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RANDOMIZED CLINICAL TRIAL

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Effect of guava and vitamin C supplementation on experimental gingivitis: A randomized clinical trial

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Abstract

Objective: To study the effect of guava and synthetic vitamin C on the development of gingival inflammation during experimental gingivitis.

Material and methods: Participants were randomly assigned to three groups supplemented daily with either 200 g guava, 200 mg synthetic vitamin C or water. The study included a 14 days pre-experimental period with oral hygiene instructions, scaling, prophylaxis and supplementation. Thereafter, experiment gingivitis was initiated, while continuing supplementation. At baseline, Day 7 and Day 14 of experimental gingivitis, Plaque Index (PII) and Gingival Index (GI) were assessed. During the entire study, dietary fruit/vegetables intake was minimal.

Results: PII increased in guava, vitamin C and control group (ΔPII: 1.30, 1.61 and 1.79, respectively). However, the guava group developed significantly less plaque compared to the control group. The GI increase in both guava and vitamin C group was significantly less than the increase in the control group (ΔGI: 0.10, 0.24 and 0.87, respectively).

Conclusion: In a population of young nonsmoking adults, consumption of either 200 g guava/day or 200 mg synthetic vitamin C/day, prior to and during the oral hygiene abstention period, has a preventive effect on the development of experimental gingivitis as compared to the control group that developed the usual amount of experimental gingivitis.

KEYWORDS

experimental gingivitis, guava fruit, supplementation, vitamin C

1 | INTRODUCTION

Humans and other primates have lost the ability to synthesize vitamin C and must rely on the diet for proper function of the human body (Carr & Frei, 1999). Therefore, insufficient consumption of vegetables and fruits, the two major sources of vitamin C, can lead to depletion or deficiency states of the vitamin (Taylor, Hampl, & Johnston, 2000; Wrieden et al., 2000). A total lack of vitamin C in the diet causes the deficiency disease scurvy (Levine, 1986), and the relationship between necrotizing ulcerative gingivitis and vitamin C deficiency is well-known (Melnick, Alvarez, Navia, Cogen, & Roseman, 1988). Although there is little evidence on the basis of well-defined gingivitis populations, it may be suggested that individuals with gingivitis have lower plasma vitamin C levels than healthy controls (Gokhale, Acharya, Patil, Trivedi, & Thakur, 2013), that the degree of gingival inflammation is directly related to plasma vitamin C levels (Leggott, Robertson, Rothman, Murray, & Jacob, 1986) and that vitamin C supplementation, either by diet or supplements, can reduce gingival bleeding (Gokhale et al., 2013; Jacob et al., 1987; Leggott et al., 1986). It is interesting to note that, as far as we are aware, in only one study, the experimental gingivitis model was used to study the effect of vitamin C supplementation on the development of gingival inflammation (Vogel et al., 1986). On the basis of their results, it was impossible to conclude that vitamin C supplementation had any positive effect, that is a reduction of the development of gingival inflammation. This

may have been due to the relatively high plasma vitamin C levels at baseline.

Up to a few decades ago, the recommended daily allowance (RDA) of vitamin C was 60 mg/day (National Research Council, 1989). The current RDA of vitamin C is 90 mg and 75 mg per day for man and women, respectively (Institute of Medicine US, 2000). The RDA is defined as the average daily dietary intake level that is sufficient to meet the nutrient requirement of nearly all healthy individuals in a group. Based on the evidence from human metabolic, pharmacokinetic and observational studies in combination with Phase II RCTs, Frei, Birlouez-Aragon, and Lykkesfeldt (2012) concluded that 200 mg per day is the optimum dietary intake of vitamin C for the majority of the adult population to maximize the vitamin's potential health benefits with the least risk of inadequacy or adverse health effects.

Vitamin C in supplementation studies can either be given by means of synthetic vitamin C in tablets or by vitamin C in fresh fruits. Vitamin C tablets have the advantage that it is easy to study the pharmacokinetics and that a possible effect can be attributed to the given vitamin C dose. In contrast, vitamin C rich fruit provides also a number of other micronutrients and phytochemicals that may influence for example the bioavailability of vitamin C (Padayatty & Levine, 2016). In addition, these components may have beneficial effects by themselves. A recent study showed that the number of

Clinical Relevance

Scientific rationale for the study: To assess the impact of the consumption of healthy fruit containing high amounts of vitamin C on the development of experimental gingivitis.
Principal findings: Both guava fruit and vitamin C supplementation are beneficial in controlling experimentally induced gingival inflammation in young adults.
Practical implications: These data support the hypothesis that consumption of fruits high in vitamin C may be helpful in the prevention of gingivitis.

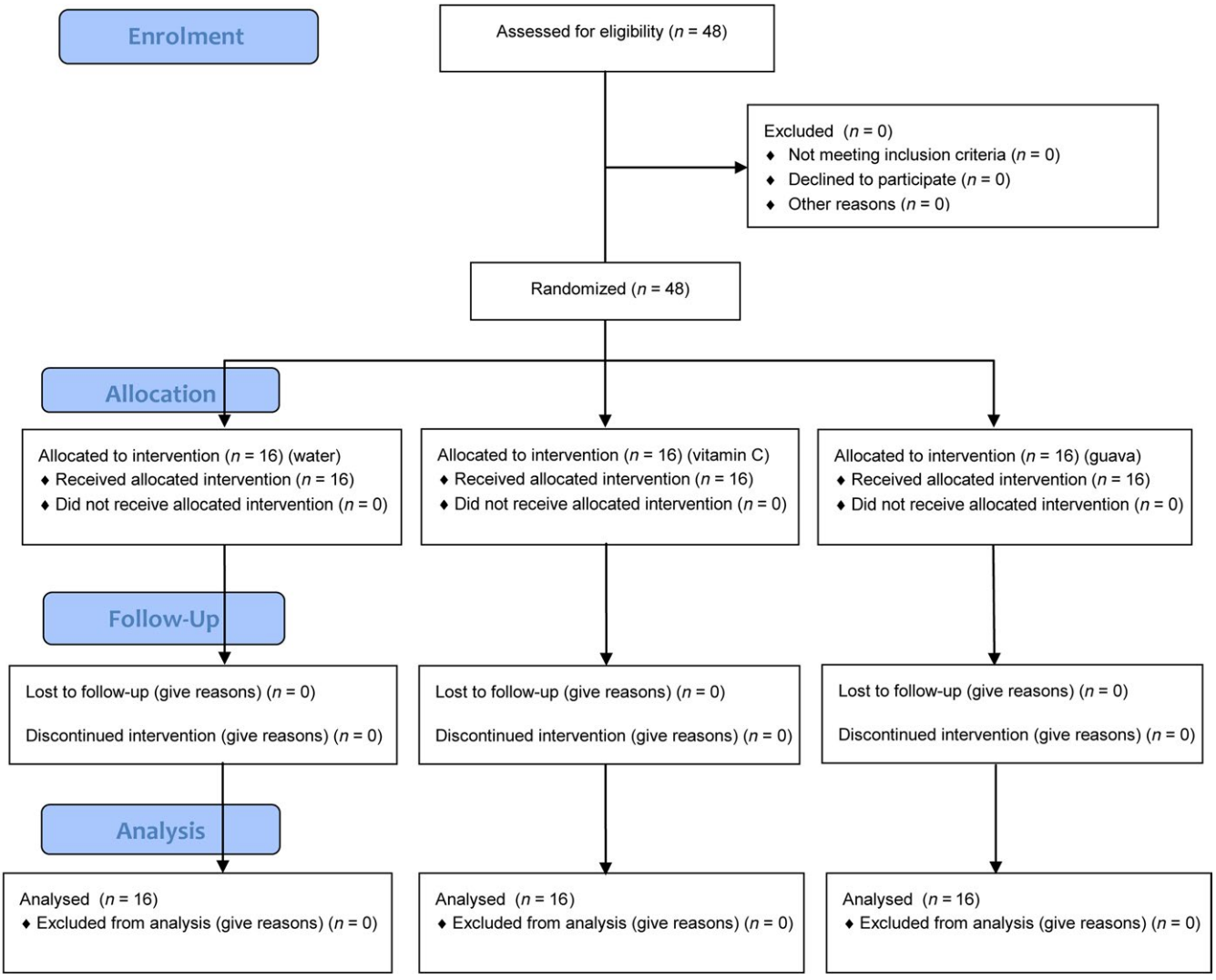

FIGURE 1 Participant flow diagram

guava fruit servings was negatively correlated with the amount of periodontal alveolar bone loss, suggesting that increased consumption of guava fruit may play a protective role in periodontitis of a malnourished population (Amaliya, Laine, Delanghe, Loos, & Van der Velden, 2015). Guava fruit is well-known for its anti-inflammatory, antimicrobial, antioxidant, antidiarrheal and antimutagenic properties. The important constituents of guava are vitamins, tannins, phenolic compounds, flavonoids, essential oils, sesquiterpene alcohols and triterpenoid acids (Barbalho et al., 2012). Moreover, guava fruit contains almost four times the amount of vitamin C compared to oranges (USDA, 2016). On the basis of the above, it can be hypothesized that guava fruit consumption may have a good potential to support gingival health.

The purpose of the present investigation was to study the effect of guava fruit and synthetic vitamin C on the development of gingival inflammation during experimental gingivitis. Three groups were investigated, a guava fruit group, a synthetic vitamin C group and a control group.

2 | MATERIAL AND METHODS

The study design was a single blind, randomized controlled trial. The participants consisted of 48 first year undergraduate students from the Dental School, Universitas Padjadjaran (UNPAD)—Bandung, West Java, Indonesia (Figure 1). The students were living in private dorms at the campus in Jatinangor where undergraduate dental education is provided and where the study was carried out. Inasmuch no previous investigations were available of the effect of guava consumption on experimental gingivitis, it was not possible to base the sample size on a power calculation. After receiving an in-depth explanation of the study proceedings and possible adverse events, candidates who volunteered to participate were asked to sign a written informed consent form. The study protocol was approved by The Ethics Committee in Hasan Sadikin Hospital Bandung—West Java Indonesia (1279/UN6.C1.3.2/KEPK/PN/2016). Participants were selected according to the following inclusion criteria: (1) 18–25 years of age, (2) nonsmokers, (3) no probing pocket depth ≥4 mm at any site and bleeding on probing <10%. Exclusion criteria for subjects were: (1) having systemic diseases, (2) being pregnant or giving breastfeeding, (3) having physical or mental handicaps that may interfere with an adequate oral hygiene, (4) having a history of drug abuse, (5) using nonsteroidal or steroidal anti-inflammatory drugs, analgesic or antibiotics within 6 weeks before the study, (7) having untreated carious lesions, crowns or orthodontic appliances in the lower jaw.

The study included a 14 days pre-experimental period, in which the participants received detailed oral hygiene instructions, thorough scaling, prophylaxis and supplementation. Participants were randomly assigned to one of 3 groups, each consisting of 16 students. Randomization was performed in the traditional Indonesian way. The name of each group was written, in required numbers, on a small piece of paper which was rolled up and put into an opaque bottle with lockable small opening. For each participant, the bottle was shaken with closed cap by a research assistant. After opening, one paper came out and determined the group allocation of the participant. As supplement, group I (Guava group) received about 200 grams guava (Psidium guajava L.) fruit cv. Susuputih once a day, while group II (Vitamin C group) was given a glass of water and a tablet of 200 mg synthetic vitamin C (IPI®, PT. Supra Ferbindo Farma, Cikarang—Indonesia), and group III (Control group) a glass of water only (without knowing what was in the water); groups are further referred to as guava, vitamin C and control group. The provided guavas were cultured in Majalengka, West Java and students consumed both flesh and skin part of the fruit. The 200 grams guava contained circa 260 mg vitamin C (Dondy, Sabari, & Syaifullah, 1991). All supplementation during the entire study period, including the weekends, was performed under supervision of the research assistant who was also responsible for the concealed assignment of the group allocation. Furthermore, all participants were asked to consume during the experimental period as little fruit and vegetables as possible and to keep a diary in which on every day all consumed fruits and vegetables had to be noted in terms of type, amount (pieces/cup) and preparation (raw/cooked). At the end of each week all information was handed over to the research assistant. By means of the National Nutrient Database for Standard Reference (USDA, 2016) the total amount of vitamin C dietary intake during the 4 weeks experimental period was calculated of raw consumed fruit and vegetables. In addition, the research assistant recorded age, sex and body mass index (BMI kg/m^2) of the participants. To determine under-/overweight, the World Health Organization (WHO) classification system for Asians was used (WHO Expert Consultation, 2004): severe underweight (<16), moderate underweight (16–16.9 kg/m^2), mild underweight (17–18.49 kg/m^2), normal (18.5–24.9 kg/m^2), preobese (25–29.9 kg/m^2), obese class I (30–34.9 kg/m^2).

After the 14 days pre-experimental period, including supplementation, the experimental gingivitis period started. All participants were asked to refrain from any oral hygiene measures of all teeth in the lower jaw for a period of 14 days, while continuing cleaning of the teeth in the upper jaw as usual. To avoid cleaning of the teeth in the lower jaw when brushing the teeth in the upper jaw, the teeth in the lower jaw were covered by a customized soft acrylic guard. The supplementation was continued until the end of the experimental gingivitis period.

The clinical parameters were assessed at the start of experimental gingivitis (Day 0) as well as on Day 7, and Day 14 of the experimental gingivitis period. The clinical parameters included the Plaque Index (PII) and the Gingival Index (GI) (Löe, 1967). The bleeding component of the GI was assessed in the way of the force controlled Angulated Bleeding Index (Van der Weijden et al., 1994). A Hawe click-probe (Kerr Dental, Orange, CA, USA) was held at an angle of approximately 60° to the long axis of the tooth and in contact with the sulcular soft tissue, stretching the gingiva with a controlled lateral force of 0.25 N. All measurements were carried out under the same conditions by one calibrated examiner who was blinded

regarding the group allocation throughout the study, from the start of the pre-experimental supplementation to the end of the experimental gingivitis period. Reproducibility measurements in 10 other dental students, 8 hours apart, showed an intraclass correlation coefficient of 0.98 and 0.82 for plaque and gingivitis measurements, respectively. Clinical parameters were assessed at the mesial, mid, and distal surfaces from the buccal and lingual aspect of all the teeth in lower jaw except the third molars. After the experimental period, subjects received a thorough oral prophylaxis, resumed their habitual oral hygiene procedures and were released from dietary restrictions.

2.1 | Data analysis

Descriptive statistics and data analyses were carried out by U.V.d.V., who was blinded for the group allocation, with SPSS version 20.0 (SPSS, IBM, New York, NY, USA). Demographic characteristics between study groups were analysed by means of one-way analysis of variance (ANOVA) and Chi-Square test where appropriate. Mean values for PII and GI were calculated per individual. In addition, as part of the analysis, the GI scores were dichotomized into no or slight gingivitis without bleeding (GI scores 0 and 1) and marked gingivitis with bleeding (GI scores 2 and 3), referred to as bleeding gingivitis score (BGS 0 or 1, respectively). The primary outcome was GI at Day 14, secondary outcomes were PII and BGS at Day 14. Both primary and secondary outcomes were tested also on Day 7. Data were analysed according to the intention-to-treat (ITT) analysis. Changes of parameters were analysed in two ways: (i) Changes over time within experiment groups were analysed with analysis of variance (ANOVA) for repeated measures adjusted for confounding factors age, gender and amount of daily dietary vitamin C intake per kg body weight during the 4-week experimental period. (ii) Differences in changes between experimental groups over time were analysed with a linear mixed model analysis including regimen variable and session variable, adjusting for the particular outcome variable at baseline and the potential confounding factors. Five models were employed: model 1: an unadjusted, model 2: adjusted for age and sex, model 3: adjusted for age, sex and BMI, model 4: adjusted for amount of daily dietary vitamin C intake per kg body weight and model 5: adjusted for age, sex and amount of daily dietary vitamin C intake per kg body weight. In addition, tests for differences between the 2 supplementation groups included analyses with adjustment for total amount of daily vitamin C intake (dietary and supplemented combined) per kg body weight. p-values <0.05 were considered statistical significant.

3 | RESULTS

All participants completed the pre-experimental and supplementation part of the study. The mean age of the 48 participants was 19.8 years without significant differences between groups and ranged in all 3 groups between 18 and 22 years. The study population consisted of 19 males and 29 females which was unbalanced distributed over the 3 study groups (Table 1). On average the BMI of the groups amounted to about 21 (kg/m^2) without significant differences between groups. Nevertheless, in the control group the BMI ranged from moderate underweight to obese class I whereas in the guava group the BMI ranged from severe underweight to preobese (Table 1). Also, no differences could be assessed in terms of body weight. The results of the amount of dietary vitamin C consumed by the 3 groups during the 4-week experimental period are presented in

TABLE 1 Demographic characteristics of the three study groups

	Experimental groups			
	Control	Vitamin C	Guava	
Variable	N = 16	N = 16	N = 16	p-value
Age (year ± SD)	19.6 ± 1.6	20.2 ± 1.3	19.5 ± 1.4	0.22
Sex (male/female)	2/14	11/5	6/10	0.005
BMI (kg/m^2)	21.37 ± 4.03	21.32 ± 2.72	20.88 ± 3.05	0.90
BMI categories[a]				
Underweight (N)				
Severe	–	–	1	0.45
Moderate	1	–	–	
Mild	1	3	1	
Normal weight (N)	12	12	12	
Overweight (N)				
Preobese	1	1	2	
Obese class I	1	–	–	
Body weight (kg)	59.44 ± 14.13	54.25 ± 4.87	54.94 ± 9.14	0.31

Note. [a]BMI cut-off points (kg/m^2): severe underweight (<16), moderate underweight (16–16.9), mild underweight (17–18.49), normal (18.5–24.9), preobese (25–29.9), obese class I (30–34.9).

TABLE 2 Daily mean (*SD*) dietary, dietary plus supplemented and total vitamin C intake per kilogram body weight during the 4-week study period

Variable	Experimental groups			*p*-value
	Control *N* = 16	Vitamin C *N* = 16	Guava *N* = 16	
Dietary vitamin C (mg) intake per day	6.92 ± 7.89	5.49 ± 7.43	4.55 ± 6.10	0.65
Dietary + supplemented vitamin C (mg) intake per day	6.92 ± 7.89	205.51 ± 7.42	264.55 ± 6.09[a]	<0.0001

Note. [a]significant different from vitamin C group ($p < 0.001$).

Table 2. It can be seen that the dietary consumption of participants amounted to about 5–6 mg vitamin C per day, without significant differences between groups. The mean total vitamin C intake per day amount to 6.92 mg in the control group and to significant higher values in the vitamin C and guava group (205.51 and 264.55 mg/day, respectively).

The pre-experimental period in which the participants received oral hygiene instructions, scaling, prophylaxis and supplementation resulted in comparable low PII, GI and BGS values at Day 0. During the 2 weeks of oral hygiene abstention, PII increased significantly in all 3 groups (Table 3). Further analysis showed that the guava group developed less plaque than the control group (PII: 1.33 versus 1.77, respectively), whereas no significant difference could be assessed between plaque development of the vitamin C group compared to the control group (Table 3). This result was found for all statistical models (Supporting Information Table S1a).

With regard to gingival inflammation, GI increased significantly in both the vitamin C and the control group, whereas no increase in the guava group could be assessed (GI: 0.23, 0.87 and 0.10, respectively, Table 3). Statistical comparison of the 3 groups showed that the GI increase during the entire 14 days experimental period as well as during the second week was less in both the guava and vitamin C group compared to the control group (Table 3, Supporting Information Table 1b). A statistically significant difference between the guava and vitamin C group, in terms of GI increase during the 14 days experimental period was present according to the unadjusted statistical model and when adjusting only for amount of daily dietary vitamin C intake per kg body weight. This significant difference was lost in the other models (Supporting Information Table 1b). For gingival inflammation in terms of BGS, a significant increase could be assessed only in the control group (28.36). Comparison of the 3 groups showed that during the entire 14 days experimental period as well as during the second week both the guava and vitamin C group developed lower BGS than the control group (Table 3, Supporting Information Table 1c). In terms of BGS no statistically significant differences could be assessed between the guava and vitamin C group (Supporting Information Table 1c).

Analyses including adjustment for total amount of daily vitamin C intake (dietary and supplemented combined) per kg body weight showed no differences between the guava and vitamin C group in terms of plaque and gingival inflammation development.

It is interesting to note that in the guava group one participant, despite the guava supplementation, developed an extremely high GI value (Day 0: 0.32, Day 7: 0.38, Day 14: 1.39) and BGS (Day 0: 8.9%, Day 7: 14.2%, Day 14: 48.4%) compared to the others in that group. This participant appeared to be a 22-year-old male with a BMI of 14.3 (kg/m^2).

4 | DISCUSSION

Results of the present study showed that both guava and synthetic vitamin C supplementation can have an inhibitory effect on the development of gingival inflammation during experimental gingivitis in a group of nonsmoking young adults. Smoking individuals were excluded because several studies demonstrated a reduced bleeding response in experimental gingivitis trials (Bergström & Preber, 1986; Danielsen, Manji, Nagelkerke, Fejerskov, & Baelum, 1990; Lie, Timmerman, Van der Velden, & Van der Weijden, 1998). As far as we are aware, only one study investigated the effect of vitamin C supplementation on experimental gingivitis previously, but found no effect (Vogel et al., 1986). In that study a daily dose of 1,500 mg vitamin C supplementation was used in subjects, having prior to the study a daily dietary vitamin C intake amounting to almost 200 mg. As has been suggested, subjects already saturated with vitamin C through their daily diet will efficiently excrete any surplus and are therefore highly unlikely to benefit from further vitamin C supplementation (Lykkesfeldt & Poulsen, 2010). Therefore it is not surprising that Vogel et al. (1986) found no effect of vitamin C supplementation on response to experimental gingivitis. Hence, in the present study participants were asked to consume as little fruit and vegetables as possible. This advice was well followed, as can be seen by the low dietary vitamin C intake of about 5 mg per day during the 4 weeks experimental period. This implies that the students in the control group actually participated in a kind of vitamin C depletion experiment. In a depletion and supplementation study in young healthy adults (Leggott et al.,1986), first a 2 weeks period of 60 mg/day vitamin C supplementation was introduced to allow body levels of the vitamin to stabilize. This was followed by 4 weeks of 5 mg/day vitamin C intake provided by basal diet. Results showed significant increases of bleeding sites during the third and fourth week of that period (Leggott et al., 1986). With regard to the present experimental gingivitis study, this would imply that the

TABLE 3 Mean values (*SD*) of the clinical parameters during 14 days of experimental gingivitis

Variable	Time in days	Control group (*N* = 16)	Within control group difference	Vitamin C group (*N* = 16)	Within vitamin C group difference	Guava group (*N* = 15)	Within Guava group difference
	0	0.50 ± 0.27		0.42 ± 0.22		0.61 ± 0.32	
			1.71 ± 0.38		1.65 ± 0.54		1.28 ± 0.55[a]
	7	2.21 ± 0.52[1]		2.07 ± 0.46[1]		1.89 ± 0.54[1]	
			0.07 ± 0.41		−0.04 ± 0.30		0.02 ± 0.61[a]
Plaque Index	14	2.28 ± 0.45[1]		2.03 ± 0.44		1.91 ± 0.54[1]	
Increase	0–14	1.79 ± 0.35[5]		1.61 ± 0.51[5]		1.30 ± 0.63[5,a]	
	0	0.15 ± 0.12		0.20 ± 0.18		0.19 ± 0.13	
			0.59 ± 0.30		0.15 ± 0.22[b]		−0.02 ± 0.07[b]
	7	0.74 ± 0.31[1]		0.35 ± 0.23[3]		0.17 ± 0.12	
			0.28 ± 0.33		0.09 ± 0.21[b]		0.12 ± 0.28[b]
Gingival Index	14	1.02 ± 0.30[1,4]		0.44 ± 0.22[1,4]		0.29 ± 0.33	
Increase	0–14	0.87 ± 0.29[5]		0.23 ± 0.28[5,b]		0.10 ± 0.30[b]	
	0	2.67 ± 3.15		4.01 ± 5.99		2.68 ± 3.95	
			9.04 ± 10.81		2.80 ± 7.44		0.58 ± 3.49
	7	11.71 ± 11.66[2]		6.81 ± 7.70		3.26 ± 3.63	
			19.32 ± 21.67		1.07 ± 7.48[b]		4.20 ± 10.25[b]
Bleeding Gingivitis Score (%)	14	31.03 ± 21.55[4]		7.88 ± 7.20		7.46 ± 11.51	
Increase	0–14	28.36 ± 21.40[5]		3.87 ± 8.10[b]		4.78 ± 8.89[b]	

Notes. [1]significant different from Day 0 ($p < 0.0001$), [2]significant different from Day 0 ($p < 0.01$)), [3]significant different from Day 0 ($p < 0.05$), [4]significant different from Day 7 ($p < 0.008$), [5]significant increase over time ($p < 0.005$), [a]significant different from increase in control group ($p < 0.05$), [b]significant different from increase in control group ($p < 0.001$), Analysis of [1-5] by means of ANOVA for repeated measures and [a,b]by means of linear mixed model analysis adjusted for age, sex and amount of daily dietary vitamin C intake per kg body weight.

increase in gingivitis in the control group could be partly attributed to vitamin C depletion. However, this seems not the case here; 30% bleeding sites after 14 days of experimental gingivitis in the control group (all nonsmokers) is not particular high and comparable to the results of other studies using a 14 days experimental gingivitis model (Giannopoulou, Cappuyns, & Mombelli, 2003; Lie et al., 1998; Slawik et al., 2011). Moreover, the study of Lie et al. (1998), employing an identical way of provoking bleeding and calculating the bleeding score, found also 30% bleeding in nonsmokers after 2 weeks of experimental gingivitis.

In the present study, one participant of the guava group had a BMI score of 14.3, which is a severely malnourished condition (BMI < 16.5, WHO Expert Consultation, 2004). It has been shown that such a condition in otherwise healthy individuals is related with increased plasma levels of TNFα, Th-1 cytokines IFN-γ, IL-2 and IL-12 as well as Th-2 cytokines IL-4, IL-5 and IL-13. Although neutrophil capacity to phagocytose is not impaired, the capacity to produce reactive oxygen species is impaired. It was suggested that these dysfunctional immune responses displayed in healthy severely malnourished individuals might contribute to increased susceptibility to infectious diseases (Takele et al., 2016). This suggestion is supported by the present finding that despite guava consumption this subject developed 48% BGS.

Several studies have shown in the experimental gingivitis model that gingival inflammation drives plaque formation (Daly & Highfield, 1996; Goldman, Abelson, Mandel, & Chilton, 1974; Hillam & Hull, 1977; Ramberg, Lindhe, Dahlen, & Volpe, 1994). This phenomenon could also explain the finding in the present study that, in contrast to the control group, the guava group failed to develop significant gingival inflammation during the 2 weeks of experimental gingivitis and consequently developed less plaque. On the other hand, although the vitamin C group developed significantly less gingival inflammation compared to the control group, the vitamin C group did not develop significantly less plaque. Another explanation for the reduced plaque development in the guava group may be a local effect of guava components. Guava contains a number of flavonoids including quercetin (Bhagwat & Haytowitz, 2015). It has been shown that quercetin has antibacterial activities against the growth of a number of oral bacteria including *Streptococcus mutans*, *Streptococcus sanguis* and *Streptococcus sobrinus* (Shu et al., 2011), possibly contributing to the reduced plaque development in the guava group.

On the basis of GI measurements that failed to show a significant increase in gingival inflammation in the guava group, it may be suggested that guava supplementation has a greater preventive effect against development of gingival inflammation than pure vitamin C. Such a phenomenon could not be confirmed by the BGS. In an apparent way, the changes in colour and oedema preceded the development of bleeding on angulated probing. This is in accordance with the structure of the GI in which a score 1 is given to sites with mild inflammation that do not bleed when a probe is run along the soft tissue wall of the entrance of the gingival crevice (Löe, 1967). In a large experimental gingivitis study, including 96 participants, Trombelli et al. (2004) showed clear differences in the behaviour of the GI without the bleeding component and the Angulated Bleeding Index (AngBI) during experimental gingivitis. Development of inflammation during the first 2 weeks as measured by means of the AngBI was slower when compared to the GI. However, in the period of 14 to 21 days the GI doubled whereas the AngBI tripled. Therefore, it is likely that, to show more clearly differences between guava and pure vitamin C against development of gingival inflammation, the experimental gingivitis should have lasted for 3 weeks instead of the present 2 weeks.

An important aspect of the present study is that all participants were asked to consume during the entire experimental period of 4 weeks as little fruit and vegetables as possible. As mentioned before, this advice was well followed resulting in about 5–6 mg vitamin C per day. Therefore, the present results have to be interpreted with care because it is unclear whether the same inflammatory preventive effect of vitamin C supplementation would be found in subjects who comply with the current RDA of vitamin C, that is 90 mg and 75 mg per day for man and women, respectively.

A limitation of the study is the relatively short period of experimental gingivitis which did not allow to make firm conclusions regarding the greater inflammatory preventive effect of guava compared to vitamin C alone. In addition, it should be remembered that this study was carried out in a group of nonsmokers. Therefore, it is currently unknown to what extent the present results can be extrapolated to smokers.

In conclusion, in a population of young nonsmoking adults consuming 5 mg vitamin C per day during 4 weeks, consumption of either 200 g guava/day or 200 mg synthetic vitamin C/day prior to and during the oral hygiene abstention period has a preventive effect on the development of experimental gingivitis as compared to the control group that developed the usual amount of experimental gingivitis.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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