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Original Article

Alleviative effects of deep-seawater drinking water on hepatic lipid accumulation and oxidation induced by a high-fat diet

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Abstract

Background: Hepatic steatosis is defined as excessive amounts of triglyceride and other fats inside liver cells and has become an emergent liver disease in developed and developing countries.
Methods: Deep seawater (DSW)300, DSW900, and DSW1500 drinking waters were formulated via a combination of reverse osmosis and electrodialysis. Hamsters on a high-fat diet were assigned to drink the following solutions: (1) normal distilled water, (2) DSW300, (3) DSW900, or (4) DSW1500. Serum, liver, and fecal biochemical values, expression of hepatic genes related to fatty-acid homeostasis, as well as liver antioxidative levels were measured after a 6-week feeding period. Additionally, hematoxylin and eosin staining was used to investigate the liver histopathology.
Results: Serum/liver lipids, liver sizes, liver malondialdehyde content, and serum aspartate aminotransferase and alanine aminotransferase of high-fat diet hamsters were reduced ($p < 0.05$) by drinking DSW, while daily fecal lipid and bile acid outputs were increased ($p < 0.05$). DSW drinking water maintained ($p < 0.05$) higher liver glutathione and Trolox equivalent antioxidant capacity levels. Although hepatic sterol regulatory element-binding protein-1c, acetyl-CoA carboxylase, fatty acid synthase, and malic enzyme gene expression were not ($p > 0.05$) altered, DSW drinking water upregulated ($p < 0.05$) hepatic peroxisome proliferator-activated receptor-alpha, retinoid X receptor alpha, and uncoupling protein-2 gene expression in high-fat diet hamsters. The lipid droplets in livers were also reduced in DSW-drinking-water groups as compared to those only drinking distilled water.
Conclusion: DSW shows a preventive effect on development of hepatosteatosis induced by a high-fat diet.

Keywords: deep-seawater drinking water; lipid homeostasis; liver lipid peroxidation; nonalcoholic hepatosteatosis

1. Introduction

A hypercaloric dietary habit easily results in increased body weight, serum lipids, and hepatic lipid accumulation. Hepatic steatosis is defined as excessive amounts of triglyceride and other fats inside liver cells and has become an emergent liver disease with prevalence estimated to be 20−30% the in general population of Western countries.[1,2] Generally, hepatic

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steatosis is always coupled with other diseases, i.e. obesity, diabetes, and hyperlipidemia.[3] In addition, increased liver lipid accumulation causes lipid peroxidation, further advancing liver damage. Hence, the clinical implications of hepatic steatosis are mostly due to its potential to cause a chronic inflammation and then progress to cirrhosis, liver failure, and hepatocellular carcinoma.

Deep seawater (DSW) designates water from 200 m below the surface of the sea. DSW is characterized by high purity, low temperature, high nutrients, and minerals, and has recently been tested in trials as a multifunctional material for the food, agricultural, cosmetic, and medical fields. DSW has also been reported to contain high levels of minerals, such as magnesium (Mg), calcium (Ca), and potassium (K) compared with surface and middle seawater.[4,5] It was reported that DSW (hardness: 1000 ppm) shows an amelioration of body-weight increase, blood sugar, and serum lipids in *ob/ob* mice.[6] Recently, the health effects of DSW from sea water below 700 m in the outer sea of Hua-Lien County, Taiwan have been demonstrated.[7] It was also reported that electrodialysis (ED)-treated DSW (ED DSW; hardness: 4685.90 ppm) could decrease serum lipids and improve blood cholesterol profile.[7] However, from the viewpoint health food development, ED DSW contains very high concentrations of minerals that may cause dangers for heart or kidney. Hence, we have formulated drinking waters from DSW (hardness: 300, 900, and 1500 ppm) via a combination of reverse osmosis (RO) and ED, and observed that those DSW drinking waters could upregulate expression of hepatic low-density-lipoprotein receptor and cholesterol-7α-hydroxylase genes to lower serum cholesterol levels, and concurrently decrease serum lipid oxidative levels.[8]

Normalizing serum lipids is also known to be a way to hinder the occurrence of hepatic steatosis. The sources of body triglycerides are mainly diet and *de novo* synthesis. Hepatic fatty-acid homeostasis in mammals is regulated by fatty-acid biosynthesis (i.e. sterol regulatory element-binding protein-1c, SREBP-1c; acetyl-CoA carboxylase, ACC; fatty acid synthase, FAS; malic enzyme, ME)[9] and energy expenditure (peroxisome proliferator-activated receptor-alpha, PPAR-α; retinoid X receptor alpha, RXRα; uncoupling protein-2, UCP2).[10–12] Although we have demonstrated that our formulated Mg-rich DSW drinking water upregulates low-density-lipoprotein receptor and cholesterol-7α-hydroxylase gene expression, which is partially related to a decrease in serum cholesterol in hamsters fed a high-fat/cholesterol diet hamsters,[8] the information regarding effects of those Mg-rich DSWs on other regulators of fatty-acid homeostasis in livers was quite limited. Additionally, a high-fat dietary habit results in hepatic lipid peroxidation when hepatic malondialdehyde (MDA) contents are increased, thus increasing probabilities of hepatic inflammation and steatosis.[13,14] Trolox equivalent antioxidant capacity (TEAC) and glutathione (GSH) are often used to evaluate antioxidative levels in biological systems.[14,15] Lowered lipid peroxidation could alleviate liver damage in a high-fat-diet-induced hepatotoxicity. Therefore, the present study investigated whether DSW drinking water in high-fat diet hamsters could: (1) regulate hepatic fatty-acid homeostasis; (2) improve hepatic antioxidative levels; and (3) attenuate hepatic damage.

2. Methods

2.1. Manufacture of DSW drinking waters

The collection and processing procedures of drinking water from DSW were according to our previous method.[8] Original DSW samples were collected from a depth of approximately 618 m in Chisingtan Bay, Hua-Lien County, Taiwan at the same time. Collected original DSW was treated with RO and ED to reduce the mineral contents, especially sodium (Na). DSW drinking waters (300, 900, and 1500 ppm) were formulated via a combination of RO and ED. DSW drinking waters were also pasteurized (80°C, 60 seconds) and immediately stored at −20°C until fed to the hamsters. The mineral contents of DSW drinking waters were analyzed using an inductively optical emission spectrometer (JY ULTIMA 2000, Horiba, France) and are shown in Table 1.

2.2. Animals and diets

The animal usage and protocol were reviewed and approved by the National Taiwan University Animal Care Committee. Thirty-two male Golden Syrian hamsters aged 5 weeks were purchased from the National Laboratory Animal Center (National Science Council, Taipei, Taiwan). Two hamsters were housed in each cage in an animal room at 22 ± 2°C with a 12 hour/12 hour light/dark cycle and fed standard chow diets (Laboratory Rodent Diet 5001, 5% lipid/0% cholesterol) with distilled water for 1 week. After the acclimation period, all hamsters were fed a high-fat diet, which was formulated as 92.8% (w/w) chow diets supplemented with 7% (w/w) butter and 0.2% (w/w) cholesterol.

At the beginning of experiment, two hamsters per cage were randomly assigned to one of the following groups: (1) normal distilled water (NDW); (2) 300 ppm DSW drinking water (DSW300); (3) 900 ppm DSW drinking water (DSW900); or (4) 1500 ppm DSW drinking water (DSW1500). All hamsters were fed the high-fat diets and assigned drinking solutions (NDW, DSW300, DSW900, or DSW1500) *ad libitum* for 6 weeks.

Table 1
Mineral contents of different drinking solutions.

	NDW	DSW300	DSW900	DSW1500
Na (mg/L)	0.49	212.00	305.00	380.00
K (mg/L)	0.10	13.00	20.48	27.80
Ca (mg/L)	0.28	12.50	48.00	65.00
Mg (mg/L)	0.38	68.00	188.00	330.60
Hardness (ppm)	2.26	310.05	890.80	1517.96

Hardness (ppm) = Ca (mg/L) × 2.5 + Mg (mg/L) × 4.1.[26]
DSW300 = 310.05 ppm deep-seawater drinking water; DSW900 = 890.80 ppm deep-seawater drinking water; DSW1500 = 1517.96 ppm deep-seawater drinking water; NDW = normal distilled water.

2.3. Serum, heart, liver, kidney, and feces

At the end of experiment, all feed was removed 12 hours before euthanasia by CO_2, after which the heart, liver, and kidney from each hamster were removed and weighed. The liver was stored at −80°C for further analyses. Blood samples were also collected by intracardiac puncture. Sera were separated from blood samples by centrifugation at 3000*g* for 10 minutes, and then stored at −80°C for further analyses. Feces were collected from each cage 24 hours before the end of the experiment and stored at −20°C for further analyses.

2.4. Serum biochemical values, liver lipids, and fecal lipids/bile acid

Serum total cholesterol (TC) and triglyceride (TG), and liver damage indices [aspartate aminotransferase (AST) and alanine aminotransferase (ALT)] were determined using commercial enzymatic kits with the SPOTCHEM EZ SP-4430 automated analyzer (ARKRAY, Inc., Kyoto, Japan). Liver TC/TG and fecal TC/TG/bile acid concentrations were measured using the technique of a previous study.[14] Briefly, hepatic or fecal lipid was extracted by chloroform and methanol (2:1, v/v). The extract was dried under N_2 and resuspended in isopropanol. Triglyceride, cholesterol, and bile acid concentrations were measured using commercial kits (Randox Laboratories Ltd., Crumlin, Antrim, UK) and multiplied with daily fecal weight (g) to obtain daily fecal cholesterol, triglyceride, and bile acid outputs.

2.5. Hepatic MDA and GSH contents, and TEAC

A 0.5 g sample of liver was homogenized on ice in 4.5 mL phosphate-buffered saline (PBS, pH 7.0, containing 0.25M sucrose) and centrifuged at 12,000*g* for 30 minutes. The supernatant was collected for further analyses. The protein content in the supernatant was measured according to the procedures of a Bio-Rad protein assay kit (Cat#: 500-0006; Bio-Rad Laboratories, Inc., Hercules, CA, USA) and using bovine serum albumin as a standard.

The hepatic MDA and GSH contents were measured according to procedures described by Fang et al.[16] The hepatic MDA content was calculated by taking the extinction coefficient of MDA to be 1.56×10^5 $M^{-1}cm^{-1}$ at 535 nm by using a UV Visible Spectrophotometer (Model T60; PG Instruments Ltd., Wibtoft, Leicestershire, UK). Due to the unique thiol compound in GSH, 2,2-dithiobisnitrobenzoic acid (DTNB) is commonly used for thiol assay. The hepatic GSH content was calculated the GSH content by taking the extinction coefficient of 2-nitro-5-thiobenzoic acid (NTB) to be 1.36×10^4 M^{-1} cm^{-1} at 412 nm by using a UV Visible Spectrophotometer (PG Instruments Ltd.). Hepatic TEAC was analyzed according to a method described by Chang et al.[14] A free radical, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid$^+$) ($ABTS^+$), can be generated by mixing ABTS (100 μM) with H_2O_2 (50 μM) and peroxidase (4.4 U/mL). The TEAC value was expressed as a scavenging capacity against $ABTS^+$. Briefly, 0.25 mL of mixture of ABTS, H_2O_2, and peroxidase, and 1.5 mL dd H_2O were mixed well and placed under a dark room. After 30 minutes, 0.25 mL diluted liver homogenate (1%, v/v) was then added. Absorbance was measured at 734 nm after the interaction of sample solution for 10 minutes. The decrease in absorption at 734 nm after the addition of reactant was used to calculate the TEAC value. A standard curve was plotted for Trolox on scavenging $ABTS^+$ capacity and was calculated as the TEAC. A higher TEAC value of a sample results in stronger antioxidant activity.

2.6. Hepatic gene expressions

Total RNA was isolated from the stored frozen liver tissues by using the protocol described by RNeasy Mini Kits (Qiagen, Valencia, CA, USA). Reverse transcription was carried out with 2 μg total RNA, 8 μL reaction buffer, 2 μL dNTPs, 4.8 μL $MgCl_2$, 4 μL Oligo-dT (10 pM), and 200 U RTase (Promega, Madison, WI, USA) with diethyl pyrocarbonate H_2O in a final volume of 40 μL at 42°C for 1 hour. After heat inactivation, 1 μL cDNA product was used for PCR amplification. The appropriate primers of target genes were designed for hamster SREBP-1c (GenBank no.: U09103), ACC (GenBank no.: AF356089), FAS (GenBank no.: AF356086), ME (GenBank no.: NM008615), PPAR-α (GenBank no.: NM001113418.1), RXRα (GenBank no.: NM_002957), UCP2 (GenBank no.: NM_011671.4), and GAP DH (GenBank no.: XR031141) as follows: SREBP-1c sense 5′-GGTGGGCACTGAGGCAAAGC-3′, antisense 5′-CGCACACAGGGCTAGGCGGG-3′; ACC sense 5′-GGTGGTGGCATTGAAGGAGC-3′, antisense 5′-CCACATAAGAGTTGGGAGAC-3′; FAS sense 5′-AGCCCCTCAAGTGCACAGTG-3′, antisense 5′-CACGTGTATGCCCTGGCGCC-3′; ME sense 5′-TCAGACAGTGCTGTTCCAGG-3′, antisense 5′-GTTCATGGGCAAACACCTCT-3′; PPAR-α sense 5′-GGACAAGGCCTCAGGGTACC-3′, antisense 5′-CCACCATCTTGGCCACAAGC-3′; RXRα: sense 5′-TGCTGCGGGCAGGCTGGAATGA-3′, antisense 5′-GTCAG CACCCGATCAAAGAT-3′; UCP2: sense 5′-TCCCTTGCCACTTCACTTCT-3′, antisense 5′-GCTGCTCATAGGTGACAAACA-3′; GAPDH sense 5′-GACCCCTTCATTGACCTCAAC-3′, antisense 5′-GGAGATGATGACCCTTTTGGC-3′. The sizes of reaction products were: for SREBP-1c, 412 bp; ACC: 362 bp; FAS, 347 bp; ME: 191 bp; PPAR-α, 421 bp; RXRα, 154 bp; UCP2, 239 bp; GAPDH, 264 bp. GAPDH was used as an internal control in all reactions. The PCR amplifications were performed under conditions using a DNA thermal cycler (ASTEC PC-818, ASTEC Co., Ltd., Fukuka, Japan) under the following conditions: SREBP-1c and FAS: 35 cycles of 94°C for 1 minute, 55°C for 1 minute, and 72°C for 2 minutes followed by 10 minutes at 72°C; ACC and ME: 40 cycles of 94°C for 1 minute, 56°C for 1 minute, and 72°C for 2 minutes followed by 10 minutes at 72°C; PPAR-α: 25 cycles of 94°C for 1 minute, 55°C for 1 minute, and 72°C for 2 minutes followed by 10 minutes at 72°C; RXRα and UCP2: 38 and 41 cycles, respectively, at 94°C for 1 minute, 55°C for 1 minute, and 72°C for 2 minutes followed by 10 minutes at 72°C; GAPDH: 25 cycles of 94°C for 1 minute, 52°C for 1 minute, and 72°C for 2 minutes followed by 10 minutes at 72°C. The final products were subjected to electrophoresis on a 2% agarose gel

and detected by ethidium bromide staining under UV. The relative expression levels of the mRNAs of the target genes were normalized using the GAPDH internal standard.

2.7. Histopathological analysis

For histopathological study, the liver tissues were placed in formalin for no longer than 24 hours and were fixed in neutral-buffered formalin solution, dehydrated in graded alcohol, cleared in xylene, and embedded in paraffin. These blocks were later sectioned using a microtome, dehydrated in graded alcohol, embedded in paraffin section, and stained with hematoxylin and eosin.

2.8. Statistical analysis

The experiment was conducted using a completely random design. Data were analyzed using analysis of variance. A significant difference was identified at 0.05 probability level and differences between treatments were tested using the least significant difference test. All statistical analyses of data were performed using SAS software (SAS Institute, Inc., Cary, NC, USA).

3. Results

3.1. Weight gain, organ sizes, serum/liver lipids, liver histological analysis, liver damage, and daily fecal lipid/bile acid output

No significant differences ($p > 0.05$) in weight gain (g and %), and heart and kidney sizes were observed among groups, but liver sizes of high-fat diet hamsters were reduced ($p < 0.05$) by drinking DSWs (Table 2). Decreased ($p < 0.05$) serum/liver lipids (TC and TG) of high-fat diet hamsters were also observed after 6 weeks of drinking DSWs (Table 2). Based on the histological examination of hamsters' livers, there was accumulation of lipid drops in liver cells in high-fat diet hamsters, but drinking DSWs apparently decreased them (Fig. 1A vs. 1B–1D). Regarding the liver damage indices, serum AST values of high-fat diet hamsters were also attenuated ($p < 0.05$) by drinking DSW300, -900, and -1500 groups; however, lower ($p < 0.05$) serum ALT values were observed in both DSW900 and DSW1500 groups compared to the NDW group (Table 2). Additionally, drinking DSWs increased ($p < 0.05$) daily fecal cholesterol, triglyceride, and bile acid outputs of high-fat diet hamsters while DSW1500 group had the most ($p < 0.05$) fecal bile acid output followed by DSW900, DSW300, and NDW groups (Table 3).

3.2. Lipid antioxidative status and fatty-acid homeostasis in livers

MDA levels were used to evaluate the oxidative level in livers, and GSH and TEAC were used to assess the antioxidative levels in livers. High-fat diet hamsters drinking DSW drinking waters had lower ($p < 0.05$) liver MDA contents when compared to those only drinking NDW (Fig. 2A). In hepatic GSH levels, although only high-fat diet hamsters drinking DSW1500 had higher ($p < 0.05$) GSH levels than those drinking NDW, there was a tendency toward higher GSH levels in high-fat diet hamsters drinking DSW300 and DSW900 compared to NDW (Fig. 2B). Regarding the total antioxidative level in livers, the TEAC values in high-fat diet hamsters were elevated ($p < 0.05$) by drinking DSWs (Fig. 2C). Expression of genes related to fatty-acid homeostasis (SREBP-1c, ACC, FAS, ME, PPAR-α, RXRα, and UCP2) of hamsters after 6 weeks of feeding, are illustrated in Fig. 3. In fatty acid biosynthesis, DSWs did not ($p > 0.05$) alter SREBP-1c, ACC, FAS, and ME gene expression. However, DSWs (especially DSW1500) upregulated ($p < 0.05$) PPAR-α, RXRα, and UCP2 gene expression, which triggers β-oxidation of fatty acids in livers.

4. Discussion

Generally, high-fat diets significantly increase body and liver weights, leading to obesity, hyperlipidemia, and hepatic

Table 2
Weight gain, serum lipids, organ sizes, liver lipids, and liver damage indices of the experimental hamsters.

Group	NDW	DSW300	DSW900	DSW1500
Weight gain (g)	24.19 ± 1.32[a]	24.47 ± 1.36[a]	22.33 ± 1.70[a]	22.10 ± 2.09[a]
Weight gain (%)	30.34 ± 1.76[a]	29.78 ± 1.91[a]	27.76 ± 2.03[a]	27.19 ± 2.59[a]
Heart size (g/100 g body weight)	0.42 ± 0.01[a]	0.40 ± 0.01[a]	0.40 ± 0.00[a]	0.41 ± 0.01[a]
Kidney size (g/100 g body weight)	0.95 ± 0.02[a]	0.95 ± 0.01[a]	0.95 ± 0.02[a]	0.97 ± 0.02[a]
Liver size (g/100 g body weight)	4.42 ± 0.05[a]	4.07 ± 0.05[b]	3.97 ± 0.04[b]	4.05 ± 0.03[b]
Serum				
Cholesterol (mg/dL)	222.30 ± 6.45[a]	181.48 ± 4.24[b]	176.51 ± 1.39[b]	148.29 ± 3.94[c]
Triglyceride (mg/dL)	211.36 ± 3.00[a]	165.5 ± 93.29[b]	161.13 ± 7.30[b]	159.12 ± 6.78[b]
Liver				
Cholesterol (mg/g tissue)	32.71 ± 1.19[a]	20.97 ± 0.83[b]	20.18 ± 0.98[bc]	17.93 ± 0.77[c]
Triglyceride (mg/g tissue)	28.73 ± 0.95[a]	18.90 ± 0.44[b]	19.06 ± 0.45[b]	18.82 ± 0.56[b]
Liver damage index				
AST (U/L)	68.13 ± 2.70[a]	58.29 ± 2.88[b]	55.50 ± 3.34[b]	57.00 ± 3.64[b]
ALT (U/L)	124.88 ± 3.24[a]	119.43 ± 2.64[ab]	113.75 ± 3.79[b]	109.13 ± 4.40[b]

Values are means ± SEM ($n = 8$). Mean values with different letters within each test parameter differ significantly ($p < 0.05$).

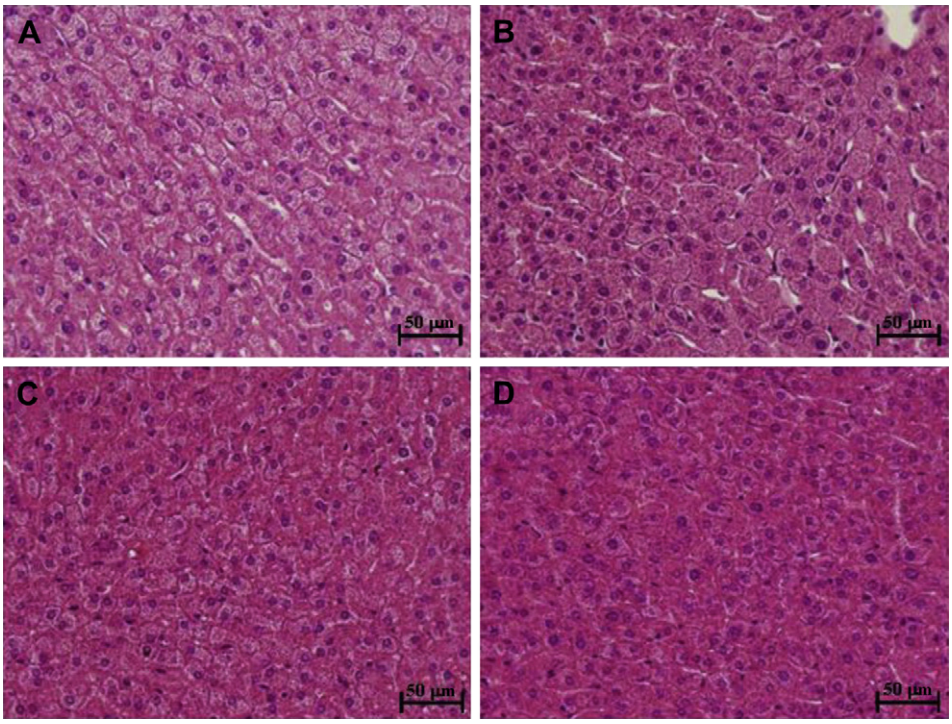

Fig. 1. Histological appearance of the liver section of the experimental hamsters (200×). Histological appearance of hematoxylin and eosin-stained liver sections from the experimental hamsters. (A) NDW, (B) DSW300, (C) DSW900, (D) DSW1500. Scale bar, 50 μm.

steatosis.[13,14,17] DSW is clean and rich in minerals, such as Ca, Mg, and K.[7] Our formulated DSW drinking waters are also rich in Mg, Ca, and K (Table 1). Mg and Ca supplementations can lower serum lipids and body weight/fat.[18,19] Although no difference in weight gain (g and %) among groups was observed during the experimental period, there was a tendency toward lower weight gain (g and %) in high-fat dietary hamsters drinking DSW drinking waters, especially DSW900 and DSW1500 (Table 2). Moreover, the hypolipidemic effect of DSW was mainly attributed to its high Ca and Mg contents.[7,20] However, because of high mineral contents in the original DSW, especially Na, we treated it via RO and ED to reduce minerals, and then formulated Mg-rich DSW300 (hardness: 325 ppm), DSW900 (hardness: 859 ppm), and DSW1500 (hardness: 1570 ppm; Table 1). A high-salt/-Na dietary habit can cause hypertension and renal hypertrophy or even renal fibrosis.[21] However, those DSW drinking waters did not alter heart rate, systolic blood pressure, diastolic blood pressure, or mean arterial pressure of hamsters during the feeding period.[8] No differences were observed in kidney and heart sizes during the 6 weeks of feeding, implying that our formulated DSW drinking waters did not harm the kidney or heart (Table 2).

The reduced liver cholesterol content can be partially accounted for by higher cholesterol secretion into bile, thus leading to a depletion of the intrahepatic pool of cholesterol; furthermore, a higher fecal lipid excretion was also related to the lower serum lipid level, thus alleviating the hepatic lipid accumulation.[14] Therefore, it is speculated that the lipid-lowering effect of DSWs (DSW300, -900, and -1500) on liver lipids is highly associated with increased daily fecal cholesterol, triglyceride, and bile acid excretions (Tables 2 and 3; Fig. 1). Regarding the hepatoprotection of DSW, Yoshioka et al investigated the therapeutic effects of DSW (hardness 28, 300, and 1200) on hyperlipidemic rabbits.[22] They reported no decreases of serum AST and ALT values in those hyperlipidemic rabbits receiving DSWs for 4 weeks, but the degree of lipid accumulation in livers was found to be less in DSW-treated groups via histological examination. However, our data showed that the liver damage indices (ASL and ALT values) were lowered in high-fat diet hamsters drinking DSWs, and less lipid accumulation in livers was observed (Table 2; Fig. 1). This difference may be due to the different animal models (rabbits vs. hamsters) and different treatments of experiments (hyperlipidemic rabbits with normal diets for 4 weeks vs. hamsters with high-fat/cholesterol diets for 6 weeks).

Liver antioxidative status is highly related to liver health.[13,15,23] MDA is often used to evaluate the oxidative

Table 3
Daily fecal cholesterol, triglyceride, and bile acid outputs of the experimental hamsters.

Group	NDW	DSW300	DSW900	DSW1500
Fecal cholesterol (mg/hamster/day)	0.82 ± 0.13^{b}	1.89 ± 0.19^{a}	1.95 ± 0.35^{a}	2.11 ± 0.39^{a}
Fecal triglyceride (mg/hamster/day)	18.01 ± 0.62^{b}	22.38 ± 1.31^{a}	21.20 ± 0.82^{a}	20.84 ± 0.51^{a}
Fecal bile acid (mg/hamster/day)	0.23 ± 0.05^{c}	0.54 ± 0.04^{b}	0.56 ± 0.06^{b}	0.71 ± 0.05^{a}

Values are means ± SEM ($n = 4$). Mean values with different letters within each test parameter differ significantly ($p < 0.05$).

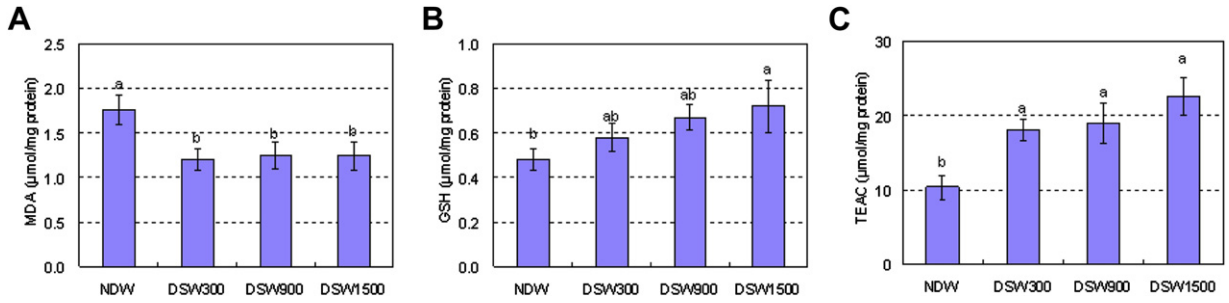

Fig. 2. Hepatic MDA, GSH, and TEAC of the experimental hamsters. Values are means ± SEM ($n = 8$). Bars with different letters differ significantly ($p < 0.05$).

stress in biological systems.[8,13,15,23,24] A high-fat or hypercaloric dietary habit easily increases liver lipid accumulation and causes oxidative stress in the body, further enhancing liver MDA contents and liver damage. Hence, the liver MDA content could be an indicator to represent the liver peroxidation status why liver GSH and TEAC were used to evaluate liver antioxidative levels. Interestingly, drinking DSW decreased liver MDA contents and maintained higher liver GSH and TEAC levels, which could result from less hepatic lipid accumulation (Figs. 1 and 2; Table 2). Moreover, higher liver MDA contents are always coupled with liver damage, which further increases serum AST and ALT values.[13–15,23] Similar results were also observed in the present study, where drinking DSWs alleviated liver MDA contents, thus resulting in lower serum AST and ALT values of high-fat dietary hamsters (Fig. 2A; Table 2).

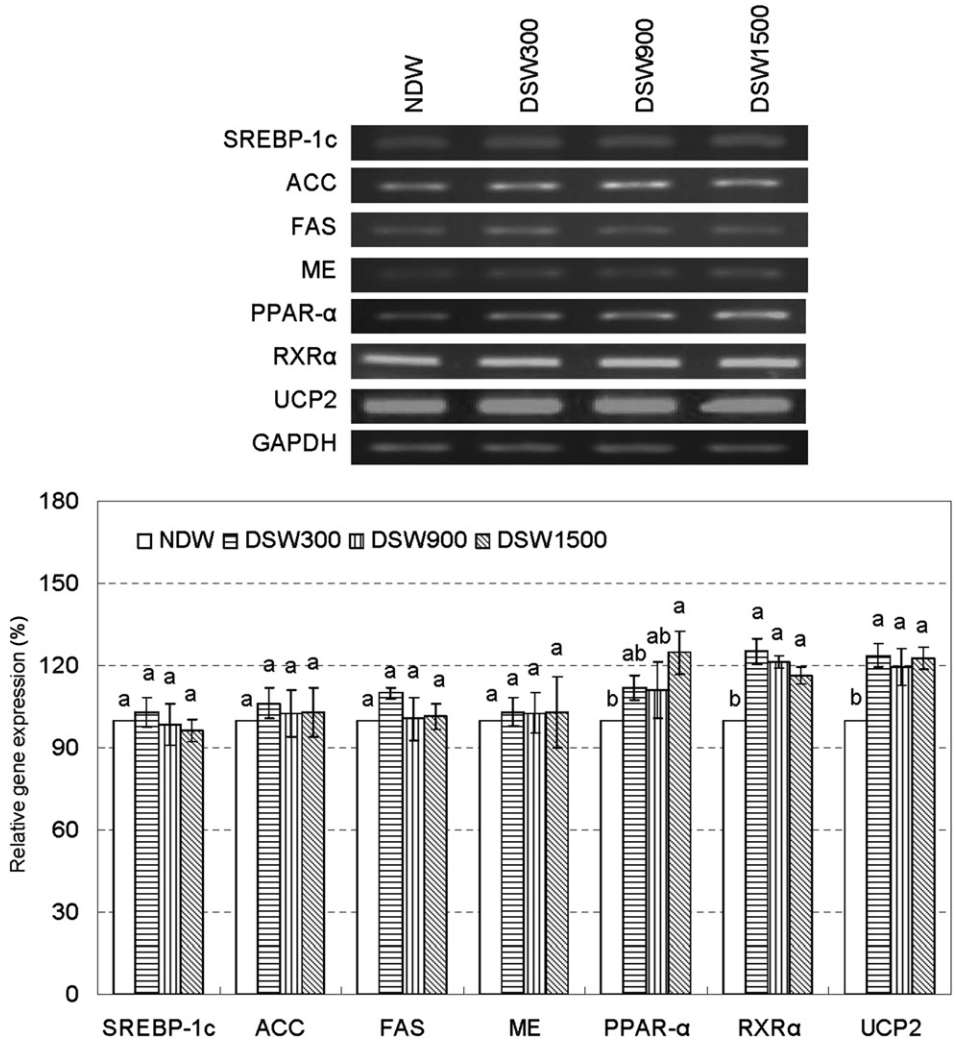

Fig. 3. Relative mRNA expressions of sterol regulatory element-binding protein-1c (SREBP-1c), acetyl CoA carboxylase (ACC), fatty acid synthase (FAS), malic enzyme (ME), peroxisome proliferator-activated receptor-alpha (PPAR-α), retinoid X receptor alpha (RXRα), and uncoupling protein-2 (UCP2) in the experimental hamsters. Values are means ± SEM ($n = 8$). Bars with different letters within each target gene differ significantly ($p < 0.05$).

Hepatic fatty-acid homeostasis in mammal animals is regulated by fatty-acid biosynthesis and energy expenditure. SREBP-1c, ACC, FAS, and ME are key proteins and enzymes in charge of fatty-acid biosynthesis.[9] In addition, the upregulation of expression of PPAR-α, RXRα, and UCP2 genes results in higher β-oxidation of fatty acids in livers, thus decreasing the development of hepatic steatosis.[10−12] RXRα is a transcription factor and can form a heterodimer with PPAR-α, thus promoting PPAR-α target gene expression. It was reported that a PPAR-α agonist, Wy14,643, could restore the DNA binding activity of PPAR-α/RXRα, inducing PPAR-α target genes to stimulate the rate of β-oxidation of fatty acids, and prevent fatty livers in ethanol-fed mice.[10] Based on current data, although no differences in SREBP-1c, ACC, FAS, and ME gene expression were detected among groups, expression of PPAR-α, RXRα, and UCP2 was upregulated by drinking the DSW formulations. That means that the rate of β-oxidation of fatty acids in livers was stimulated by DSW drinking waters and corresponded to the lower lipid accumulation in livers of high-fat diet hamsters (Figs. 1 and 3; Table 2). Additionally, it was also reported that PPAR-α can enhance catalase activity and reduce oxidative stress,[25] which is also in line with higher PPAR-α expression and lower lipid peroxidation status in livers of high-fat diet hamsters drinking DSWs (Figs. 2 and 3).

Our previous study illustrated that DSW drinking water can decrease serum lipids and keep higher serum antioxidative status in high-fat diet hamsters.[8] In the present study, we again demonstrated that formulated DSW drinking water is characterized as a hepatoprotective effect against a high-fat diet as well. On the basis of current data, the protective effects of DSWs against a high-fat diet are explained by: (1) lower lipid accumulation in livers due to increased daily fecal lipid/bile acid outputs; (2) upregulation of genes related to β-oxidation of fatty acids, i.e. PPAR-α, RXRα, and UCP2; and (3) lower lipid peroxidation status in livers. Therefore, DSW warrants application as a hepatoprotective ingredient against nonalcoholic hepatosteatosis induced by a high-fat diet in the future.

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