Deep-Sea Water Improves Cardiovascular Hemodynamics in Kurosawa and Kusanagi-Hypercholesterolemic (KHC) Rabbits

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Deep-sea water is rich in minerals, *e.g.*, Mg, Ca, and K which have been considered to be associated with prevention of cardiovascular disease. We investigated the effect of deep-sea water on cardiovascular hemodynamics in Kurosawa and Kusanagi-Hypercholesterolemic (KHC) rabbits. Deep-sea water was pumped in the offing of Cape Muroto in Japan and the mineral constituents were refined to a degree of hardness of 1000. Twenty four 4-month-old KHC rabbits were given refined deep-sea water ($n=12$) and tap water ($n=12$) for 6 months. Pressure and flow waves at the ascending aorta were recorded under pentobarbital anesthesia. Systolic, diastolic, pulse and mean arterial pressures and total peripheral resistance were significantly lower in the deep-sea water group than in the control group. There were no significant differences in changes in serum lipid levels, plasma renin and angiotensin converting enzyme activities and electrolyte levels except for Mg^{2+} after the feeding of the water between the two groups. A slight increase in serum Mg^{2+} level in the deep-sea water group may not account for the inhibition of mild hypertension. From our results, we conclude that deep-sea water could improve cardiovascular hemodynamics, even though the factors which affect the blood pressure are still unknown.

Key words deep-sea water; Mg^{2+}; cardiovascular hemodynamics; Kurosawa and Kusanagi-Hypercholesterolemic rabbit; renin-angiotensin system; mild hypertension

Water is essential to life processes and has a number of significant functions. The ingredients of drinking water as well as nutrients in foodstuffs are now considered important to people's health. Deep-sea water in particular has started to receive attention for its rich inorganic nutrients such as Mg, Ca, and K[1,2] which are due mainly to less photosynthesis of plant plankton and much organic decomposition. In addition to the beneficial effects of these minerals on the cardiovascular system, unknown effects of some ultratrace elements or unknown substances in deep-sea water may be found in future. Some scientific evidences of therapeutic or preventive effects of deep-sea water have been reported recently. Deep-sea water improved mineral imbalances and atopic eczema/dermatitis syndrome in humans[2] and was effective in the prevention of hyperlipidemia and atherosclerosis in cholesterol-fed rabbits.[3,4] We expect that the continuous intake of deep-sea water could prevent hypertension as well as hypercholesterolemia, because investigators have revealed that oral supplementation of calcium[5,6] or magnesium[7,8] to hypertensive patients lowered blood pressure in addition to reducing the serum total cholesterol level.[7]

Hypercholesterolemia and hypertension occur due to genetic as well as dietary factors. The Kurosawa and Kusanagi-Hypercholesterolemic (KHC) rabbit is an animal model of spontaneous hypercholesterolemia (Type IIa) and atherosclerosis established by inbreeding a mutant of the Japanese White rabbit discovered by Japan Laboratory Animals, Inc. in 1985.[9] The KHC rabbit is deficient in LDL-receptors in the liver and shows an abnormally increased LDL-cholesterol level without unusual deposits of fat in some organs.[9] Atherosclerosis develops in the aortic arch and around bifurcations of the main branch arteries by the age of 3 months.[9] We also reported previously that young adult KHC rabbits aged 10—12 months showed mild hypertension compared to age-matched normal rabbits in addition to hypercholesterolemia, though atherosclerotic lesions were relatively in the early-stage.[10,11] The mild hypertension is thought to be independent of hypercholesterolemia.[11] Intervention of either hypercholesterolemia or hypertension is considered to be inadequate to prevent progression of atherosclerosis and related cardiovascular events if hypertension and hypercholesterolemia coexist.[12] Ca and Mg in deep-sea water could suppress absorption of cholesterol in the small intestine in cholesterol-fed rabbits, which might contribute to anti-hypercholesterolemic action of deep-sea water.[3,4] Therefore, it is appropriate to use spontaneous hypercholesterolemic rabbits.

In the present study, we investigated the effect of the intake of refined deep-sea water at a degree of hardness of 1000 for 6 months on hypercholesterolemia and mild hypertension in KHC rabbits aged 4 months, at which time atherosclerosis starts to occur in the ascending aorta and around orifices of branch arteries, by measuring serum and plasma biochemical parameters, blood pressure and cardiovascular hemodynamics.

MATERIALS AND METHODS

Refinement of Deep-Sea Water Deep-sea water was pumped from approximately 344 m below sea level in the offing of Cape Muroto in Kochi Prefecture in Japan. The deep-sea water was industrially divided into two components; salt-rich concentrated water and salt-poor freshwater using a reverse osmosis membrane as described previously.[1] A great amount of NaCl was removed from the salt-rich con-

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centrated water by heat and concentration, and then brine containing a small amount of NaCl was obtained. The brine was dissolved in the salt-poor fresh water to adjust mineral constituents to a degree of hardness of 1000. The refined deep-sea water was filtered for disinfection and bottled by Ako Kasei Co., Ltd. Tap water was supplied by Fukushima City Waterworks Department and sterilized using an ultraviolet sterilizer just before feeding to the rabbits. Levels of major and trace elements in the refined deep-sea water and tap water were determined by the similar methods reported previously.[13—15] Contents of Na, K, Ca and Mg were determined using inductively coupled plasma atomic emission spectrometry (ICP-AES). Concentration of P was measured using absorption photometry. Contents of Fe, Cu, Zn and Mn were determined using coupled plasma mass spectrometry (ICP-MS). Level of Se was measured using hydride ICP-MS.

Animals and Breeding Twenty-four male KHC rabbits aged 4 months were purchased from Japan Laboratory Animals, Inc., Tokyo, Japan), and then left for about 1 week to acclimatize to the breeding environment. The rabbits were divided into two groups; control ($n=12$) and deep-sea water ($n=12$) groups, with equal serum total cholesterol levels in the two groups. Refined deep-sea water and sterilized tap water were freely given to the KHC rabbits in both groups *via* feeding bottles (500 ml/d at maximum) for 6 months. The rabbits were kept in an air-conditioned room at a room temperature of 22—25 °C, relative humidity of 50—60% and light and dark cycle of 12L (light)/12D (dark) and given a cholesterol-free commercial rabbit food RC-4 (Oriental Yeast Co., Tokyo, Japan) at 100 g/d/animal. Food and water intakes were measured 3 d running once a month. Body weight (BW) was measured once a month. All the experiments had been approved by the Experimental Animal Committee of Fukushima Medical University and performed in line with the Guidelines for Animal Care and Handling of the Japanese Association for Laboratory Animal Science.

Measurement of Serum and Plasma Chemical Parameters Blood was sampled from the posterior earlobe artery in the morning at the beginning and end of drinking of the deep-sea water after overnight fastening. Blood was drawn into a tube containing EDTA-2Na to obtain plasma. The plasma and serum were stored at −85 °C after centrifugation at 3000 rpm for 10 min at 4 °C. Serum total cholesterol (T-Chol) and high-density lipoprotein cholesterol (HDL-Chol), triglyceride (TG) and glucose (Glu) levels were analyzed by enzymatic methods using an automatic analyzer (AU-5232, Olympus Corporation, Tokyo, Japan). Serum Na^+, K^+ and Cl^- levels were measured using an ion selective electrode. Serum Ca^{2+} and Mg^{2+} levels were determined using O-CPC[16] and Xylidyl Blue II[17] methods, respectively. The activity of serum angiotensin converting enzyme (ACE) was assayed by Kasahara's method.[18] Plasma renin activity[19] and concentrations of plasma Ang I and Ang II were measured using a radioimmunoassay.[20] Plasma adrenalin (Adr) and noradrenalin (NorAdr) levels were determined using high performance liquid chromatography (HPLC) (HLC-725 CAII, TOSOH Corporation, Tokyo, Japan).[21]

Measurements of Cardiovascular Parameters The rabbits were anesthetized by the intravenous injection of pentobarbital sodium (Nembutal, Abbott Laboratories, Abbott Park, IL, U.S.A.) at a dose of 0.12 mmol/kg (30 mg/kg body weight), fixed supine and intubated through tracheotomy. Prior to the catheterization, catheter tip was immersed into the physiological saline at 37 °C for about 30 min to minimize drifting of sensitivity of micromanometers. A catheter with a micromanometer at the tip (SPS-330, Millar Instrument Inc., Dallas, TX, U.S.A.) was advanced from the left common carotid artery and fixed at the origin of the ascending aorta. The chest was opened carefully to avoid the pneumothorax with normal breathing maintained. A flow probe 8 mm in diameter was attached to the ascending aorta. Pressure and flow waves at the ascending aorta were simultaneously measured using a polygraph system (Polygraph 360 System, NEC San-Ei, Inc., Tokyo, Japan) and an ultrasonic flow meter (Transonic T206, Transonic Systems Inc., Ithaca, NY, U.S.A.) and recorded in the computer (PowerBook G4, Apple Computer, Inc., Cupertino, CA, U.S.A.) through an analogue-to-digital converter (PowerLab System /8s, AD Instruments, Inc., Sydney, Australia) at intervals of 0.25 ms after a stable recording had been established.

Analysis of Cardiovascular Parameters The computer-stored data on pressure and flow waves were analyzed for 20 successive waves. Mean arterial pressure (MAP) and cardiac output (CO) were calculated as an integrated mean value of original pressure and flow waves within each cardiac cycle. Heart rate (HR) was determined from peak-to-peak intervals of pressure waves. Total peripheral vascular resistance (TPR) was calculated as MAP/CO. Stroke volume (SV) was determined as a mean value for 1 min by dividing CO by HR.

Determination of Lesioned Area of the Aorta The rabbits were euthanized with an overdose of pentobarbital sodium. The aorta was removed from just above the aortic valve to the bifurcation of the common iliac arteries and cut open longitudinally. The measurement of lesioned area was similar to that reported previously.[22] The traced outline of the aorta and borderline of the plaques were digitized by an image analysis system (LUZEX-FS, Nireco Corporation, Tokyo, Japan) through a CCD-video camera. The lesioned and lesion-free areas were displayed in black and white, respectively. Percent lesioned area (PLA) was calculated as a ratio of total lesioned area to total surface area of the aorta.

Statistical Procedure The difference in each serum and plasma chemical parameter and BW before and after the feeding of the water was calculated in the control (Δd_1) and deep-sea water (Δd_2) groups. The values before and after the feeding in each parameter were compared using the Wilcoxon matched-pair signed-rank test. The difference (Δd) between Δd_1 and Δd_2 in each parameter was compared using the Mann–Whitney test, when a significant difference was observed in the Wilcoxon matched-pair signed-rank test in the control and/or deep sea-water groups. Since there was a variation in plasma renin activity, serum ACE activity and plasma Ang I and II levels between the control and deep-sea water groups before the feeding of the water, the difference in them between the two groups was also compared using the Mann–Whitney test. Plasma Adr and NorAdr levels after the feeding of the water were tested using the Mann–Whitney test. Differences in water intake each month were analyzed with Scheffe's multi-comparison test after a two-way analysis of variance. Cardiovascular parameters and PLA were compared using the Mann–Whitney test between the two groups. $p=0.05$ was taken as statistically significant.

RESULTS

Water Intake, Food Intake and Body Weight Table 1 shows levels of elements and microelements in the refined deep-sea water and tap water. Refined deep-sea water at a degree of hardness of 1000, was rich in minerals such as Mg, Ca, and K, concentrations of which were by far higher than those in the tap water. Mg had the highest concentration (200 mg/l) of any mineral. The concentration of Na in the deep-sea water was about 3.5-fold that in the tap water. Concentrations of P, Fe, Cu, Zn, Mn and Se were lower in the deep-sea water than tap water.

Water intake varied from 282 to 331 ml/d in average throughout the experiment (Table 2). All animals ate up the food in any month (100 g/d/animal). Though water intake had decreased by about 10% at 4 months in the two groups, there was no significant difference in water intake between the two groups in any month for 6 months.

Table 3 indicates total daily intake of elements and microelements from diet and water. The commercial rabbit food contained sufficient elements and microelements to maintain normal biological functions. The daily intake of most elements and microelements was accordingly almost the same in the deep-sea water and control groups except for Na and Mg. Total daily intake of Na and Mg in the deep-sea water group was increased by about 10% and 17% respectively, compared to that in the control group.

Hemodynamic Parameters and Lesioned Area in the Aorta Table 4 lists hemodynamic parameters and lesioned area in the control and deep-sea water groups. Systolic (SAP) ($p=0.000$), diastolic (DAP) ($p=0.002$) and pulse (PP) ($p=0.040$) pressures and MAP ($p=0.000$) were significantly lower in the deep-sea water group than in the control group. TPR was significantly low in the deep-sea water group ($p=0.008$), whereas there were no significant differences in CO ($p=0.332$), SV ($p=0.847$), HR ($p=0.567$) and PLA ($p=0.478$) between the two groups.

Serum and Plasma Chemical Parameters Table 5 shows BW, and serum lipid and glucose levels in the control and deep-sea water groups. Serum T-Chol, HDL-Chol, TG and Glu levels decreased from the onset of drinking of deep-sea water or tap water for 6 months in the two groups. BW rose by about 0.5 kg after the feeding of the water in the two groups. There were no significant differences in these parameters between the two groups after the feeding of the test water for 6 months. Table 6 compares serum electrolyte concentrations in the control and deep-sea water groups. Serum Na^+, K^+, Cl^-, Ca^{2+} and Mg^{2+} levels were almost constant in

Table 1. Levels of Elements and Microelements in the Deep Sea Water and Tap Water

Elements/Group	Content in water	
	Deep-sea water	Tap water
Na (mmol/l)	3.2	0.9
K (mmol/l)	1.8	0.1
Ca (mmol/l)	1.8	0.4
Mg (mmol/l)	8.2	0.1
P (mmol/l)	0.1	0.3
Fe (nmol/l)	0.2	186.2
Cu (μg/l)	0.1	2.3
Zn (μg/l)	0.2	7.1
Mn (μg/l)	0.08	0.4
Se (μg/l)	0.005	0.020

Table 2. Change in Water Intake after the Feeding of the Test Water for 6 Months

Months	1	2	3	4	5	6
Control	307±114	330±119	311±96	282±101	291±76	341±70
Deep-sea water	312±94	323±95	331±105	288±107	315±83	316±90
p value	0.766	0.879	0.631	0.888	0.455	0.445

Water intake, ml; values are means±standard deviations.

Table 3. Total Daily Intake of Elements and Microelements from Diet and Water

Elements	Diet	Total daily intake	
		Deep-sea water +diet	Tap water +diet
Na (mg/d)	150.0	173.7	156.9
K (mg/d)	1910.0	1932.1	1911.1
Ca (mg/d)	1410.0	1432.7	1414.9
Mg (mg/d)	360.0	424.0	361.1
P (mg/d)	560.0	560.0	560.0
Fe (mg/d)	22.1	22.1	22.1
Cu (μg/d)	1.06	1.06	1.06
Zn (μg/d)	4.92	4.92	4.92
Mn (μg/d)	6.92	6.92	6.92
Se (μg/d)	0.39	0.39	0.40
Ca/Mg	3.92	3.38	3.92

Levels in the diet were based on the analytical values from the Oriental Yeast, Co., Ltd., Tokyo, Japan. Total daily intake per animal from diet and water was estimated from mean water (320 ml/d) and food (100 g/d) intake.

Table 4. Changes in Cardiovascular Parameters and Lesioned Area after the Feeding of the Test Water

Parameters	Control	Deep-sea water	*p* value
SAP (mmHg)	139.2±7.2	128.3±4.4	0.000
DAP (mmHg)	111.7±5.3	105.0±2.9	0.002
PP (mmHg)	27.5±5.1	23.3±3.8	0.040
MAP (mmHg)	128.3±6.7	118.9±3.5	0.000
CO (ml/min)	346.0±29.3	351.4±18.4	0.332
TPR (mmHg/ml/min)	0.37±0.03	0.34±0.01	0.008
HR (beat/min)	255.1±26.3	260.5±23.0	0.562
SV (ml)	1.4±0.2	1.4±0.1	0.847
PLA (%)	57.5±15.6	51.7±16.8	0.478

SAP, systolic arterial pressure; DAP, diastolic arterial pressure; PP, pulse pressure; MAP, mean arterial pressure; CO, cardiac output; TPR, total peripheral vascular resistance; HR, heart rate; SV, stroke volume; PLA, percent lesioned area. Values are means±standard deviations.

Table 5. Changes in Serum Lipid and Glucose Levels after the Feeding of the Test Water for 6 Months

Parameters	Control group			
	Before	After	Δd_1	p value[a)]
BW (kg)	2.6±0.3	3.1±0.3	0.5±0.2	0.000
T-Chol (mmol/l)	19.53±2.77	15.87±0.18	−3.66±0.56	0.000
HDL-Chol (mmol/l)	0.21±0.04	0.18±0.02	−0.03±0.02	0.035
TG (mmol/l)	2.50±0.75	1.83±0.67	−0.67±0.09	0.000
Glucose (mmol/l)	7.02±0.99	6.49±1.54	−0.53±0.56	0.390

Parameters	Deep-sea water group				
	Before	After	Δd_2	p value[a)]	p value[b)]
BW (kg)	2.7±0.4	3.2±0.3	0.5±0.4	0.001	0.551
T-Chol (mmol/l)	19.59±2.25	15.81±1.78	−3.78±0.47	0.000	0.887
HDL-Chol (mmol/l)	0.23±0.07	0.19±0.03	−0.04±0.05	0.080	0.799
TG (mmol/l)	2.49±0.53	1.77±0.31	−0.72±0.21	0.000	0.932
Glucose (mmol/l)	7.38±1.04	6.71±0.56	−0.67±0.48	0.101	

BW, body weight; T-Chol, total cholesterol; HDL-Chol, high-density lipoprotein cholesterol; TG, triglyceride. Values are means±standard deviations. Δd_1 and Δd_2 are the difference in each parameter after the feeding of the water in the control and deep-sea water groups, respectively. *a*) p value; Wilcoxon matched-pair signed-rank test for each parameter before *versus* after the feeding of the water. *b*) p value; Mann–Whitney test for difference between Δd_1 and Δd_2 when the difference in each parameter before *versus* after the feeding of the water was significant in the control and/or deep-sea water groups.

Table 6. Changes in Serum Electrolyte Levels after the Feeding of the Test Water for 6 Months

Parameters	Control group			
	Before	After	Δd_1	p value[a)]
Na^+ (mmol/l)	139.92±3.15	139.75±3.29	−0.17±3.67	0.883
K^+ (mmol/l)	3.75±0.47	3.68±0.23	−0.07±0.35	0.543
Cl^- (mmol/l)	102.50±2.40	102.33±1.80	−0.17±1.86	0.772
Ca^{2+} (mmol/l)	3.32±0.13	3.18±0.10	−0.14±0.11	0.002
Mg^{2+} (mmol/l)	0.88±0.07	0.83±0.07	−0.05±0.08	0.049

Parameters	Deep-sea water group				
	Before	After	Δd_2	p value[a)]	p value[b)]
Na^+ (mmol/l)	137.25±3.98	139.58±2.40	2.33±4.25	0.096	
K^+ (mmol/l)	3.82±0.35	3.70±0.23	−0.12±0.42	0.379	
Cl^- (mmol/l)	100.58±2.98	103.08±1.50	2.50±2.84	0.014	0.060
Ca^{2+} (mmol/dl)	3.35±0.14	3.18±0.08	−0.18±0.14	0.002	0.319
Mg^{2+} (mmol/dl)	0.87±0.09	0.90±0.07	0.03±0.10	0.201	0.039

Values are means±standard deviations. See the legend for Table 5.

the two groups before and after the drinking of the test water. The changes in serum Na^+, K^+, Cl^- and Ca^{2+} levels after the feeding of the test water were not significantly different between the two groups, but the difference in the Mg^{2+} level between before and after the feeding of the test water showed a slight but significant increase in the deep-sea water group compared with that in the control group, which was within a normal range.

Table 7 illustrates plasma hormone levels relating to cardiovascular parameters. There were no significant differences in renin and ACE activities and plasma Ang I and II levels between the control and deep-sea water groups before the feeding of the water. Renin and ACE activities and Ang II level tended to decrease after the feeding of deep-sea water or tap water in the two groups. The difference in the Ang II level before and after the feeding of the water in the deep-sea water group was about twice that in the control group, though it was not statistically significant. Change in the values of renin and ACE activities, and Ang I and Ang II levels after the drinking of the test water did not differ significantly between the control and deep-sea water groups. There were no significant differences in plasma adrenalin (Adr) and noradrenalin (NorAdr) levels between the two groups after the feeding of deep-sea water or tap water.

DISCUSSION

Effects of Deep-Sea Water on Food and Water Intake Water intake decreased by 10% at 4 months in both rabbit groups, but this was not significant between the two groups. Food intake did not change in the two groups (almost 100 g/animal/d). There were no ill health such as diarrhea or dysorexia and no abnormal finding in liver and renal function tests among the rabbits (Katsuda *et al.*, unpublished data).

Table 7. Changes in Plasma Hormone Levels after the Feeding of the Test Water for 6 Months

Parameters	Deep-sea water group				
	Before	After	Δd_1	p value[a)]	p value[b)]
Renin activity (ng/ml/h)	2.93±1.17	2.34±1.15	−0.59±0.88	0.443	0.047
ACE activity (IU/l)	108.6±21.3	70.8±9.4	−37.8±18.4	0.198	0.000
Ang I (pmol/l)	1232.2±657.2	1388.3±922.2	156.3±1121.3	0.590	0.653
Ang II (pmol/l)	15.63±7.79	11.88±5.16	−3.75±8.96	0.799	0.193
Adr (ng/ml)	—	0.03±0.03	—		
NorAdr (ng/ml)	—	0.21±0.16	—		

Parameters	Deep-sea water group				
	Before	After	Δd_2	p value[b)]	p value[c)]
Renin activity (ng/ml/h)	3.68±2.07	2.62±1.84	−1.06±1.92	0.093	0.755
ACE activity (IU/l)	118.1±14.7	75.6±7.8	−42.5±10.9	0.000	0.219
Ang I (pmol/l)	1463.6±824.0	1267.4±540.5	−196.2±734.8	0.395	
Ang II (pmol/l)	18.58±12.44	11.09±7.04	−7.49±11.27	0.050	0.551
Adr (ng/ml)	—	0.04±0.02	—		
NorAdr (ng/ml)	—	0.20±0.15	—		

ACE, angiotensin converting enzyme; Ang I, angiotensin I; Ang II, angiotensin II; Adr, adrenalin; NorAdr, noradrenalin. Values are means±standard deviations. Δd_1 and Δd_2 are the difference in each parameter after the feeding of the water in the control and deep-sea water groups, respectively. *a*) p value; Mann–Whitney test for each parameter between the control and deep sea-water groups before the feeding of the water. *b*) p value; Wilcoxon matched-pair signed-rank test for each parameter before *versus* after the feeding of the water. *c*) p value; Mann–Whitney test for difference between Δd_1 and Δd_2 when the difference in each parameter before *versus* after the feeding of the water was significant in the control and/or deep-sea water groups, Adr and NorAdr after the feeding of the water were compared between the control and deep-sea water groups using the Mann–Whitney test. There were no significant differences in both Adr ($p=0.131$) and NorAdr ($p=0.928$) between the two groups after the feeding of the water.

The decrease in water intake may have been due to seasonal fluctuation.

Effects of Deep-Sea Water on Hypercholesterolemia and Atherosclerosis Serum T-Chol and TG levels declined significantly after 6 months in the two groups. We also observed previously in KHC rabbits that serum T-Chol and TG levels decreased gradually with age (Katsuda, unpublished data). Shiomi *et al.*[23] reported that the plasma cholesterol level showed age-related change in Watanabe heritable hyperlipidemic (WHHL) rabbits, which was related to an increase in the cholesterol catabolic rate as well as a decrease in the rate of secretion of very low density lipoprotein (VLDL) cholesterol. The age-related change in the serum cholesterol level would be due to a similar mechanism because both the KHC and WHHL rabbit strains are a LDL-receptor-deficient animal model.[9,23]

Yoshikawa *et al.*[3] demonstrated that deep-sea water inhibited the accumulation of lipid and permeation of macrophages in the arterial wall in rabbits fed 1% cholesterol for 4 weeks. Miyamura *et al.*[4] also reported that among rabbits fed 1% cholesterol for 12 weeks, there was less LDL cholesterol in plasma and less cholesterol accumulated in the arterial wall in the deep-sea water group than in the control group. They proposed as a possible anti-atherosclerotic mechanism that the activity of glutathione peroxidase, an antioxidative enzyme, increased due to the administration of deep-sea water and that Ca and Mg suppressed the absorption of cholesterol in the small intestine. In the present study, there was no significant difference in changes in serum T-Chol and TG levels before and after ingestion of the water between the control and deep-sea water groups. The discrepancy would arise partly from the difference in the mechanism of hypercholesterolemia between cholesterol-fed and heritable hypercholesterolemic rabbits. The precise mechanism by which deep-sea water affects in cholesterol's metabolism and accumulation in the wall should be elucidated in the future.

Effects of Deep-Sea Water on Hemodynamics In the present study, the feeding of deep-sea water for 6 months had a preventive effect on mild hypertension in young KHC rabbits. The decrease in blood pressure was accompanied by a significant decrease in TPR, without any changes in CO. The mild hypotensive effect of deep-sea water is considered to be due mainly to the decrease in TPR.

Since few atheromatous plaques were observed in the main branch arteries in the two groups, the significant decrease in TPR in the deep-sea water group would be due to vasodilation rather than pathological changes in arterioles. In addition, there was no marked difference in PLA and circumferential histological findings in the ascending, proximal thoracic and proximal abdominal aortas between the two groups, suggesting that progress of atherosclerosis was not different between the two groups.

Effects of Plasma Hormones on Hemodynamics in the Rabbits Fed Deep-Sea Water The renin-angiotensin system (RAS) plays an important role in pressor response in the recovery of hypotension and in long-term AP control.[24,25] The plasma RAS is activated in hypertensive patients.[26] Recently, investigators have revealed that Ang II was also involved in the progression of atherosclerosis within the vascular wall by stimulating the production of superoxide anion and expression of some inflammatory cytokines in the intima and SMCs.[27—31] In the present study, the change in plasma renin and ACE activities, and Ang I and Ang II levels after the feeding of the water did not differ significantly between the two groups, which suggests that the RAS was little associated with the hypotensive effect of refined deep-sea water. In addition, plasma renin and ACE activities decreased significantly after the feeding of the water for 6 months in both groups. These results may be partially associated with age-related changes though detailed mechanism is unknown in

the present study. Plasma Adr and NorAdr levels were almost the same in the two groups after 6 months on the deep-sea water, and so contributed little to the blood pressure level in the deep-sea water group.

Effects of Minerals on Hemodynamics A shortage of magnesium is associated with cardiovascular disease.[32,33] It has been thought that magnesium, as well as calcium, might be involved in the physiological regulation of vascular tone, whereas perturbations in cellular Mg^{2+} homeostasis had a potentially pathophysiologic role in hypertension in animals with genetic and experimentally induced hypertension and patients with essential hypertension.[34,35] Magnesium acts in an antagonistic fashion against calcium in the cell membrane.[36] Mg^{2+} functions as a natural blocker of Ca^{2+} in the cell membrane. The intracellular concentration of Mg^{2+} has been reported to decrease in smooth muscle cells due to the antagonistic effects of Mg^{2+} on Ca^{2+}, such as increased Na^{+} and Mg^{2+} exchange or reduced ATPase activities.[37,38] Kisters *et al.*[36,39] also demonstrated that the ratio of calcium to magnesium in the cell membrane was elevated in patients with primary hypertension, which could be an atherogenic risk factor for hypertensives. A positive correlation between mortality from ischemic heart disease and the ratio of calcium to magnesium in diet has been proven in several countries.[40,41] Magnesium supplementation could contribute to lower blood pressure in hypertensive patients.[7,8]

In the present study, the serum Mg^{2+} concentration showed a slight but significant increase in the deep-sea water group after 6 months. The serum Mg^{2+} level, however, was normal in the two groups before and after the feeding of Mg^{2+}-rich deep-sea water, which may contribute little to the decrease in blood pressure level. The amount of Ca and Mg in the commercial diet was 1410 and 360 mg/100 g, which was sufficient for daily intake. The total daily intake of Ca and Mg was respectively, about 1432.7 and 424.0 mg/d in the deep-sea water group and 1414.9 and 361.1 mg/d in the control group, when the average water intake was estimated as 320 ml/d (Table 3). The ratio of Ca to Mg in total diet and water was respectively, 3.92 and 3.38 in the deep-sea water and control groups (Table 3). The increase in daily Mg intake (17.5%) and decrease in dietary Ca to Mg ratio may be partly associated with the increased serum Mg^{2+} level in the deep-sea water group. However, there were no significant correlations between serum Mg^{2+} and MAP levels in the deep-sea ($r=-0.12$, $p=0.71$) and tap water ($r=0.53$, $p=0.12$) groups after the feeding of the water. Serum Mg^{2+} and MAP levels distributed in a relatively normal narrow range, which might partly explain why there was no relationship between them. It would be difficult to attribute a preventive effect of deep-sea water on mild hypertension due to a slight but significant increase in serum Mg^{2+}. The water more than 200 m below the sea is cold and receives little sunshine. Minerals are utilized little for photosynthesis. Plant plankton is broken down by organisms mainly into mineral salts and unidentified side products. It is possible that an unknown substance preventing hypertension is present in deep-sea water.

Microelements such as Cu, Zn, and Mn have important biological functions mainly in the scavenging of superoxide, by Cu/Zn-superoxide dismutase (SOD) and Mn-SOD in the endothelium.[42] Though levels of P, Fe, Cu, Zn, Mn, and Se in the deep-sea water were low compared with those in the tap water, the difference in the daily intake of these elements and microelements was negligibly small between the deep-sea water and control groups (Table 3). The content of Fe in the refined deep-sea water was about 1/930 of that in the tap water, though Fe level in the deep-sea water before the refinement was about 10—50 times as large as that of the refined water.[43] Fe may be eliminated mostly in the process of refinement using a reverse osmosis membrane. However, the amounts of elements and microelements in the commercial rabbit food are designed to be sufficient for maintaining biological functions. An ameliorating effect of microelements on hypercholesterolemia or hypertension may have been masked by the microelement-rich commercial diet. It is still possible that some ultratrace elements help to improve hemodynamics.

Thus, deep-sea water is a well-balanced mineral resource, and is beneficial for the prevention and treatment of mild hypertension. The precise mechanism suppressing mild hypertension and the candidate substances in deep-sea water that remedy hemodynamics remain to be elucidated.

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