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Daily consumption of monoglucosyl-rutin prevents high-fat diet-induced obesity by suppressing gastric inhibitory polypeptide secretion in mice

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ABSTRACT

Background: Alpha monoglucosyl-rutin (4^{G}-α-D-glucopyranosyl rutin, αMR) has been shown to stimulate antioxidant defenses and anti-glycation. We evaluated the effects of αMR on body weight gain in mice. This study aimed to investigate the effects of αMR supplementation on diet-induced excess weight gain in mice. Also assessed alterations in insulin, leptin, interleukin 6 (IL-6), resistin, tumor necrosis factor α (TNFα), gastric inhibitory polypeptide (GIP), ghrelin,

and glucagon to elucidate the mechanism underlying anti-obesity effects.

Methods: Male C57BL/6J mice were divided into four groups: Control low-fat diet, low-fat diet + 0.5% αMR, high-fat diet, and high-fat diet + 0.5% αMR. Blood chemistry, hepatic lipids, and serum metabolic hormones and cytokines were evaluated after 4 and 13 weeks.

Results: After 6 weeks, the high-fat diet group gained more weight than the low-fat diet group. Supplementing the high-fat diet with αMR suppressed weight gain by week 13. Visceral fat weight was higher in the high-fat diet group on weeks 4 and 13, while αMR supplementation inhibited increase on week 13 but not on week 4. Serum levels of gastric inhibitory polypeptide were higher in the high-fat-diet group than in the low-fat-diet group. αMR supplementation inhibited this elevation and regulated levels of serum leptin and hepatic triglycerides.

Conclusion: For the first time, we demonstrated how daily consumption of αMR inhibits diet-induced visceral fat accumulation by regulating the secretion of gastric inhibitory polypeptide, which thereby prevents excess weight gain. Therefore, αMR may be a promising potential functional food.

Keywords: Anti-obesity, gastric inhibitory polypeptide, mouse, alpha monoglucosyl-rutin; quercetin

BACKGROUND

The analysis of life insurance data reveals how obesity is associated with increased death rates, as obesity involves highly elevated risks of adverse health outcomes such as type 2 diabetes mellitus and cardiovascular disease [1, 2]. Obesity can be categorized either as total obesity (body mass index ≥ 25.0 kg/m^2) and severe obesity (body mass index ≥ 30.0 kg/m^2).

The prevalence of obesity worldwide has been estimated to reach 18% for men and surpass 21% for women by 2025, with severe obesity surpassing 6% in men and 9% in women in developed countries [3]. The economic burden of obesity-related diseases has been estimated to be around $48–66 billion/year in the US and £1.9–2 billion/year in the UK by 2030 [4]. Therefore, prevention of excess body weight gain and obesity is a high research priority.

Weight gain results from a chronic positive energy balance, occurring when energy expenditure is lower than energy intake. Consequently, management of daily food consumption is important for controlling weight. Studies have reported the probability that our daily foods contain several ingredients that protect against obesity by inhibiting lipid absorption and accumulation or promoting energy combustion.

Green tea catechins, cacao oligomeric procyanidins, and glucomannan have been shown to reduce lipid absorption [5-8]. Resveratrol, a natural polyphenolic compound found in berries, has been shown to regulate lipid metabolism through AMP-activated protein kinase α (AMPKα), silent information regulator 2 homolog 1 (Sirt1), and the peroxisome proliferator-activated receptor γ (PPARγ) pathway in zebrafish [9]. Chili pepper capsaicin has been reported to activate transient receptor potential cation channel sub-family V member 1 (TRPV1) channels and reduce energy expenditure directly by activating brown adipose tissue [10]. These data suggest that daily consumption of foods rich in such compounds might contribute to preventing weight gain.

Another potential compound is quercetin (Figure 1A), one of the major flavonoids, which has recently been reported to exhibit anti-obesity activity and regulate lipid metabolism in diabetes mellitus [11-13]. The majority of quercetin is found in plants and exclusively in *O*-glycoside forms, but not in the aglycone form [14]. Accordingly, research on the effects of quercetin should assess glucosides in addition to quercetin aglycone itself.

Rutin (quercetin-3-*O*-rutinoside, Figure 1B) is one of the major quercetin glucosides, and is widely distributed in plant foods such as buckwheat seeds, coriander, and nalta jute [14, 15]. However, its bioavailability has been reported to be lower than that of quercetin aglycone [16]. Alpha monoglucosyl-rutin (4^{G}-α-D-glucopyranosyl rutin, αMR, Figure 1C), where glucose is added using glycosyltransferase, has been suggested as an alternative [17]. The absorption rate of αMR in rats was approximately 4.5 times that of quercetin aglycone [18]. Additionally, αMR exhibited stronger radioprotective properties *in vitro* [19]. αMR was also reported to inhibit azoxymethane-induced colon carcinogenesis, stimulate the antioxidant defense system, and suppress glycation in rats [20-22]. Despite these findings, data on the effects of αMR are still insufficient.

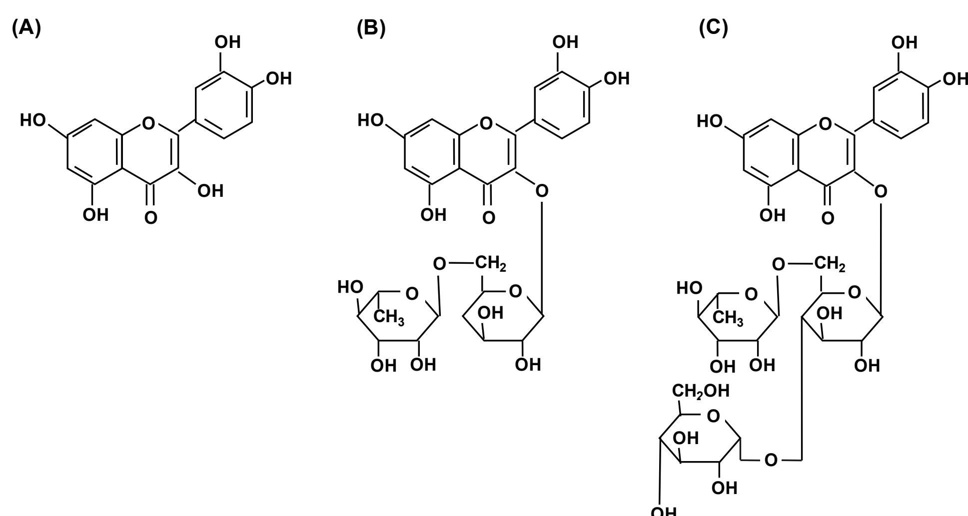

Figure 1. Structure of quercetin (A), rutin (B), and monoglucosyl-rutin (C)

MATERIALS AND METHODS

Toyo Sugar Refining Co. Ltd. (Tokyo, Japan) donated the quercetin glycoside, αMR, used in this study. According to the data from EU-Material Safety data sheet, the toxicological information of αMR was following: LD_{50} oral (mouse), >42,000 mg/kg; skin and eye irritation, none. Quercetin was obtained from Sigma-Aldrich (St. Louis, MO, USA). The control low-fat diet (LFD) consisted of laboratory chow (MF, Oriental Yeast Co. Ltd., Tokyo, Japan). Oriental Yeast Co. Ltd. prepared an MF-based high-fat diet (HFD) and 0.5% αMR-containing LFD and HFD (Table 1). The water used was ultra-pure. All other reagents were of the highest grade available.

Table 1. Composition of animal diets

Ingredients	LFD	LFD+αMR	HFD	HFD+αMR
		(%)		
Water	7.9	7.9	7.9	7.9
Crude proteins	23.1	23.1	23.1	23.1
Crude lipids	5.1	5.1	30.0	30.0
Crude ash	5.8	5.8	5.8	5.8
Crude fibers	2.8	2.8	2.8	2.8
Soluble nitrogen-free products	55.3	54.8	30.4	29.9
αMR	—	0.5	—	0.5
Energy (kcal/g)[1]	359.5	357.5	484.0	482.0

αMR, α-monoglucosyl rutin; LFD, low-fat diet (control); HFD, high-fat diet.
[1]Energy values were calculated using Atwater factors; 4 kcal/g for soluble nitrogen-free products and crude protein, and 9 kcal/g for crude lipid.

Institutional approval of the study protocol

All procedures for animal experiments were conducted according to the guidelines for the care and use of laboratory animals of the University of Miyazaki (Miyazaki, Japan). The experimental protocol was registered under the number 2014-005-4.

Animal experiments

Male C57BL/6J mice were obtained from Japan SLC (Shizuoka, Japan) and housed in an air-conditioned room (temperature: 23 ± 1°C; humidity: 55 ± 5%) under a 12-h dark/light cycle (light on between 9:00 and 21:00) with free access to deionized water and laboratory chow. After 1-week of acclimatization, the animals were submitted to two studies according to these protocols:

Protocol I: Ninety-six 5-week-old mice were randomly divided into the following four groups: LFD; LFD + 0.5% αMR; HFD; HFD + 0.5% αMR (Table 1). Each group was sub-divided into two groups. After 4 weeks, the first sub-group was sacrificed. After 13 weeks, the second sub-group was sacrificed. Each animal was anesthetized with isoflurane between Zeitgeber time (ZT) 11 and ZT13. In this study, ZT0 represents the time when the light was turned on at the start of the light period. Accordingly, the light period ran from ZT0 to ZT12 and the dark period ran from ZT12 to ZT24. In this study, six mice per sub-group were sacrificed after a 6-h fast, while six other mice were sacrificed without fasting. Blood samples were taken from the abdominal vein into serum Capiject tubes (Terumo Medical Corporation, Somerset, NJ, USA) under anesthesia. Serum fractions were obtained by centrifugation (3,500 × *g*, 90 sec) after standing at room temperature for 30 min and were stored at −80°C until analysis. The liver, kidney, thymus, spleen, and a section of visceral fat (peritestis) were weighed. To eliminate the technical variations between prosectors, the same researchers collected each organ. One liver section was stored at −80°C for lipid analysis after flash-freezing in liquid nitrogen.

Protocol II: Six 8-week-old mice were randomly divided into three groups. Quercetin (500 μmol/10 mL/kg body weight), αMR (500 μmol/10 mL/kg), or vehicle solvent (deionized water, 10 mL/kg) were orally administrated after a 12-h fast according to our previous methods [23]. The mice were decapitated one h after treatment under anesthesia with isoflurane and trunk blood was collected into heparin-treated Capiject tubes. Plasma fractions were separated by

centrifugation at 3,500 × *g* for 90 sec and acidified by the addition of 15 μL formic acid to 1 mL plasma. After adding 5 μL of 100 mM ascorbic acid, the plasma was stored at −80°C for analysis.

Blood chemistry

Serum biochemical parameters (triglycerides, total cholesterol, high-density lipoprotein [HDL] cholesterol, free cholesterol, phospholipids, and non-esterified fatty acids) were analyzed using test kits obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All standards were used in accordance with the manufacturer's instructions. Non-HDL cholesterol amounts were calculated using the following formula:

$$\text{Non-HDL cholesterol} = \text{total cholesterol} - \text{HDL cholesterol}$$

Hepatic lipid analysis

Lipids from liver samples were extracted according to modified methods described previously [24-26]. Briefly, 200-mg aliquots of the liver samples were homogenized with 1 mL of 50 mM sodium acetate. Then, 6 mL of chloroform-methanol (2:1 [vol/vol]) was added and the mixture was incubated at 40°C for 30 min. A 500-μL aliquot of the organic phase was dried with a centrifugal concentrator (CC-105, Tomy Seiko Co., Ltd., Tokyo, Japan). The residues were dissolved in 200 μL of 10% Triton X-100 containing isopropyl alcohol. Triglycerides, total cholesterol, and phospholipid levels were analyzed using Wako kits.

Determination of serum metabolic hormone and cytokine levels

A multiplex biometric enzyme-linked immunosorbent assay-based immunoassay containing dye microsphere conjugate with a monoclonal antibody specific for a target protein was used according to the manufacturer's instructions. We used a Mouse Metabolic Hormone panel (MMHMAG-44KK-9, Millipore, Billerica, MA, USA) for the simultaneous detection and quantitation of GIP, IL-6, insulin, leptin, monocyte chemoattractant protein 1 (MCP-1), resistin, TNFα, ghrelin, and glucagon. We also used a Mouse Adiponectin Single Plex panel (MADPNMAG-70K-1, Millipore). Cytokine levels were determined using a multi-analyte profile and the MAGPIX system (Millipore).

Extraction of quercetin metabolites from plasma

The extraction procedure was carried out according to our previous method [23] with some modifications. Aliquots (800 µL) of frozen plasma samples from two mice were loaded onto Bond Elut Plexa (30 mg) extraction cartridges (Agilent Technologies, Santa Clara, CA, USA), which were equilibrated with 0.01 M oxalic acid. After washing the cartridge with 2 mL of 0.01 mM oxalic acid, quercetin metabolites were eluted with 1 mL of methanol containing 0.5% trifluoroacetic acid. The eluate was evaporated to dryness using a centrifugal concentrator. The residue was then dissolved in 100 µL of methanol containing 0.5% trifluoroacetic acid, filtered with a 0.2-µm membrane filter (Millex-LG, Millipore), and analyzed by high-performance liquid chromatography (HPLC), as described below.

HPLC-diode array detector (DAD)

Quercetin, αMR, and their metabolites were analyzed by HPLC in combination with DAD as described in our previous report [14] with small modifications. The HPLC system employed was a JASCO LC-NetII/ADC system control program (Tokyo, Japan) equipped with a ChromNAV chromatography data station, PU-2089 Plus pump, AS-2057 Plus autosampler, CO-2060 Plus column oven, and MD-2018 Plus DAD system monitoring all 200–600 nm wavelengths. The column, Capcell Pak UG120 (3.0 mm i.d. × 150 mm, 3 µm, Shiseido Co. Ltd., Tokyo Japan), was used at 40°C. Linear gradient elution was performed with solution A, which was composed of 50 mM sodium phosphate (pH 3.3) and 10% methanol, and solution B, comprised of 70% methanol, delivered at a flow rate of 0.4 mL/min as follows: Initially 100% of solution A; 70% A for 10 min; 65% A for 15 min; 60% A for 5 min; 50% A for 5 min; for another 10 min, 0% A; maintaining at 0% A for 20 min. The injection volume for the extract was 4 µL.

Statistical analysis

Statistical analysis was conducted using StatView for Windows (version 5.0, SAS Institute, Cary, NC, USA). One-way analysis of variance (ANOVA) was used. The Tukey-Kramer method was applied for multiple comparisons between groups. Results were considered significant if the probability of error was <5%.

RESULTS

Food intake and body and organ weights

After 6 weeks, the animals from the HFD group exhibited significantly higher body weight gain than the LFD group (Figure 2). On weeks 10 and 13, weight gain in the HFD + αMR group was significantly lower than weight gain in the HFD group and did not differ from weight gain in the LFD group. During the experiments, mice in both the HFD and HFD + αMR groups consumed similar amounts of dietary energy (12.7 and 13.0 kcal/mouse/day, respectively); consumption was lower in the LFD group (11.1 kcal/mouse/day) (Table 2). Visceral fat weight increased significantly in the HFD group on weeks 4 and 13 (Table 2); supplementation of the HFD with αMR reduced visceral fat weight on week 13 but not on week 4.

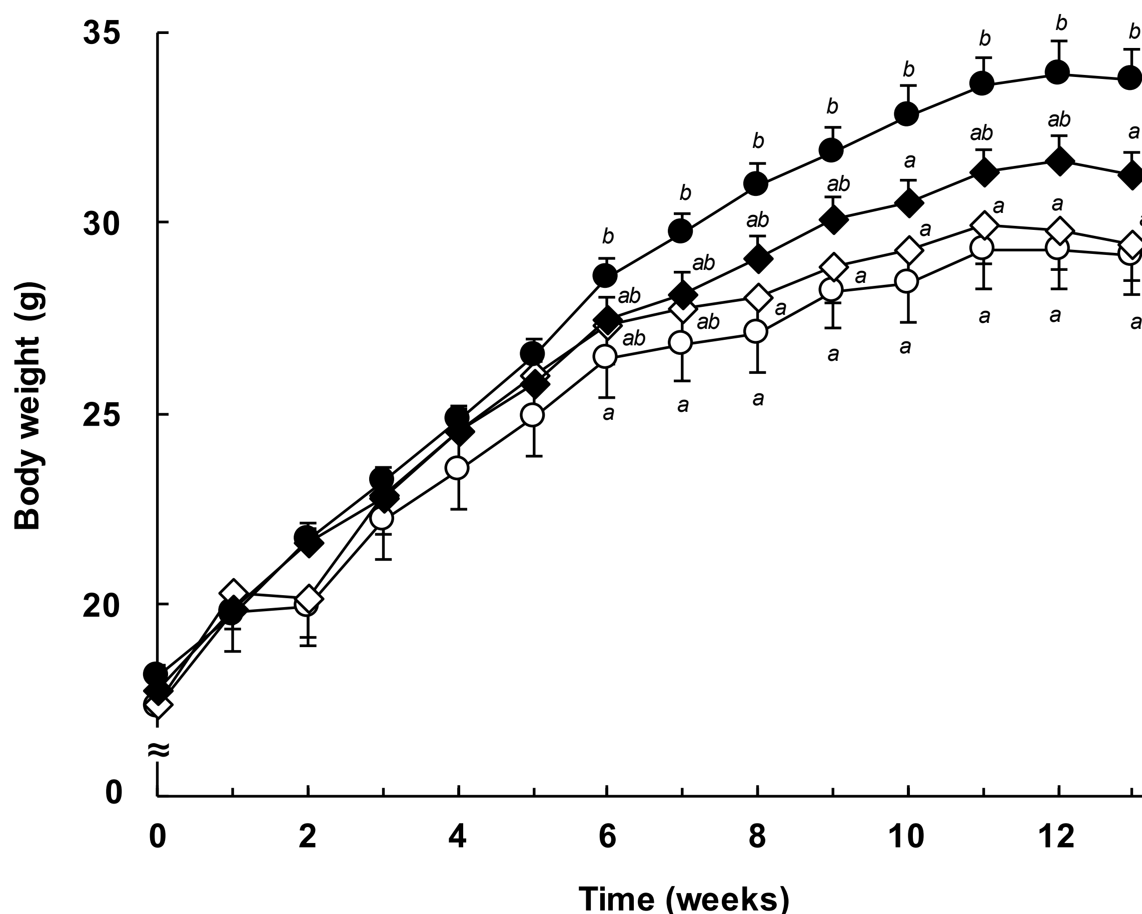

Figure 2. Effects of α-monoglucosyl-rutin (αMR) on body weight gain in mice

Mice were divided into four groups: Control low-fat diet (LFD, ○), LFD + 0.5% αMR (◇), high-fat diet (HFD, ●), and HFD + αMR (◆). Data indicate mean ± SE. Different superscript letters indicate significant differences ($p < 0.05$, Tukey-Kramer test). The groups did not differ until week 5.

Table 2. Effects of α-monoglucosyl rutin (αMR) administration on body weight, food consumption, and organ weights in mice

	LFD	LFD+αMR	HFD	HFD+αMR
Body weight (g)[1]				
Initial (week 0)	17.4±0.2[a]	17.4±0.2[a]	18.1±0.3[a]	17.8±0.3[a]
Middle (Week 4)	22.2±0.3[a]	22.9±0.3[a]	23.3±0.4[a]	22.8±0.4[a]
Final (week 13)	29.1±0.5[a]	29.5±0.7[a]	33.8±0.8[b]	31.2±0.6[a]
Food consumption				
kcal/mice/day	11.1±0.7[a]	11.4±1.0[a]	12.7±0.4[b]	13.0±1.0[b]
Relative organ weight (g/100 g body weight)				
Middle (week 4)				
Liver	4.95±0.29[a]	4.80±0.17[ab]	4.43±0.24[ab]	4.08±0.15[b]
Kidney	1.27±0.09[a]	1.23±0.06[a]	1.33±0.07[a]	1.19±0.04[a]
Spleen	0.24±0.01[a]	0.25±0.01[a]	0.35±0.05[a]	0.27±0.01[a]
Visceral fat	1.29±0.11[a]	1.52±0.10[a]	2.76±0.34[b]	2.67±0.27[b]
Final (week 13)				
Liver	3.92±0.17[ac]	4.17±0.13[a]	3.24±0.05[bc]	3.48±0.06[c]
Kidney	1.17±0.03[a]	1.10±0.02[a]	0.93±0.02[b]	1.06±0.06[ab]
Spleen	0.26±0.01[ab]	0.25±0.01[ab]	0.19±0.01[a]	0.28±0.03[b]
Visceral fat	1.37±0.10[a]	1.81±0.17[ac]	4.76±0.23[b]	3.09±0.65[cd]

LFD, control low-fat diet; HFD, high-fat diet. Data indicate mean ± SE.
[1]Initial (week 0) and middle (week 4) groups consisted of 24 mice each. Final (week 13) groups consisted of 12 mice each. Different superscript letters indicate significant differences ($p < 0.05$, Tukey-Kramer test).

Serum lipid parameters

On both week 4 and week 13, total cholesterol, HDL cholesterol, free cholesterol, and phospholipid levels were significantly higher in mice from the HFD group than in LFD group mice regardless of whether the animals were fasted prior to analysis (Figure 3A–D). Non-HDL cholesterol and triglyceride levels were also significantly higher on week 13 in HFD mice that were not fasted (Figure 3E and F). Increases in total cholesterol and non-HDL cholesterol levels were inhibited by αMR supplementation on week 13 but not on week 4.

Hepatic lipid levels

We evaluated major hepatic lipids such as triglycerides, total cholesterol, and phospholipids. Only hepatic triglyceride levels increased significantly in HFD mice that were not fasted on week 13 (Figure 4A). αMR supplementation inhibited this increase. Levels of total cholesterol and phospholipids did not differ between the LFD and HFD groups (Figure 4B and C).

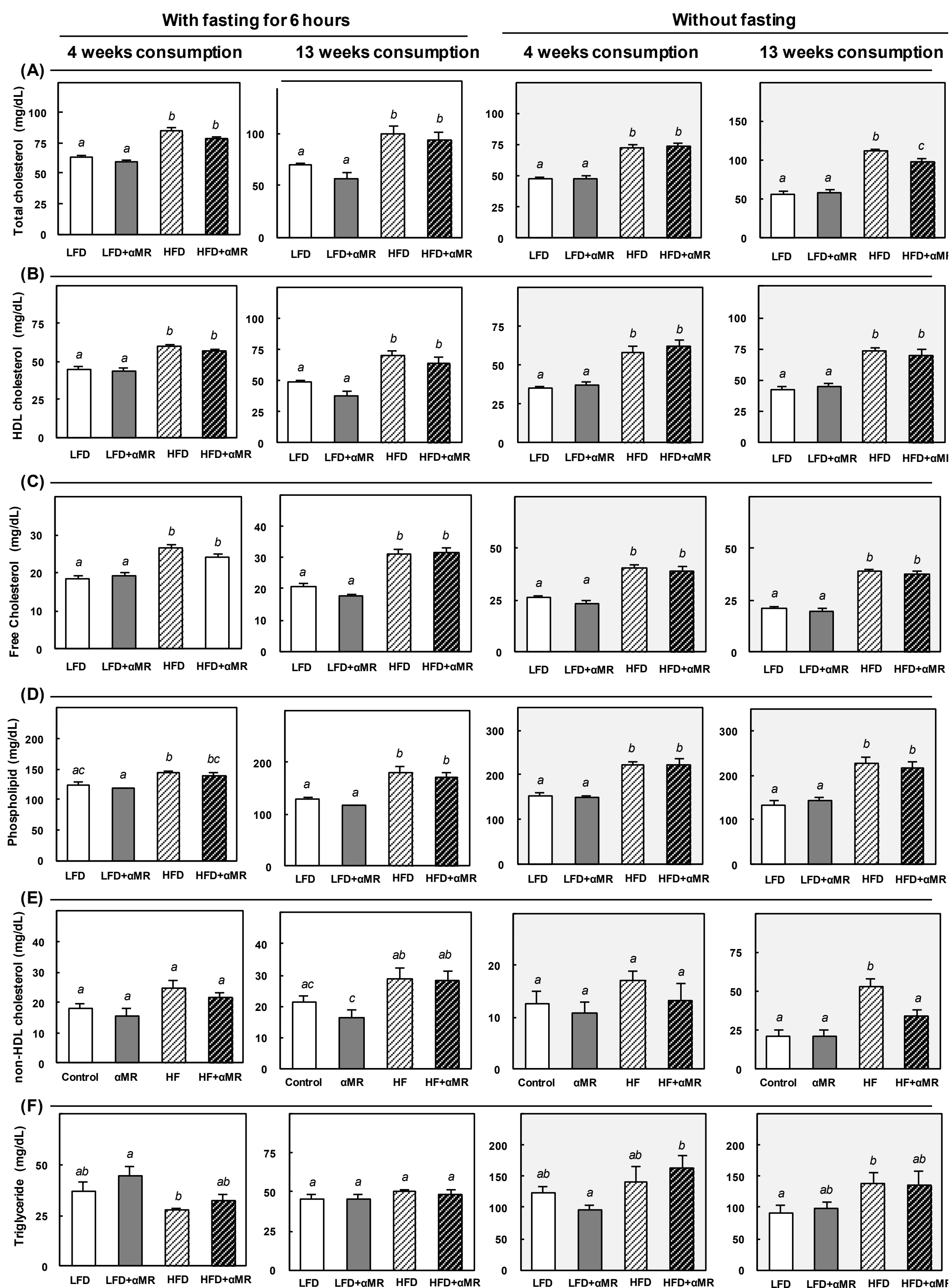

Figure 3. Effects of α-monoglucosyl rutin (αMR) on serum biochemical parameters in mice

Mice were divided into four groups: Control low-fat diet (LFD), LFD + 0.5% αMR, high-fat diet (HFD), and HFD + 0.5% αMR. Twelve mice from each group were sacrificed on week 4, and the other 12 mice were sacrificed on week 13. Mice were sacrificed either following

a 6-h fast or without fasting. Data indicate serum lipid amounts (mean ± SE, n = 6). (A) Total cholesterol. (B) High-density lipoprotein (HDL) cholesterol. (C) Free cholesterol. (D) Phospholipids. (E) Non-HDL cholesterol. (F) Triglycerides. Superscript letters indicate significant differences ($p < 0.05$, Tukey-Kramer test).

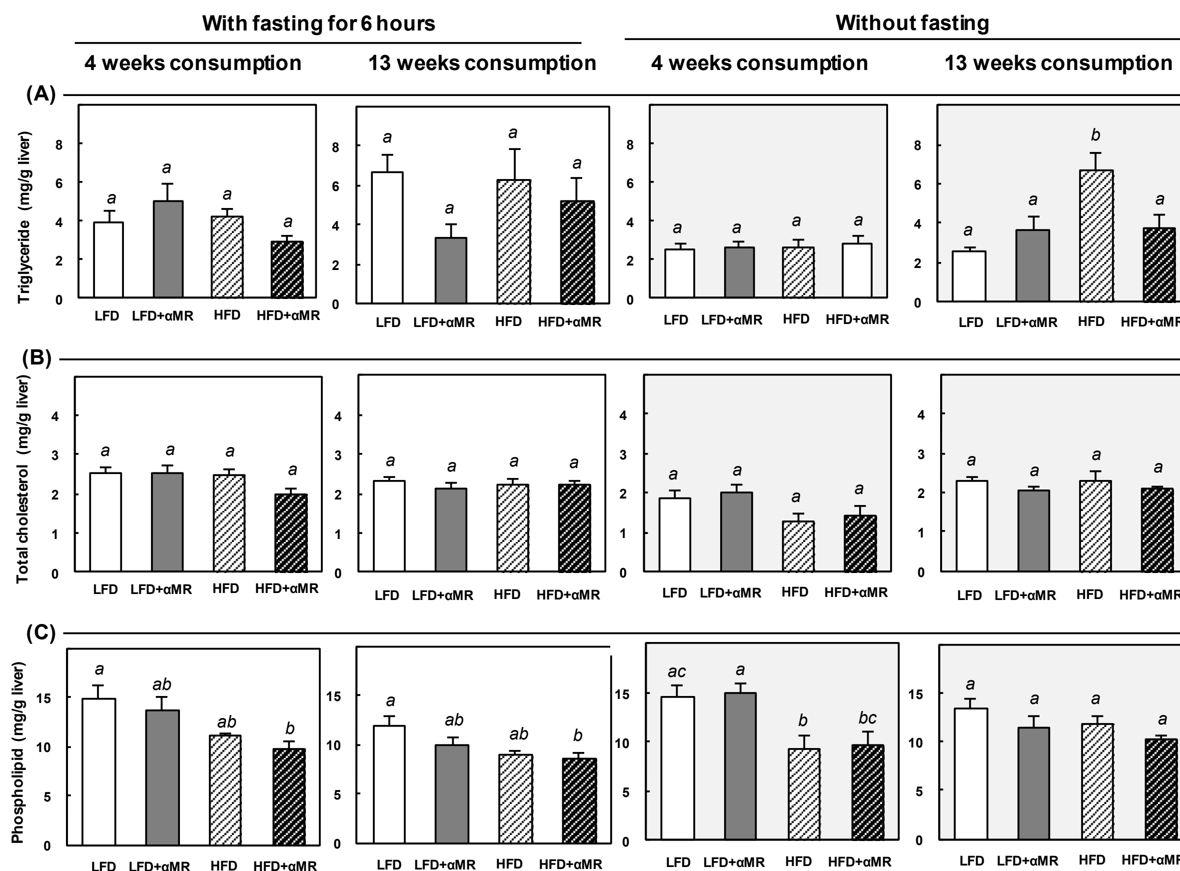

Figure 4. Effects of α-monoglucosyl-rutin (αMR) on hepatic lipid levels in mice

Mice were divided into four groups: Control low-fat diet (LFD), LFD + 0.5% αMR, high-fat diet (HFD), and HFD + 0.5% αMR. Twelve mice from each group were sacrificed on week 4, and the other 12 mice were sacrificed on week 13. Mice were sacrificed either following a 6-h fast or without fasting. Data indicate hepatic lipid amounts (mean ± SE, n = 6). (A) Triglycerides. (B) Total cholesterol. (C) Phospholipids. Superscript letters indicate significant differences ($p < 0.05$, Tukey-Kramer test).

Determination of serum metabolic hormone and cytokine levels

We analyzed serum levels of adiponectin, GIP, IL-6, insulin, leptin, MCP-1, resistin, TNFα,

ghrelin, and glucagon in mice after a 13-week consumption of test diets without fasting. Ghrelin and glucagon values were under the detection limits.

Serum GIP and leptin levels were significantly upregulated in the HFD group (Figure 5A and B), but supplementation with αMR inhibited the increase. Levels of the other six hormones and cytokines did not differ between the LFD and HFD groups (Figure 5C–H).

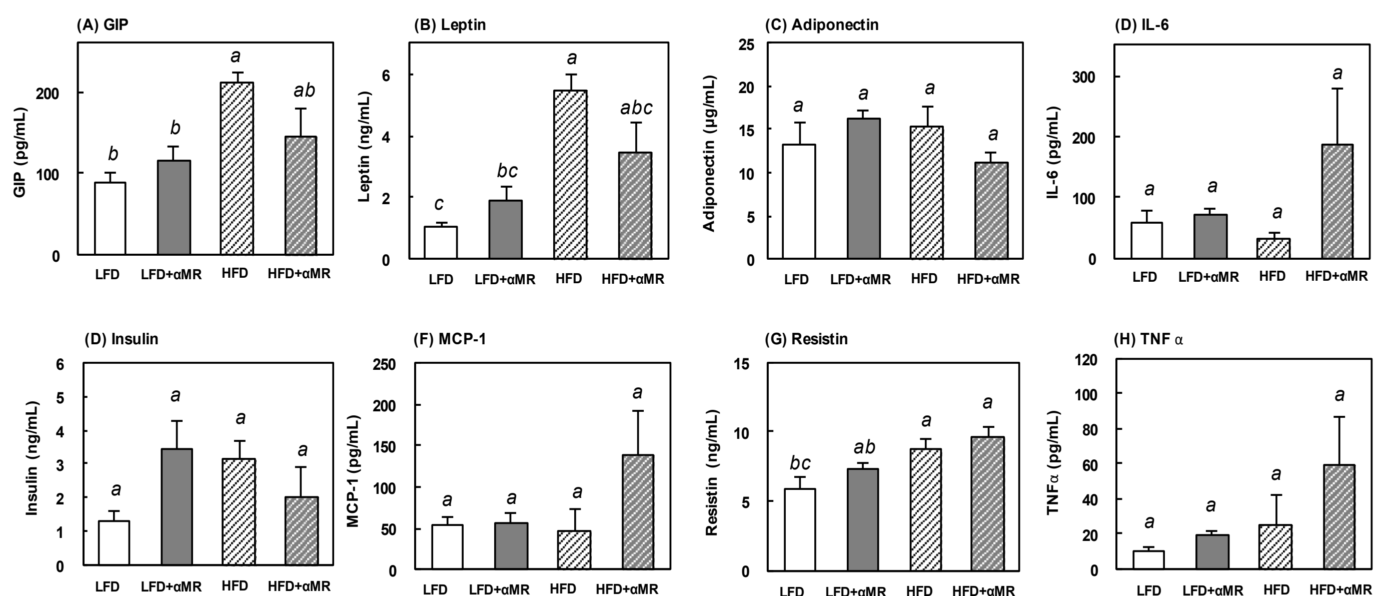

Figure 5. Effects of α-monoglucosyl-rutin (αMR) on serum parameters in mice

Serum levels of hormones and cytokines following a 13-week treatment with a low-fat diet (LFD), LFD supplemented with αMR, high-fat diet (HFD), or HFD supplemented with αMR. (A) Adiponectin. (B) Gastric inhibitory polypeptide (GIP). (C) Interleukin 6 (IL-6). (D) Insulin. (E) Leptin. (F) Monocyte chemoattractant protein 1 (MCP-1). (G) Resistin. (H) Tumor necrosis factor α (TNFα). Data indicate mean ± SE (n = 6). Superscript letters indicate significant differences ($p < 0.05$, Tukey-Kramer test).

αMR absorption

Figure 6 shows typical HPLC chromatograms at 370 nm of plasma samples collected from mice administered vehicle solvent, quercetin, or αMR. Major peaks did not appear in the plasma from vehicle control mice.

More than 10 major peaks were detected in plasma collected from mice treated with quercetin and αMR. The retention times of these peaks did not conform to those of quercetin

or αMR, indicating that the compounds were poorly absorbed in their intact forms. The peaks detected in plasma from both groups indicated the typical flavonoid spectrum and the chromatogram profiles were similar.

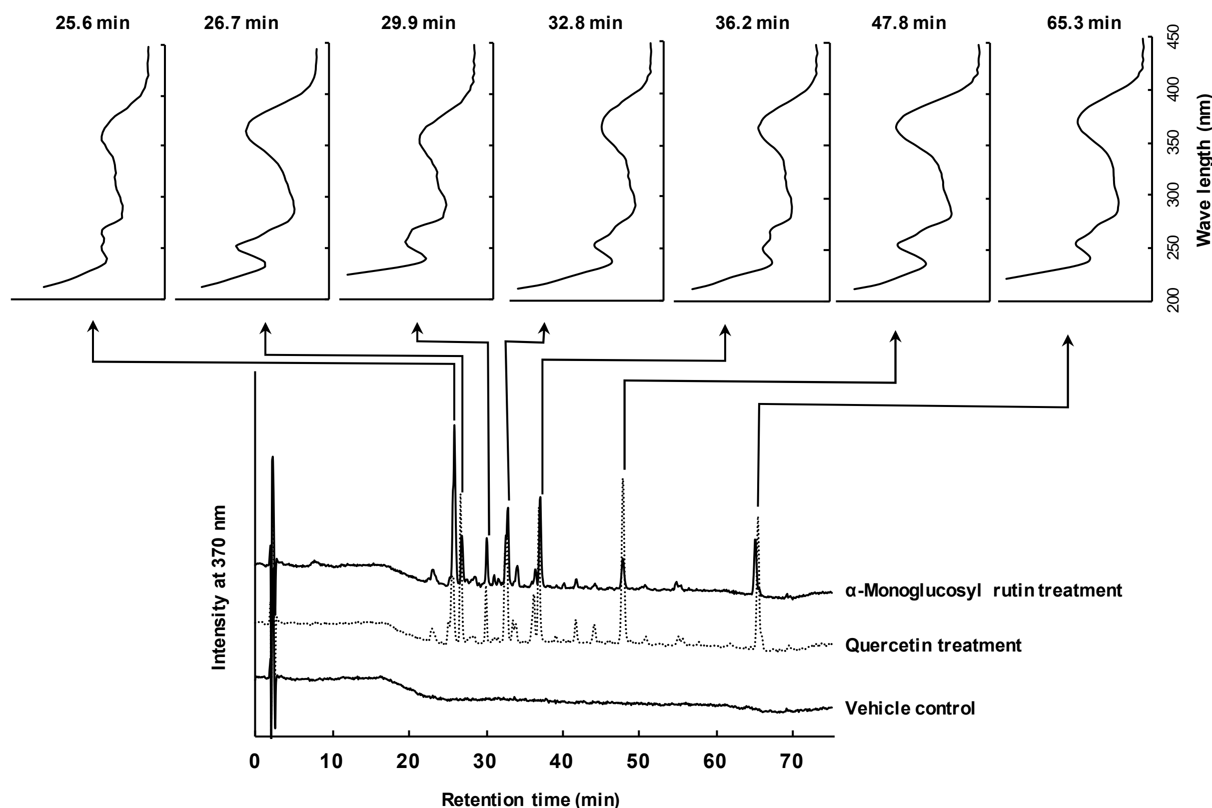

Figure 6. Typical high-performance liquid chromatography (HPLC) readouts (370 nm)

α-Monoglucosyl rutin or quercetin were orally administered to male C57BL/6 mice following a fast (500 μmol/kg body weight). Control mice received vehicle solvent. After 1 h, blood samples were collected, and quercetin and its metabolites in plasma fractions were analyzed using HPLC-diode array detection.

DISCUSSION

Obesity has become a worldwide concern, especially within developed countries, and is accordingly a significant topic of research. In this study, we evaluated the effects of αMR on HFD-induced obesity in mice. In a preliminary unpublished study using HFD containing 0.1% and 0.5% αMR, we discovered that excess weight gain induced by HFD was significantly inhibited by consumption of 0.5% αMR (Supplemental Figure 1). Consumption of 0.1% αMR inhibited weight gain, but not significantly. Therefore, we used 0.5% αMR in this study. The HFD group gained more weight than the LFD group after 6 weeks. αMR supplementation

significantly decreased weight gain on weeks 10 and 13. The HFD and HFD + αMR groups consumed similar amounts of dietary energy during the study, while the LFD group consumed lower amounts. Visceral fat weight was significantly higher in the HFD group on both week 4 and week 13, while αMR supplementation of the HFD significantly inhibited visceral fat weight on week 13 but not on week 4.

Weight gain is caused by a chronic positive energy balance [2]. The primary cause of obesity is increased energy intake, especially consumption of fat in high proportions [27, 28]. Functional foods that inhibit lipid absorption may slow or suppress weight gain. We found that serum cholesterol levels increased following consumption of HFD, while supplementation with αMR prevented such an increase on week 13. We could not assess the effects of αMR consumption on serum triglyceride levels on week 4 in the HFD group, but elevated serum triglycerides in corn oil-fed mice were not inhibited by αMR (Supplemental Figure 2). On week 13, we observed that αMR supplementation prevented increases in serum cholesterol and non-HDL cholesterol levels in HFD-fed mice. These alterations were not evident at the earlier analytical stage (week 4), in which visceral fat accumulation was observed without concurrent increases in body weight. Consequently, αMR consumption did not affect the initial stage of lipid accumulation, in which excess weight gain was not observed. Daily consumption of αMR appeared to regulate lipid accumulation, thereby preventing excess weight gain.

One of the putative mechanisms of αMR's anti-obesity effects may be its activity in the gastrointestinal tract. There is evidence from studies that the gastrointestinal tract secretes several peptide hormones related to obesity, such as GIP, which is secreted from duodenal endocrine K cells during stimulation by nutrients such as fats and glucose [29]. GIP has been shown to stimulate pancreatic islet β cells and accelerate insulin secretion by binding to the GIP receptor [30]. GIP has also appeared to exert direct physiological effects on lipid metabolism; accordingly, reducing GIP levels has been shown to restrict weight gain in HDF-fed mice [31]. Therefore, dietary ingredients that attenuate GIP action may be effective in preventing obesity. Fukuoka and colleagues reported that supplementation with triterpene alcohol and sterol preparation from rice bran decreased the fat-promoted plasma GIP response in HFD-fed mice and prevented weight gain, along with regulating plasma leptin, hepatic triglycerides, and fat weight [32]. In our study, serum GIP in the HFD group was significantly higher than in the LFD group, regardless of whether the animals were fasted prior to analysis. Supplementation with αMR maintained serum GIP at near-control levels. Visceral fat weight,

serum leptin, and insulin levels were also altered in a manner similar to the results reported by Fukuoka et al. [32], indicating that αMR may affect duodenal endocrine K cells and regulate GIP secretion. However, its detailed mechanism of action requires further study. In this study, we evaluated the serum metabolic hormone and cytokine levels only under the fed state. Some hormone and cytokine secretion are reported to be different during fed and fasted states [33, 34]. Consequently, the effects of αMR on GIP response might change under fasting conditions.

Consumed αMR has been reported to be absorbed into the bloodstream [16, 18], a result which we confirmed in the present study. A detectable amount of intact αMR was not discovered in the plasma fraction. However, there were multiple quercetin metabolites, with a pattern similar to that of quercetin aglycone. According to previous reports, they may include quercetin 3,4'-di-*O*-β-glucoside, quercetin 3-*O*-β-glucoside and quercetin 4'-*O*-β-glucoside [35]. Our future study should confirm their exact structures. Our results indicated that αMR may exert its anti-obesity effects via circulating quercetin metabolites, perhaps by regulating the AMPKα-Sirt1-PPARγ pathway and TRPV1 channels [9, 10]. We are designing studies to investigate these effects.

CONCLUSION

The present study demonstrated for the first time that daily consumption of αMR inhibits diet-induced visceral fat accumulation by regulating GIP secretion, consequently preventing excess weight gain. Therefore, αMR may be a potential functional food with weight loss effects. More detailed studies analyzing the mechanism of action involved should be conducted in the future, in preparation for human trials.

List of Abbreviations: αMR, alpha monoglucosyl-rutin; DAD, diode array detector; GIP, gastric inhibitory polypeptide; HDL, high-density lipoprotein; HFD, high-fat diet; HPLC, high-performance liquid chromatography; IL-6, interleukin 6; LFD, low-fat diet; MCP-1, monocyte chemoattractant protein 1; TNFα, tumor necrosis factor α; ZT, Zeitgeber time.

Competing Interests: There are no conflicts of interest to declare.

Authors' Contributions: H.S., D.Y., Y.H., and K.S. designed the research. M.T. provided test monoglucosyl-rutin and supported the research. D.Y., W.T., and H.S. conducted the research.

M.S. performed statistical analyses. D.Y. and H.S. wrote the manuscript. H.S. had primary responsibility for the final content. All authors read approved the final version of the manuscript.

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