Continuous partially hydrolyzed guar gum intake reduces cold-like symptoms: a randomized, placebo-controlled, double-blinded trial in healthy adults

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Abstract. – OBJECTIVE: Partially hydrolyzed guar gum (PHGG), a water-soluble dietary fiber produced by the controlled partial enzymatic hydrolysis of guar gum beans, has various physiological roles. PHGG is expected to influence the immune function and prevent infections. The objective of this study was to examine the effect of continuous ingestion of PHGG for 12 weeks on the development of cold-like symptoms.

PATIENTS AND METHODS: A placebo-controlled, double blind, randomized, parallel-group comparative study was conducted. 96 healthy Japanese adults received 5.2 g PHGG or placebo daily for 12 weeks. Cold-like symptoms were assessed based on patient diary, and the levels of short-chain fatty acids (SCFAs) in stool and blood immune markers at baseline and at weeks 6 and 12.

RESULTS: The cumulative number of “no symptoms” days for all symptoms was significantly larger in the PHGG than in the placebo group. The result of the analysis by severity of cold-like symptoms also showed significant differences, with the PHGG group having a lower severity of cold-like symptoms. Propionic acid at weeks 6 and 12 and n-butyric acid and total SCFAs at week 12 were significantly higher in the PHGG than in the placebo group. The Interferon-γ level was significantly lower at week 6 in the PHGG than in the placebo group.

CONCLUSIONS: PHGG intake may affect immune function and suppress cold-like symptoms through the production of SCFAs in healthy adults.

Key Words:  
PHGG, Short-chain fatty acids, Butyric acid, Cold-like symptoms, Immune function.

Introduction

As Japan is facing a super-aged society, there is a growing interest in healthy lifestyle. It is extremely important to maintain and promote health through food. The pandemic of coronavirus disease 2019 has affected many people in Japan. In this context, there is an increasing demand among consumers to improve and maintain their immunity by cultivating healthy dietary habits. However, the immunostimulatory and immune function-maintaining effects of various diets have not been sufficiently investigated in humans, highlighting the need to accumulate scientific data by conducting human intervention studies.

PHGG is a water-soluble dietary fiber obtained from the enzymatic degradation of guar gum in the endosperm of guar beans (Cyamopsis tetragonolobus), a leguminous plant cultivated in northern India and Pakistan. The usefulness of PHGG has been demonstrated in terms of improvement in the intestinal flora1 and bowel movement4,5 and inhibition of diarrhea2 as well as postprandial blood glucose levels6.

PHGG is expected to influence the immune function and prevent infections. A previous animal study7 revealed the stimulatory effect of PHGG on the innate immunity through the production of SCFAs in the intestine, leading to the inhibition of intestinal Salmonella infection. In addition, an in vitro study8 suggested the possibility of preventing an infection against influenza virus associated with PHGG consumption. A
Effect of partially hydrolyzed guar gum on cold-like symptoms

Clinical, retrospective, observational study\(^9\) of 522 patients in rehabilitation or long-term care wards showed that the incidence of influenza was significantly lower after continuous intake of PHGG. However, no randomized controlled trials have examined the effects of PHGG on cold-like symptoms, although there are studies that suggest the effects of PHGG on immunity and prevention of infections by pre-clinical study and observational study in clinical.

The objective of this placebo-controlled, randomized, parallel-group comparative study was to examine the effect of continuous ingestion of PHGG for 12 weeks on the development of cold-like symptoms mediated by its immune regulatory functions.

Patients and Methods

This study was conducted in compliance with the ICH-GCP and the Declaration of Helsinki (revised at the World Medical Association's General Assembly in Fortaleza in October 2013). The feasibility and ethical and scientific validity of this clinical study were reviewed and approved by the Bioethics Committee of Hokkaido Information University to conduct the study at the Health Information Science Center of Hokkaido Information University. The study was conducted based on the test plan approved by the Ethics Review Committee (approval date: 25 November 2020; approval number: 2020-29), and was registered with UMIN (approval number UMIN000042753; 15 December 2020).

Subject

A total of 100 subjects who met the inclusion criteria, but not the exclusion criteria, listed below were considered eligible for the study.

Inclusion Criteria

1. A person who fully understood the significance, content, and objective of this study and provided written informed consent to participate.
2. A Japanese man or woman aged 20 years to < 65 years at screening.
3. A person with a relatively high total score of 18 items related to psychological stress in the Brief Job Stress Questionnaire.

Exclusion Criteria

1. A person who is under treatment, medication, or lifestyle guidance by a physician for any autoimmune, chronic inflammatory, or allergic disease.
2. A person who is to receive influenza vaccination during the period from the screening test to the end of intake.
3. A patient with serious cerebrovascular disease, heart disease, liver disease, kidney disease, gastrointestinal disease, or infection requiring notification.
4. A person with a history of major gastrointestinal surgery such as gastrectomy, gastrointestinal suture, and intestinal resection.
5. A person with a marked abnormality in blood pressure, physical measurements, or blood tests.
6. A person with severe anemia.
7. A pre- or post-menopausal woman with a marked change in physical conditions.
8. A person with a risk of allergic symptoms caused by drugs, foods (especially soybean or peanut), or white birch pollen.
9. Regular use of drugs (antibiotics, immunosuppressants, anti-inflammatory drugs, antirheumatic drugs, antihistamines, anti-allergic drugs, Lactobacillus preparations, etc.), health foods, or supplements (containing constituents of mushrooms, seaweeds, nucleic acids, yeasts, lactic acid bacteria, etc.) that may affect immune function.
10. A heavy smoker, alcoholic, or person with an extremely irregular lifestyle.
11. A woman who donated 400 mL of blood within 16 weeks, a man who donated 400 mL of blood within 12 weeks, and any person who donated 200 mL of blood within 4 weeks or blood components within 2 weeks before the start of test food intake.
12. A woman who is pregnant, may be pregnant, or is breastfeeding.
13. A person who is currently participating in another clinical study or has participated in another clinical study within 4 weeks.
14. A person who is judged ineligible by the investigator.

Test Food

A sachet containing 5.2 g of PHGG (7.2 g) (Isocal\(^\text{®}\) Fiber, Nestlé Health Science Company, Nestlé Japan Ltd. Tokyo, Japan) and a sachet containing dextrin (7.2 g) were prepared as the test and control foods, respectively. Each test food was individually packaged with plain aluminum to ensure the blinding of subjects and persons.
who performed the interventions. Table I lists the nutritional composition of each test food.

**Study Design and Food Intake Method**

This was a randomized, double blind, placebo-controlled, parallel-group comparative study. The screening test was conducted from December 15 to 21, 2020. Subjects were assigned to two groups as follows by the allocation manager: the PHGG group received the test food and the placebo group received the placebo food by the stratified permuted block method using sex, age distribution, influenza vaccination status in the 2020/2021 season, and the total score of 18 items related to psychological stress in the Brief Job Stress Questionnaire as stratification factors. Subjects lived their life as usual and were exposed to the normal risk of developing a cold in daily life.

The subjects took one sachet of the test or control food per day by dissolving it in a drink, etc., during a meal every day for 12 weeks. There were no restrictions on the intake time. The intake started from January 24 to 26, 2021 and ended during the period from April 17 to 19, 2021.

**Evaluation**

The primary endpoint was cold-like symptoms, and the secondary endpoints were secretory immunoglobulin A (IgA) levels, natural killer (NK) cell counts, CD4+ and CD8+ T cell counts, Treg cell count, serum immunoglobulin G (IgG) and cytokines (interferon [IFN]-γ and interleukin [IL]-10) levels, and SCFAs in stool. Cold-like symptoms, such as runny nose, plugged nose, sore throat, cough, general malaise, arthralgia, chill, constipation, and diarrhea were evaluated on an 8-point scale using the diary as follows: “No symptoms at all (0),” “Almost no symptoms (1),” “Very few symptoms (2),” “Slight symptoms (3),” “Apparent symptoms (4),” “Strong symptoms (5),” “Very strong symptoms (6),” and “Severe symptoms (7),” based on the WURSS-21 (Wisconsin Upper Respiratory Symptom Survey-21). The WURSS-21 has been validated and confirmed to perform well as an illness-specific health-related quality-of life questionnaire outcomes instrument10. In addition to the evaluation items of WURSS-21, constipation and diarrhea were also evaluated in this study. Responses to cold-like symptoms (runny nose, plugged nose, sore throat, cough, general malaise, arthralgia, chills, constipation, and diarrhea) of “1” or higher were defined as symptomatic, and the scores were calculated. For constipation and diarrhea, the subjects were asked to respond as symptomatic even if they did not have symptoms associated with a cold. The secondary endpoints were measured by collecting blood, stool and saliva samples at baseline, week 6, and week 12. The subjects were asked to collect saliva at home and keep it cool, and the saliva was analyzed on the same day. Secretory salivary IgA was measured by enzyme immunoassay. Blood samples were collected at the examination site, kept at room temperature, and analyzed on the same day for blood tests. Serum IgG was measured by turbidimetric immunoassay. IFN-γ and IL-10 was determined by immunoassay using Ella (Protein Simple Japan Co., Ltd.). NK cell count, CD4+ T cell count, CD8+ T cell count, and Treg cell count were measured by flow cytometry. Blood and saliva tests, except IFN-γ and IL-10, were performed at the Sapporo Clinical Laboratory Inc. (Hokkaido, Japan). IFN-γ and IL-10 were examined at Hokkaido Information University (Hokkaido, Japan). A fixed amount of stool samples was weighed into a bead tube, suspended in an extraction solution, and then heat-treated (85°C, 15 min). After crushing by beads, the samples were centrifuged (18,400 x g, 10 min), and the supernatant was filtered through a membrane filter with a pore size of 0.20 µm to make the sample solution. The concentration of SCFAs in the samples was analyzed by HPLC (pH-buffereed post-column electroconductivity detection).

<table>
<thead>
<tr>
<th>Table I</th>
<th>Nutritional ingredients per sachet (7.2 g) of the test and control foods.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Test food (PHGG)</td>
</tr>
<tr>
<td>Calorie</td>
<td>kcal</td>
</tr>
<tr>
<td>Proteins</td>
<td>g</td>
</tr>
<tr>
<td>Lipids</td>
<td>g</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>g</td>
</tr>
<tr>
<td>Sodium chloride equivalent</td>
<td>g</td>
</tr>
<tr>
<td>PHGG</td>
<td>g</td>
</tr>
</tbody>
</table>

...
The fecal SCFAs measurement was performed at TechnoSuruga Laboratory Co., Ltd. (Shizuoka, Japan).

For the analysis of SCFAs, the levels of acetic, propionic, and n-butyric acids and the total amount of these SCFAs in stool samples were measured before the start of intake and at weeks 6 and 12 after the start of intake of the test food.

**Statistical Analysis**

The target sample size of this study was determined with reference to a previous study. Assuming that the cumulative incidence of “No symptoms” for cold-like symptoms after consumption of the food was 92% in the test food group and 90% in the placebo food group, the number of cases required to assure the analysis result of the chi-square test at a one-sided significance level of 5% and power of 90% was 41 subjects per group. We also estimated that the proportion of subjects expected to discontinue the study and to be excluded from the analysis was within 15% and determined the target sample size to be 50 subjects per group.

The cumulative incidence of “No symptoms” for cold-like symptoms was calculated using the following formula:

\[
\text{Cumulative incidence of "No symptoms" (\%)} = \frac{\text{Number of responses of "No symptoms"}}{\text{Total number of responses}} \times 100
\]

Total number of responses = 84 days × 50 people = 4,200

Efficacy analyses were performed on patients with PPS (Per Protocol Set). In the diary of cold-like symptoms, which were included as the primary endpoint, the presence of “Almost no symptoms (1)” or higher was defined as symptomatic for each individual symptom item and the total of all items (all symptoms), and the cumulative number of days with the onset of symptoms was calculated for each group to perform statistical analysis using the chi-square test. For the amount of SCFAs in stool samples, the actual values at each measurement point were used to perform an unpaired Student’s t-test. For secondary endpoints other than SCFAs in stool, changes from baseline to week 6 and week 12 were calculated in each group and statistical analysis was performed using an unpaired Student’s t-test. A p-value less than 0.05 was considered statistically significant. SPSS version 25.0 (IBM Japan, Ltd., Tokyo, Japan).

**Results**

One subject dropped out because of hospitalization. In addition, we excluded three subjects for whom the evaluation was affected by changes in daily life during the study. Therefore, finally, 96 subjects were included in the analysis (Figure 1). The backgrounds of the subjects in each group are shown in Table II. No adverse events related to the test food were observed during the study period.

**Cold-Like Symptoms**

The cumulative number of days of “no symptoms” for runny nose, plugged nose, general malaise, arthralgia, chills, constipation, and diarrhea as well as for all the symptoms was significantly higher in the PHGG group than in the placebo group (Table III).

**SCFAs in Stool**

Given the insufficient amount of stool samples collected, each 1 subject at evaluation time points

| Table II. Subject demographics at baseline. |
|----------------|----------------|
| Item                  | PHGG group \(n = 49\) | Placebo group \(n = 47\) | p-value |
| Male/female           | 14/35            | 14/33            | 0.896   |
| Age (years old)       | 54.2±6.5         | 54.0±6.7         | 0.881   |
| Height (cm)           | 161.6±8.9        | 162.0±9.0        | 0.811   |
| Weight (kg)           | 56.4±9.7         | 56.5±12.3        | 0.949   |
| BMI (kg/m²)           | 21.5±2.4         | 21.4±3.3         | 0.839   |
| Body fat percentage (%) | 28.0±6.0      | 26.9±6.4         | 0.387   |
| Total score of the Brief Job Stress Questionnaire (points) | 24.0±8.1 | 24.0±7.7 | 1.000   |
| Influenza vaccination rate (%) | 36.7 | 34.0 | 0.783 |

Mean ± SD. Unpaired Student’s t-test and chi-square test were used to calculate p-values between groups.
before the start of intake and at weeks 6 and 12 of intake in the placebo group were excluded from the efficacy analysis set. Hence, propionic acid levels were significantly higher in the PHGG group than in the placebo group at week 6 (mean ± standard error, 1.094 ± 0.086 vs. 0.824 ± 0.061, p = 0.012) and week 12 (1.128 ± 0.086 vs. 0.882 ± 0.083, p = 0.039) as well as the levels of n-butyric acid at week 12 (0.934 ± 0.086 vs. 0.711 ± 0.063, p = 0.040), and total SCFAs at week 12 (4.382 ± 0.311 vs. 3.512 ± 0.244, p = 0.030) (Figure 2).

**Immunological Indicators**

Two subjects from the placebo group and one subject from the PHGG group were excluded from the efficacy analysis set, owing to their use of antibiotics and vaccination during the study period. Secretory salivary IgA levels, NK cell counts, Treg cell counts, and serum IgG levels increased from before the start of intake or were maintained in the PHGG group as compared to those in the placebo group, but no significant differences were noted between the groups. The amount of change in IFN-γ at week 6 was significantly lower in the PHGG group than in the control group (mean ± standard error, -0.452 ± 0.186 vs. 0.059 ± 0.168, p = 0.045) (Figure 3).

**Discussion**

The effects of PHGG on the onset of cold-like symptoms were investigated in this randomized,
Effect of partially hydrolyzed guar gum on cold-like symptoms

Table III. Results for cold-like symptoms.

<table>
<thead>
<tr>
<th>Item</th>
<th>Group</th>
<th>Cumulative number of days of &quot;no symptoms&quot;</th>
<th>Cumulative number of days of &quot;apparent symptoms&quot;</th>
<th>Rate of no symptoms (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Runny nose</td>
<td>PHGG</td>
<td>3,999</td>
<td>117</td>
<td>97.2</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>3,791</td>
<td>157</td>
<td>96.0</td>
<td></td>
</tr>
<tr>
<td>Plugged nose</td>
<td>PHGG</td>
<td>4,050</td>
<td>66</td>
<td>98.4</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>3,843</td>
<td>105</td>
<td>97.3</td>
<td></td>
</tr>
<tr>
<td>Sore throat</td>
<td>PHGG</td>
<td>4,050</td>
<td>66</td>
<td>98.4</td>
<td>0.133</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>3,867</td>
<td>81</td>
<td>97.9</td>
<td></td>
</tr>
<tr>
<td>Cough</td>
<td>PHGG</td>
<td>4,062</td>
<td>54</td>
<td>98.7</td>
<td>0.777</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>3,899</td>
<td>49</td>
<td>98.8</td>
<td></td>
</tr>
<tr>
<td>General malaise</td>
<td>PHGG</td>
<td>4,078</td>
<td>38</td>
<td>99.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>3,873</td>
<td>75</td>
<td>98.1</td>
<td></td>
</tr>
<tr>
<td>Arthralgia</td>
<td>PHGG</td>
<td>4,111</td>
<td>5</td>
<td>99.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>3,904</td>
<td>44</td>
<td>98.9</td>
<td></td>
</tr>
<tr>
<td>Chill</td>
<td>PHGG</td>
<td>4,086</td>
<td>30</td>
<td>99.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>3,873</td>
<td>75</td>
<td>98.1</td>
<td></td>
</tr>
<tr>
<td>Constipation</td>
<td>PHGG</td>
<td>3,335</td>
<td>781</td>
<td>81.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>2,885</td>
<td>1,063</td>
<td>73.1</td>
<td></td>
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<tr>
<td>Diarrhea</td>
<td>PHGG</td>
<td>3,791</td>
<td>325</td>
<td>92.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>3,525</td>
<td>423</td>
<td>89.3</td>
<td></td>
</tr>
<tr>
<td>All symptoms</td>
<td>PHGG</td>
<td>2,968</td>
<td>1,148</td>
<td>72.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>2,444</td>
<td>1,504</td>
<td>61.9</td>
<td></td>
</tr>
</tbody>
</table>

Chi-square test was used to calculate p-values between groups.

double blind, placebo-controlled, parallel-group comparative study on healthy adult men and women who took PHGG or dextrin (placebo) daily for 12 weeks. The results showed that the proportion of subjects without cold-like symptoms, including runny nose, plugged nose, general malaise, arthralgia, chills, constipation, diarrhea, and all symptoms, was significantly higher in the PHGG group than in the placebo group. The percentage of people with cold-like symptoms was not high, therefore the significant difference between the groups was observed, but the difference was small. Therefore, the effect of PHGG on cold-like symptoms may be expected. In addition, the PHGG group showed significantly higher values than the placebo group for propionic acid at weeks 6 and 12, n-butyric acid at week 12, and total amount of SCFAs at week 12.

It has been reported that soluble dietary fiber such as PHGG increases the production of immunoglobulin in spleen and mesenteric lymph node lymphocyte in rats. In addition, in vitro studies showed that PHGG dose-dependently suppressed the production of IL-6 and IFN-γ against influenza virus H1N1-induced inflammation in macrophage cells. PHGG is fermented by enteric bacteria in the intestine to produce SCFAs. In particular, butyric acid is known to be produced at higher levels than other dietary fibers. SCFAs produced by enteric bacteria regulate immune functions through their receptors. In vivo and in vitro study results suggest that SCFAs prevent infection through their effects on immune functions. Changes were observed in immunological indicators of both the innate and acquired immune systems attributable to the increased levels of SCFAs in the intestine following intake of PHGG; however, the difference between the groups was statistically significant only for the changes in IFN-γ. This observation may be associated with the apparent lack of statistical power.

Innate immunity functions as the first defense system against infection, and secretory salivary IgA and NK cells contribute to immunity. In this study, the change in salivary IgA level did not greatly differ between the two groups, but the level was maintained without any decrease at week 12 in the PHGG group as compared to that in the placebo group. The production of secretory IgA is increased by butyric acid, and the effect of acetic acid on CD4+ T cells, which supports the production of IgA, enhances not only the production but also the function of IgA. In this study, the level of n-butyric acid was significantly higher at week 12 in the PHGG group than in the placebo group. The level of acetic acid tended to increase at week 12 in the PHGG group and that of IgA was maintained without any decrease at week 12.
Figure 2. Ingestion of PHGG increase the SCFAs. All data are expressed as mean ± standard error. \( p \) values between groups were calculated by unpaired Student’s \( t \)-test. † \( p < 0.1 \), * \( p < 0.05 \).

Figure 3. Ingestion of PHGG effects on immunological indicators. All data are expressed as mean ± standard error. \( p \)-values between groups were calculated by unpaired Student’s \( t \)-test. † \( p < 0.1 \), * \( p < 0.05 \).
in the PHGG group. Therefore, one may suggest that the increase in butyric acid and acetic acid level after the intake of PHGG affected IgA production. The increase in the number of NK cells also tended to be higher in the PHGG group (no statistically significant difference), suggesting its positive effect on the innate immunity.

Considering the acquired immunity, CD4+ T cell count showed a more consistent increasing trend in the PHGG group and CD8+ T cell count tended to decrease in the placebo group but was maintained up to week 12 in the PHGG group. In addition, the amount of blood IgG, which acts on the secondary immune response, consistently decreased in the placebo group but tended to increase in the PHGG group at week 6. Although the level of IgG decreased at week 12 in the PHGG group, the degree of reduction was smaller than that in the placebo group. Previous studies with mice infected with influenza virus have shown that the administration of SCFAs before virus inoculation increased blood IgG levels16 and oral administration of butyric acid increased the number of CD8+ cells37.

The number of Treg cells, which inhibit excessive immune responses, tended to increase at weeks 6 and 12 in both groups, but the effect was more pronounced in the PHGG group. Intestinal butyric acid may affect Treg cell count, as reported in a study with mice, suggesting that there is a similar possibility in humans24. It is possible that the immune response was appropriately controlled by the action of Treg cells, which affected the onset of cold-like symptoms. In this study, the level of IFN-γ, an inflammatory cytokine of PHGG group, also significantly decreased at week 6 and tended to decrease at week 12 compared to placebo group, suggestive of the effect of Treg action. The increase of CD4+ T cells and CD8+ T cells did not cause the elevation of IFN-γ, which may have been suppressed by the increased Treg cells. IFN-γ level is known to increase in response to viral infections25, and the behavior of IFN-γ may also represent a low viral infection. A previous study26 with mice demonstrated how IFN-γ production contributed to an increased incidence of influenza virus infection, and the fact that IFN-γ production significantly decreased in the PHGG group is considered to be a meaningful effect. In addition, it has been reported27 that Treg cells change into follicular T cells in Peyer’s patches to promote the production of IgA. Therefore, PHGG is thought to exert effects on the innate immunity.

As described above, the onset of cold-like symptoms was suppressed in the PHGG group owing to the activation of the innate and acquired immunity and Treg-mediated immunity, consistent with a significantly higher production of SCFAs.

A limitation of this study is that no statistically significant results except for IFN-γ were obtained for immunological indices to discuss the mechanism underlying the decreased incidence of cold-like symptoms after PHGG consumption owing to the lack of statistical power. In addition, the immune response in humans is extremely complex, and the infection itself has a major impact on changes in immune indices, which has generated some unlinked immune markers. However, consistent results were obtained in terms of the amount of SCFAs, major innate and acquired immune markers, and cold-like symptoms, which are clinical determinants. Therefore, it may be considered that cold-like symptoms are suppressed by the effect of SCFAs on immune function.

Conclusions

This placebo-controlled, double-blind, randomized, parallel-group comparative study of 96 healthy adults suggested that the intake of PHGG may maintain immune functions and suppress the development of cold-like symptoms through the production of SCFAs by intestinal flora.

Acknowledgments

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Authors’ Contribution

Conflicts of Interest
This study was sponsored by NHS (Nestlé Health Science Company, Nestlé Japan Ltd). Seigo Sakai, Yuki-kazu Kamada, Hiroki Takano and Masaki Ichikawa are employees of NHS. Masaya Sasaki has received consultancy fees and honoraria for speaking at symposia from NHS.

Data Availability Statement
The data presented in this study are available on request from the corresponding author.

Informed Consent
Informed consent was obtained from all individual participants included in the study.

References


