figure 2. Similar to plaque changes, a significant calculus reduction effect over time associated with substance use was observed. The differences in calculus reduction between groups during the first 4 weeks were not significant. During the second 4 week period, however, a significant difference was seen between HA and placebo groups ($P = 0.029$) and a trend towards a difference between LA and placebo groups ($P = 0.078$). For the total eight-week period the calculus reductions in both LA and HA groups were statistically significant compared to placebo ($P = 0.015$ and $P = 0.001$, respectively). The results remained as baseline plaque levels were introduced as a co-variable in the analyses.

Discussion

This is the first study to demonstrate that a food supplement containing the brown algae *Ascohyllum nodosum* SW1313 (ProDen PlaqueOff), taken daily, significantly reduces the amount of established supragingival plaque and calculus in humans. Given that the tablets were wax-coated and swallowed, it would appear that the effect of plaque and calculus resulted from systemic absorption of algal components, not a purely local effect.

The higher concentration of the algae had a greater effect than the lower one, suggesting a dose-dependent response. This is further substantiated by the observation that a greater reduction was seen in the second clinical phase when the dose was increased from two to three tablets daily.

The results are consistent with those of a pilot study (unpublished) in 30 persons, conducted over 16 weeks and where four tablets were taken daily. The reduction in plaque and calculus was 86% and 87%, respectively.

The reduction of plaque in the placebo group at the four-week examination reflected an improvement in oral hygiene during the first part of the study, making difficult any firm conclusion about the true reducing effect of the active agent during the first four weeks.

However, at the eight-week examination the plaque level of the placebo group remained unchanged whereas the levels observed in the verume groups continued to decrease.

Three mechanisms may account for the reduction in plaque accumulation observed. Among the many components of AN, three have previously been shown to affect plaque accumulation when used topically: phenols (11-14), iodine (31) and sulphate (9, 10). The assumption that the high content of the sulphate containing fucoidan in AN is a probable reason to the plaque reduction is in line with the results of previous studies. They demonstrated that furanone had a strong desorption activity against mutans streptococci pre-absorbed to saliva-coated hydroxyl apatite. Furanone like fucoidan is a sulphated

polysaccharide but is extracted from a red seaweed. In rats funoran reduced the colonization of *S. cricetus* and the caries scores compared to a control group (32). Added to chewing gums funoran reduced plaque formation in man (33). It is assumed that the sulphate groups of funoran interfered with bacterial adhesion to proteins of the pellicle in those studies.

However, the administration of the active agent used in those studies precludes the possibility to judge if the effect was systemic or local.

Prevention of calculus formation has been demonstrated after topical application of zinc (16-18), silica (19) and pyrophosphates (5, 20, 21). AN contains rather high concentrations of all these except pyrophosphate. However the sulphate in AN is a structural analog of pyrophosphate (34, 35) and may often replace it.

About 35 per cent of AN dry weight consists of highly acid sulphate esters and the absorption and mobilisation of these compounds may contribute to an increased sulfation of acid salivary components such as glycosaminoglycans (GAGs). The acidic nature of these components exhibit calcium-binding properties, which in turn would reduce the concentration of calcium available for precipitation (34-37). Although AN was taken by the subjects as a food supplement and a direct topical effect is ruled out, one or more of these simple molecules may be secreted in the saliva following absorption.

The observed dissolution of calculus is more difficult to relate to current knowledge of the dental science. However, in urological studies sulphated compounds have been used in order to prevent or eliminate urinary stones (38-40) *in vitro* and *in vivo* in animals and man. The mechanism is believed to result from acidification of the urine.

Hydroxyapatite is generally resistant to demineralisation but acids produced by oral bacteria can dissolve it. The critical pH for dissolution of hydroxyapatite in dental enamel is pH5,5 in persons with high salivary calcium and phosphate concentrations (41). Apatite is the most abundant structure but calculus precipitations may also contain brushite, whitlockite,

octacalcium phosphate, moneitite and calcite, all having a higher solubility than hydroxyapatite (43).

A theoretical explanation to the observed dissolution of calculus is an acidification of salivary pH due to an increased GAG concentration caused by AN consumption. That would be a similar mechanism as when acidification of urine dissolves the kind of renal stones (45) which have a similar composition as dental calculus. The lowering of pH from 6,5 to 5,75 increased the dissolution rate by 35 per cent in that study. It is reasonable to believe that certain parts of calculus may dissolve at a higher pH values than hydroxyl apatite (pH 5,5) due to the presence of more soluble calcium phosphates and carbonates in calculus.

Maybe the simplest explanation to the dissolution of calculus is acids from microorganisms in plaque. The bacterial composition of dental plaque should be the same as in plaque covering dental calculus. Thus after consumption of fermentable carbohydrates a pH drop should occur on the calculus surface having the same magnitude as on the enamel surface. Consequently one may expect that a pH drop in plaque covering calculus will dissolve calculus and particularly if parts of the calculus contain calcium precipitations that dissolve at a higher pH than hydroxyl apatite in dental enamel. Due to that difference the dissolution of calculus will start earlier during a pH drop and stop later during recovery of pH to normal values. The significance of oral microorganisms for the dissolution of dental calculus is supported by previous observations that rinsing with the antibacterial solution Chlorhexidine increased the formation of calculus (44, 45).

Future studies will be needed in order to explain the mechanisms behind the results of this study but one conclusion seems inevitable; the effects were caused by a systemic distribution of dietary nutrients.

It may also be concluded from the present observations that daily consumption of tablets made of brown algae (ProDen PlaqueOff) reduces the amounts of dental plaque and calculus already after a few weeks. The rate of the reduction seems to be dose dependent.

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Table 1. Average composition of the brown algae *Ascophyllum Nodosum*.

Major components	%	Minor minerals	mg/kg
N-free extractives	45-60	P	1500
Alginic acid	20-26	I	500-1200
Phenol	5-15	Fe	150-1000
Mannitol	5-8	Zn	50-200
Laminaran	2-5	B	40-100
Fucoidan	10-15	Mn	10-50
Crude fibre	8	Ba	15-50
Crude protein	5-10	Co	1-10
Major minerals	mg/kg	Cu	1-10
S	35000	Se	4
Cl	37000	Ni	2-5
Na	35000	Mo	0,3-1
Lipid	30000		
K	25000		
Ca	20000		
Mg	7000		
Vitamins mg/kg			
C	500-2000	D	4
E	150-300	K	10
A(provit)	30-60	Thiamin	1-5
Niacin	10-30	Biothin	0,1-0,5
Riboflavin	5-10	Folacin	0,1-0,5

Table 2. Variation of plaque over time. Mean and standard deviation

	Baseline		4 weeks		8 weeks	
	Mean	SD	Mean	SD	Mean	SD
Algae high	4.72	1.71	3.17	1.61	1.62	1.41
Algae low	5.01	1.70	3.90	2.05	2.56	2.02
Placebo	4.26	1.54	3.40	1.71	3.40	2.07
	F = 1.6, P>0.05		F = 1.2, P>0.05		F = 6.8, P = 0.002	

Table 3. Variation of supragingival calculus over time. Mean and standard deviation

	Baseline		4 weeks		8 weeks	
	Mean	SD	Mean	SD	Mean	SD
Algae high	3.07	1.16	2.31	1.01	1.65	1.04
Algae low	3.33	1.69	2.89	1.51	2.40	1.53
Placebo	3.43	1.26	3.16	1.42	3.50	1.31
	F = 0.6, P>0.05		F = 3.4, P = 0.037		F = 16.2, P = 0.000	

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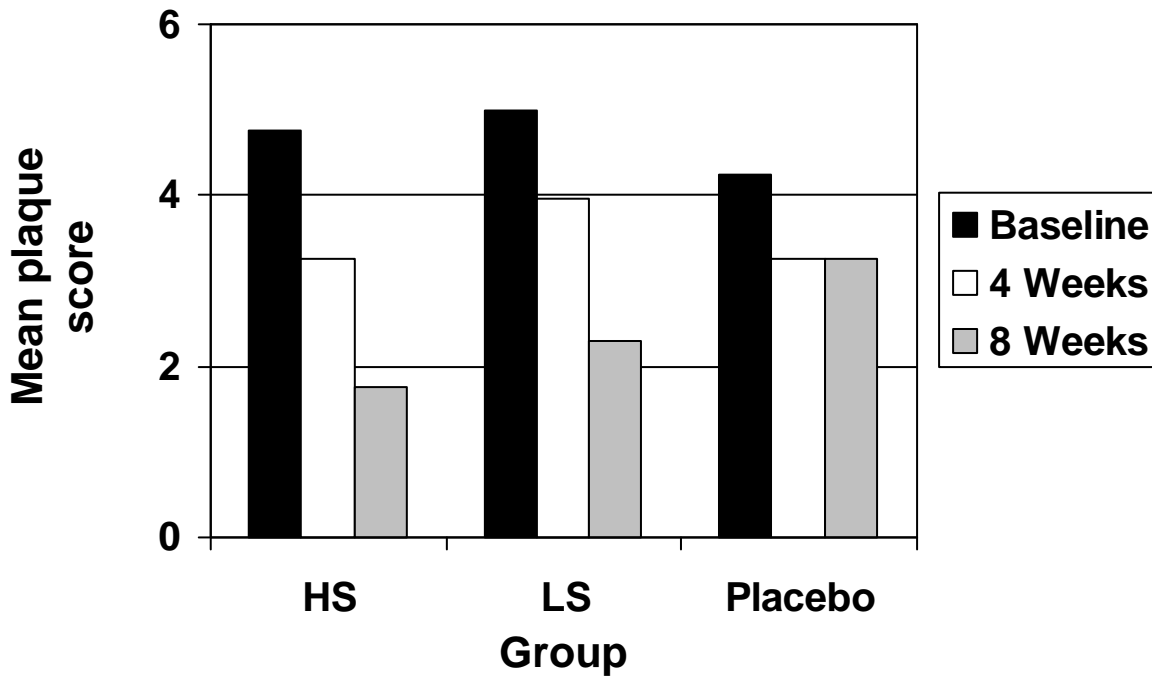
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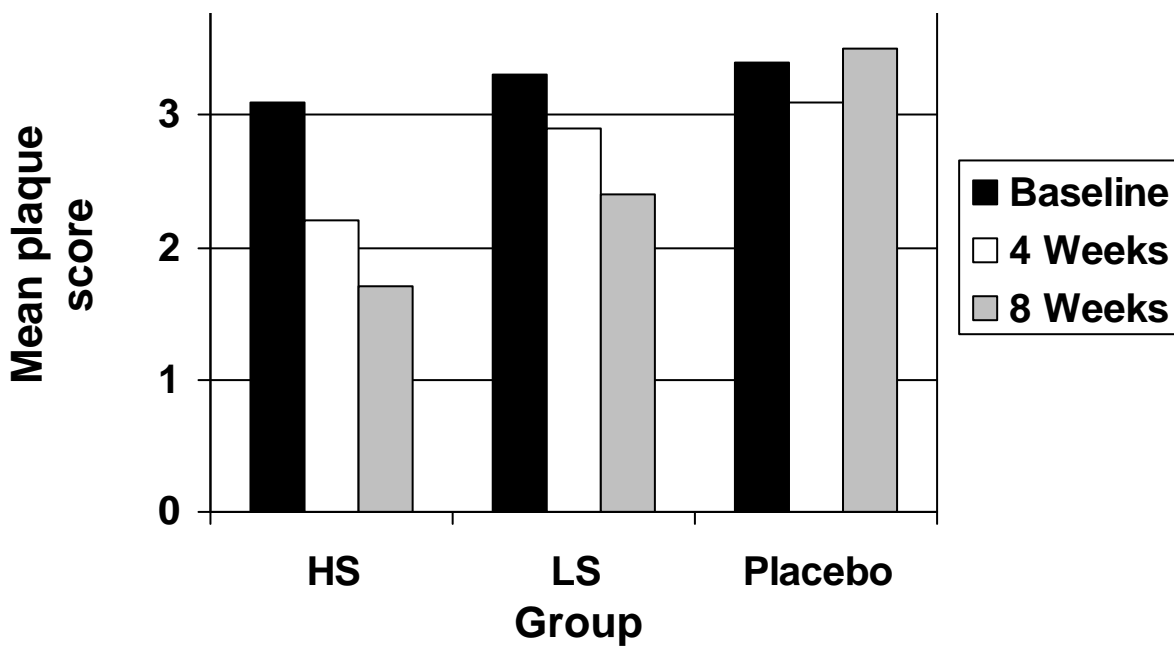
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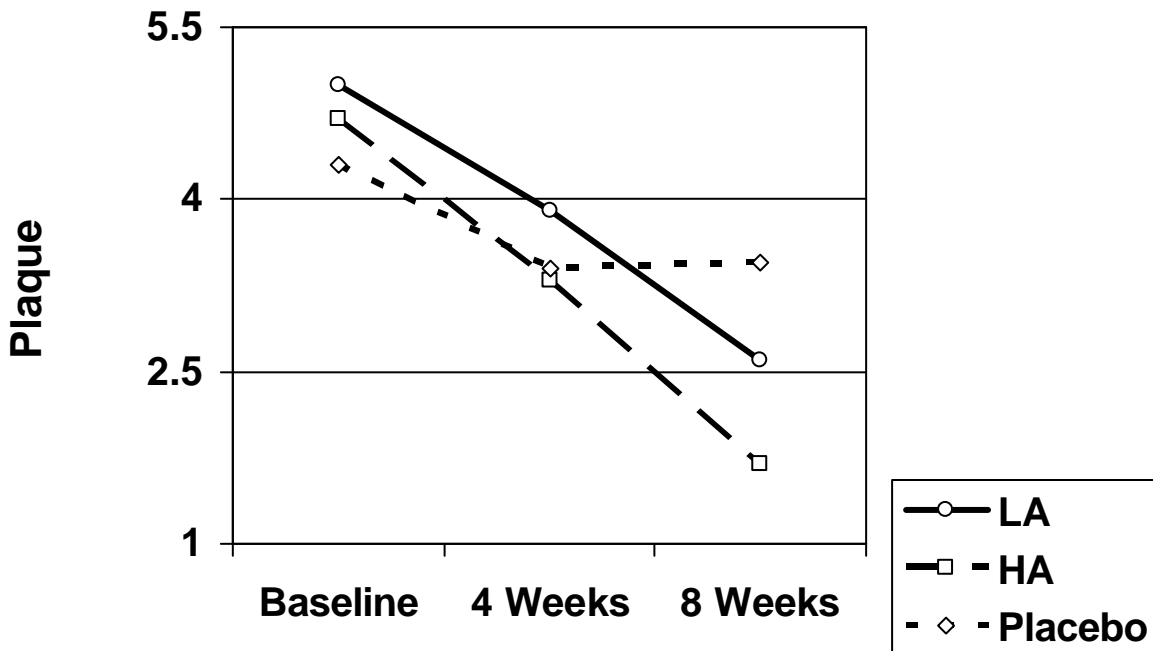
Mean plaque score related to amount of seaweed and time



Mean calculus score related to amount of seaweed and time



Plaque Change over Time
2-way interaction
 $F(4,170)=3.98; p<.0041$



Change in Calculus over Time
2-way interaction
 $F(4,170)=3.36; p<.0112$

