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Effect of Deep Sea Water on the Exercise-Induced Fatigue of Rats

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ABSTRACT

This study is to investigate the effect of deep sea water (DSW) on the exercise-induced fatigue of rats. The exhaustive exercise test on the treadmill and the measurement of biochemical values related to fatigue were conducted after the two-week feeding to male Wistar rats with different hardnesses DSW (DRO, D100, D600) in different dosages (6, 12, 30 mL/kg·d). It was found that the exhausting time and the ratio of lactic acid elimination to lactic acid increment from the experiment groups were significantly better than those from the control group. The BUN level of rats fed with D100 in a dosage of 30 mL/kg·d and D600 in three dosages distinctly was lower than that of the control group. The liver glycogen content in the group that the rats fed with D100 in dosages of 6 and 30 mL/kg·d and D600 in a dosage of 30 mL/kg·d showed significant difference compared with control group. However, the weight gain, kidney-body weight ratio, spleen-body weight ratio, and total plasma protein level in the rats of experimental groups showed no significant difference from those of control group. It suggested that endurance, adaptation for exercising load and accelerating elimination in fatigue of rats were improved when fed with higher hardness and dosage of DSW.

Key words: deep sea water, hardness, exhaustive exercise, exercise-induced fatigue

INTRODUCTION

Deep sea water (DSW) means the seawater from more than 200-meters depth below sea level. As the seawater is exploited practically, DSW possesses some characteristics such as low temperature, abundant and rich mineral, non-pathogen, and stable water property. The applicable scope of DSW is being extensive in different fields, such as aquatic farming, agricultural cultivation, food addition and processing, beverage manufacture, healthy and organic food, drug and cosmetics, tourism and leisure, refrigeration and air conditioning, and thermoelectric generation technology, etc. In the manufacture of foods and beverages, DSW can facilitate the fermentation of fermentable foods such as Sake, soybean sauce, Miso, and bread. Beside, it is commonly used for production of various potable water, fruit juice, alcohol, and carbonated beverage[1-5].

Tsuchiya *et al.*[6] compared changes in plasma lactate and pyruvate concentrations after taking a bath in DSW, surface seawater, and tap water heated to 42°C for 9 healthy young men. In the DSW bathing, plasma lactate and pyruvate concentrations showed no significant changes after bathing or after 60 min of bathing. However, in surface seawater and tap water bathing, plasma lactate and pyruvate concentrations were significantly changed for different measured periods. It was suggested that DSW was the mildest water to the human body among three kinds of water. Yoshioka *et al.*[5] reported that the values of biochemical indicators and organic histopathology of rabbits fed with DSW were no difference from those of control group (distilled water). However, the reductions of serum total cholesterol (TC) and low density lipoprotein cholesterol (LDLC) of hyperlipemia rabbits fed DSW with hardness 28, 300 and 1200, were more significant than those of the control group. Hence, the minerals in DSW were suggested to play the role in reducing TC and LDLC. Kimura *et al.*[7] reported that the male Wistar rats fed DSW with different magnesium (Mg^{2+}) contents of 200, 600 and 1000 ppm. The DSW groups with higher Mg^{2+} (600 and 1000 ppm) significantly resulted in 18% and 15% reduction of TC when compared to the control group, respectively. Furthermore, the DSW groups with higher Mg^{2+} also led to the improvement of uric acid and urea nitrogen metabolism in plasma. Tsuchiya *et al.*[4] also reported

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that effects of diluted desalted DSW on hematologic and blood chemical values in female BALB/c mice. There was no evidence of acute or subacute effect of diluted desalted DSW. The values of hematologic and blood chemical indicators of experimental group were similar to those of control group, meaning desalted DSW was as safe as purified water for drinking. Hataguchi *et al*.[8] reported the effect of drinking DSW (hardness 1000) on hair minerals in 33 patients with atopic eczema/dermatitis syndrome (AEDS). After drinking DSW for 6 months, the level of essential minerals, potassium was significantly decreased, while selenium (Se) was contrast. However, the level of the toxic minerals mercury and lead were significantly decreased. Further, the skin symptoms were improved in 27 out of 33 patients with skin diseases. Nagai *et al*.[9] reported that the DSW with 200 ppm Mg^{2+} could effectively delay cataract onset in the Shumiya cataract rat (SCR). The opacification and Ca^{2+} level of lens in cataract SCR administered with 200 ppm Mg^{2+} DSW was lower than those of control group.

The fatigue is defined as a body fails to maintain an operation at a regular level and each organ in a body does not preserve its normal processing skill[10,11]. It also means that the vitality of one person is reduced since the operational muscle is unable to keep its physical strength or skill indispensable and anticipative due to excessive activity of a body. The mechanism of generation of exercise-induced fatigue includes energy exhaustion (glycogen, ATP and creatine phosphate depletion), muscle acidosis, metabolic product accumulation (lactate, ADP, phosphate, ammonia, inosine monophosphate and so furth), excitabilities of central nervous system, spinal cord, neuromuscular junction and muscle membrane[10-12], and oxidative stress[13-15]. According to endurance experiments, some biochemical indicators including blood urea nitrogen (BUN), glycogen, lactate dehydrogenase, blood lactate acid, blood glucose, and non-esterified fatty acid, are available for judging the function of food in anti-fatigue[13-16].

Owing to exercise, a great quantity of electrolytes such as sodium, potassium, chlorine, and magnesium will be lost through perspiration[10]. Moreover, because enzymes responsible for energy metabolism of muscles in exercise demand participation of more zinc, most endurance-type athletes may have low blood zinc[17,18]. For a person who has lost electrolytes after exercising for a long period, he (she) ought to consider complementing electrolytes to restore his (her) physical strength[12]. Therefore, properly drinking DSW with rich minerals can make up loss of various electrolytes attributing to exercise and restore normal functions of human body.

The objective of this study is to investigate effect of DSWs on exercise-induced fatigue of rats. The male Wistar rats were fed with DSWs in different dosages and different hardnesses for two weeks and then proceeded with an exhaustive exercise test on a treadmill. Meanwhile, some biochemical indicators including BUN, glycogen, and blood lactic acid that are related to exhaustive exercise were used to evaluate the effects of DSW on exercise-induced fatigue of rats.

MATERIALS AND METHODS

I. *Materials*

The DSW was supplied by Stone & Resource Industry R&D Center (Hualien, Taiwan) and pumped from seawaters of 618 meters below sea level in Hualien, Taiwan. Figure 1 showed the process flow chart of DSW. The DSW was passed through an ultrafiltration set and a reverse osmosis set to get reverse osmosis treated deep sea water (DRO). The hardness of DRO was about 25 ppm. Then, the DRO was adjusted with divalent salt fraction to get hardness of 100 ppm (D100) and 600 ppm (D600) DRO. The contents of major minerals for three types of DSW were listed in Table 1. The 7-week-old male Wistar rats were purchased from BioLASCO Taiwan Co., Ltd. (Taipei, Taiwan). BUN kits, liquid glucose kits, lactic acid kits, and total protein kits were purchased from Randox Laboratories Ltd., (Antrim, United Kindom), and other agents from Sigma Chemical Co. (St. Louis, Mo. USA).

II. *Experimental Animal*

The 7-week-old male Wistar rats weighting of 180-200 g were raised in the Experimental Animal Center of College of Life Sciences, National Taiwan Ocean Univer-

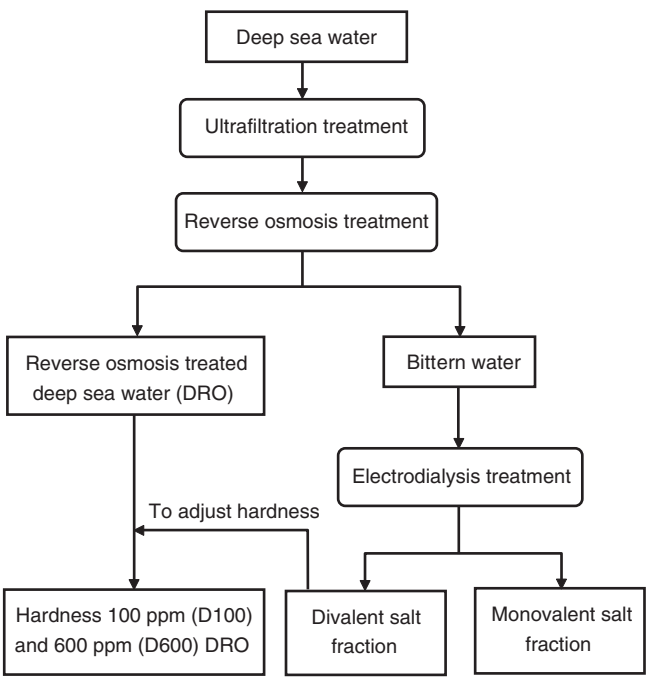

Figure 1. The flow chart of deep sea water processing.

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Table 1. The mineral concentration (ppm) of reverse osmosis treated deep sea water (DRO), DRO with hardness of 100 ppm (D100), and DRO with hardness of 600 ppm (D600), and common reverse osmosis water (RO) from fresh water

Mineral	RO	DRO	D100	D600
Na	0.930	1.102	27.40	159.0
K	0.007	---	0.460	2.910
Ca	---	---	3.120	18.10
Mg	---	---	19.20	113.0
Zn	---	---	0.002	0.003
Se*	---	---	0.333*	1.998*

--- too low to be detected.
* The concentration unit of Se is ppt while other minerals are ppm.

Table 2. The relationship between gradient, speed, and enduring time of treadmill for exhaustive exercise

Gradient (°)	Speed (mph)	Enduring time (min)
0	8.2	3
5	15.2	3
10	19.3	3
10	26.8	3
12.5	26.8	3
12.5	30.3	3
15	30.3	3
15	35.4	3
15	40.0	3
15	43.8	3

sity, Taiwan. The indoor temperature and relative humidity in the Experimental Animal Center was set at 23 ± 1°C and 60 ± 1%, respectively. The light cycle was divided to 12-hr daytime (6:00 to 18:00) and 12-hr night. All rats were bred over one week after the purchase, the dysplastic rats were excluded and the remaining ones were grouped randomly. The number of rats in each group was greater than 8 and all rats had a similar weight, about 230 g apiece, so there was no difference in the average weight for each group. General forage (Laboratory Rodent Diet MF18, Oriental Yeats Ltd., Japan) and reverse osmosis (RO) water (Millipore RiOs^{TM}, Billerica, MA, USA) were supplied for rats in this experiment. From the second week, these rats were separated into 10 groups by different kinds and dosages of DSW or RO (control group). The DRO, D100, and D600 were fed in three dosages (6, 12, 30 mL/kg·d) to rats of experimental groups by stomach intubation for two weeks at seventeen o'clock every day, but only one dose (6 mL/kg·d) for control group. Biochemical indicators of exercise-induced fatigue and exhaustive exercise on a treadmill for all tested rats were then examined.

This study was carried out under the approval of the Institutional Animal Care and Use Committee at College of Life Sciences, National Taiwan Ocean University.

III. *Exhaustive Exercise Test on a Treadmill*

After grouping (from the second week), all rats were trained to fit the test of the treadmill (rat/mice treadmill T306E, Singa, Taipei, Taiwan). These rats were daily trained for 5 min on the treadmill in 30 min after the stomach intubated with DRO, D100, D600 or RO. The speed and gradient of running belt in the treadmill are showed in Table 2. In the experiment, the rat was put on the treadmill equipped with an electric shock zone at the rear part in 30-60 min after the stomach intubation. When the rat was running on the machine, the speed and gradient of running belt in the treadmill was increasingly adjusted until the rat was exhausted (Table 2). Once the rat fell into the electric shock zone and could not run further after several electric shocks, the rat was judged as exhaustion and the running time was recorded[11,19].

IV. *Measurement of Biochemical Values of Swimming Rats*

The biochemical indicators including BUN, glycogen, blood lactic acid, and total plasma protein quantity that are related to exercising exhaustion were used to evaluate the effect of DSW on exercise-induced fatigue of swimming rats.

BUN test: after 30 min of stomach intubation, the rat without any heavy burden was put into water at 30°C for 45 min swimming and then was anaesthetized by ether and the blood of rat was collected from the tail. The determination of exercised time was due to BUN, a later-stage product of exercise metabolism. The plasma of blood samples were collected by centrifugation (7000 rpm, 20 min) from blood and examined for plasma

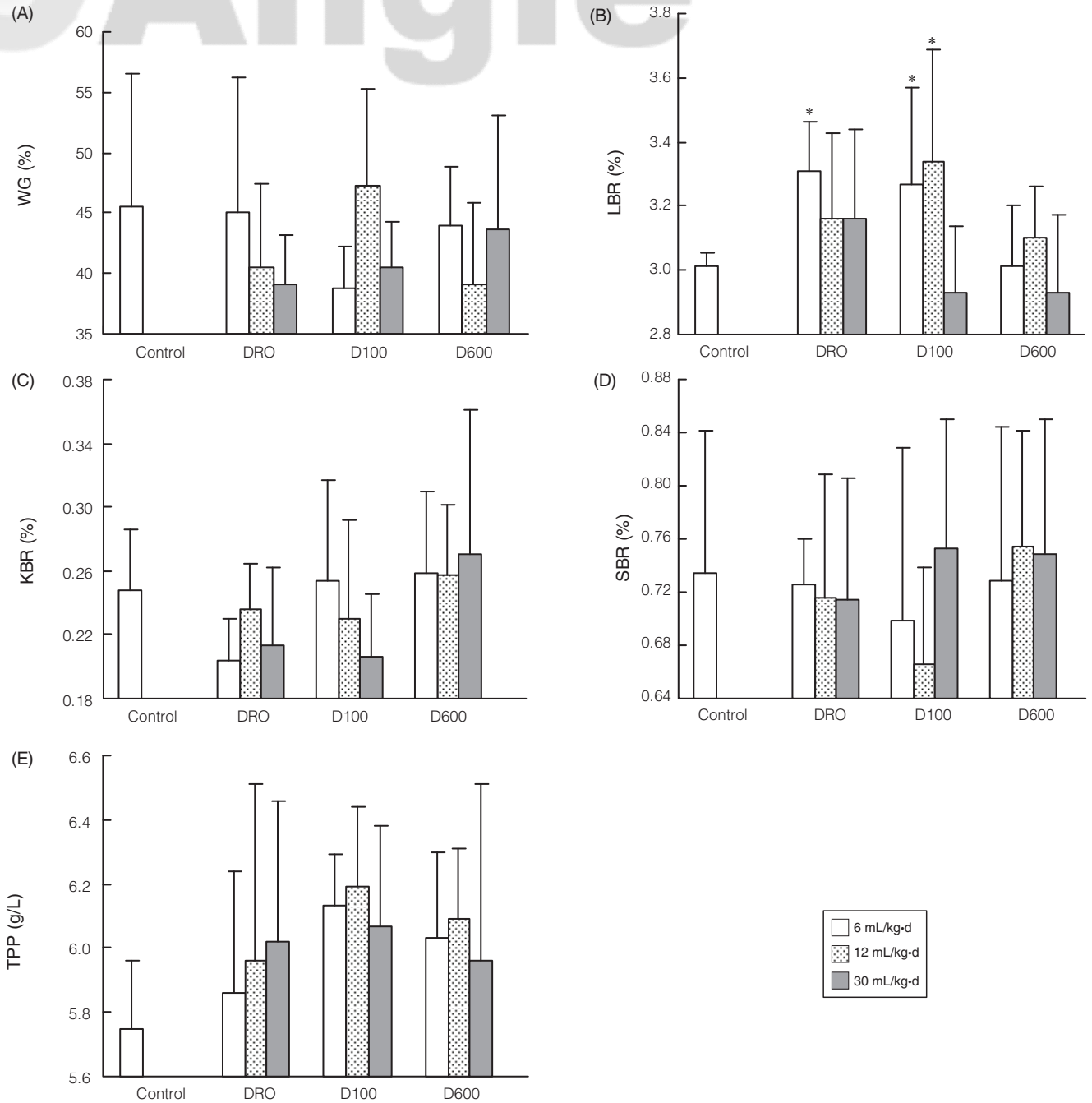

Figure 2. (A) The weight gain (WG); (B) liver-body weight ratio (LBR); (C) kidney-body weight ratio (KBR); (D) spleen-body weight ratio (SBR); (E) total plasma protein (TPP) of male Wistar rats fed with different hardness DSW (DRO, D100, D600) in different dosages for two weeks. *Data were significantly different from those of controls ($p < 0.05$).

biological characteristics. Heparin (500 IU/mL) as anticoagulant was added 20 μL in each sample. The sample was mixed with a kit of blood urea nitrogen (BUN) and the absorbance was measured with the enzyme-linked immunosorbent assay (ELISA) reader (μQuant, BIO-TEK Instruments, Kontron, USA) at 600 nm, then the BUN of blood plasma was obtained.

Serum lactic acid test: after 30 min of stomach intubation, the rat blood was collected from the tail. Then, the rat without any heavy burden was put into water swimming at 30°C for 10 min. The determination of exercised time was due to lactic acid which is former-stage product of exercise metabolism. Subsequently the rat blood was collected again after swimming and after 20 min of rest. The serum was collected by centrifugation at 3000 rpm for 10 min. The serum was mixed with lactic acid kit for 10 min and the absorbance at 550 nm was measured for conversion of the blood lactic acid content by contrasting

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it with the standard sample. The formula of lactic acid increment (LAI, %) and lactic acid elimination (LAE, %) are as follows:

LAI = (The difference in lactic acid concentration between before and after swimming)/(the lactic acid concentration before swimming) × 100%

LAE = (The difference in lactic acid concentration between after swimming and after 20 min rest)/(the lactic acid concentration after 20 min rest) × 100%

Glycogen test: after 5 weeks of DSWs or RO water feeding, the rats were sacrificed after 30 min of stomach intubation. The liver, kidney, and spleen of rats were collected and soaked in phosphate buffer. These organs were blotted with tissue paper to remove excess water and weighted. In addition, the liver was taken out and pH 4.2 citric acid buffer was added to mix. Then amyloglucosidase for decomposition of glycogen to glucose was added for the concentration test. When the sample was incubated with the liquid glucose kit over 10 min at 15-20°C, the absorbance at 500 nm was measured and the conversion of glycogen content could be acquired by contrasting it with the standard sample.

Total plasma protein quantity: the rats were sacrificed after 30 min of stomach intubation. The bloods of rats were collected and the total protein quantity was determined. The blood serum was incubated with the protein of blood plasma kit 30 min at 20-25°C and the absorbance was measured at 546 nm for conversion of the plasma protein content by contrasting it with the standard sample.

V. *Statistics Analyses*

Statistical analyses were performed using SPSS (version 12.0) for Windows. All data are expressed as the mean ± standard deviation (SD) from 8 different animals. Statistical significance was determined by Student's *t*-test for comparison of the control and treatment groups.

RESULTS AND DISCUSSION

I. *Effect of DSW on Weights, Organs, and Total Plasma Protein Quantities of Rats*

Figure 2 shows rats' weight increment, ratio of the liver weight to the body weight (liver-body weight ratio, LBR), ratio of the kidney weight to the body weight (kidney-body weight ratio, KBR), ratio of the spleen weight to the body weight (spleen-body weight ratio, SBR), and changes of the total plasma protein content when rats fed with different hardness DSW (DRO, D100, D600) in different dosages (6, 12, 30 mL/kg·d) and RO water (in a dosages of 6 mL/kg·d as the control group) for two weeks. Prior to the gavage, the average weight of rats in all groups was about 230 g and there was no difference among groups. After two weeks of stomach intubation, the average weight increment rate of rats in all groups was 38.7-47.3% and there was no significant difference according to statistical analyses.

After two weeks of feeding with three types of DSW, the KBR and SBR of rats were almost similar to those of control group (Figure 2). Additionally, there was no significant difference in total plasma protein contents between any experimental groups and control group. However, the LBR of rats fed with DRO in dosage of 6 mL/kg·d and D100 in dosages of 6 & 12 mL/kg·d, respectively was larger than that of control group. Therefore, the weight gain, kidneys, and spleens of male Wistar rats were unaffected after the rats were treated with DSW, while the effect of DSW on liver of rat needs more research further.

Our results are similar to those of other reports[4,5,9]. The growth curve of SCRs fed with DSW was almost the same as that of administered purified water[9]. The organic histopathology, such as liver, kidney, stomach, duodenum, large intestine, and main artery bow of normal male rabbits (1.8-2.0 kg) fed with 150 mL/d DSWs (desalinated water, hardness 28, 300, and 1200) for 4 weeks was found to be of no difference from that of distilled water group[5]. The weight gain of female BALB/c mice fed with diluted desalted DSW for 12 weeks was found to be also of no difference from that of control group[4].

II. *Effect of DSW on the Running Endurance of Rats*

Figure 3 shows the changes of running exhausting time when the rats were fed with three different hardnesses of DSW in different dosages and RO water for two week. According to results shown in Figure 3, DSW of three different hardnesses can promote the endurance of rats in exercise test. The exhausting time is 323.4-476.1

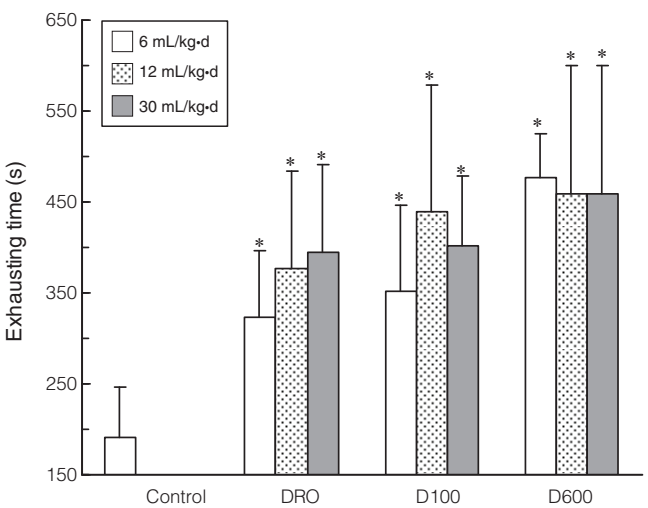

Figure 3. Effect of the hardness and dosage of DSW (DRO, D100, D600) on the exhausting time of male Wistar rats fed with DSW for two weeks. *Data were significantly different from those of controls (p<0.05).

seconds when the rats were fed with any types of DSW, which is obviously superior to that (191.0 seconds) of control group. As shown in Table 1, DSW contains more variety of minerals than RO water does, and these various minerals have higher concentration that may contribute to promote the endurance of exercise for rats.

Figure 4 indicates that the effect of the amount of divalent salt by stomach intubation on the exhausting time performed by rats. The correlation equation of the amount of divalent salt and the exhausting time is y = 37.01 Ln(x) + 391.33 (R^2 = 0.88). The exhausting time of rats was increasing due to more amount of divalent salt was fed in. It means that the supplied DSW with a high hardness or a large dosage enhanced rats' running endurance. Accordingly, the phenomenon that the promotion of rats' running endurance comes from ingestion of more electrolytes may be the loss of electrolytes because of a great deal of exercise (treadmill training) by rats in advance[10,12]. Thus, by means of the stomach intubation of DSW with high hardness or high dosages, the rats, which acquired more electrolytes to complement lost electrolytes in previous exercise, perform well in the endurance of exercise. In addition, the Zn and Se of DSW may contribute the improvement of the endurance of exercise. Zn is a kind of coenzyme for muscle enzymes at energy metabolism[17,20]. Se is a potent free radical scavenger and can thus decrease oxidative stress and improve endurance of exercise[13].

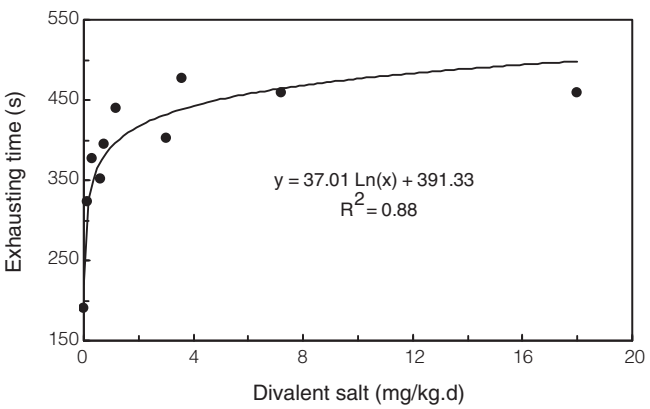

Figure 4. The effect of the amount of divalent salt by stomach intubation on the exhausting time performed by rats.

III. *Effect of DSW on the Biochemical Indicator of Blood Plasma in Rats*

Figure 5 demonstrates the effect of the hardness and dosage of DSW on the BUN and liver glycogen of male Wistar rats fed with DSW for two week. It shows that BUN levels (21.51-26.70 mg/dL) of rats in the experimental groups are lower than that (27.15 mg/dL) of control group (Figure 5A). According to statistical analyses, BUN levels of rats fed with D100 in a dosage of 30 mL/kg·d and D600 in three types of dosages are significantly lower than that of control group. The BUN content of the animals ascended in virtue of increment in work or exercising load. Based on this perception, BUN of laboratory animals has poor adaptation to the increase of exercising load obviously[16]. This result indicates that the rats fed with DSW in high hardness and high dosage could adapt to larger exercising load and the production of BUN would slow down.

As shown in Figure 5B, the liver glycogen contents of rats in the experimental groups after exercise are 94.8-222.4 mg/dl that presented a fluctuant change. Through statistical analyses, the liver glycogen content in the group that was fed with D100 in a dosage of 6 mL/kg·d was significant higher as compared with control group, but the rats fed with D100 in a dosage of 30 mL/kg·d and D600 in a dosage of 30 mL/kg·d showed significant lower compared with control group. Therefore, DSW with high

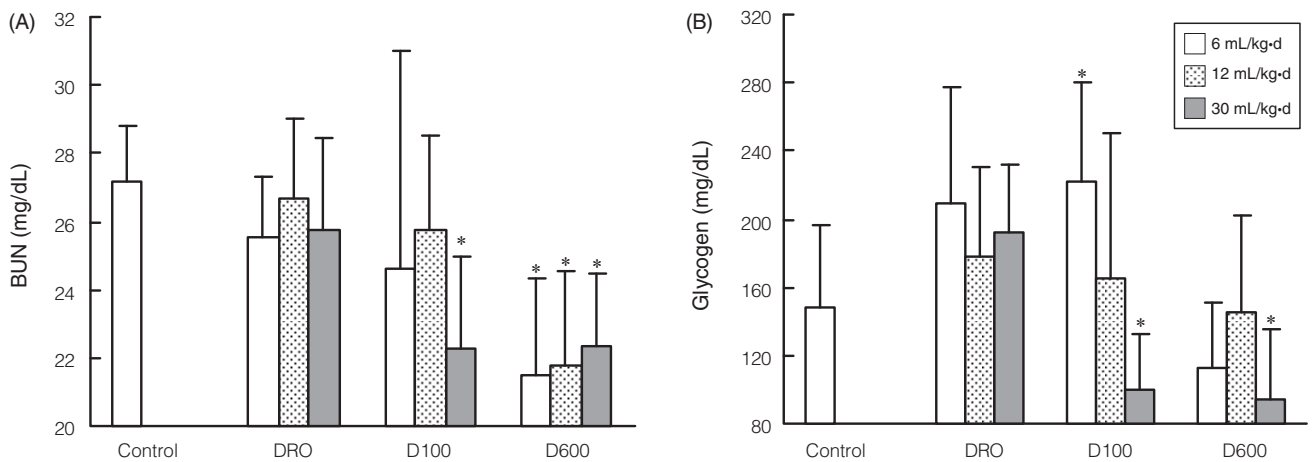

Figure 5. (A) Blood urea nitrogen (BUN) and (B) liver glycogen of male Wistar rats fed with different hardness DSW (DRO, D100, D600) in different dosages for two weeks. *Data were significantly different from those of controls ($p < 0.05$).

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hardness and dosage seem to have no good effect on the increment of the liver glycogen contents of rats. This result has a great resemblance to the effect of desalted DSW on the female BALB/c mice[4].

Figure 6A shows serum lactic acid increment (LAI) after exercise and serum lactic acid elimination (LAE) after rest for rats fed with DSW for two weeks. The results show that the LAIs (29.18-40.23%) of rats in all experimental groups are higher than that (27.94%) of control group. Through statistical analyses, the rats fed with D600 in three types of dosage have significantly higher LAI than control group. This result is consistent with that of glycogen content above. Lactic acid is a metabolism product from glycogen and glucose. The glycogen sparing effect could be slower utilization of glycogen, resulting in the improvement of endurance exercise performance[13]. However, these results indicate that DSW neither improves glycogen content nor exerts a better glycogen sparing effect.

Figure 6B indicates the LAE (11.99-23.55%) of the experimental groups are greater than that (9.21%) of control group. According to statistical analyses, all data of the experimental groups are significantly greater than that of control group except for the group that the rats fed with DRO water in a dosage of 6 mL/kg·d. The ratio of elimination to increment should be compared if the LAI and the LAE of the experimental group are significantly higher than those of the control group, because a higher ratio of experimental group than that of control group means anti-fatigue efficacy[21]. As shown in Figure 6C, all ratios (0.38 to 0.80) of the LAE/LAI for the experimental groups are greater than that (0.33) of control group. Because the accelerated metabolism rate of anaerobic energy at intense exercise of a body results in accumulation of enormous lactic acid in muscles[10,12], lactate dehydrogenase catalyzes lactic acid to become pyruvate that enters the citric acid cycle for energy generation[16]. From results shown in the LAE, the accumulation of lactic acid descended through a metabolism owing to a 20 min rest after a rat's exercise. For those rats of the experiment groups that ingest DSW, their LAE is superior to that of rats in the control group. Based on this fact, it can be inferred that zinc, a component of lactate dehydrogenase, in DSW plays an important role in energy metabolism[17,20]. Thus, because of stronger activation of lactate dehydrogenase, the rats in the experiment groups

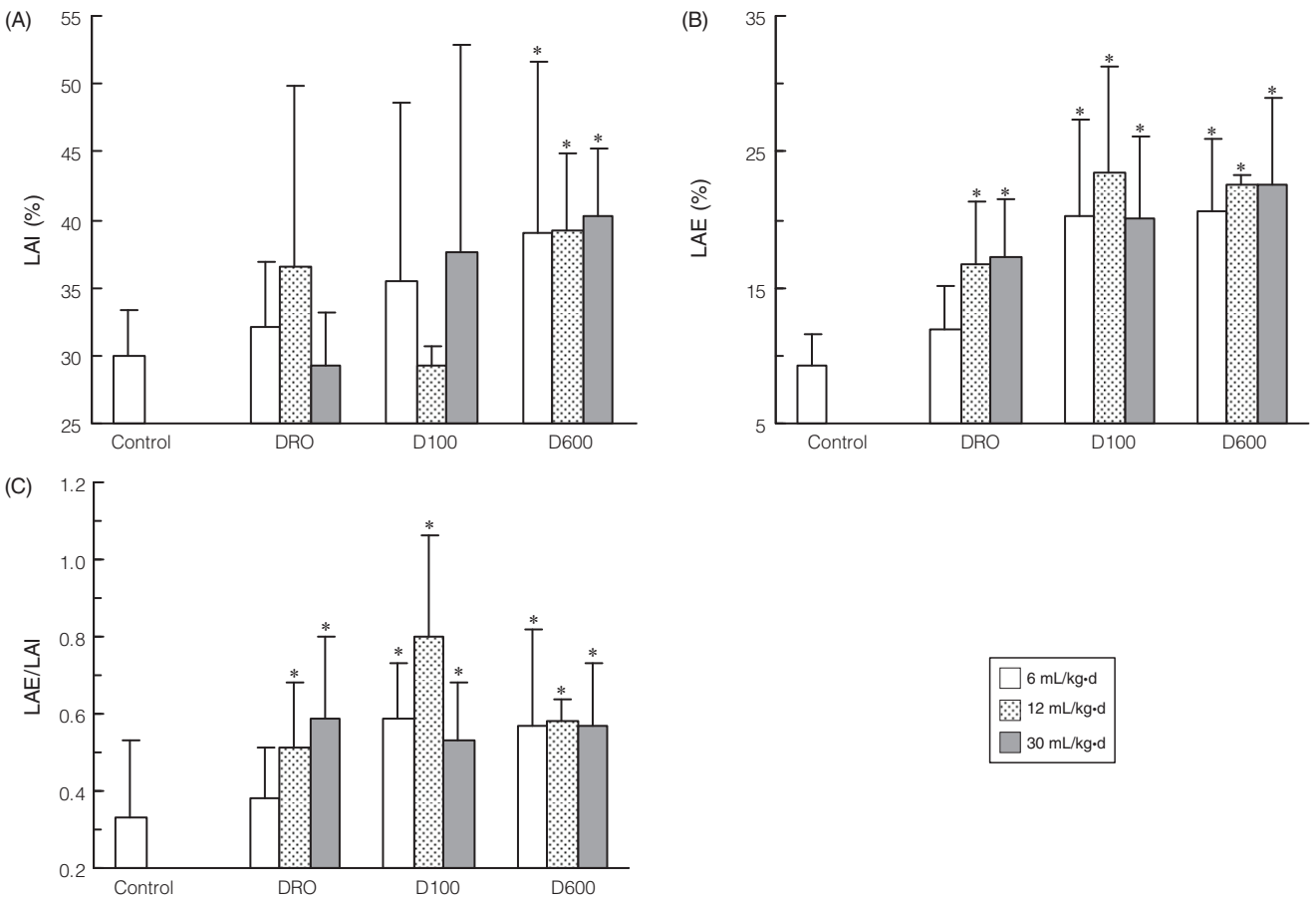

Figure 6. (A) The lactic acid increment (LAI) after exercise, (B) lactic acid elimination (LAE) after rest, and (C) LAE/LAI for male Wistar rats fed with different hardness DSW (DRO, D100, D600) in different dosages for two weeks. *Data were significantly different from those of controls ($p < 0.05$).

have higher LAE that led to a higher LAE/LAI ratio.

CONCLUSIONS

After the two-week breeding of male Wistar rats with deep seawater in different dosages and different hardness and general RO water, some indicators such as weight increment, kidney-body weight ratio, spleen-body weight ratio, and total plasma protein content measured in rats of the experiment groups did not show any significant difference. As regards to the exhausting time and the ratio of lactic acid elimination to lactic acid increment, the experiment groups were significantly better to those from the control group. The BUN level of rats fed with D100 in a dosage of 30 mL/kg·d and D600 in three dosages distinctly was smaller than that of the control group. The liver glycogen content in the group that were fed with D100 in dosages of 6 and 30 mL/kg·d and D600 in a dosage of 30 mL/kg·d showed significant different compared with the control group. Summarizing the above results, it is suggested that endurance, adaptation for exercising load and accelerating elimination in fatigue of rats could be improved when fed with DSW of higher hardness and quantity.

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