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

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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 distiled 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-7α-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.

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 shumiya 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

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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), 324.5 ppm deep-seawater drinking water (DSW300), 858.5 ppm deep-seawater drinking water (DSW900), and 1569.0 ppm DSW drinking water (DSW1500)] are shown in Table 1.

Table 1
pH values and mineral contents of different waters.

	NDW[B]	DSW300[B]	DSW900[B]	DSW1500[B]
pH	6.89	6.92	7.09	7.25
Na (mg/L)	0.61	260	300	350
K (mg/L)	0.15	12.8	13.5	13.8
Ca (mg/L)	0.32	15.0	40.0	70.0
Mg (mg/L)	0.41	70.0	185	340
Mg/Ca	1.28	4.67	4.63	4.86
Hardness (ppm)[A]	2.5	325	859	1570

[A] Hardness (ppm) = Ca (mg/L) × 2.5 + Mg (mg/L) × 4.1 (Miyamura et al., 2004).
[B] 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.

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 distiled 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 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). 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:

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

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

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.

	NDW	DSW300	DSW900	DSW1500
Body weight (g)[A]				
Initial weight	81.1 ± 1.65a	79.9 ± 1.50a	79.1 ± 3.62a	81.4 ± 1.21a
Final weight	107 ± 2.39a	107 ± 2.09a	103 ± 3.31a	105 ± 3.04a
Relative organ size (g/100 g body weight)[A]				
Liver	4.40 ± 0.06a	4.08 ± 0.05bc	3.94 ± 0.04c	4.16 ± 0.06b
Heart	0.43 ± 0.01a	0.40 ± 0.01ab	0.39 ± 0.01b	0.42 ± 0.01ab
Epididymal adipose tissue	1.82 ± 0.08a	1.91 ± 0.07a	1.85 ± 0.05a	1.75 ± 0.07a
Food intake (g/hamster/day)	7.48 ± 0.08b	7.99 ± 0.15a	7.78 ± 0.11ab	8.10 ± 0.12a
Water intake (ml/hamster/day)	11.8 ± 0.25c	14.7 ± 0.27ab	14.0 ± 0.38b	14.9 ± 0.33a

[A] Values are means ± SEM (n = 12). Mean values with different letters within each test parameter indicate a significant difference ($p < 0.05$).