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Article

Deep Sea Water Modulates Blood Pressure and Exhibits Hypolipidemic Effects via the AMPK-ACC Pathway: An *in Vivo* Study

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Abstract: Deep sea water (DSW), originally pumped from the Pacific Rim off the coast of Hualien County (Taiwan), and its mineral constituents, were concentrated by a low-temperature vacuum evaporation system to produce a hardness of approximately 400,000 mg/L of seawater mineral concentrate. The primary composition of this seawater mineral concentrate was ionic magnesium (Mg^{2+}), which was approximately 96,000 mg/L. Referring to the human recommended daily allowance (RDA) of magnesium, we diluted the mineral concentrate to three different dosages: 0.1 × DSW (equivalent to 3.75 mg Mg^{2+}/kg DSW); 1 × DSW (equivalent to 37.5 mg Mg^{2+}/kg DSW); and 2 × DSW (equivalent to 75 mg Mg^{2+}/kg DSW). Additionally, a magnesium chloride treatment was conducted for comparison with the DSW supplement. The study indicated that 0.1 × DSW, 1 × DSW and 2 × DSW decreased the systolic and diastolic pressures in spontaneous hypertensive rats in an eight-week experiment. DSW has been shown to reduce serum lipids and prevent atherogenesis in a hypercholesterolemic rabbit model. Our results demonstrated that 1 × DSW

and 2 × DSW significantly suppressed the serum cholesterol levels, reduced the lipid accumulation in liver tissues, and limited aortic fatty streaks. These findings indicated that the antiatherogenic effects of DSW are associated with 5′-adenosine monophosphate-activated protein kinase (AMPK) stimulation and the consequent inhibition of phosphorylation of acetyl-CoA carboxylase (ACC) in atherosclerotic rabbits. We hypothesize that DSW could potentially be used as drinking water because it modulates blood pressure, reduces lipids, and prevents atherogenesis.

Keywords: deep sea water; atherosclerosis; HMG-CoA reductase; AMP-activated protein kinase; acetyl-CoA carboxylase

1. Introduction

Approximately 50% of all deaths from cardiovascular diseases (CVD) in Western countries are due to coronary heart disease (CHD), and the primary cause of CHD is atherosclerosis. It is hypothesized that atherosclerosis begins when the endothelium becomes damaged, thereby allowing low-density lipoprotein cholesterol (LDL-C) to accumulate on the artery wall. Subsequently, lipid accumulation, foam cell development, and vascular smooth muscle cell proliferation occur, and finally, the arteries become narrowed and hardened [1]. Hypercholesterolemia and high levels of LDL cholesterol are two important stimuli that regulate the pathogenesis of atherosclerosis [1,2]. Steinberg *et al*. demonstrated that oxidized LDL (oxLDL) is a key element involved in atherosclerotic plaque formation and atherogenicity [3]. When oxLDL is uptaken by vascular scavenger receptors, the transformation of macrophages into foam cells is triggered in atherosclerotic lesions [4]. A strong correlation between hypertension and CHD is widely under investigation. Several pathophysiologic mechanisms link both hypertension and CHD. Hypertension induces endothelial dysfunction, exacerbates the atherosclerotic process and contributes to make the atherosclerotic plaque more unstable [5].

An effective and safe drug to hypercholesterolemia would be beneficial for the prevention of atherosclerosis. Clinically, HMG-CoA reductase (HMGCR) inhibitors (*i.e.*, statins) are commonly prescribed for hyperlipidemia. Statins reduce the incidence of CHD by 23%–34% and mortality by 20%–42% [6]. Because there are limited cases of statin-induced rhabdomyolysis, it is essential to develop new and safer lipid-lowering agents.

5′-Adenosine monophosphate-activated protein kinase (AMPK) plays an important role in regulating the different phases of intermediary metabolism, which include glucose transport, gluconeogenesis, glycogenolysis, lipolysis, and sterol synthesis [7,8]. AMPK, a key cellular sensor for energy homeostasis, consists of a catalytic subunit (α) and two regulatory subunits (β, γ), which bind to form a functional kinase [9]. AMPK is activated by metabolic stress or exercise that reduces cellular energy levels, which indicates an increase in the AMP/ATP ratio due to the depletion of ATP. Therefore, AMPK advances the ATP-generating pathway and reduces the ATP-consuming pathway [10]. Under fasting conditions, the activated AMPK regulates several intracellular metabolic systems to generate energy or reduce energy depletion. Predominantly, the metabolic modifications include the acceleration of lipid catabolism via the suppression of ACC [11], the inhibition of cholesterol synthesis via the

depressed activity of HMGCR [12], and decreased fatty acid *de novo* biosynthesis via the suppression of fatty acid synthase [13]. For that reason, AMPK protein may be considered to be a pharmacological target for the management of hyperlipidemia or atherosclerosis.

Characterized by its clarity, sanitary quality, plentiful nutrients, particularly rich in ionic magnesium (Mg^{2+}), calcium (Ca^{2+}), and potassium (K^{+}), deep sea water (DSW) has received attention for its potential in various disease treatments, including attenuation of hyperlipidemia, atherosclerosis [14], hypertension [15], and dermatitis syndrome [16]. Recently, DSW has been widely investigated for its therapeutic or preventive effects in CVD. DSW has demonstrated its efficacy on lowering total cholesterol (TC) and LDL-C levels in hypercholesterolemic human subjects [14]. DSW administered in hypercholesterolemic rabbits exhibited lipid-lowering effects [17,18].

Epidemiological studies have demonstrated that the serum Mg^{2+} level is inversely correlated with the formation of atherosclerosis [19]. The Mg^{2+} supplement lowers the serum cholesterol and triglyceride levels and attenuates the atherosclerotic process in rabbits that are fed a high cholesterol diet [20,21]. These research studies indicated that dietary Mg^{2+} prevents the development of atherosclerosis in cholesterol-fed rabbits by inhibiting the lipid accumulation in the aortic wall [22]. Another study by Kishimoto *et al.* indicated that the Mg^{2+} supplement could inhibit fat absorption and improve postprandial hyperlipidemia in healthy subjects [23]. The co-administration of Ca^{2+} and Mg^{2+} significantly enhanced the cholesterol-lowering effects from plant sterols [24]. However, the Mg supplement appears to only produce a small, but clinically significant, reduction in blood pressure. A future prospective, large-scale, randomized trial should be conducted to further explore these results [25].

The purpose of the present study was to assess whether DSW from the Pacific Rim off of Hualien County could modulate systolic artery pressure (SAP) and diastolic artery pressure (DAP), reduce serum lipid levels, and prevent atherosclerosis formation. This study also investigated whether the molecular mechanisms underlying the lipid-lowering effects of DSW are associated with the AMPK-ACC pathway activation.

2. Results and Discussion

2.1. Body Weight Changes in Spontaneous Hypertensive Rats (SHRs)

Table 1 shows the changes in body weights of spontaneous hypertensive rats (SHRs) in the control, Lasix, 10% $MgCl_2$, and DSW-treated groups. The body weights increased by approximately 189 g, 179 g, and 183 g after the administration with 0.1 × DSW, 1 × DSW, and 2 × DSW, respectively (equivalent to 3.75 mg/kg, 37.5 mg/kg, and 75 mg/kg Mg content). There were no significant differences in body weights between the DSW-treated and the normal control groups after the four- and eight-week study.

Table 1. Changes in body weights of spontaneous hypertensive rats in the control, Lasix, 10% $MgCl_2$, and deep sea water (DSW)-treated groups. * $p < 0.05$ compared to week 0.

Groups	Week 0 (g)	Week 2 (g)	Week 4 (g)	Week 8 (g)
Control	133 ± 10	189 ± 7 *	269 ± 9 *	300 ± 11 *
10 mg/mL, Lasix	131 ± 6	178 ± 12 *	261 ± 7 *	326 ± 5 *
10% $MgCl_2$	122 ± 13	167 ± 8 *	255 ± 17 *	312 ± 22 *
0.1 × DSW (3.75 mg/kg/day)	131 ± 4	175 ± 9 *	260 ± 10 *	320 ± 14 *
1 × DSW (37.5 mg/kg/day)	133 ± 8	179 ± 18 *	264 ± 15 *	312 ± 13 *
2 × DSW (75 mg/kg/day)	130 ± 7	167 ± 4 *	255 ± 2 *	313 ± 8 *

2.2. DSW Lowers the Blood Pressures of Spontaneous Hypertensive Rats

Tables 2 and 3 show the SAP and DAP in the SHRs of control, Lasix, 10% $MgCl_2$, and DSW-treated groups. The SAP and DAP were significantly lower in the 1 × DSW- and 2 × DSW-treated groups than in the control group at the end of the four-week treatment. However, the SAP and DAP were significantly lower even at the lowest concentration (0.1 × DSW) than in the control group in the eight-week study. Additionally, our results demonstrated that 10% $MgCl_2$ significantly lowered the SAP and DAP in SHRs. Previous studies have indicated that feeding DSW pumped from Cape Muroto (Kochi Prefecture, Japan) demonstrated a preventive effect on mild hypertension in Kurosawa and Kusanagi-Hypercholesterolemic rabbits [15]. Our results, similarly, showed that DSW pumped from the Pacific Rim off the Hualien County (Hualien County, Taiwan) exhibited effects of lowering the SAP (Table 2) and DAP (Table 3) in SHRs. An earlier study indicated that Mg supplement might lower blood pressure by suppressing the adrenergic activity and, likely, natriuresis [26]. Another study showed that Mg^{2+} supplement corrected hypertension in mineralocorticoid-salt hypertensive animals by reducing the vascular tone [27].

Table 2. SAP changes in spontaneous hypertensive rats administered with Lasix, 10% $MgCl_2$, and DSW-treated groups. * $p < 0.05$ compared to week 0; $^{\#}$ $p < 0.05$ compared to the control group in the same time point.

Groups	Week 0 (mm Hg)	Week 2 (mm Hg)	Week 4 (mm Hg)	Week 8 (mm Hg)
Control	127 ± 6	164 ± 8 *	211 ± 8 *	244 ± 22 *
10 mg/mL, Lasix	134 ± 10	162 ± 17 *	193 ± 4 *	178 ± 14 *,#
10% $MgCl_2$	135 ± 9	165 ± 11 *	181 ± 12 *,#	181 ± 3 *,#
0.1 × DSW (3.75 mg/kg/day)	131 ± 15	174 ± 7 *	226 ± 7 *	188 ± 12 *,#
1 × DSW (37.5 mg/kg/day)	125 ± 14	180 ± 8 *	176 ± 7 *,#	156 ± 16 *,#
2 × DSW (75 mg/kg/day)	126 ± 11	173 ± 7 *	162 ± 6 *,#	171 ± 18 *,#

Table 3. DAP changes in spontaneous hypertensive rats from treatments administered to the control, Lasix, 10% $MgCl_2$, and DSW-treated groups. * $p < 0.05$ compared to week 0; # $p < 0.05$ compared to the control group in the same time point.

Groups	Week 0 (mm Hg)	Week 2 (mm Hg)	Week 4 (mm Hg)	Week 8 (mm Hg)
Control	81 ± 8	137 ± 9 *	150 ± 11 *	177 ± 19 *
10 mg/mL, Lasix	84 ± 12	138 ± 10 *	129 ± 5 *	129 ± 14 *,#
10% $MgCl_2$	87 ± 11	115 ± 12 *	107 ± 13 *,#	124 ± 15 *,#
0.1 × DSW (3.75 mg/kg/day)	92 ± 12	132 ± 6 *	143 ± 10 *	121 ± 21 *,#
1 × DSW (37.5 mg/kg/day)	82 ± 6	128 ± 7 *	105 ± 5 *,#	112 ± 7 *,#
2 × DSW (75 mg/kg/day)	91 ± 9	134 ± 9 *	103 ± 7 *,#	120 ± 10 *,#

2.3. Body Weight Changes in New Zealand White Rabbits

Table 4 presents the changes in the body weights of rabbits in the control, 0.5% cholesterol, 0.01% lovastatin (Lova), 10% $MgCl_2$, and DSW-treated groups. The body weights increased by approximately 0.97 kg, 0.82 kg, and 0.82 kg after the administration of 0.1 × DSW, 1 × DSW, and 2 × DSW, respectively. There were no significant differences in the body weights between the DSW-treated and the normal control groups after eight-week studies.

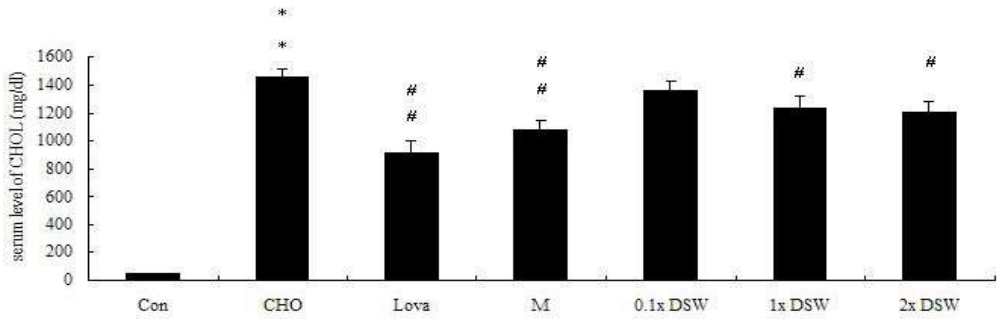
Table 4. Changes in the body weights of rabbits in the control, 0.5% cholesterol, 0.01% lovastatin, 10% $MgCl_2$, and DSW-treated groups.

Group	Control	0.5% Cholesterol	0.01% Lovastatin	10% $MgCl_2$	0.1 × DSW	1 × DSW	2 × DSW
Initial weight (kg)	2.37 ± 0.54	2.47 ± 0.48	2.46 ± 0.50	2.65 ± 0.32	2.21 ± 0.65	2.17 ± 0.46	2.23 ± 0.41
Final weight (kg)	3.12 ± 0.33	3.19 ± 0.20	3.02 ± 0.34	3.23 ± 0.22	3.18 ± 0.48	2.99 ± 0.33	3.05 ± 0.25

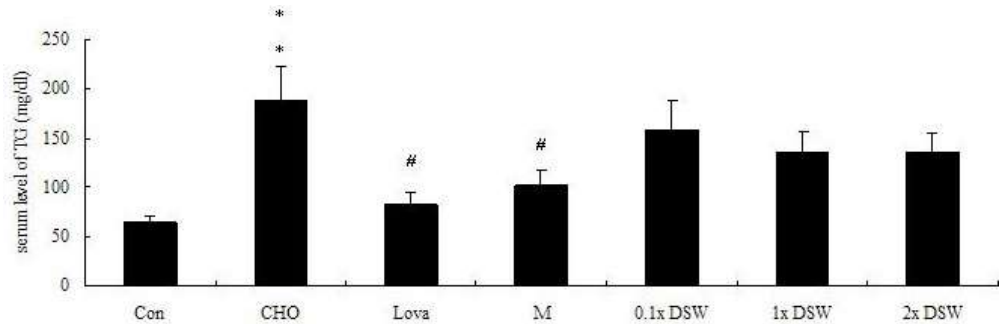
2.4. Down-Regulatory Effect of Deep Sea Water (DSW) on Serum Total Cholesterol (TC)

The serum chemical parameters were examined to assess whether DSW could reduce the serum lipid profiles. Our results indicated that the plasma TC, triglyceride (TG), and LDL-C levels were improved after eight weeks of a 0.5% cholesterol diet (Figure 1a–c). In total, 1 × DSW, and 2 × DSW significantly improved the TC level by 1.18-, and 1.21-fold, respectively. The results have been consistent with findings in other studies [28,29], and the results were similar to the effects in the $MgCl_2$ and lovastatin groups (Figure 1a).

Figure 1. Serum chemical parameters were calculated in the high-fat-fed rabbit model (0.5% cholesterol) after an eight-week experiment. Control group (Con), 0.5% cholesterol diet (CHO), 0.5% cholesterol diet with 0.01% lovastatin (Lova), 0.5% cholesterol diet with a 10% $MgCl_2$ (M), 0.5% cholesterol diet with 0.1 × DSW (0.1 × DSW), 0.5% cholesterol diet with 1 × DSW (1 × DSW), and 0.5% cholesterol diet with 2 × DSW (2 × DSW). CHOL, total cholesterol (**A**); TG, triglyceride (**B**); HDL, high-density lipoprotein (**C**); LDL, low-density lipoprotein (**D**). * $p < 0.05$; ** $p < 0.01$ compared to the control group; $^{\#}$ $p < 0.05$; $^{\#\#}$ $p < 0.01$ compared to the cholesterol group.



(**A**)



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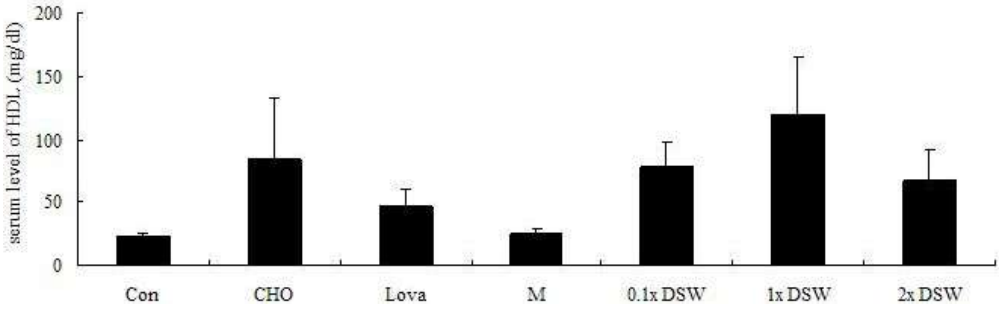

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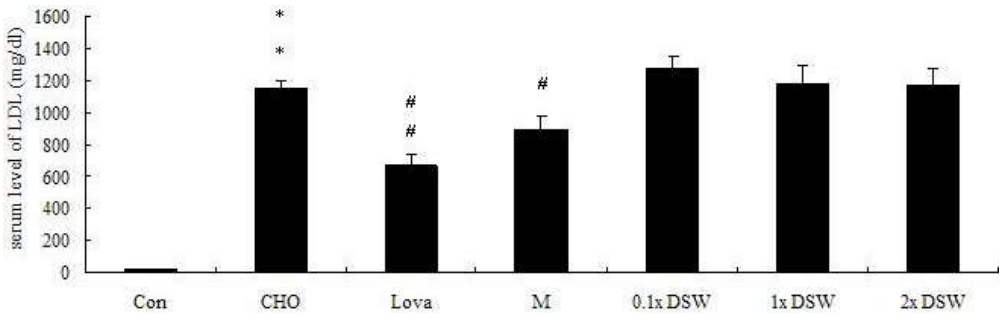


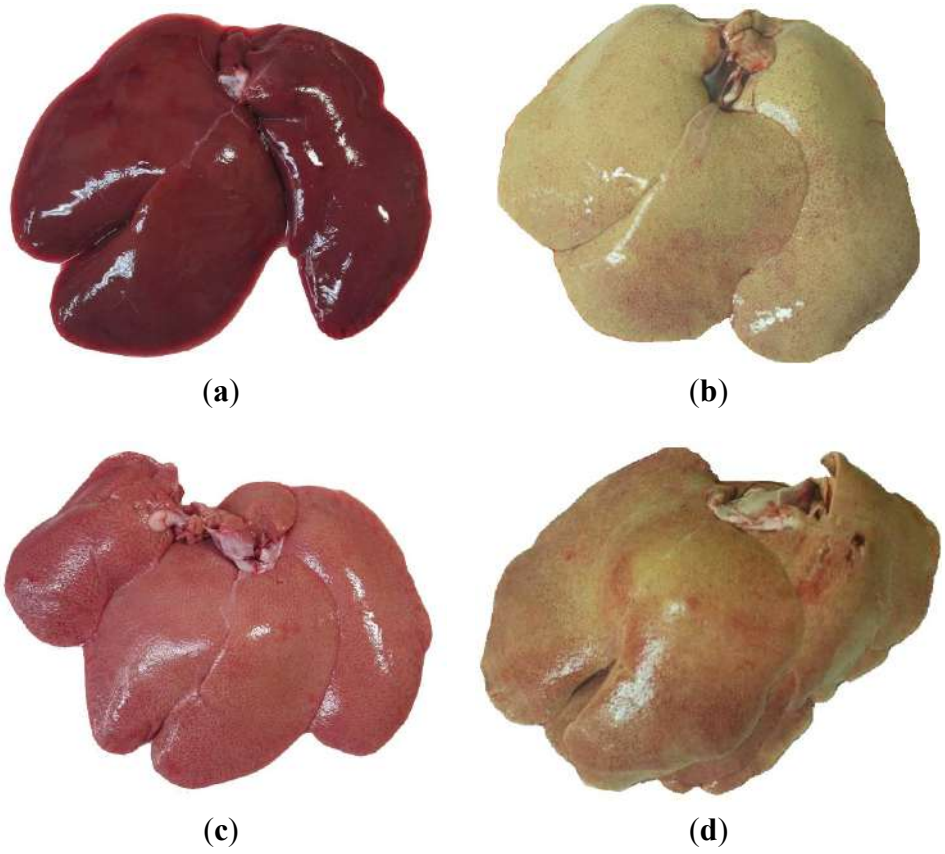
(**D**)

Statins have been used in large randomized trials targeting lipid-lowering therapy and reduced risk of cardiovascular death, non-fatal MI, and stroke [30]. Statins inhibit HMGCR, thus reducing the production of cholesterol in the liver and up-regulating the LDL receptors to uptake LDL-C into the liver. Additionally, the Mg supplement decreases the levels of aortic cholesterol, and particularly cholesteryl ester in mice [31].

2.5. Down-Regulatory Effect of DSW on Fatty Liver Status and Lipid Accumulation

A histopathological analysis of liver cryosections demonstrated that 0.5% cholesterol diet induced a phenomena-like fatty liver after eight-week administration (Figure 2b). Remarkably, 1 × DSW, and 2 × DSW reduced the severity of cholesterol diet-induced fatty liver (Figure 2f,g). This result is consistent with the result from the $MgCl_2$ group (Figure 2d). A 0.5% cholesterol diet increased oil droplets accumulation within the liver tissue (Figure 3b); this effect was markedly reversed by 1 × DSW and 2 × DSW supplement (Figure 3f,g), which was similar to the results of the $MgCl_2$ group (Figure 3d).

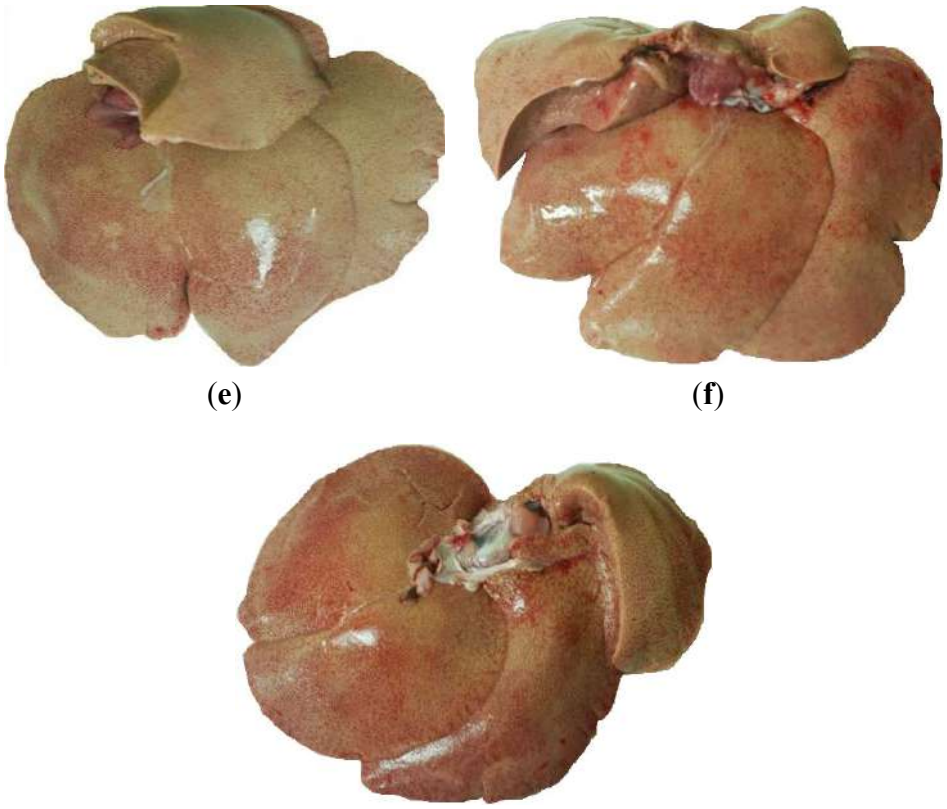
Figure 2. Photographs of liver appearance in the high-fat-fed rabbit model (0.5% cholesterol) after an eight-week study. (**a**) Control group; (**b**) 0.5% cholesterol diet; (**c**) 0.5% cholesterol diet with 0.01% lovastatin; (**d**) 0.5% cholesterol diet with a 10% $MgCl_2$; (**e**) 0.5% cholesterol diet with 0.1 × DSW; (**f**) 0.5% cholesterol diet with 1 × DSW; and (**g**) 0.5% cholesterol diet with 2 × DSW.



(**a**) (**b**)

(**c**) (**d**)

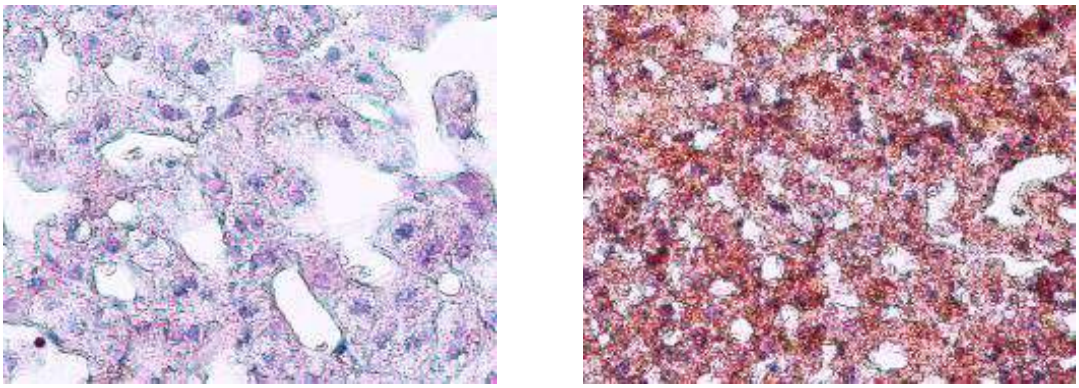
Figure 2. *Cont.*



(**e**) (**f**)

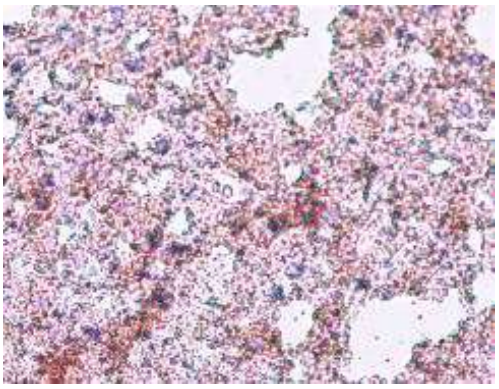
(**g**)

Figure 3. Histopathochemical examination of liver tissues in the hypercholesterolemic rabbit model after the eight-week study. (**a**) Control group (Con); (**b**) 0.5% cholesterol diet (CHO); (**c**) 0.5% cholesterol diet with 0.01% lovastatin (Lova); (**d**) 0.5% cholesterol diet with a 10% $MgCl_2$ (M); (**e**) 0.5% cholesterol diet with 0.1 × DSW (0.1 × DSW); (**f**) 0.5% cholesterol diet with 1 × DSW (1 × DSW); (**g**) 0.5% cholesterol diet with 2 × DSW (2 × DSW); (**h**) densitometric analyses of (**a**–**g**). ** $p < 0.01$ compared to the control group; $^{\#\#}$ $p < 0.01$ compared to the cholesterol group.



(**a**) (**b**)

Figure 3. *Cont.*



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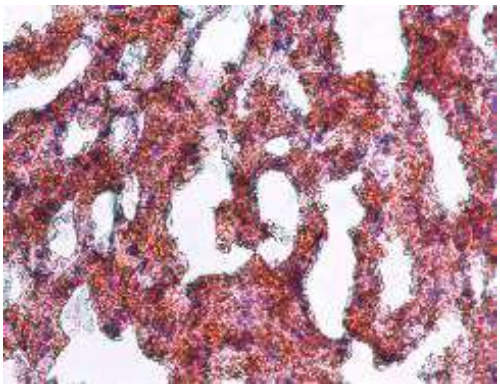

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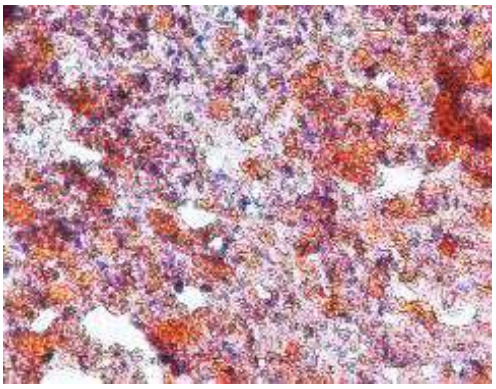
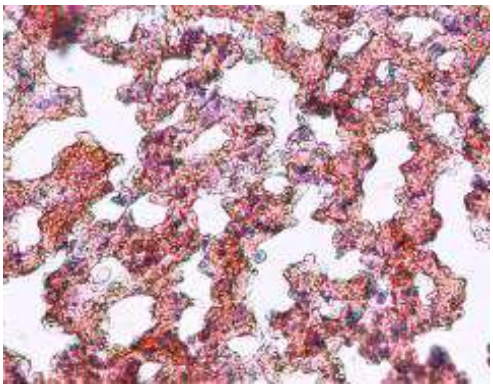
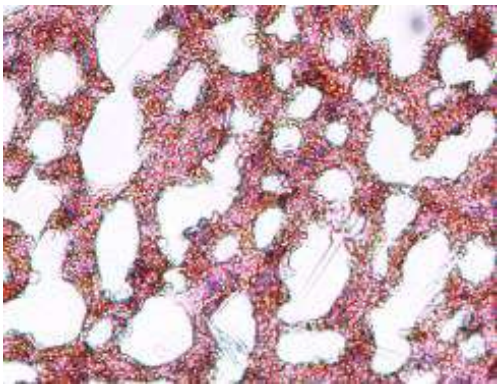


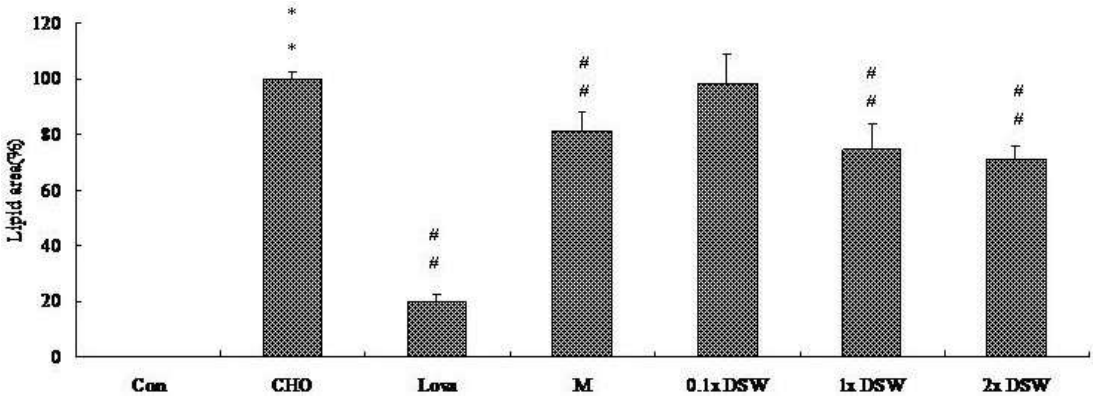
(**e**)



(**f**)



(**g**)



(**h**)

2.6. Down-Regulatory Effect of DSW on Fatty Streak Lesions

Previously, Pan *et al.* reported the methods for the quantification of atherosclerotic lesions in the aortic root [29]. To determine the effect of DSW on the fatty streak formation, six-week-old male New Zealand white rabbits were fed with the 0.5% cholesterol diet for eight weeks. The fatty streak lesions stained with Sudan IV in the aorta root were used to determine if DSW could reduce the formation of atherosclerosis plaque (Figure 4). This study demonstrated that a 0.5% cholesterol diet considerably increased the aortic fatty streak lesions compared to the control group (Figure 4b). Additionally, 37.5 mg/kg and 75 mg/kg of DSW significantly reduced the intensity of the fatty streaks on the aorta intima compared to the CHO group (Figure 4f,g), which was similar to the $MgCl_2$ group (Figure 4d). This study supports the hypothesis that inadequate intake of Mg results in an increase in atherosclerotic plaque development in rabbits [32].

Figure 4. Histopathochemical examination of aortic fatty streak lesions in the hypercholesterolemic rabbit model after the eight-week study. (**a**) Control group (Con); (**b**) 0.5% cholesterol diet (CHO); (**c**) 0.5% cholesterol diet with 0.01% lovastatin (Lova); (**d**) 0.5% cholesterol diet with a 10% $MgCl_2$ (M); (**e**) 0.5% cholesterol diet with 0.1 × DSW (0.1 × DSW); (**f**) 0.5% cholesterol diet with 1 × DSW (1 × DSW); (**g**) 0.5% cholesterol diet with 2 × DSW (2 × DSW); (**h**) densitometric analyses of (**a**–**g**). ** $p < 0.01$ compared to the control group; $^{\#\#}$ $p < 0.01$ compared to the cholesterol group.

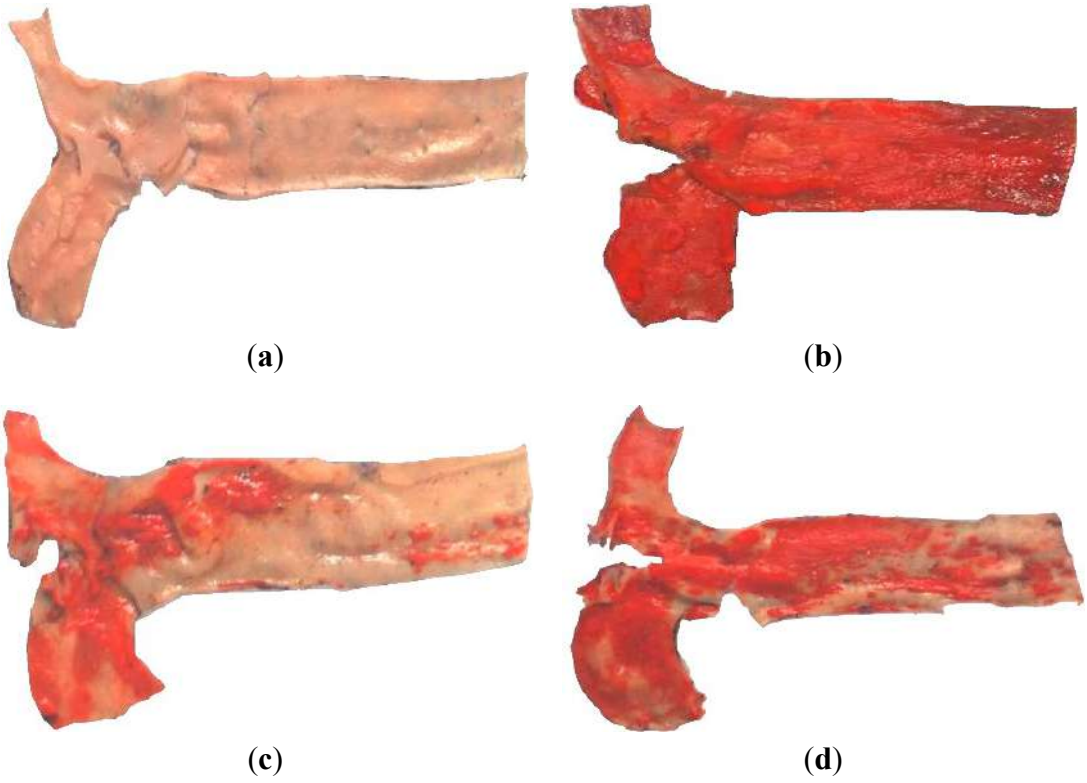
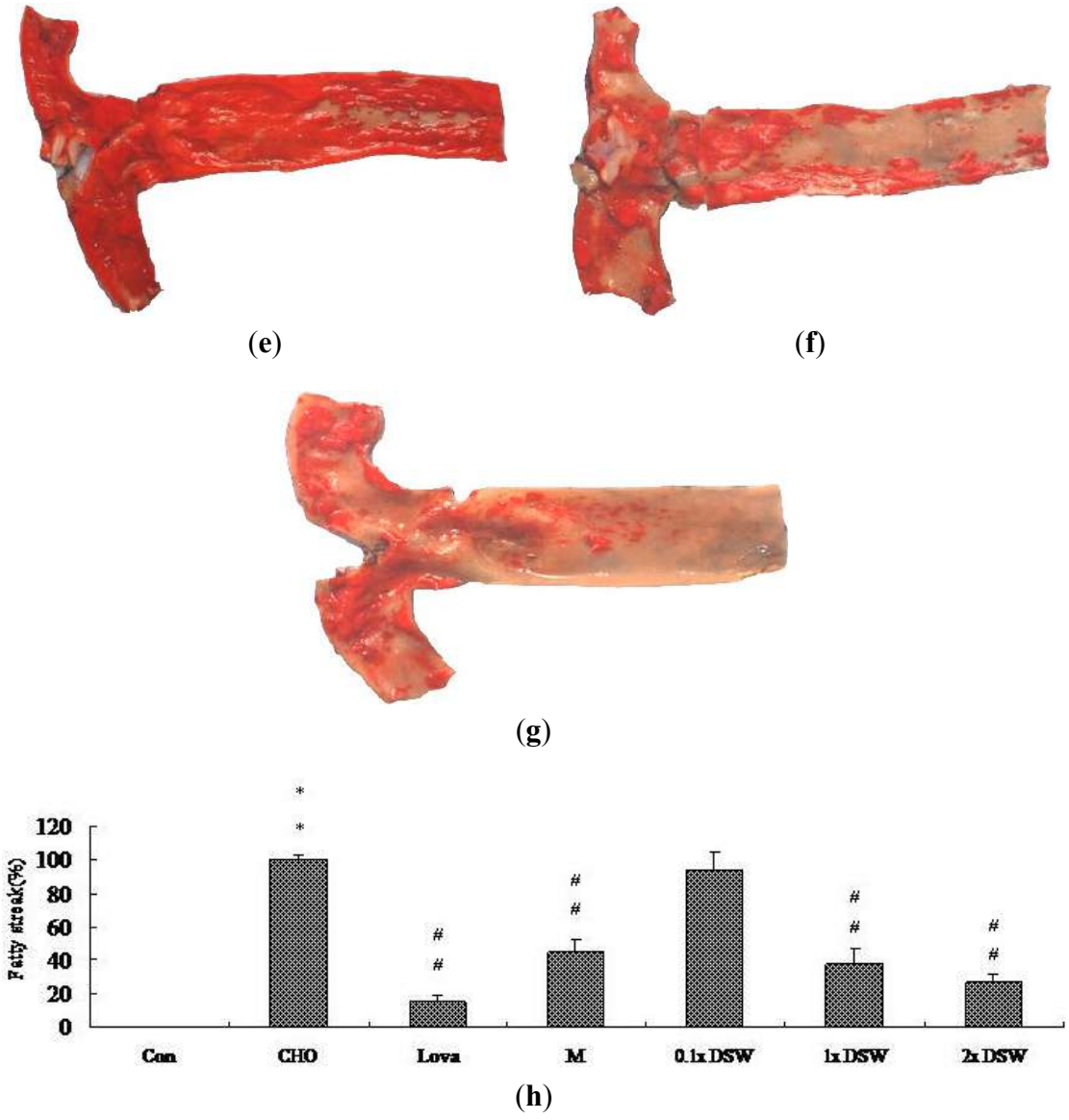


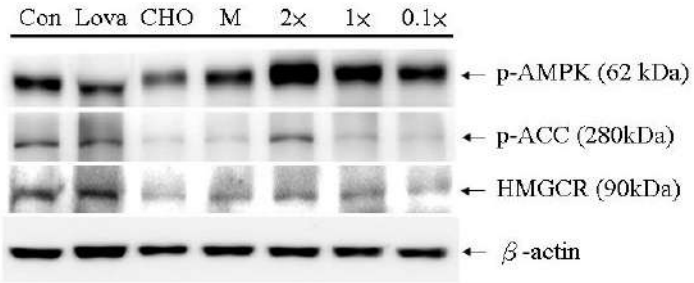
Figure 4. *Cont.*



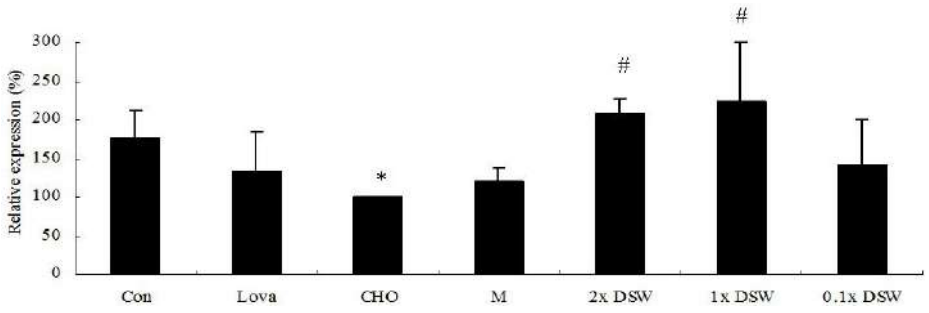
2.7. Lipid-Modulating Effect of DSW and Lipid Metabolism-Associated Proteins

To determine how DSW affects the lipid-lowering effects, we investigated lipid metabolism-associated proteins, such as AMPK, ACC, and HMGCR. Our results demonstrated that the protein expression of AMPK phosphorylation, ACC phosphorylation, and HMGCR were significantly decreased after an eight-week treatment with the 0.5% (w/w) cholesterol diet (Figure 5). The addition of 1 × DSW and 2 × DSW returned the expression of these proteins to near basal levels compared to the cholesterol group, which showed a similar result as in the lovastatin group.

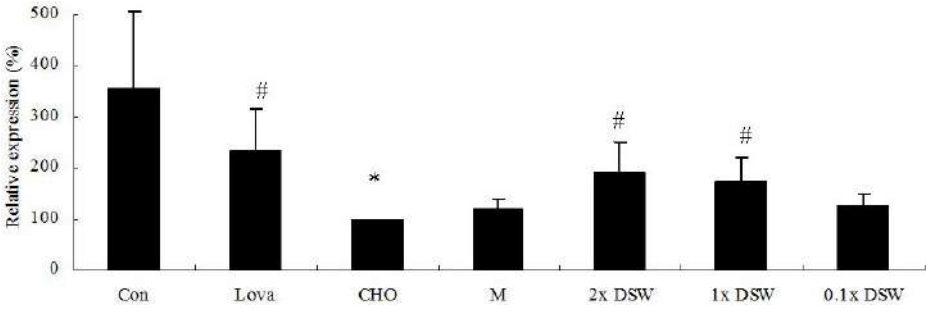
Figure 5. Protein expression of lipid metabolism associated molecules in the hypercholesterolemic rabbit model (n = 8 per group) after the eight-week study. (**A**) Control group (Con); 0.5% cholesterol diet (CHO); 0.5% cholesterol diet with 0.01% lovastatin (Lova); 0.5% cholesterol diet with a 10% $MgCl_2$ (M); 0.5% cholesterol diet with 0.1 × DSW (0.1 × DSW); 0.5% cholesterol diet with 1 × DSW (1 × DSW); and 0.5% cholesterol diet with 2 × DSW (2 × DSW); (**B**) densitometric analyses of (**A**). * $p < 0.05$ compared to the control group; # $p < 0.05$ compared to the cholesterol group.



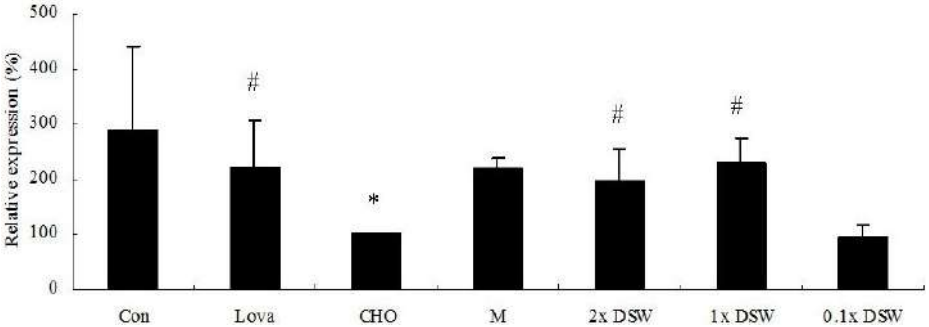
(**A**)



p-AMPKα



p-ACC



HMGCR

(**B**)

Our results first demonstrated that DSW might lower the lipid profiles in the rabbits via the AMPK-ACC pathways (Figure 5). AMPK plays an important role in lipid metabolism via the inhibition of HMGCR and ACC, thereby leading to fatty acid oxidation and inhibiting the production of cholesterol. Many studies have indicated that modulating the AMPK pathways significantly affects lipid metabolism. Platycodin D, saponins from the roots of *Platycodon grandiflorum*, increased the phosphorylation of AMPK and ACC in high-fat-diet-fed rats and activated AMPK via SIRT1/CaMKKβ in HepG2 cells, which was verified by the use of specific inhibitors [33]. Resveratrol has been proven to avoid liver fat accumulation induced by high-fat diet by increasing fatty acid oxidation and decreasing lipogenesis mediated via AMPK/SIRT1 signaling [34]. Si *et al.* demonstrated that *in vivo* administration of a novel synthesized indole compound improved the serum TG levels and decreased lipid accumulation in the livers of db/db mice [35]. Our results showed that DSW significantly stimulates AMPK and ACC phosphorylation (Figure 5B). An earlier study indicated that activated AMPK phosphorylate and consequently inactivated HMGCR [12]. However, our results showed that the total protein expression of HMGCR was upregulated by the DSW supplement (Figure 5B). We might speculate that these lower levels of sterols and non-sterol metabolites derived from mevalonate negatively regulated HMGCR. Additionally, HMGCR may be suppressed by cholesterol in the mammalian cells [36–39]. Therefore, this upregulated HMGCR protein, after an eight-week DSW supplement, might be initiated by their low levels of serum cholesterol (Figure 1a).

In addition to the beneficial effects of these minerals on the cardiovascular system, unknown effects of several ultratrace elements or unknown substances in DSW may be found in the future. Several inorganic trace substances, such as boron, rubidium, and vanadium, demonstrate higher concentrations in DSW. A boron-containing compound inhibits cholesterol biosynthesis by suppressing HMG-CoA reductase gene expression in hepatocytes [40].

3. Experimental Section

3.1. Materials and Production of the DSW

DSW was acquired from the Pacific Rim at a depth of 662 m, five kilometers off of Hualien County, Taiwan. We used DSW to conduct a series of procedures, including filtration, reverse osmosis, and concentration, as described by Fu *et al.* (2012) [14]. This concentrated DSW (deep ocean minerals; LC-90K) had a hardness of 400,000 mg/L, and the Mg content was 96,000 mg/L. The concentrated DSW from the deep ocean had been identified and compared with surface sea water (Table 5). The DSW used in this experiment was pasteurized, bottled, and provided by Taiwan Yes Deep Ocean Water Co., Ltd., Hualien, Taiwan.

Table 5. Mineral contents of surface sea water and DSW used in this study were analyzed by SGS Taiwan Ltd. LC-90K indicates liquid concentrate with content of Mg^{2+} over 90,000 ppm.

	Surface Sea Water (mg/L)	Deep Sea Water (DSW) LC-90K (mg/L)
Na	10,800	7240
K	392	10,400
Ca	411	39
Mg	1290	96,100
Sr	8.1	0.17
B	4.45	320
Fe	0.003	0.25
Li	0.17	11.7
Cu	0.0009	0.22
Co	0.0004	0.26
Mo	0.01	0.62
Ni	0.0066	0.11
Cr	0.0002	0.087
Rb	0.12	1.2
Si	2.9	0.5
V	0.002	1.2
F	13	21.8
Br	67.3	5400
I	0.064	5.5

The antibody against phosphor-ACC and phospho-AMPKα were obtained from Cell Signaling Technology, Inc., Beverly, MA, USA. The primary antibodies against β-actin and anti-HMGCR were purchased from Abcam, Cambridge, MA, USA, and Millipore/Upstate, Bedford, MA, USA, respectively. HRP-labeled secondary antibodies against mouse IgG and rabbit IgG were acquired from Santa Cruz Biotechnology, Santa Cruz, CA, USA. All other reagents were purchased from Sigma-Aldrich, St. Louis, MO, USA.

3.2. Animal Experimental Design

All animal care was conducted according to the institutional animal ethical guidelines of the China Medical University. The animals were housed in cages and given *ad libitum* access to food and water and maintained on a 12-h light/dark cycle.

3.2.1. Measurements of Blood Pressure and Heart Rate in SHRs

Sixty spontaneous hypertensive rats (250–300 g) were purchased from BioLasco Taiwan Co., Ltd., Nankang, Taiwan. The animals sustained one week of adaptation; subsequently, they were randomly divided into six groups and fed the following diets for eight weeks until they were euthanized: A control group; 10 mg/mL of Lasix (Lasix group); 10% (w/w) $MgCl_2$ supplement ($MgCl_2$ group); 0.1 × DSW (0.1 × DSW group, equivalent to 3.75 mg/kg Mg); 1 × DSW (1 × DSW group, equivalent to 37.5 mg/kg Mg); and 2 × DSW (2 × DSW group, equivalent to 75 mg/kg Mg). All animals were kept in cages on a

12-h day/night cycle. The SBP, DBP, and HR were monitored in conscious animals by the tail-cuff method (MK-2000ST; Muromachi, Japan).

3.2.2. Lipid-Lowering Effects

Fifty-six male New Zealand white rabbits (1500–2000 g) were purchased from the Animal Health Research Institute (Council of Agriculture, Executive Yuan, Tainan, Taiwan). Animals sustained one week of adaptation; subsequently, they were randomly divided into seven groups and fed the following diets for eight weeks: A regular diet (control group) (Fwu Sow Ind., Taichung, Taiwan); 0.5% cholesterol diet alone (CHO group); 0.5% cholesterol diet with 0.01% (w/w) lovastatin supplement (Lova group) (Yung Shin Pharm. Ind., Taipei, Taiwan); 0.5% cholesterol diet with a 10% (w/w) $MgCl_2$ supplement ($MgCl_2$ group); 0.5% cholesterol diet with 0.1 × DSW (0.1 × DSW group); 0.5% cholesterol diet with 1 × DSW (1 × DSW group); and 0.5% cholesterol diet with 2 × DSW (2 × DSW group). The daily feeding amount for each rabbit was 50 g/kg body weight per day. At the beginning and end of the eight-week study, the rabbits were anesthetized by an intramuscular injection of Zoletil 50® (1 mL/kg) (Virbac Ltd., Carros, France), and the blood samples were harvested. Finally, the aortas (from the aortic arch to the bifurcation of the iliac arteries) and whole livers were collected from the rabbits after they were sacrificed for additional histopathological and western blotting assays.

3.3. Measurement of Serum Chemical Parameters

This study was based on our previous study [29]. The rabbits were fasted for 12 h; the blood samples were collected from the marginal ear veins of rabbits into BD Vacutainer™ EDTA blood collection tubes. The plasma was separated by centrifugation at 3000 rpm at 4 °C for 10 min. We measured the changes in blood chemistry parameters, including the serum levels of LDL, high-density lipoprotein (HDL), TC, TG, AST, and ALT (CheChang Co., Ltd., Taichung, Taiwan).

3.4. Cryosectioning of Liver Tissues

Based on our previous study [29], we perfused the rabbit liver tissues with normal saline and fixed in 10% (v/v) formalin neutralized solution (J.T. Baker, Inc., Phillipsburg, NJ, USA) for 24 h. Subsequently, the tissues were embedded in Tissue-Tek® OCT Compound (Sakura Finetek Inc., Torrance, CA, USA). The embedded tissues were cut into 10 μm-thick slices and stained with Sudan IV and hematoxylin (Merck, Darmstadt, Germany). The slices were washed with pure water for one minute to remove the OCT compound, washed with 50% (v/v) ethanol for 30 s, and then stained with 2% (w/v) Sudan IV for one hour. After additional washing with 50% (v/v) ethanol and pure water for two minutes, the slices were counterstained with hematoxylin. Photographs were obtained using a microscope equipped with a 10-fold magnification objective and quantified using an Alpha Imager 2200 documentation system (Alpha Innotech, Santa Clara, CA, USA). The manifestation of fatty liver progression was presented as the percentage of the area of oil droplets to the total liver tissues (cells).

3.5. Aortic Fatty Streak Staining

This study was based on our previous published research [29]. We opened the aortas longitudinally to expose the intimal surface and rinsed gently with normal saline. The aortas were incubated in 2% (w/v) Sudan IV, rinsed with several concentrations (100%, 90%, 80%, 70%, and 60%) of ethanol for one minute, and rinsed with pure water. The photographs were obtained using a digital camera (Nikon D80, Tokyo, Japan) and quantified using an Alpha Imager 2200® documentation system (Alpha Innotech, Santa Clara, CA, USA). The progression of the fatty streak lesions was presented as the percentage of the stained area to the total area.

3.6. Western Blot

Based on our previous study [41], we extracted the proteins from the frozen liver tissues that were subjected to SDS-PAGE under reducing conditions on 10% acrylamide gels and transferred to polyvinylidene fluoride (PVDF) membranes by electroblotting. After a blockade of nonspecific binding sites, the membranes were incubated with primary antibodies (1:1000 dilution), followed by horseradish peroxidase-conjugated secondary antibodies (1:2000 dilution). The protein expression was visualized using SuperSignal® West Pico Chemiluminescent Substrate (Thermo Scientific, Rockford, IL, USA), and the luminescence signal was acquired and analyzed using a Fujifilm LAS-4000® system (Tokyo, Japan). The amount of p-AMPKα, p-ACC and HMGCR were expressed relative to the amount of β-actin.

3.7. Statistical Analysis

All values are expressed as the mean ± standard deviation (SD). The data were compared using a one-way analysis of variance (ANOVA) to evaluate the differences among multiple groups. $p < 0.05$ was considered to be statistically significant.

4. Conclusions

Our experimental study demonstrated that the DSW supplement from the Pacific Rim off of Hualien County (Taiwan) can attenuate mild hypertension (Tables 2 and 3), reduce serum TC (Figure 1), decrease lipid accumulation in tissues (Figures 2 and 3), and diminish aortic fatty streak lesions (Figure 4). Moreover, the lipid-lowering effects of the DSW may be partially mediated by the activation of AMPK/ACC molecular signaling (Figure 5). The liver damage, evidenced by the plasma levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), was not noticeable with the DSW supplement at the end of an eight-week study (data not shown). The highest concentration (2 × DSW) treatment had no effects on the serum AST and ALT (data not shown). Our data showed that the 0.5% cholesterol diet led to a 4.22-fold increase in the tissue MDA content compared to the control group and that 1 × DSW and 2 × DSW supplements reduced the MDA content by 1.18- and 1.21-fold, respectively (data not shown).

These results suggest that DSW may have the potential to be developed as a hypotensive and lipid-lowering therapeutic agent or medicinal health food for the prevention or treatment of cardiovascular diseases, such as atherosclerosis.

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Conflict of Interest

All of the authors declared no conflict of interest.

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Review Article

Potential Health Benefits of Deep Sea Water: A Review

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Deep sea water (DSW) commonly refers to a body of seawater that is pumped up from a depth of over 200 m. It is usually associated with the following characteristics: low temperature, high purity, and being rich with nutrients, namely, beneficial elements, which include magnesium, calcium, potassium, chromium, selenium, zinc, and vanadium. Less photosynthesis of plant planktons, consumption of nutrients, and organic decomposition have caused lots of nutrients to remain there. Due to this, DSW has potential to become a good source for health. Research has proven that DSW can help overcome health problems especially related to lifestyle-associated diseases such as cardiovascular disease, diabetes, obesity, cancer, and skin problems. This paper reviews the potential health benefits of DSW by referring to the findings from previous researches.

1. Introduction

Water is generally defined as a liquid which is shaped by the container that it is filled in and is able to have many variants of colours. It is the crucial component for all living things. For instance, humans need water for many functions such as to regulate body temperature, enhance body metabolism, and provide minerals that are essential to the body. There are many sources of water, such as surface water, aquifer, spring, and seawater. Meanwhile, deep sea water (DSW) can also be a good water source. It is beneficial as it could supply minerals that are essential to health. DSW commonly refers to seawater that is pumped up from a depth of over 200 m. It is usually associated with the following characteristics: low temperature, high purity, and being rich in nutrients, namely, beneficial elements [1]. Its location being far from solar radiation results in it having minimal to no bacteria activities. Less photosynthesis of plant plankton, consumption of nutrients, and lots of organic decomposition causes abundant nutrients to remain there. The abundance of inorganic material becomes higher when the depth of the seawater is increased. These characteristics have derived attention for research regarding DSW especially for its numerous beneficial minerals, which include magnesium (Mg), calcium (Ca), potassium (K), chromium (Cr), selenium (Se), zinc (Zn), and vanadium (V) [1, 2]. DSW is claimed to be high in minerals compared to other sources of water [2].

People usually consume drinking water that is in the form of bottled drinking water (such as mineral water), filtered tap water, or boiled tap water. Drinking water sold by suppliers is expected to contain good nutrient content and be safe to be consumed, because the suppliers possess a production license from the authorities. Surprisingly, some drinking water that is available in the market has been reported to have low mineral content [3]. This is possibly due to the common process drinking water undergo such as reverse osmosis and filtration, which removes the mineral contents inside it. Mineral water, which does not undergo the extensive process

needed, is completely taken from groundwater and gains mineral ions from its sources such as rocks. It is also reported to contain low minerals [3]. However, the mineral contents in the water may vary with the geographical locations and the treatment process that it has gone through. Promisingly, DSW can offer plenty of minerals for the production of drinking water, and other DSW by-products. The production of refined DSW usually involves a desalination process, followed by a mineralization process. A high concentration of mineral salts in DSW though will commonly be processed through means such as reverse osmosis, electrodialysis, or low vacuum temperature in order to produce a safe concentration of water for consumption [1, 4, 5].

DSW has been acquired from many countries with sources of it that are accessible to land. This include Korea, Japan, Taiwan, China, and the USA [1, 2, 6, 7]. Most of those countries conducted researches regarding the health effects that can be attained from the consumption of DSW. As a result, the production of products such as deep sea drinking water (DSDW) became available from those countries. DSDW is claimed as a drinking water which can promote health, since it does not contain carbohydrate, fat, protein, and other bioactive materials which potentially cause adverse health effects, instead of providing valuable minerals to health. Despite being produced for drinking water, it is also used for a variety of purposes such as for food products, cosmetics, aquaculture, and agriculture [8]. Thus, due to the availability of numerous minerals, many researches have been conducted regarding it, in order to discover its benefits to health. By conducting literature reviews, the findings regarding the potential health benefits of DSW applications have been compiled and discussed in this paper.

2. Minerals in Deep Sea Water

DSW contains many types of minerals, such as Mg, Ca, Cl, Na, K, Se, and V, as shown in Table 1 [8]. In fact, DSW is more abundant in minerals compared to surface seawater [6]. The example of the difference between the amount of minerals among surface seawater and DSW is shown in Table 2. DSW is a good nutrient source and could be claimed as a nutrients provider, since the minerals contained inside it provide many benefits to health. For instance, Mg is significant for many physiological processes in the body such as for energy metabolism and enzyme functions [9]. Mg is able to reduce lipids accumulation in the aorta of subjects that has high cholesterol intake [10]. Besides that, Mg is beneficial to people who have cardiovascular disease as it can reduce the potential of a heart attack by dilating the blood vessels and stopping spasms in the heart muscles and vessel walls [11]. It is also able to reduce the risk of obesity, diabetes, and asthma [1, 12]. Drinking water, which has high Mg content, has shown higher inhibitory effects in the adipocyte differentiation, which means that the synthesis of fat cells is able to be slowed down by Mg [13]. Ca is one of the major minerals for humans. It has many benefits to health such as for bone development and density and acts as the pivotal cofactor for several enzymes needed for energy metabolism. Adequate intake of Ca can help reduce the risks of cardiovascular disease, obesity, and some forms of cancers [1, 9, 12]. A high Ca diet is able to increase lipolysis and preserve thermogenesis during caloric restriction, in a way that markedly accelerates weight loss [14]. Cr is an essential nutrient that is required for carbohydrates and lipids metabolism [15, 16]. It has antioxidant properties which are useful for expanding cell life [17]. V has the potential for reducing lipids and has shown effectiveness in inhibiting adipocyte differentiation of the fat cells [18]. There are lots of benefits of other elements in DSW to health, which remain to be elucidated, particularly for the trace elements. The total amount of each element contained in DSW has been estimated [8], based upon the average concentration of each element in DSW. The total volume of DSW of 1.35×10^{18} m^3 is shown in Table 1.

3. Potential Benefits of Deep Sea Water to Health

Many researchers and scientists have done studies about DSW, particularly about refined or balanced DSW. The minerals in it have been proven to improve many health problems. The potential health benefits of DSW are described below by providing some of the mechanisms involved. The findings that have been reviewed in this paper are significant, and comparisons have been made between the treated group and the control group.

3.1. Improvement of the Cholesterol Profiles. The most promising benefits that can be attained from DSW intake are that it is able to improve the cholesterol profiles in the serum and liver, respectively. Its applications have reduced the levels of triglyceride (TG), non-high-density lipoprotein cholesterol (non-HDL-C) levels, and total cholesterol (TC) in the serum and liver of animal models, respectively [4–6, 19–22, 24]. Drinking water produced from DSW which contains Mg of 600 and 1000 ppm, is able to decrease cholesterol levels by 18% and 15%, respectively [22]. Interestingly, a study of DSW consumption by hypercholesterolemic individuals proved that it could reduce TC and low density lipoproteins (LDL) and decreased lipid peroxidation in those subjects. The mechanisms for the improvement of cholesterol profiles are associated with the upregulation of hepatic low density lipoprotein receptor and cholesterol-7a-hydroxylase (CYP7A1) gene expressions, which are involved in cholesterol catabolism. A DSW intake resulted in a higher faecal cholesterol and bile acid excretions, thus decreasing the TC levels [5]. DSW decreases the lipid contents of hepatocytes through the activation of AMP-activated protein kinase, inhibiting the synthesis of cholesterol and fatty acid [19]. The details of respective studies are described in Table 3.

3.2. Protection from Cardiovascular Problems. DSW provides protection from cardiovascular diseases by decreasing the TC, TG, atherogenic index, and malondialdehyde (MDA) levels, while increasing the serum trolox equivalent antioxidant capacity (TEAC). The molecular mechanism of its cardiovascular protection is via upregulation of hepatic low density lipoprotein receptors (LDL receptors) and CYP7A1 gene expressions [5]. The cardioprotective effects of it were

Table 1: Total amount of elements in deep sea water [8].

Element	Total (10^6 ton)
CI	26,120,000,000
Na	14,550,000,000
Mg	1,728,000,000
S	1,312,000,000
Ca	556,000,000
K	538,000,000
Br	90,000,000
C	36,000,000
N	11,700,000
Sr	10,500,000
B	6,100,000
O	3,800,000
Si	3,800,000
F	1,900,000
Ar	840,000
Li	240,000
Rb	160,000
P	84,000
I	78,000
Ba	20,000
Mo	14,000
U	4,300
V	2,700
As	1,600
Ni	650
Zn	470
Kr	420
Cs	413
Cr	271
Sb	270
Ne	216
Se	209
Cu	202
Cd	94
Xe	89
Fe	40
Al	40
Mg	27
Y	22
Zr	20
TI	17
W	13
Re	11
He	10
Ti	8.8
La	7.6
Ge	2.4
Nb	<7
Hf	4.6
Nd	4.4
Ta	<3
Ag	2.7
Co	1.6
Ga	1.6
Er	1.6
Yb	1.6
Dy	1.5
Gd	1.2
Pr	0.9
Ce	0.9
Se	0.9
Sm	0.8
Sn	0.7
Ho	0.5
Lu	0.3
Be	0.3
Tm	0.3
Eu	0.2
Hg	0.2
Rh	0.1
Te	0.1
Pd	0.008
Pt	0.07
Bi	0.04
Au	0.03
Th	0.02
In	0.01
Ru	<0.006
Os	0.003
Ir	0.0002

Table 1: Continued.

further proven, when its application can reduce abnormal cardiac architecture and apoptosis and enhance insulin-like growth factor-1 receptor (IGF-1R) cardiac survival signalling [25]. DSW can also improve cardiovascular hemodynamics in the study conducted by Katsuda et al. [2]. More details about the protective effects of DSW on the cardiovascular system are described in Table 4.

3.3. Prevention from Atherogenesis. Atherogenesis is the formation of plaque in the inner lining of an artery, which deposits fatty substances, cholesterol, cellular waste products, calcium, and other substances. Treatment with DSW was able to prevent the atherogenesis process [6, 21]. DSW with the hardness of 300, 900, and 1500 had significantly decreased the atherogenic index [(TC − HDL-C)/HDL-C]

Table 2: Amount of elements in the surface seawater and deep sea water [6].

Type of element	Surface seawater (mg/L)	Deep sea water (mg/L)
Na	10800	7240
K	392	10400
Ca	411	39
Mg	1290	96100
Sr	8.1	0.17
B	4.45	320
Fe	0.003	0.25
Li	0.17	11.7
Cu	0.0009	0.22
Co	0.0004	0.26
Mo	0.01	0.62
Ni	0.0066	0.11
Cr	0.0002	0.087
Rb	0.12	1.2
Si	2.9	0.5
V	0.002	1.2
F	13	21.8
Br	67.3	5400
I	0.064	5.5

[5]. Antiatherogenic effects of DSW are associated with 5-adenosine monophosphate-activated protein kinase (AMPK) stimulation and the consequent inhibition of phosphorylation of acetyl-CoA carboxylase (ACC) [6]. AMPK plays an important role in lipid metabolism via the inhibition of 3-Hydroxy-3-methyl-glutaryl-CoA reductase (HMGCR) and ACC and then inhibits the production of cholesterol. The details of these studies are described in Table 5. Prevention of atherogenesis may avoid severe health problems, including coronary heart disease and stroke. DSW has antiatherogenic properties due to the existence of many beneficial mineral ions such as Mg and Ca in it. Hence, it could be widely promoted to enhance cardiovascular protection.

3.4. Reduction of Blood Pressure. DSW could improve cardiovascular hemodynamics and reduce blood pressure [2, 6, 20]. Hypertensive rats that were treated with DSW for eight weeks had lower blood pressure than the control group [20]. Reduced fats and blood lipids, such as in the artery, may be associated with the reduced blood pressure. Although DSW used in the study contains pretty much salt, the blood pressure did not increase. In another study [5], DSW application did not affect the blood pressure. Moreover, DSW can also prevent thrombotic disorder by suppressing the release of type-1 plasminogen activator inhibitor from the human vascular endothelial cells [7]. Lots of minerals combination in the DSW, such as Mg, Ca, and Na are associated with a reduced blood pressure. Na content may induce hypertension, though Mg supplement might lower the blood pressure by suppressing the adrenergic activity and, likely, natriuresis [46]. It is interesting that high Mg content can lower blood pressure in the presence of sodium. The details of these respective studies are described in Table 6.

3.5. Protection from Obesity. DSW has antiobesity properties and has been proven to reduce fat and body weight [1, 27, 29, 45]. It has been recognized as possible antiobesity therapeutics from nature [47]. The research has reported that DSW was significantly able to reduce lipids accumulation in the in vitro and in vivo models. Study with obese mice elucidated that DSW with hardness of 1000 was able to reduce body weight by 7%. It also increased the plasma protein levels of adiponectin and decreased plasma protein levels of resistin, RBP4, and fatty acid binding protein [1, 29]. The results suggest that the antiobesity activities were mediated by modulating the expression of obesity-specific molecules. The expression of key adipogenic genes such as peroxisome proliferator-activated receptor-γ (PPARγ), CCAAT/enhancer-binding protein-α (C/EBPα), and adipocyte protein-2 (aP2) was suppressed, and the expression of glucose transporter 4 (GLUT4) was increased by its application [1, 27]. The magnificent effects of DSW on obesity were further proven when it stimulated mitochondrial biogenesis, the component which controls the release of energy associated with lipid metabolism [26]. Mg and Ca ions play a role as the main active components to reduce fats. However, DSW that has the same hardness of 1000 with drinking water which only contains Mg and Ca ions has showed small different effects in the obesity finding [13]. Thus, this hypothesized that Mg and Ca are not the main factors to reduce fats, as the roles of many elements in DSW remain to be elucidated. However the available findings on the clinical study showed that there is no significant difference of TG level and body weight, between treated subjects and controls [4]. More clinical studies are warranted. The detailed mechanisms involved regarding the effects of DSW on obesity-specific molecules are described in Table 7.

3.6. Treatment for Diabetes. DSW was able to improve glucose intolerance and suppress hyperglycaemia which indicated its ability to treat diabetes [26, 27, 29]. Its application had recovered the size of the pancreatic islets of Langerhans and increased the secretion of glucagon and insulin. Through quantitative reverse transcription polymerase chain reaction, DSW showed improvement results regarding the expression of hepatic genes involved in glycogenolysis and glucose oxidation. Whereas in muscles, glucose uptake, β-oxidation, and glucose oxidation were increased by its supplementation [29]. DSW increased the phosphorylation of IRS-1, LKB1, AMPK, and mTOR, which are signalling molecules related to lipid and glucose metabolism [27]. Moreover, blood glucose in treated mice was reduced by its application [27, 29]. The plasma glucose levels in DSW-fed mice were substantially reduced by 35.4%, compared to the control mice group [1]. The antidiabetic properties of it were associated with the existence of mineral ions such as Mg and Ca. The details of these studies are described in Table 8.

3.7. Treatment for Skin Problems. DSW is also capable of treating skin problems. In a study involving patients with

Table 3: Effects of deep sea water on cholesterol levels.

Type of study model	Experimental method [subject (age/weight), treatment dosage, duration of treatment]	Major activity	Mechanism of action	Reference
In vivo study	High fat diet (HFD) male Wistar rats (200–220 g), DSW 1,000 hardness, ad libitum, 4 weeks	Increased the level of HDL-C.	ND.	[19]
In vivo study	Cholesterol-fed diet (CFD) male New Zealand white rabbits (1500–2000 g) fed diet containing 3.75, 37.5, and 75 mg/kg of Mg, DSW 1410 hardness, 8 weeks	Improved plasma total cholesterol (TC), triglyceride (TG), and LDL-C levels.	Improved the protein expression of AMPK phosphorylation, ACC phosphorylation, and HMGCR.	[6]
In vivo study	High cholesterol diet (HCD) ICR mice (7 weeks), reverse osmosis (RO DSW) (44.6 hardness), electrodialysis (ED DSW) (4685.9 hardness) and 10% (v/v) dilution with ddH_2O 10% DSW (544.2 hardness), ad libitum, 8 weeks	Reduced the level of TG, TC, and non-high-density lipoprotein cholesterol (non-HDL-C) levels in the serum and liver of animal models, respectively.	Increase in daily faecal lipid of TG and TC and bile acid outputs.	[20]
In vivo study	HFD Hamster (5 weeks), DSW 300, 900, 1500 hardness, ad libitum, 6 weeks		Increase in daily faecal lipid of TG and TC and bile acid outputs. Upregulated hepatic low-density-lipoprotein receptor (LDL receptor) and cholesterol-7a-hydroxylase (CYP7A1) gene expressions.	[5]
Type of study model	Male hyperlipidemia rabbits (1.8–2.0 g), DSW 1200 hardness, 150 ml/d, ad libitum, 12 weeks	Reduced plasma TC and plasma LDL cholesterol level. Increased plasma HDL cholesterol.	ND.	[21]
In vivo study	Male Wistar rats (90 g), DSW containing 200, 600, and 1000 mg/L of Mg, ad libitum, 4 weeks	Attenuated plasma TC.	ND.	[22, 23]
Clinical study	Hypercholesterolemic individuals (23 men and 19 women), DSW (1410 hardness), supplemented 1050 mL daily, 6 weeks	Decreased serum TC and low-density lipoprotein cholesterol (LDL-C).	Decreased lipid peroxidation in hypercholesterolemic subjects.	[4]
Clinical study	CFD and hyperlipemia male Japanese rabbits, DSW hardness of 28, 300, and 1200, 150 ml/d, ad libitum, 4 weeks	Reduced TC and LDL-C levels in hyperlipemia rabbits. Prevented increase of TC and LDL-C levels in CFD rabbits.	ND.	[24]

ND: not described.

atopic eczema/dermatitis syndrome (AEDS) treated with DSW, the improvement of skin symptoms such as inflammation, lichenification, and cracking of the skin was observed [31]. AEDS patients typically exhibit an imbalance of various essential minerals in hair, and some have toxic minerals present. From that study, DSW intake has restored the essential minerals such as Se and reduces the levels of toxic minerals such as mercury and lead in the treated patients. In another study, the intake of DSW has reduced allergic skin responses and serum levels of total IgE, Japanese cedar pollen-specific IgE, interleukin-4 (IL-4), IL-6, IL-13, and IL-18 in the patients with allergic rhinitis, compared to the distilled water intake which fails to give those effects [32]. In vivo study revealed that DSW can recover the atopic skin lesion by improving the skin symptoms such as edema, erythema, dryness, itching, transepidermal water loss (TEWL), decreased epidermal thickness, and infiltration of inflammatory cells. Its application can reduce allergic responses when reduction of total IgE levels and histamine released were recorded. It also inhibited upregulation of IgE, histamine, and proinflammatory cytokines (tumor necrosis factor α (TNF-α), IL-1β, and IL-6) in the serum. Downregulated CD4+/CD8+ ratio in spleen lymphocyte by 10% CDSW was also observed. Its application can reduce the expression of IL-4 and IL-10 from Th2 cells in the 10% CDSW-treated group [30]. The details of these studies are described in Table 9.

Table 4: Effects of deep sea water on cardiovascular protection.

Type of study model	Experimental method [subject (age/weight), treatment dosage, duration of treatment]	Major activity	Mechanism of action	Reference
In vivo study	HCD ICR mice (7 weeks), reverse osmosis-DSW 44.6 hardness, Electrodialysis-DSW 4685.9 hardness, 10% DSW 544.2 hardness, 8 weeks	Reduced abnormal cardiac architecture, apoptosis in left ventricle (LV). Increased cardiac survival signalling components in LV of mice. Change in Fas and mitochondrial-dependent apoptotic components in LV of mice. Change in apoptosis related proteins and cardiac apoptotic cells in LV of mice.	Decreased LV diameter, LV thickness, and ratio of thickness to diameter in hearts. Increased insulin-like growth factor-1 receptor, phosphoinositide-3-kinases, and p-AKT/AKT ratio. Decreased the protein products of TNF-α in LV of mice. Decreased levels of Fas, cytochrome c, cleaved caspase-9, t-Bid, and cleaved caspase-3. Decreased Bak and increased antiapoptotic proteins, including Bcl-XL and ratio of p-Bad to Bad. Decreased TUNEL-positive cardiac cells.	[25]
In vivo study	High fat/cholesterol-fed (HFCD) male Syrian Golden hamster (5 weeks), DSW 300, 900, and 1500 hardness, ad libitum, 6 weeks	Decreased levels of serum TC, TG, atherogenic index, and malondialdehyde.	Increase in daily faecal lipid of TG and TC and bile acid outputs. Upregulated hepatic low-density-lipoprotein receptor (LDL receptor) and cholesterol-7a-hydroxylase (CYP7A1) gene expressions. Increase of serum trolox equivalent antioxidant capacity (TEAC).	[5]
In vivo study	Kurosawa and Kusanagi-Hypercholesterolemic (KHC) rabbits (4 months), DSW 1000 hardness, 500 ml/d, 6 months	Improved cardiovascular hemodynamics.	Lowered systolic, diastolic pulse, mean arterial pressures, and total peripheral resistance.	[2]

Table 5: Effects of deep sea water on atherosclerosis.

Type of study model	Experimental method [subject (age/weight), treatment dosage, duration of treatment]	Major activity	Mechanism of action	Reference
In vivo study	CFD male New Zealand white rabbits (1500–2000 g) fed diet contain 3.75, 37.5, and 75 mg/kg of Mg, DSW 1410 hardness, 8 weeks	Reduced serum lipids, prevented atherogenesis, and suppressed serum cholesterol levels. Reduced lipids accumulation in liver tissues, and limited aortic fatty streaks.	Improved protein expression of AMPK phosphorylation, ACC phosphorylation, and HMGCR.	[6]
In vivo study	Male hyperlipidemia rabbits (1.8–2.0 g), DSW 1200 hardness, 150 ml/d, ad libitum, 12 weeks	Suppressed lipid deposition on the inner wall of the aorta. Suppressed foam cell formation.	Reduced plasma TC, plasma LDL cholesterol, and LPO. Increased plasma HDL cholesterol. Increased glutathione peroxidase (GPx) activity. Decreased plasma lipid peroxide (TBARS) value.	[21]
In vivo study	CFD and hyperlipemia male Japanese rabbits, DSW hardness of 28, 300, and 1200, 150 ml/d, 4 weeks	Reduced TC and LDL-C levels in hyperlipemia rabbits. Prevented increase of TC and LDL-C levels in CFD rabbits. Reduced lipid accumulation in liver and permeation of macrophages in CFD rabbits.	ND.	[24]

ND: not described.

Table 6: Effects of deep sea water on blood pressure.

Type of study model	Experimental method [subject (age/weight), treatment dosage, duration of treatment]	Major activity	Mechanism of action	Reference
In vivo study	Spontaneous hypertensive rats (250–300 g) fed diet containing 3.75, 37.5, and 75 mg/kg of Mg, DSW 1410 hardness, ad libitum, 8 weeks	Decreased blood pressure.	Decreased systolic and diastolic pressure.	[6]
In vivo study	Kurosawa and Kusanagi-Hypercholesterolemic (KHC) rabbits (4 months), DSW 1000 hardness, 500 ml/d, 6 months	Decreased blood pressure.	Lowered systolic, diastolic pulse, and mean arterial pressure and total peripheral resistance.	[2]

3.8. Protection from Hepatic Problems. High fat diets may cause problems to hepatic systems. DSW is able to give protection for hepatic problems. In a study by Chen et al. [33], it has decreased the lipid accumulation in livers, which are associated with the increase in daily faecal lipid and bile acid outputs. The hepatic antioxidative levels were also improved by its application, which were proven by the high capacity levels of liver glutathione and trolox equivalent antioxidant. DSW was able to regulate hepatic fatty acid homeostasis by upregulating genes related to b-oxidation of fatty acids, which are hepatic peroxisome proliferator-activated receptor-alpha, retinoid X receptor-alpha, and uncoupling protein-2 gene expression. Its application can attenuate hepatic damage, which is proven by reduced lipid peroxidation status in livers, which might be related to reducing hepatic malondialdehyde (MDA) content [33]. The liver damage indices which are aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are also reduced by its application. The details of these studies are described in Table 10.

3.9. Treatment for Fatigue. DSW can restore fatigue and improve exercise workload. Its application has promoted the endurance and reduced exhaustive period of rats in an exercise test [34]. The ratios of lactic acid elimination to lactic acid increment were improved in DSW treated rats. The study showed low blood urea nitrogen (BUN) level of rats fed with D100 in a dosage of 30 mL/kg·d and D600 in dosages of 6, 12, and 30 mL/kg·d, respectively. As a result, the liver glycogen content had increased in the rats fed with D100 in a dosage of 6 mL/kg·d. Study regarding effects of DSW on human shows significant findings as well. DSW is able to accelerate recovery from physical fatigue of people, following an exhaustive physical challenge [35]. The findings suggested that DSW which has enriched contents of boron, magnesium, lithium, and rubidium may complement and enhance the molecular and cellular complexity of human during exercise, eradicate exercise-induced muscle damage, and strengthen antioxidant capability against oxidative stress. The details of these studies are described in Table 11.

3.10. Treatment for Stomach Ulcer. DSW can reduce ulcer area as well as apoptotic signalling in acetic acid-induced duodenal ulcers. It had upregulated antioxidant and enhanced Bcl-2 and thioredoxin reductase 1 expression in a study that used rats [36]. In that study, DSW ingestion provides intestinal protection via the antioxidant and antiapoptotic mechanisms of selenium. The details of this study are described in Table 12.

3.11. Prevention of Cancer. DSW is potential to prevent cancer. Its application can inhibit human breast cancer cell lines' migratory ability in a wound-healing assay. The inhibitory effects of DSW on breast cancer invasion/metastasis that uses MDA-MB-231 cells appears to be mediated through TGF-β and Wnt5a signalling, resulting in attenuated expression of CD44 [37]. In a study that uses the noninvasive MCF-7 cells, DSW treatment resulted in the inhibition of TPA-induced migration and MMP-9 activity with a concomitant decrease in mRNA levels of MMP-9, TGF-β, Wnt5a, and Wnt3a [37]. DSW also improves the quality of green tea prepared with it, in which it enhanced the production of epigallocatechin gallate (EGCG), which could potentially act as an inhibitor for N-nitrosation, which can induce mutagenic and cell damaging reactions [38]. The details of the studies regarding effects of DSW on cancer are described in Table 13.

3.12. Improvement in Antibacterial Activity. DSW has promising effects on antibacterial activity. The findings of its antibacterial activities were proven in the studies using the in vitro, in vivo, and clinical model as described in Table 14.

3.13. Treatment for Cataract. DSW application can delay cataract development [40, 41]. This effect is associated with the presence of Mg and Ca content in DSW. The details of these studies are described in Table 15.

3.14. Recovery from Osteoporosis. DSW has therapeutic potential on osteoporosis. DSW at hardness 1000 showed significant increase in proliferation of osteoblastic cell (MC3T3). In the in vivo study that uses DSW for 4 months, bone mineral density (BMD) was strongly enhanced followed by the significantly increased trabecular numbers through micro-CT examination. Biochemistry analysis showed that serum alkaline phosphatase (ALP) activity was decreased. BMSCs treated with DSW showed increase of osteogenic differentiation markers such as BMP2, RUNX2, OPN, and OCN and enhanced colony forming abilities, compared to the control group. The results demonstrated the regenerative potentials of DSW on osteogenesis, showing that it could potentially be applied in osteoporosis therapy as a complementary and alternative medicine (CAM). The details of these studies are described in Table 16.

Table 7: Effects of deep sea water on obesity.

Type of study model	Experimental method [subject (age/weight), treatment dosage, duration of treatment]	Major activity	Mechanism of action	Reference
In vitro study	C2C12 cells, DSW 100, 500, 1000, 1500, and 2000 hardness, indicated time of 0, 1, 2, and 3 days	Increased mitochondrial biogenesis and function.	Enhanced gene expression of peroxisome proliferator-activated receptor gamma coactivator 1 α (PGC-1a), nuclear respiratory factor-1 (NRF-1), and mitochondrial transcription factor A (TFAM); mitofusin-1/2 (MFN1/2) and dynamin-related protein 1 (DRP1) for mitochondrial fusion; optic atrophy 1 (OPA1) for mitochondrial fission; translocase of outer mitochondrial membrane 40 (TOMM40) and translocase of inner mitochondrial membrane 44 (TIMM44) for mitochondrial protein import; carnitine palmitoyltransferase 1α (CPT1α) and medium-chain acyl-CoA dehydrogenase (MCAD) for fatty acid oxidation; and cytochrome c (CytC) for oxidative phosphorylation. Increased mitochondria staining, citrate synthase (CS) activity, CytC oxidase activity, NAD+ to NADH ratio, and the phosphorylation of signalling molecules such as AMPK and sirtuin 1 (SIRT1).	[26]
In vitro study	3T3-L1 cells, DSW 100, 500, and 1000 hardness, 3 days	Decreased lipid accumulation.	Reduced expression mRNA levels of PPARγ and C/EBPα and protein levels of fatty acid binding protein and adiponectin.	[13]
In vivo study	HFD C57BL/6J mice (6 weeks), DSW 500, 1000, and 2000 hardness, ad libitum, 20 weeks	Enhanced mitochondrial biogenesis in muscles.	Improved mitochondrial DNA (mtDNA) content in the muscles of HFD-induced obese mice. Enhanced expression of PGC-1α, NRF1, and mtTFA. Enhanced estrogen-related receptor α (ERRα), PPARα, and PPARδ.	[26]
In vivo study	HFD C57BL/6J mice (6–26 weeks), DSW 500, 1000, and 2000 hardness, ad libitum, 20 weeks	Suppressed body weight gain. Inhibited increase in adipocyte size. Suppressed the expression of adipogenic, lipogenic, lipolytic, and proinflammatory cytokine genes. Increased the expression of adipokines and b-oxidation genes in fat.	Suppressed mRNA expression of key adipogenic genes such as PPARγ, C/EBPα, and aP2. Increased the expression of GLUT4, adiponectin, and leptin. Decreased the expressions of IL-6 and TNF-a. Decreased the expressions of sterol regulatory element-binding protein 1c (SREBP1c) and fatty acid synthase (Fas), which are involved in lipogenesis; adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL), which are involved in lipolysis. Increased the expression of MCAD and CPT1α, which are involved in b-oxidation. Increased phosphorylation of IRS-1, LKB1, AMPK, and mTOR in fat.	[27]
In vivo study	Male C57BL/6J ob/ob mice, DSW 1000 hardness, ad libitum, 84 days	Decreased body weight gain by 7%. Reduced plasma glucose levels by 35.4%.	Increased glucose disposal. Increased plasma protein levels of adiponectin. Decreased plasma protein levels of resistin, RBP4, and fatty acid binding protein. Increased GLUT4 and AMP-activated protein kinase levels in skeletal muscle tissue. Decreased PPARγ and adiponectin in adipose tissue.	[1]

4. Effects of Deep Sea Water in the Liver and Kidney Status

From the available studies, DSW hardness which ranges from 0 to 1500 had caused no damage to liver and kidneys. In a study, through in vivo and clinical subjects, ALT, AST, and BUN levels showed that there is no significant difference between treated subjects and the controls. The details of these respective studies are described in Table 17.

5. Functional Deep Sea Water with Other Substances

DSW is very beneficial to health. Its uses are applied to many DSW by-products. For example, it can enhance the antibacterial activity of yogurt [44]. The green tea leaves that were soaked in DSW had an increase in the antioxidant and catechin properties [38]. These findings increase the value of DSW as a health-promoting water. Combination of DSW

Table 8: Effects of deep sea water on diabetes.

Type of study model	Experimental method [subject (age/weight), treatment dosage, duration of treatment]	Major activity	Mechanism of action	Reference
In vitro study	Differentiated C2C12 cells, DSW 100, 500, 1000, 1500, and 2000 hardness, 1 hr	Increased glucose uptake.	Stimulated the phosphorylation of IRS-1, LKB1, AMPK, and mTOR and improved impaired phosphorylation of these molecules.	[28]
In vitro study	Matured 3T3-L1 cells, DSW 500, 1000, and 2000 hardness, 1 hr	Increased glucose uptake.	Increased AMPK phosphorylation in 3T3-L1 pre- and mature adipocytes. Stimulated phosphoinositol-3-kinase and AMPK pathway-mediated glucose uptake.	[29]
In vivo study	Streptozotocin- (STZ-) induced diabetic male ICR mice (4–9 weeks), DSW 1000, 2000, and 4000 hardness, ad libitum, 4 weeks	Improved impaired glucose tolerance. Regulated blood glucose levels by inhibited glucose production and enhanced glucose uptake via regulation of gene expression.	Increased adiponectin and leptin levels and reduced the levels of the proinflammatory cytokines IL-6 and TNF-α. Improved architecture of pancreatic islets of Langerhans and enhanced insulin secretion from β-cells. Stimulated the phosphorylation of IRS-1, LKB1, AMPK, and mTOR and improved impaired phosphorylation of these molecules in muscle. Downregulated the expression of phosphoenolpyruvate carboxykinase (PEPCK) and glucose 6-phosphatase (G6Pase), both of which are required for gluconeogenesis; glucokinase (GK) and citrate synthase (CS), both of which are required for glucose oxidation; and liver glycogen phosphorylase (LGP), which is required for glycogenolysis. Upregulated glycogen synthase (GS) expression. Upregulated the expression of GLUT1 and GLUT4 in skeletal muscle, which are required for glucose transport; glucokinase and citrate synthase, which are required for glucose oxidation; and acyl-CoA oxidase (ACO), CPT1α, and MCAD, which are required for β-oxidation.	[28]
In vivo study	HFD-induced diabetic male C57BL/6J mice (6–25 weeks), DSW 500, 1000, and 2000 hardness, ad libitum, 20 weeks	Improved impaired glucose tolerance. Suppressed the expression of hepatic genes involved in glucogenesis, glycogenolysis, and glucose oxidation. Increased glucose uptake, β-oxidation, and glucose oxidation in muscle. Improved impaired AMPK phosphorylation in the muscles and livers.	Recovered size of the pancreatic islets of Langerhans and increased the secretion of insulin and glucagon. Increased adiponectin levels. Decreased IL-6 and TNF-α levels. Downregulated the expression of PEPCK and G6Pase for gluconeogenesis; GK and CS for glucose oxidation; and LGP for glycogenolysis. Upregulated the expression of GS for glycogenesis. Upregulated the GLUT1 and GLUT4 for glucose transport, GK and CS for glucose ACO, CPT1α, and MCAD for β-oxidation in skeletal muscle. Increased the expression of SIRT family proteins such as SIRT1, SIRT4, and SIRT6.	[29]

Table 8: Continued.

Type of study model	Experimental method [subject (age/weight), treatment dosage, duration of treatment]	Major activity	Mechanism of action	Reference
In vivo study	Male C57BL/6J ob/ob mice, DSW 1000 hardness, ad libitum, 84 days	Reduced glucose levels in plasma by 35.4%.	Increased glucose disposal. Increased adiponectin levels in plasma. Decreased plasma protein levels of resistin, RBP4, and fatty acid binding protein. Increased GLUT4 and AMP-activated protein kinase levels in skeletal muscle tissue.	[1]

Table 9: Effects of deep sea water on skin diseases.

Type of study model	Experimental method [subject (age/weight), treatment dosage, duration of treatment]	Major activity	Mechanism of action	Reference
In vivo study	Male NC/Nga mice (6 weeks), 2% concentrated DSW (CDSW) (7958.6 hardness), 10% CDSW (39793 hardness), 200 μL of test samples, five times per week, six weeks	Reduced severity of symptoms in the skin lesions, such as edema, erythema, dryness, itching, and transepidermal water loss (TEWL). Decreased epidermal thickness and infiltration of inflammatory cells.	Inhibited upregulation of IgE, histamine, and proinflammatory cytokines (TNF-α, IL-1β, and IL-6) in the serum. Downregulated CD4+/CD8+ ratio in spleen lymphocyte by 10% CDSW. Reduced the expression of IL-4 and IL-10 from Th2 cells in the 10% CDSW-treated group.	[30]
Clinical study	33 patients (mean age 26 years, range 1–50 years, 13 male and 20 female subjects), DSW 1000 hardness, 500 ml/day, 6 months	Improved skin symptoms. Balanced certain minerals in the body.	Improved skin symptoms such as inflammation, lichenification, and cracking in skin. Restored essential minerals such as Se and reduced the level of toxic minerals such as mercury and lead.	[31]
Clinical study	50 patients with allergic rhinitis (age 22–50 years), DSW 1000 hardness, 500 ml/day, 3 weeks	Improved skin symptoms.	Reduced allergic skin responses and serum levels of total IgE, Japanese cedar pollen-specific IgE, IL-4, IL-6, IL-13, and IL-18.	[32]

with *Sesamum indicum* leaf extract (SIE) had prevented high fat diet-induced obesity, through AMPK activation in the visceral adipose tissue [45]. Furthermore, DSW has advantages for the development of functional fermentation food. The main factors of its increased health properties are due to it being able to increase functional metabolite production, intrinsic health functions of DSW, and the microbial use of mechanisms of converting the absorbed inorganic ions into highly bioavailable organic ions for the human body [48]. The detailed reviews regarding effects of DSW applications for the development of functional fermentation food are explained by Lee [48]. The detailed studies of functional deep sea water with other substances are described in Table 18.

6. Discussion and Conclusion

DSW originates from deep levels of the sea, which are far from contamination except for the natural occurrence of hazardous chemicals such as arsenic and mercury. It will usually undergo a process such as desalination to make it suitable for a particular purpose such as drinking water. The hardness of DSW of up to 1500 caused no cytotoxicity effects in the in vitro study [13]. However, the maximum hardness of it for human consumption should be remarked. The hardness values of water were estimated according to the following equation:

$$\text{Hardness} = \text{Mg}\left(\frac{\text{mg}}{\text{L}}\right) \times 4.1 + \text{Ca}\left(\frac{\text{mg}}{\text{L}}\right) \times 2.5; \quad (1)$$

see [49].

The probability of physical, chemical, or bacteriological contaminants present in the drinking water has triggered compulsory actions by most authorities to ensure that the water is subjected to appropriate treatments prior to being supplied. This includes the step of adding chlorine into the drinking water as a treatment. However, chlorine causes an unpleasant taste and raises health concerns such as cancer due to its ability to accumulate within the body [50–52]. Nowadays, it is becoming a trend to supply drinking water, through a vending machine that has the reverse osmosis

Table 10: Effects of Deep Sea Water on Hepatic Protection.

Type of study model	Experimental method [subject (age/weight), treatment dosage, duration of treatment]	Major activity	Mechanism of action	Reference
In vitro study	HepG2 cells, DSW 200, 400, 600, 800, and 1000 hardness, 24 hr	Decreased lipids accumulation.	Inhibited the activity of HMGCR by 30.2%. Increased the phosphorylation level of AMPK by 15.2%. Reduced p68 of SREBP-1 levels by 55%. DSW of hardness 600, 800, and 1,000 increased p68 levels of SREBP-2 by 12, 42, and 80%, respectively. DSW of hardness 600, 800, and 1,000 increased level of CYP7A1 by 41, 115, and 162%, respectively. DSW of hardness 1,000 increased Apo AI content by 20.3%.	[19]
In vivo study	HFD male Wistar rats (200–220 g), DSW 1,000 hardness, ad libitum, 4 weeks	Decreased levels of TC and TG in liver. Improved liver function.	Decreased serum levels of AST and ALT.	[19]
In vivo study	HFD C57BL/6J mice (6–26 weeks), DSW 500, 1000, and 2000 hardness, ad libitum, 20 weeks	Suppressed the expression of genes involved in lipogenesis and cholesterol synthesis; and increased the expression of genes related to b-oxidation in liver. Improved severe liver steatosis. Regulated mitochondrial biogenesis and function in liver.	Decreased the expression of Fas and acetyl-CoA carboxylase 1 (ACC1), which are involved in lipogenesis, and liver X receptor a (LXR a), and 5-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoAR), which are involved in cholesterol metabolism. Increased the expression of MCAD and CPT1α, which are involved in b-oxidation. Increased the phosphorylation of IRS-1, LKB1, AMPK, and mTOR in liver. Increased expression of PGC1a, NRF1, Tfam, and mtDNA content in liver.	[27]
In vivo study	HFD male Golden Syrian hamsters (5 weeks), DSW 300, 900, and 1500 hardness, ad libitum, 6 weeks	Decreased lipids accumulation in liver. Regulated hepatic fatty acid homeostasis. Improved hepatic antioxidative levels. Attenuated hepatic damage.	Increased daily faecal lipid and bile acid outputs. Upregulated genes of hepatic PPARα, retinoid X receptor-alpha, and uncoupling protein-2 (UCP-2) gene expression. Maintained higher liver glutathione and TEAC levels. Reduced lipid peroxidation status (MDA content) in liver.	[33]

system, from the treated wastewater and from the treated water pipeline. The process of water treatment will commonly cause reduction or loss of minerals. The increase in the availability of treated drinking water through processes such as reverse osmosis and chlorination should be put in high concern. Chlorine is not good for health. Furthermore, low nutrient in the drinking water can pose as a health threat to people that have nutrient deficiency. The desalinated DSW is usually added or concentrated with minerals, by the process of dilution, blending, or mixing it with concentrated minerals from the DSW [2, 4, 19, 53]. These mineralized methods of desalinated seawater have been a popular method. Therefore, the desalinated DSW will normally regain its minerals that might have been lost through the desalination process again, compared to the packaged drinking water, which has lost most of its minerals through water treatments. Thus, DSDW is able to have numerous minerals constituents in the water compared to the common mineral water sources such as aquifer, which only contain minerals that originated readily from the source. It can be claimed that the mineral contents in the DSW are greater than in the groundwater sources.

Through the impressive findings of DSW benefits to health, it is suggested that its utilization should be promoted widely. The nutrients deficiency of population in a region could be provided with DSW. Adequate nutrient contents in the drinking water supply can contribute to a healthy population status in the area of supply. Areas which have lack of nutrient contents in the water supply are linked with the deficiency of nutrients among their populations. Nutritious water supply is crucial for the people. Prevalence of cardiovascular mortality and sudden death is 10% to 30% greater in the soft water areas, which has low Mg or Ca ions, compared to the hard water areas that have high Mg or Ca ions in the water supply [54]. Intake of hard water

Table 11: Effects of deep sea water on fatigue.

Type of study model	Experimental method [subject (age/weight), treatment dosage, duration of treatment]	Major activity	Mechanism of action	Reference
In vivo study	Exercise-induced fatigue male Wistar rats, DSW 100, and 600 hardness, dosages (6, 12, and 30 mL/kg·d)	Promoted the endurance of rats in exercise test. Reduced exhaustive period.	Improved the ratio of lactic acid elimination to lactic acid increment. Reduced BUN level of rats fed with D100 in a dosage of 30 mL/kg·d and D600 in dosages of 6, 12, and 30 mL/kg·d. Increased liver glycogen content in rats group fed with D100 in a dosage of 6 mL/kg·d.	[34]
Clinical study	12 healthy male volunteers (age 24 ± 0.8 years; height 171.8 ± 1.5 cm; weight 68.2 ± 2.3 kg; VO_2max 49.7 ± 2.2 ml·kg^{-1}·min^{-1}), randomized, double-blind, placebo-controlled, DSW 710 hardness, fatiguing exercise conducted for 4 hr at 30°C	Accelerated recovery from physical fatigue.	Complete recovery of aerobic power within 4 hr. Elevated muscle power above placebo levels within 24 hr. Increased circulating creatine kinase (CK) and myoglobin; indicatives of exercise-induced muscle damage, were completely eliminated, in parallel with attenuated oxidative damage.	[35]

Table 12: Effects of deep sea water on stomach ulcer.

Type of study model	Experimental method [subject (age/weight), treatment dosage, duration of treatment]	Major activity	Mechanism of action	Reference
In vivo study	Female Wistar rats (220–250 g weight), DSW 600 (41 mL/day), DSW 1200 (39 mL/day), 1 week	Reduced ulcer area as well as apoptotic signalling in acetic acid-induced duodenal ulcers. DSW influenced oxidative stress genes expression. Upregulated antioxidant and antiapoptotic genes and downregulated proapoptotic gene expression by DSW of hardness 600 and 1200, respectively.	Increased pH value, scavenging H_2O_2 , and HOCl activity and reduced ORP value. Enhanced Bcl-2 and thioredoxin reductase 1 expression. DSW1200 activated the expression of flavin-containing monooxygenase 2 (Fmo2), Gpx1, Gpx5, Gpx6, glutathione reductase (Gsr), nitric oxide synthase 2, inducible (Nos2), thioredoxin reductase 1 (Txnrd1), superoxide dismutase 1 (Sod1), some antioxidant-related genes, peroxiredoxin 4 (Prdx4), and selenoprotein P plasma 1 (Sepp1). DSW600 and DSW1200 upregulated Txnrd1 and Bcl-2 and downregulated Bax, caspase 3, and PARP in duodenal cells. DSW 600 upregulated expression of apoptosis-inducing factor, mitochondrion-associated 1 (Aifm1), DNA-damage-inducible, alpha (Gadd45a), myeloid cell leukemia sequence 1 (Mcl 1), and X-linked inhibitor of apoptosis (Xiap). DSW 600 downregulated expression of apoptosis inhibitor 5 BCL2-associated athanogene (Api5), cell death-inducing DFFA-like effector b (Ciedb), cytochrome c, and somatic (Cycs), Fas (TNF receptor superfamily, member 6), growth arrest and mitogen activated protein kinase 1 (Mapk1), PYD and CARD domain containing (Pycard). DSW 1200 upregulated expression of Fas, Gadd45a, and Mcl1. DSW 1200 downregulated expression of Aifm1, Api5, Bag1, Cideb, Cycs, and Pycard.	[36]

Table 13: Effects of deep sea water on cancer.

Type of study model	Experimental method [subject (age/weight), treatment dosage, duration of treatment]	Major activity	Reference
In vitro study	MDA-MB-231 cells, DSW 200, 400, 800, and 1500 hardness, 2-3 days	Inhibited cells' migratory ability in a wound-healing assay, mediated through TGF-β and Wnt5a signalling, resulting in attenuated expression of CD44.	[37]
In vitro study	Noninvasive MCF-7 cells, DSW 200, 400, 800, and 1500 hardness, 2-3 days	Inhibited TPA-induced migration and MMP-9 activity with a concomitant decrease in mRNA levels of MMP-9, TGF-β, Wnt5a, and Wnt3a.	[37]
	Green tea leaves were soaked in desalinated DSW at 75°C for 10 min	Increased nitrite scavenging activity from 31.33 ± 0.05 to 37.12 ± 0.42%. Increased overall amounts of catechins.	[38]
	Salmonella Typhimurium TA98 and TA100, Ames test, methanol extract of kochujang added with sea tangle and deep sea water salts (SDK), 200 μg/plate	71.4% inhibitory effect on the mutagenesis induced by 4NQO against TA98 strain. 56.1% and 83.6% inhibitions on the mutagenesis induced by 4NQO and MNNG against TA100 strain.	[39]

Table 14: Effects of deep sea water on cataract.

Type of study model	Experimental method [subject (age/weight), treatment dosage, duration of treatment]	Major activity	Mechanism of action	Reference
In vivo study	Male Shumiya cataract rat (5–15 weeks), DSW (Mg^{2+}, 200 mg/L, Ca^{2+}; 71 mg/L), 9-10 weeks	Delayed cataract development.	Reduced less opaque and nitric oxide (NO) levels.	[40]
In vivo study	Male Shumiya cataract rat (5–15 weeks), DSW containing Mg of 50, 200, and 1000 mg/L, respectively, 9-10 weeks	Delayed cataract onset.	Mg suppressed Ca influx into the lens.	[41]

Table 15: Effects of deep sea water on antibacterial activity.

Type of study model	Experimental method [subject (age/weight), treatment dosage, duration of treatment]	Major activity	Reference
In vitro study	Five types ratio of DSW containing magnesium : calcium (Mg : Ca) ratios of 1 : 2 (A), 1 : 1 (B), 3 : 1 (C), 1 : 0 (D), and 0 : 1 (E) at different concentrations to give levels of hardness of 100, 250, 500, and 1000; produced 20 types of samples Sixteen *H. pylori* strains, clinical isolates were obtained from patients with gastric cancer, gastric ulcer, and normal gastric mucosa	Inhibited bacterial growth and mobility.	[42]
In vitro study	Sheep blood, *H. pylori* obtained from gastric biopsy specimens of peptic ulcer patients, 3 to 5 days	DSW hardness of 1200 and 2400 inhibited growth of *H. pylori* strains by 20% and 60%, respectively.	[36]
In vivo study	Male Mongolian gerbils (4 weeks), DSW at 5 different Mg/Ca ratios (hardness of 1000) were administered for 2 weeks	Decreased amount of *H. pylori* colonized in stomach by treatment with 2 types of DSW ratio which are C and D. Anti-*H. pylori* effects were observed in ≥90% of subjects.	[42]
Clinical study	Healthy subjects infected with *H. pylori*, DSW at 5 different Mg/Ca ratios (hardness: 1000), 1 L/daily, 10 days	Reduced Δ13 C values.	[42]

Table 16: Effects of deep sea water on osteoporosis.

Type of study model	Experimental method [subject (age/weight), treatment dosage, duration of treatment]	Major activity	Reference
In vitro study	Osteoblastic cell (MC3T3), DSW 50, 1000, and 2000 hardness, 3 days	Increased cells proliferation.	[43]
In vitro study	Bone marrow stromal cells (BMSCs), DSW 1000 hardness, 3 days	Enhanced colony forming abilities.	[43]
In vivo study	Ovariectomized (OVX) SAMP8 mice (4 months), DSW 1000 hardness, 5.2 mL/day, 4 months	Enhanced bone mineral density. Increased trabecular numbers through micro-CT examination. Decreased serum alkaline phosphatase (ALP). Increased osteogenic differentiation markers such as BMP2, RUNX2, OPN, and OCN.	[43]

Table 17: Effects of deep sea water in the liver and kidney status.

Type of study model	Experimental method [subject (age/weight), treatment dosage, duration of treatment]	Major activity	Reference
In vivo study	HFD male Wistar rats (200–220 g), DSW 1,000 hardness, ad libitum, 4 weeks	Improved liver function by the decrease of serum levels of AST and ALT.	[19]
In vivo study	HFD male Golden Syrian hamsters (5 weeks), DSW 300, 900, and 1500 hardness, ad libitum, 6 weeks	Attenuated serum AST values in hamsters drinking DSW 300, 900, and 1500. Lower serum ALT values in hamsters drinking DSW 900 and DSW 1500.	[33]
In vivo study	CFD male New Zealand white rabbits (1500–2000 g) fed diet containing 3.75, 37.5, and 75 mg/kg of Mg, DSW 1410 hardness, 8 weeks	No differences were observed in values of AST and ALT.	[6]
In vivo study	Male hyperlipidemia rabbits (1.8–2.0 g), DSW 1200 hardness, 150 ml/d, ad libitum, 12 weeks	No differences were observed in values of AST and ALT.	[21]
Clinical study	Hypercholesterolemic individuals (23 men and 19 women), DSW (1410 hardness), supplemented 1050 mL daily, 6 weeks	No significant difference of ALT, AST, and BUN levels between treated subjects and controls.	[4]

has potential to decrease the risks of cardiovascular disease [55]. The importance of mineral contents in the drinking water is proven, when its intake is able to reduce calcium oxalate stone in the kidney of people that consume drinking water rich in minerals such as Mg, Ca, and bicarbonate [56–58]. In contrast, consumption of low calcium content in the drinking water has resulted in the hip fracture incident in the Norwegian population [59]. Instead of epidemiological studies, researchers have identified the importance of mineral water content in the experimental studies. According to the study, the rabbits and men which consumed water with low mineral contents have higher risks of cardiovascular disease, compared to the group that consumed water with high mineral content [60]. The miracle of water to cure diseases has progressively been discussed. One of the mechanisms of mineral water to treat diseases is through the existence of minerals which are capable of activating the aquaporin genes, which are responsible for transporting water within the cells [61]. Lack of aquaporin gene activation has been linked to many disease occurrences [62]. Minerals in the DSW are plenty and thus could be a major factor in curing diseases.

Some areas may have lack of nutrients in the soil and crops, which may pose as health threats to its consumers. The soil provides minerals to the plants, and through the plants the minerals go to the animals and humans [63]. Referring to the chain, it is a health threat to people that usually rely on the crops and animals as their main nutrients provider. For instance, nutrient deficiency in the land of South Africa was associated with many diseases occurrences such as thyroid, iodine deficiency disorders (IDD), Mseleni Joint Disease (MJD), HIV-AIDS, and Mg insufficiency [64]. Besides that, the groundwater could be contaminated with man-made activities including the industries, agriculture, and logging. These could pose as a threat to the residents that use groundwater as a source for drinking water. For instance, agricultural activities have caused an increase in the

Table 18: Effects of functional deep sea water with other substances.

Type of study model	Experimental method [subject (age/weight), treatment dosage, duration of treatment]	Major activity	Mechanism of action	Reference
In vivo study	Outbred albino female ICR mice (20–26 g), yogurt containing DSW, 10.3 g hardness of $CaCO_3$/L, 8 weeks	Increased populations of intestinal lactic acid bacteria. Decreased the activity of serum AST and ALT. Reduced TC, TC to HDL-C ratio, TAG, and HDL-C in serum.	ND.	[44]
In vivo study	HFD-induced obesity ICR (4 weeks), DSW, and DSW + 125 mg/kg SIE (DSS), ad libitum, treated with SIE once per day for 8 weeks	Reduced body weights in the DSW group by 3.95% and in the DSS group by 8.42%, respectively. Decreased plasma glucose levels in the DSW group by 14.9% and in the DSS group by 36.4%, respectively. Decreased serum levels of glucose, TAG, and leptin. Decreased insulin resistance index (HOMA-IR) values for the DSS-treated group by 38.2%.	Decreased size of the epididymal white, retroperitoneal white, and scapular brown adipose tissue. Increased levels of phosphorylated AMPK and its substrate and ACC in mice epididymal adipose tissues. Upregulated the expression levels of lipolysis-associated mRNA, PPAR-α, cluster of differentiation 36 (CD36), and energy expenditure-associated mRNA and UCP2 and CPT1 epididymal adipose tissues. Suppressed the expression of SREBP1 at the mRNA level.	[45]
In vivo study	Green tea leaves were soaked in desalinated DSW at 75°C for 10 min	Increased antioxidant activity.	Increased 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activities by 83.98% and increased reducing power by 15%. Increased nitrite scavenging activity from 31.33 ± 0.05 to 37.12 ± 0.42%. Increased amounts of catechins and caffeine.	[38]

ND: not described.

nitrate concentration of groundwater in the area of Machang, Malaysia, resulting from the fertilizer application [65]. DSW which is far from man-made contamination could provide a safe water source. DSW is rarely polluted, has no or slight bacteria existence, and is very pure [2, 8, 66].

Furthermore, nutrients deficiency among the people was also associated with the types of daily food intake. For instance, the regular consumption of phytate content foods had caused the zinc deficiency among Korean people [67]. Phytate impairs the zinc bioavailability. Thus, choosing the right foods is crucial for nutrient intake. Dynamic activities in today's life had caused the tendency for people to choose fast, instant, and easy prepared foods. These kinds of foods usually contain a small amount of nutrients, which is not the most promising source of nutrients intake. Minerals in food may also be lost during cooking [68–70]. In a nutshell, nutrients intake should not solely rely on food intake. DSW has lots of minerals to supply, and could be provided in the form of health drinks or water supply, as an alternative to maintain nutrients source. The roles of minerals in the water to heal disease and maintain health has already been recognized. Water can be classified into a few categories based on its total salt content, its mineral biological activity, and its ion mineral composition [71]. The effort to put DSW as a water source that is beneficial for health should be enhanced. The studies regarding types of mineral water have also been progressively carried out. Examples of these studies can be referred to Astel et al. [72], included in the discussion about the types of minerals available in the water, types of available water treatments, associated regulations, and therapeutic potentials of mineral water. The study to classify DSW into particular types of water based on the types of production should be established, as there is a great therapeutic potential about it yet to be discovered.

Ideally, countries with the accessibility to pump up water from DSW should consider maximizing the use of it. Perhaps, the only limitations are the technology provider and cost of production, rather than reachable sources to the DSW itself. Technologies that are involved may include desalination, low vacuum temperature, and ocean thermal energy conversion

(OTEC). OTEC is a kind of technology which could produce water as a by-product from its process, without the extensive cost [73]. There are many great findings from the studies regarding DSW applications in the in vitro models such as using 3T3L-1 cells, and in the in vivo models such as using mice, and rabbits. However, the potential health benefits of its applications in the clinical studies are not widely established. Hence, the study of its applications especially to the human health should be conducted more. DSW is worthy of further investigations and could be developed as medicated water in the prevention and treatment of many health problems, especially lifestyle-related diseases.

Competing Interests

The authors declare that there are no competing interests.

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Health Effects of Drinking Water Produced from Deep Sea Water: A Randomized Double-Blind Controlled Trial

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Abstract: Global trends focus on a balanced intake of foods and beverages to maintain health. Drinking water (MIU; hardness = 88) produced from deep sea water (DSW) collected offshore of Muroto, Japan, is considered healthy. We previously reported that the DSW-based drinking water (RDSW; hardness = 1000) improved human gut health. The aim of this randomized double-blind controlled trial was to assess the effects of MIU on human health. Volunteers were assigned to MIU (n = 41) or mineral water (control) groups (n = 41). Participants consumed 1 L of either water type daily for 12 weeks. A self-administered questionnaire was administered, and stool and urine samples were collected throughout the intervention. We measured the fecal biomarkers of nine short-chain fatty acids (SCFAs) and secretory immunoglobulin A (sIgA), as well as urinary isoflavones. In the MIU group, concentrations of three major SCFAs and sIgA increased postintervention. MIU intake significantly affected one SCFA (butyric acid). The metabolic efficiency of daidzein-to-equol conversion was significantly higher in the MIU group than in the control group throughout the intervention. MIU intake reflected the intestinal environment through increased production of three major SCFAs and sIgA, and accelerated daidzein-to-equol metabolic conversion, suggesting the beneficial health effects of MIU.

Keywords: health effect; deep sea water (DSW)-based drinking water; body maintenance; short-chain-fatty-acid; sIgA; daidzein-to-equol conversion; intestinal microbiota

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1. Introduction

The utilization of deep sea water (DSW) has expanded to the energy, agriculture, food, cosmetics, and public health fields [1]. DSW obtained from depths of >200 m is characterized by high purity, stability at low temperatures, high mineral concentrations, and the presence of bioactive nutritional species [2]. Bottled commercial DSW-based drinking water produced by different methods such as desalinization, is currently available on the market; this commercial product is gaining popularity due to its potential benefits to human health, as confirmed by various animal studies [3–6]. However, clinical trials are required to clarify the safety and validity of the effects of DSW-based drinking water on human health.

Previous clinical trials using DSW-based drinking water have confirmed that DSW-based drinking water (RDSW; hardness, 1000 mg/L of Ca/Mg) has various beneficial effects on human health, for example with regard to hemorheology, allergies, immunology, infectious diseases (e.g., anti-*Helicobacter pylori* activity), and the intestinal environment [7–12]. For example, a recent clinical trial reported that drinking RDSW improved human health due to the increased production of short-chain fatty acids (SCFAs) in the intestinal environment and urinary isoflavones [12].

The intestinal environment comprises the microbiota, microbiota-derived metabolites, and ingesta, and includes the microbe–microbe and host–microbe interactions, which play a fundamental role in human health [13]. Healthy foods and beverages, including probiotics and supplements, are widely consumed to maintain and support the intestinal environment and microbiota [14–17]. Recently, fecal microbiota transplantation has been included in the treatment of autoimmune diseases, hepatitis, metabolic syndromes, and mental disorders [18–21] via modulation of the gut microbiota [22,23]. Gut microbes produce various metabolites, such as isoflavones, that are beneficial to human health [24–26]. Isoflavone and other metabolite contents vary among individuals due to differences in the intestinal environment, including microbial identity and activity, stability, and variations in the concentrations of endogenous compounds that modulate biotransformation pathways [27,28]. Equol, which is produced from daidzein by gut microbes, is one of the most physiologically active isoflavones. However, only 30–50% of the human population produces equol; a regional difference exists due to the frequency of soybean consumption. Current research has focused on manipulating the gut environment to enhance equol production.

The purpose of the present study was to assess whether DSW-based drinking water (MIU; hardness, 88) could modulate intestinal microbe biomarkers in healthy adults.

2. Materials and Methods

2.1. Clinical Study Design

This randomized double-blind controlled trial was designed to compare the intestinal environment of individuals in response to drinking MIU vs. mineral water using a self-administered questionnaire and stool and urine sample analysis. The study was conducted in Muroto, Kochi, Japan, from 2018 to 2020. The study protocol, although severely restricted in terms of time and budget, was approved by the Ethics Committees of Kochi University (approval no. 28–93) and the International University of Health and Welfare (approval no. 18-lo-100) and was conducted in accordance with the ethical standards described in the 1964 Declaration of Helsinki and its later amendments. The questionnaire and stool and urine samples were collected before and after the intervention.

2.2. Participants

The study cohort included 114 healthy adults residing in Muroto, Kochi, Japan, who agreed to participate and submitted a signed consent form (Figure 1). Potential participants with any current illness, those using any prescription or commercial drugs or dietary supplements, and pregnant women were excluded from the study. Of the 107 healthy adults who met the inclusion criteria, 82 who correctly completed the questionnaire were randomly divided into 2 groups: the MIU group (n = 41) and the mineral water (control) group (n = 41). The characteristics of the study participants are presented in Table 1. There were no significant differences in terms of age, sex, body mass index, and biomarker concentrations between the 2 groups (Mann–Whitney U test).

Table 1. Preintervention characteristics of the participants from the 2 groups.

	MIU						Mineral Water (Control)					
	Total (*n* = 41)		**male (*n* = 17)**		**female (*n* = 24)**		**Total (*n* = 41)**		**male (*n* = 19)**		**female (*n* = 22)**	
Age (year)	43	(33–53)	47	(33–52)	42.5	(32–53)	42	(33–57)	37	(37–58)	47	(37–58)
BMI(Kg/m^2)	22.6	(20.7–26.4)	23.5	(22.5–27.2)	21.7	(20.3–23.2)	22.9	(21.5–25.5)	22.4	(21.3–25.1)	23.2	(21.9–26.4)
sIgA (μg/g)	408	(209–651)	394	(202–538)	492	(207–678)	555	(169–1042)	449	(159–1016)	614	(21.9–26.4)
Putrefaction (μg/g)												
Phenol	1.3	(0.50–6.70)	4.6	(1.07–13.45)	0.8	(0.45–5.20)	1.4	(0.60–7.20)	2	(0.72–13.55)	1.3	(0.45–5.90)
p-Cresol	28.2	(9.15–69.45)	19.7	(6.00–65.50)	40.7	(10.07–81.30)	59.2	(21.20–90.98)	57.4	(23.80–111.42)	60.65	(19.70–78.90)
4-Ethylphenol	2.3	(1.63–4.15)	2.7	N/A	2.3	N/A	1.7	(0.70–2.90)	1.7	(1.50–7.80)	0.7	(0.70–2.47)
Indol	19.4	(11.80–31.75)	19.4	(9.32–32.37)	19.45	(12.60–31.25)	22.8	(11.85–35.90)	30.4	(15.20–41.70)	17.3	(10.00–27.60)
Skatol	2.75	(1.20–7.80)	2.8	(1.27–7.02)	2.7	(0.57–12.12)	4.8	(1.35–10.00)	2.7	(0.09–0.24)	5.8	(1.40–10.00)
SCFA (mg/g)												
Succinic acid	0.19	(0.09–0.47)	0.21	(0.11–0.36)	0.16	(0.08–0.50)	0.12	(0.08–0.24)	0.14	N/A	0.11	(0.07–0.24)
Lactic acid	0.19	(0.08–0.68)	0.23	(0.13–0.82)	0.12	(0.07–0.44)	0.11	(0.08–0.17)	0.08	(0.07–0.15)		N/A
Formic acid		N/A		N/A		N/A		N/A		N/A		N/A
Acetic acid	3.19	(1.85–4.03)	2.72	(1.65–3.77)	3.27	(2.16–4.04)	2.63	(1.97–3.37)	2.63	(1.90–3.65)	2.59	(1.99–3.69)
Propionic acid	1.02	(0.76–1.28)	1.01	(0.72–1.28)	1.04	(0.79–1.35)	1.12	(0.87–1.57)	1.38	(0.88–1.66)	1.07	(0.85–1.45)
Isobutyric acid	0.16	(0.12–0.20)	0.19	N/A	0.13	(0.11–0.19)	0.15	(0.13–0.19)	0.17	(0.130–0.21)	0.14	(0.130–0.155)
Butyric acid	0.77	(0.54–1.27)	0.73	(0.54–1.05)	0.78	(0.53–1.31)	0.84	(0.54–1.48)	1.03	(0.56–1.80)	0.81	(0.52–1.23)
3-Methylbutanoic acid	0.2	(0.14–0.26)	0.18	(0.14–0.30)	0.21	(0.14–0.25)	0.2	(0.15–0.27)	0.21	(0.16–0.33)	0.2	(0.130–0.25)
Valeric acid	0.21	(0.13–0.31)	0.25	(0.18–0.30)	0.18	(0.12–0.32)	0.17	(0.14–0.29)	0.22	(0.18–0.38)	0.15	(0.13–0.20)
Urine Isoflavones												
Daidzein (mg/g-Cre)	0.9	(0.37–2.15)	0.88	(0.57–1.36)	0.92	(0.36–2.70)	0.79	(0.33–1.60)	0.9	(0.48–1.34)	0.76	(0.19–1.71)
Genistein (mg/g-Cre)	1.11	(0.41–2.10)	1.11	(0.41–1.59)	1.12	(0.54–2.41)	1.03	(0.49–1.76)	1.15	(0.75–2.04)	0.87	(0.46–1.43)
	Total (*n* = 20)		male (*n* = 7)		female (*n* = 13)		Total (*n* = 12)		male (*n* = 8)		female (*n* = 4)	
Equol (mg/g-Cre)	0.5	(0.00–1.73)	0.21	(0.00–5.52)	0.57	(0.26–1.52)	0.98	(0.35–2.14)	1.9	(1.08–2.72)	0.32	(0.19–0.48)
Equol (g/g-Da)	0.72	(0.00–1.72)	0.73	(0.00–3.59)	0.72	(0.15–0.88)	1.82	(0.37–3.01)	2.4	(1.06–4.76)	0.31	(0.15–0.93)
Equol (g/g-E + D)	0.42	(0.00–0.63)	0.42	(0.00–0.75)	0.42	(0.13–0.47)	0.63	(0.27–0.74)	0.71	(0.50–0.83)	0.23	(0.13–0.40)

N/A, less than *n* = 6; The data was shown as median and IQR in parentheses; non-parametric analysis (Mann-Whitney U test).

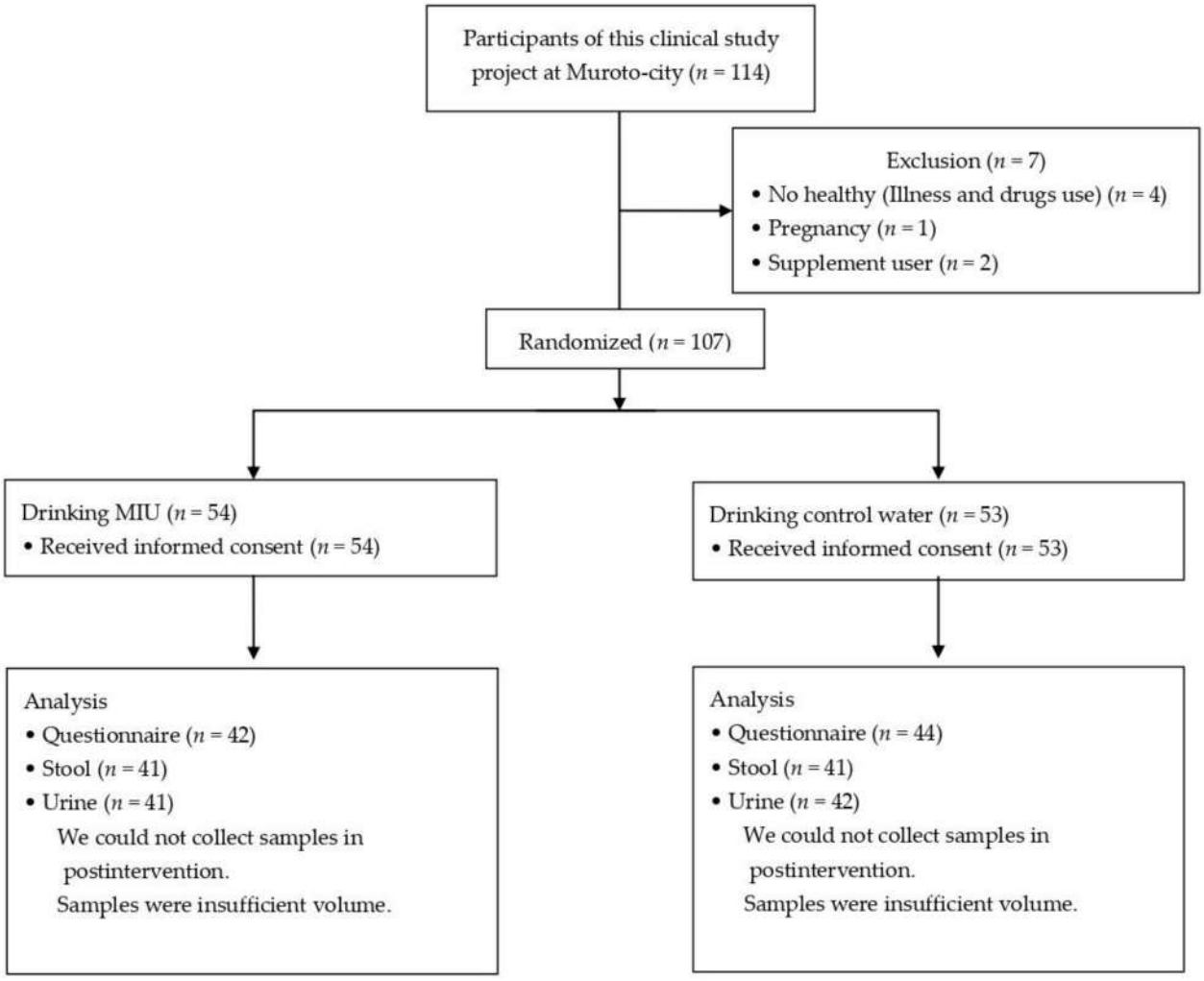

Figure 1. Flow diagram of this clinical study. A total 107 healthy adults were enrolled from Muroto, Kochi, Japan. Potential participants with any current illness, those using any prescription or commercial drugs or dietary supplements, and pregnant women were excluded. Participants in the experimental group consumed MIU (hardness, 88) and those in the control group consumed mineral water (hardness, 0–20).

2.3. Ingestion Schedule

The study participants in the MIU group consumed bottled MIU water (Dydo-miu; hardness, 88; Dydo-Takenaka Beverage Co., Ltd., Kochi, Japan) (Supplementary Table S1), whereas those in the control group consumed mineral water (hardness, 0–20). The most popular mineral water consumed in Japan was used. Neither of the types of water had calories, proteins, fats, carbohydrates, or vitamins. Both bottled waters were commercially available in Japan and the labels were changed to mask the type of water. The participants were instructed to consume 1 L of the assigned water type daily for 12 weeks.

2.4. Evaluation

A self-administered questionnaire was implemented to assess the general health status of the participants. We analyzed the following fecal biomarkers: secretory immunoglobulin A (sIgA), 5 putrefactive products (phenol, *p*-cresol, 4-ethylphenol, indole, and skatole), and 9 SCFAs (succinic, lactic, formic, acetic, propionic, isobutyric, butyric, 3-methylbutanoic, and valeric acids) at TechnoSuruga Laboratory Co., Ltd. (Shizuoka, Japan) [29,30]. Three urinary isoflavones (genistein, daidzein, and equol) were measured in the urine samples. Nonparametric analysis was conducted to assess the differences in these biomarkers before

and after the intervention. Based on the changes in the biomarker concentrations throughout the intervention period, multiple logistic regression analysis was performed to evaluate the relationship between the water type and biomarkers.

2.5. Self-Administered Questionnaire

A total of 86 participants, including 4 who did not submit urine and fecal samples, answered the questions regarding general gut health and eating habits (i.e., constipation (evacuation frequency, incomplete evacuation, straining at stool, dyschezia, etc.), abdominal discomfort, medication use, and consumption of unusual foods and beverages). Constipation was defined in accordance with the guidelines of the World Gastroenterology Organization [31].

2.6. Measurement of Fecal and Urine Samples

Measurements of the samples were taken as previously described [12]. Fecal sample analyses were performed at TechnoSuruga Laboratory Co., Ltd. [29,30].

2.6.1. Fecal sIgA

Here, 0.1 g of each fecal sample suspended in a mixture containing 0.1 mM perchloric acid and 3% phenol was heated and vortexed, followed by centrifugation (15,350× *g*, 10 min) according to previous protocol [12]. The supernatant was collected and filtered (pore size, 0.45 µm) to measure sIgA and SCFA contents. sIgA levels were measured using the Human IgA ELISA Quantitation kit (E80–102; Bethyl Laboratories Inc., Montgomery, TX, USA) and a microplate reader (Varioskan Flash; Thermo Fisher Scientific, Waltham, MA, USA).

2.6.2. Fecal Putrefactive Products

For this, 0.1 g of each fecal sample suspended in 2.5 mL of phosphate buffer including 0.4 mg/L of 4-isopropylphenol as an internal standard was employed to the previous procedures [12]. Briefly, 1 mL of the supernatant was dehydrated and purified with 3 cartridges such as sodium sulfate drying cartridge (Bond Elut LRC; Agilent Technologies, Tokyo, Japan), C18 cartridge (Smart SPE C18-30; AiSTI Science, Wakayama, Japan), and PSA cartridge (Smart SPE PSA-30; AiSTI Science).

The levels of indole and phenol were determined by a single quadrupole gas chromatograph–mass spectrometer (QP-2010; Shimadzu, Kyoto, Japan) equipped with a capillary column (Inert cap WAX; GL Science, Tokyo, Japan). Helium was used as the carrier gas. The injector and interface temperatures were maintained at 240 °C and 230 °C, respectively. For the analysis, 1 µL of the extract was subjected to the splitless mode. The mass spectrometer was operated in the electron impact ionization mode at 70 eV. The measurements were recorded, and data were obtained from the selected ion-monitoring mode for quantification.

2.6.3. Analysis of Intestinal Microbiota

The fecal samples suspended in a buffer containing 4 M guanidium thiocyanate, 100 mM Tris-HCl, and 40 mM ethylenediaminetetraacetic acid were pulverized as previously described [12]. Following this, DNA was extracted from the suspension using the Magtration System 12GC and GC series MagDEA DNA 200 (Precision System Science Co., Ltd., Matsudo, Japan). The final DNA concentration (10 ng/µL) was subjected to the analysis of the microbial community structure by terminal restriction fragment length polymorphism and next-generation sequencing using the MiSeq system (Illumina, San Diego, CA, USA) at TechnoSuruga Laboratory Co., Ltd. [29,30,32]. Bioinformatic analysis was performed using the Ribosomal Database Project (RDP) Multiclassifier tool and Metagenome@KIN software (World Fusion Co., Ltd., Tokyo, Japan) based on data from bacterial species as determined by RDP taxonomic analysis.

2.6.4. Urinary Isoflavones

Measurement of urinary isoflavones (genistein, daidzein, and equol) was performed according to the previous procedures [12]. Briefly, the mixture containing 800 µL urine, 80 µL 1 M sodium acetate and 8 µL β-glucuronidase/sulfatase solution was hydrolyzed, followed by addition of 80 µL of propyl 4-hydroxybenzoate as an internal standard, and then the analytes were extracted. A 20 µL of the residue dissolved in 400 µL of methanol was subjected to a high-performance liquid chromatography system (Shimadzu Co., Ltd., Koto, Japan) and evaluated under the previous conditions [12]. The detection limit of urinary isoflavones was as follows; 100 ng/mL for genistein and daidzein, and 200 ng/mL for equol. The urinary isoflavones were corrected with urinary creatinine (expressed as mg/g-Cre). The equol was corrected for the presence of daidzein (equol/daidzein expressed as g/g-D). The metabolic efficiency of daidzein-to-equol conversion was calculated as equol/ equol + daidzein (expressed as g/g-E + D) [33].

2.7. Statistical Analysis

Throughout the study, we basically performed statistical analysis with nonparametric analyses unless otherwise indicated whenever necessary. The normality test was performed using the Kolmogorov–Smirnov method to assess the normal distribution. Differences in the preintervention biomarker concentrations between the MIU and mineral water (control) groups were identified by the Mann–Whitney U test. There were no significant differences in the baseline characteristics of the participants among the two groups (Table 1). The measured values of all biomarkers of the 2 groups before and after the intervention were compared using the Wilcoxon signed-rank test ($p < 0.05$) as appropriate (Table 2). Fecal formic acid was excluded from the statistical analysis due to the limited number of samples. Based on the differences in the changes to fecal biomarker concentrations throughout the intervention period, multiple logistic regression analysis was performed to evaluate the relationship between the water types and fecal biomarkers ($p < 0.05$). Multiple logistic regression analysis was performed using adequate data excluding extreme values. Participants with detectable ($\geq$ 200 ng/mL) and undetectable equol levels were classified as equol producers and equol nonproducers, respectively. Equol nonproducers were excluded from the statistical analysis for equol level assessment. Daidzein-to-equol conversion efficiency and relative abundance of equol-producing bacteria detected in the equol producers were assessed using the Wilcoxon signed-rank test. All analyses were performed with BellCurve for Excel ver. 3.20 (Social Survey Research Information Co., Ltd., Tokyo, Japan).

Table 2. The values of fecal biomarkers in the 2 intervention groups.

	MIU (*n* = 41)				**Mineral Water (Control) (*n* = 41)**			
	Preintervention		**Postintervention**		**Preintervention**		**Postintervention**	
sIgA (µg/g)	408	(209–651)	515	(319–1039)	555	(169–1042)	479	(215–893)
Putrefaction (µg/g)								
Phenol	1.30	(0.50–6.67)	1.75	(0.85–6.20)	1.40	(0.60–7.20)	1.63	(0.75–4.70) ↑ *
p-Cresol	28.19	(9.15–69.45)	42.56	(12.60–95.72)	59.18	(21.20–90.97)	44.80	(18.22–105.77)
4-Ethylphenol	2.32	(1.62–4.15)	1.92	(0.70–2.20)	1.68	(0.70–2.90)	2.24	(0.75–8.45)
Indol	19.36	(11.80–31.75)	21.44	(14.57–41.10) ↑ *	22.79	(11.85–35.90)	18.78	(8.50–28.45)
Skatol	2.76	(1.20–7.80)	1.98	(1.30–6.85)	4.81	(1.35–10.00)	5.50	(2.30–14.75)
SCFA (mg/g)								
Succinic acid	0.19	(0.09–0.46)	0.11	(0.080–0.220) ↓ **	0.12	(0.08–0.23)	0.14	(0.085–0.345) ↑ *
Lactic acid	0.19	(0.08–0.68)	0.12	(0.10–0.24) ↓ *	0.11	(0.08–0.17)	0.17	(0.08–0.53)
Formic acid	0.24	N/A	0.18	N/A	0.25	N/A	0.25	N/A
Acetic acid	3.19	(1.85–4.02)	3.00	(1.94–4.21)	2.63	(1.97–3.66)	1.99	(1.72–3.20) ↓ **
Propionic acid	1.02	(0.75–1.28)	1.19	(0.81–1.53)	1.12	(0.87–1.57)	0.99	(0.67–1.26) ↓ **
Isobutyric acid	0.15	(0.12–0.20)	0.15	(0.12–0.20)	0.15	(0.13–0.19)	0.15	(0.12–0.21)
Butyric acid	0.77	(0.54–1.26)	0.89	(0.51–1.11)	0.84	(0.54–1.48)	0.61	(0.33–1.04) ↓ **
3-Methylbutanoic acid	0.19	(0.14–0.26)	0.20	(0.14–0.33)	0.20	(0.15–0.27)	0.25	(0.17–0.34)
Valeric acid	0.21	(0.13–0.31)	0.20	(0.14–0.27)	0.17	(0.14–0.29)	0.21	(0.15–0.32)

Table 2. *Cont.*

	MIU (n = 41)				Mineral Water (Control) (n = 41)			
	Preintervention		Postintervention		Preintervention		Postintervention	
Urine Isoflavones	(n = 20)				(n = 12)			
Equol (mg/g-Cre)	0.50	(0.00–1.73)	1.90	(0.50–5.53)	0.98	(0.35–2.14)	1.30	(0.14–2.00)
Equol (g/g-Da)	0.72	(0.00–1.72)	2.74	(0.53–4.06)	1.82	(0.37–3.01)	0.31	(0.00–2.60)
Equol (g/g-E + D)	0.42	(0.00–0.63)	0.73	(0.34–0.80) ↑ #	0.63	(0.27–0.74)	0.25	(0.17–0.80)

*, $p < 0.05$; **, $p < 0.01$; #, $p < 0.1$; N/A, less than $n = 6$; The data was shown as median and IQR in parentheses.; non-parametric analysis (Wilcoxon rank sum test); ↑: increase; ↓; decrease.

3. Results

3.1. Self-Administered Questionnaire

The questionnaire (n = 86) revealed that three and two participants in the MIU and control groups, respectively, suffered from constipation as per the guidelines of the World Gastroenterology Organization prior to the study [31]. Drinking water ameliorated the symptoms of constipation in all (100%) and none (0%) of the individuals in the MIU and control groups, respectively. Improvement in constipation was observed only in the MIU group, although the small number of samples limited the suitability of the definition of constipation.

3.2. Analysis of Fecal sIgA, Putrefactive Products, and SCFAs

The values of fecal biomarkers throughout the intervention period are summarized in Table 2. Overall, in the postintervention period, the sIgA concentration increased in the MIU group and decreased in the control group compared with the concentrations in preintervention period. Thus, the preintervention data of the two subgroups were analyzed in detail to evaluate the sustainable effect on intestinal immune status between the low and high sIgA subgroups with the median (<500 and ≥500 μg/g, respectively). The postintervention sIgA concentration significantly increased in both low-value subgroups. Conversely, the sIgA concentration significantly decreased in the high-value subgroup of the control group but remained unchanged in the high-value subgroup of the MIU group(Figure 2). Among putrefactive products in the postintervention period, indol significantly increased in the MIU group and phenol significantly increased in the control group.

Differences in the changes in SCFAs concentrations throughout the intervention period were analyzed using the Mann–Whitney U test (Table 2). In few minor SCFAs, an increase/decrease of amounts was observed. In particular, the levels of succinic acid and lactic acid decreased in the postintervention period in the MIU group but not in the control group. However, overall, the differences in the changes in the nine SCFAs among the two groups were similar. On the other hand, the total amounts of the three major SCFAs (acetic, propionic, and butyric acids) slightly increased during the postintervention period in the MIU group. A decrease was only observed in the control group ($p < 0.1$, Wilcoxon signed-rank test), with a 23% difference between the two groups (Figure 3a). The populations of the responders whose concentrations of three major SCFAs increased in the postintervention period were significantly higher in the MIU group than in the control group, as determined by the Chi-squared test (Figure 3b). Notably, there were no significant differences between males and females.

Based on the differences in the changes to the fecal biomarker concentrations throughout the intervention period, multiple logistic regression analysis was performed to evaluate the relationship between the two types of water and fecal biomarkers. The results revealed that MIU significantly affected only one biomarker (butyric acid). In addition, MIU more noticeably impacted the intestinal concentrations of the three major SCFAs. Among the 82 participants, formic acid was detected in the range of 0.01–0.02 mg/mL (limit of detection, 0.01 mg/mL) in relatively few samples, suggesting that only minute amounts of formic acid are produced in the human intestine.

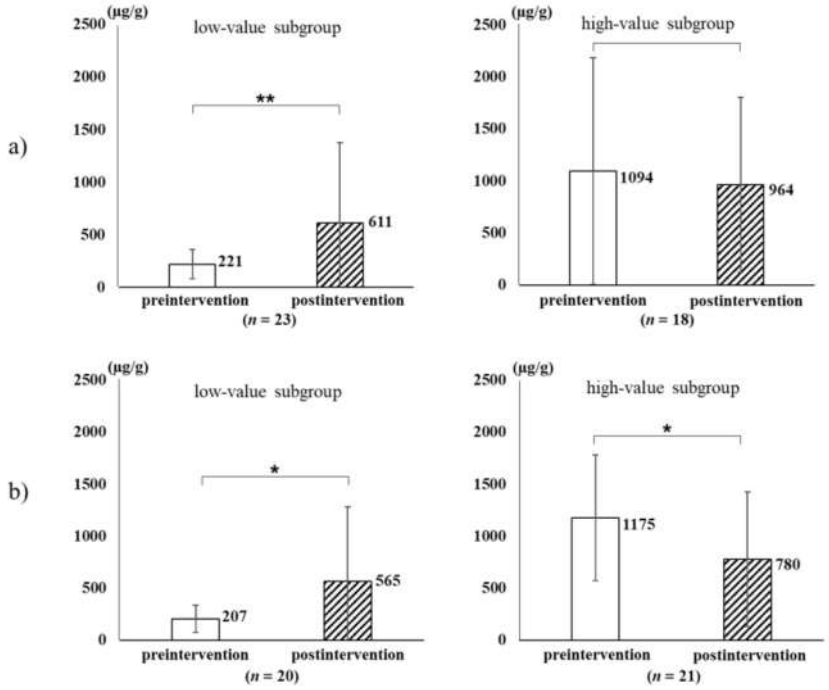

Figure 2. Differences in the changes to sIgA concentrations throughout the intervention period in the MIU (**a**) and control (**b**) groups. Participants in the MIU and control groups was further classified into 2 subgroups: low and high sIgA preintervention levels (<500 vs. ≥500 μg/g). The concentration of sIgA throughout the intervention period significantly increased in both low-value subgroups irrespective of the water type. However, sIgA significantly decreased in the high-value subgroup of the control group but remained unchanged in the MIU group. Open bar, preintervention; hatched bar, postintervention. Bar depicts standard deviation. * $p < 0.05$; ** $p < 0.01$.

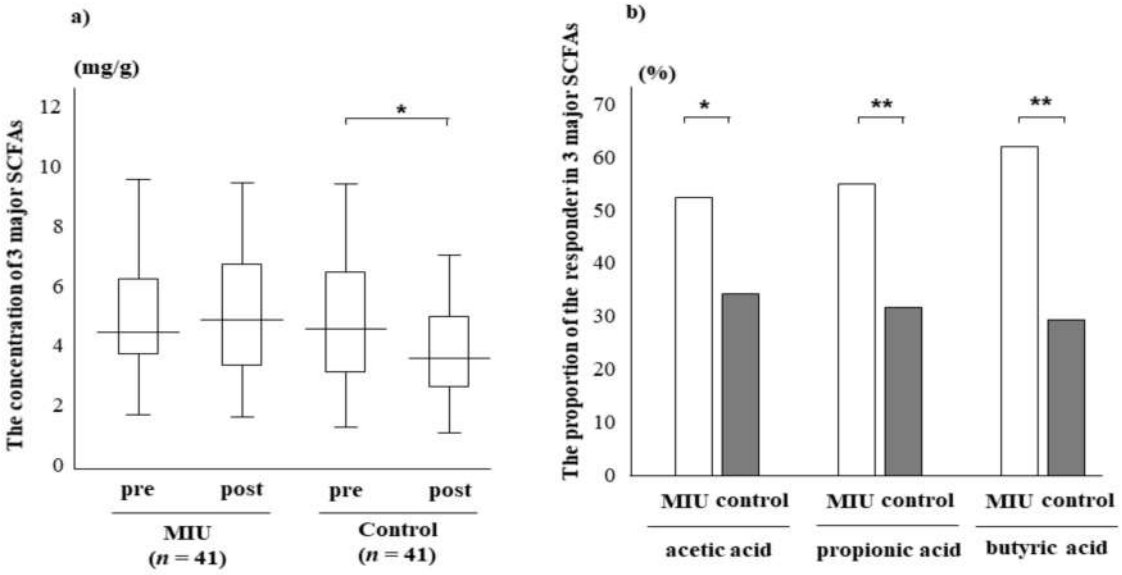

Figure 3. Effect of MIU or control water on fecal biomarker concentrations of 3 SCFAs (acetic acid, propionic acid, and butyric acid) throughout the intervention period. (**a**) The concentrations of the SCFAs decreased in the control group (* $p < 0.1$). There was a 23% difference between the 2 groups. The top and bottom of each box indicate the 25th and 75th percentiles, and the solid line within the box is a median. Whiskers depict the minimum and maximum values. pre, preintervention; post, postintervention, (**b**) The proportions of responders were significantly higher in the MIU group than in the control group. * $p < 0.05$; ** $p < 0.01$.

3.3. Analysis of Urinary Isoflavones

The results of urinary isoflavone analysis of the 41 and 42 participants in the MIU and control groups, respectively, are presented in Figure 1 and Table 1. The focus of this analysis was the differences in the changes to equol concentrations, which is among the most physiologically active isoflavones [34]. Throughout the intervention period, urinary equol was detected in 20 and 12 participants (who were identified as equol producers) in the MIU and control groups, respectively. Overall, equol was identified in 38.6% (32/83) of participants. Interestingly, among the 32 equol producers, 8 participants (6 in the MIU group and 2 in the control group, respectively) became equol producers during the intervention period.

If urinary isoflavones were not detected in a sample, the value was considered 0. The equol value was corrected with creatinine (mg/g-Cre) and daidzein (g/g-D). In addition, the metabolic efficiency of daidzein-to-equol conversion was calculated as equol/equol + daidzein (g/g-E + D), as mentioned above. The changes in equol concentrations throughout the intervention period between the two groups are presented in Table 2. All three evaluations revealed increased equol concentrations in the MIU group. The metabolic efficiency of daidzein-to-equol conversion significantly increased in the MIU group compared with the control group ($p < 0.1$, Wilcoxon) (Figure 4).

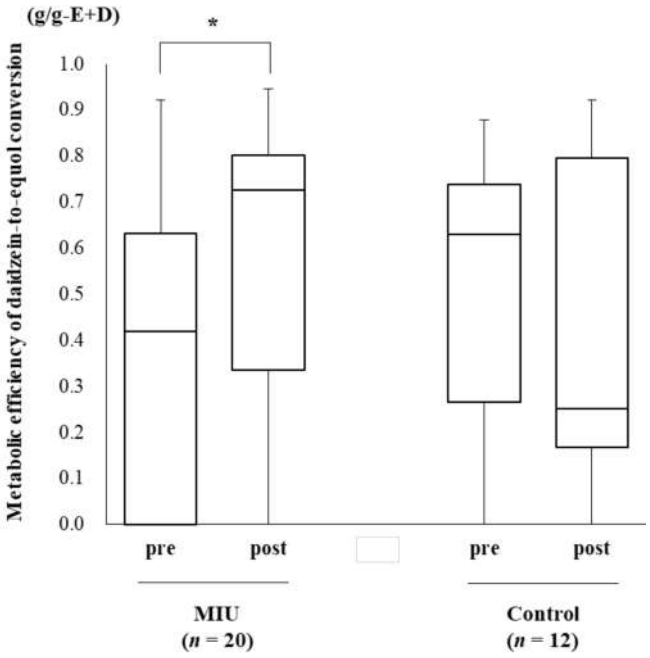

Figure 4. Effect of MIU or control water on the metabolic efficiency of daidzein-to-equol conversion throughout the intervention period. The metabolic efficiency of daidzein-to-equol conversion was significantly prompted in the MIU group. The top and bottom of each box indicate the 25th and 75th percentiles, and the solid line within the box is the median. Whiskers depict the minimum and maximum values. pre, preintervention; post, postintervention, * $p < 0.1$.

3.4. Analysis of Fecal Microbiota in Equol Producers

Fecal microbiota analysis of the 32 equol producers identified 15 equol-producing bacteria (Table 3) [34,35]. The metabolic efficiency of daidzein-to-equol conversion was significantly greater in the equol producers in the MIU group as compared to the control group throughout the intervention period. Thus, relative abundance of equol-producing bacteria detected in the equol producers was compared before and after drinking MIU. Of 15 equol-producing bacteria, the median of relative abundance of *Bacteroides ovatus* especially increased from 0.064% (IQR, 0.021–0.262%) to 0.126% (0.034–0.217%) without statistical significance (Wilcoxon signed-rank test).

Table 3. Detection of equol-producing bacteria in the 32 subjects whose urinary equol levels were detected in this study.

Adlercreutzia equolifaciens	*Asaccharobacter celatus*	*Bacteroides ovatus*
Bifidobacterium animalis	*Bifidobacterium breve*	*Bifidobacterium longum*
Finegoldia magna	*Lactobacillus graminis*	*Lactobacillus intestinalis* *
Lactobacillus mucosae	*Lactobacillus sakei*	*Pediococcus pentosaceus* *
Slackia equolifaciens	*Slackia isoflavoniconvertens*	*Streptococcus intermedius*

Twenty and 12 from MIU and control groups, respectively. * Not detected in control group.

Furthermore, the differences in intestinal microbes were analyzed, except for 15 known equol-producing bacteria in the 8 participants who became equol producers during the intervention period. Intestinal microbiota analysis was able to be performed in 5 of the 8 participants (4 in the MIU group and 1 in the control group, respectively). The results revealed that the relative abundances of *Blautia wexlerae* and *Streptococcus cristatus* increased throughout the intervention period in the five equol producers (Table 4).

Table 4. List of the increased bacteria detected postintervention in 5 equol producers.

Blautia wexlerae	*Streptococcus cristatus*	
Increased bacteria detected in 4 of 5 equol producers		
Blautia faecis	*Butyricicoccus desmolans*	*Clostridium aldenense*
Clostridium bolteae	*Eggerthella lenta*	*Enterococcus avium*
Eubacterium hallii	*Fusobacterium varium*	*Gemella sanguinis*
Lactococcus lactis	*Murimonas intestini*	*Ruminococcus lactaris*
Solobacterium moorei		

4. Discussion

Current world trends are focused on the balanced intake of foods and beverages to promote human health. However, the quality of products currently available in the market is questionable because of a lack of clinical studies. RDSW (hardness, 1000) is reported to improve the intestinal environment [12]. In this study, the average of total amount of the three SCFAs slightly increased in MIU (hardness, 88); however, MIU mainly increased sIgA production as a fecal biomarker. It also improved the metabolic efficiency of daidzein-to-equol conversion and, subsequently, the intestinal environment. Intriguingly, MIU significantly induced sIgA secretion, and the increased level was continuously maintained irrespective of the sIgA level in the preintervention period. However, this was not seen in the control group, indicating that MIU sustainably maintains the intestinal immune status with inducible sIgA. Furthermore, the metabolic efficiency of daidzein-to-equol conversion was accelerated in the MIU group. These findings were not observed in a previous clinical study with RDSW [12]. We previously found increased concentrations of five SCFAs in the RDSW. Thus, the influences differed even with similar DSW-based drinking water, which was likely due to the hardness and manufacturing process. The reference values of the constituents (i.e., putrefaction, SCFA, isoflavones) measured in this study are not defined at present. New investigations could assess these effects on immune and inflammatory responses, gut microbiota, and microbial products in healthy adults. However, at least in healthy persons, the increased constituents (IgA and SCFAs) are considered as beneficial to the body and not a disadvantage. We found no adverse events during this clinical study.

It is generally accepted that isoflavones can ameliorate the symptoms of various syndromes and diseases, including cancers. In particular, the physiological activities of equol produced by bacterial daidzein conversion in the intestine can reduce the risk of several diseases [34]. Epidemiological evidence suggests that equol production (~30–50% worldwide) is related to environmental factors, dietary habits, and gut microbes that convert daidzein to equol (daidzein-to-equol conversion). To date, a limited number of bacteria capable of daidzein-to-equol conversion have been identified [34,35]. Thus, further studies are warranted to identify foods, beverages, and as-yet unidentified bacteria capa-

ble of daidzein-to-equol conversion to improve the intestinal environment and maintain human health.

In this study, MIU increased the metabolic efficiency of daidzein-to-equol conversion, which benefited human health by improving the intestinal environment. Of the 15 equol-producing bacteria detected in 32 subjects, the relative abundance of *B. ovatus* especially increased in the MIU group without statistical significance, suggesting that MIU intake influenced the equol-producing bacteria, including *B. ovatus*. Furthermore, fecal microbiota analysis of five participants who became equol producers throughout the intervention period demonstrated that the proportions of *B. wexlerae* and *S. cristatus* had significantly increased in the postintervention period. *Blautia* spp., which is dominant in the human intestine, can ameliorate the symptoms of inflammatory and metabolic diseases and improve antibacterial activity [36,37]. These physiological activities of probiotics are beneficial to human health [38]. In particular, *B. wexlerae* reduces inflammation associated with obesity-related complications and produces acetic acid as a final product of glucose fermentation [38,39]. The relative abundance of *B. wexlerae* in the postintervention period was observed in all five equol producers, suggesting that *B. wexlerae* could be involved in equol production. However, no study has investigated the relationship between equol production and *Blautia* spp. in the intestine. Hence, further studies are needed to investigate the contribution of *B. wexlerae* to equol production and/or the metabolic efficiency of daidzein-to-equol conversion. Previous studies have reported that *S. cristatus* peptides repressed expression of the virulence genes of *Porphyromonas gingivalis*, which had an inhibitory effect on the oral microbiota [40,41]. The physiological function of *S. cristatus* is mostly unknown; thus, in vitro studies are warranted to investigate the activities of *S. cristatus* in the intestinal environment to clarify the connection with equol. In addition, to evaluate the improvement of intestinal microbiota, all 82 fecal samples collected during the preintervention period were subjected to PCR analysis with specific primers for the amplification of methicillin-resistant *Staphylococcus aureus* (MRSA) [42]. The results demonstrated that three participants in the MIU group were healthy MRSA-carriers and all three were free of MRSA postintervention, indicating that MIU eventually cleared MRSA from the intestine, although the results were limited by the small number of MRSA carriers (data not shown). Furthermore, an increased concentration of indol was observed postintervention in the MIU group. Indol is a metabolite produced from tryptophan by intestinal microbiota, and this thus suggests that MIU intake influenced the intestinal microbiota/condition.

The health effects of isoflavones, including equol, are dependent on the quantity and bioavailability of absorbed nutrients [43]. Isoflavones are converted to aglycones (daidzein and genistein) from glycones (daidzin and genistin) by enzymes (i.e., β-glucosidase) in intestinal microbes/conditions. The aglycones absorbed in the body physiological function. Thus, we measured urinary isoflavones (daidzein, genistein, and equol) to evaluate the health effects. The lifestyle factors of the participants were strictly monitored to assess dietary habits and the consumption of unusual foods, beverages, and supplements throughout the study period. MIU probably influenced not only the metabolic efficiency of daidzein-to-equol conversion but also the absorption efficiency via the intestinal environment. An improvement of constipation was suggested in the MIU group only, although no significant difference was observed due to the small number of participants fitting the definition of constipation. Taken together, the findings of this study indicate that MIU intake induces these biomarkers in the intestinal environment.

5. Conclusions

This clinical study revealed that a long-term intervention with MIU mainly induced sIgA production and increased the metabolic conversion of daidzein-to-equol, suggesting an adaptation of the host/microbe which influences human health.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/nu14030581/s1, Table S1: Nutrition facts of Dydo-MIU, hardness, 88 (100 mL).

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Institutional Review Board Statement: The study protocol, although severely restricted in terms of time and budget, was approved by the Ethics Committees of Kochi University (approval no. 28–93) and the International University of Health and Welfare (approval no. 18-lo-100) and was conducted in accordance with the ethical standards described in the 1964 Declaration of Helsinki and its later amendments.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

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