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Ameliorating Effects of Pulverized Sesame Powder Containing Lignocellulose and Resistant Protein on Postprandial Triglyceride Elevation

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Abstract The insoluble fraction of the pulverized sesame powder (commercially available as LipiSmart®), which consisted of lignocellulose and resistant protein as active compounds, has been demonstrated to attenuate postprandial triglyceride (TG) elevation in rats presumably by reducing the intestinal absorption of micellized lipids. The present study aims at evaluating inhibitory effects of the pulverized sesame powder on the postprandial elevation of serum TGs in healthy individuals. A total of 83 normolipidemic healthy participants were exposed and assessed in this randomized, placebo-controlled, double-blind, two-arm crossover study. The participants were randomly allocated to either the pulverized sesame powder-placebo sequence or the placebo-pulverized sesame powder sequence. After an oral intake of 500 mg of the pulverized sesame powder or placebo following a standardized high-fat meal, postprandial concentrations of TG and several other lipidemic parameters were measured. For the per protocol subjects (n=77), no significant differences in postprandial TG responses or other lipidemic parameters were found between treatment arms. However, for the participants who tended to have heightened postprandial responses to dietary fat intake (i.e., baseline-corrected TG level≧83 mg/dL when placebo was ingested), intake of the pulverized sesame powder led to significant reduction of baseline-corrected TG concentration at 6h after meal (37.4 vs. 47.3 mg/dL, p-value=0.03) and incremental area under the curve (iAUC) of TG (409.2 vs. 451.4 mg/dL·h, p-value=0.04). These results suggest that the pulverized sesame powder containing lignocellulose and resistant protein represent a new dietary solution to reduce the postprandial elevation of serum TG for healthy individuals, particularly for those who tend to have heightened postprandial responses to dietary fat intake.

Keywords: *Insoluble dietary fiber, Lignocellulose, Luminacoid, Pulverized sesame powder, Resistant protein, Postprandial triglyceride-lowering effect*

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1. Introduction

Sesame (*Sesamum indicum* L.) is one of the oldest oilseed crops widely cultivated in the world with an annual global production of sesame seeds exceeding 6 million metric tons in 2021 [1]. While sesame seeds are primarily used for oil production, they are also extensively used in various processed foods with their health benefits being increasingly recognized [2]. Sesame seeds are rich in bioactive substances that are known to confer a broad range of health benefits. Among these bioactive substances, in particular, vitamin E, monounsaturated fatty acids, dietary fibers and lignans have been reported to exert blood lipid-lowering effects in animal models and human subjects [3]. The byproduct of sesame oil pressing or extraction is known as defatted sesame seeds. As the lipid-soluble components, such as vitamin E and monounsaturated fatty acids, are removed during oil pressing or extraction, the resulting defatted fraction is primarily composed of proteins and total dietary fiber, most of which is insoluble fiber [4]. The insoluble fiber of defatted sesame seeds is comprised of cellulose, hemicellulose and lignin [5], the crosslinking of which form lignocellulose and plant cell walls. Several reports showed that dietary fiber can enhance fecal bulk and reduce gastrointestinal transit time [6,7]. The proteins in defatted sesame seeds were shown to primarily consist of proteins that are insusceptible to proteolytic digestive enzymes [8,9], known as resistant protein. The proteins in sesame seeds are mainly 11S globulins (α-globulin) and

2S albumins (β-albumin). 2S albumins, in particular, are highly resistant to digestion by pepsin in simulated gastric fluid [8,9]. Resistant proteins in soybean and buckwheat have been reported to have cholesterol-lowering effects owing to their excellent capacity to capture bile acid and enhancing the fecal excretion of dietary cholesterol [10]. These findings highlight the great potential of defatted sesame seeds, as a rich source of dietary lignocellulose and resistant proteins, in modulating gastrointestinal health and postprandial handling of dietary lipids. Traditionally, defatted sesame seeds are used as feeds for ruminant livestock and poultry or to make compost; however, in fact, most of defatted sesame seeds are not being sufficiently utilized [4]. Therefore, as part of efforts to pursue the Sustainable Development Goals (SDGs), there is an urgent need to explore the added value of defatted sesame seeds.

High fasting TG level in the blood (> 150 mg/dL) is a known risk factor for cardiovascular diseases (CVD) and one of the diagnostic criteria of metabolic syndrome and often observed in patients with type2 diabetes [11]. Although blood TGs are traditionally measured in the fasting state, in recent years, high postprandial TG level (measurements are taken within 8 hours after meal) has been increasingly recognized as a causative factor for CVD, in particular atherosclerotic CVD, and accumulating evidence indicates that postprandial TG level is more strongly associated with cardiovascular events than fasting TGs, suggesting the prominence of a proatherogenic milieu induced by elevated postprandial TGs [12,13]. Therefore, development of medicines and functional foods that can reduce postprandial lipemia has attracted increasing attention in recent years. Curbing postprandial TG spikes represents a straightforward, effective and safe strategy to attenuate postprandial lipemia. For instance, orlistat, a gastrointestinal lipase inhibitor, has demonstrated beneficial effects on post prandial lipemia in obese, dyslipidemia and type2 diabetic patients. However, the gastrointestinal adverse effects of orlistat, such as oily stools, diarrhea and abdominal pain, limit its use by healthy people for the prevention and routine management of dyslipidemia and related conditions [14,15]. As safe alternatives to orlistat, several functional foods, such as indigestible dextrin and globin peptide, have been shown to be effective in reducing postprandial TG excursion [16,17]. The recommended dose of indigestible dextrin to exert postprandial TG-lowering effect is 5 g per meal, which is substantially higher than those of typical functional foods, and its application is restricted to dietary supplement and beverages [16]. Comparatively, globin peptide is consumed at a lower dose (1 g per meal), but the fact that it is derived from porcine may restrict the scope of its application due to religious concerns [17]. These facts highlight a need for novel functional foods with greater potency against postprandial lipemia and a broader scope of applications. Plant-based materials, including sesame seeds, which are widely available and generally face no religious restrictions, represent a rich and diverse source of functional foods in this regard.

Recently, we have reported that the insoluble fraction of pulverized defatted sesame seeds, which is primarily composed of lignocellulose and resistant proteins, acts as a suppressor of postprandial TG spikes. Specifically, a significant decrease in postprandial TG concentration was observed in rats administrated with a single oral dose of 45 mg/kg body mass of the insoluble fraction following a high-fat diet challenge [18]. The human equivalent dose (HED) was estimated to be 5.8 mg/kg or 348 mg for an adult of 60 kg, according to Equation 1 in Nair and Jacob [19]. We hence speculate that the insoluble fraction of pulverized sesame seeds can exert postprandial TG-lowering effects in humans at a lower dose comparing to indigestible dextrin or globin peptide. We have also found that the insoluble fraction of pulverized sesame seeds was capable of reducing the absorption of the lipids micellized by bile acids in vitro. We consider that this may be one of the mechanisms that underlie the postprandial TG-lowering effects of the insoluble fraction in vivo [18].

It is known that postprandial blood TG elevation increases the risk of atherosclerosis and coronary heart disease [13]. From a preventive perspective, this study targeted normolipidemic healthy individuals and conducted a single-dose intake. Based on our findings described above, this study was intended to further investigate the effects of the insoluble fraction of pulverized sesame seeds on postprandial lipidemia in healthy individuals, especially the participants who tended to have heightened postprandial responses to dietary fat intake in order to ameliorate the risk of arteriosclerosis. In this study, despite targeting healthy individuals, a subgroup analysis was conducted to examine the suitability of pulverized sesame powder for different types of participants based on their baseline-corrected TG concentrations, which are regarded as the most direct indicator of postprandial TG responses. Participants were divided into two groups based on their postprandial responses, with some showing higher postprandial blood TG levels, while others exhibited lower levels. This approach was used to further investigate the effects of pulverized sesame powder on postprandial hyperlipidemia in different subgroups.

Generally, the median value is used as the cut-off value in clinical trials on subgroup analysis [20]. Therefore, we conducted subgroup analysis based on the median value of the baseline-corrected TG concentrations and evaluated the changes in postprandial TG concentrations or other lipid parameters.

2. Materials and Methods

2.1. Preparation of Ethanolic Extracts of the Pulverized Sesame Powder and its Pepsin-Pancreatin Digests

The ethanolic extract of the pulverized sesame powder was prepared as following methods; 450 g of the pulverized sesame powder (LipiSmart®, Mitsui DM Sugar, Tokyo, Japan) was mixed with 9 L 50% ethanol and extracted at 50°C for 3h. The mixture was vacuum filtrated and the filtrate was lyophilized after removal of the solvent by rotary evaporation. This procedure yielded 85.8 g (19.1% based on dry mass) extract of the pulverized sesame powder. One g of the ethanolic extract was completely dissolved in 100 mL of distilled water by agitation for 5 min and by sonication for 30 min. The pH

of the solution was then adjusted to 2.0 by using 2M hydrochloric acid (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan). The pepsin-digested ethanolic extract of the pulverized sesame powder (pepsin digestion) was prepared by reacting 80 mL of the pH-adjusted solution with 48 μL 20 mg/mL working solution of pepsin (Sigma-Aldrich, St. Louis, United States) at 37°C with gentle agitation for 2h. pH of the solution was adjusted to 8.5 using 3M sodium hydroxide (FUJIFILM Wako Pure Chemical Corporation) and 60 mL of the pepsin-digestion of the pulverized sesame powder was further digested with 72 μL pancreatin (FUJIFILM Wako Pure Chemical Corporation) at a working concentration of 200 mg/mL at 37°C with gentle agitation for 2h. The pepsin-pancreatin digestion products were lyophilized after being heated in boiling water for 30 min.

2.2. In Vitro Lipase Inhibitory Assay

The lipase inhibitory activities of the extract and digestion products of the pulverized sesame powder were measured in vitro following the method as previously reported [21] with modifications. The lipase derived from the intestinal acetone powders of rats (Sigma-Aldrich) was reconstituted in 0.1 M citrate buffer (pH 6.0) to a concentration of 1 mg/mL. The lipase inhibition assay was then carried out using Lipase Kit S (SB Bioscience, Osaka, Japan) as per manufacturer's instructions.

2.3. Clinical Study

2.3.1. Ethical Statement

This study complies with the Declaration of Helsinki and the protocol was approved by the Ethics Committees of the Yoga Allergy Clinic (committee No. 21000023, Tokyo, Japan). Written informed consent was obtained from all participants prior to study entry. The study is registered at UMIN Clinical Trials Registry (UMIN-CTR) (UMIN ID: 000044700).

2.3.2. Participants

Participants were recruited from June 12th to 19th and from November 2nd to 15th, 2021 on a voluntary basis. Healthy Japanese individuals who were 20–65 years of age with a normal fasting TG level (less than 150 mg/dL) based on the health inspection reports issued within a year and were able to understand the research requirements and give informed consent were enrolled in this trial. Individuals meeting any of the following criteria (by review of medical history and participant intake) were excluded: (a) diagnosed with malignancies, heart failure or myocardial infarction; (b) any current or previous treatment for dyslipidemia, diabetes, liver disorders, kidney disorders, cerebrovascular disorders, atrial fibrillation, arrhythmia, rheumatism, hypertension, etc.; (c) frequent use of dietary supplements or functional foods including traditional Chinese medicines that might alter lipid metabolism; (d) known hypersensitivity to any of the foods or their excipients used in this study; (e) current pregnancy or breastfeeding or planning pregnancy; (f) other conditions deemed unsuitable for participation by the physician. Use of medicines for minor illnesses was permitted and provided that their uses were properly documented.

2.3.3. Study Design and Protocol

Due to the capacity constraint of the facility, the study was conducted in two sessions: Session One from June 21 to July 8 with the participants recruited from June 12 to June 19 and Session Two from November 19 to December 2 with the participants recruited from November 2 to 15, 2021.

In this randomized, double-blind, placebo-controlled, two-arm crossover trial, the participants were randomly allocated to either the pulverized sesame powder-placebo sequence or the placebo-pulverized sesame powder sequence. The two treatments of each sequence were separated by a one-week washout period. The allocation was performed by using Excel-generated random numbers and blinded to the participants, investigators and outcome assessors. Allocation information was concealed in sequentially numbered, opaque sealed envelopes until the participants were entered into the trial.

The treatment was 500 mg pulverized sesame powder or 500 mg maltodextrin (placebo). Food coloring and starch wrapping were used to make the appearance and taste of the pulverized sesame powder and the placebo indistinguishable. The nutritional composition of the pulverized sesame powder and the placebo is provided in Table 1. The nutritional composition, except lignocellulose and resistant protein, of the pulverized sesame powder and the placebo was determined by Japan Food Research Laboratories (Osaka, Japan). Quantification of lignocellulose and resistant protein was carried out by Japan Inspection Association of Food and Food Industry Environment (Tokyo, Japan) following the previously reported method with modifications [18]. Detailed methods are provided in the supplemental material.

The participants were provided with an identical meal as dinner and instructed to fast—no consumption of any food or drink except water—from 9 pm on the day before each treatment until 9 am on the day of treatment. The participants were also required to refrain from alcohol use and strenuous exercise on the day before each treatment. Moreover, all participants were instructed to maintain their habitual patterns of diet and physical activity, and not to consume any dietary supplements (except the routinely used ones) other than the pulverized sesame powder or placebo throughout the trial. Any use of medicines, dietary supplements or functional foods were recorded on a daily basis.

The participants were instructed to take the pulverized sesame powder or the placebo with drinking water and then to consume the standardized high-fat meal within 15 min. The standardized meal consisted of one Japanese hamburger steak, two butter breads and one piece of hashed potatoes with a macronutrient breakdown of 43.8 g fat, 62.8 g carbohydrate, 27.6 g protein and a total caloric content of 750 kcal. Venous blood samples were collected before the pulverized sesame powder or placebo and the standardized high-fat meal were consumed (baseline) and at 2, 3, 4, and 6 hours after intake of the meal. Adverse events were monitored by participant self-reports.

The primary endpoints of this study include the differences in TG concentration at different postprandial

time points, the area under the curve (AUC) and the incremental area under the curve (iAUC) of TGs between treatments. The secondary endpoints include the differences in the concentrations of remnant-like lipoprotein particle (RLP) cholesterol, beta lipoproteins (βLPs), low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, total cholesterol, free fatty acids (FFAs), and phospholipids at different postprandial time points between treatments.

Table 1. Nutritional composition of the pulverized sesame powder and the placebo

	Nutritional value per serve (500 mg)	
	Experimental food	Placebo
Carbohydrate (g)	0.17	0.46
Protein (g)	0.20	0.01
Fat (g)	0.05	<0.01
Water (g)	0.02	0.03
Ash (g)	0.06	0.01
Sodium (mg)	0.03	2.19
Energy (kcal)	1.94	0.188
Lignocellulose (g)	0.09	<0.001
Resistant protein (g)	0.04	<0.001

Table 2. Baseline characteristics of the participants

Characteristics	All exposed (n=83)	All analyzed (n=77)	ΔTG ≧83 mg/dL
Gender, n (%)			
Male	42 (51%)	37 (48%)	23 (59%)
Female	41 (49%)	40 (52%)	16 (41%)
Age (years)	41.2 ± 9.7 (24~64)	41.4 ± 9.9 (24~64)	43.6 ± 10.8 (25~64)
Height (cm)	166.0 ± 8.2	166.1 ± 8.0	167.8 ± 8.0
Weight (kg)	62.7 ± 10.7	62.4 ± 10.6	65.3 ± 11.9
Body Mass Index (BMI, kg/m^2)	22.7 ± 2.8	22.5 ± 2.9	23.1 ± 3.1
Fasting lipidemic parameters			
TG (mg/dL)	73.8 ± 35.8	71.3 ± 33.6	89.4 ± 33.9
RLP-cholesterol (mg/dL)	3.4 ± 1.7	3.3 ± 1.5	4.0 ± 1.5
FFAs (μEq/L)	502.8 ± 182.8	490.7 ± 180.3	482.8 ± 159.8
Total cholesterol (mg/dL)	205.5 ± 34.4	203.6 ± 34.4	207.1 ± 29.0
Phospholipids (mg/dL)	206.9 ± 29.8	204.9 ± 28.6	208.9 ± 26.2
βLPs (mg/dL)	299.8 ± 82.5	292.7 ± 77.4	319.8 ± 71.5
HDL cholesterol (mg/dL)	69.1 ± 14.3	69.3 ± 14.1	67.2 ± 14.9
LDL cholesterol (mg/dL)	118.3 ± 30.4	116.7 ± 29.1	119.7 ± 25.4

TG, triglyceride; RLP-cholesterol, remnant-like lipoprotein particle cholesterol; FFAs, free fatty acids; βLPs, beta lipoproteins; HDL, high density lipoprotein; LDL, low density lipoprotein.

2.3.4. Blood Sampling and Analysis

Blood samples were collected from the veins in the antecubital fossa into vacuum blood collection tubes (Terumo, Osaka, Japan). The blood samples were allowed to clot at ambient temperature from 30 min. Serum was separated from the clot by centrifugation and stored at -80ºC for analysis at a later date. TG concentrations were analyzed by using an enzymatic colorimetric method with commercial reagents

(Pure Auto TG-N, Sekisui Medical, Tokyo, Japan) and an automatic analyzer (Labospect008, Hitachi, Tokyo, Japan). RLP cholesterol and FFAs were quantitatively determined by a homogeneous assay (MetaboLead RemL-C; Kyowa Medex, Tokyo, Japan) and an enzymatic assay (NEFA-HR (2), FUJIFILM Wako Pure Chemical Corporation), respectively, using a JCA-BM8040 analyzer (JEOL, Tokyo, Japan). βLPs were measured using a turbidimetric assay (Clinimate β-L, Sekisui Medical). LDL cholesterol and total cholesterol were analyzed with enzymatic colorimetric assays (Cholestest LDL and Cholestest CHO, Sekisui Medical). HDL cholesterol was determined following a direct method (Cholestest N HDL, Sekisui Medical). Quantitative analysis of phospholipids was based on the choline oxidase-DAOS (N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-3,5dimethoxyaniline sodium salt) method using commercial reagents (L-Type Phospholipids, FUJIFILM Wako Pure Chemical Corporation). These assays were conducted using the Hitachi automatic analyzer (Hitachi, Tokyo, Japan) as well.

2.3.5. Statistical Analysis

The primary aim of this study was to achieve a statistical power of 80% to detect a difference of 0.3 standard deviations (SD) in serum TG concentration between the pulverized sesame powder and placebo arms of the crossover trial. Accordingly, a sample size of approximately 71 participants was determined, assuming a dropout rate of 15%. Postprandial concentrations of TGs and other lipemic parameters were presented as both absolute values and baseline-corrected values (denoted as Δ). Differences between the pulverized sesame powder arm and the placebo arm in these parameters at sequential postprandial time points were analyzed by using two-tailed, paired t-tests. The postprandial variations in TGs were integrated as the AUC, which was calculated using the absolute values of postprandial TG concentrations following the trapezoidal rule, while iAUC was calculated using the baseline-corrected values. Differences in AUC or iAUC of TGs between the two arms were compared using two-sided, paired t-test. The per protocol (PP) set was analyzed. Moreover, the primary and secondary endpoints associated with the participants who tend to have heightened postprandial responses to dietary fat intake (i.e., defined as the subjects whose baseline-corrected postprandial TG levels after consumption of the placebo fell within the half of greater values) were also assessed. The statistical analysis plan was and the conditions for excluding randomized and exposed subjects were specified prior to unblinding. All data are presented as mean ± SD. A value of $p<0.05$ was considered statistically significant. Statistical analysis was performed using SPSS ver. 24.0 (IBM, N.Y., USA)

3. Results

3.1. Lipase Inhibitory Activity of the Ethanolic Extract of Pulverized Sesame Powder and its Enzyme-Treated Digests

Lipase inhibitory activities of the ethanolic extract and the pepsin-digests and pepsin-pancreatin-digested of ethanolic extract of pulverized sesame powder were quantitatively evaluated in vitro at three concentrations,

which equivalent to 0.3, 1.0 and 3.0 mg/mL of the ethanolic extract. As shown in Figure 1, the ethanol extract exhibited strong inhibitory activity against lipase with an IC_{50} of 0.76 mg/mL. The pepsin-digests of ethanolic extract showed reduction of lipase inhibitory activity comparing to the the ethanolic extract of pulverized sesame powder at all three concentrations with an IC_{50} of 1.47 mg/mL. Further treatment of the pepsin-digests of ethanolic extract with pancreatin resulted in minimal or no lipase inhibition.

3.2. Subject Demographics

85 eligible healthy volunteers were enrolled in the study and randomized. 42 participants were allocated to receive the pulverized sesame powder followed by the placebo, while 43 were assigned to receive the placebo followed by the pulverized sesame powder. Before the first treatment, one participant from each sequence withdrew due to inconvenience to participate; as a result, 41 (21 male and 20 female) and 42 participants (21 male and 21 female) for each sequence respectively received both treatments and were assessed (Figure 2). Baseline characteristics of the participants are presented in Table 2. After sorting the baseline-corrected postprandial TG concentrations in association with placebo intake, considering the individual variations in digestion, absorption and metabolism of lipids described in the discussion section, the 50% cut-off value of TG concentration was revealed to be 83 mg/dL. The participants whose TG concentrations were higher or equal to 83 mg/dL after consuming the placebo were considered individuals who tended to have heightened postprandial responses to dietary fat intake and their baseline characteristics were summarized in Table 2 as well. Two participants were excluded from outcome analysis from each sequence due to fasting TG level >150 mg/dL on both days of treatment and assessment, and one was excluded from each sequence due to abnormal postprandial TG responses (i.e., highly fluctuated TG levels, suspected hyperlipidemia). Therefore, 77 subjects (the PP set) were analyzed for the primary and secondary endpoints (Figure 2).

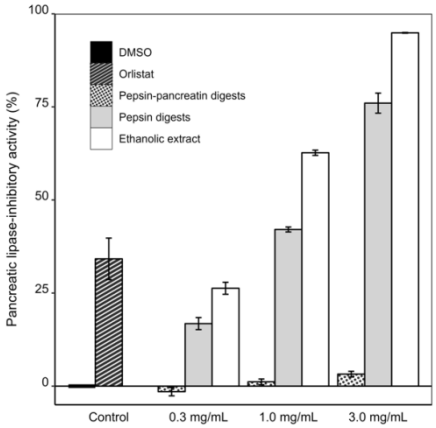

Figure 1. Lipase inhibitory activities of ethanolic extract of pulverized sesame powder and its pepsin or pepsin-pancreatin-digests. DMSO and orlistat were used as each negative and positive control. Data represent the mean ± SD.

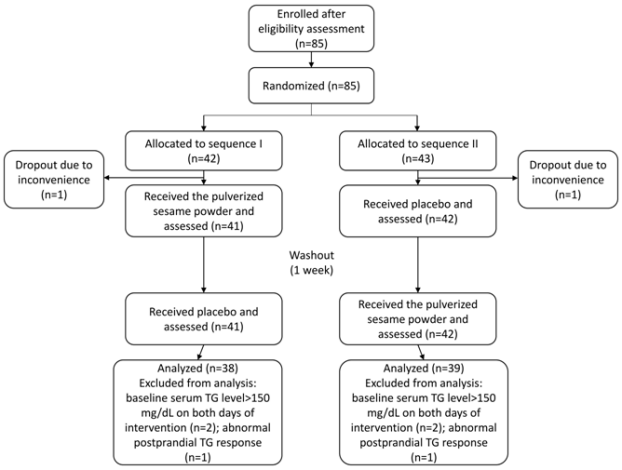

Figure 2. The schematic diagram of the design and conduct of the trial

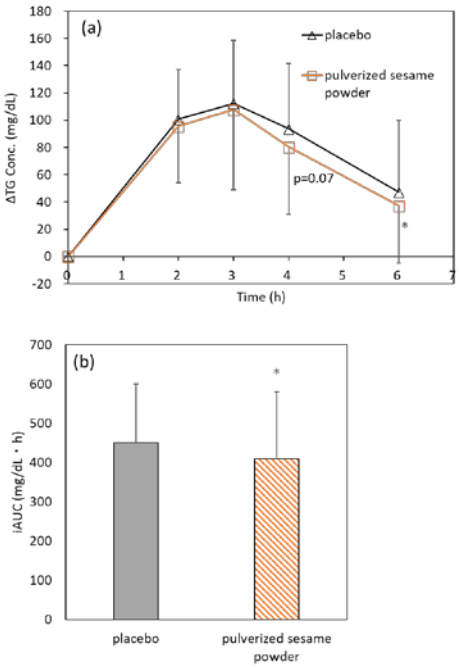

Figure 3. Postprandial TG responses following high-fat meals in the participants with baseline-corrected TG concentrations (Δ TG) ≧ 83 mg/dL (a) Time course of the baseline-corrected postprandial serum TG concentrations (Δ TG) after intake of pulverized sesame powder or placebo (n=39). (b) iAUCs of postprandial Δ TGs (n=39). Data represent the mean ± SD. Statistical analysis by two-tailed, paired t-tests. *p-value<0.05.

3.3. Effects of Pulverized Sesame Powder on Postprandial TG Responses

The effects of the pulverized sesame powder on postprandial TG responses following a high-fat meal in healthy individuals were investigated by comparing the TG concentrations, the baseline-corrected TG concentrations, and the AUC and iAUC of TGs of the pulverized sesame powder arm with those of the placebo arm. For the PP subjects, no significant difference in postprandial TG responses was observed between arms (Table 3). On the other hands, for top half of the subjects whose baseline-corrected TG levels were ≧83 mg/dL when placebo was ingested, intake of the pulverized sesame meal led to a significant decrease (37.4

vs. 47.3 mg/dL, p-value=0.03) in baseline-corrected TG concentration at 6h after consumption of the high-fat meal. A tendency of reduction in baseline corrected TG concentration was also observed at 4h (p-value=0.07). Moreover, intake of the pulverized sesame powder also resulted in significant reduction of TG iAUC (409.2 vs. 451.4 mg/dL · h, p-value=0.04) (Figure 3). Carryover effects and period effects of this trial were insignificant (t-tests, p=0.36 and p=0.12, respectively).

3.4. Effects of Pulverized Sesame Powder on other Postprandial Lipidemic Parameters

For the PP subjects, the effects of the pulverized sesame powder on other postprandial lipidemic parameters following a high-fat meal were insignificant (Table 3).

Similarly, for the subjects with baseline-corrected TG levels ≧83 mg/dL, no difference in all other lipidemic parameters was observed except that the RLP cholesterol concentration at 6h tended to lower for the pulverized sesame powder arm comparing to the placebo arm (5.5 vs. 6.1 mg/dL, p-value=0.09) (Table 4).

Table 3. Postprandial serum concentrations of TG and other lipemic parameters (per protocol set n=77)

Parameter	Intervention	Serum concentration				
		0h	2h	3h	4h	6h
TGs (mg/dL)	Placebo	70.2 ± 32.6	147.0 ± 60.4	146.1 ± 76.4	134.6 ± 72.7	105.2 ± 62.9
	Pulverized sesame powder	73.2 ± 33.6	149.3 ± 63.1	151.1 ± 81.7	132.4 ± 72.8	105.1 ± 54.2
RLP cholesterol (mg/dL)	Placebo	3.2 ± 1.4	5.0 ± 2.4	5.5 ± 3.0	5.5 ± 3.3	4.7 ± 2.7
	Pulverized sesame powder	3.3 ± 1.5	5.1 ± 2.4	5.6 ± 3.1	5.5 ± 3.1	4.6 ± 2.4
FFAs (μEq/L)	Placebo	472.6 ± 187.1	399.6 ± 172.8	434.5 ± 209.7	530.7 ± 229.9	712.2 ± 198.7
	Pulverized sesame powder	469.1 ± 160.3	400.4 ± 196.6	440.3 ± 231.7	526.7 ± 232.7	725.7 ± 172.4
Total cholesterol (mg/dL)	Placebo	201.0 ± 35.2	192.0 ± 31.4	192.0 ± 31.4	194.0 ± 31.2	198.0 ± 31.2
	Pulverized sesame powder	202.0 ± 35.0	193.0 ± 30.9	192.0 ± 31.2	195.0 ± 31.6	197.0 ± 32.0
Phospholipids (mg/dL)	Placebo	205.0 ± 28.2	207.0 ± 26.9	210.0 ± 27.1	215.0 ± 27.8	221.0 ± 27.6
	Pulverized sesame powder	206.0 ± 28.3	207.0 ± 26.7	210.0 ± 27.3	214.0 ± 27.8	219.0 ± 28.1
βLPs (mg/dL)	Placebo	289.0 ± 74.5	346.0 ± 82.7	339.0 ± 93.4	332.0 ± 91.5	310.0 ± 84.1
	Pulverized sesame powder	293.0 ± 76.4	350.0 ± 88.5	346.0 ± 101.0	330.0 ± 96.4	312.0 ± 83.8
HDL cholesterol (mg/dL)	Placebo	70.0 ± 14.0	64.0 ± 12.7	63.0 ± 12.8	64.0 ± 12.9	66.0 ± 13.4
	Pulverized sesame powder	69.0 ± 14.5	64.0 ± 13.3	63.0 ± 13.0	64.0 ± 13.1	66.0 ± 13.9
LDL cholesterol (mg/dL)	Placebo	115.0 ± 28.5	108.0 ± 26.8	108.0 ± 26.6	109.0 ± 26.3	112.0 ± 27.2
	Pulverized sesame powder	115.0 ± 28.2	109.0 ± 26.0	108.0 ± 26.1	109.0 ± 26.2	112.0 ± 26.2

Data represent the mean ± SD. Statistical analysis by two-tailed, paired t-tests. TG, triglyceride; RLP-cholesterol, remnant-like lipoprotein particle cholesterol; FFAs, free fatty acids; βLPs, beta lipoproteins; HDL, high density lipoprotein; LDL, low density lipoprotein.

Table 4. Postprandial serum concentrations of other lipidemic parameters of the participants with ΔTG≧83 mg/dL (n=39)

Parameter	Intervention	Serum concentration				
		0h	2h	3h	4h	6h
RLP cholesterol (mg/dL)	Placebo	3.9 ± 1.5	6.4 ± 2.4	7.4 ± 3.0	7.5 ± 3.4	6.1 ± 3.0
	Pulverized sesame powder	3.9 ± 1.6	6.2 ± 2.5	7.2 ± 3.3	7.0 ± 3.4	5.5 ± 2.6 (p-value=0.09)
FFAs (μEq/L)	Placebo	459.2 ± 175.7	420.7 ± 194.3	495.6 ± 255.9	602.7 ± 261.9	764.9 ± 203.9
	Pulverized sesame powder	472.5 ± 126.9	438.5 ± 241.7	503.5 ± 279.9	608.3 ± 244.2	758.2 ± 187.1
Total cholesterol (mg/dL)	Placebo	203.1 ± 28.2	194.2 ± 24.6	193.2 ± 24.4	196.1 ± 25.0	198.4 ± 24.6
	Pulverized sesame powder	205.5 ± 29.4	196.8 ± 25.3	196.4 ± 26.2	198.6 ± 25.8	200.0 ± 25.2
Phospholipids (mg/dL)	Placebo	208.6 ± 26.6	211.2 ± 24.6	214.8 ± 25.3	220.7 ± 26.4	224.7 ± 25.6
	Pulverized sesame powder	209.2 ± 25.8	211.5 ± 24.2	216.0 ± 25.8	220.3 ± 25.5	222.8 ± 25.6
βLPs (mg/dL)	Placebo	312.6 ± 70.3	389.1 ± 73.2	391.1 ± 83.0	379.0 ± 85.3	341.9 ± 85.6
	Pulverized sesame powder	317.6 ± 69.7	390.0 ± 84.4	393.3 ± 99.3	370.8 ± 94.0	340.1 ± 81.9
HDL cholesterol (mg/dL)	Placebo	67.5 ± 15.0	60.9 ± 13.3	59.6 ± 12.8	60.6 ± 13.2	63.4 ± 14.2
	Pulverized sesame powder	67.6 ± 15.6	61.8 ± 14.0	60.5 ± 13.5	61.1 ± 13.5	63.1 ± 14.3
LDL cholesterol (mg/dL)	Placebo	117.0 ± 22.8	109.7 ± 21.3	108.4 ± 21.4	110.1 ± 21.2	113.5 ± 22.4
	Pulverized sesame powder	118.9 ± 24.0	111.4 ± 22.1	110.5 ± 22.0	112.2 ± 22.7	115.4 ± 22.3

Data represent the mean ± SD. Statistical analysis by two-tailed, paired t-tests. *p-value<0.05. RLP-cholesterol, remnant-like lipoprotein particle cholesterol; FFAs, free fatty acids; βLPs, beta lipoproteins; HDL, high density lipoprotein; LDL, low density lipoprotein

4. Safety

No clinically relevant adverse events or withdrawal were observed following intake of the pulverized sesame powder.

5. Discussion

Previously we reported that the insoluble fraction of pulverized sesame powder, which was rich in lignocellulose and resistant protein, was effective in reducing postprandial TG excursion in high-fat diet-challenged rats [18]. In this study, we aimed at further evaluating the clinical efficacy of the insoluble fraction of pulverized sesame powder in attenuating the postprandial elevation of serum TG in healthy individuals. Because the insoluble fraction used in the animal study was prepared using reagents not intended for food use, the unprocessed pulverized sesame powder was used for human subjects in this study. In addition to the insoluble fraction, the unprocessed pulverized sesame powders also contain lipids, soluble dietary fibers, carbohydrates, peptides and digestible proteins. Since some of these components have been suggested to cause gastrointestinal lipase inhibition, which also leads to reduction of dietary fat absorption and postprandial elevation of TGs [22], The presence of these components had the potential to activity as lipase inhibitory substances within the gastrointestinal tract.

Therefore, we first examined whether these components could cause lipase inhibition after entering the gastrointestinal tract by measuring the lipase inhibitory activities of the digestive enzyme-treated pulverized sesame powder in vitro. Firstly, pulverized sesame powder was extracted with 50% ethanol. As expected, the 50% ethanolic extract exhibited substantial lipase inhibitory

activity in a concentration-dependent manner. The ethanolic extract was then treated with pepsin and the pepsin-treated extract exhibited reduced lipase inhibitory activity comparing to the ethanolic extract at all three concentrations (Figure1). Further digestion of the pepsin-treated extract with pancreatin led to near-zero lipase inhibition (Figure1). These results indicated that the digestion products of these components would not contribute to the attenuation of postprandial TG spikes. The pulverized sesame powder also contained 5% soluble dietary fiber. Therefore, in this study, 25 mg soluble dietary fiber per person per meal was consumed in 500 mg of the pulverized sesame powder. Because the soluble dietary fiber does not affect postprandial TG responses unless 5 g or more per meal is consumed [23], the effects of the soluble dietary fiber could be considered almost negligible. Considering the above, lignocellulose and resistant protein are believed to play a significant role as the primary active components in pulverized contributing to the inhibition of postprandial increase in blood triglycerides

After ruling out the possible effects of these components on postprandial TG responses, the trial was carried out based on the hypothesis that the pulverized sesame powder was effective in lowering the postprandial elevation of serum TG with its activity primarily hinging on its lignocellulose and resistant protein-rich insoluble fraction. It is known that the absorption and digestion rates of lipids vary significantly between individuals [24]. Hussain et al. and Hartmann et al. reported that during a 5-day control period in which subjects consumed very similar diets, the fecal fat excretion ranged from 1.62 to 5.4 g/day and from 1.32 to 5.52 g/day, respectively [25-26]. As these subjects were carefully managed in clinical trials, these results revealed the significantly individual variations even though identical or very similar diets were consumed. In the present study, a large individual variation in lipid absorption was assumed to be the case. In addition, the physiological activities of foods or food ingredients are generally weaker than those of pharmaceuticals, but they are well known to be less likely to cause side effects and easier to use in daily life. Therefore, when using the physiological activity of food or food ingredients for the purpose of health promotion, it is necessary to determine the target health condition in detail. In accordance with the blood glucose challenge reported in Ishida et al., we conducted additional analyses of the top half of the subjects as sorted by the ΔTG values when placebo was ingested [27]. Analysis of the primary and secondary endpoints of the trial revealed no significant differences in postprandial TG responses or other lipidemic parameters between the pulverized sesame powder arm and the placebo arm for the per protocol subjects (77 subjects analyzed). On the contrary, for the participants who tended to have heightened postprandial responses to dietary fat intake (39 subjects analyzed), consumption of the pulverized sesame powder led to significant reduction of baseline-corrected TG concentration at 6h after meal and TG iAUC (Figure 3). A moderate decrease in postprandial RLP cholesterol was also observed, indicating an attenuated status of TG-rich lipoproteins [23]. Previously, we have demonstrated that the insoluble fraction of pulverized sesame powder, which was primarily comprised of lignocellulose and resistant protein, was capable of adsorbing the lipids micellized by bile acids in vitro [18]. Bile salts are a class of polarized steroids that play an indispensable role in digestion and absorption of dietary fats by solubilizing dietary fats in the hydrophilic milieu of intestinal tract and thus enhancing the efficiency of lipase [28]. We hence speculate that the insoluble fraction functions in the same way in the intestine as it does in vitro. That is, bile salts and dietary fats are both captured by the insoluble fraction and excreted into the feces without undergoing absorption, leading to postprandial TG increases to a lesser extent. Bile salts also form mixed micelles with free fatty acids, the products of dietary fat digestion, so as to transport them to the surface of enterocytes [28]. Therefore, the increased excretion of bile salts caused by the insoluble fraction may also serve as a mechanism that underlies the reduced postprandial TG elevation. The postprandial TG responses of an individual depend on multiple factors, one of which is dietary fat absorption rate. Dietary fat absorption rate is in turn determined by the availability of fats and physiological, lifestyle and genetic factors that vary among individuals. For individuals who have inherently lower fat absorption rates, their fat absorption rates may be poorly associated with the availability of fats. This may explain why no significant TG-lowering effects of the pulverized sesame powder were observed in the participants whose blood TGs tended to be less responsive

to oral fat intake in this study, while the effects were significant for those who tended to have heightened postprandial TG responses. However, further investigations are still needed to clarify the causes of the discrepancies between these populations.

This study has several limitations. Firstly, the observed effects were limited only to subjects whose postprandial blood triglyceride levels tend to rise significantly. Therefore, further investigations are needed to assess the effects on other subjects with different health conditions. Secondly, the study was conducted with a single-dose administration, which restricts the evaluation of long-term effects. Therefore, additional clinical tests for long-term effect are required. Taking the above two points into consideration, future studies are warranted to examine the long-term effects of the pulverized sesame powder in a broader range of participants.

Luminacoid, according to Japanese Association for Dietary Fiber Research (JDF), is explained as a class of food-derived nondigestible and nonabsorbable substances that exert their beneficial physiological effects via the digestive tract [29]. Our previous study revealed that the insoluble fraction of pulverized sesame powder, which was primarily comprised of lignocellulose and resistant protein, was capable of adsorbing the lipids micellized by bile acids in vitro, suggesting that the insoluble fraction may act in a similar fashion in the intestinal tract to disrupt the digestion of dietary fats and thereby reduce TG absorption. These findings indicate the great potential of pulverized sesame powder as a luminacoid for the management of the postprandial elevation of serum TG for healthy individuals, particularly for those who tend to have heightened postprandial responses to dietary fat intake. Given the accumulating evidence that older individuals are more likely to exhibit a greater rise in postprandial TGs [30, 31], the pulverized sesame powder may represent a new solution to ameliorate postprandial the postprandial elevation of serum TG for the elderly population.

6. Conflict of Interest

Hiroaki Yamada, Sayo Morita, Xuan Li, Akiko Ishida, Yusuke Yamashita, Takashi Kometani and Young-Il Kim are employees of Pharma Foods International Co., Ltd. Toma Furuta is employee of Mitsui DM Sugar Co., Ltd. Pharma Foods International Co., Ltd. and Mitsui DM Sugar Co., Ltd. sponsored this study.

Supplementary Material

Quantification of Lignocellulose and Resistant Protein in Pulverized Sesame Powder

30 g of pulverized sesame powder was crushed in a food processor. 10 g of the crushed powder was extracted with 250 mL of petroleum ether (FUJIFILM Wako Pure Chemical Corporation) with constant agitation for 30 min at room temperature. The mixture was then filtrated with glass filter and the residues were further extracted with 220 mL of petroleum ether for 30 min at room temperature. The resulting residues were collected after filtration and dried at elevated temperature (105°C) for 1 hr. 1.6 g of the dried residues and 80 mL of 0.08 M phosphate buffer (pH 6.0) were mixed and heated to 95°C, at which point 160 μL of α-amylase (Sigma–Aldrich) was added to the mixture and agitated for 30 min. The residues were further digested sequentially with 160 μL of amyloglucosidase from Aspergillus niger (Sigma–Aldrich) at pH 4.3, 60°C for 30 min, 6.4 mg of Pepsin from porcine gastric mucosa (Sigma–Aldrich) at pH 2.0, 37°C for 4 h, and 64 mg of pancreatin from hog pancreas (FUJIFILM Wako Pure Chemical Corporation) at pH 8.0, 37°C for 16 h. 2M hydrochloric acid (FUJIFILM Wako Pure Chemical Corporation) and 3M sodium hydroxide (FUJIFILM Wako Pure Chemical Corporation) were used for pH adjustment. The reaction was quenched by heating the mixture at 95°C in a water bath. The resulting residues were collected by filtration using a glass filter with 1 g of celite and successively washed twice with 15 mL of water, twice with 95% ethanol (FUJIFILM Wako Pure Chemical Corporation), twice with acetone (FUJIFILM Wako Pure Chemical Corporation). The insoluble fraction was finally obtained by drying the above residues at 105°C, overnight. The proteins in the insoluble fraction are considered as resistant protein, and their content was determined by following the Kjeldahl method. Ash content was measured by incinerating a weighed sample of the insoluble fraction at 525°C for 5h and determining the weight of the inorganic matter remaining. The content of lignocellulos was determined by subtracting the content of resistant protein and the content of ash from 100%.

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