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Effects of Deep Ocean-Derived Magnesium-Enhanced Water on Metabolic Diseases with Microbiome Changes

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Running title: Mg-Enhanced Water, metabolic diseases and Microbiome

Abstract

Aims: To investigate the effects of magnesium (Mg) from deep ocean sources, we conducted a randomized clinical trial involving adults with hypertension, diabetes, or hyperlipidemia.

Methods : Subjects consumed either Mg-enriched water (MEW) or a placebo (80 or 6 mg of Mg per 2 L/day, respectively) for 4 weeks. We examined the detoxifying effects of MEW on environmental toxicants, including polycyclic aromatic hydrocarbons (PAHs) and oxidative stress, and its impact on gut microbiome composition (N=30; 49.26 ± 9.55 yrs).

Results: Most subjects consumed less Mg than the RDA, enabling their participation in the trial. Despite limitations in serum Mg measurement to assess Mg intake, MEW intake led to improvements in body mass index (BMI), insulin levels, triglycerides, glucose-BMI, and fatigue. Regardless of Mg content, water consumption reduced urinary levels of 1-hydroxypyrene, a major PAH metabolite, and malondialdehyde, an oxidative stress biomarker. Moreover, the MEW group exhibited greater diversity in gut microbiome composition than the placebo group. Notably, MEW kept the abundance of *Clostridium*, *Dorea*, or *Desulfovibrio*, indicating a balanced Mg intake.

Conclusion: MEW (80 mg of Mg/day) appears safe for RDA and effective for preventing CVD or T2DM, as evidenced by gut microbiome and biomarker outcomes.

Keywords: Magnesium, Deep Ocean, Metabolic diseases, Biological monitoring, Microbiome

1. Introduction

Magnesium (Mg) is an essential mineral crucial for maintaining homeostatic functions in vital organs such as the heart, muscles, and nervous system [1]. Approximately 94–95% of Mg is distributed intracellularly, with 5% found in extracellular fluid and less than 1% in blood serum [2]. Acting as a co-enzyme for approximately 300 enzymes involved in the tricarboxylic acid cycle (TCA cycle), Mg plays a pivotal role in producing energy in the form of ATP [3], making it integral for physiological, biochemical, and cellular processes that regulate cardiovascular function. This includes the modulation of vascular smooth muscle tension, endothelial cell function, and myocardial excitability [4]. Epidemiological studies have consistently linked low serum Mg levels with metabolic disorders, such as diabetes, cardiovascular diseases (CVDs), and coronary artery calcification [5-6], underscoring the importance of meeting the recommended dietary allowance (RDA) for Mg intake.

In Korea, the RDA for Mg is set at 280 mg/day for women and 350 mg/day for men [7]. Nuts, seeds, legumes, whole-grain cereals, leafy vegetables or water are well-recognized dietary sources of Mg [8]. However, many individuals may fall short of RDA due to dietary irregularities, consumption of soft drinks, alcohol, or coffee to inhibit Mg intake [8]. Particularly, western diets are generally rich in refined foods that are very poor in Mg, having a very low content of whole grains and green vegetables. In addition, cooking and the refining processes may consistently diminish the Mg content present in the food, since a significant amount of Mg is lost during these procedures [9]. Conversely, excessive Mg levels, often associated with kidney failure, can lead to adverse effects such as nausea, vomiting, low blood pressure, and muscle weakness, posing life-threatening risks [2]. Recent animal studies have indicated that improper Mg intake can alter the microbiome, increasing the risk of type 2 diabetes mellitus (T2DM), obesity, and inflammatory enteritis through changes in bacteria such

as *Desulfovibrio*, *Dorea*, and *Clostridia* [10].

To address Mg deficiency, Mg-enhanced water (MEW) offers an alternative solution due to its ease of ingestion and high Mg absorption rate, compared to other formulations like tablets [11]. Our study aimed to determine whether MEW could help maintain RDA levels and prevent metabolic diseases, such as CVDs or diabetes. We also investigated whether MEW could detoxify environmental triggers of metabolic diseases, such as polycyclic aromatic hydrocarbons (PAHs) and PAH-induced oxidative stress, exemplified by malondialdehyde (MDA). Finally, we examined the impact of MEW on gut microbiome composition to elucidate potential metabolic mechanisms. Thus, our clinical trial may provide the safe dosage of Mg for RDA and the mechanisms by which Mg influences the microbiome in relation to metabolic disorders, offering a novel approach.

2. Materials and Methods

2.1. Clinical trial

We conducted a double-blind, parallel, and randomized clinical trial (Fig. 1). The data coordinating center determined the allocation of placebo and MEW. Participants (N=30) with prehypertension or borderline diabetes (HbA1c ≥ 5.6%), who visited the Suwon-ICOOP Yeongtong medical center (Suwon City, Gyeonggi Province, Korea) for regular checkups, were recruited for this study. They were assigned to drink either placebo water (6 mg of Mg/2 L/day) or MEW (approx. 80 mg of Mg/2L/day: Supplement Table 1), sourced from microplastic-free deep ocean water in Goseong, East Sea, Korea (≥ 200 m in depth). The water was provided by iCOOP Natural Dream Company (Goseong-gun, Gangwon Province, Korea). Participants filled out a diet diary for 4 weeks and completed a questionnaire covering lifestyle and physical information, including age, housing, exercise, tobacco smoking, alcohol consumption, fatigue,

and thirst. The status of fatigue and thirst was assessed using the fatigue severity scale [12] and the thirst distress scale for patients with heart failure [13], respectively. These scores were reverse-scored for uniformity with the questionnaire. Participants provided urine, feces, and blood samples. Vital signs, cardio-ankle vascular index (CAVI), and ankle-brachial index were measured with VaSera VS-1000 (Fukuda Denshi, Japan) to assess arterial stiffness and stenosis or obstruction.

All of biospecimens were collected before and after the trial. Morning preprandial blood and urine specimens were collected with 3 ml of EDTA-containing V-tubes (AB Medical, Jangsung, Korea) ™ - or 5 ml of serum separation gel tubes, i.e., AMPULAB™ evacuated blood collection tubes (Soyagreentec Co. Ltd., Hwaseong-si, Korea) and 15 ml of conical tubes, respectively and kept at -20°C until analyzed. Following the manufacturer's protocol, the subjects collected feces in DNA/RNA Shield™ Fecal collection tube (Zymo Research, Irvine, CA). This study was approved by Sookmyung Women's University Research Ethics Committee (KCT0009526), and written informed consent was obtained from all participants. We excluded the people, who had calorie changes during the experiment and did not take the test materials for over 1 week.

2.2. Dietary analyses

The subjects filled out diet diaries during the trial. We calculated each nutrient and calories per meal and per day from the diaries, using Can-Pro 5.0 (The Korean Nutrition Society, Seoul, Korea) and the mobile Noom program (Noom, Inc., New York, NY, US), respectively.

2.3. Mg depletion score

The Mg depletion score (MDS), proposed by Fan *et al*. [14] as a predictor of Mg deficiency instead of the Mg tolerance test, was calculated. MDS aggregates four factors: diuretic use (current use for 1 point), proton-pump inhibitors (PPIs) use (current use for 1 point), kidney function (estimated glomerular filtration rate ≤ 60 mL/min/1.73 m^2 for 1 point), and alcohol consumption (heavy drinker for 1 point).

2.4. Analysis of urinary 1-hydroxypyrene and MDA

We analyzed two CVD risk factors: urinary 1-hydroxypyrene (1-OHP), an exposure biomarker for PAHs, and MDA, an oxidative stress biomarker, using high-performance liquid chromatography with fluorescence detection (HPLC/FLD), following our previously established methods [15]with minor modifications.

For 1-OHP analyses, 50 μL of supernatant, prepared from 200 μL of urine using the aforementioned method [15], was injected into an HPLC/FLD system consisting of an Agilent HPLC 1290 Infinity (Agilent Technologies, Santa Clara, CA), FLD detector (Agilent Technologies), and a YMC-Triart C18 column (150 mm × 4.6 mm, 5 μm; YMC Co. Ltd., Kyoto, Japan). The mobile phases, conditions, and excitation and emission wavelengths were consistent with our previous method [15].

For MDA analysis, 20 μL of supernatant, prepared from 50 μL of urine using the aforementioned method [15], was injected into an HPLC/FLD system comprising a YL9111 HPLC binary pump (Younglin Co., Seoul, Korea), YL9150 autosampler (Younglin Co., Seoul, Korea), and FP-4025 FLD (Jasco, Tokyo, Japan). The levels of thiobarbituric acid-reactive substances (TBA-MDA adducts) were determined at specific excitation and emission

wavelengths. The column and mobile conditions were consistent with our previous method [15].

Urinary analytes were adjusted for creatinine and analyzed using an automatic biomedical analyzer (HITACHI 7020, Tokyo, Japan). The same automatic biomedical analyzer was utilized to analyze hematological indicators.

2.5. Gut-microbiome analyses

DNA extraction from stool specimens was performed following the protocol of the PureLink Microbiome DNA Purification Kit (Thermo Fisher Scientific, Waltham, MA), along with in-house optimized methods. The microbial DNA extracted served as a template for PCR amplification of seven hypervariable regions of the 16S bacterial gene (V2-4-8 and V3-6, 7–9) [16]. Amplification of 16S rRNA was carried out using the Ion 16S Metagenomics Kit (Thermo Fisher Scientific) with two primer pools. PCR conditions included an initial denaturation step at 95°C for 10 min, followed by 25 cycles of denaturation at 95°C for 30 s, annealing at 58°C for 30s, and extension at 72°C for 20 s, with a final extension at 72°C for 10 min. PCR amplification was performed using a PCR Thermal Cycler Dice Touch (Takara Bio Inc., Shiga, Japan). The integrity of the PCR amplicon was assessed by electrophoresis on a LabChip GX Touch 24 (PerkinElmer, Waltham, MA). Library preparation was conducted with the Ion Plus Fragment Library Kit, Ion Xpress Barcode Adapters 1-16 Kit, and Ion Xpress Barcode Adapters 17-32 Kit (Thermo Fisher Scientific). Purification was carried out with HiAccubead (AccuGene, Incheon, South Korea). The prepared library was quantified into 200–300 bps using LabChip GX Touch 24 (PerkinElmer). Subsequently, the library was loaded onto an Ion 520 Chip (Thermo Fisher Scientific) using the Ion Chef System (Thermo Fisher Scientific),

followed by sequencing on the Ion S5 XL System (Thermo Fisher Scientific). Operational taxonomic units were assigned using Ion Reporter software.

To validate the stool analyses, we utilized the ZymoBIOMICS Microbial Community Standard (D6300, Zymo Research, Irvine, CA) as a regulatory grade reference material consisting of 10 inactivated microorganisms [17].

2.6. Statistical analyses

The Shapiro–Wilk W Test was employed to assess distributional normality in biomarkers. Pearson's product moment or Spearman's rank correlation analyses were conducted to explore relationships between dietary factors and CVD risk factors. ANOVA or Kruskal–Wallis tests were employed to identify differences in biomarker levels between MEW treatment and placebo groups. Multiple regression analyses were performed to investigate associations among biomarkers and CVD risk factors. Statistical significance was defined at $P < 0.05$. JMP ver.4 (SAS Institute, Cary, NC, USA) was utilized for the statistical analyses.

3. Results

3.1. Characteristics of subjects

Along with baseline measurements, biomarkers, most vital signs, or hematological indicators did not significantly vary between the placebo and MEW groups before the trial (Table 1). All subjects (N=30; 49.26 ± 9.55 yrs; men, 50%) had prehypertension or hypertension, overweight, or diabetes. The demographical features of the participants and the changes of biomarkers by the trial were described in Table 1.

3.2 Mg intake

During the trial, the daily calorie intake changed for some subjects (N = 14), prompting their exclusion from future analyses. When assessing Mg intake from the diet before the trial, most subjects consumed less Mg (90.68 ± 48.46 mg/day) than the RDA of 280–350 mg/day. Thus, the MEW treatment (80 mg/day) appeared safe, ensuring Mg intake remained within the RDA for the subjects.

Although the MEW treatment did not significantly increase serum Mg levels, there was a somewhat positive association between Mg daily intake and the gap in serum Mg levels before and after the trial (Fig. 2A). Additionally, we investigated whether there was any inhibition of Mg intake associated with MDS. As depicted in Fig. 2B, there was a somewhat negative effect of MDS on the changes in serum Mg levels before and after the trial. While MDS did not entirely account for the inhibition of Mg intake, our results suggest that MDS may partially reflect it.

Furthermore, the ratio of serum Ca to Mg ranged from 3.91 to 5.05 (4.53 ± 0.33) before the trial and 3.91 to 5.16 (4.46 ± 0.29) after the trial. Although not significant, the ratio of serum Ca to Mg showed some improvement in the MEW group (Table 1).

3.3. Changes in biomarkers

Relative to before the trial, the MEW group showed improvements in body mass index (BMI) and insulin levels. Additionally, carotid artery vascular index (CAVI-L) and triglyceride (TG) levels exhibited some improvement with MEW ($0.05 < Ps < 0.1$). Conversely, urinary 1-OHP and MDA levels, which are potential risks of CVD, were not improved by MEW with or without creatinine modification (Table 1). However, water intake (2 L/day) regardless of Mg amounts improved urinary MDA (median before the trial, 1.77 ng/mL; after, 1.00 μM, $P < 0.01$ by Wilcoxon Signed-Rank) and 1-OHP levels (median before the trial, 0.12 ng/mL; after, 0.01

ng/mL, P < 0.001 by Wilcoxon Signed-Rank). Therefore, proper water drinking may help prevent environmental CVD risks. Additionally, thirst improved regardless of treatment (P = 0.02), while there were no differences in thirst between the placebo and MEW groups (P = 0.47). However, fatigue was significantly improved with MEW (Fig. 3).

3.3. Changes in gut-microbiome

Gut DNA samples were amplified by 106 940 sequences (average, 25 317 sequences), providing sufficient sequences from all samples. Before the trial, the total number of identified bacteria in the placebo and treatment groups was 596 363 and 484 602, respectively, with the placebo group accounting for approximately 23% more. Alpha-diversity analysis before the trial revealed mixed diversity among placebo and MEW subjects (Fig. 4A). After the trial, most MEW subjects belonged to a highly diverse group with low individual variations compared to the placebo (Fig. 4B). Further analysis of alterations in richness (Chao1) and evenness (Shannon) showed no significant differences between the two groups during the trial (P = 0.68 and 0.29 by paired T-test), respectively.

The most prevalent bacterial family observed in both groups was *Bacteroidaceae*, constituting approximately 33.2% and 31.2% in the placebo and MEW groups, respectively. Conversely, *Enterobacteriaceae*, the second most common in the placebo group (11.8%), was significantly lower at 1.8% in the MEW group. However, *Prevotellacea*e, often more prevalent in non-Western populations due to its association with high fiber and low-fat diets [18], was approximately three times more common in the MEW group compared to the placebo group (14.7% vs. 5.2%), although these trends were not statistically significant (P = 0.18, 0.06 by U test, respectively).

In terms of individual genus comparison, the proportions of *Clostridium* were significantly altered by MEW (Fig. 4C). Specifically, the relative abundances of *Clostridium*, *Dorea*, and *Alistipes* remained stable or somewhat decreased in the MEW group, while they increased in the placebo group. The abundance of *Desulfovibrio* remained stable in the MEW group but decreased in the placebo group (P = 0.14). A somewhat negative association was observed between *Dorea* and *Desulfovibrio* (P = 0.22). Additionally, *Ruminococcus*, *Veillonella*, and *Paraprevotella* showed somewhat decreased relative abundances in the MEW group, whereas they remained unchanged in the placebo group. Notably, *Veillonellaceae*, known to produce hydrogen in obese individuals, increases energy accumulation through the fermentation of plant polysaccharides [19] and utilizes lactic acid to produce propionic acid, an inducer of inflammation, in children with type 1 diabetes [20]. Considering their prevalence among all subjects, *Clostridium* and *Dorea* appear to be more sensitive bacterial indicators of MEW effects than others (Fig. 4C and D).

4. Discussion

4.1 Mg intake

Over a 5-year period (2016–2021), there was a decline in daily Mg intake and the Ca to Mg ratio in Korea [21]. Concurrently, the prevalence of T2DM has increased by approximately 28%, with people with diabetes, exhibiting a two-fold higher risk of CVD complications [22]. Given the involvement of Mg in glucose and lipid metabolism [19], we focused on Mg intake as a potential factor [21]. As Mg is a trace essential metal, it's crucial to consider both deficiency and excess for safety. Therefore, we assessed Mg intake from the diet before the trial and found that most subjects consumed less than the RDA of Mg. Consequently, we implemented the MEW treatment (80 mg of Mg/day). Upon estimating Mg intake from both

diet and the trial, the range varied from 9.3–302 mg/day, within the normal dose range based on serum Mg levels (range 1.8–2.50 mg/dL, 2.13 ± 0.16 mg/dL, after the trial; Table 1). However, serum Mg levels did not significantly differ between the two groups post-trial (Table 1). Thus, we focused MDS, i.e., inhibitors of Mg absorption. Serum Mg levels are commonly used to diagnose Mg deficiency; however, they may not accurately reflect Mg levels throughout the body [14]. This is because over 80% of Mg in the blood is regulated by filtration and reabsorption in the kidneys to maintain homeostasis [21]. Additionally, factors such as medication (e.g., diuretics, PPIs) and alcohol consumption can impact Mg tolerance and depletion [14]. In our study, the gap in serum Mg levels post-trial showed somewhat positive and negative associations with Mg intake and MDS, respectively (Fig. 2A and B). Therefore, the positive association between calculated Mg intake and the gap in serum Mg levels post-trial confirms compliance with the MEW treatment.

The reference ranges for the Ca-Mg ratio in plasma vary, with the China Adult Chronic Disease and Nutrition Surveillance (2015) suggesting a range of 2.41–3.44 [23], while a recent Chinese study proposed a range of 2.36–3.66 for Chinese adults over 45 years old (N = 337) [24]. Optimal Ca-Mg ratios from the diet have been suggested as 1.70–2.60, based on studies from China and the United States [25]. In a National Health and Nutrition Examination Survey in the USA (2007–2014), the ratio of Ca to Mg intake was 3.3 among over 20, 000 subjects [26]. In our study, the median ratio of Ca to Mg in plasma was 4.45, with no difference observed before and after the trial, while the ratio from the diet during the trial was 2.388. Although the subjects showed a higher ratio of Ca to Mg in plasma, they maintained a proper ratio from the diet during the trial. Many people suspect Ca interferes Mg effects on CVDs [27], however, our subjects were in the normal ranges of Ca/Mg. We did not find harmful effects of the ratio of Ca to Mg on CVD biomarkers in the present study.

4.2. Changes in clinical biomarkers by MEW

We focused on alterations in biomarkers of CVD or diabetes. Results showed relative differences in BMI and insulin levels due to the trial (Table 1). Additionally, CAVI-L and TG levels were somewhat improved by MEW (Ps = 0.08; Table 1). Among the risk factors of CVD, associations were observed between the relative differences in TG and BMI or insulin (P = 0.03 and 0.007, respectively). According to recent studies, the TG glucose (TyG)-BMI can simultaneously capture several clinical variables and more closely reflect insulin resistance than the index alone [28-29]. When the TyG-BMI was < 231.66, Han et al. showed that it reflected the risk of progression from prediabetes to diabetes in Chinese patients with prediabetes [29]. We calculated the TyG-BMI as Ln [1 / 2 fasting plasma glucose (mg/dL) × fasting TGs (mg/dL)] * BMI (kg/m^2)] in these subjects and found that relative differences in the TyG-BMI index were improved before and after the trial by MEW (placebo, before, 228.3 ± 52.02, after, 234.19 ± 12.85, differences, –5.89 ± 16.13; MEW, 219.39 ± 34.97, 211.84 ± 8.41, 7.55 ± 9.78, respectively; P = 0.008).

In this study, sufficient water intake (2 L/day) led to reduced levels of urinary MDA and 1-OHP regardless of Mg amounts. While water alone may not directly reduce these levels in the body, adequate hydration can support natural detoxification processes, indirectly aiding in their reduction through PAH dilution or promoting urination to reduce PAH body burden [30].

Additionally, Mg deficiency can induce oxidative stress by increasing reactive oxygen species (ROS) production [31] and modulating the oxidation and anti-oxidation system of thigh muscles, partly through affecting ROS production [32]. Symptoms of Mg deficiency, such as general fatigue and muscle weakness, are well-documented [33]. Deep ocean mineral water,

containing soluble elements, has been shown to expedite recovery from physical fatigue in Taiwanese young adults [34]. The microplastic-free MEW somewhat improved fatigue used in this study (Fig. 3). It may be related to the nutraceutical formulation of MEW, liquid water, as well as other nutraceutical trace elements abundant in deep oceans, such as Cu, Co, and Mo [35].

For the effects of Mg on CVDs and diabetes, there were some controversies. For example, a 20-year longitudinal American study revealed a significant inverse association between Mg intake and CVD or T2DM biomarkers, such as levels of hs-CRP, IL-6, fibrinogen, and HOMA-IR [36]. A current meta-analysis also showed that Mg supplementation significantly reduced fasting plasma glucose in people with T2DM (9 studies ; 95% CI, −0.80 to −0.00) and at high risk of T2DM (3 studies ; 95% CI, −0.62 to −0.07) after a 2-hour oral glucose tolerance test [37]. However, some clinical trials showed no correlation between Mg intake and metabolic syndromes [38-39]. These studies were performed with quite high dose of Mg, compared to this study, i.e., 350-360 mg/day vs. 80 mg/day, respectively. For Mg dose issues, a current metanalysis concluded that higher intake of dietary Mg, each additional intake of 100 mg/d of dietary Mg, was associated with a reduced risk of all-cause and cancer mortality, but not CVD mortality [40]. Although various epidemiological studies support beneficial effects of Mg on metabolic diseases [5-6], some factors, such as dose, duration, compliance, etc., in clinical trials may affect the results.

4.3. Effects of MEW on gut-microbiome

The health effects of Mg on the gut microbiome in humans are not fully understood. However,

a recent study in male Wistar rats showed higher diversity in control or low Mg groups compared to the high Mg group. Co-exclusion patterns were observed between the high Mg hub (6,000 mg/kg; *Desulfovibrio* and *δ-Proteobacteria*), low-Mg (60 mg/kg; *Dorea* and *Clostridia*), and control (1, 000 mg/kg; *Prevotella* and *Bacteroidia*) groups [10]. In our study, the placebo group exhibited an increase in *Clostridium* and *Dorea*, potentially indicating a low Mg pattern. However, the MEW group did not show an increase in *Desulfovibrio*, associated with hydrogen sulfide production detected in T2DM, obesity, and inflammatory bowel syndrome [41], but rather an increase in *Prevotellaceae*, reflecting control diet [10] and often linked with desirable health measures, such as reduced visceral fat and improved glucose metabolism [18].

Furthermore, the diversity of the microbiome was somewhat higher in the MEW group compared to the placebo (Fig. 4A and B). Diversity of gut microbiome is pivotal as current research highlights the relationship between it and metabolic syndromes. Two population-based cohort studies revealed that poor control of blood glucose levels and systemic inflammation were associated with lower gut microbiome diversity [41-42]. Mg shows positive impacts on gut microbiome composition and metabolism of vitamins B1 and D in patients with metabolic syndrome, T2DM, and obesity [44]. Thus, the fact that MEW improved BMI, insulin levels, triglycerides, glucose-BMI, and fatigue in this study may be affected by microbiome- modulation.

Our study includes some study limitations or application for the future studies. The first one is the small sample size. To overcome this limitation, we analyzed various biomarkers in a double blinded, randomized and placebo-controlled clinical trial. Thus, our study provides various data for future enlarged studies to confirm the results. Second one is the period issue

to observe end points, such as HbA1c, which acts as a surrogate marker of glucose concentration during the preceding 8–12 weeks [43]. Due to the continuous turnover of erythrocytes (120 days), only 50% of an HbA1c value may represent glucose exposure in the preceding 30 days [43]. As we performed the trial for 4 weeks to get the best compliance among the subjects, our results of HbA1c may reflect half of the treatment effects. In our study, MEW alleviated 12-hour fasting insulin levels rather than HbA1c by the treatment (Table 1). Thirdly, to find safe dose within RDA, we could not compare various doses of Mg. Thus, we expect our data with 80 mg of Mg /day can be used as the safe dose for the future studies.

5. Conclusion

Mg (80 mg/day) in MEW, derived from microplastic-free deep ocean sources, is deemed safe for RDA and effective for preventing metabolic diseases with improvement of CVD or diabetic biomarkers with microbiomic diversity in the middle-aged Koreans at high risk for CVDs or T2DM.

Author contributions

Kang H.: Writing - Original Draft/Investigation. Lee U.J. : Formal analyses and clinical trial. Park B. Y.: Recruit of Subjects and administration. Kim M.: Literature review and Writing. Yang M.: Conceptualization, Writing - Review and Editing, Supervision, and Funding acquisition.

Non-Conflicts of interest statement

There were no conflicts of interest to declare.

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Table 1. **Changes in Biomarkers by the trial**

Biomarkers	Before			After			Comparison		Diagnosis
	Placebo (N = 9)	MEW (N = 21)	P[a]	Placebo (N = 9)	MEW (N = 21)	P[a]	P[b]	P[c]	
Characteristics:									
Age (yrs)	46.56 ± 9.23	50.43 ± 9.67	0.32	46.56 ± 9.23	50.43 ± 9.67				
Sex: N of Man (%)	3 (33.33)	12 (57.14)	0.23	3 (33.33)	12 (57.14)				
Vital Signs:									
Systolic BP (mmHg)	143.56 ± 14.67	145.71 ± 14.94	0.72	137.89 ± 16.03	134.33 ± 15.86	0.58	0.16	0.14	Prehypertension, 130–139
Diastolic BP (mmHg)	88.22 ± 12.55	86.48 ± 17.07	0.79	85.56 ± 13.12	79.95 ± 16.07	0.37	0.23	0.14	Prehypertension, 80–89
BMI (kg/m^2)	26.69 ± 4.03	25.50 ± 3.51	0.51	26.70 ± 4.01	25.08 ± 3.55	0.28	0.04*	0.04	Overweight, 25–29.9
CAVI-R	6.58 ± 1.08	7.15 ± 0.83	0.12	6.92 ± 0.93	7.17 ± 0.96	0.52	0.25	0.24	Normal, ≤ 7.9
CAVI-L	6.52 ± 1.10	7.10 ± 0.68	0.09	6.96 ± 0.99	7.01 ± 0.91	0.89	0.08	0.09	
ABI-R	1.16 ± 0.10	1.17 ± 0.09	0.87	1.15 ± 0.07	1.16 ± 0.09	0.71	0.89	0.98	Normal, 0.91–1.29
ABI-L	1.14 ± 0.08	1.16 ± 0.08	0.51	1.15 ± 0.09	1.14 ± 0.09	0.80	0.50	0.42	

Lipid profile:									
TC (mg/dL)	199.67 ± 36.62	176.62 ± 42.42	0.17	198.56 ± 35.98	170.38 ± 40.67	0.08	0.50	0.17	Normal < 200
TG (mg/dL)	138.89 ± 160.05	120.76 ± 59.30	0.46	148.00 ± 88.82	108.67 ± 55.50	0.26	0.08	0.53	Normal < 150
HDL-C (mg/dL)	57.22 ± 15.16	55.42 ± 9.21	0.69	54.22 ± 12.14	53.83 ± 10.27	0.93	0.61	0.86	Normal ≥ 40
LDL-C (mg/dL)	119.67 ± 44.31	101.86 ± 39.30	0.28	119.67 ± 32.20	99.71 ± 37.74	0.18	0.79	0.37	Normal ≤ 130
Apolipoprotein B (mg/dL)	102.56 ± 29.50	91.11 ± 27.42	0.31	103.22 ± 25.70	89.60 ± 29.27	0.24	0.58	0.22	Normal < 100
HS-CRP (mg/L)	1.45 ± 1.34	0.70 ± 0.58	0.12	0.89 ± 0.61	0.59 ± 0.42	0.09	0.56	0.16	Normal, 1.0–3.0
Diabetic profile:									
HbA1c-NGSP (%)	5.93 ± 0.76	5.82 ± 0.38	0.91	5.81 ± 0.55	5.76 ± 0.40	0.96	0.78	0.38	Normal ≤ 5.6%
Glycoalbumin (μmol/L)	13.33 ± 2.39	13.48 ± 1.92	0.86	12.96 ± 1.93	13.45 ± 2.06	0.54	0.39	0.46	Normal, 11–16%
Insulin (μU/mL)	7.98 ± 3.83	7.32 ± 3.32	0.75	10.96 ± 8.38	6.48 ± 3.44	0.06	0.02*	0.01	Normal, 5–15

Mg levels :									
Serum Mg (mg/dL)	2.07 ± 0.14	2.12 ± 0.16	0.44	2.10 ± 0.10	2.16 ± 0.18	0.38	0.89	0.90	Normal, 1.58–2.55
Serum Ca/Mg	4.47 ± 0.29	4.55 ± 0.35	0.51	4.49 ± 0.27	4.45 ± 0.31	0.76	0.35	0.24	Reference value, 2.41–3.44
Environmental risks :									
Urinary 1-OHP (ng/ml)	0.09 ± 0.15	0.17 ± 0.18	0.21	0.04 ± 0.11	0.11 ± 0.13	0.07	0.62	0.47	Average[d], 0.24–0.76
Urinary 1-OHP (μg/g Cre)	0.18 ± 0.36	0.68 ± 1.72	0.20	0.44 ± 1.31	0.47 ± 1.11	0.08	0.88	0.94	
Oxidative stress :									
Urinary MDA (μM)	1.94 ± 0.73	1.89 ± 1.12	0.92	1.16 ± 1.25	1.42 ± 1.25	0.29	0.50	0.64	Average[d], 0.07–0.12
Urinary MDA (μg/g Cre)	147.59 ± 68.41	299.11 ± 297.61	0.32	213.01 ± 128.57	257.70 ± 216.80	0.79	0.14	0.24	

[a] differences between placebo and MEW before or after the trial

[b] differences in changes of biomarkers before and after the trial between placebo and MEW, i.e., relative differences

[c] relative differences, which were adjusted for age and sex

[d]Based on the database of Goodbeing Co., Ltd. (Seoul, Korea) (https://goodbeing.center)

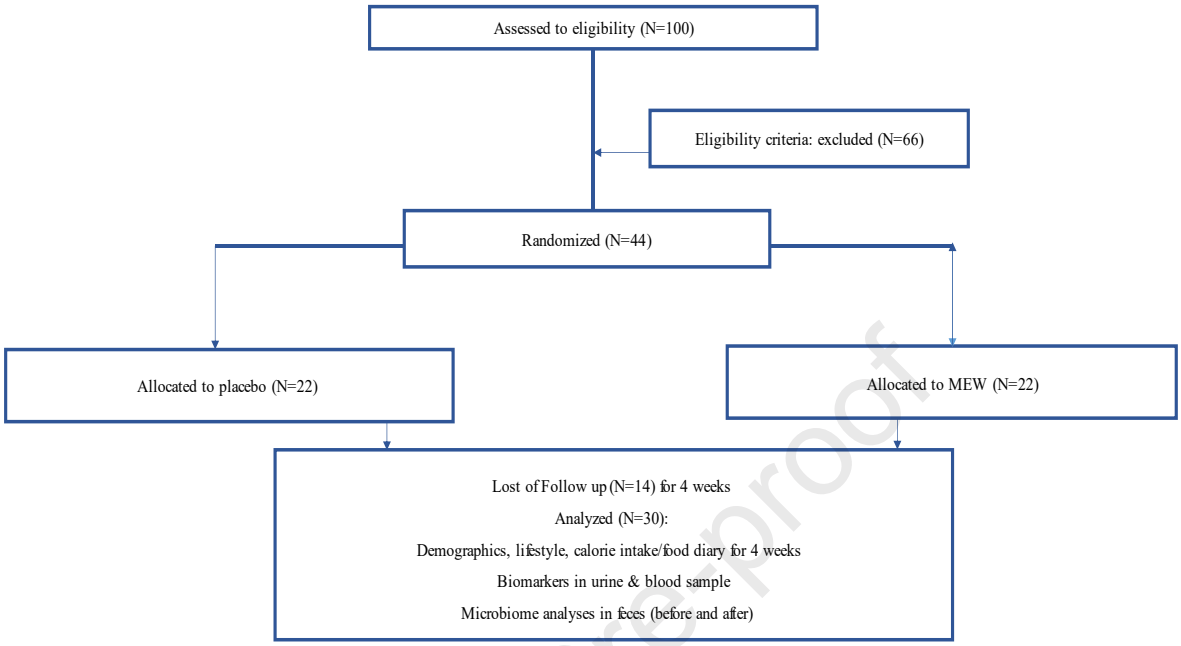
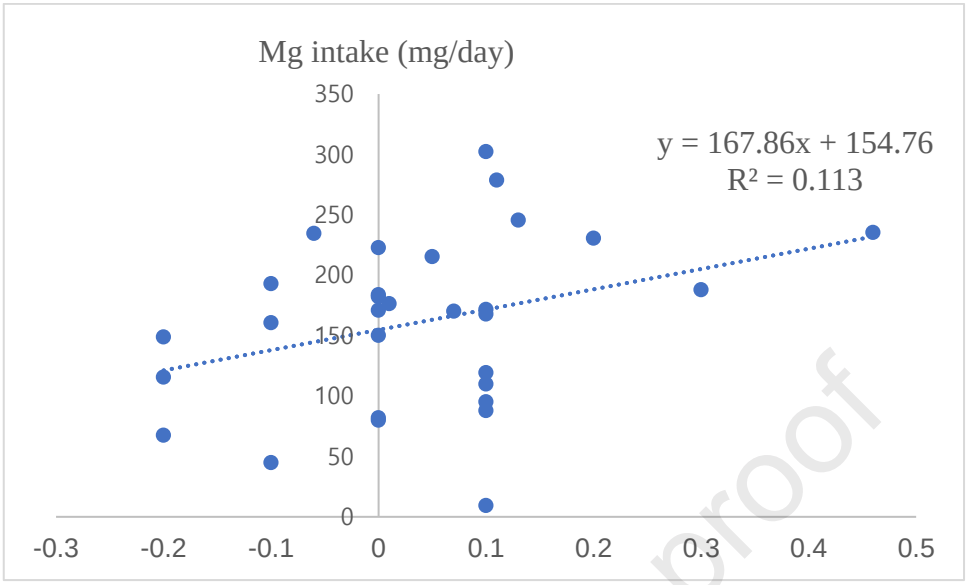


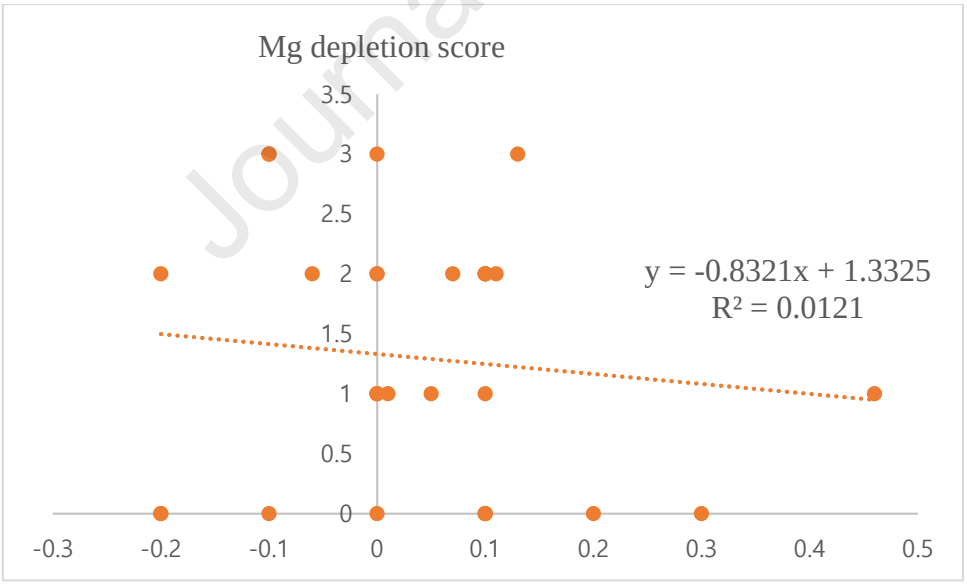
Fig. 1. Study flow through a parallel randomized double-blinded trial of two groups, placebo and MEW: Samples were collected before and after the trial.

A.



Changes in serum Mg (mg/dL)

B.



Changes in serum Mg (mg/dL)

Fig. 2. Relationship between changes in serum Mg levels and Mg Intake (A) or Mg depletion score (MDS) (B): The X-axes represent the changes in serum Mg levels before and after the

trial, i.e., [Mg] after the trial – [Mg] before the trial, while the Y-axis in A indicates the cumulative Mg intake from the sum of daily intake and MEW or placebo (80 or 6 mg of Mg /day, respectively), P = 0.07 for the association between x and y. In B, the Y-axis represents MDS, P = 0.56 for the association between x and y.

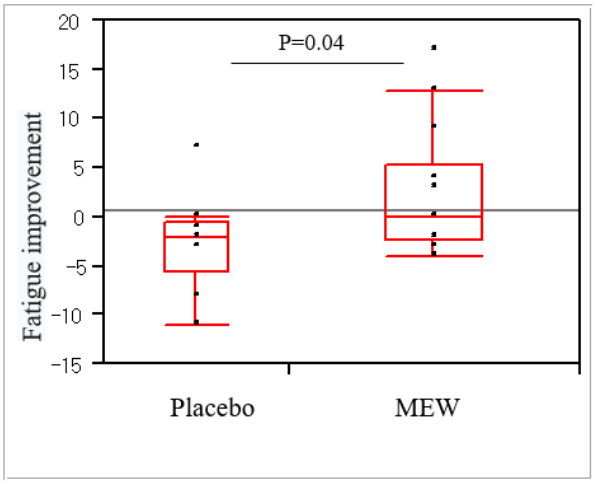
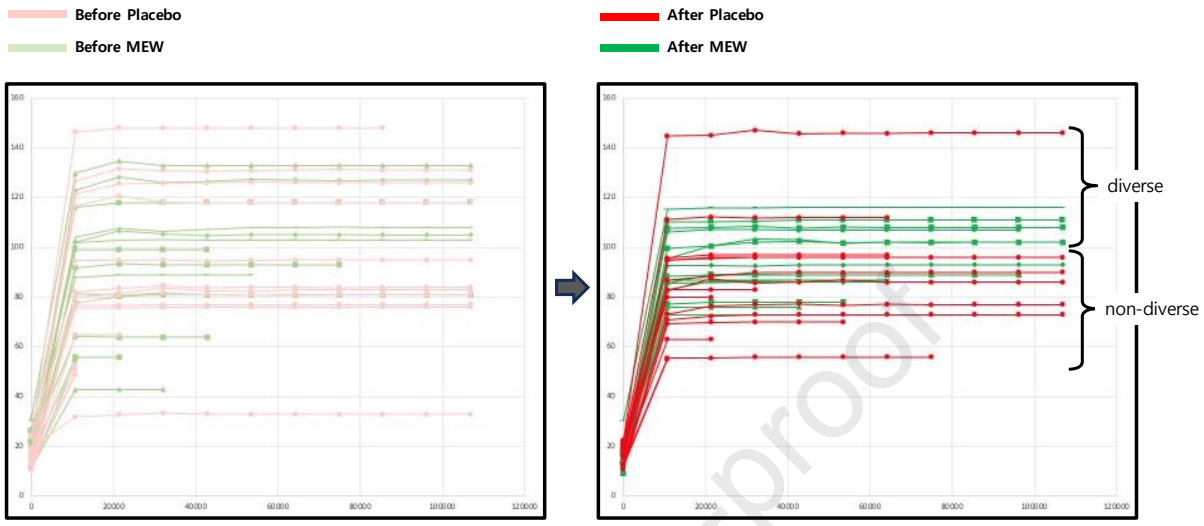


Fig. 3. Improvement in fatigue with MEW: The Y-axis represents the change in fatigue levels (after-before). Fatigue levels were rated on a scale from 1 to 5, ranging from very tired to less tired. The maximum total score for the 10 fatigue status items is 50, indicating the most energetic state.

A. B.



C. D.

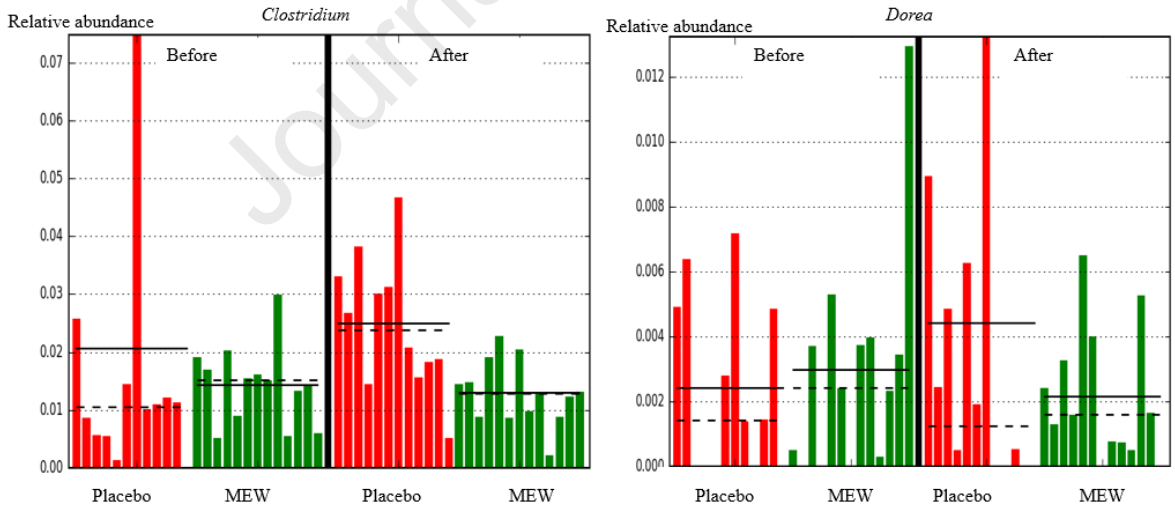


Fig. 4. Effects of MEW on Gut Microbiome: A and B mean alpha diversity (chao) before and after the trial, respectively. X-axis = sequence, Y-axis = Rarefied chao1. C and D mean differences in relative abundance of *Clostridium* ($P < 0.01$) and *Dorea* ($P = 0.21$) between placebo and MEW, respectively.

Highlights :

- Mg-enriched water (80 mg of Mg /day) is safe within recommended dietary allowance.
- Mg-enriched water may prevent metabolic diseases with microbiome diversity.
- Mg-enriched water alleviates body mass index, insulin levels, triglycerides, and fatigue.

Declaration of interests

☐ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☒ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Mihi Yang reports financial support was provided by the iCOOP Natural Dream Company. Mihi Yang reports a relationship with the iCOOP Natural Dream Company that includes: funding grants.

If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.