



Positive influence of gut microbiota on the effects of Korean red ginseng in metabolic syndrome: a randomized, double-blind, placebo-controlled clinical trial

Eunhak Seong¹ · Shambhunath Bose¹ · Song-Yi Han¹ · Eun-Ji Song² · Myeongjong Lee¹ · Young-Do Nam² · Hojun Kim¹

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Abstract

Background Ginseng, a traditional herbal medicine, has been used for thousands of years to treat various diseases including metabolic syndrome (MS). However, the underlying mechanism(s) of such beneficial actions of ginseng against MS is poorly understood. Emerging evidence indicates a close association of the host gut microbiota with MS. The present study was conducted to examine, whether the beneficial effects of Korean red ginseng (KRG) against MS could be influenced by gut microbial population and whether gut microbial profile could be considered a valuable biomarker for targeted treatment strategy for MS in compliance with the predictive, preventive, and personalized medicine (PPPM / 3PM).

Methods This clinical study was a randomized, double-blind, placebo-controlled trial evaluating the effects of KRG treatment for 8 weeks on patients with MS. The anthropometric parameters, vital signs, metabolic biomarkers, and gut microbial composition through 16S rRNA gene sequencing were assessed at the baseline and endpoint. The impact of KRG was also evaluated after categorizing the subjects into responders and non-responders, as well as enterotypes 1 and 2 based on their gut microbial profile at the baseline.

Results Fifty out of 60 subjects who meet the MS criteria completed the trial without showing adverse reactions. The KRG treatment caused a significant decrease in systolic blood pressure (SBP). Microbial analysis revealed a decrease in *Firmicutes*, *Proteobacteria*, and an increase in *Bacteroidetes* in response to KRG. In patient stratification analysis, the responders showing marked improvement in the serum levels of lipid metabolic biomarkers TC and LDL due to the KRG treatment exhibited higher population of both the family *Lachnospiraceae* and order *Clostridiales* compared to the non-responders. The homeostasis model assessment-insulin resistance (HOMA-IR) and insulin level were decreased in enterotype 1 (*Bacteroides*-abundant group) and increased in enterotype 2 (*Prevotella*-abundant group) following the KRG treatment.

Conclusion In this study, the effects of KRG on the glucose metabolism in MS patients were influenced by the relative abundances of gut microbial population and differed according to the individual enterotype. Therefore, the analysis of enterotype categories is considered to be helpful in predicting the effectiveness of KRG on glucose homeostasis of MS patients individually. This will further help to decide on the appropriate treatment strategy for MS, in compliance with the perspective of PPPM.

Keywords Korean red ginseng · Herbal medicine · Metabolic syndrome · Patient stratification · Blood serum · Biomarker panel · Molecular pathways · Oxidative stress · Inflammation · ROS detoxification · Lipid metabolic biomarkers · Hyperlipidemia · Glucose homeostasis · Insulin level · Anthropometric parameters · Vital signs · Fat mass · BMI · Gender · Predictive preventive personalized medicine (PPPM / 3PM) · Individual enterotype · Clinical trial · Gut microbiome profile · Drug response · Treatment strategy · Blood pressure · 16S rRNA gene sequencing · HOMA-IR

✉ Young-Do Nam
youngdo98@kfri.re.kr

✉ Hojun Kim
kimklar@dongguk.ac.kr

Extended author information available on the last page of the article

Introduction

Metabolic syndrome (MS) represents a group of clinical disorders, including obesity (central adiposity), insulin resistance, hyperglycemia, glucose intolerance, dyslipidemia, and

hypertension [1–3]. Being a major risk factor for several diseases, such as type 2 diabetes (T2D), neurological disorders, cardiovascular disease (CVD), stroke, non-alcoholic steatohepatitis, polycystic ovary syndrome, and cancer [2], MS is now considered a serious global public health problem and it is becoming a major cause of morbidity and mortality in the world [4]. Therefore, MS appears to be a crucial challenge for the coming years [5]. According to the definition of MS from the International Diabetes Federation, the National Cholesterol Education Program, and the Adult Treatment Panel criteria, the prevalence of MS in the USA is estimated to be 22% to more than 30% [6]. Among adults, 12–37% of the Asian population, 12–63% of the African population, and 12–26% of the European population are affected by MS [7]. The WHO estimated that worldwide, more than 1.9 billion adults, 18 years and older were overweight in 2016. Among them, over 650 million were obese. More than 340 million children and adolescents aged 5–19 were overweight or obese in that year. Furthermore, 38 million children under the age of 5 were overweight or obese in 2019 [8]. A similar trend is also seen in the prevalence of diabetes. In 2013, 382 million people were found suffering from diabetes, and it has been predicted that the incidence of diabetes will increase abruptly to 600 million cases by 2045 [9, 10]. A nomogram prediction of the 3-year risk of diabetes based on analyses of the data of a clinical study on the healthy mainland China residents revealed 10.71% and 11.02% occurrences of this disease in 5557 participants (training cohorts) and 1870 participants (validation cohorts), respectively [11]. Furthermore, the WHO estimated that 17.9 million people died from cardiovascular diseases (CVDs) in 2016, representing 31% of all global deaths, and predicted that CVDs would persist over the next decade as the major cause of death [12].

Albeit, the detailed molecular mechanisms behind the onset and progression of MS are not completely elucidated, accumulating evidence indicates the involvement of oxidative stress (OS), a consequence of a decrease in the antioxidant systems and an increase in the production of reactive oxygen species (ROS), in this multifactorial disorder [13, 14]. Increased oxidative stress in accumulated fat is an early initiator of MS. The redox state in adipose tissue appears to be a potentially promising therapeutic target for obesity-associated MS [15]. Additionally, OS plays an important role in the onset and development of diabetes complications related to microvessels and coronary vessels. Diabetes mellitus triggers the production of excessive mitochondrial peroxide in vascular endothelial cells and the myocardium, leading to the activation of the main pathways involved in the pathogenesis of complications [16, 17]. Furthermore, a number of studies have revealed that MS is associated with a state of low-grade inflammation, characterized by abnormal proinflammatory cytokine production, augmented acute-phase reactants, and the activation of a network of inflammatory signaling pathways [13, 14, 18].

Accumulating evidence indicates that the microbiome, as well as probiotics and prebiotics, modulate the functions of distant organs, the mechanisms of which are well elucidated in the literature [19]. In adult humans, approximately 10^{14} bacterial cells are present in the intestine which are ten times more than the total body cells of the host [20, 21]. The gut microbes play an important role in metabolism and health [22] as evident by animal and human studies exhibiting a remarkable microbial influence on nutrient absorption, energy utilization and storage, immunologic system, and metabolic disease [23–28]. Accordingly, in the precision nutrition research field, the gut microbiome appears to be a potential tool for predicting and validating the personalized responses to diet and variability of human metabolism [29, 30]. A previous *in vivo* study reported that compared to lean mice, *ob/ob* animals had a 50% decrease in the abundance of *Bacteroidetes* and a proportional increase in *Firmicutes* indicating that obesity affects the gut microbial diversity [31]. In keeping with this, a clinical study showed that the relative proportion of *Bacteroidetes* decreased in obese participants compared to lean subjects. This proportion increased with weight loss driven by two types of low-calorie diet, further supporting that obesity has a microbial component that might have potential therapeutic applications [32].

An imbalance in the gut microbial communities (dysbiosis) might be associated with an elevation in the aberrant metabolite concentrations that disrupt the GLP-2-mediated tight junction integrity of the intestine, leading to an increase in the permeability of the gut epithelium. This allows the microbial lipopolysaccharide (LPS), trimethylamine (TMA), and other metabolites to enter the circulation, causing chronic inflammation of the liver and adipose tissue that triggers the onset and development of insulin resistance and other conditions associated with MS [33, 34]. Furthermore, gut microbial perturbations and related dysbiosis are associated with the progression and pathogenesis of CVD, including atherosclerosis, hypertension, and heart failure [33, 35]. Accordingly, various strategies, including diet, herbal medicines, probiotics, prebiotics, postbiotics, antibiotics, and fecal microbial transplantation (FMT), have been employed to maintain the balance in the gut microbial composition to improve type 2 diabetes, non-alcoholic fatty liver disease (NAFLD), atherosclerosis, CVD, and cancer [36, 37]. Furthermore, emerging evidence indicates that the combined application of inorganic prebiotics and probiotics has great therapeutic potential to treat hypercholesterolemia [38]. It has been found that cholesterol metabolism is sensitive to the microbial profiles and the setup of appropriate probiotics, immunobiotics, and prebiotics is critical for a personalized clinical set [38]. Therefore, it is conceivable that the prebiotics-mediated modulation of the gut microbiota can be an important therapeutic strategy for predictive, preventive, and personalized medicine (PPPM) to combat the onset and development of obesity and MS.

Korean ginseng (*Panax ginseng* Meyer, Araliaceae), a traditional herbal medicine, has been used as a therapeutic agent to treat various diseases for thousands of years [17]. Korean red ginseng (KRG) is produced by steaming and drying fresh ginseng to potentiate its therapeutic activities [39]. Accumulating evidence suggests that ginseng, which is composed of multiple ingredients, possesses antioxidant, anti-inflammatory, antiapoptotic, and immunostimulant properties and exerts central nervous system effects, neuroprotective, immunomodulatory, and anticancer effects [40]. More specifically, as a potent natural antioxidant, ginseng effectively suppresses the inflammatory response in acute or chronic inflammation [41]. In parallel, growing evidence suggests that KRG is a potent therapeutic agent against MS disorders, including obesity, dyslipidemia, diabetes, and CVD [39]. For example, in vivo studies using rat and mouse models reported that ginseng or its components improved the glucose and lipid metabolism in MS [42–45]. Furthermore, our earlier clinical studies on South Korean obese women showed that a KRG treatment reduced the weight, BMI, waist-hip ratio, daily food intake, the Korean version of obesity-related quality of life scale [46, 47]. Another clinical study demonstrated the hypolipidemic action of a *Panax ginseng* extract on the lipid metabolism in humans, which was associated with a decrease in total cholesterol (TC), triglycerides (TG), and low-density lipoprotein (LDL) cholesterol, and an increase in high-density lipoprotein (HDL) cholesterol [48]. Furthermore, previous clinical trials in South Korea reported the anti-diabetic properties of KRG because it decreased the serum glucose and whole blood glucose levels, C-peptide, fasting insulin level, HOMA-IR, and current perception threshold of the lower extremities in type 2 diabetic patients [39]. Another clinical study reported that 12 weeks of supplementation with the selected KRG preparation maintained good glycemic control and improved the oral glucose tolerance test-plasma glucose, and plasma insulin regulation in well-controlled, type 2 diabetes [49]. Ginseng also exerted potent anti-stress effects in in vivo studies [41]. Moreover, accumulating clinical evidence indicates the beneficial impact of KRG on CVD. More specifically, the applications of KRG or its active constituents showed a marked decrease in central systolic and diastolic blood pressure (BP), end-systolic pressure, area under the systolic/diastolic BP curve, central and brachial mean arterial pressure, brachial systolic and diastolic BP [39]. Furthermore, ginseng suppresses the oxidative stress induced by ischemia, a major event causing stroke-induced tissue damage [41]. Previous in vivo studies reported that ginseng reduced the levels of ROS in myocardial tissue and improved blood circulation, facilitating the maintenance of cardiac function [41].

Growing evidence suggests that KRG and its components have a reciprocal interrelationship with the host's gut microbiota. Thus far, approximately 200 substances have

been isolated from ginseng which include ginsenosides, polysaccharides, polyacetylenes, antioxidative aromatic compounds, amino acids, volatile oils, alkaloids, lignanes, and other nitrogen compounds (adenosine, acidic peptide, radiation protective protein, and immunocyte generative protein) [50, 51]. The major bioactive ingredients of *P. ginseng* are ginsenosides, which are triterpene saponins [51, 52]. In a rat model, it was found that the consumption of KRG powder increased the number of total ileal microbiota and *Lactobacillus* strains significantly compared to the control group [53]. Using the same animal model, it was shown that the ginseng extract affected the gut microbiota structure with a decreased abundance of TM7 and an increased population of *Proteobacteria*, *Methylobacteriaceae*, *Parasutterella*, and *Sutterella* [54]. A previous study reported that a treatment with ginseng saponins shaped the murine gut microbiome by enhancing the abundance of *Akkermansia muciniphila* and *Parabacteroides distasonis* [55]. In one of our clinical studies conducted on obese middle-aged Korean women showed that the order of dominance of the host's gut microbial genera were changed from *Blautia*, *Bifidobacterium*, and *Anaerostipes* to *Bifidobacterium*, *Blautia*, and *Faecalibacterium* after KRG administration [47]. Furthermore, in NAFLD patients, KRG exerted beneficial effects that were associated with a decrease in the relative abundance of *Firmicutes*, whereas an increase was noted in the placebo group [56].

Overall, the abovementioned findings show that ginseng can modulate the gut microbial composition of the host, including those suffering from MS. Several lines of evidence indicate that gut microbes, in turn, enhance the therapeutic potential of ginseng. Our previous clinical study reported that treating of middle-aged obese Korean women with a ginseng extract affected weight loss and the gut microbiota, and that its anti-obesity effects differed according to the gut microbial composition prior to ginseng intake [47]. The gut microbes are enriched with enzymes that convert hydrophilic ginsenosides to hydrophobic compounds, making them easily absorbable. This enhances the bioavailability of ginsenosides and potentiates their efficacy [57, 58]. Taking account of the abovementioned beneficial effects of KRG, reciprocal relationship between KRG and gut microbiome, and the definition of prebiotics as “a substrate that is selectively utilized by host microorganisms conferring a health benefit” [59], it is conceivable that KRG could be considered a potential prebiotic candidate [60].

PPPM, also considered “medicine of the future,” is an integrated approach addressing an individualized patient profile, predictive diagnosis, and targeted prevention. It is a new paradigm to provide both “health care” and “disease care,” combining the advantages of individual bio/medical fields and technologies and amalgamating a multi-professional collaboration [61]. In addition to personalized patient profiling, an analysis of the complex interactions between

the host and gut microbes using integrated multiomics technology and patterning gut microbiota-based translational biomarkers would be vital in addressing an effective PPMM approach for MS [10, 62, 63]. Previous studies reported that the differences in the drug responses depend on the gut microbial composition at the baseline [47, 64]. Accordingly, the drugs could be administered to the “right” person and targeting the gut microbiota appears to be a potential option for improving the pharmacological effects of drugs. Analyzing the drug effects individually after stratifying the patients according to the gut microbial composition, such as “enterotypes,” is one of the key strategies to address PPPM [61, 65]. “Enterotypes” is a concept that categorizes the gut microbial population into three clusters determined by the composition of the predominant species, such as *Bacteroides*, *Prevotella*, and *Ruminococcus* [66]. Evaluating the pharmacological impact of KRG according to the gut microbial composition, such as enterotypes, will be helpful in cost-effective use of KRG in the future.

This study evaluated the beneficial effects of KRG in improving the MS-related markers of subjects, as well as how these effects were influenced by gut microbial composition at the individual level. The outcome of this study could be used to evaluate the compatibility of the application of KRG as personalized medicine for MS based on the information on the response of individual patient’s enterotype, thereby establishing a targeted treatment strategy.

Materials and methods

Study design

The study was a single-center, randomized, double-blind, placebo-controlled clinical trial conducted on participants with MS. After 66 volunteers were screened, 60 participants who satisfied the criteria of this study were enrolled and assigned randomly to the KRG group ($n = 30$) or placebo group ($n = 30$) using a computerized procedure. The participants of the KRG and placebo groups received KRG and placebo capsules, respectively, for 8 weeks. All subjects visited the hospital for initial screening and at the 0th, 2nd, 4th, 6th, and 8th weeks of their KRG treatment for their assessment. At each visit, the subjects were scrutinized to determine if changes in their background medications had been made and the possible adverse effects of KRG.

Participants

Sixty subjects, both male and female, were registered. The inclusion criteria were as follows: (1) adults aged 19 to 65; (2) those who had 3 or more of the following MS features (i) waist circumference (WC) male > 90 cm, female > 85 cm, (ii)

TG ≥ 150 mg/dL or those taking medications for hyperlipidemia, (iii) HDL male < 40 mg/dL, female < 50 mg/dL, (iv) fasting glucose ≥ 100 mg/dL or those taking medications for diabetes, and (v) systolic blood pressure (SBP) ≥ 130 mm Hg or diastolic blood pressure (DBP) ≥ 85 mm Hg or those taking medication for hypertension); (3) those who had been explained the outline of this clinical trial and signed a written consent form. The exclusion criteria were as follows: (1) those with endocrine diseases that could affect the body weight, such as hypothyroidism and Cushing’s syndrome; (2) those with heart diseases (heart failure, angina, and myocardial infarction) or a history of stroke or temporary ischemic heart attack; (3) those with a malignant tumor or lung disease; (4) those diagnosed with liver dysfunction [serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) > 2.5 times above the normal upper limit]; (5) those with cholelithiasis; (6) those showing serum creatinine level > 2.0 mg/dL; (7) those with narrow angle glaucoma; (8) those with a neurological or psychological disorder-related medical history or present illness; (9) those with a history of eating disorders, such as anorexia nervosa or bulimia nervosa; (10) those who had a history of taking drugs without prescription (especially, medicines that can affect the body weight, such as appetite suppressants, laxatives, and oral steroids), thyroid hormones, amphetamines, cyproheptadine, phenodiazin in the last 3 months; (11) those receiving weight-loss agents that might have an impact on central nervous system; (12) those receiving prohibited treatments (insulin, antidepressants, antiserotonins, barbiturates, antipsychotics, and drugs with a high potential for abuse); (13) those with anatomical changes, such as resection, and therefore, had difficulties in the measurement of body parameters; (14) those who were surgically operated for weight loss; (15) pregnant women, lactating women, women of childbearing potential who had a pregnancy plan or who did not agree to the selection of appropriate contraceptive methods (e.g. oral contraceptives, hormone transfer, intrauterine device (IUD), spermicide barrier method, condoms, abstinence); (16) those who participated in another clinical trial within the last month; (17) those who had lost more than 10% of their body weight within last 6 months; (18) those who were judged by the sub-investigator to be unable to follow the study’s protocol; and (19) those who had taken antibiotics or probiotics within the past month.

Intervention

The KRG and placebo groups received KRG capsules (Korean red ginseng powder 100%, 6000 mg/day) and placebo capsules (4200 mg/day), respectively, for 8 weeks. Each KRG capsule contained the ginsenosides Rg1 (2.90 mg/g), Re (1.48 mg/g), Rf (0.76 mg/g), Rg2s (0.19 mg/g), Rb1 (4.94 mg/g), Rc (1.98 mg/g), Rb2 (1.53 mg/g), Rd (0.31 mg/g), Rg3s

(0.24 mg/g), Rg3r (0.14 mg/g), and Rh1 (0.23 mg/g). The placebo capsules, which were similar to the KRG capsules in shape and flavor, contained crystalline cellulose 70%, maltodextrin 28.37%, magnesium stearate 0.5%, silicon dioxide 1%, and food color 0.124%. The KRG and placebo capsules were provided by Korea Ginseng Corporation (Seoul, Republic of Korea).

Randomization and blinding strategies

Group allocation to either the KRG group or placebo group was based on randomization. Subjects were randomized in blocks of 4 using an online random code generation tool (http://www.jerrydallal.com/random/random_block_size_r.htm). Before being provisioned, KRG and placebo capsules were packed and labeled according to random code. All participants, pharmacists, and investigators remained blinded to the allocation until the end of the study. Subjects could not recognize the difference between the KRG and placebo capsules due to the similarity in their shape.

Assessment

The vital signs, body weight, total fat mass, fat percentage, body mass index (BMI), and WC of the participants were measured at each visit (0th, 2nd, 4th, 6th, and 8th week). The obesity-related quality of life of the subjects was evaluated at the 0th and 8th week [67], and fecal samples were collected. Blood samples were collected when volunteers were screened and at the 0th and 8th week of the KRG treatment. The sera were separated from the blood after clotting and centrifuging at 3000 rpm for 15 min at 4 °C.

Anthropometry

Vital signs, such as blood pressure and heart rate, were measured using an automatic digital sphygmomanometer (Easy X 800; SELVAS Healthcare, Daejeon, Republic of Korea). Body compositions, such as the fat mass and fat percentage, were determined using a bioelectrical impedance analysis method (Inbody 720; Biospace, Seoul, Republic of Korea).

Blood chemistry

At weeks 0 and 8 of the trial, biochemical tests were performed to determine the serum levels of insulin, glucose, TG, TC, and HDL using a Cobas 8000 modular analyzer (Roche, Branford, CT, USA). Low-density lipoprotein (LDL) was estimated using the Friedewald equation [68]. The homeostasis model assessment-insulin resistance (HOMA-IR) was determined using the following equation

$$\text{HOMA-IR} = (\text{Glucose}(\text{mg/dL}) \times \text{Insulin}(\mu\text{IU/mL})) \div 405$$

Sequencing of the 16S rRNA gene amplicon of fecal microbiota

Bacterial DNA was isolated from fecal samples using a QIAamp stool DNA mini kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. The PCR of the V1-V3 region of the 16S rRNA gene was performed using a C1000 Touch thermal cycler equipped with a 96-deep-well reaction module (Bio-Rad, Hercules, CA, USA). The PCR products were purified using a LaboPass PCR purification kit (Cosmogenetech Inc., Seoul, Republic of Korea). The amplicons of each sample were pooled in equimolar amounts, and the resulting mixture was purified using AMPure XP beads (Agencourt Bioscience, Beverly, MA, USA). The genomic DNA in the samples was quantified using a PicoGreen dsDNA assay kit (Invitrogen, Carlsbad, CA, USA). The mixed amplicons were amplified on sequencing beads using emulsion PCR. The sequencing reactions were performed on a Roche/454 GS Junior system (454 Life Sciences, Branford, CT, USA) according to the manufacturer's instructions.

Enterotype analysis

RStudio (RStudio Inc., Boston, MA, USA; version 3.6.2), a powerful, free, open-source programming language for statistical analyses and graphics, was used to categorize the enterotypes. For this, the samples were clustered using the partitioning around medoids (PAM) clustering algorithm [69], and an optimal number of clusters was validated using the Calinski-Harabasz index [70]. The sample distributions were calculated based on the relative gut microbial genus abundances using the Jensen-Shannon divergence (JSD) distance metric and visualized using a PCoA plot. All R codes used were obtained from the MetaHIT consortium [66].

Sequence analysis

The obtained sequence reads were filtered by eliminating the low-quality reads (average quality score < 20 or read length < 300 bp). The processed sequences and the operational taxonomic units (OTUs) were clustered using the open reference OTU picking method according to the Quantitative Insights into Microbial Ecology (QIIME) pipeline-version 1.9.1 [71]. For profiling taxa and finding differences in microbiota abundances between the KRG and placebo groups, a linear discriminant analysis effect size (LEfSe) assessment was performed using an online software tool (<http://huttenhower.sph.harvard.edu/galaxy>). For this evaluation, the threshold of the logarithmic linear discriminant analysis

(LDA) score was set to >2.0 , and the alpha value of the factorial Kruskal–Wallis test among the classes was set to <0.05 .

Statistical analysis

The results are presented as mean \pm standard deviation. A pre vs. post-treatment comparison of the variables within each study group (KRG or placebo) was performed using a paired *t*-test or Wilcoxon signed-rank test. An independent *t*-test or Wilcoxon's rank-sum test was used to compare the KRG and placebo groups. All statistical analyses were performed using SPSS version 18.00 for Windows (SPSS Inc., Chicago, IL, USA). The strength of the relationship between the parameters was evaluated using the two-tailed Pearson's correlation test; *p*-values <0.05 were considered significant.

Results

Participants, anthropometric and biochemical parameters, vital signs, and adverse reactions

Among the 66 participants screened, 60 subjects fulfilled the study criteria and were registered for the trial. The selected volunteers were assigned randomly to the KRG group ($n=30$) or the placebo group ($n=30$). Fifty subjects (KRG, $n=25$, placebo, $n=25$) ultimately completed the

study (Fig. 1). At the end of the trial, an analysis of the stool microbiota was accomplished for 17 and 16 subjects from the KRG and placebo groups, respectively. This analysis could not be performed for the rest of the participants due to the inadequate amount of fecal samples (Fig. 1). No significant inter-group differences in the anthropometric and biochemical parameters and vital signs were observed at the baseline (Table 1). Furthermore, no adverse effects of KRG on the subjects were observed throughout the entire trial period.

KRG administration improved SBP and led to shifts in gut microbiota

There were no significant differences in the body composition, anthropometric and biochemical parameters related to MS in the subjects between the pre and post-treatments of KRG (Table 2). In addition, these parameters were similar in the placebo and KRG groups at the end of the study. On the other hand, the SBP was observed significantly lower in the subjects exposed to KRG ($p=0.026$). Furthermore, marked changes in the gut microbial communities were observed in the subjects at phylum, family, and genus levels in response to the KRG treatment. At the phylum level, although the changes were not significant, the relative abundance of the microbiota was increased from $68.87 \pm 17.43\%$ to $73.88 \pm 21.00\%$ ($p=0.156$) in

Fig. 1 Study flow chart depicting the entire processes of the clinical trial

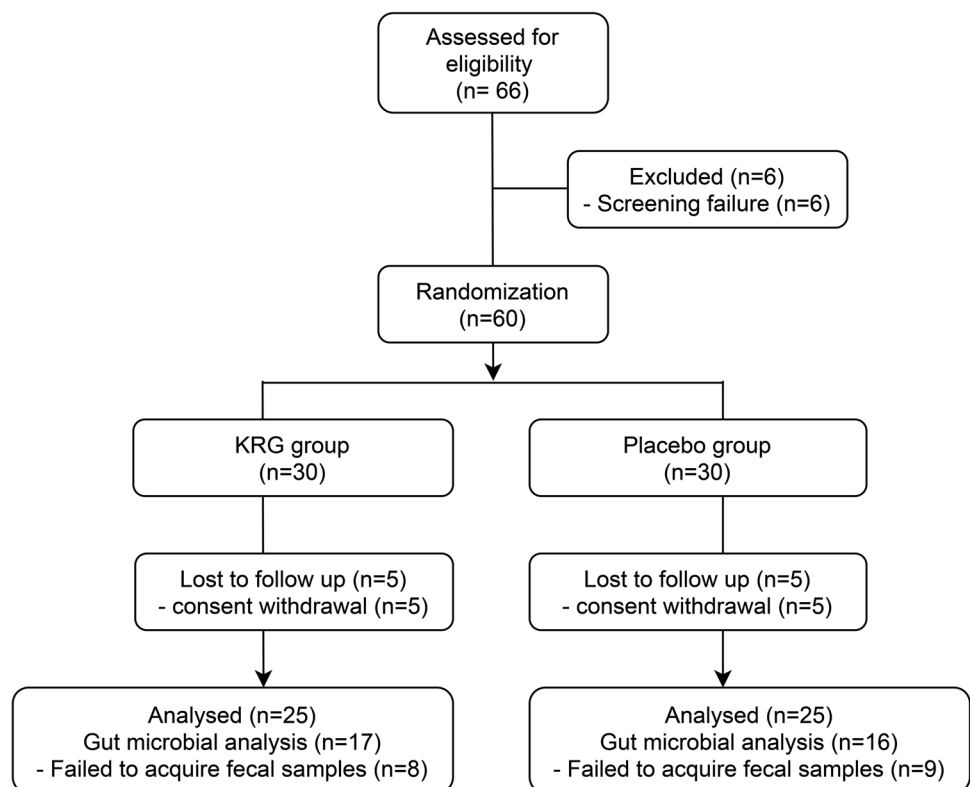


Table 1 Baseline characteristics of the subjects

| Variables | KRG | Placebo | <i>p</i> -value ‡ |
|-------------------------------|----------------|----------------|-------------------|
| Gender (male/female) | 8/17 | 8/17 | 1.000† |
| Age (years) | 53.60 ± 8.45 | 49.84 ± 10.56 | 0.171 |
| Height (cm) | 162.48 ± 9.19 | 162.68 ± 7.22 | 0.932 |
| HOMA-IR | 3.01 ± 1.38 | 3.66 ± 3.38 | 0.930 |
| Insulin (μIU/mL) | 10.82 ± 4.73 | 12.04 ± 8.87 | 0.823 |
| Glucose (mg/dL) | 112.52 ± 21.16 | 117.72 ± 22.83 | 0.299 |
| TG (mg/dL) | 150.72 ± 57.51 | 166.36 ± 69.61 | 0.547 |
| TC (mg/dL) | 195.76 ± 39.71 | 186.72 ± 32.51 | 0.383 |
| HDL (mg/dL) | 52.52 ± 11.25 | 55.74 ± 9.75 | 0.107 |
| LDL (mg/dL) | 129.84 ± 40.20 | 116.48 ± 36.61 | 0.225 |
| Body weight (kg) | 74.30 ± 12.68 | 72.57 ± 16.31 | 0.676 |
| BMI (kg/m ²) | 28.16 ± 4.47 | 27.22 ± 4.74 | 0.337 |
| WC (cm) | 95.90 ± 7.93 | 95.32 ± 9.29 | 0.503 |
| Fat mass (kg) | 26.22 ± 8.06 | 25.20 ± 10.12 | 0.273 |
| Fat percentage (%) | 35.10 ± 7.26 | 34.14 ± 7.48 | 0.646 |
| SBP (mmHg) | 132.36 ± 12.42 | 127.32 ± 15.62 | 0.213 |
| DBP (mmHg) | 77.40 ± 8.09 | 78.08 ± 13.57 | 0.831 |
| Pulse rate (/min) | 78.16 ± 11.42 | 79.32 ± 14.64 | 0.756 |
| CRP (mg/dL) | 0.13 ± 0.17 | 0.25 ± 0.45 | 0.189 |
| Number of MS factors | 3.60 ± 0.58 | 3.40 ± 0.65 | 0.243 |
| Obesity-related questionnaire | 29.64 ± 6.89 | 27.96 ± 9.82 | 0.487 |

Data are expressed as mean ± SD; †*p*-value obtained from a chi-square test; ‡*p*-value obtained from an independent *t*-test, Wilcoxon's rank-sum test; **p* < 0.05, ***p* < 0.01; HOMA-IR, homeostatic model assessment-insulin resistance; TG, triglycerides; TC, total cholesterol; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; PR, pulse rate; MS, metabolic syndrome

Bacteroidetes (B) and decreased from 29.47 ± 17.72% to 25.33 ± 20.84% (*p* = 0.332) in *Firmicutes* (F). In contrast, the population of phylum *Proteobacteria* had declined significantly from 1.27 ± 1.33 to 0.64 ± 0.72 (*p* = 0.023). Microbes representing more than 1% of the total population were considered for comparative analysis at the genus level. Among the dominant communities, although the changes were not significant, the relative abundance of *Bacteroides* decreased from 47.41 ± 27.09% to 41.93 ± 28.78% in response to the KRG treatment (*p* = 0.155). In contrast, the relative abundance of *Prevotella* increased from 13.29 ± 21.27% to 23.01 ± 30.19% (*p* = 0.286) (Fig. 2a). For a better understanding of the impact of KRG on the gut microbial communities, LEfSe analysis was performed to determine the differences in the distributional patterns of the gut microbes between the placebo and KRG groups. The results showed that after 8 weeks of KRG or placebo administration, *Bacteroidetes*, *Bacteroidia*, and *Bacteroidales* were the dominant gut microbial population at the phylum, class, and order levels, respectively, in the KRG group (Fig. 2b). In contrast,

phylum *Firmicutes*, order *Bifidobacteriales*, family *Bifidobacteriaceae*, and genus *Bifidobacterium* were the dominant gut microbial communities in the placebo group.

Impact of KRG on the serum glucose and lipid parameters differed depending on the gut microbial composition at the baseline

Next, we evaluated the influence of gut microbial population at baseline on the subjects' response to the KRG treatment. For this, the participants were sorted into responder and non-responder groups based on the degree of improvement of the metabolic parameters HOMA-IR and serum levels of insulin, TC, and LDL in response to KRG treatment. Subjects who ranked among the top and bottom 6 responders, were categorized as the responder and non-responder groups, respectively (Table 3). The gut microbial profile of the responders exhibiting marked improvement in HOMA-IR upon exposure to KRG showed that at the family level, the abundance of *Bacteroidaceae* (*p* = 0.002) was significantly higher than that of the non-responders while the populations of *Prevotellaceae* (*p* = 0.004), *Ruminococcaceae* (*p* = 0.025), and *Rikenellaceae* (*p* = 0.016) were significantly lower (Fig. 3a). Among the genera, *Bacteroides* (*p* = 0.002) was significantly higher in the responders than the non-responders, whereas *Prevotella* (*p* = 0.004) and the family *Ruminococcaceae* (*p* = 0.010) were significantly lower (Fig. 3b). On the other hand, the gut microbial communities of the responders demonstrating noticeable improvement in the serum insulin level after the KRG treatment showed that at the family level, the population of *Bacteroidaceae* (*p* = 0.002) was significantly higher in the responders than the non-responders, while that of *Prevotellaceae* (*p* = 0.004), *Ruminococcaceae* (*p* = 0.025), *Rikenellaceae* (*p* = 0.016), and *Alcaligenaceae* (*p* = 0.010) were significantly lower (Fig. 3c). Furthermore, among the genera, *Bacteroides* (*p* = 0.002) was significantly higher in the responders than the non-responders, whereas *Prevotella* (*p* = 0.027) and the family *Ruminococcaceae* (*p* = 0.048), *Sutterella* (*p* = 0.010), and *Odoribacter* (*p* = 0.024) were significantly lower (Fig. 3d). The pattern of the gut microbial distribution of the responders, who exhibited marked improvement in the serum levels of TC and LDL due to the KRG treatment, showed that the population of both the family *Lachnospiraceae* and order *Clostridiales* were significantly higher than the non-responders (all *p* < 0.05, Fig. 3e–h).

Effects of KRG on the blood glucose parameters differed depending on the enterotype

The participants of the KRG group were categorized into two enterotypes, enterotypes 1 and 2, according to the abundance of *Bacteroides* and *Prevotella* at the baseline to

Table 2 Comparison of the changes in the anthropometric and biochemical parameters as well as the vital signs between the KRG and placebo groups

| Variables | KRG (n = 25) | | | Placebo (n = 25) | | | p-value‡ |
|-------------------------------|----------------|----------------|---------|------------------|----------------|---------|----------|
| | Initial | Final | Within† | Initial | Final | Within† | |
| HOMA-IR | 3.01 ± 1.38 | 3.09 ± 1.90 | 0.882 | 3.66 ± 3.38 | 3.93 ± 3.40 | 0.510 | 0.528 |
| Insulin (μIU/mL) | 10.82 ± 4.73 | 10.75 ± 5.69 | 0.840 | 12.04 ± 8.87 | 12.49 ± 8.15 | 0.412 | 0.467 |
| Glucose (mg/dL) | 112.52 ± 21.16 | 114.28 ± 25.34 | 0.210 | 117.72 ± 22.83 | 118.72 ± 30.06 | 0.688 | 0.503 |
| TG (mg/dL) | 150.72 ± 57.51 | 157.44 ± 58.95 | 0.563 | 166.36 ± 69.61 | 146.64 ± 59.07 | 0.115 | 0.479 |
| TC (mg/dL) | 195.76 ± 39.71 | 191.40 ± 37.07 | 0.380 | 186.72 ± 32.51 | 176.36 ± 32.71 | 0.010 | 0.135 |
| HDL (mg/dL) | 52.52 ± 11.25 | 51.80 ± 13.91 | 0.537 | 55.74 ± 9.75 | 53.68 ± 9.23 | 0.132 | 0.145 |
| LDL (mg/dL) | 129.84 ± 40.20 | 124.96 ± 39.33 | 0.241 | 116.48 ± 36.61 | 109.00 ± 36.77 | 0.066 | 0.145 |
| Body weight (kg) | 74.30 ± 12.68 | 74.58 ± 13.01 | 0.384 | 72.57 ± 16.31 | 72.37 ± 16.39 | 0.367 | 0.600 |
| BMI (kg/m ²) | 28.16 ± 4.47 | 28.46 ± 4.47 | 0.014* | 27.22 ± 4.74 | 27.13 ± 4.71 | 0.292 | 0.240 |
| WC (cm) | 95.90 ± 7.93 | 95.35 ± 7.94 | 0.167 | 95.32 ± 9.29 | 94.04 ± 8.79 | 0.005* | 0.331 |
| Fat mass (kg) | 26.22 ± 8.06 | 26.24 ± 8.15 | 0.864 | 25.20 ± 10.12 | 25.29 ± 10.06 | 0.891 | 0.318 |
| Fat percentage (%) | 35.10 ± 7.26 | 35.06 ± 7.54 | 0.896 | 34.14 ± 7.48 | 34.40 ± 7.43 | 0.328 | 0.755 |
| SBP (mmHg) | 132.36 ± 12.42 | 126.80 ± 12.87 | 0.026* | 127.32 ± 15.62 | 124.88 ± 17.27 | 0.249 | 0.658 |
| DBP (mmHg) | 77.40 ± 8.09 | 75.84 ± 7.51 | 0.415 | 78.08 ± 13.57 | 75.72 ± 11.41 | 0.159 | 0.965 |
| Pulse rate (/min) | 78.16 ± 11.42 | 78.28 ± 9.84 | 0.657 | 79.32 ± 14.64 | 77.32 ± 12.82 | 0.257 | 0.977 |
| CRP (mg/dL) | 0.13 ± 0.17 | 0.21 ± 0.31 | 0.315 | 0.25 ± 0.45 | 0.22 ± 0.31 | 0.988 | 0.869 |
| Number of MS factors | 3.60 ± 0.58 | 3.32 ± 0.80 | 0.106 | 3.40 ± 0.65 | 3.08 ± 0.95 | 0.033* | 0.395 |
| Obesity-related questionnaire | 29.64 ± 6.89 | 29.44 ± 7.12 | 0.869 | 27.96 ± 9.82 | 27.60 ± 7.71 | 0.500 | 0.186 |

Data are expressed as mean ± SD; †p-value obtained from a paired *t*-test, Wilcoxon signed-rank test; ‡p-value obtained from an independent *t*-test, Wilcoxon's rank-sum test; **p* < 0.05, ***p* < 0.01; HOMA-IR, homeostatic model assessment-insulin resistance; TG, triglycerides; TC, total cholesterol; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; MS, metabolic syndrome; CRP, C-reactive protein

understand the influence of the gut microbial composition on the mode of action of KRG further. The relative abundance of *Bacteroides* was significantly higher in enterotype 1 than enterotype 2 (*p* < 0.001), whereas that of *Prevotella* was significantly higher in enterotype 2 than enterotype 1 (*p* < 0.001) (Fig. 4). In contrast, the anthropometric parameters, such as BMI, WC, and fat mass, were significantly lower in enterotype 1 than enterotype 2 at the baseline (*p* < 0.05, Table 4). When the delta values (differences between post- and pre-treatment of KRG) of the anthropometric, body composition, and biochemical parameters were compared between the enterotypes, a decreasing trend in HOMA-IR was observed in enterotype 1 compared to enterotype 2 (*p* = 0.070, Table 5). Furthermore, the delta value of the serum insulin level was significantly lower in enterotype 1 than enterotype 2. (*p* = 0.026).

Certain gut microbial strains have strong links to MS markers

Fifty-nine gut microbial strains correlated with several biomarkers of MS (*p* < 0.05) (Fig. 5). Among them, *Lachnospiraceae* showed a positive correlation with HOMA-IR, BMI, and the serum levels of insulin and glucose. *Acidaminococcus* and *Holdemania* were positively correlated with

the HOMA-IR and serum insulin concentration. *Actinomyces* demonstrated a positive correlation with the HOMA-IR, serum glucose level, and BMI. In contrast, *Anaerostipes* was negatively correlated with the HOMA-IR and serum insulin concentration.

Discussion

This study was a single-center, randomized, double-blind, placebo-controlled clinical trial conducted to evaluate the beneficial impact of KRG on MS and determine if the gut microbial composition and enterotypes could influence these effects.

Influences of KRG administration on MS-related markers

Although the molecular mechanisms underlying MS are not fully understood, OS and inflammatory insults are actively involved in MS [13]. OS, which is imposed due to an imbalance between ROS production and biological systems' ability to detoxify the reactive intermediates or to repair the resulting damages. This condition plays an important role in MS and MS-related diseases [13, 72]. Specifically, enhanced

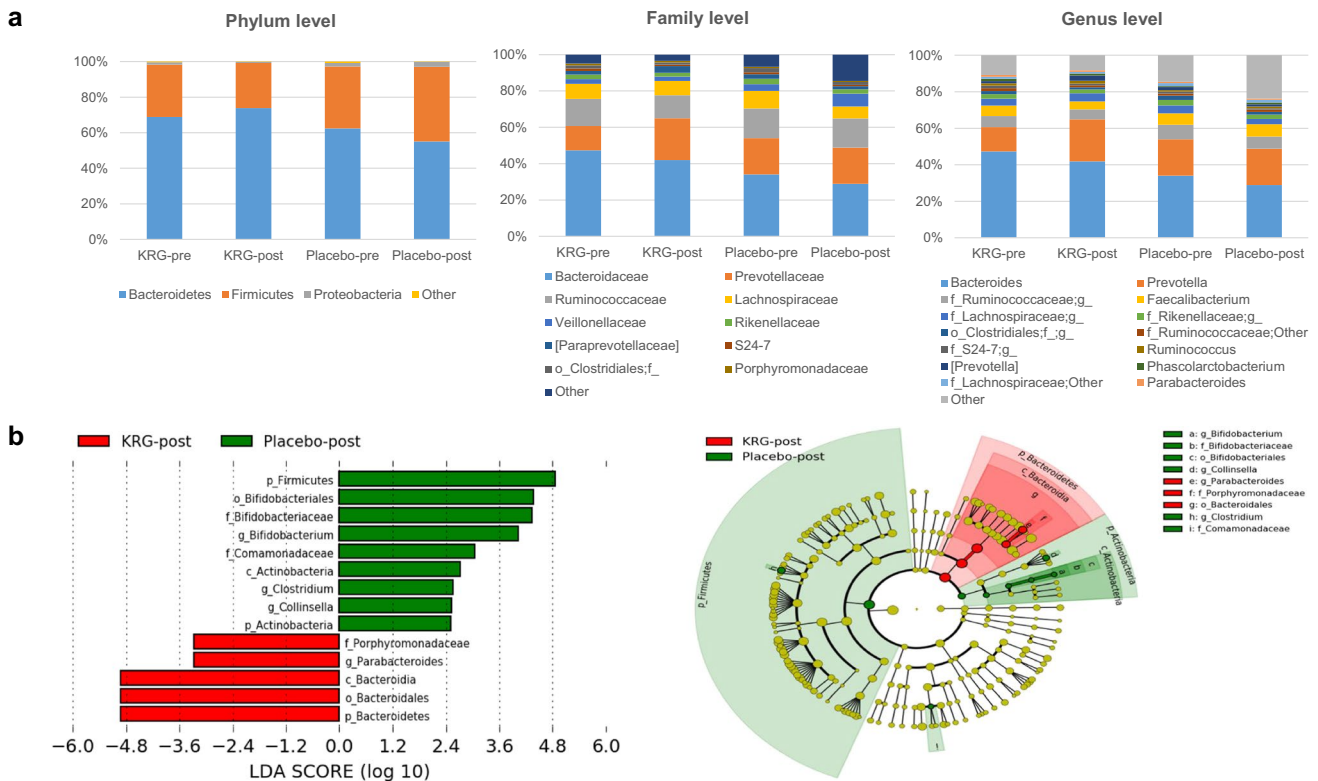


Fig. 2 Effects of KRG or placebo treatments on the gut microbiota. **a** Comparison of the relative abundances of the gut microbiota at phylum, family, and genus levels between before and after KRG, placebo

administration; **b** comparison of the dominant gut microbial taxa between the KRG and placebo groups at the termination of the study, as shown by LEfSe

oxidative damage reflected by reduced α -tocopherol and vitamin C levels, decreased antioxidant protection and superoxide dismutase (SOD) activity, as well as increased malondialdehyde concentration, lipid peroxidation, protein carbonyls, and xanthine oxidase activity are strongly correlated with MS occurrence [13, 73]. Fat accumulation correlates with systemic oxidative stress in both humans and mice. The generation of ROS increases selectively in adipose tissue of obese mice, accompanied by upregulated expression of NADPH oxidase and downregulated expression of antioxidative enzymes. In contrast, in cultured adipocytes, increased levels of fatty acids trigger oxidative stress via NADPH oxidase activation, and oxidative stress causes dysregulated production of adipocytokines (fat-derived hormones), including adiponectin. The contribution of OS to MS is supported further by the fact that in obese mice, treatment with a NADPH oxidase inhibitor, which reduces ROS production in the adipose tissue, attenuated the dysregulation of adipocytokines, and improved diabetes, hyperlipidemia, and hepatic steatosis [15]. Accumulating evidence also indicates that obesity and MS are associated with a low-grade, chronic (smoldering) state of inflammation characterized by augmented circulating levels of free fatty acids, increased

chemoattraction of immune cells (such as macrophages), as well as elevated concentrations of various proinflammatory cytokines including interleukin 1 β (IL-1 β), IL-8, monocyte chemoattractant protein-1 (MCP-1), tumor necrosis factor α (TNF α), and prothrombotic mediator plasminogen activator inhibitor-1 (PAI-1), and biomarkers of inflammation (e.g. C-reactive protein) [13, 18]. Furthermore, dysregulated inflammation with impaired wound healing is a characteristic feature of diabetes [74].

Several animal and human studies have reported that ginseng and its major active components have antioxidant effects, which are mediated mainly through the activation of antioxidant enzymes, such as SOD, catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), and glutathione-S-transferases (GSTs), as well as the attenuation of lipid peroxidation and MDA production [17]. Accordingly, ginseng has therapeutic potential in the prevention and treatment of a wide range of diseases that are associated with oxidative stress such as types 1 and 2 diabetes, cancer, rheumatoid arthritis, heart disease, schizophrenia, Parkinson’s disease, and Alzheimer’s disease [17]. Furthermore, growing evidence suggests that the anti-obesity effects of KRG and ginsenosides are exerted via the regulation of lipid synthesis

Table 3 Alignment of the subjects according to the improvement of the HOMA-IR, insulin, TC, and TG after KRG administration

| Participants | Δ HOMA-IR | Participants | Δ Insulin | Participants | Δ TC | Participants | Δ LDL |
|--------------|------------------|--------------|------------------|--------------|-------------|--------------|--------------|
| S-15* | -2.78 | S-15* | -10.85 | S-64* | -53 | S-44 | -43 |
| S-44 | -1.51 | S-44 | -5.44 | S-44 | -49 | S-64* | -39 |
| S-37 | -1 | S-20 | -3.92 | S-32 | -42 | S-51 | -39 |
| S-18 | -1 | S-37 | -3.73 | S-15* | -39 | S-32 | -33 |
| S-20 | -0.91 | S-18 | -3.62 | S-28 | -28 | S-15* | -23 |
| S-01* | -0.86 | S-53 | -3.29 | S-51 | -26 | S-28 | -23 |
| S-53 | -0.82 | S-45* | -3.22 | S-54* | -22 | S-54* | -14 |
| S-46* | -0.78 | S-01* | -3 | S-02 | -12 | S-02 | -14 |
| S-45* | -0.59 | S-46* | -2.43 | S-25 | -9 | S-25 | -14 |
| S-23 | -0.52 | S-23 | -1.77 | S-23 | -7 | S-12* | -11 |
| S-25 | -0.4 | S-25 | -1.46 | S-45* | -6 | S-22* | -10 |
| S-36 | -0.22 | S-36 | -0.5 | S-46* | -6 | S-23 | -6 |
| S-52 | 0.02 | S-12* | -0.04 | S-22* | -1 | S-18 | -2 |
| S-12* | 0.04 | S-52 | 0.26 | S-18 | 3 | S-45* | -1 |
| S-51 | 0.25 | S-02 | 0.73 | S-12* | 3 | S-39 | -1 |
| S-02 | 0.39 | S-22* | 1.67 | S-63 | 8 | S-46* | 2 |
| S-22* | 0.46 | S-51 | 1.73 | S-39 | 10 | S-63 | 11 |
| S-63 | 0.64 | S-63 | 2.31 | S-53 | 11 | S-53 | 12 |
| S-54* | 0.88 | S-54* | 2.37 | S-61 | 18 | S-20 | 13 |
| S-64* | 0.96 | S-39 | 2.68 | S-36 | 19 | S-52 | 16 |
| S-39 | 1.29 | S-64* | 3.51 | S-52 | 19 | S-08 | 16 |
| S-61 | 1.74 | S-28 | 4.04 | S-20 | 21 | S-61 | 18 |
| S-08 | 1.96 | S-08 | 5.82 | S-01* | 22 | S-01* | 19 |
| S-32 | 2.21 | S-61 | 6.98 | S-37 | 25 | S-37 | 21 |
| S-28 | 2.56 | S-32 | 9.44 | S-08 | 32 | S-36 | 23 |

The degree of improvement was evaluated according to the decrease in size of each index value, and it was considered that smaller the delta value, greater the improvement. Participants who failed to isolate bacterial DNA sample were marked with “*” and excluded from the microbiological analysis. Δ means delta value of each item. *HOMA-IR*, homeostatic model assessment-insulin resistance; *TC*, total cholesterol; *LDL*, low-density lipoprotein cholesterol

and adipocyte differentiation, which are driven by several mechanisms: downregulated expression of lipogenesis and adipogenesis factors (peroxisome proliferator-activated receptor (PPAR γ), CCAAT enhancer-binding protein alpha (C/EBP α), fatty acid-binding protein 4 (FABP4), acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), sterol regulatory element-binding protein 1 (SREBP-1C), stearoyl-coenzyme A desaturase 1 (SCD1), and perilipin) in adipose tissue; upregulated expression of glucose transporter type 4 (GLUT4) and PPAR γ ; increased phosphorylation of AMP-activated protein kinase (AMPK) in the skeletal muscle [39, 75]. On the other hand, the effects of KRG and ginsenosides against diabetes and insulin resistance were found to be associated with the following: increased phosphorylation of Akt and AMPK; decreased phosphorylation of signal transducer and activator of transcription 5 (STAT5); and increased expressions of insulin receptor (IR), glucose transporter type 1 (GLUT1), PPAR γ , and lipoprotein lipase (LPL) [39, 75]. Furthermore, as stated before, MS is associated with a state of low-grade inflammation, and ginseng has been

found to exert strong inhibitory effects on the key players in the inflammatory cascade, such as the p38 mitogen-activated protein kinase (p38 MAPK) pathway, nuclear factor κ B (NF- κ B), inducible NOS (iNOS), and proinflammatory cytokines such as TNF α , interleukin (IL)-1 β (IL-1 β), IL-6, IL-12, IL-18, and interferon-gamma (IFN- γ) as well as the inflammatory markers CRP and cyclooxygenase-2 (COX-2) [39, 76, 77].

Although the previous randomized clinical trials (RCTs) showed improvement of the glucose and lipid parameters, such as HbA1c, fasting blood glucose, and serum LDL levels in type 2 diabetic patients in response to the ginseng treatment [78, 79], the administration of the subjects with MS to KRG did not grossly elicit significant improvement in the glucose and lipid profiles in the present study. On the other hand, a significant decrease in SBP (-5.56 ± 11.71 , $p=0.026$) and a slight change in DBP were noted in the subjects exposed to KRG at a dose of 6000 mg/day for 8 weeks. This agrees with a previous RCT showing a reduction in SBP in pre-hypertensive subjects in response to treatment with

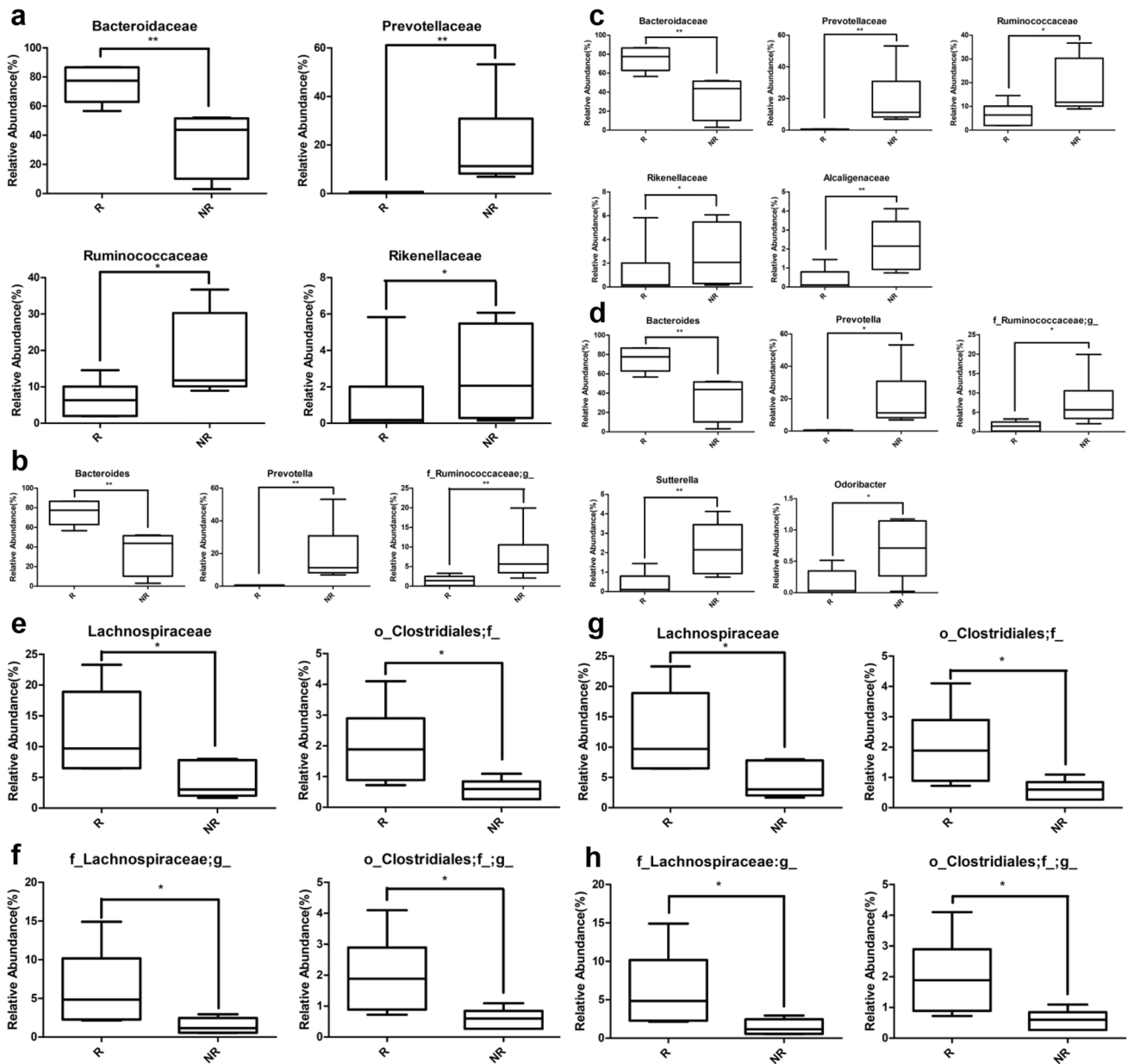


Fig. 3 Comparison of the relative abundances of gut microbial strains between the responder and non-responder groups at the baseline. **a** Strains that showed a significant difference in the HOMA-IR (at family level); **b** strains that showed a significant difference in the HOMA-IR (at genus level); **c** strains that showed a significant difference in the serum insulin level (at family level); **d** strains that showed a significant difference in the serum insulin level (at genus level); **e** strains that showed a significant difference in the serum TC level (at family

level); **f** strains that showed a significant difference in the serum TC level (at genus level); **g** strains that showed a significant difference in the serum LDL level (at family level); **h** strains that showed a significant difference in the LDL (at genus level); HOMA-IR, homeostatic model assessment-insulin resistance; TC, total cholesterol; LDL, low-density lipoprotein cholesterol; R, responder; NR, non-responder; * $p < 0.05$, ** $p < 0.01$

KRG at a dose of 5000 mg/day for 12 weeks [80]. Previous studies reported that compared to DBP, SBP is related more closely to the risk of cardiovascular disease [81] and that a 2 mm Hg decrease in SBP among middle-aged people would result in a 10% and 7% decrease in the risks of mortality from stroke and ischemic heart disease, respectively, as

well as a decrease in other cardiovascular complications [82]. Based on this, it is conceivable that in the present study, the decrease in SBP following KRG administration is a beneficial outcome with clinical significance. Indeed, many ginsenosides have vasodilating effects and appeared to be potential candidates for the treatment of hypertension [83].

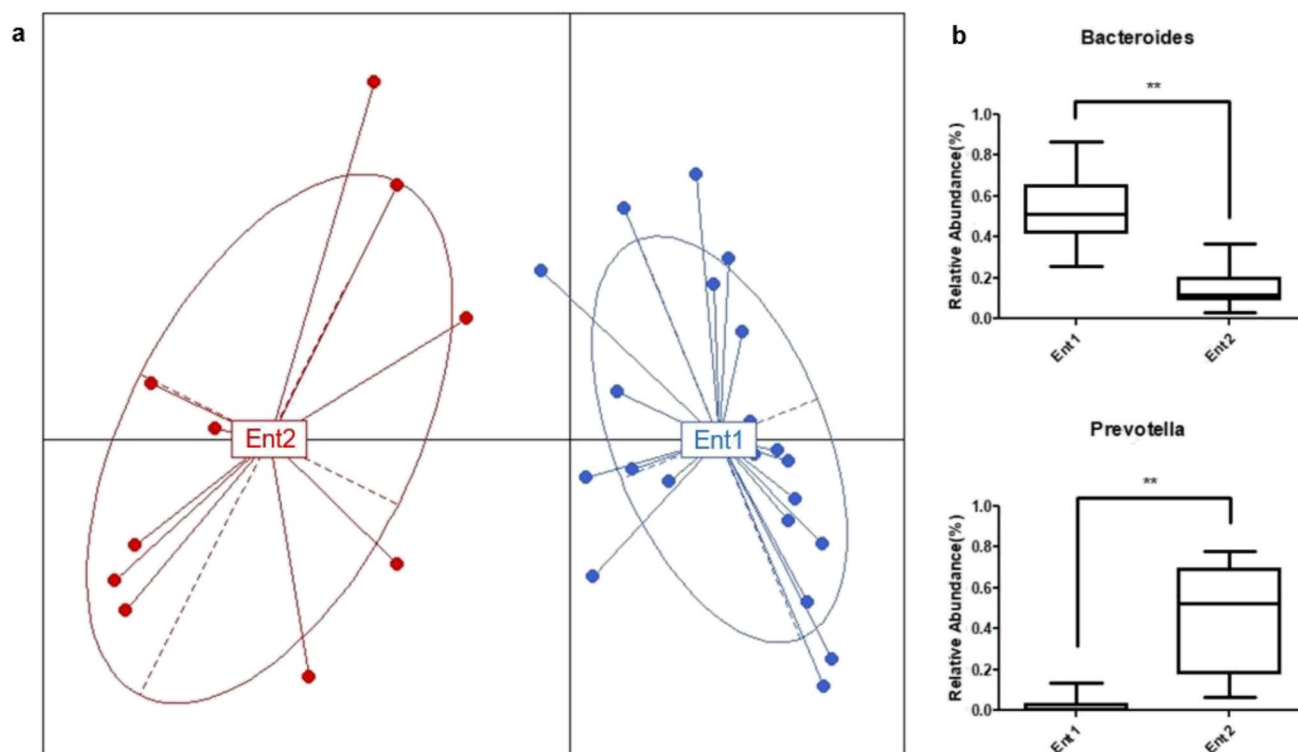


Fig. 4 Analysis of enterotypes. **a** Profiling of the enterotypes. The red and blue colors represent the participants belonging to enterotypes 2 and 1, respectively. Ent1, enterotype 1; Ent2, enterotype 2. **b** Enterotype-specific dominant strains

Table 4 Baseline characteristics of the subjects according to the enterotype in the KRG group

| Variables | Enterotype 1 (n = 12) | Enterotype 2 (n = 5) | p-value [†] |
|--------------------------|--------------------------|-------------------------|----------------------|
| HOMA-IR | 2.80 ± 1.53 | 2.66 ± 1.08 | 0.861 |
| Insulin (μIU/mL) | 9.68 ± 4.42 | 9.29 ± 2.87 | 0.860 |
| Glucose (mg/dL) | 114.58 ± 24.34 | 113.40 ± 22.91 | 0.879 |
| TG (mg/dL) | 143.16 ± 33.39 | 150.20 ± 64.11 | 0.767 |
| TC (mg/dL) | 202.91 ± 47.36 | 188.60 ± 46.18 | 0.576 |
| HDL (mg/dL) | 53.41 ± 12.27 | 47.50 ± 7.99 | 0.341 |
| LDL (mg/dL) | 137.58 ± 47.39 | 126.40 ± 41.06 | 0.653 |
| Body weight (kg) | 69.35 ± 10.93 | 79.30 ± 13.86 | 0.134 |
| BMI (kg/m ²) | 26.59 ± 3.13 | 31.46 ± 3.12 | 0.011* |
| WC (cm) | 93.08 ± 7.87 | 102.4 ± 8.33 | 0.045* |
| Fat mass (kg) | 23.07 ± 5.92 | 31.16 ± 4.03 | 0.020* |
| Fat percentage (%) | 33.27 ± 6.42 | 39.70 ± 4.73 | 0.063 |
| SBP (mmHg) | 129.58 ± 10.57 | 132.40 ± 15.58 | 0.669 |
| DBP (mmHg) | 76.50 ± 6.69 | 76.20 ± 7.19 | 0.935 |
| Pulse rate (/min) | 74.33 ± 10.39 | 78.00 ± 5.91 | 0.476 |
| CRP (mg/dL) | 0.095 ± 0.100 | 0.128 ± 0.048 | 0.130 |

Data are expressed as the mean ± SD; [†]p-value obtained from an independent *t*-test, Mann Whitney *U*-test; **p* < 0.05, ***p* < 0.01; *HOMA-IR*, homeostatic model assessment-insulin resistance; *TG*, triglycerides; *TC*, total cholesterol; *HDL*, high-density lipoprotein cholesterol; *LDL*, low-density lipoprotein cholesterol; *BMI*, body mass index; *WC*, waist circumference; *SBP*, systolic blood pressure; *DBP*, diastolic blood pressure; *CRP*, C-reactive protein

Table 5 Comparison of the delta values of the anthropometric and biochemical parameters as well as the vital signs between enterotypes 1 and 2

| Variables | Enterotype 1 (n = 12) | Enterotype 2 (n = 5) | p-value [†] |
|--------------------------|--------------------------|-------------------------|----------------------|
| HOMA-IR | -0.08 ± 1.25 | 1.13 ± 0.92 | 0.070 |
| Insulin (μIU/mL) | -0.82 ± 3.55 | 4.02 ± 4.03 | 0.026* |
| Glucose (mg/dL) | 0.00 ± 7.30 | 3.80 ± 5.17 | 0.310 |
| TG (mg/dL) | 17.42 ± 54.54 | 10.60 ± 66.70 | 0.828 |
| TC (mg/dL) | 0.00 ± 24.56 | -1.40 ± 25.90 | 0.921 |
| HDL (mg/dL) | -0.50 ± 8.01 | -0.30 ± 2.61 | 0.958 |
| LDL (mg/dL) | -2.58 ± 22.79 | -2.80 ± 21.37 | 0.986 |
| Body weight (kg) | 0.40 ± 1.63 | -0.66 ± 1.70 | 0.273 |
| BMI (kg/m ²) | 0.27 ± 0.43 | 0.02 ± 0.83 | 0.423 |
| WC (cm) | -0.33 ± 1.85 | -2.10 ± 2.33 | 0.116 |
| Fat mass (kg) | 0.47 ± 1.67 | -1.22 ± 2.68 | 0.133 |
| Fat percentage (%) | 0.37 ± 1.59 | -0.96 ± 2.62 | 0.213 |
| SBP (mmHg) | -4.00 ± 11.48 | -6.60 ± 10.06 | 0.667 |
| DBP (mmHg) | -3.00 ± 8.71 | -2.60 ± 3.85 | 0.721 |
| Pulse rate (/min) | 1.83 ± 9.63 | -1.60 ± 8.91 | 0.499 |
| CRP (mg/dL) | 0.18 ± 0.34 | 0.00 ± 0.08 | 0.130 |

Data are expressed as the mean ± SD; [†]p-value obtained from an independent *t*-test, Mann Whitney *U*-test; **p* < 0.05, ***p* < 0.01; *HOMA-IR*, homeostatic model assessment-insulin resistance; *TG*, triglycerides; *TC*, total cholesterol; *HDL*, high-density lipoprotein cholesterol; *LDL*, low-density lipoprotein cholesterol; *BMI*, body mass index; *WC*, waist circumference; *SBP*, systolic blood pressure; *DBP*, diastolic blood pressure; *CRP*, C-reactive protein

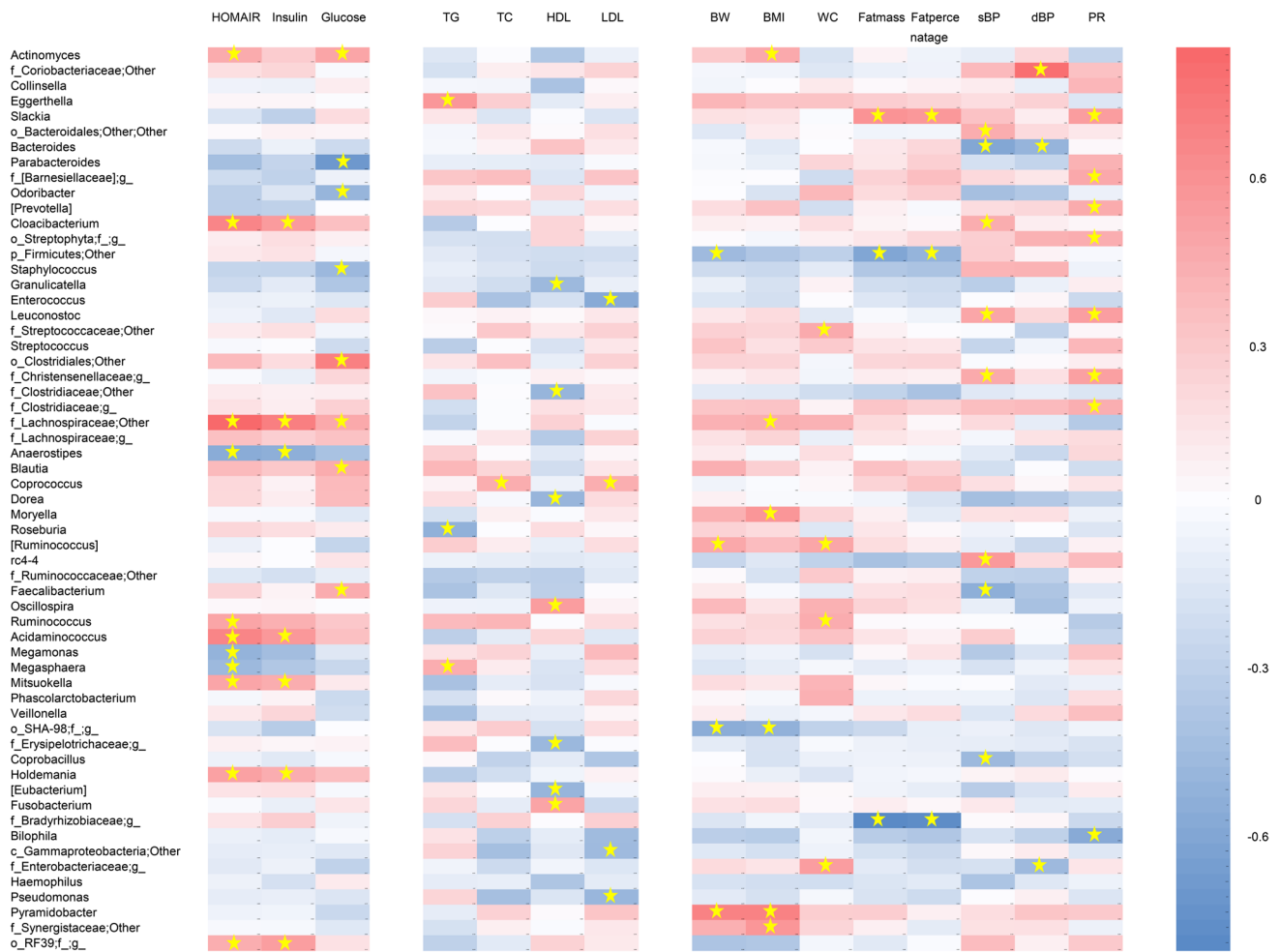


Fig. 5 Correlation between the gut microbiota and metabolic markers. Heatmap showing the correlation between the changes in the gut microbiota at the genus level and alterations in metabolic markers. The red and blue colors represent the positive and negative relationships, respectively. * $p < 0.05$; BMI, body mass index; WC, waist

circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; PR, pulse rate; HOMA-IR, homeostatic model assessment-insulin resistance; TG, triglycerides; TC, total cholesterol; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein

Reciprocal interaction of KRG with gut microbiota: potential use of KRG as prebiotics

The gut microbiota plays crucial roles in the host’s metabolic functions, such as vitamin production, amino acid biosynthesis, bile acid transformation, lipid metabolism, fermentation of non-digestible substrates, and production of short chain fatty acids (SCFAs) [84]. An imbalance in the gut microbial communities (dysbiosis) represented by lower-diversity and higher abundance of pathogenic species is associated with MS and related diseases, including obesity, insulin resistance, type 2 diabetes, hypertension, and cardiovascular disease [33–35, 85–89]. Accumulating evidence suggests the therapeutic potential of ginseng and its active constituents to improve MS, which is mediated by restoration of the

gut microbial composition, amelioration of the gut barrier function, and regulation of metabolites of gut flora [90]. In the present study, the 16S rRNA sequencing data of the gut microbial analyses revealed changes in the relative abundances of several bacterial strains in response to the KRG treatment. In particular, an increase in *Bacteroidetes* and a decrease in *Firmicutes* were observed at the phylum level, but these changes were statistically insignificant. Consistent with this, LEfSe also revealed a remarkable difference in the distributional pattern of the dominant microbial species between the KRG and placebo group. Accumulating evidence suggests that a higher F/B ratio in the gut is related to the increased storage of energy by the host’s adipose tissue, which is mediated by promoting energy extraction [91]. In an earlier study, an increased gut F/B ratio in HFD-fed or

ob/ob mice was reversed in response to the treatment with ginseng saponins [55]. Furthermore, in the present study, the abundance of *Proteobacteria* decreased significantly after KRG administration. An increase in *Proteobacteria* is associated with gut microbial dysbiosis and diseases with an inflammatory phenotype [92, 93]. Therefore, it is conceivable that the reduction in F/B ratio and *Proteobacteria* population caused by KRG would have a beneficial impact on energy and gut microbial homeostasis. In a previous clinical study, silymarin-treated NAFLD patients showed an increased abundance of *Proteobacteria* upon exposure to KRG, but they exhibited a declining trend in the F/B ratio [56]. The differences in the pathophysiological conditions of the subjects and treatment regimens between these two studies might account for these discrepancies. Furthermore, the outcomes of correlation analysis elucidating the relationship between the gut microbiomes and MS-related biomarkers are in parallel with previous studies. For example, high abundances of *Lachnospiraceae* were positively correlated with metabolic disturbances [94]. *Acidaminococcus* was positively linked to the parameters for glucose and insulin, while *Holdemania* was correlated with the clinical indicators of an impaired lipid and glucose metabolism [95]. *Actinomyces* was abundant in obese adolescent subjects [96]. A negative correlation was noted between *Anaerostipes* and the metabolic markers, such as the fasting glucose and insulin resistance index levels [97].

The gut microbes do not only exhibit changes in their abundance in response to ginseng treatment, rather, in turn, they enhance the therapeutic potential of this herb. One of our earlier clinical studies showed that the anti-obesity effects of ginseng extract on middle-aged obese Korean women differed according to the gut microbiota composition prior to ginseng intake [47]. In the host, the bioavailability of ginsenosides is low because they are hydrophilic compounds and difficult to absorb. On the other hand, the metabolism of ginsenosides by the gut microbial enzymes make these compounds more polar, which facilitates their absorption [57, 58]. In vitro models showed that various biotransformations, such as deglycosylation, hydration, esterification are the major metabolic pathways of ginsenoside driven by the gut microbiota [98, 99]. In humans, compound K (CK) is one of the leading products of the gut microbial-mediated metabolism of orally administered ginsenosides, such as Rb1, Rb2, Rc, and Rd [100]. This metabolite has more potent pharmacological effects, such as anti-inflammatory and anti-diabetic activities, compared to the parent ginsenosides [58]. Taken together, it is conceivable that the reciprocal interaction between KRG and gut microbiota plays a vital role in the beneficial effects exerted by KRG.

The term “prebiotic” was first defined in 1995 as “a non-digestible food ingredient that beneficially affects the host

by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health” [101]. In 2016, the definition of prebiotic was modified as “a substrate that is selectively utilized by host microorganisms conferring a health benefit.” Several indigestible dietary carbohydrates, such as oligofructose, galactooligosaccharides, lactulose, and inulin (long chain β -fructan), are shown to be prebiotics because they selectively promote the growth of several beneficial gut bacteria (probiotics) and simultaneously maintain the homeostasis of the gut microbial community along with host health [102, 103]. Accordingly, it has been found that among the ginseng constituents, the polysaccharide fractions especially, sulfated acidic polysaccharides, have prebiotic-like properties, such as sustaining the balance in the gut microbial population, stimulating the growth of beneficial probiotic flora, including *Lactobacillus* and *Bacteroides* species, and enhancing the absorption of metabolites [104–107]. The treatment of hyperlipidemic rats with red ginseng acidic polysaccharide (RGAP) reduced the serum and hepatic levels of TG and increased the activity of LPL in the serum, a key hydrolytic enzyme of lipid molecules in lipoprotein [108]. Taking account of the abovementioned beneficial impact of KRG and its reciprocal interaction with the host’s gut microbiome, KRG is considered a potentially promising prebiotic candidate [60].

Patient stratification strategy for KRG use: enterotypes as potential biomarkers for MS

The differences in the intestinal microbial communities between the responder and non-responder groups were analyzed at the baseline to understand the influence of bacterial strains on the beneficial action of KRG further. The responders who had a higher abundance of *Bacteroides* and *Ruminococaceae* and a lower population of *Prevotella* compared to the non-responders showed marked improvement in HOMA-IR and serum insulin level after the KRG treatment. The responders who had a higher abundance of *Lachnospiraceae* and *Clostridiales* than the non-responders demonstrated decreases in the serum TC and LDL levels after the KRG treatment. These findings are in keeping with a comparative analysis of the gut microbiota in people with different degrees of ginsenoside metabolism activities. The study showed that *Bacteroides*, *Ruminococcus*, and *Clostridiales* were higher in the groups with fecal activity metabolizing ginsenoside Rb1 to CK [109].

The recent advances in PPPM have led to a paradigm shift from the uniform treatment strategy for a particular disease to personalized health care by considering the characteristics of each patient [61]. Over the last few years, predictive medicine has attracted considerable attention as a promising therapeutic approach to meet the need for increasing the

crisis of diseases, including MS using technologies, such as personalized profiling, biomarker patterning, multiomics, big data, and machine learning [10, 11, 74, 110–112]. Emerging evidence indicates that the use of personalized data, including the gut microbial profile, is helpful to predict individual glycemic responses and to lower the post-meal blood glucose level [113]. Drugs affect the human gut microbiota, and the gut microbiota facilitates the effects of many drugs [114]. Enterotype, a concept that categorizes individuals according to their gut microbial profile, helps analyze the correlation between the gut microbiota and the overall functioning of the body. This facilitates the strategic application of precision medicine for many diseases, including MS [64, 65, 115]. The enterotypes are independent of age, gender, cultural background and geography [114]. Thus far, three enterotypes of bacterial patterns have been traditionally reported: *Bacteroides*-rich (enterotype 1), *Prevotella*-rich (enterotype 2), and *Ruminococcus*-rich (enterotype 3) [66]. Functional compositions and properties including coenzyme, lipid, amino acid, and carbohydrate metabolism show differences among the enterotypes owing to their different microbial profile [116]. Furthermore, in MS patients, there are several associations between species abundance of gut microbiota and metabolic parameters that are enterotype-specific [115]. Because the microbial enterotypes have different digestion capacities and substrate preferences, individuals with different enterotypes would respond differently to specific diets and medications [114]. Indeed, many clinical studies confirmed differential metabolic or pharmacological responses of the subjects to diets, dietary products or dietary supplements, probiotics or drugs correlating with specific gut enterotypes [65, 114, 117].

In enterotype analyses, the anthropometric parameters, such as BMI, WC, and fat mass, in the KRG group were significantly lower in enterotype 1 (*Bacteroides*-rich) than enterotype 2 (*Prevotella*-rich) at the baseline, which is in agreement with a previous study showing a significantly lower basal body weight and BMI in the low *Prevotella*-to-*Bacteroides* (P/B) group compared to the high P/B group [118]. Furthermore, in the present study, enterotype 1 responded more effectively in improving the serum insulin level to KRG than enterotype 2. *Bacteroides* metabolizes polysaccharides and oligosaccharides and provides nutrition and vitamins to the host and other intestinal microbial communities; hence, they positively impact human health [119]. Accumulating evidence suggests that *Bacteroides* is negatively associated with type 2 diabetes. It has been found that this bacterial genus plays a beneficial role in the glucose metabolism in humans and experimental animals by improving glucose intolerance and insulin resistance [120]. Emerging evidence also suggests that *Bacteroides* is involved in the metabolism of ginsenosides through deglycosylation, oxygenation, and hydrolysis [57, 121]. Rc, Rg3, Re, and Rb1 are

potentially transformed into CK, and protopanaxadiol, Rh1, Rh2, and F1 by the action of enzymes of *Bacteroides* [109, 122–124]. Therefore, the greater effectiveness of KRG in the glucose metabolism in enterotype 1 than enterotype 2 is due to the improved pharmacokinetic properties of ginsenosides contributed mainly by *Bacteroides*.

Limitations

This study had some limitations. First, the factors that can modulate the intestinal floral communities, such as diet [125], sleep [126], and exercise [127], were not controlled completely. Second, because MS is a cluster of diseases, including hyperlipidemia, hypertension, and type 2 diabetes, various classes of drugs have already been taken by a large number of participants of the KRG (15 out of 30) and placebo groups (16 out of 30) to combat those conditions. This might mask the effects of KRG because many of the metabolic parameters are improved by these background medications in both groups. Furthermore, these background medications might also interact with KRG. Third, both enterotypes 1 and 2 encompassed only a small number of participants. Therefore, the statistical power might not be sufficient to validate the outcomes of enterotype analyses. Fourth, the ginsenoside metabolites and short chain fatty acids were not measured to analyze the effects of the gut microbiota, and the phenotype biomarkers, such as visceral fat, were not estimated. It has been found that the excessive visceral fat accumulation is associated with insulin resistance and multiple risk factors for MS [128, 129]. Earlier studies reported that visceral fat area (VFA) was superior to BMI and waist circumference to predict MS and consequently, various cut-off values of VFA have been developed according to race, gender, and age [130–133]. Furthermore, it has been shown that VFA could be an independent phenotype marker of MS in type 2 diabetic patients [134]. Based on the abovementioned points, we opined that after the KRG treatment, the trial should be continued with routine analyses of the parameters and markers until the gut microbial profile is restored to its initial condition. Therefore, a further follow-up trial without the abovementioned limitations should be carried out in the future.

Conclusion and expert recommendations

To the best of our knowledge, this is the first randomized, double-blind, placebo-controlled clinical trial evaluating the effects of KRG on patients with MS, especially from the context of PPPM. The results showed that the administration of KRG capsules at a dose of 6000 mg/day for 8 weeks elicited clinically meaningful improvement in SBP. Gut microbial analysis revealed a decrease in *Firmicutes* and

Proteobacteria, and an increase in *Bacteroidetes* due to the abovementioned treatment. Moreover, the patients' response to KRG was influenced by the gut microbial composition. In patient stratification analysis, the responders who had a higher abundance of *Lachnospiraceae* and *Clostridiales* than non-responders showed decreases in serum TC and LDL levels. Further differentiation of the subjects into two enterotypes showed that *Bacteroides* might play a role in the beneficial impacts of KRG on the parameters related to the glucose metabolism, particularly the HOMA-IR and serum insulin level.

In this study, a reciprocal interaction between the gut microbiota and KRG was observed. Taking account into the abovementioned interactions and the beneficial impact of KRG on the host, ginseng is thought to be a potentially promising prebiotics candidate. Additionally, the findings on the impact of KRG on blood glucose parameters of the participants with MS can be interpreted from the perspective of personalized medicine that the effects of KRG on the glucose metabolism vary depending on the enterotypes. Therefore, it is conceivable that having information on the enterotype in advance will be helpful in predicting the effectiveness of KRG on glucose homeostasis of MS patients at an individual level. This will also help to establish a targeted treatment strategy for MS, in compliance with the concept of PPPM.

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Availability of data and material Data can be provided upon request from the journal.

Code availability Related information was provided in the manuscript.

Declarations

Ethics approval and consent to participate This trial was performed at Dongguk University-Ilsan Oriental Medical Hospital (Ilsan, Gyeonggi-do, Republic of Korea) from February 27, 2019, to February 21, 2020. The Institutional Review Board of the Hospital approved this study (approval number: DUIOH 2018–12-004). The study protocol was also registered in the Clinical Research Information Service online registration system (registration number: KCT0004823). It was conducted according to the principles of the Declaration of Helsinki. Voluntary

written consent was obtained after providing a detailed explanation regarding the research to the participants.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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Authors and Affiliations

Eunhak Seong¹ · Shambhunath Bose¹ · Song-Yi Han¹ · Eun-Ji Song² · Myeongjong Lee¹ · Young-Do Nam² · Hojun Kim¹ 

¹ Department of Rehabilitation Medicine of Korean Medicine, Dongguk University, Gyeonggi-do 10326, Republic of Korea

² Research Group of Healthcare, Korea Food Research Institute, Wanju-gun 55365, Republic of Korea