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Monoglucosyl rutin, a flavonoid glycoside, improves low-density lipoprotein-cholesterol levels in healthy adults: A randomized controlled trial

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ABSTRACT

Background: Atherosclerotic cardiovascular disease (ASCVD) continues to be a significant contributor to global mortality, impacting over 523 million individuals worldwide. Dyslipidemia stands as one of the foremost risk factors for ASCVD. Thus, prioritizing the reduction of low-density lipoprotein cholesterol (LDL-C) levels is essential in mitigating cardiovascular complications.

Objective: This study aimed to evaluate the lipid-lowering activity of a dietary supplement containing monoglucosyl rutin (MR) in individuals with low to mild hypercholesterolemia.

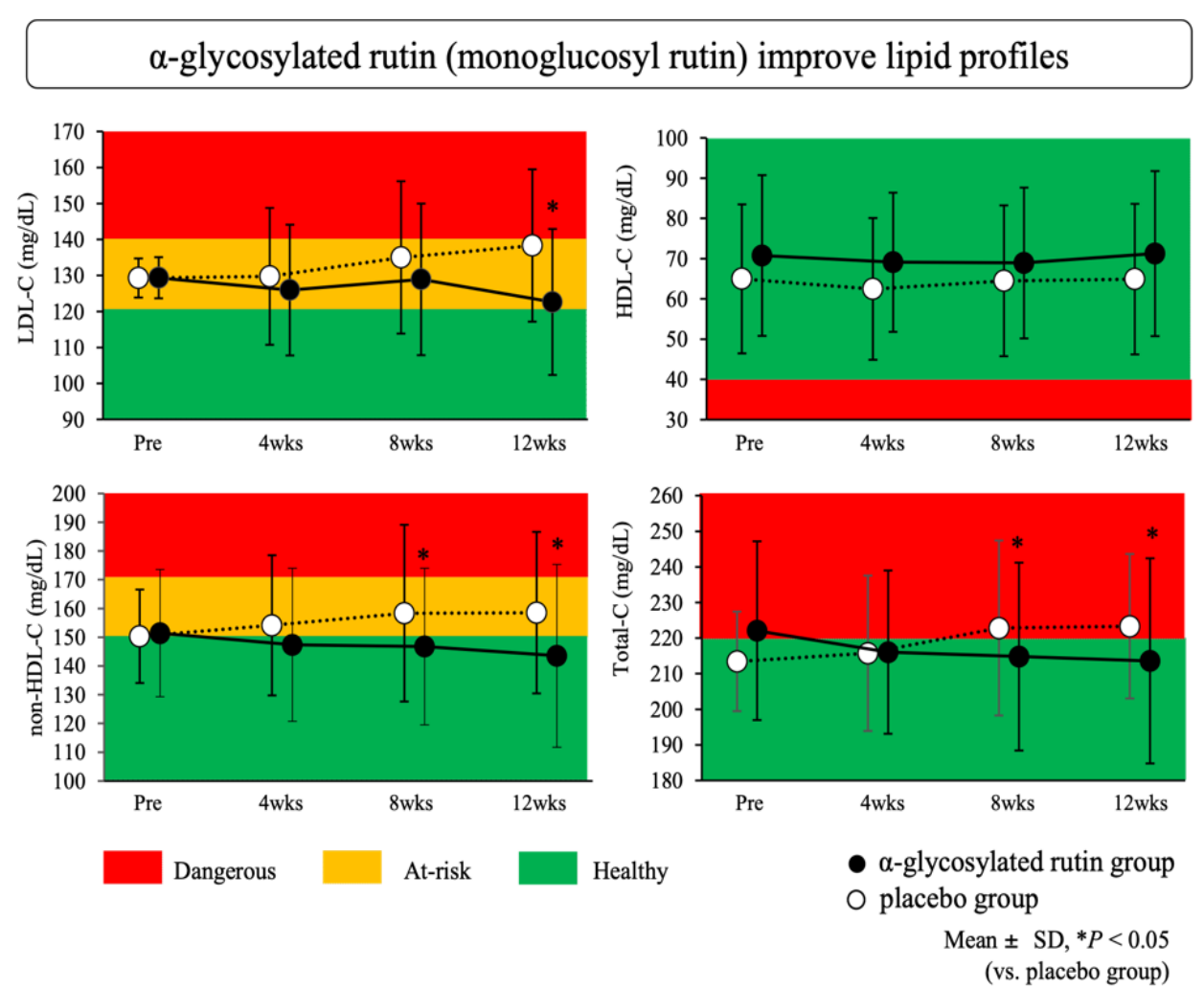
Methods: This was a randomized, placebo-controlled, double-blind, parallel-group study conducted from April 20 to December 24, 2022. The study population included 56 healthy Japanese adult participants with LDL-C levels between 120–139 mg/dL who were randomly allocated to either the MR or placebo groups (n = 28/group) using a computerized random number generator. 200 mg of MR or placebo divided into 3 tablets was given daily; participants were instructed to take 1 tablet with water after each meal for 12 weeks. The main focus was on measuring the serum LDL-C level as the primary outcome, with additional attention given to secondary outcomes such as serum high-density lipoprotein cholesterol (HDL-C), total cholesterol, and non-HDL-C levels. The study also evaluated the percentage of participants achieving serum LDL-C levels below 120 mg/dL after the 12-week intervention. Assessments were conducted after 4, 8, and 12 weeks of intervention.

Results: There were 54 (27 in each group) participants in the per-protocol set (PPS) and 53 (placebo group, 26; MR group, 27) participants in the modified PPS (mPPS). A statistically significant group difference in serum LDL-C levels was observed ($P < 0.05$), with a 5.0% decrease from preintervention to after 12 weeks. The reduction in LDL-C levels coincided with statistically significant decreases in total cholesterol levels, non-HDL-C levels, and the LDL-C/HDL-C ratio. During the study period, there was no adverse event or concern about the safety of MR.

Conclusions: MR has potential as a preventive or therapeutic tool for improving improve long-term health and reducing cardiovascular morbidity.

Keywords: monoglucosyl rutin, flavonoid glycoside, low-density lipoprotein cholesterol, hyperlipidemia, lipid profile

Trial registration number: UMIN000047790



Graphical Abstract

INTRODUCTION

Cholesterol is a foundational element in the synthesis of vitamin D, steroid hormones, and bile acids, while also playing a critical role as a structural component within cell membranes. Despite its crucial role in the body, abnormal levels of cholesterol can lead to pathological conditions such as arteriosclerosis [1]. Cholesterol is transported in the blood inside lipoprotein particles that have different functions [2]. In particular, elevated levels of low-density lipoprotein cholesterol (LDL-C) have been linked to the heightened likelihood of developing atherosclerotic cardiovascular disease (ASCVD) [3] and coronary heart disease [4]. High density lipoprotein cholesterol (HDL-C) facilitates the transportation of cholesterol from peripheral tissues back to the liver. The LDL-C/HDL-C ratio is reportedly highly predictive of ASCVD risk in mild-to-moderate hypercholesterolemic individuals [5].

One of the parameters utilized in diagnosing dyslipidemia is LDL-C, where levels ranging from 120 to 139 mg/dL are classified as borderline hyper-LDL cholesterolemia, and those equal to or exceeding 140 mg/dL are categorized as hyper-LDL cholesterolemia in Japan [6]. Despite advancements in the treatment and prevention of dyslipidemia, prescription medicines are limited to chemical compounds that are associated with side effects. Statins, which are first-line drugs, may cause headache, nausea, and muscle pain [7] and even had a nocebo effect in some patients [8]. Therefore, treatment with functional foods in combination is also recommended [9].

Rutin, a polyphenolic flavonoid, is frequently found in various plant-based sources including buckwheat [10], tea, apples [11], and asparagus [12]. It has antioxidant properties and can potentially prevent lifestyle-related diseases such as obesity, heart disease, atherosclerosis, and hypertension [13]. However, the use of rutin (sometimes called vitamin P) has been largely limited because of its low water solubility and bioavailability [14]. Enzymatic treatment (glycosylation) of rutin has been shown to eliminate the problem of low water solubility and bioavailability. Notably, α-glycosylated rutin (4G-α-D-glucopyranosyl rutin, monoglucosyl rutin [MR]) is approximately 30,000-fold more water soluble than rutin. It is currently marketed as αG Rutin PS™ and is widely used in beverages and functional foods for its high verbality and stability. MR is metabolized to rutin and then quercetin by digestive enzymes [15], suggesting that rutin is functional in MR. Thus, the previous reports on rutin-related health function [16,17] are expected to be applicable for MR as well. In fact, we reported a reduction in visceral fat and suppression of postprandial blood glucose levels in humans by MR [18,19].

In this study, we examined the effect of 12 weeks of continuous MR intake on reducing LDL-C in healthy adults with LDL-C levels ranging from 120–139 mg/dL.

METHODS

Study design: The randomized, placebo-controlled, double-blind, parallel-group study conducted at Takara Clinic, Medical Corporation Seishinkai (Tokyo, Japan), received approval from the independent ethical committee on April 20, 2022 (Approval number: 2204-00334-0048-18-TC). This investigation adhered to the principles outlined in the Declaration of Helsinki (2013) and meticulously observed the Ethical Guidelines for Medical and Biological Research Involving Human Subjects, as stipulated by the Ministry of Education, Culture, Sports, Science, and Technology, alongside the Ministry of Health, Labor, and Welfare, and the Ministry of Economy, Trade, and Industry of Japan. The research protocol was duly registered with the University Hospital Medical Information Network Clinical Trials Registry (UMIN000047790). Recruitment of participants spanned from April 20 to September 13, 2022, with the study itself conducted from June 2 to December 24, 2022.

Study population: The study included healthy individuals whose health status is confirmed based on physical measurements, urinalysis, and blood test data. The following eligibility criteria were then met for inclusion.

The study's inclusion criteria encompassed: (a) individuals aged ≥20 years, both men and women, deemed to be in good health; (b) LDL-C levels falling within the range of 120–139 mg/dL during screening

(pre-intervention; Pre); (c) approval for participation granted by the principal investigator. Conversely, the exclusion criteria consisted of: (a) a medical history involving malignant tumors, heart failure, or myocardial infarction; (b) utilization of a pacemaker or implantable cardioverter defibrillator; (c) ongoing treatment for chronic ailments (e.g., cardiac arrhythmia, liver or kidney failure, cerebrovascular disorders, rheumatism, diabetes mellitus, dyslipidemia, hypertension, or any other chronic condition); (d) regular consumption of "Foods for Specified Health Uses," "Foods with Function Claims," or other functional foods/beverages; (e) consistent usage of medications, including herbal supplements and remedies; (f) allergic reactions to study substances or associated products; (g) pregnancy, breastfeeding, or plans for pregnancy during the study duration; (h) recent infection with novel coronavirus-19; (i) participation in another clinical study within 28 days prior to consenting for this study; and (j) determination of ineligibility by the principal investigator for participation in this specific study. Participants were enlisted via an online platform (https://www.go106.jp/) managed by ORTHOMEDICO Inc. (Tokyo, Japan). Upon receiving thorough details about the study, participants provided electronic consent through the network before their involvement. There was no involvement of sponsors or funding entities in this study. Assessments were carried out at two collaborating medical facilities: Takara Clinic (Medical Corporation Seishinkai, Tokyo, Japan), and Nerima Medical Association, Minami-machi Clinic (Tokyo, Japan). In the event of an adverse occurrence, the principal investigator promptly undertook requisite measures and evaluated the feasibility of participants' continued participation in the study, while also considering the activation of emergency procedures. Additionally, the principal investigator was responsible for evaluating and documenting any potential linkages between adverse events and the test substance in written reports. On examination days, interviews and dietary assessments employing the Calorie and Nutrition Diary (CAND) [20] were administered to ascertain participants' health status. Additionally, participants were instructed to maintain a logbook documenting their daily routines, including consumption of test substances, alterations in physical well-being, and medication usage.

Intervention: Participants consumed either the MR or placebo tablet as the test food. For the intervention group, the daily dose was set at 200 mg of MR (product name αG Rutin PS™ by Toyo Sugar Refining Co., Ltd. Tokyo, Japan) divided into 3 tablets. The placebo tablets contained dextrin instead of αG Rutin PS™. Participants were instructed to take one tablet with water after each meal. The intervention period was 12 weeks. The MR and placebo tablets could not be distinguished by color, smell, or flavor at the time of the ethics review. αG Rutin PS™ is considered a functional food as defined by the Functional Food Center [21,22].

Outcomes: Effective and safety evaluations were conducted at Pre, and after 4 weeks (4wks), 8 weeks (8wks), and 12 weeks (12wks) of intervention. The primary outcome was measured serum LDL-C levels at 12wks. The following parameters were set as secondary outcomes: (a) serum LDL-C levels at 4wks and 8wks, (b) serum total cholesterol (Total-C) levels, serum HDL-C levels, serum non-HDL-C levels, and LDL-C/HDL-C ratio at 4wks, 8wks, and 12wks, and (c) percentage of participants with serum LDL-C levels less than 120 mg/dL at 12wks.

(1) Lipid profiles

After overnight fasting, blood samples were collected from all participants in the morning. Measurements of serum LDL-C, total-C, and HDL-C levels were conducted at LSI Medience Inc. (Tokyo, Japan). These measurements were performed by enzymatic methods using the lipid test kits MetaboLead LDL-C, Determiner C-TC, and MetaboLead HDL-C (Minaris Medical Co., Ltd., Tokyo, Japan) on automatic analyzers JCA-BM8060 (JEOL Ltd., Tokyo, Japan) and LABOSPECT 008 α (Hitachi High-Tech Corporation., Tokyo, Japan). Non-HDL-C was calculated using the following formula: Non-HDL-C = T-Cho − HDL-C.

Sample size: No previous study has evaluated the effects of a 12-week intervention using only MR on serum LDL-C levels. The difference in effect between the MR and placebo groups was hypothesized to be large (an effect size of $d = 0.80$) [23]. At a significance level (α) of 0.05 and statistical power ($1-\beta$) of 0.80, the sample size was calculated to be 52 participants (n = 26 in each group). To accommodate potential dropouts and noncompliance with the protocol during the study period, this figure is augmented by 10%, resulting in a total of 56 participants (n = 28 in each group).

Enrollment, randomization, and blinding: Among 258 participants who provided informed consent in the study, 56 satisfied the eligibility criteria according to the principal investigator. The test foods (MR tablets and placebo tablets) were presented to the contract research organization (CRO, ORTHOMEDICO Inc.) by Toyo Sugar Refining Co., Ltd. The CRO's test food dispatcher provided the food identification numbers to the allocation manager, who was not directly implicated in the study, after determining that the test foods were unidentifiable and that the screening test data had been entered and verified. The allocation was by stratified block random allocation with serum LDL-C levels (a) between 120–129 mg/dL and (b) between 130–139 mg/dL as the stratification factor. The allocation manager randomly allocated 28 participants to the MR and placebo groups according to a computer-generated allocation table. Then, the allocation manager created a list containing codes which do not identify the test foods and provided this list only to the test food dispatcher in the CRO. The dispatcher then sent the test foods to the study participants. All study site personnel were blinded to the allocation, including the sponsor, principal investigator, co-investigator, test food dispatcher (e.g., study project co-director, study operation director, monitoring director, statistical analyst/responsible person), study site personnel, ethics committee members, and contract clinical laboratories. The allocation table was sealed and stored by the allocation manager in a secure location until the analysis participants and statistical analysis methods were determined.

Statistical analysis: All statistical analyses conducted in this study were two-sided, with statistical significance defined at 5%, and no adjustments made for multiple comparisons. Data analyses were executed using Windows SPSS version 23.0 (IBM Japan, Ltd., Tokyo, Japan).

Demographic characteristics were collated based on the participants under analysis. These characteristics encompassed age, height, body weight, body mass index, blood pressure, and serum lipid profile (including LDL-C, Total-C, HDL-C, non-HDL-C, and the LDL-C/HDL-C ratio) for each group. The primary and secondary outcomes are presented as the mean ±standard deviation (SD), except for the percentage of participants whose serum LDL-C levels were 120 mg/dL at 12wks. Group differences are presented as the difference values of the mean at pre-intervention or estimated marginal mean (EMM) after intervention and its 95% confidence interval (CI). The baseline values of both primary and secondary outcomes were determined from measurements taken prior to intervention and compared between groups using Welch's t-test. Subsequently, the measured values of primary and secondary outcomes at 4 weeks, 8 weeks, and 12 weeks were subjected to analysis utilizing a linear mixed model. Covariates included baseline values, while factors such as time, group, group-time interaction, baseline value-time interaction, and individual participants were also analyzed. Intergroup comparisons (percent difference and its 95% CI) were performed for participants whose serum LDL-C levels were 120 mg/dL at 12wks, and Fisher's exact test was used for the analysis. The datasets in this study were defined at the time of study design as follows. First, the intention to treat group included all participants who provided

informed consent and were included in the study. Second, the full analysis set includes the entire enrolled population set except: (a) participants who did not receive the intervention to which they were assigned, (b) participants who did not meet the inclusion criteria (i.e., participants with a confirmed diagnosis of any disease or those violating well-defined, objective, and important selection or exclusion criteria), (c) participants who never received any intervention after allocation, and (d) participants with no post-allocation data. Third, the per protocol set (PPS) included the full analysis set which excluded the following: (a) participants with an intake rate of < 80% for the test food, (b) participants with significant behavior that undermines the reliability of the study results, such as missing diary records, (c) participants who were reassessed to have met the exclusion criteria, (d) participants who had violated any of the study compliance rules during the study period, (e) participants in which the participant consumed food or drugs that are expected to significantly affect the study results during the study period, (f) participants who engaged in activities significantly different from the lifestyle at the time of study enrollment, and (g) participants in whom there were clear reasons for exclusion.

RESULT

Demographics: Figure 1 shows the study flowchart. Although all participants received the allocated test foods, 2 of them in the placebo group missed follow-up at either 8wks or 12wks. Incidentally, a participant who failed to show up for the examination at 8wks came at 12wks.

The datasets used for effective evaluation were PPS and modified PPS (mPPS). The PPS data excluded participants who have incomplete diaries that prevents the calculation of accurate intake rates of the study foods. On the other hand, the mPPS data from the PPS excluded participants who did not come at 12wks (n = 1, from the placebo group). Therefore, there were 54 (27 in each group) and 53 (26 in placebo group, 27 in. MR group) participants in the PPS and mPPS datasets, respectively.

Table 1 summarizes the participants 'background factors.

Lipid profiles: Initially (Prior), there were no discernible disparities noted among the groups in terms of serum levels of LDL-C, Total-C, HDL-C, non-HDL-C, and the LDL-C/HDL-C ratio (as depicted in Figure 2 and detailed in Table 2). As the investigation concluded (at 12 weeks), a statistically significant revelation unfolded: individuals in the MR cohort exhibited markedly diminished LDL-C levels in contrast to those in the placebo cohort (placebo group: 138.3 ±21.2 mg/dL; MR group: 122.6 ±20.3 mg/dL; EMM Group difference: -15.4 mg/dL; 95% CI: -26.3 to -4). Moreover, the MR group had a statistically significantly lower serum non-HDL-C levels compared with the placebo group at 8wks and 12wks (placebo vs. MR group; 8wks: 158.4 ±30.7 vs. 146.8 ±27.3 mg/dL, group difference of EMM: −12.1 mg/dL, 95%CI: −23.5 to −0.6; P = 0.039; 12wks: 158.6 ±28.1 vs. 143.5 ±31.8 mg/dL, group difference of EMM: −16.3 mg/dL, 95%CI: −27.8 to −4.9, P = 0.006; Figure 2c). Furthermore, the MR group showed a statistically significantly lower serum Total-C levels compared with the placebo group at 8wks and 12wks (placebo vs. MR group; 8 weeks: 222.8 ±24.6 vs. 214.8 ±26.4 mg/dL, group difference of EMM: −13.4 mg/dL, 95%CI: −24.8 to −2.0, P = 0.022; 12 weeks: 223.3 ±20.3 vs. 213.6 ±28.8 mg/dL, group difference of EMM: −15.1 mg/dL, 95%CI: −26.5 to −3.7, P = 0.010; Figure 2d). While no statistically significant group difference in HDL-C was observed (Figure 2b), the LDL-C/HDL-C ratio was statistically significantly lower in the MR group compared with the placebo group (Table 2). In addition to the blood lipid profile values, a statistically significantly higher percentage of participants the MR group had serum LDL-C levels < 120 mg/dL at 12wks compared with the placebo group (placebo vs. MR group; 19.2% vs. 48.1%) (Table 3

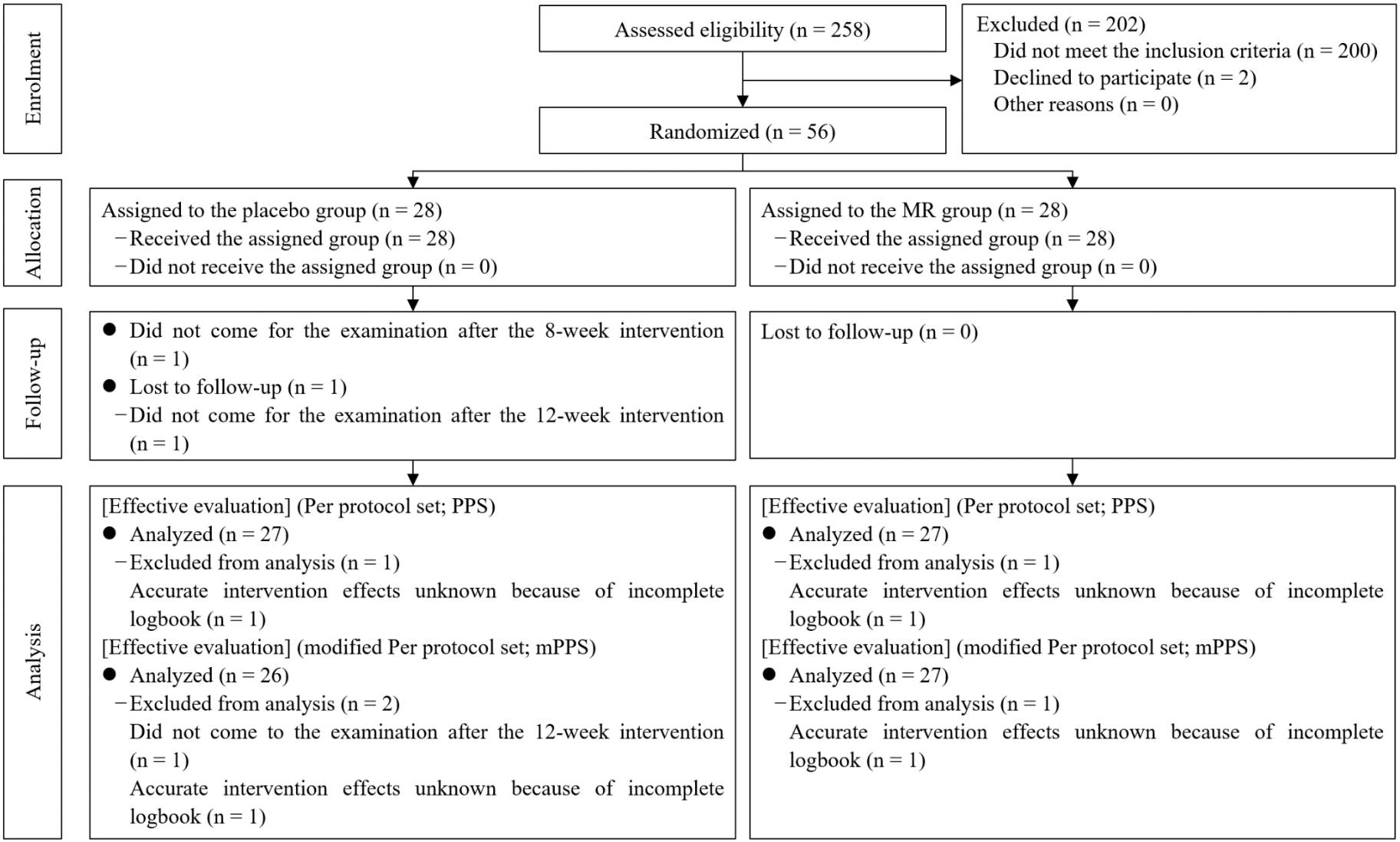


Figure 1. Flowchart of the participants in this study.

Table 1. Demographic characteristics

Items (unit)		ITT, SAF		PPS		mPPS	
		Placebo group (n = 28)	MR group (n = 28)	Placebo group (n = 27)	MR group (n = 27)	Placebo group (n = 26)	MR group (n = 27)
Sex	Male	13 (46.4%)	12 (42.9%)	12 (44.4%)	11 (40.7%)	12 (46.2%)	11 (40.7%)
	Female	15 (53.6%)	16 (57.1%)	15 (55.6%)	16 (59.3%)	14 (53.8%)	16 (59.3%)
Age (years)	Mean (SD)	45.9 (11.2)	44.8 (8.2)	46.2 (11.3)	44.4 (8.0)	46.3 (11.5)	44.4 (8.0)
	Median	46.0	45.0	46.0	45.0	46.5	45.0
	Min–Max	25–71	29–59	25–71	29–59	25–71	29–59
Height (cm)	Mean (SD)	163.5 (9.7)	163.3 (9.3)	162.7 (9.0)	163.0 (9.4)	163.0 (9.1)	163.0 (9.4)
	Median	163.85	160.80	162.90	158.50	163.85	158.50
	Min–Max	146.2–184.1	150.1–178.5	146.2–179.0	150.1–178.5	146.2–179.0	150.1–178.5
Body weight (kg)	Mean (SD)	64.6 (18.3)	61.9 (12.9)	62.9 (16.3)	61.7 (13.1)	63.2 (16.5)	61.7 (13.1)
	Median	64.05	61.90	63.90	60.70	64.05	60.70
	Min–Max	38.0–116.0	43.0–95.4	38.0–116.0	43.0–95.4	38.0–116.0	43.0–95.4
BMI (kg/m²)	Mean (SD)	23.8 (4.8)	23.0 (3.2)	23.5 (4.5)	23.0 (3.2)	23.6 (4.6)	23.0 (3.2)
	Median	22.85	22.55	22.60	22.40	22.75	22.40
	Min–Max	16.4–36.2	15.9–31.3	16.4–36.2	15.9–31.3	16.4–36.2	15.9–31.3
Systolic blood pressure (mmHg)	Mean (SD)	116.0 (15.1)	113.5 (11.5)	115.4 (15.1)	113.4 (11.7)	115.2 (15.3)	113.4 (11.7)
	Median	113.5	111.5	113.0	111.0	112.5	111.0
	Min–Max	87–157	92–142	87–157	92–142	87–157	92–142
Diastolic blood pressure (mmHg)	Mean (SD)	75.5 (10.5)	74.4 (9.1)	74.8 (10.0)	74.4 (9.3)	74.5 (10.1)	74.4 (9.3)
	Median	75.0	75.0	75.0	75.0	74.5	75.0
	Min–Max	51–100	55–92	51–100	55–92	51–100	55–92
LDL-C (mg/dL)	Mean (SD)	129.5 (5.4)	129.3 (5.6)	129.3 (5.4)	129.4 (5.7)	129.1 (5.4)	129.4 (5.7)
	Median	129.50	129.50	129.00	130.00	128.50	130.00
	Min–Max	120.0–139.0	120.0–139.0	120.0–139.0	120.0–139.0	120–139	120–139
HDL-C (mg/dL)	Mean (SD)	63.7 (19.4)	70.3 (19.8)	65.0 (18.5)	70.8 (20.0)	65.5 (18.7)	70.8 (20.0)
	Median	63.50	66.00	65.00	69.00	65.00	69.00
	Min–Max	29.0–107.0	42.0–104.0	34.0–107.0	42.0–104.0	34–107	42–104
Total-C (mg/dL)	Mean (SD)	214.0 (14.1)	221.7 (24.7)	213.4 (14.0)	222.1 (25.1)	213.3 (14.2)	222.1 (25.1)
	Median	213.00	213.50	213.00	214.00	212.00	214.00
	Min–Max	190.0–250.0	185.0–301.0	190.0–250.0	185.0–301.0	190-250	185–301
LDL-C/HDL-C ratio	Mean (SD)	2.2 (0.8)	2.0 (0.5)	2.1 (0.6)	2.0 (0.5)	2.1 (0.6)	2.0 (0.5)

Items (unit)		ITT, SAF		PPS		mPPS	
		Placebo group (n = 28)	MR group (n = 28)	Placebo group (n = 27)	MR group (n = 27)	Placebo group (n = 26)	MR group (n = 27)
	Median	2.10	2.00	2.00	1.90	2.00	1.90
	Min–Max	1.2–4.7	1.3–2.9	1.2–3.7	1.3–2.9	1.2–3.7	1.3–2.9
non-HDL-C (mg/dL)	Mean (SD)	150.3 (16.3)	151.4 (22.2)	148.4 (13.1)	151.3 (22.6)	147.8 (12.9)	151.3 (22.6)
	Median	149	147.5	149	147	148.5	147
	Min–Max	123–201	136–259	123–174	136–259	123–174	136–259

Data presented as mean, standard deviation (SD), median, minimum (Min), and maximum (Max), except for sex. Data in the gender row indicate the corresponding number of relevant participants.

BMI, body mass index; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; Total-C, total cholesterol

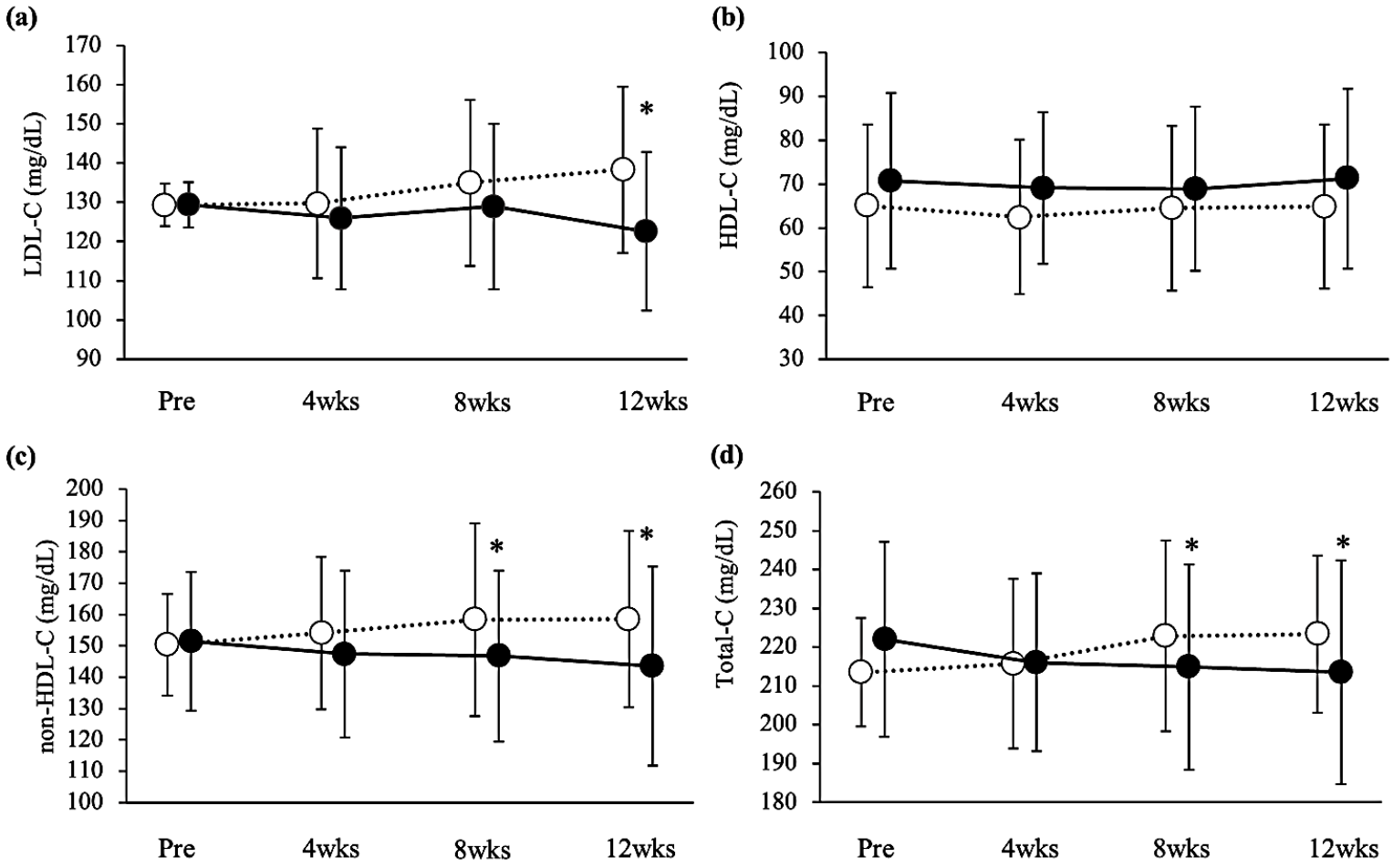


Figure 2. Changes in lipid profile pre- and post-intervention.

Data are presented as mean standard deviation (SD). The closed circles and solid line denote the MR group (n = 27). The open circles and dashed line denote the placebo group (Pre and 4wks: n = 27; 8wks and 12wks: n = 26). (a), LDL-C; (b), HDL-C; (c), non-HDL-C; (d), Total -C. *P < 0.05 (vs. placebo group)

Pre, pre-intervention; 4wks, 4 weeks of intervention; 8wks, 8 weeks of intervention; 12wks, 12 weeks of intervention; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; Total-C, total cholesterol.

Table 2. LDL-C/HDL-C ratio

Time point	Placebo group			MR group			Between-group comparison	
	n	Mean (SD)		n	Mean (SD)		Difference (95%CI)	*P* value
Pre	27	2.1 (0.6)		27	2.0 (0.5)		0.2 (–0.5 to0.1)	0.259
4wks	27	2.2 (0.7)		27	2.0 (0.6)		0.1 (–0.4 to 0.1)	0.338
8wks	26	2.3 (0.8)		27	2.0 (0.8)		0.1 (–0.3 to 0.2)	0.684
12wks	26	2.3 (0.9)		27	1.9 (0.7)		0.3 (–0.5 to 0.0)	0.034*

Data are presented as mean and standard deviation (SD). *$P < 0.05$.
Pre, pre-intervention; 4wks, 4 weeks of intervention; 8wks, 8 weeks of intervention; 12wks, 12 weeks of intervention; CI, confidence interval.

Table 3. Participants with serum LDL-C levels < 120 mg/dL at 12 weeks

Number of cases (%) in each group					Between-group comparison	
Placebo group (n = 26)			MR group (n = 27)		Difference (95%CI)	*P* value
5	(19.2%)		13	(48.1%)	28.9% (–54.4 to –3.4)	0.042*

*$P < 0.05$; CI, confidence interval

Adverse events: Continuous ingestion of the test foods did not result in any adverse effects during the clinical study.

DISCUSSION

In this study, we examined the effect of MR on serum LDL-C reduction among participants with an LDL-C of 120–139 mg/dL, who are considered to be at high risk for ASCVD.

CVDs and cardiovascular events are major contributors to disability and the main leading causes of death worldwide [24]. The number of patients surged from 271 million in 1990 to 523 million in 2019, almost doubling over a span of 29 years. Dyslipidemia is highlighted as a prominent risk factor for ASCVD, stroke, and mortality, as emphasized in the Guidelines for the Prevention of Atherosclerotic Disease jointly established by the American College of Cardiology (ACC) and the American Heart Association (AHA) [25]. LDL-C has been well-documented as a reliable marker for ASCVD because of its role in causing atherosclerosis [2] via the formation and growth of coronary atherosclerotic plaques [26] in the vessel wall, which is associated with LDL-C oxidation.

In this study, it was observed that the MR group exhibited markedly reduced serum LDL-C levels compared to the placebo group following the 12-week intervention. Moreover, both serum Total-C and serum non-HDL-C levels were notably lower compared to the placebo group after 8 and 12 weeks. Additionally, the LDL-C/HDL-C ratio demonstrated a significant decrease in the MR group compared to the placebo group after the 12-week intervention period. Furthermore, the MR group had a significantly higher percentage of participants with LDL-C < 120 mg/dL after the 12-week intervention compared with the placebo group (48.1% vs. 19.2%). Several studies have reported that lower serum LDL-C levels lead to a lower absolute risk of ASCVD [27–30].

The effect of MR intake on the reduction of serum LDL-C levels may involve three pathways: 1) regulation of lipid metabolism via its effect on gastric inhibitory polypeptide (GIP), 2) inhibition of chylomicron synthesis, and 3) reduction of cholesterol synthesis via inhibition of 3- hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase.

The effect of MR on GIP has been reported in a study using mice [31], suggesting that MR regulates the

synthesis of GIP by K cells in the gastrointestinal tract. Several studies have reported that healthy dietary patterns such as traditional Japanese diet are associated with low LDL-C levels [32–33]. Quercetin, the aglycone released from MR in the intestinal track, binds to C/EBPβ and suppresses ApoB, the major apoprotein of LDL-C [34]. An *in vitro* study by Suganya *et al.* [35] revealed the antidyslipidemic property of rutin, mediated by its inhibition of HMG-CoA reductase. Therefore, MR is predicted to inhibit cholesterol synthesis and reduced serum LDL cholesterol levels via an inhibitory effect on HMG-CoA reductase.

CVDs are generally treated with statins and other HMG-CoA reductase inhibitors. Some patients experience adverse events that are associated with statin use, leading to either discontinuation or dose reduction, while a nocebo effect has been reported to occur in statin-intolerant patients who are reluctant to undergo treatment [8]. As there may be limitations to pharmacologic treatment alone, some researchers recommend combining functional foods with therapeutic drugs [36,37]. Because the MR used in this study demonstrated a significant lowering effect on serum LDL-C levels, it has potential as a treatment adjunct as a functional food in combination with therapeutic drugs or alone, which can ultimately help to reduce the global burden of CVD-related morbidity and mortality.

One limitation of this study was that it was not conducted with participants regularly consuming a high-fat and high-caloric diet. By considering various dietary patterns, more detailed evidence on the effect of MR on lipid metabolism may be obtained.

Conclusions: A 12-week intake of MR (200 mg/day) significantly lowered serum LDL-C levels, suggesting that MR intake could prevent the occurrence and development of atherosclerosis, as well as reduce the risk of heart disease and stroke.

List of abbreviations: ASCVD, atherosclerotic cardiovascular disease; CVDs, cardiovascular diseases; MR, monoglucosyl rutin; LDL-C, low density lipoprotein cholesterol; HDL-C, High density lipoprotein cholesterol; Total-C, total cholesterol; Pre, pre-intervention; 4wks, 4 weeks; 8wks, 8 weeks; 12wks, 12 weeks; CAND, Calorie and Nutrition Diary; BMI, body mass index; SD, standard deviation; EMM, estimated marginal mean; SE, standard error; 95%CI, 95% confidence interval; PPS, per protocol set; mPPS, modified PPS; GIP, gastric inhibitory polypeptide; HMG-CoA, 3- hydroxy-3-methylglutaryl coenzyme A

Competing interests: The sponsor of the present study, Toyo Sugar Refining Co., Ltd., assigned ORTHOMEDICO Inc. to conduct the study. Yushi Hashizume and Mahamadou Tandia were affiliated with Toyo Sugar Refining Co., Ltd.

Authors' contributions: Yushi Hashizume drafted the manuscript. Yushi Hashizume and Mahamadou Tandia designed the study and interpreted the results.

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