High Levels of *Akkermansia muciniphila* Growth Associated With Spring Water Ingestion Prevents Obesity and Hyperglycemia in a High-fat Diet-Induced Mouse Model

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**Abstract**

**Background:** Type 2 diabetes may be alleviated by mineral water (MW) ingestion. We investigated whether spring water (SW) (a kind of mixed MW) ingestion influences metabolic parameters via alteration of the gut microbiota in high-fat diet (HFD)-fed mice.

**Method:** We divided 32 C57/BL mice into 4 equal groups: normal diet with tap water (Control), high-fat diet with tap water (HFD), normal diet with SW (SW), and high-fat diet with SW (HFD + SW). During this experiment, we checked the body weight (BW) with fasting blood sugar (FBS) every week and all mice were sacrificed in the 17th week to observe serological markers, internal organs, and composition of gut microbiota.

**Results:** The BW of HFD-fed mice was significantly higher than that of mice fed an HFD + SW diet in the early period of the experiment. Fasting blood glucose (FBG) in the HFD group showed a fluctuating pattern compared to the HFD + SW group, and the area under the curve (AUC) value of the oral glucose tolerance test (OGTT) was significantly greater in the HFD group than in the HFD + SW group. Serologic markers were not significantly different between the HFD and HFD + SW groups. Histologically, the most severe fatty changes in the liver were observed in the HFD group. Lastly, the gut levels of *Akkermansia muciniphila* were 100-fold higher in the HFD + SW group than in the HFD mice.

**Conclusion:** These findings indicate that SW ingestion, and the associated high levels of *A. muciniphila* growth in the gut, may improve the early stage of obesity and ameliorate HFD-induced hyperglycemia.

**Keywords**
type 2 diabetes, obesity, mice, *Akkermansia muciniphila*, spring water

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**Introduction**

Type 2 diabetes mellitus (T2DM) and obesity affect a significant proportion of the adult population worldwide. These diseases are caused by excessive caloric intake and insulin resistance, eventually leading to dysfunction of several organs such as the kidneys, heart, blood vessels, and eyes.¹

Recently, many researchers have focused on dysbiosis of the intestinal microbiota and endotoxemia in metabolic diseases.²⁻⁴ The microbiota composition of healthy individuals is different from that of obese and diabetic individuals. Several kinds of foods or drugs may alter the gut microbiota with a positive or negative effect.⁵,⁶ In several clinical investigations, either vitamins or minerals have been used for the management of metabolic syndromes.⁷,⁸ This is because micronutrients including macro elements (like calcium and magnesium), and trace

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elements (like zinc and fluoride [F]) are essential for glycemic control in diabetes. The control of blood glucose by minerals might be dependent on the concentrations of the minerals and their relative composition.10-14 There are a few reports that suggest that the underlying mechanism for the observed improvement in metabolic parameters associated with mineral water (MW) consumption has to do with compositional changes in the intestinal microbiota. They demonstrated improvement of hyperglycemia in diabetic patients drinking bicarbonate-rich spring water (SW), leading to high growth of gut bacteria, specifically those in the family Christensenellaceae, of the Firmicutes phylum. SW possesses many kinds of minerals: not just small amounts of diabetes-alleviating minerals, including potassium, calcium, magnesium, zinc, and magnesium, but also other ones unrelated to diabetes. The composition of SW varies according to regional differences and chemical characteristics of the water.16

Thus, we investigated whether the ingestion of weak alkaline bicarbonate SW, the most common form of SW, in the Republic of Korea, could affect the metabolic parameters and internal organs in a high-fat diet (HFD)-induced mouse model of obesity, specifically via alteration of the gut microbiota.

Materials and Methods
Reagents
The SW utilized in this study, which is the most common type of SW in Korea with respect to mineral composition (described in Table 1), was obtained from Dukgu Oncheon, located in Gyeongsangbuk-do, Republic of Korea. The mice were provided with freshly supplied SW, which was first filtered through a 0.45 µm filter paper and stored at 4 °C.

Table 1. Comparative Mineral Compositions of Spring Water (SW) and tap Water.

| Component | SW (mg/L) | Tap water (mg/L) |
|-----------|-----------|-----------------|
| K         | 0.6       | 0.2             |
| Na        | 41        | 0.7             |
| Ca        | 3         | 1               |
| Mg        | 0.01      | 0.1             |
| Cl        | 4.4       |                 |
| SO₄²⁻     | 5.2       |                 |
| CO₃²⁻     | 5.8       |                 |
| HCO₃⁻     | 89.7      |                 |
| Sr         | 34        |                 |
| F         | 10.3      |                 |
| Fe         | 0.02      |                 |
| Mn         | -         |                 |
| Li         | 0.08      |                 |
| Sr         | 0.03      |                 |
| Zn         | 0.01      |                 |
| Al         | 0.016     |                 |
| PbSiO₂     | 0.03      | 32.7            |

Abbreviations: F, fluoride; Pb, lead.

Analysis of gut Microbial Composition
Fecal samples (~4 mg) were immediately suspended in a solution containing 100 mM Tris HCl (pH 9.0), 40 mM Tris

Animal Studies
All the experimental procedures were approved by the Committee of Animal Ethics of Uijeongbu St. Mary’s Hospital (UJA2017-06A). Thirty-two 4-week-old male mice (C57BL/6J) were purchased from The Jackson Laboratory (Seoul, Korea). All the mice were bred freely until the experiment, fed a chow diet, and provided with tap water for 1 week prior to the initiation of the experiment. An HFD containing 45% fat, and a chow diet were both supplied by Research Diets Inc. (NJ, USA) for 17 weeks. The mice were randomly and evenly divided into 4 groups (Control [n = 8], HFD [n = 8], HFD + SW [n = 8], and SW [n = 8] groups), and fed ad libitum with either standard chow or HFD. About 5 mL of either SW or tap water per 20g body weight (BW) was supplied to each group.

Fasting Blood Glucose and Oral Glucose Tolerance Test
After fasting for 8 h, blood samples were collected from the tail vein and the fasting blood glucose (FBG) levels were measured using a Gluco Navii Link 0.3 (SD BIOSENSOR). After oral administration of 2 g/kg of glucose following 8 h of fasting, the oral glucose tolerance test (OGTT) was performed. Briefly, the blood glucose levels were determined in blood samples obtained from the tail vein at 0, 30, 60, 90, and 120 min after glucose administration.

RNA Extraction and PCR Method for Bile Acid-Related Receptor Gene Expression Analysis
To study gene expression of bile acid receptors, total RNA was extracted from the colon using TRizol reagent (Invitrogen). One µg/µL of the isolated RNA was subjected to reverse transcription using a cDNA synthesis kit (Invitrogen). The mRNA levels of farnesoid X receptor (FXR), vitamin D receptor (VDR), liver X receptor (LXR-α), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were quantified using a quantitative real-time polymerase chain reaction (PCR), the iTaqTM Universal SYBR Green Supermix (Bio-Rad Korea), and a CFX96 real-time PCR detection system (Bio-Rad Korea). The primers F (5’-GCCACAGATTTCTCTCCTGC-3’) and R (5’-CAGTTCTCCCTGCTGACCA-3’) were used to amplify the FXR gene. The primers F (5’-GGTAGAGGGGGCAGGGTAGA-3’) and R (5’-CAGTGTGGGCTGACATTCCCTA-3’) were used to amplify the VDR gene. The primers F (5’-AGGAGGTGCATCTGCACCAA-3’) and R (5’-CTCTCAGTGTCTTGAC-3’) were used to amplify the LXR-α gene. The primers F (5’-CCCATTGTTGTCTGGGTTG-3’) and R (5’-GTTAGGCGCTGCGTCTG-3’) were used to amplify the GAPDH gene.
The BW of the mice in the HFD group was significantly higher than that of the mice in the HFD + SW group in the early stage of the experiment ($P < 0.05$); the BW of the mice in the HFD /HFD + SW groups was $34.02 \pm 1.12/31.95 \pm 1.74$, $36.87 \pm 1.55/34.29 \pm 2.01$, $38.36 \pm 1.91/35.64 \pm 2.52$, $39.96 \pm 1.85/37.00 \pm 3.08$, and $42.90 \pm 1.18/40.02 \pm 3.11$ g on the second, third, fourth, fifth, and seventh weeks, respectively. The BW was not significantly different between the HFD and HFD + SW groups on the sixth week, and in the very early (beginning to second week) and late periods (8th week to 17th week) of the experiment (Figure 1). These data suggest that ingestion of SW while consuming a HFD may prevent BW gain in the early period.

The FBG levels, which were also measured every week, showed a fluctuating pattern (100-300 mg/dL) in the HFD group compared to the other 3 groups (100-200 mg/dL) (Figure 2a). The OGTT was performed by checking blood glucose levels every 30 min for 2 h on the last day. The area under the curve (AUC) values were $22775 \pm 153$, $35220 \pm 867$, $25785 \pm 1322$, and $23240 \pm 90$ in the Control, HFD, HFD + SW, and SW groups, respectively, ($P < 0.001$). Thus, the AUC value was significantly higher in the HFD group than in the other 3 groups (Figure 2b). It might be presumed that HFD-induced hyperglycemia would decline substantially after ingestion of SW, but it still did not reach the control levels. The plasma levels of insulin, leptin, and monocyte chemoattractant protein 1 (MCP-1) were significantly higher in the HFD and HFD + SW groups than in the Control and SW groups ($P < 0.05$); however, there was little difference between the HFD and HFD + SW groups. The serum levels of insulin, leptin, and MCP-1 were $1004 \pm 711.6$, $3515 \pm 1843$, $2439 \pm 1119$ and $2438 \pm 1438$, $7910 \pm 5993$, $6895 \pm 5226.7$, and $57.5 \pm 8.1$, $114.9 \pm 46.3$, $92.1 \pm 30.2$ pg/mL in the Control, HFD, HFD + SW groups, respectively (Table 2). Other blood markers, including interleukin 6 (IL-6), resistin, and tumor necrosis factor-alpha (TNF-α), were not significantly different among the 4 groups (Table 2).

**Results**

**BW, FBG, and Other Serologic Markers Associated With Metabolic Diseases**

There was little difference in the initial BW of the 4 groups. While the BW of the mice in the Control and SW groups increased gradually from 28 to 32 g during the study period, the BW of the mice in the HFD and HFD + SW groups increased rapidly from the 1st week to the 10th week to 40 g, and then reached 45 g by the end of the experiment (Figure 1). The BW of the mice in the HFD group was significantly higher than that of the mice in the HFD + SW group in the early stage of the experiment ($P < 0.05$): the BW of the mice in the HFD /HFD + SW groups was $34.02 \pm 1.12/31.95 \pm 1.74$, $36.87 \pm 1.55/34.29 \pm 2.01$, $38.36 \pm 1.91/35.64 \pm 2.52$, $39.96 \pm 1.85/37.00 \pm 3.08$, and $42.90 \pm 1.18/40.02 \pm 3.11$ g on the second, third, fourth, fifth, and seventh weeks, respectively. The BW was not significantly different between the HFD and HFD + SW groups on the sixth week, and in the very early (beginning to second week) and late periods (8th week to 17th week) of the experiment (Figure 1). These data suggest that ingestion of SW while consuming a HFD may prevent BW gain in the early period.

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**Changes in the Weight of the Internal Organs and Liver**

Ingestion of HFD + SW might affect the internal organs by altering the accumulation of fat and various minerals. The weight of the liver in the HFD group was the highest among the 4 groups ($P < 0.05$) (Figure 3a): $1.32 \pm 0.14$, $2.14 \pm 0.24$, $1.79 \pm 0.44$, and $1.20 \pm 0.11$ g in the Control, HFD, HFD +
SW, and SW groups, respectively. The weight of white fat in the SW group was the lowest among the 4 groups ($P < 0.05$) and there was no significant difference between the HFD and HFD + SW groups: 1.22 ± 0.24, 1.64 ± 0.39, 1.51 ± 0.25, and 0.68 ± 0.28 g in the Control, HFD, HFD + SW, and SW groups. There was also no significant difference in the weight of other organs between the HFD and HFD + SW groups (Figure 3a). The liver function test revealed that all parameters, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALK-P), and lactate dehydrogenase (LDH), were not significantly different between the HFD and HFD + SW groups (Table 2). However, there was a significant difference in AST, ALT, and LDH levels between the HFD (with or without SW) and the non-HFD groups (Table 2). Histologically, the HFD group showed more severe fat infiltration into the liver than the other 3 groups, but there was no significant difference between the HFD and HFD + SW groups: the grade of steatosis of the liver was grades 2 (5/8, 62.5%) and 3 (3/8, 37.5%) in the HFD group, and grades 1 (3/8, 37.5%), 2 (3/8, 37.5%), and 3 (2/8, 25.0%) in the HFD + SW group (Figure 3b, 3c).

**Blood Lipid Profiles, Inflammatory Markers, and Mineral Toxicity**

In the serologic lipid profiles, the levels of total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were significantly higher in the HFD groups, irrespective of SW ingestion, than in the non-HFD groups (Control and SW), but were not different between the HFD and HFD + SW groups, or between the Control and SW groups (Table 2). The triglyceride (TG) levels were also not significantly different between the 4 groups (Table 2). All serum electrolytes were not significantly different between the 4 groups, except iron, which was significantly higher in the Control group than in the SW group (Table 2).
Expression of Bile Acid-Related Receptors in the Large Intestine

In the large intestine, the gene expression levels of bile acid-related receptors, including FXR, VDR, LXR-α, and GAPDH were not significantly different between the 4 groups.

Changes in gut Microbiota Associated With the Ingestion of SW’ in the Mice-Fed HFD

Using next-generation sequencing (NGS) of the gut microbiota, the levels of A. muciniphila, a species in the phylum Verrucomicrobia, were 100-fold higher in the HFD + SW group than in the HFD group (Figure 4). According to the gut microbiota analysis, the ratio of Bacteroides to Firmicutes was not different among the 4 groups as follows: Control (49.5/51.5, 0.96), HFD (42.5/60.0, 0.70), HFD + SW (39.0/56.0, 0.69), and SW (45.5/62.5, 0.72).

Discussion

T2DM is regarded as a disorder closely associated with obesity, hypertension, and hypercholesterolemia. In the present study, we propose that the ingestion of SW, a mixed MW, might prevent HFD-induced hyperglycemia and obesity in the HFD-induced mouse model, by altering the composition of gut microbiota. Although several clinical and animal studies have investigated the improvement of metabolic parameters associated with MW consumption, few studies have examined the effects of MW consumption on gut microbiota. One study found heavy growth of Christensenellaceae in the stool of HFD-fed mice having ingested SW. In the present study, we used SW, a type of MW obtained from the hot spring of Dukgu, Gyeongsangbuk-do, Republic of Korea. It is the most common type of SW in Korea, with a weakly alkaline character and some silicate. However, it also contains high amounts of minerals, such as lead (Pb), and fluoride (F), making it inappropriate for a drinking beverage due to its toxicity (Table 1). For usage as a daily beverage, it would be necessary to make it suitable for drinking to reduce toxicity. Although we could not check for Pb and F in the mice having ingested SW, no deaths of experimental mice occurred in this study, and the tested serological mineral levels were little different between the 4 groups, except for the elevation of serum iron observed in the Control group (Table 2). This result requires further investigation in the future.

In this study, the reduction in BW gain was significant in the HFD + SW group during the first 6 weeks. The BW gap between the HFD and HFD + SW groups was observed in the Control group (Table 2). This result requires further investigation in the future.

Table 2. Serological Parameters in Blood of Experimental Mice.

| Group     | Control | HFD | HFD + SW | SW | P value |
|-----------|---------|-----|----------|----|---------|
| LFT       | AST     | 23.5 ± 0.7 | 111.0 ± 29.3 | 97.4 ± 33.3 | 37.0 ± 6.4 | <.05** |
|           | ALT     | 12.5 ± 0.7 | 99.3 ± 27.6 | 81.9 ± 45.1 | 15.8 ± 2.8 | <.05** |
|           | ALP     | 92.0 ± 0.0 | 96.4 ± 16.1 | 95.6 ± 27.2 | 106.3 ± 8.3 | ns     |
|           | LDH     | 163.5 ± 171.8 | 517.1 ± 266.6 | 438.9 ± 114.2 | 116.0 ± 57.5 | <.05** |
| Lipid     | TC      | 94.0 ± 4.2 | 234.1 ± 24.9 | 207.4 ± 44.9 | 85.5 ± 13.1 | <.05** |
|           | TG      | 34.0 ± 5.7 | 43.8 ± 19.4 | 34.1 ± 6.1 | 32.0 ± 12.0 | ns     |
|           | HDL-C   | 60.0 ± 2.8 | 81.4 ± 5.5 | 77.1 ± 7.5 | 53.5 ± 4.8 | <.05*** |
|           | LDL-C   | 4.5 ± 0.7 | 16.6 ± 2.9 | 15.0 ± 5.5 | 2.8 ± 0.5 | <.05*** |
| DM markers (pg/mL) | Resistin | 5382.2 ± 2911.9 | 11375.6 ± 9745.3 | 7635.8 ± 5459.5 | 4074.9 ± 4751.8 | ns     |
|           | Insulin | 1004 ± 712 | 3515 ± 1843 | 2440 ± 1119 | 552. ± 223 | <.05**** |
|           | Leptin  | 2438 ± 1438 | 7910 ± 5993 | 6895 ± 5227 | 199 ± 109 | <.05**** |
|           | TNF-α   | 14.3 ± 6.1 | 17.5 ± 6.9 | 12.4 ± 4.4 | 20.9 ± 0.0 | ns     |
|           | MCP-1   | 57.5 ± 18.1 | 114.9 ± 46.3 | 92.1 ± 30.2 | 49.6 ± 33.9 | <.05*** |
| Electrolytes | Mg      | 25.4 ± 3.1 | 24.8 ± 2.1 | 23.2 ± 4.1 | 16.5 ± 3.8 | ns     |
|           | Fe      | 182.0 ± 7.1 | 162.1 ± 35.3 | 147.0 ± 40.1 | 116.5 ± 30.4 | <.05**** |
|           | Na      | 154.0 ± 1.4 | 153.1 ± 1.5 | 153.4 ± 1.8 | 151.8 ± 1.0 | ns     |
|           | K       | 9.2 ± 0.3 | 8.7 ± 0.6 | 9.0 ± 0.9 | 11.2 ± 1.4 | ns     |
|           | Cl      | 106.5 ± 0.7 | 107.6 ± 1.2 | 107.8 ± 1.0 | 109.3 ± 2.2 | ns     |
|           | CA      | 11.3 ± 0.6 | 11.5 ± 0.4 | 11.5 ± 0.2 | 10.9 ± 0.4 | ns     |

Abbreviations: ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; DM, diabetes mellitus; HFD, high-fat diet; HFD + SW, high-fat diet + spring water; HDL-C, high-density lipoprotein cholesterol; LFT, liver function test; LDL-C, low-density lipoprotein cholesterol; LDH, lactate dehydrogenase; MCP-1, monocyte chemoattractant protein-1; ns, nonspecific; SW, spring water; TC, total cholesterol; TG, triglyceride; TNF-α, tumor necrosis factor-α.

*P < 0.05: HFD + SW versus SW, **P < 0.05: HFD versus HFD + SW, ***P < 0.05: C versus HFD + SW, ****P < 0.05: C versus SW.
composition of the original SW by manipulating its composition would be necessary. Hence, SW ingestion may be regarded as a useful supplement in addition to the conventional management of obesity. Hitherto, there have been 3 different opinions regarding the influence of MW on the control of BW, where its consumption has been associated with weight gain, weight loss, and no change in weight. These conflicting results may result from the difference in mineral composition of the SW consumed. Further, these studies did not investigate the relationship between the change in weight and gut microbiota alteration.

In the Control, SW, and HFD + SW groups, the FBG levels showed a relatively narrow fluctuating pattern during the experimental period. The levels were maintained at ~100 to 200 mg/dL, whereas the FBG of the HFD + SW group showed a highly fluctuating value between 100 and 300 mg/dL. The OGTT performed on the last day of this experiment also revealed a higher AUC value in the HFD group than in the other 3 groups (P < 0.001) (Figure 2). These findings are consistent with previous reports, which demonstrated MW-associated weight loss and better homeostatic model assessment of insulin resistance (HOMA-IR) and OGTT. These results suggest the possibility that SW could be utilized as a tool for the stabilization of blood glucose by SW ingestion during the management of diabetes. Furthermore, these outcomes suggest that long-term intake of SW may prevent glucose intolerance or hyperglycemia in an HFD mouse model. The mechanism of lowering blood glucose may be associated with changes in the gut microbiota, and with the function of minerals, acting in a way similar to the way insulin transports glucose to cells. Other serological parameters related to diabetes such as insulin, leptin, and MCP-1, were significantly more elevated in the HFD group, regardless of SW ingestion. However, there was no significant difference between the HFD and HFD + SW groups. Furthermore, other serological parameters, including resistin, TNF-α, and IL-6, were not
some amount of silicate (32.7 mg/L). Even if there are few cholesterol levels.5,7,8,14,24 Bile acid metabolism is strongly associated with metabolic disorders since bile acid is necessary for the synthesis of cholesterol.25 The SW used in our study had some amount of silicate (32.7 mg/L). Even if there are few reports about the relation of silicate directly with glycemic control, silicate has the characteristic of a bile acid-binding property to decrease serum cholesterol as a primary precursor of bile acid.10,25-28 Thus, we quantified the amount of bile acid in the stool of mice ingesting SW, but there was no significant difference among the 4 groups (unpublished data). Further studies will be required to elucidate the mechanism for controlling blood glucose between silicate and bile acids and to elucidate the mechanism of SW for metabolic effects. Previous reports, with either vitamins or minerals, used in the management of metabolic syndromes, did not evaluate bile acid and gut microbiota of the stool.8 It was known that the gene expression associated with bile acid-related receptors in the intestine is related to bile acid circulation, cholesterol metabolism, and obesity.25,29 Thus, we examined the gene expression of bile acid-related receptors in the large intestine: FXR, VDR, and LXR-α. However, there was little difference in the expression of all the bile acid-related genes between the 4 groups.

We analyzed the gut microbiota composition to clarify the influence of SW ingestion on the population. Interestingly, we found heavy residence (approximately 100-fold higher) of A. muciniphila in the HFD + SW group compared with the HFD group (Figure 4). A. muciniphila is a Gram-negative, anaerobic bacterium that colonizes the mucus layer of the human gastrointestinal tract.30-33 Mucin is a mandatory factor for the growth of A. muciniphila, which was recently discovered in various studies to possess anti-obesity and anti-diabetic functions through the tightening of the mucosal barrier, making the barrier impermeable to commensal bacteria. It is presumed that large numbers of resident A. muciniphila might increase the factors acting against the metabolic syndrome indicated in this study. There have been few reports in association with MW ingestion, leading to the overgrowth of gut microbiota, specifically of Christensenellaceae species, which is related to lowering hyperglycemia.18 In this study the SW contained highly confluent minerals like Na, K, Ca, Mg, Cl, SO4, HCO3, Fe, Mn, and Zn, while Dukgu Oncheon SW possessed a high amount of F, Zn, and SiO2 (Table 1). The difference in mineral composition between the 2 SWs might have had an impact on the growth of a different type of microbiome. Purified tap water contains a scanty amount of Na, K, Ca, and some amount of Mg relative to SW, which did not make the serum level more elevated than that in the tap water groups (Table 2). Further studies are necessary to elucidate the mechanism of microbiota change in association with mineral components. Lastly, the proportion of Bacteroides to Firmicutes was not different between the HFD and HFD + SW groups. Further studies to possess anti-obesity and anti-diabetic functions will be required to elucidate the mechanism for controlling blood glucose between silicate and bile acids and to elucidate the mechanism of SW for metabolic effects.

As our results demonstrated, cholesterol levels with the exception of TG levels, were significantly lower in the non-HFD group than in the HFD group (Table 2). However, SW ingestion in the HFD group did not significantly decrease serum cholesterol levels. Previously, there have been conflicting positive or negative correlations between MW intake and cholesterol levels.5,7,8,14,24 Bile acid metabolism is strongly associated with metabolic disorders since bile acid is necessary for the synthesis of cholesterol.25 The SW used in our study had some amount of silicate (32.7 mg/L). Even if there are few reports about the relation of silicate directly with glycemic control, silicate has the characteristic of a bile acid-binding activity to decrease serum cholesterol as a primary precursor of bile acid.10,25-28 Thus, we quantified the amount of bile acid in the stool of mice ingesting SW, but there was no significant difference among the 4 groups (unpublished data). Further studies will be required to elucidate the mechanism for controlling blood glucose between silicate and bile acids and to elucidate the mechanism of SW for metabolic effects. Previous reports, with either vitamins or minerals, used in the management of metabolic syndromes, did not evaluate bile acid and gut microbiota of the stool.8 It was known that the gene expression associated with bile acid-related receptors in the intestine is related to bile acid circulation, cholesterol metabolism, and obesity.25,29 Thus, we examined the gene expression of bile acid-related receptors in the large intestine: FXR, VDR, and LXR-α. However, there was little difference in the expression of all the bile acid-related genes between the 4 groups.

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As our results demonstrated, cholesterol levels with the exception of TG levels, were significantly lower in the non-HFD group than in the HFD group (Table 2). However, SW ingestion in the HFD group did not significantly decrease serum cholesterol levels. Previously, there have been conflicting positive or negative correlations between MW intake and cholesterol levels.5,7,8,14,24 Bile acid metabolism is strongly associated with metabolic disorders since bile acid is necessary for the synthesis of cholesterol.25 The SW used in our study had some amount of silicate (32.7 mg/L). Even if there are few reports about the relation of silicate directly with glycemic control, silicate has the characteristic of a bile acid-binding activity to decrease serum cholesterol as a primary precursor of bile acid.10,25-28 Thus, we quantified the amount of bile acid in the stool of mice ingesting SW, but there was no significant difference among the 4 groups (unpublished data). Further studies will be required to elucidate the mechanism for controlling blood glucose between silicate and bile acids and to elucidate the mechanism of SW for metabolic effects. Previous reports, with either vitamins or minerals, used in the management of metabolic syndromes, did not evaluate bile acid and gut microbiota of the stool.8 It was known that the gene expression associated with bile acid-related receptors in the intestine is related to bile acid circulation, cholesterol metabolism, and obesity.25,29 Thus, we examined the gene expression of bile acid-related receptors in the large intestine: FXR, VDR, and LXR-α. However, there was little difference in the expression of all the bile acid-related genes between the 4 groups.

We analyzed the gut microbiota composition to clarify the influence of SW ingestion on the population. Interestingly, we found heavy residence (approximately 100-fold higher) of A. muciniphila in the HFD + SW group compared with the HFD group (Figure 4). A. muciniphila is a Gram-negative, anaerobic bacterium that colonizes the mucus layer of the human gastrointestinal tract.30-33 Mucin is a mandatory factor for the growth of A. muciniphila, which was recently discovered in various studies to possess anti-obesity and anti-diabetic functions through the tightening of the mucosal barrier, making the barrier impermeable to commensal bacteria. It is presumed that large numbers of resident A. muciniphila might increase the factors acting against the metabolic syndrome indicated in this study. There have been few reports in association with MW ingestion, leading to the overgrowth of gut microbiota, specifically of Christensenellaceae species, which is related to lowering hyperglycemia.18 In this study the SW contained highly confluent minerals like Na, K, Ca, Mg, Cl, SO4, HCO3, Fe, Mn, and Zn, while Dukgu Oncheon SW possessed a high amount of F, Zn, and SiO2 (Table 1). The difference in mineral composition between the 2 SWs might have had an impact on the growth of a different type of microbiome. Purified tap water contains a scanty amount of Na, K, Ca, and some amount of Mg relative to SW, which did not make the serum level more elevated than that in the tap water groups (Table 2). Further studies are necessary to elucidate the mechanism of microbiota change in association with mineral components. Lastly, the proportion of Bacteroides to Firmicutes was not different between the HFD and HFD + SW groups.

In conclusion, our study reports that long-term consumption of SW may have the potential to improve obesity and hyperglycemia as a result of the associated changes in the intestinal microbiome.

Author’s Contribution
E.J.K., T.S.S., H.H.C, H.Y.O., and S.H.P. performed the investigation. J.O.J., J.S.S., and S.S.C. performed analysis of gut microbial
composition. Y.C.C provided resources. E.J.K and H.S.C analyzed the data and wrote the first draft of the manuscript. T.S.S and H.S.C reviewed and edited the manuscript. H.K.L. and H.S.C. conceptualized and supervised the study and acquired funding. H.S.C. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for its integrity and the accuracy of the data analysis.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical Approval

This study was approved by the Committee of Animal Ethics of Uijeongbu St. Mary’s Hospital (UJA2017-06A).

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Informed Consent

Not applicable, because this article does not contain any studies with human or animal subjects.

Trial Registration

Not applicable, because this article does not contain any clinical trials.

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References

1. Lotfy M, Adeghte J, Kalasz H, Singh J, Adeghte E. Chronic complications of diabetes mellitus: a mini review. Curr Diabetes Rev. 2017;13(1):3-10. doi: 10.2174/157339981266151016101622
2. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature. 2006;444(7122):1027-1031. doi: 10.1038/nature05414
3. Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Microbial ecology: human gut microbes associated with obesity. Nature. 2006;444(7122):1022-1023. doi: 10.1038/4441022a
4. Cani PD, Bibiloni R, Knauf C, et al. Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. Diabetes. 2008;57(6):1470-1481. doi: 10.2337/db07-1403
5. Leeming ER, Louca P, Gibson R, Menni C, Spector TD, Le Roy CI. The complexities of the diet-microbiome relationship: advances and perspectives. Genome Med. 2021;13(1):10. doi: 10.1186/s13073-020-00813-7
6. Forslund K, Hildebrand F, Nielsen T, et al. Disentangling type 2 diabetes and metformin treatment signatures in the human gut microbiota. Nature. 2015;528(7581):262-266. doi: 10.1038/nature15766
7. Farvid MS, Siassi F, Jalali M, Hosseini M, Saadat N. The impact of vitamin and/or mineral supplementation on lipid profiles in type 2 diabetes. Diabetes Res Clin Pract. 2004;65(1):21-28. doi: 10.1016/j.diabres.2003.11.009
8. Gunasekara P, Hettiarachchi M, Liyanage C, Lekamwasam S. Effects of zinc and multiminer al vitamin supplementation on glycemic and lipid control in adult diabetes. Diabetes Metab Syndr Obes. 2011;4:53-60. doi: 10.2147/DSMO.S16691
9. Siddiqui K, Bawazeer N, Joy SS. Variation in macro and trace elements in progression of type 2 diabetes. ScientificWorldJournal. 2014;2014:461591. doi: 10.1155/2014/461591
10. Quatrini S, Pampaloni B, Brandi ML. Natural mineral waters: chemical characteristics and health effects. Clin Cases Miner Bone Metab. 2016;13(3):173-180. doi: 10.11138/ccmbm/2016.13.3.173
11. Naumann J, Biehler D, Lüty T, Sadagiani C. Prevention and therapy of type 2 diabetes—what is the potential of daily water intake and its mineral nutrients? Nutrients. 2017;9(8):914. doi: 10.3390/nu9080914
12. Desmarchelier C, Borel P, Lairon D, Maraninchi M, Valéro R. Effect of nutrient and micronutrient intake on chylomicron production and postprandial lipemia. Nutrients. 2019;11(6):1299. doi: 10.3390/nu11061299
13. Pereira CD, Severo M, Araújo JR, et al. Relevance of a hypersaline sodium-rich naturally sparkling mineral water to the protection against metabolic syndrome induction in fructose-fed Sprague-Dawley rats: a biochemical, metabolic, and redox approach. Int J Endocrinol. 2014;2014:384583. doi: 10.1155/2014/384583
14. Costa-Vieira D, Monteiro R, Martins MJ. Metabolic syndrome features: is there a modulation role by mineral water consumption? A review. Nutrients. 2019;11(5):1141. doi: 10.3390/nu11051141
15. Murakami S, Goto Y, Ito K, et al. The consumption of bicarbonate-rich mineral water improves glycemic control. Environ Health Insights. 2014;2014:824395. doi: 10.1155/2014/824395
16. Lee CM, Hamm SY, Lee CW, Choi SJ, Chung SY. Characteristics of South Korea’s geothermal water in relation to its geological and geochemical feature. J Soil Groundw Environ. 2014;19(2):25-37. doi: 10.7857/JSGE.2014.19.2.025
17. Bedossa P. Pathology of non-alcoholic fatty liver disease. Liver Int. 2017;37(suppl 1):85-89. doi: 10.1111/liv.13301
18. El-Sewify MM, Sadik NA, Shaker OG. Role of sulfurous mineral water and sodium hydrosulfide as potent inhibitors of fibrosis in the heart of diabetic rats. Arch Biochem Biophys. 2011;506(1):48-57. doi: 10.1016/j.abb.2010.10.014
19. Corradini SG, Ferri F, Mordenti M, et al. Beneficial effect of sulphate-bicarbonate-calcium water on gallstone risk and weight control. World J Gastroenterol. 2012;18(9):930-937. doi: 10.3748/wjg.v18.i9.930
20. Schoppen S, Pérez-Granados AM, Carbajal A, et al. A sodium-rich carbonated mineral water reduces cardiovascular risk in postmenopausal women. J Nutr. 2004;134(5):1058-1063. doi: 10.1093/jn/134.5.1058
21. Obici S, Rossetti L. Minireview: nutrient sensing and the regulation of insulin action and energy balance. *Endocrinology.* 2003;144(12):5172-5178. doi: 10.1210/en.2003-0999

22. Newsholme P, Cruzat V, Arfuso F, Keane K. Nutrient regulation of insulin secretion and action. *J Endocrinol.* 2014;221(3):R105-R120. doi: 10.1530/JOE-13-0616

23. Zabolotnaya IB. [The prospects for the application of the natural and preformed therapeutic factors in the treatment of non-alcoholic fatty liver disease]. *Vopr Kurortol Fizioter Lech Fiz Kult.* 2016;93(4):42-48. doi: 10.17116/kurort2016442-48

24. Aslanabadi N, Habibi Asl B, Bakhshalizadeh B, Ghaderi F, Nemati M. Hypolipidemic activity of a natural mineral water rich in calcium, magnesium, and bicarbonate in hyperlipidemic adults. *Adv Pharm Bull.* 2014;4(3):303-307. doi: 10.5681/apb.2014.044

25. Ma H, Patti ME. Bile acids, obesity, and the metabolic syndrome. *Best Pract Res Clin Gastroenterol.* 2014;28(4):573-583. doi: 10.1016/j.bpg.2014.07.004

26. Peluso MR, Schneeman BO. A food-grade silicon dioxide is hypolipidemic in the diet of cholesterol-fed rats. *J Nutr.* 1994;124(6):853-860. doi: 10.1093/jn/124.6.853

27. Belyakova LA, Besarab LN, Roik NV, et al. Designing of the centers for adsorption of bile acids on a silica surface. *J Colloid Interface Sci.* 2006;294(1):11-20. doi: 10.1016/j.jcis.2005.06.081

28. Hansen M, Sonne DP, Mikkelsen KH, Glaud LL, Vilsbøll T, Knop FK. Bile acid sequestrants for glycemic control in patients with type 2 diabetes: a systematic review with meta-analysis of randomized controlled trials. *J Diabetes Complications.* 2017;31(5):918-927. doi: 10.1016/j.jdiacomp.2017.01.011

29. Schaap FG, Trauner M, Jansen PL. Bile acid receptors as targets for drug development. *Nat Rev Gastroenterol Hepatol.* 2014;11(1):55-67. doi: 10.1038/nrgastro.2013.151

30. Li J, Lin S, Vanhoutte PM, Woo CW, Xu A. *Akkermansia muciniphila* protects against atherosclerosis by preventing metabolic endotoxemia-induced inflammation in Apoe−/− mice. *Circulation.* 2016;133(24):2434-2446. doi: 10.1161/CIRCULATIONAHA.115.019645

31. Ottman N, Huuskonen L, Reunanen J, et al. Characterization of outer membrane proteome of *Akkermansia muciniphila* reveals sets of novel proteins exposed to the human intestine. *Front Microbiol.* 2016;7:1157. doi: 10.3389/fmicb.2016.01157

32. Shen W, Shen M, Zhao X, et al. Antiobesity effect of capsaicin in mice fed with high-fat diet is associated with an increase in population of the gut bacterium *Akkermansia muciniphila*. *Front Microbiol.* 2017;8(272):272. doi: 10.3389/fmicb.2017.00272

33. Derrien M, Vaughan EE, Plugge CM, de Vos WM. *Akkermansia muciniphila* gen nov, sp nov, a human intestinal mucin-degrading bacterium. *Int J Syst Evol Microbiol.* 2004;54(5):1469-1476. doi: 10.1099/ijsem.0.02873-0