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The effects of cyclodextran on plaque accumulation in healthy Japanese adults: A randomized, placebo-controlled, double-blind, parallel-group comparison study

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ABSTRACT

Background: Cyclo-isomalto-oligosaccharides (CI) have been reported to inhibit glucosyltransferases (GTF) in vitro. GTF is an enzyme related to oral plaque synthesis. However, there are few clinical studies to investigate the effect of CI on oral plaque via inhibiting GTF activity.

Objective: The purpose of this study was to evaluate the inhibitory effect of consuming CI-Dextran mix on plaque accumulation in healthy Japanese adults.

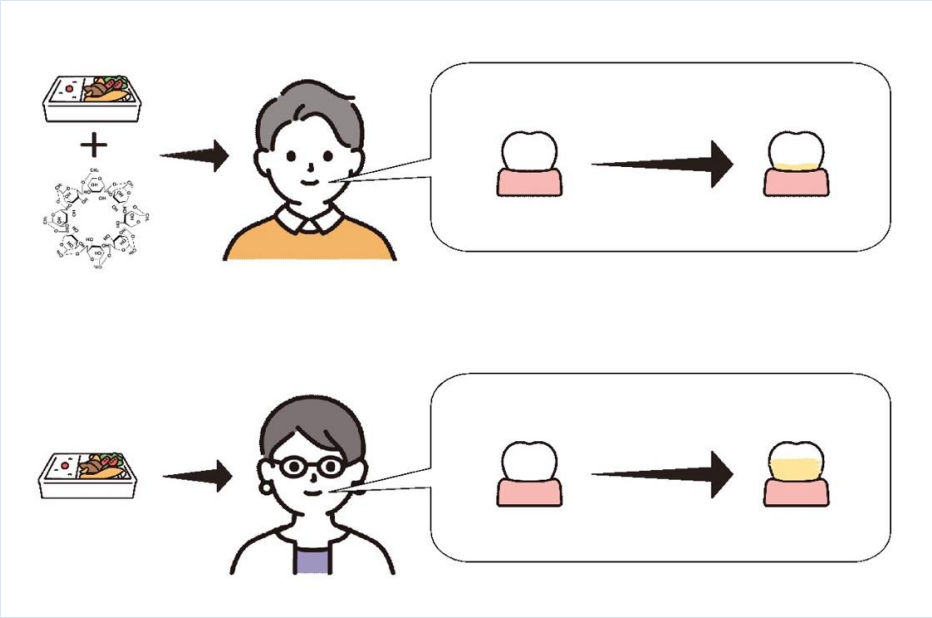
Methods: This randomized, placebo-controlled, double-blind, parallel-group comparison study was conducted from May 26, 2022, to September 16, 2022. Individuals who agreed to participate in the study were randomly assigned to the CI-Dextran mix group with 60 mg/day (n = 22), the CI-Dextran mix group with 600 mg/day (n = 11), or the placebo group (n = 22). The intervention period was three days, and the outcome of this study was the plaque index (PII), an indicator of plaque accumulation.

Results: Our results showed that the CI-Dextran mix group (600mg/day) had significantly lower post-intervention PII values than the placebo group in a full analysis set (FAS). In individuals identified as prone to plaque accumulation among the FAS, both CI-Dextran mix groups showed significantly lower PII values than the placebo group. No adverse events were observed during the study period, and consumption of the test food under the study conditions was considered safe.

Conclusions: Our results clearly indicated that a high dose of CI-Dextran mix (600 mg/day) could significantly reduce plaque formation in healthy Japanese adults, while a marginal reduction was noted for the group taking a low dose (60 mg/day). In particular, intake of both doses (CI-Dextran mix 60 mg/day and 600 mg/day) in individuals who were prone to plaque accumulation, could significantly inhibit plaque formation.

Keywords: CI-Dextran mix, cyclodextran, plaque inhibition, functional carbohydrates, isomaltooligosaccharide, Randomized Controlled Trial

Trial registration: UMIN000047901. **Foundation:** Nissin Sugar Co., Ltd.



INTRODUCTION

Dental plaque, a biofilm formed by oral microorganisms, is the primary cause of dental caries, gingivitis, and periodontal disease. These conditions are considered lifestyle-related diseases which are preventable through the maintenance of appropriate dental health behaviors and habits. “Health Japan 21 (the second term)”, which was published by the Japanese government, provides a basic direction for comprehensive implementation of national health promotion, and has “Dental and Oral health” as one of its main goals [1].

In general, a wide variety of microorganisms reside in the oral cavity, which in turn associate with one another to form a biofilm (plaque). The process of plaque formation consists of the following steps: plaque is a highly adhesive substance formed by insoluble glucan and *mutans streptococci*. *Streptococcus mutans* and *Streptococcus sobrinus* have the ability to synthesize insoluble glucans in the oral cavity using sucrose as a substrate, and the resulting *mutans streptococci* bind to the tooth surface [2]. First, *S. mutans* adhere to the tooth surface by hydrophobic bonds between the protein antigen c on the surface layer of *S. mutans* and salivary components in the pellicle, which is the organic film derived from saliva and salivary glycoproteins [3]. Next, glucans are synthesized from sucrose by glucosyltransferases (GTFs) produced by the bacteria themselves, causing *S. mutans* to aggregate through

glucans [4]. Glucans also bind to the surface proteins of *S. mutans*, forming glucan-binding proteins, which subsequently enhance the adhesion between *S. mutans* and the tooth surface, facilitating biofilm (plaque) accumulation [5].

Plaque is often recognized as a feel rough on the teeth [6,7] before brushing, and the oral microflora can be disturbed by excessive sugar intake and inadequate oral care, thereby promoting biofilm development [8]. In mature plaque, the acid produced by *S. mutans* sucrose metabolism demineralizes the tooth surface, leading to the development of caries [9]. Furthermore, excessive plaque accumulation in the oral cavity is known to cause gingivitis and periodontitis [10,11]. While the definitive link between periodontitis and systemic diseases remains under investigation, numerous studies have independently identified periodontopathogenic bacteria and the ensuing immune-inflammatory response as contributory factors in the development of various conditions. These include, but are not limited to, diabetes, atherosclerotic cardiovascular disease, chronic obstructive pulmonary disease, Alzheimer's disease, chronic kidney disease, and rheumatoid arthritis [12–14]. Moreover, other studies have shown that the number of teeth present may be related to functional impairment [15], and tooth loss may be lessened with a lower plaque index [16]. In addition, caries and periodontal disease are the main causes of tooth extraction in Japan [17]. The above studies suggest that it is very important to maintain a healthy oral environment, especially by reducing plaque accumulation, in order to prevent the development of dental caries, gingivitis, and periodontitis, thereby reducing the risk of systemic disease and the likelihood of tooth loss.

In recent times, there has been a growing interest in functional foods as a strategy for mitigating disease risk [18,19]. Among these functional foods is the CI-Dextran mix, which incorporates cyclo-isomalto-oligosaccharides (CI). Nissin Sugar Co., Ltd. employs a cyclization enzyme reaction on dextran to produce the CI-Dextran mix. CI, the main component of the CI–Dextran mix, has been suggested to inhibit GTFs in both in vitro and in vivo studies [2,20]. For example, an in vitro study by Asaumi et al. confirmed that CI could diminish the inter-fungal cohesion of plaques formed by S. mutans in a dose-dependent manner [21]. The inhibitory effect of CI on GTFs has been thought to occur as a result of CI-binding to the active sites of GTF as a substrate, preventing the transfer of glucose residues generated from sucrose [22,23]. This hypothesis is also supported by Asaumi et al. [21], in a study that demonstrated CI as a potent inhibitor of GTFs. Depending on the degree of polymerization, CI can consist of 7–12 glucose units (CI-7 to CI-12), and GTFs inhibition has been confirmed for all these cases [24]. Therefore, it is expected that foods containing a CI–Dextran mix may reduce plaque accumulation by interfering with glucan synthesis and weakening inter-mutans streptococci binding forces in plaques. However, to the best of our knowledge, there have not been any previous studies verifying the inhibitory effect of consuming a CI-Dextran mix on plaque accumulation. Therefore, the purpose of the present study was to evaluate the inhibition of plaque accumulation due to the consumption of a CI–Dextran mixes in Japanese adults.

METHODS

Study design: The Ethics Committee of Takara Clinic (Medical Corporation Seishinkai, Tokyo, Japan) conducted an ethical review of the protocol for this randomized, placebo-controlled, double-blind, parallel-group comparison study, which was subsequently approved on May 25, 2022 (Approved no. 2205-03379-0047-2E-TC). In addition, this study was registered with the clinical trial registration database (University hospital medical information network in Japan clinical trials registry, registration no. UMIN000047901). This study was also conducted in accordance with the latest Helsinki Declaration and the ethical guidelines for medical and

biological research involving human subjects in Japan.

Study participants: The target population of this study was healthy Japanese adults, and the following exclusion criteria were applied: Individuals undergoing treatment for, or with a history of, malignant tumors, heart failure, or myocardial infarction; Individuals with an implanted pacemaker or implantable cardioverter defibrillator; Individuals undergoing treatment for arrhythmia, liver damage, kidney damage, cerebrovascular disease, rheumatism, diabetes, dyslipidemia, hypertension, or other chronic diseases; Individuals who regularly consume "Food for Specified Health Uses (FOSHU)," "Foods with Function Claims," medicines, including herbal medicine, and other supplements; Individuals with allergies to medicine or test food-related substances; Individuals who are pregnant, breastfeeding, or intending to become pregnant during the study period; Individuals with a current coronavirus infection; Individuals who have already participated in other clinical trials within 28 days prior to the consent form acquisition date, or those who plan to participate during the trial period; Individuals judged by the study director to be inappropriate subjects for this study; Individuals wearing dentures, false teeth, or other dental appliances such as, implants or bridges; Individuals who regularly use gargles, toothpaste, medicated toothpaste, floss, interdental brushes, and mouth rinses; Individuals who have or are suspected of having a history of Sjogren's syndrome; Individuals with dental disease and/or diseases related to salivary glands, such as xerostomia and sialolithiasis; Individuals undergoing treatment for periodontal disease, caries, or other reasons; and Individuals with a smoking habit or those who quit smoking within one year prior to the consent date.

Recruitment of participants was conducted on the monitor recruitment site "GO-TOROKU" (https://www.go106.jp/) operated by ORTHOMEDICO Inc. (Tokyo, Japan). The study contents were fully explained to individuals who agreed to participate in the study via a network or face-to-face interviews, and their consent was obtained electronically or hand-written. Study participants did not involve individuals affiliated with the sponsoring or funding company of the present study. The responsible organization for the study was the Medical Corporation Yuseikai, Horiuchi Dental Clinic (Tokyo, Japan). Blood and urine tests performed during the screening test at the time of study inclusion and safety evaluation during the intervention period were confirmed by the collaborating institutions Medical Corporation Seishinkai, Takara Clinic (Tokyo, Japan).

Additionally, the participants were asked to comply with the following instructions: Participants were to consume the test foods provided according to the prescribed usage and dosage; Participants had to maintain a consumption rate of the test food of ≥80%; Participants had to avoid binge eating and drinking and not change their lifestyle habits until the day of the final test (conducted the morning after three days of intervention); Participants were not to drink alcohol or exercise excessively from the start of the intervention period until the morning of the final test; If participants were to experience any changes in their physical condition during the study period, they were obliged to immediately contact the contract research organization (CRO) and receive instructions on their following steps; During the study period, participants had to refrain from consuming "FOSHU," "Foods with Function Claims," and other foods/drinks that are thought to have functional properties; During the study period, participants had to take thorough measures to prevent coronavirus and other infections (e.g., by thoroughly washing and disinfecting their hands, wearing a mask, etc.); and participants were not to brush their teeth, use floss, interdental brushes, and mouth rinses, or consume any foods/drinks outside of the prescribed diet from the beginning of the intervention until the morning of the final test.

Intervention: Study participants were given food in tablet form with (CI group; CI–Dextran mixes 60 mg/day [CI low-dose group] or 600 mg/day [CI high-dose group]) or without (placebo group) a CI–Dextran mix (manufactured by Nissin Sugar Co., Ltd.). The components per tablet in each intervention were as follows: the CI low-dose group contained 20 mg of CI–Dextran mix and 980 mg of excipients; the CI high-dose group contained 200 mg of CI–Dextran mix and 800 mg of excipients; and the placebo group contained 0 mg of CI–Dextran mix and 1000 mg of excipients.

Regarding the intake method and period, we referred to current evidence for foods with functional claims containing epigallocatechin gallate, whose mechanism of action is partially similar (GTFs inhibitory effect) to that of CI [20,25]. The prescribed usage and dosage were then set to one tablet at a time, three times a day, after breakfast, lunch, and dinner, and the intervention period was three days.

All test foods were in the form of tablets, and an ethical review before the beginning of the present study confirmed that both CI-containing and placebo foods could not be distinguished by color, odor, or flavor.

Previous studies were referred to determine the daily intake dose of CI–Dextran mix. Current literature has confirmed the development of anti-caries and anti-halitosis effects in human clinical trials using CI-containing gel and CI-containing spray [26]. Regarding the safety of CI, studies on single-dose toxicity, chronic toxicity, acute toxicity, subacute toxicity, and reverse mutation have been conducted, including a human clinical trial (each study was based on unpublished data). In human clinical trials using the above-mentioned CI-containing spray [26], intake of CI–Dextran mix was approximately 40 mg/day. However, this CI-containing spray also contains hop extract, which has been reported to have an inhibitory effect on plaque formation [27,28]. Thus, we conducted an in vitro study to examine whether the GTFs inhibitory effects could be attributed to CI alone (unpublished data) and set a CI–Dextran mix intake of 60 mg/day. Furthermore, no adverse events were observed in a human clinical trial in which individuals were given 2,000 mg/day or 4,000 mg/day of CI–Dextran mix for four weeks. Based on these previous studies of CI, we set the intake dose for this study accordingly, and subsequently examined the effect of consuming a CI–Dextran mixes on plaque accumulation and its dose-dependency at doses of 60 mg/day (CI low-dose group) and 600 mg/day (CI high-dose group)..

Assessment items: The test schedule is shown in Table 1. Measurement items for effectiveness evaluation were conducted on the morning after the screening test and after three days of the intervention (i.e., at day 4 after the beginning of the intervention). In addition, measurement items for safety evaluation included the incidence of adverse events during the intervention period based on a diary kept by study participants. All participants had their dental surface polished on the morning of the first day of the intervention, and they were asked to follow a specific diet prescribed by the CRO throughout the intervention period. Participants were also prohibited from brushing their teeth, including flossing, interdental brushes, and mouth rinses, until after the post-intervention examination.

Primary outcome: The plaque index (PlI) was the primary outcome of this study, and the evaluation method was based on the report published by Terajima *et al.* [29] Intraoral images of the upper and lower jaws and the labial surfaces of teeth numbered 1–3 on the left and right sides were obtained. Additionally, the observation area of teeth was the cervical of the labial side and the bottom, center, and top when the tooth surface of the labial side was divided horizontally into three parts (from the cervical to the tooth tip). The average PlI for each tooth was calculated using a modified version matching the Quigley and Hein's plaque scoring system [30] and

Debris index [31]. Furthermore, as the participants in this study had their calculus removed before the intervention, we compared the PII values after the intervention period to evaluate the effect of foods containing a CI– Dextran mix on plaque formation.

Table 1. Study schedule.

		Briefing	Screening test	Enrollment	Allocation Intervention period	Intervention period			
						Day 1	Day 2	Day 3	Day 4
Registration	Eligibility screen	X		X					
	Informed consent	X							
	Other procedures	X							
	Allocation				X				
Intervention	CI-Dextran mix (low dose)					←----------	----------	----------	----------→
	CI-Dextran mix (high dose)					←----------	----------	----------	----------→
	Placebo					←----------	----------	----------	----------→
	Prescribed diet					Lunch and dinner	Breakfast, lunch, and dinner	Breakfast, lunch, and dinner	Breakfast
Assessment	Plaque evaluation								X
	Calculus removal					X			
	Dental examination								
	Physical measurement	X	X						
	Urinalysis		X						
	Blood test		X						
	Medical interview		X						X
	Diary recording					←----------	----------	----------	----------→

"X" indicates the implementation time, while an arrow shape indicates the intake or recording period.

Safety evaluations: Physical measurements and urinalysis were performed to confirm the health of participants, ensuring that they did not suffer from any disease at the time of enrollment.

Physical measurements included weight, body mass index, body fat percentage, systolic blood pressure, and diastolic blood pressure, while urinalysis provided measurements of protein, glucose, pH, and occult blood. Furthermore, blood tests included white blood cell count, red blood cell count, hemoglobin, hematocrit value, platelet count, aspartate aminotransferase, alanine aminotransferase, γ-glutamyltranspeptidase, total bilirubin, total protein, urea nitrogen, creatinine, uric acid, sodium, potassium, chloride, serum amylase, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, glucose, and hemoglobin A1c (NGSP). Both urinalysis and blood tests were conducted by LSI Medience Corporation

(Tokyo, Japan).

The number of adverse events was recorded. In the event of symptoms recognized as adverse events (i.e. poor physical condition during the exam period), the investigator immediately took the necessary and appropriate action, including deciding whether the participant was eligible to participate in the study and whether the emergency key could be unlocked. In addition, the investigator was required to evaluate the relationship between adverse events and test foods, but also report the results.

To confirm the health status of study participants, we conducted interviews and a dietary survey using the Calorie and Nutrition Diary (CAND) [32] on each test day. Study participants also maintained daily diaries to record their living conditions, including intake of test foods, potential changes in their physical condition, and medication use.

Sample size: The target number of participants was set at 50, which was the maximum number that could be achieved considering our budget. In addition, we decided to increase the number of participants by five to account for dropouts during the study period, bringing the total number of participants to 55.

Selection, randomization, and blinding: Of the 75 participants who agreed to participate in this study, the principal investigator enrolled 55 based on the eligibility criteria. The test foods were provided by Nissin Sugar Co., Ltd. to the CRO. The individual in charge of shipping the test food at the CRO confirmed that the test food was indistinguishable, and both input and confirmed the screening test data. The identification number was communicated to the person responsible for allocation who was not directly involved in the study. We employed a stratified random allocation method using gender and age as the adjustment factors. The allocator used a computer-generated randomization list to include 22 participants in the placebo group, 22 participants in the CI low-dose group, and 11 participants in the CI high-dose group (assignment ratio of 2:2:1). The created allocation table was provided only to the person in charge of shipping the test food at the contracted clinical testing organization and subsequently to the study participants based on the allocation table. The principal investigator, sub-study physician, and all staff members of the medical institution conducting the study, i.e., study director, implementation management manager, monitoring person, statistical analysis person/responsible person, etc., staff of the CRO, members of the ethics committee, and clinical testing commissioning organizations were all blinded to the allocation included. The allocation table was sealed and kept by the person responsible for allocation until the cases and statistical analysis methods were fixed.

Statistical method: All statistical analyzes were performed using two-tailed tests, and the significance level was set at 5%. The software used was the SPSS ver. 23.0 (IBM Japan Ltd, Tokyo, Japan).

Primary outcomes were presented as means and standard deviations and were compared between groups using a general linear model having the group as a factor. Between-group comparisons were performed between the CI low-dose and placebo groups, and between the CI high-dose and placebo groups. All comparison data were presented as between-group differences (the value of the CI low-dose or CI high-dose group minus the placebo group) and 95% confidence intervals (CIs).

The analysis dataset was constructed based on the following definitions. The intention-to-treat set involved all participants enrolled in the present study. Next, a full analysis set (FAS) was defined when cases were excluded because they did not receive the allocated intervention, did not meet eligibility criteria, never received an intervention after allocation, and had no post-allocation data. In addition, among the FAS, the data set was a per-protocol analysis set when individuals meeting the

following criteria were excluded: (1) cases whose intake rate of the test food was less than 80%; (2) cases with behavior that affected the reliability of the test results, such as missing diary records; (3) cases found to meet the exclusion criteria after enrollment; (4) cases found to have violated compliance rules during the study period; (5) cases who took foods or medications that could be expected to significantly affect the test results; (6) cases engaged in activities that were significantly different from their lifestyle at the time of enrollment; and (7) other cases in which there were clear reasons why exclusion was deemed appropriate.

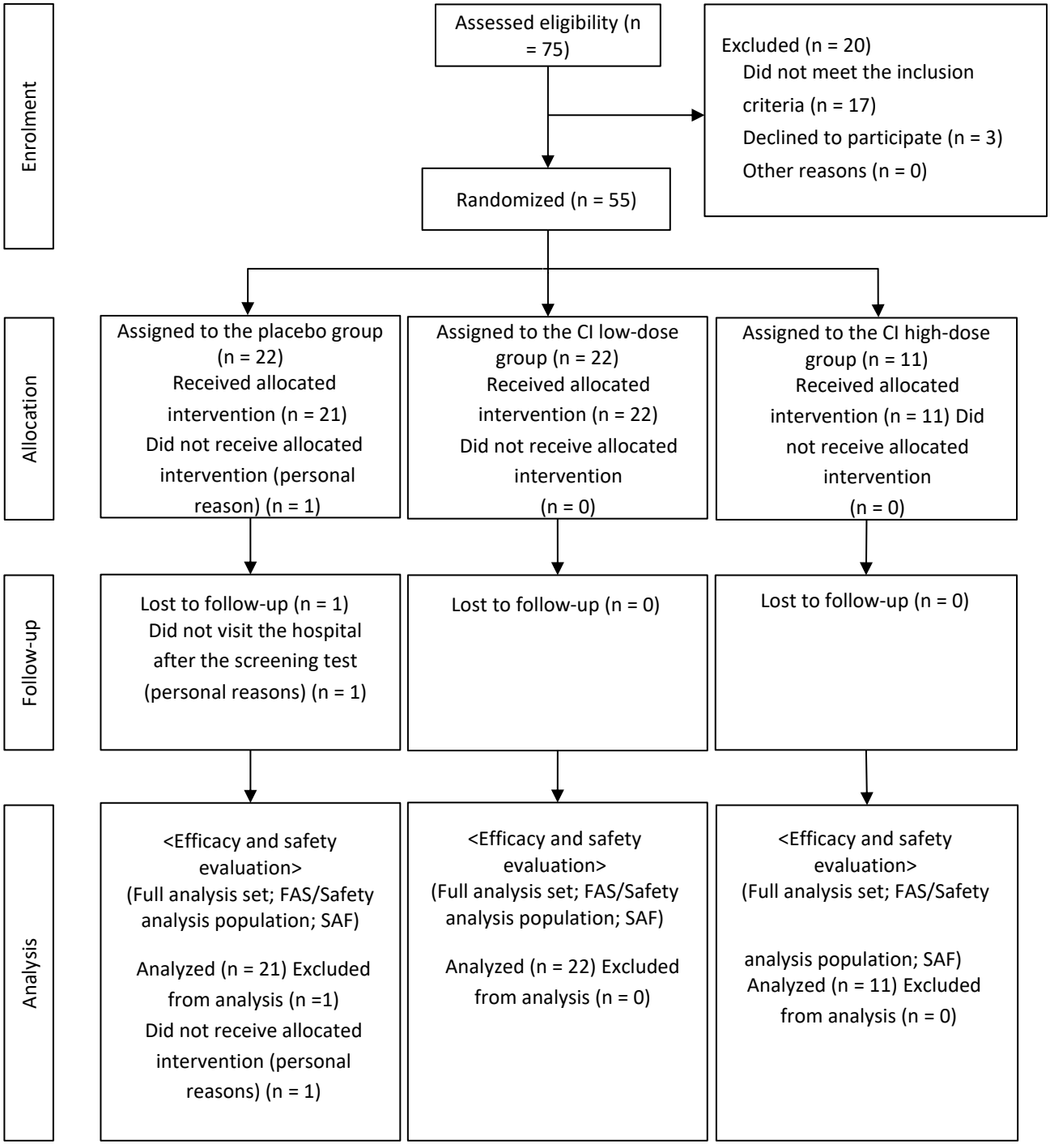


Figure 1. Flow diagram our study protocol.

Table 2. Characteristics of study participants

		ITT			FAS、SAF		
		Placebo group	CI low-dose group	CI high-dose group	Placebo group	CI low-dose group	CI high-dose group
Gender	Men	11 (50.0%)	11 (50.0%)	6 (54.5%)	11 (52.4%)	11 (50.0%)	6 (54.5%)
	Women	11 (50.0%)	11 (50.0%)	5 (45.5%)	10 (47.6%)	11 (50.0%)	5 (45.5%)
Age (years)	Mean (SD)	41.9 (12.3)	43.0 (11.8)	43.0 (12.2)	42.1 (12.6)	43.0 (11.8)	43.0 (12.2)
	Med	43.0	46.0	38.0	45.0	46.0	38.0
	Min-Max	20-64	23-63	28-63	20-64	23-63	28-63
Height (cm)	Mean (SD)	164.3 (8.2)	163.1 (8.7)	165.9 (4.8)	164.2 (8.4)	163.1 (8.7)	165.9 (4.8)
	Med	164.65	164.50	166.20	164.60	164.50	166.20
	Min-Max	142.3-177.0	143.6-174.1	157.0-175.0	142.3-177.0	143.6-174.1	157.0-175.0
Weight (kg)	Mean (SD)	59.0 (12.2)	61.7 (16.1)	64.0 (9.5)	59.4 (12.3)	61.7 (16.1)	64.0 (9.5)
	Med	55.75	59.40	64.60	57.50	59.40	64.60
	Min-Max	45.8-83.0	38.6-99.4	48.9-80.1	45.8-83.0	38.6-99.4	48.9-80.1
Body mass index (kg/m^2)	Mean (SD)	21.7 (3.5)	23.0 (4.4)	23.2 (2.9)	21.9 (3.4)	23.0 (4.4)	23.2 (2.9)
	Med	20.85	21.70	23.20	21.00	21.70	23.20
	Min-Max	16.2-28.0	15.6-32.8	18.9-28.0	16.2-28.0	15.6-32.8	18.9-28.0
Systolic blood pressure (mmHg)	Mean (SD)	114.5 (13.6)	112.8 (12.9)	117.1 (15.1)	115.0 (13.7)	112.8 (12.9)	117.1 (15.1)
	Med	113.0	111.0	117.0	113.0	111.0	117.0
	Min-Max	91-137	90-143	97-147	91-137	90-143	97-147
Diastolic blood pressure (mmHg)	Mean (SD)	72.5 (11.3)	73.1 (9.8)	75.6 (11.8)	73.1 (11.1)	73.1 (9.8)	75.6 (11.8)
	Med	71.5	72.5	75.0	72.0	72.5	75.0
	Min-Max	47-93	56-99	58-100	47-93	56-99	58-100

Gender is expressed by the number of participants and the proportion within the group, and other items are expressed as the mean, standard deviation (SD), median (Med), minimum (Min), and maximum (Max) value. ITT, intention to treat set; FAS, full analysis set; SAF, safety analysis population.

Additionally, the present study conducted an analysis that excluded individuals whose plaque accumulation volume exceeded 1 mm at the post-intervention evaluation. With respect to PII, a study investigating the average PII after three days of intervention by Simonsson *et al.* [33] reported that the PII value of individuals with a tendency to accumulate plaque was 2.6, while the PII value of individuals who did not accumulate plaque was 0.6. In addition, referring to the clinical status for each PII value shown by Volgenant *et al.* [34], PII 2 was defined as "a thin continuous band of plaque (≤1 mm) at the cervical margin of the tooth," and PII 3 was defined as "a band of plaque wider than 1 mm which, however, covered less than one-third of the crown of the tooth." By referring to the research findings of Simonsson *et al.*, Volgenant *et al.,* determined that individuals with a PI of ≥2 were prone to accumulate plaque. In contrast to Simonsson *et al.,* where the actual PII was 2.6, we considered that an individual was prone to plaque accumulation if the amount of plaque

exceeded 1 mm. Thus, we additionally verified its effectiveness in inhibiting plaque accumulation in individuals prone to plaque accumulation.

RESULTS

Analysis set: Figure 1 shows the follow-up flowchart for our study participants. Recruitment took place between May 26, 2022, and June 29, 2022, and the study period was from May 26, 2022, to September 16, 2022. After enrollment, one participant from the placebo group was excluded from the analysis because the assigned intervention was not received. The final analysis target was FAS. The characteristics of the study participants are shown in Table 2.

PII: The CI high-dose group showed significantly lower post-intervention PII values than the placebo group in FAS, while the CI low-dose and high-dose groups showed significantly lower post-intervention PII values than the placebo group in healthy participants assumed to be prone to plaque accumulation (Table 3). These results the suppressing effect of consuming foods containing high doses of CI–Dextran mixes on plaque formation in healthy Japanese adults with normal plaque accumulation. In addition, plaque formation in healthy Japanese adults who were prone to plaque accumulation was found to be suppressed by the consumption of foods containing both low and high doses of a CI–Dextran mix.

Table 3. Average plaque index per tooth

Data set		n	Mean	SD	Group differences	95%CIs	*P* value
FAS	Placebo group	21	9.1	2.2	-	-	-
	CI low-dose group	22	7.8	2.5	–1.3	–2.84 to 0.22	0.091
	CI high-dose group	11	6.7	3.0	–2.4	–4.26 to –0.54	0.013*
Subgroup with FAS and average plaque amount >1 mm per tooth	Placebo group	19	9.5	1.9	-	-	-
	CI low-dose group	21	8.0	2.4	–1.5	–3.03 to –0.01	0.048*
	CI high-dose group	11	6.7	3.0	–2.8	–4.59 to –0.99	0.003*

Data are shown as mean and standard deviation (SD). The between-group difference was the subtracted value of the placebo group from the CI low-dose group or CI high-dose group, and its 95% confidence intervals (CIs) are shown. *$P < 0.05$

Confirmation of adverse events: No adverse events were observed during the study period, and consumption of the test food under our study conditions was considered safe.

DISCUSSION

The goal of this study was to verify the inhibition of plaque accumulation due to the intake of a CI–Dextran mix (low-dose, CI-Dextran mix 60 mg/day; high-dose, CI-Dextran mix 600 mg/day) in healthy Japanese adults.

Our findings showed that the mean PII per tooth was significantly lower in the CI high-dose group than in the placebo group in the FAS. Furthermore, the mean PII per tooth was significantly lower in the CI low-dose and high-dose groups than in the placebo group in the subgroup of individuals prone to plaque accumulation, which was determined based on the decision criteria published in Simonsson *et al*. [33] and Volgenant *et al.* [34].

A study evaluating the effect of cetylpyridinium chloride mouthwash as an adjunct to tooth brushing on interdental plaque compared with a placebo in a systematic review [35] found that the use of cetylpyridinium chloride mouthwash significantly

reduced PII compared with the use of a placebo, and the mean difference between interventions and 95%CIs was –0.70 (–0.83 to –0.57). In another meta-analysis on interdental plaque scores using toothbrush bristle hardness and different bristle tip shapes [36], the PII was significantly reduced in the group using the softer tapered toothbrush compared with the group using the softer, round-tipped (non-tapered) one, and the standardized mean difference and 95 %CIs were –2.64 (–4.26 to –1.01). Furthermore, in a meta-analysis of the adjuvant effect of dentifrice on overnight plaque regeneration inhibition [37], the use of dentifrice containing ferrous fluoride or triclosan was found to be beneficial, and the mean differences in PII and 95%CIs were –0.33 (–0.49 to –0.16) and –3.15 (–4.61 to –1.69), respectively. Therefore, it can be inferred that if the group difference in PII is –0.70 to –3.15, the difference can be considered medically meaningful. In our study, the between-group differences from the placebo group and 95%CIs were as follows: the CI high-dose group in the FAS was –2.4 (–4.26 to – 0.54), the CI low-dose group in individuals prone to plaque accumulation was –1.5 (–3.03 to –0.01), and the CI high-dose group in individuals prone to plaque accumulation was –2.8 (–4.59 to –0.99). These between-group differences were within the range that is considered medically meaningful (–0.70 to –3.15). Therefore, the significant differences between groups identified in the present study can be considered medically meaningful, indicating that the CI–Dextran mix is effective in inhibiting plaque accumulation.

Both *in vitro* and *in vivo* studies have shown that CI in the CI–Dextran mix could inhibit GTFs [2,20], and it has been speculated that this inhibitory effect occurs when CI binds as a substrate to its active site, thereby preventing the transfer of glucose residues generated from sucrose [22,23]. Consequently, it was considered that the CI–Dextran mix-containing food could also reduce plaque accumulation by interfering with glucan synthesis via GTFs inhibition and by weakening the inter-*mutans streptococci* binding forces in the plaque.

Previous studies demonstrated distinct differences in salivary constituents among individuals [38], salivary secretory characteristics, which have been suggested to influence caries development [39], and bacterial diversity in saliva, which may influence plaque accumulation [40]. Moreover, since sex and aging have been reported to affect the oral microflora [41,42], the differences in salivary constituents and oral microflora in the participants of the present study could have influenced our results, and this is one of the limitations of the present study. As salivary constituents, secretory characteristics, and composition of oral flora were not set as a factor for inclusion, future studies should investigate the effect of CI in more detail by targeting individuals with similar salivary constituents, salivary secretory characteristics (e.g., salivary viscosity and salivary volume), and oral flora. In addition, since we excluded individuals having teeth with crown restorations, it is essential to verify in the future whether the inhibitory effect on plaque accumulation is also observed in this category of participants.

The target population in the present study was healthy Japanese adults, and the FAS was considered to represent healthy Japanese adults with a normal range of plaque accumulation or those who are prone to plaque accumulation. In healthy Japanese adults with a normal range of plaque accumulation, a plaque accumulation inhibitory effect was observed by taking a CI-Dextran mix of 600 mg/day. In contrast, a plaque accumulation inhibitory effect was observed in healthy Japanese adults who were more prone to plaque accumulation by taking a CI-Dextran mix of 60 mg/day and 600 mg/day. Although we need to underline the fact that our sample size was determined by our limited budget, suggesting a possible bias to our results due to the small number of participants, the present study showed that CI was associated with plaque accumulation in healthy Japanese adults. To our knowledge, this is the first study to

demonstrate the plaque accumulation inhibitory effect of CI, and we are confident that our findings will pave the way for further research studies conducted on this topic.

CONCLUSION

The present study examined the changes in the oral cavity of healthy Japanese adults who consumed a CI–Dextran mix-containing food (60 mg/day or 600 mg/day) for a period of three days. The results showed that a high-dose CI–Dextran mix (600 mg/day) could significantly reduce plaque formation, while a low-dose CI–Dextran mix (60 mg/day) revealed a marginal reduction. In particular, for individuals prone to plaque accumulation, intake of both doses (CI–Dextran mixes 60 mg/day and 600 mg/day) significantly inhibited plaque formation. Of note, our study reported no adverse events.

List of Abbreviations: GTFs, glucosyltransferases; CI, dextran; FOSHU, Food for Specified Health Uses; CRO, contract research organization; PII, plaque index; CAND, Calorie and Nutrition Diary; 95%CIs, 95% confidence intervals; FAS, full analysis set.

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