Cardiovascular protection of deep-seawater drinking water in high-fat/cholesterol fed hamsters

Chin-Lin Hsu, Yuan-Yen Chang, Chih-Hsien Chiu, Kuo-Tai Yang, Yu Wang, Shih-Guei Fu, Yi-Chen Chen

ABSTRACT

Cardiovascular protection of deep-seawater (DSW) drinking water was assessed using high-fat/cholesterol-fed hamsters in this study. All hamsters were fed a high-fat/cholesterol diet (12% fat/0.2% cholesterol), and drinking solutions were normal distilled water (NDW, hardness: 2.48 ppm), DSW300 (hardness: 324.5 ppm), DSW900 (hardness: 858.5 ppm), and DSW1500 (hardness: 1569.0 ppm), respectively. After a 6-week feeding period, body weight, heart rates, and blood pressures of hamsters were not influenced by DSW drinking waters. Serum total cholesterol (TC), triacylglycerol (TAG), atherogenic index, and malondialdehyde (MDA) levels were decreased \( p < 0.05 \) in the DSW-drinking-water groups, as compared to those in the NDW group. Additionally, increased \( p < 0.05 \) serum Trolox equivalent antioxidant capacity (TEAC), and faecal TC, TAG, and bile acid outputs were measured in the DSW-drinking-water groups. Hepatic low-density-lipoprotein receptor (LDL receptor) and cholesterol-7a-hydroxylase (CYP7A1) gene expressions were upregulated \( p < 0.05 \) by DSW drinking waters. These results demonstrate that DSW drinking water benefits the attenuation of high-fat/cholesterol-diet-induced cardiovascular disorders in hamsters.

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1. Introduction

Dietary fat is regarded as an important environmental factor associated with the incidence of metabolic syndrome, i.e., cardiovascular disease (CVD), hypertension, and obesity. Muller, Lindman, Brantsaeter, and Pedersen (2003) indicated that a high-saturated fat diet is the main cause of a high serum cholesterol level and is strongly correlated with death rates from coronary heart disease. Elevated low-density-lipoprotein cholesterol (LDL-C) is the leading cause of coronary artery disease in modern societies (Stocker & Keaney-Junior, 2004). Simons (2002) indicated that the increased levels of cholesterol or lipid profiles (total cholesterol/high-density lipoprotein cholesterol, TC/HDL-C) in the plasma is a condition called hyperlipidaemia, which enhances the risk of coronary heart disease, fatty liver disease, and carcinogenesis.

Deep seawater (DSW) designates water that flows 200 m below the surface of the sea. DSW is characterised by high purity, low temperature, high nutrients and minerals. DSW has recently been in trials as a multifunctional material for food, agricultural, cosmetic, and medical fields. DSW also has been reported to contain high levels of minerals, such as magnesium (Mg), calcium (Ca), and potassium (K) compared with surface and middle-sea water (Katsuda et al., 2008; Toyota & Nakashima, 1998). The biological functions of DSW have been investigated for various uses, including attenuating hyperlipidaemia, as well as atherosclerosis, dermatitis syndrome and allergic skin responses (Hataguchi, Tai, Nakajima, & Kimata, 2005; Kimata, Tai, & Nakajima, 2001; Miyamura et al., 2004; Ueshima, Fukao, Okada, & Matsuo, 2003; Yoshioka et al., 2003). Nagai et al. (2006) indicated that intake of Mg from DSW delays cataract development in the shumiyam cataract rat. Ouchi et al. (1990) indicated that dietary Mg prevents atherosclerosis in rabbits fed a cholesterol-enriched (1%) diet. However, the literature regarding the possible mechanism for cardiovascular protective effects of DSW drinking water against a high-fat/cholesterol diet remains unclear.

In the present study, we dug into the cardiovascular protective effect of DSW drinking water, using a Syrian Golden hamster model, because the Syrian Golden hamster has been used for...
atherosclerosis and cholesterol metabolism studies, due to its cardiovascular metabolic similarities to humans (Moghadasian, Frohlich, & Scudamore, 2002). The plasma lipoprotein profile of hamsters is similar to human lipoprotein profile, and approximately 80% of LDL-C in humans and hamsters is taken up through the LDL-receptor-related pathways (Nistor, Bulla, Filip, & Radu, 1987). Besides, the blood pressure and antioxidant status in the serum are also attributed to the cardiovascular health. Hence, the objectives of this study were to investigate the effects of DSW drinking water on cholesterol homeostasis, blood pressure, and serum antioxidant status in hamsters fed a high-fat/cholesterol diet.

2. Materials and methods

2.1. Collection of deep-seawater (DSW)

Original DSW samples were collected from a depth of approximately 618 m in Chisingtan Bay, Hua-Lien County, Taiwan at the same time. Enough selected original DSW was generously offered by Haewan Deep Seawater Resources Co. Ltd., Hua-Lien County, Taiwan. First, selected original DSW was treated via reverse osmosis (RO DSW) and electrodialysis (ED DSW) to reduce the mineral contents, especially sodium (Na). DSW drinking waters (300, 900, and 1500 ppm) were formulated with RO and ED DSW. DSW drinking waters were also treated by pasteurisation (80°C, 60 s) and immediately stored at −20°C until fed to the hamsters. The mineral contents in each sample of drinking water were analysed using an inductively coupled plasma optical emission spectrometer (JY ULTIMA 2000, Horiba, France). The pH value, major minerals, i.e., sodium (Na), potassium (K), calcium (Ca) and magnesium (Mg), as well as hardness of each different drinking water [2.5 ppm normal distiled water (NDW); (2) 324.5 ppm DSW drinking water (DSW300); (3) 858.5 ppm deep-seawater drinking water (DSW900); (4) 1569.0 ppm deep-seawater drinking water (DSW1500)] are shown in Table 1.

2.2. Animal, diets and experimental design

The animal use and protocol were reviewed and approved by Chung Shan Medical University Animal Care Committee, Taiwan. Forty-eight male Syrian Golden hamsters 5 weeks in age were housed individually in an animal room at 22 ± 2°C with a 12/12 h 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 chow diets with 12% lipid and 0.2% cholesterol. Meanwhile, hamsters were randomly divided into four different drinking water groups: (1) 2.5 ppm normal distilled water (NDW); (2) 324.5 ppm DSW drinking water (DSW300); (3) 858.5 ppm deep-seawater drinking water (DSW900); (4) 1569.0 ppm deep-seawater drinking water (DSW1500). All hamsters were fed a high-fat/cholesterol diet and assigned drinking solutions (including NDW, DSW300, DSW900 and DSW1500) ad libitum for 6 weeks. The diets were stored in a 4°C cold chamber. Body weights, food intake and water intake were measured every day for 6 weeks and summarised weekly. After an overnight fasting, blood samples were collected by an intracardiac puncture, and serum was harvested. The visceral tissues (liver, heart, and epididymal adipose tissue) were immediately excised, rinsed, weighed, and frozen in liquid nitrogen.

2.3. Determination of heart rate and blood pressure

According to a non-invasive measurement (Matoba et al., 2001), heart rate and blood pressures of hamsters were measured before the experiment and in the 3 days before the end of experiment. First, hamsters were held in a small and dark-coloured plastic holder. After about 10 min of equilibration, heart rates, as well as systolic, diastolic, and mean arterial pressures were monitored in conscious hamster by the forearm artery method with a BP Monitor MK-2000A (Muromachi Co. Ltd., Tokyo, Japan) consecutively, at least 3 times per hamster. The systolic pressure is regarded as the pressure value when the pulse signal appeared for the first time, and the mean arterial pressure is defined when the amplitude of the pulse wave is the greatest. Then, the diastolic pressure is calculated by a formula:

\[
diastolic~pressure = (3 \times \text{mean~arterial~pressure}) - \text{systolic~pressure}/3.
\]

The arithmetic mean of the values in the respective hamster represented the heart rate, as well as systolic, diastolic, and mean arterial pressures.

2.4. Determination of serum lipid parameters

Serum total cholesterol (TC), triacylglycerol (TAG) and high-density-lipoprotein cholesterol (HDL-C) were measured by using

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### Table 1

<table>
<thead>
<tr>
<th>pH values and mineral contents of different waters.</th>
</tr>
</thead>
<tbody>
<tr>
<td>NDW&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>pH</td>
</tr>
<tr>
<td>Na (mg/L)</td>
</tr>
<tr>
<td>K (mg/L)</td>
</tr>
<tr>
<td>Ca (mg/L)</td>
</tr>
<tr>
<td>Mg (mg/L)</td>
</tr>
<tr>
<td>Mg/Ca</td>
</tr>
<tr>
<td>Hardness (ppm)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Hardness (ppm) = Ca (mg/L) + Mg (mg/L) × 0.1 (Miyamura et al., 2004).
<sup>b</sup> NDW, normal distiled water; DSW300, 324.5 ppm deep-seawater drinking water; DSW900, 858.5 ppm deep-seawater drinking water; DSW1500, 1569.0 ppm deep-seawater drinking water.

### Table 2

The body weight, relative liver, heart, and epididymal adipose tissue sizes, and food and water intake of hamsters as affected by drinking different waters.

<table>
<thead>
<tr>
<th>Body weight (g)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>NDW</th>
<th>DSW300</th>
<th>DSW900</th>
<th>DSW1500</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial weight</td>
<td>81.1 ± 1.65a</td>
<td>79.9 ± 1.50a</td>
<td>79.1 ± 3.62a</td>
<td>81.4 ± 1.21a</td>
</tr>
<tr>
<td>Final weight</td>
<td>107 ± 2.39a</td>
<td>107 ± 2.09a</td>
<td>103 ± 3.31a</td>
<td>105 ± 3.04a</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Relative organ size (g/100 g body weight)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>NDW</th>
<th>DSW300</th>
<th>DSW900</th>
<th>DSW1500</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>4.40 ± 0.06a</td>
<td>4.08 ± 0.05bc</td>
<td>3.94 ± 0.04c</td>
<td>4.16 ± 0.06b</td>
</tr>
<tr>
<td>Heart</td>
<td>0.43 ± 0.01a</td>
<td>0.40 ± 0.01ab</td>
<td>0.39 ± 0.01b</td>
<td>0.42 ± 0.01ab</td>
</tr>
<tr>
<td>Epididymal adipose tissue</td>
<td>1.82 ± 0.08a</td>
<td>1.91 ± 0.07a</td>
<td>1.85 ± 0.06a</td>
<td>1.75 ± 0.07a</td>
</tr>
<tr>
<td>Food intake (g/hamster/day)</td>
<td>7.48 ± 0.08b</td>
<td>7.99 ± 0.15a</td>
<td>7.78 ± 0.11ab</td>
<td>8.10 ± 0.12a</td>
</tr>
<tr>
<td>Water intake (ml/hamster/day)</td>
<td>11.8 ± 0.25c</td>
<td>14.7 ± 0.27ab</td>
<td>14.0 ± 0.38b</td>
<td>14.9 ± 0.33a</td>
</tr>
</tbody>
</table>

<sup>a</sup> Values are means ± SEM (n = 12). Mean values with different letters within each test parameter indicate a significant difference (p < 0.05).
commercial kits (Randox Laboratories Ltd., Antrim, UK). The atherogenic index (AI) was calculated as $(TC-HDL-C)/HDL-C$ (Woo et al., 2008).

2.5. Determination of serum malondialdehyde (MDA)

The serum MDA content was measured by a 2-thiobarbituric acid reactive substances (TBARS) assay, as described by Yang et al. (2010). Serum (0.5 mL) was mixed with 0.75 mL of TBA solution in a Teflon tube, and then 4.25 mL trichloroacetic acid–HCl (TCA–HCl) reagent was added. The tube was flushed with nitrogen and closed. A blank was prepared in the same manner, but with PBS (pH 7.0) replacing the serum. The tubes were boiled for 30 min, and then cooled. The coloured solution was centrifuged at 4000g for 15 min. A clear and coloured supernatant was transferred to a cuvette, and the absorbance was measured at 535 nm using an Implen NanoPhotometer (Model 1443, Implen GmbH, Munich, Germany). The serum MDA content was calculated by taking the extinction coefficient of MDA to be $1.56 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ at 535 nm.

2.6. Trolox equivalent antioxidant capacity (TEAC)

The serum TEAC was analysed according to a method described by Hung, Fu, Shih, Lee, and Yen (2006). A free radical, ABTS+, can be generated by mixing ABTS (100 μM) with H$_2$O$_2$ (50 μM) and peroxidase (4.4 U/ml). The TEAC value was expressed as a scavenging capacity against ABTS+. Briefly, 0.25 mL of a mixture of ABTS, H$_2$O$_2$, and peroxidase, and 1.5 mL of dd H$_2$O were mixed well and placed in a dark room. After 30 min, 0.25 mL of diluted serum (1%, v/v) were then added. Absorbance was measured at 734 nm, after interaction with the sample solution for 10 min. 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, which was calculated as the TEAC. The higher the TEAC value of a sample is, then the stronger its antioxidant activity.

### Table 3

<table>
<thead>
<tr>
<th></th>
<th>NDW</th>
<th>DSW300</th>
<th>DSW900</th>
<th>DSW1500</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Initial experiment</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR (beats/min)$^\text{A}$</td>
<td>408 ± 18.7</td>
<td>404 ± 12.7</td>
<td>402 ± 23.9</td>
<td>398 ± 20.0</td>
</tr>
<tr>
<td>SBP (mmHg)$^\text{B}$</td>
<td>114 ± 6.54</td>
<td>113 ± 6.24</td>
<td>111 ± 7.94</td>
<td>111 ± 9.26</td>
</tr>
<tr>
<td>MAP (mmHg)$^\text{B}$</td>
<td>77.9 ± 5.42</td>
<td>78.1 ± 2.69</td>
<td>76.1 ± 7.02</td>
<td>84.3 ± 5.32</td>
</tr>
<tr>
<td><strong>Final experiment</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR (beats/min)$^\text{A}$</td>
<td>448 ± 7.48</td>
<td>435 ± 11.0</td>
<td>435 ± 18.2</td>
<td>435 ± 11.8</td>
</tr>
<tr>
<td>SBP (mmHg)$^\text{B}$</td>
<td>134 ± 4.10</td>
<td>129 ± 2.47</td>
<td>122 ± 6.65</td>
<td>128 ± 5.68</td>
</tr>
<tr>
<td>MAP (mmHg)$^\text{B}$</td>
<td>93.3 ± 3.34</td>
<td>95.8 ± 5.41</td>
<td>101 ± 12.6</td>
<td>97.9 ± 6.74</td>
</tr>
<tr>
<td>DBP (mmHg)$^\text{B}$</td>
<td>72.7 ± 4.90</td>
<td>72.7 ± 7.11</td>
<td>72.4 ± 5.98</td>
<td>72.8 ± 5.76</td>
</tr>
</tbody>
</table>

$^\text{A}$ Values are means ± SEM ($n = 12$). No differences in each test parameter over the test period were observed ($p > 0.05$).

$^\text{B}$ HR, heart rate; SBP, systolic blood pressure; MAP, mean arterial pressure; DBP, diastolic blood pressure.

Fig. 1. Serum (A) TC, (B) TAG, (C) HDL-C, (D) atherogenic index (AI), (E) MDA level, and (F) TEAC level of the experimental hamsters. Values are means ± SEM ($n = 12$). Different letters on data bars in each feeding period indicate significant differences ($p < 0.05$). Atherogenic index = (TC–HDL-C)/HDL-C.
2.7. Determination of faecal cholesterol, triacylglycerol, and bile acid

Faecal cholesterol, triacylglycerol, and bile acid measurements were measured according to the procedure of Tzang et al. (2009). Briefly, faecal lipids were extracted by chloroform and methanol (2:1, v/v). The extracts were dried under N2 and the resuspended in isopropanol, using an ultrasonic cleaner (Model: DC150H, Taiwan Delta New Instrument Co., Ltd., Taiwan), for an efficient dissolution. The contents of cholesterol and triacylglycerol were measured using commercial kits (Randox Laboratories Ltd.). Faecal bile acids can be determined using an enzymatic method (Randox Laboratories Ltd.).

2.8. Hepatic mRNA expressions of LDL receptor, HMG-CoA reductase, CYP7A1 and glyceraldehyde 3-phosphate dehydrogenase (GAPDH)

Total RNA was isolated from the stored frozen liver tissues by using the protocol described by RNeasy Mini Kits (Qiagen, Valencia, CA.). Reverse transcription was carried out with 2 μg of total RNA, 8 μL of reaction buffer, 2 μL of dNTPs, 4.8 μL of MgCl2, 4 μL of Oligo-dT (10 μM), and 0.5 μL of 200 U RTase (Promega, Madison, WI) with diethyl pyrocarbonate (DEPC) H2O in a final volume of 50 μL at 42 °C for 1 h. After a heat inactivation, 1 μL of cDNA product was used for a PCR amplification. The appropriate primers of target genes were designed for hamster’s LDL receptor (GenBank No.: M94387.1), HMG-CoA reductase (GenBank No.: M12705.1), CYP7A1 (GenBank No.: L04690.1), and GAPDH (GenBank No.: M94387.1), HMG-CoA reductase sense 5’-ACAGATTCAGTTC-CACGGAG-3’, antisense 5’-TGGGGACAAGAGGTTTTCAG-3’; HMG-CoA reductase sense 5’-AACTGAGAGCACAAGCAGAG-3’, antisense 5’-ATCATGTCATCAAAGGTA-3’; CYP7A1 sense 5’-TTTGCACACAAAGCATTT-3’, antisense 5’-TCCATGTCATCAAAGGTA-3’; GAPDH sense 5’-GACCCCCCTATGGACCTCAAC-3’, antisense 5’-CGAGATGATGACCCTTGGCC-3’. The size of reaction products is as follows: for LDLR, 477 bp; HMG-CoA reductase, 583 bp; CYP7A1, 497 bp; GAPDH, 264 bp. PCR was used as an internal control in all reactions. The PCR amplification was performed using a DNA thermal cycler (ASTEC PC-818, ASTEC Co., Ltd., Fukuka, Japan) under the following conditions: LDL receptor and CYP7A1: 30 cycles at 94 °C for 1 min, 51 °C for 1 min, and 72 °C for 2 min followed by 10 min at 72 °C; HMG-CoA reductase: 30 cycles at 94 °C for 1 min, 52 °C for 1 min, and 72 °C for 2 min followed by 10 min at 72 °C; GAPDH: 25 cycles at 94 °C for 1 min, 52 °C for 1 min, and 72 °C for 2 min followed by 10 min at 72 °C. The final products were subjected to electrophoresis on a 2% agarose gel and detected by ethidium bromide staining using a UV light. The relative expression levels of the mRNAs of the target genes were normalised using the GAPDH internal standard.

2.9. Statistical analysis

The experiment was conducted using a completely random design (CRD). Data was analysed using analysis of variance (ANOVA) (SAS Institute, Inc. 2002). A significant difference was used at 0.05 probability level and differences between treatments were tested using the least significant difference (LSD) test. All statistical analyses of data were performed using SAS software.

3. Results and discussion

3.1. Effects of DSW drinking water on body weight and tissue weights

Table 1 shows levels of elements and microelements in the different DSW drinking waters and normal drinking water (NDW). The hardness contents of NDW, DSW300, DSW900, and DSW1500 were 2.5, 325, 859 and 1570 ppm, respectively. The Mg/Ca ratio of NDW, DSW300, DSW900 and DSW1500 were 1.28, 4.67, 4.63 and 4.86, respectively. Additionally, there is a tendency toward to higher pH values of drinking waters with increased hardness. Due to high levels of inorganic materials and a better Mg/Ca ratio in DSW, it has been used in food, agricultural, cosmetic, and medical fields.

As shown in Table 2, after 6 weeks of feeding, body weights in the NDW, DSW300, DSW900 and DSW1500 groups were not significantly different (p > 0.05). Tsuchiya et al. (2004) indicated that body weight gain of mice in the desalted deep seawater and desalted surface seawater groups were not significantly different. The sizes of organ and epididymal adipose tissue in four groups are also depicted in Table 2. The liver sizes in the DSW300, DSW900 and DSW1500 groups were smaller than those of the NDW group (p < 0.05). Although there were no significantly statistical differences in the sizes of heart and epididymal adipose tissue
among treatments \((p > 0.05)\), an observed tendency towards smaller heart size in the DSW groups was observed, compared to the NDW group. Additionally, higher \((p < 0.05)\) food and water intakes were recorded in the DSW-drinking-water groups compared to the NDW group.

Although the data in the present study differ from our previous study (Chang et al., 2011) which indicated that drinking RO DSW (hardness: 44.6 ppm), ED DSW (hardness: 4690 ppm), and 10% DSW (hardness: 544 ppm) did not affect sizes of liver and abdominal fat pad in high-cholesterol fed mice, this difference could be explained by different rodent species (hamster vs. ICR mice) and different levels of fat/cholesterol in diets (12% fat/0.2% cholesterol vs. 5% fat/1% cholesterol). Additionally, the higher water intakes in DSW-drinking-water groups might be explained by higher mineral contents in DSW drinking water, which make hamsters thirstier.

3.2. Effects of DSW drinking water on heart rate and blood pressure

The estimation of the proportion of Americans suffering high blood pressure \((-33\%)\) is low, since hypertension has no symptoms, which may result in unawareness of their condition. Additionally, because of a concern about the effect of sodium (Na) on hypertension, effects of DSW drinking water on heart rate and blood pressure of high-fat/cholesterol-fed hamsters were investigated. There were no significant differences in the heart rate (HR), systolic blood pressure (SBP), mean arterial pressure (MAP) and diastolic blood pressure (DBP) among the four groups \((p > 0.05)\) (Table 3). Although higher Na absorption was related to hypertensive induction, hypotensive effects of magnesium (Mg) supplementation is regarded as suppressing adrenergic activity and increasing natriuresis (Itoh, Kawasaki, & Nakamura, 1997). Hence, no differences in HR, SBP, MAP, and DBP among NDW and DSW drinking groups (DSW300, 900, and 1500) is probably due to counteraction of Na and Mg contents in DSW drinking waters. These results also neutralise the concern regarding hypertensive induction of mineral contents in DSW drinking waters (DSW300, 900, and 1500).

3.3. Effects of DSW drinking waters on serum lipid profiles, atherogenic index, and oxidative stress

![Fig. 3. Hepatic (A) LDL receptor, (B) HMG-CoA reductase, and (C) CYP7A1 mRNA expression of hamsters as affected by drinking different waters. Values are means ± SEM \((n = 12)\). Different letters on data bars indicate significant differences \((p < 0.05)\).]
Drinking DSW300, DSW900, and DSW1500 showed lower 1.2 g calcium carbonate (CaCO₃) daily can reduce LDL-C/HDL-C ratio in patients with mild to moderate hypercholesterolemia. Chang et al. (2011) indicated that drinking deep-seawater lowered serum TAG and TC levels in high-cholesterol-fed ICR mice, compared with those only drinking NDW.

The serum lipid peroxidation and antioxidant levels are expressed as MDA and TEAC levels, respectively, which highly correlated with cardiovascular health condition (Yang et al., 2010). Drinking DSW300, DSW900, and DSW1500 showed lower (p < 0.05) MDA contents (14.34, 13.01 and 12.94 μmol/mL serum, respectively) when compared with the NDW group (26.04 μmol/mL serum) (Fig. 1E). TEAC value is expressed as Trolox equivalents. It was originally utilised for analyses of blood samples and other solutions (Miller, Rice-Evans, Davies, Gopnathan, & Milner, 1993). Increased serum TEAC values were only measured in the DSW900 and DSW1500 groups, as compared to that in the NDW group (p < 0.05) (Fig. 1F). The lower serum MDA level may be due to lower serum lipids (TC and TAG) (Fig. 1A, B, and E). Moreover, lower serum MDA levels could also account for higher serum TEAC levels (Fig. 1E and F).

3.4. Effects of DSW drinking waters on faecal TC, TAG, and bile acid excretion

The effects of DSW drinking waters on faecal TC, TAG, and bile acid contents in high-fat/cholesterol-fed hamsters are shown in Fig. 2. The faecal TC and bile acid contents in the DSW-drinking-water groups were significantly increased as compared to those in the NDW group (p < 0.05) (Fig. 2A and C). DSW300 and DSW1500 drinking waters showed increased faecal TAG excretions, as compared to the NDW group (p < 0.05) (Fig. 2B). A hypolipidaemic effect of divalent cations, i.e., Ca²⁺ and Mg²⁺ are accounted for the reaction with fatty acids and insoluble soap formation in the intestine, thus decreasing the absorption of dietary fat (Vaskonen, 2003). An intake of Ca and Mg-rich DSW increases faecal outputs of cholesterol and triacylglycerol (Chang et al., 2011).

3.5. Effects of DSW drinking waters on lipid metabolism related gene expression

The effects of DSW drinking waters on gene expressions of cholesterol homeostasis, i.e., LDL receptor, HMG-CoA reductase, and CYP7A1 in high-fat/cholesterol fed hamsters are shown in Fig. 3. After 6 weeks of feeding, the highest mRNA expressions of LDL receptor were observed in DSW1500 group followed by DSW900, DSW300, and NDW groups (p < 0.05) (Fig. 3A). Meanwhile, our data indicated the mRNA expressions of HMG-CoA reductase in high-fat/cholesterol fed hamsters were not influenced by different drinking waters (p > 0.05) (Fig. 3B). Besides, the mRNA expression of CYP7A1 was higher only in DSW1500 group compared to that of the NDW group (p < 0.05) (Fig. 3C). The LDL receptor plays a central role in the reduction of cholesterol levels and the prevention of coronary artery heart disease (White, Bennett, Billett, & Salter, 1997). HMG-CoA reductase plays an important role in control cholesterol biosynthesis (Kritchovsky, 1987). The synthesis of bile acids from cholesterol is regulated by CYP7A1 gene and CYP7A1 is also associated with metabolic disorders of cholesterol and bile acids (Bartley et al., 2010). Hence, upregulated LDL-receptor gene expression by DSW drinking waters could explain the lower serum TC in high-fat/cholesterol fed hamsters (Figs. 1A and 3A).

Moreover, an upregulation of CYP7A1 expression can accelerate cholesterol catabolism, which may result in higher faecal cholesterol and bile acid excretions and further decrease the serum TC level (Yang et al., 2010). Similar results to the data from Yang et al. (2010) were observed in the present study (Figs. 1A, 2B, 2C, and 3C).

4. Conclusions

The present results demonstrated that the DSW drinking waters (DSW300, 900, and 1500) decrease liver size, as well as serum TC, TAG, atherogenic index, and MDA levels in high-fat/cholesterol fed hamsters. We also observed that DSW drinking water is also able to increase the serum TEAC, as well as faecal TC, TAG, and faecal bile acid contents in high-fat/cholesterol fed hamsters. In the gene expressions related to cholesterol homeostasis, DSW drinking waters upregulated LDL receptors and CYP7A1 gene expressions, but did not affect the HMG-CoA reductase gene expression. Moreover, despite its sodium content, DSW drinking waters did not influence heart rate and blood pressure in high-fat/cholesterol-dietary hamsters. These results provide initial evidence that DSW drinking water may be useful for cardiovascular protection in a high-fat/cholesterol diet.

Acknowledgements

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