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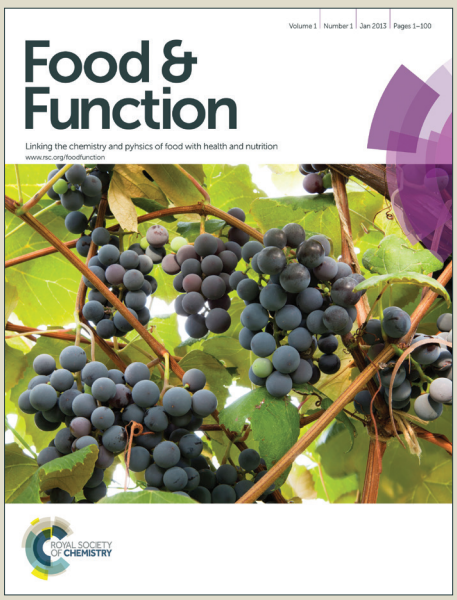

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Effect of dietary α-lipoic acid, betaine, L-carnitine, and swimming on the obesity of mice induced by high-fat diet

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Abstract

To evaluate the effect of supplementation, at 300 mg/kg body weight (BW), with the antioxidants α-lipoic acid (AL), betaine (BT), L-carnitine (LC), and the combination of these and exercise on obesity induced by a 9-week high-fat diet (HFD) in mice. Healthy 5-week-old male C57BL/6J mice were divided into 9 groups: 1) CON, control group fed with a commercial mice chow containing 10% crude fat; 2) HFD, high fat diet group fed with a commercial mice chow containing 60% crude fat; 3) HFD-AL, HFD group fed with AL; 4) HFD-BT, HFD group fed with BT; 5) HFD-LC, HFD group fed with LC; 6) HFD-SW, HFD with swimming as an exercise; 7) HFD-SWAL, HFD-AL with swimming; 8) HFD-SWBT, HFD-BT with swimming, and 9) HFD-SWLC, HFD-LC with swimming. The BW of mice with LC and swimming reduced the increase of BW after 9 weeks. Relative adipose tissue weights were reduced by the combinations of antioxidant supplementation and swimming. Levels of serum glucose and leptin levels were reduced in HFD-SWLC group when compared with HFD group. Serum triglyceride and total cholesterol and size of adipose were also decreased in the HFD-LC and HFD-SWLC groups. These results show that LC at a dose of 300 mg/kg BW was the most effective for reducing fat accumulation in mice with HFD for 9 weeks. In addition, exercise should be given in combination to enhance the BW reduction and serum lipid level.

Key words: α-lipoic acid, betaine, L-carnitine, antiobesity, high-fat diet

1. Introduction

Obesity is prevalent worldwide and induces health risks such as heart disease, type II diabetes, sleep problems, cancer, and osteoarthritis[1]. The extent of obesity caused by diet varies according to the length of the feeding trial, kinds and levels of dietary fat, and/or the presence of other modifications in dietary ingredients. Chronic exposure to a high-fat diet induces the generation and reception by the brain of meal-related signals that control energy metabolism and of adiposity-indicating signals that regulate food intake and metabolism and then result in obesity[2]. Orlistat and sibutramine are currently approved two antiobesity medications for long-term use.[4] Both drugs reduce intestinal fat absorption by inhibiting pancreatic lipase, whereas sibutramine decreases appetite by inhibiting deactivation of the brain neurotransmitters norepinephrine, serotonin, and dopamine.[1,3] However, these kinds of drugs have been reported to have important side effects and a low efficacy[1]. For example, the sibutramine was withdrawn from the US market due to the concerns about cardiovascular disease.[4]

Among the well-known antioxidants, α-lipoic acid (AL), betaine (BT), and L-carnitine (LC) are antioxidants with specific functions, although BT and LC are not as strong as AL. AL has been used as a dietary antioxidant at doses in the range of 100–200 mg/day since the 1990s.[5] It is generated naturally in most cell types; function as an antiobesity compound[6] and a co-factor for essential enzymes for energy metabolism; and is found mostly in animal tissues with high metabolic rates such as the heart, kidney, liver, and muscle.[5,7] BT is a choline metabolite and produced by choline oxidase in the liver. Administration of BT has been reported to decrease hepatic steatosis caused by either carbon tetrachloride or ethanol.[8] LC is a naturally occurring material for the regulation of energy metabolism in mammals. It is produced by the body, and it was reported that the LC content in the m. pectoralis of humans

is 516 ± 48 mg/kg (w/w).[9] Skeletal muscles are the major reservoir of LC in the body, with a concentration of at least 50–200 times higher than that in blood plasma.[10] Furthermore, LC is available in the diet, mainly from animal products,[11] whereas fruit and vegetables contain negligible quantities. LC is essential for the transport of long-chain fatty acids across the mitochondrial membrane for subsequent fat degradation and energy production. In addition, recent work concluded that high doses of LC modulate glucocorticoid receptor function and might mimic some of the biological activities of glucocorticoids such as stimulating lipolysis in adipose tissue.[12]

Although these antioxidants have antioxidative and fat-reducing effects, no study has evaluated the effect of their administration in combination with exercise on obesity. Exercise has a positive impact on body mass loss and insulin sensitivity[13] and high-intensity exercise has a major impact on fat oxidation during post-exercise period.[14] Especially, swimming is the best exercise for obese individuals, pregnant women, and elderly people because it provides low damage in bone, joint, and muscle during exercise.[15,16] Therefore, in this study, we investigate the effect of supplementation of AL, BT, and LC, and their combinations with exercise on obesity induced by a high-fat diet in mice.

2. Materials and Methods

2.1. Chemicals

AL, BT, and LC were purchased from Sigma Co. (USA). All chemicals used in this study were of analytical grade.

2.2. Animals and treatments

Male C57BL/6J (4-week-old) mice were obtained from Samtaco (Seoul, Korea). Before

the beginning of the study, the mice were allowed to adapt to the environment for 7 days, and healthy mice were selected for the experiment. As shown in Table 1, healthy 5-week-old mice were randomly allocated into 9 diet groups with 18 pens and 5 mice per each pen: (1) control (CON) group (n = 10), comprising animals fed with a commercial mice chow containing 10% crude fat; (2) high-fat diet (HFD) group, comprising animals fed with a commercial mice chow containing 60% crude fat; (3) high-fat diet with AL (HFD-AL) group, comprising mice fed with 300 mg/kg body weight (BW) of AL; (4) high-fat diet with BT (HFD-BT) group, comprising mice fed with 300 mg/kg BW of BT; (5) high-fat diet with LC (HFD-LC) group, comprising mice fed with 300 mg/kg BW of LC; (6) high-fat diet with exercising (swimming) group (HFD-SW); (7) high-fat diet with AL and swimming group (HFD-SWAL); (8) high-fat diet with BT and swimming group (HFD-SWAL); and (9) high-fat diet with LC and swimming group (HFD-SWLC). The mice were allowed free access to food and water, and kept in plastic cages. BT and LC were dissolved in tap water. AL (37.5 mg) was dissolved in 50 μL ethanol. The ethanol was evaporated by N_2 gas. After adding distilled water with respect to the sample concentration, AL in the distilled water was homogenized using an ultrasonic processor (Sonics VCX-130, Chemical Instruments AB, Sweden). They were oral supplemented by gavage at a dose of 300 mg/kg BW every day at the same time for 9 weeks.[2] Swimming program was conducted by a previous method[17] with slight modification. For adaptation to the exercise program, mice were made to swim for 10, 20, 30, 40, and 50 min gradually for 1 week. Thereafter, mice in the swimming groups were made to swim at a water temperature of 32–36°C for 5 days a week and 1 h per day for 9 weeks (Fig. 1). The experimental procedure used in this study met the protocol of the Animal Care and Use Committee of the Kangwon National University, Korea (KIACUC-13-0004).

2.3. Physiological analysis

Mortality, feed intake, behaviour, and general conditions were observed daily. BW was measured weekly, and feed efficiency was determined by the ratio of the increase of BW per total feed intake for 9 weeks. Faeces of mice were collected 48 h before sacrifice and kept at -75°C until use. Diethyl ether was used to anaesthetise the mice, and blood was collected via heart puncture and centrifuged at 150 × *g* for 15 min (Avanti Centrifuge J-20XP; Beckman Coulter, USA) to separate serum. After the removal of blood, organs and fat, such as the lungs, liver, spleen, kidney, testis, and epididymal fat were dissected. The organs and fat were washed with cold saline, and dried with paper towels. The organs were weighed, frozen in liquid nitrogen, and kept in a deep freezer (-75°C) until use.

2.4. Blood composition

Serum triglyceride, total cholesterol, high-density lipoprotein (HDL)-cholesterol, glucose, and blood urea nitrogen (BUN) were measured using an automatic biochemical analyser (ADVIA 2400; Siemens, USA). Insulin was measured using ^{125}I-labelled antibody and Packard Cobra Gamma Counter (BIOSOURCE, Belgium). Leptin content in serum was assayed using mouse Leptin ELISA Kit (Crystal Chem Inc., USA). In addition, serum low-density lipoprotein (LDL)-cholesterol was calculated using Friedewald's method, as follows: LDL = (Total-C) - [(HDL-C) + (TG/5)].[18] Atherogenic index (AI) was calculated using Fiordaliso's equation: AI = ([Total-C] - [HDL-C])/[HDL-C]. [19]

2.5. Liver fat and lipid composition

Fat in dissected mice liver was extracted according to Folch's method, with slight modification, to determine the triglyceride and total cholesterol levels.[20] Briefly, 0.5 g of liver

was added to 25 mL Folch's solvent (mixture of chloroform/methanol, 2:1) and homogenised. The mixture was centrifuged at 3000 rpm for 10 min and filtered using Whatman No. 1 filter paper. Five millilitres of 0.88% KCl was added into the filtrates and left for 24 h to separate into 2 layers. Chloroform in the lower layer was separated and concentrated using N_2 gas. Concentrated fat was dissolved in 1 mL ethanol, and its triglyceride, total cholesterol, and HDL-cholesterol contents were determined using a commercial analysis kit (Asan Pharmacy, Korea).

2.6. Faecal cholesterol and total lipids

The collected faeces were dried in a drying oven (60°C) for 2 h. Briefly, 0.5 g of faeces were added to 25 mL Folch's solvent and homogenized. The mixture was centrifuged at 3000 rpm for 10 min and filtered using Whatman No. 1 filter paper. Five millilitres of 0.88% KCl was added to the filtrates and left for 24 h to separate into 2 layers. Chloroform in the lower layer was separated and concentrated using N_2 gas. Concentrated fat was dissolved in 1 mL ethanol, and its triglyceride, total cholesterol, and HDL-cholesterol contents were determined using a commercial analysis kit (Asan Pharmacy, Korea).

2.7. Histological characteristics of liver and epididymal adipose tissue

Liver and epididymal fat were dissected from mice and fixed in 10% formalin in phosphate buffered saline (pH 7.4) for 2–3 days. The fixed liver and epididymal fat were embedded in paraffin and sliced into 4-μm-thick sections by using Rotary Microtome (Microm HM340E; Thermo Scientific, USA). The organ tissue slices were stained with haematoxylin & eosin. Stained images were obtained using an optical microscope (Olympus BX 50; Olympus Optical Ltd., Japan) and a digital camera (Olympus DP72, Japan). Twenty randomly selected

epididymal fat images per section were digitally captured (magnification, ×200), and the size of the fat was analysed using the image analysis program Image-Pro Plus ver. 5.0 (Media Cybernetics, USA).

2.8. Statistical analysis

All data collected were subjected to one-way analysis of variance (ANOVA) according to the general linear model procedures of SAS software (SAS Institute Inc., Cary, NC, USA). Mean values and standard error were reported. When analysis of variance indicated a significant treatment effect, Duncan's multiple range test was used to compare the mean values, and $p < 0.05$ was considered statistically significant.

3. Results and Discussion

3.1 BW change and feed efficiency

The C57BL/6 mice were chosen in this study due to their tendency to develop obesity and other signs similar to human metabolic syndrome when fed high-fat diets.[21] The administration of high-fat diet caused an increase in body mass, hyperinsulinemia associated with insulin resistance, and high serum total cholesterol and triglycerides as expected in these animals.[21-23] An increase in body weight can be recognized after as little as 2 weeks, the diet induced phenotype becomes most apparent after more than 4 weeks of high fat feeding.[24]

A large amount of abdominal fat was seen in the HFD group; however, combinations of antioxidant supplementation (AL, BT, and LC at 300 mg/kg BW) and exercise significantly decreased both the amount of fat and BW (Fig. 1). Mice that were fed high-fat diets showed greater cumulative weight gain (0.21 g/day) than the groups that were fed the control diet.

The BW increase per day was highest in mice in the HFD group (0.21 g/day); however, supplementation of AL, BT, and LC without exercise significantly decreased the rate of BW increase (Table 1). In addition, combinations of those antioxidants and exercise (swam for 1 h/day, 5 days/wk) more effectively reduced the BW to a level similar to the normal control group. AL is well known to have anti-obesity effect of inhibiting food intake and growth.[6] It was reported that 240 subjects aged 18 to 65 years with a body mass index (BMI) $\geq$ 30 kg/m^2 were significantly decreased by supplementation of AL.[1] They suggested that in both the 1200 and 1800 mg/d AL groups, averaged body weight decreased significantly from baseline, starting as early as 4 weeks. AL is well known to be able to improve insulin sensitivity and prevents vascular dysfunction and fatty liver in obese rats.[25] BT is an organic osmolyte found in various foods such as spinach, beets, and whole grains.[26] Effect of BT on regulation of body composition is controversial. The chronic administration of BT has shown to decrease adipose mass and increase muscle mass in pigs.[27] However, BT did not improve human body composition in obese, sedentary subjects on a 500 kcal/day caloric deficit following 12 weeks of administration.[28] On the other hand, the effects of betaine on body composition, strength and power may be most apparent as administration occurs for several weeks accompanied by a resistance training program.[29] Feed efficiency was the highest in the HFD group, but was decreased in all of the treatment groups. Kim and Park[2] also demonstrated that rat fed a high-fat diet showed a significantly high food efficiency ratio for 9 weeks. The administration of LC increased weight gain, reduced carcass fat, and improved feed conversion ratio in weaning pigs.[30] In addition, it was suggested that LC might reduce fat deposition in favour of protein deposition.[31] These are supported by our results that the BW of mice fed HFD-LC was higher than that of controls ($p < 0.05$), which means that LC reduced fat and increased the body protein of mice.

205 *3.2. Relative organ weight*

206 Relative organ weight and fat weight were changed with supplementation of antioxidants and
207 exercise, and these results are shown in Table 3. Organ weight assay is crucial to assess
208 general toxicity because any change in organ weight is a sensitive indicator of toxicity.[32]
209 Especially, the liver is a target organ because most toxicants enter the body through the
210 gastrointestinal tract, and after absorption, the toxicants are carried by the hepatic portal vein
211 to the liver.[32] Liver weight relative to BW was significantly reduced in the HFD group;
212 however, the liver weight of the HFD-LC and HFD-SW groups recovered to that of the
213 control group. The liver weight of mice in the HFD-SWAL, SWBT, and SWLC groups
214 showed no significant difference. The weight of the kidney from mice fed AL, BT, and SW
215 was significantly heavier than mice fed HFD, whereas the spleen weight showed no
216 significant difference. Supplementation of HFD significantly reduced the testis weight
217 relative to BW; however, the testis weight of the HFD-BT and HFD-SW groups increased to
218 as much as the weight of the control group. Weights of retroperitoneal fat and epididymal fat
219 of the mice fed HFD, which are usually used as indicators of obesity, were increased up to
220 40.8% and 48.8%, respectively, compared with controls. This result is supported by the study
221 of Kim and Park[2]. The weights of those adipose tissues in mice fed antioxidants and in mice
222 with swimming were decreased compared with the HFD group ($p < 0.05$), especially in the
223 HFD-BT and HFD-SWLC groups. It was reported that supplementation of high-fat diets for a
224 long time increases oxidative stress in a variety of tissues,[33] whereas antioxidant
225 supplementation prevents many diseases caused from HFD. LC administration beneficially
226 affects markers of recovery from exercise stress.[34,35]
227
228 *3.3. Blood glucose, BUN, insulin, and leptin*
229 The blood glucose, BUN, insulin, and leptin levels of mice are shown in Table 4. When the

stomach is empty, the blood glucose level of mice in the HFD group was 263.0 mg/dL, and this group showed the highest level among all tested groups ($p < 0.05$). However, administration of AL and BT significantly reduced the blood glucose level, and the reduction rate was higher with the combination of exercise. However, no significant difference was found in insulin levels and BUN.

Leptin is a cytokine that not only suppresses appetite and insulin synthesis and secretion but also increases energy expenditure and insulin sensitivity.[36,37] Leptin is produced in proportion to the adipose tissue contents; it works in the hypothalamic nuclei to reduce food intake and is associated with type I and type II diabetes.[36,38,39] The highest leptin level was seen in mice fed HFD (26.4% higher than the control group). The leptin level of mice fed antioxidants and mice with swimming was significantly decreased when compared with that of HFD mice; especially, mice in the HFD-SWLC group showed similar leptin level as the controls, suggesting that the combination of LC administration and exercise is most effective in reducing the leptin level.

3.4 Serum lipid profile

The serum triglyceride level (TG), total cholesterol (TC), and atherosclerosis index (AI) of mice are shown in Table 5. Mice of all treatment groups showed a lower TG level compared with mice in the HFD group, and the values are equivalent to that of the control, except for the mice in the AL supplementation group. The TC of mice in all treatment groups was significantly reduced compared with that of mice in the HFD group, although the value is not equivalent to that of the control group. The HDL-cholesterol level of mice was not affected by either antioxidant supplementation or swimming. However, the LDL-cholesterol level was significantly reduced by the combinations of antioxidant supplementation and swimming ($p < 0.05$). The AI value was significantly higher in mice of the HFD group, whereas the value

was significantly reduced in mice of the BT, LC, SW, SWBT, and SWLC groups ($p < 0.05$). This result indicates that administration of BT and LC or swimming alone, and the combination of BT and LC and swimming were effective to regulate serum LDL- cholesterol of mice fed high fat diet. The serum lipid profile is a valuable metabolic factor to identify in obesity. Usually, the incidence of cardiovascular diseases and diabetes are associated with high levels of total TG, TC, and LDL-cholesterol.[40] The decrease of lipids in the serum may be due to leptin, as leptin secretion levels are positively correlated with the extent of triglyceride stored in adipocytes.[41,42] TC, TG, LDL-cholesterol, free fatty acid and lipase in serum decreased with the increase of LC in the broiler feed. [43]

3.5. Hepatic lipid profile

The effect of supplementation of AL, BT, LC, and swimming on hepatic triglyceride (TG) and cholesterol levels of mice fed a high-fat diet is shown in Table 6. In the HFD group, hepatic triglyceride and total cholesterol were significantly higher than that of control. Whereas supplementation of antioxidants reduced those values, when those supplementations were combined with swimming (SWLC group), the hepatic triglyceride and cholesterol levels decreased further to a value equivalent to that of the control ($p < 0.05$). The hepatic HDL-cholesterol level of mice in the HFD group showed the lowest value, and the combination of AL, BT, and LC supplementation and swimming significantly increased the value to up to that of the control group. Obesity alters the function of adipocytes, including increasing the adipocyte mass, insulin secretion, and release free fatty acid in the blood, which increases the amount of TG stored in the liver.[44,45] The levels of TG and TC in the HFD group were greatly increased; however, the levels of TG and TC were significantly decreased by dandelion leaf extract (DLE) supplementation.[44] It was suggested that DLE supplementation has a preventive effect on HFD-induced lipid accumulation in the liver by controlling the

metabolism of free fatty acid through the antioxidative luteolin and chlorogenic acid contents of DLE. In the present study, TG and TC were significantly reduced in the HFD-SWLC group, and this suggests that the supplementation of antioxidative LC also modulates free fatty acid metabolism.

3.6. Excretion of faecal lipids

The effect of supplementation of AL, BT, LC, and swimming on the faecal TG and cholesterol levels of mice fed HFD is shown in Table 7. Increased excretion of TG and cholesterol through faeces is one of the major anti-obesity mechanisms in mouse body lipid regulation. The TG level was the lowest in mice in the control group Mice in the HFD group fed a diet containing 60% fat showed 40.06 mg/dL triglyceride in faeces, whereas the HFD-SWLC group showed significantly higher triglyceride contents in faeces (53.97 mg/dL). This result suggests that the combination of LC supplementation and swimming significantly increased the triglyceride excretion in faeces. The total cholesterol content in faeces of mice in the BT, LC, SWBT, and SWLC groups was higher than in mice in the HFD group when swimming and supplementation of BT and LC were applied at the same time. The HDL-cholesterol contents in faeces of mice in the SWBT and SWLC groups were significantly lower, whereas the LDL-cholesterol contents in faeces of mice in the BT, LC, SWBT, and SWLC groups were significantly higher. This indicated that the combination of BT and LC supplementation with swimming effectively excretes cholesterol out of the body of mice.

3.7. Histological characteristics of the liver and epididymal adipocytes

In the histological analysis, the size of adipocytes in the liver was significantly decreased in the HFD-SWLC group compared with the HFD group (Fig. 2). The size of the epididymal adipocytes in the HFD-SWLC group was even lower than that of the control group (Fig. 3).

These results suggest that the decreased plasma leptin levels after supplementation of the antioxidant LC and exercise may be attributable to the decreased lipid accumulation in the epididymal adipocyte tissue. Administration of AL, BT, and LC without exercise also significantly reduced the size of adipocyte tissue; especially, LC with exercise is more effective in reducing adipocytes. LC increases long chain fatty acid oxidation in liver and heart, which means LC could improve fat metabolism, reduce fat mass, and increase muscle mass.[46] Endurance athletes often use carnitine to boost the oxidation of fat during exercise and spare muscle glycogen.[47] Adipocyte growth and differentiation are complex processes that are distinguished by many changes in cell morphology, hormone sensitivity, and expression of genes that control lipogenesis and lipolysis.[48] Therefore, decrease of the leptin level in blood but also increase of fat metabolism after LC administration may be one of the reasons for the reduction of adipocyte growth in the liver and epididymal tissue, as shown in Table 4.

4. Summary

Administration of antioxidants such as AL, BT, and LC at 300 mg/kg BW significantly decreased the serum leptin level, LDL-cholesterol, AI, and size of epididymal adipose tissue in mice with high-fat-diet-induced obesity. Especially, LC supplementation with proper exercise was more effective in reducing BW and serum lipids. These results suggest that administration of LC at 300 mg/kg BW with exercise 5 days a week for 9 weeks could reduce the BW more effectively than administration of AL and BT at the same concentration.

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420

421 **Figure Legends**

422

423 Fig. 1. Experimental schedule of treatments and exercise

424

425 Fig. 2. Effect of α-lipoic acid (AL), betaine (BT), L-carnitine (LC) and exercise on HFD-
426 induced lipid accumulation liver tissues were fixed with 4% paraformaldehyde, embedded in
427 paraffin, and H&E stained. (X200).

428 Abbreviations : See Table 1.

429

430 Fig. 3. Histological characteristics of the epididymal adipose tissue (× 200) and average size
431 of sections of epididymal adipose (μm^2).

432 Abbreviations : See Table 1.

433

434 **Table Legends**

435

436 Table 1. Experimental design of treatments

437 Table 2. Effect of exercise and antioxidants (AL, BT, and LC) supplementation on weight
438 gain and feed efficiency ratio of C57BL/6 mice fed high fat diet

439 Table 3. Effect of exercise and antioxidants (AL, BT, and LC) supplementation on relative
440 organ and tissue weight to body weight (%) of C57BL/6 mice fed high fat diet

441 Table 4. Effect of exercise and antioxidants (AL, BT, and LC) supplementation on serum
442 glucose, BUN, insulin, and leptin contents of C57BL/6 mice fed high fat diet

443 Table 5. Effect of exercise and antioxidants (AL, BT, and LC) supplementation on serum
444 lipid profile of C57BL/6 mice fed high fat diet

445 Table 6. Effect of exercise and antioxidants (AL, BT, and LC) supplementation on the hepatic
446 triglyceride and cholesterol of C57BL/6 mice fed high fat diet

447 Table 7. Effect of exercise and antioxidants (AL, BT, and LC) supplementation on the fecal
448 triglyceride and cholesterol of C57BL/6 mice fed high fat diet

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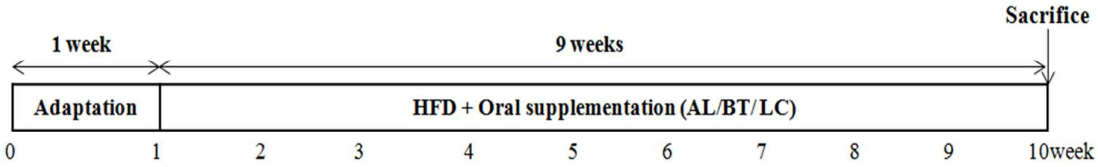

Swimming Schedule	Day 1	Day 2	Day 3	Day 4	Day 5
0 - 1 week	10 min	20 min	30 min	40 min	50 min
1 - 10 week	60 min per day				

451

452 Fig. 1.

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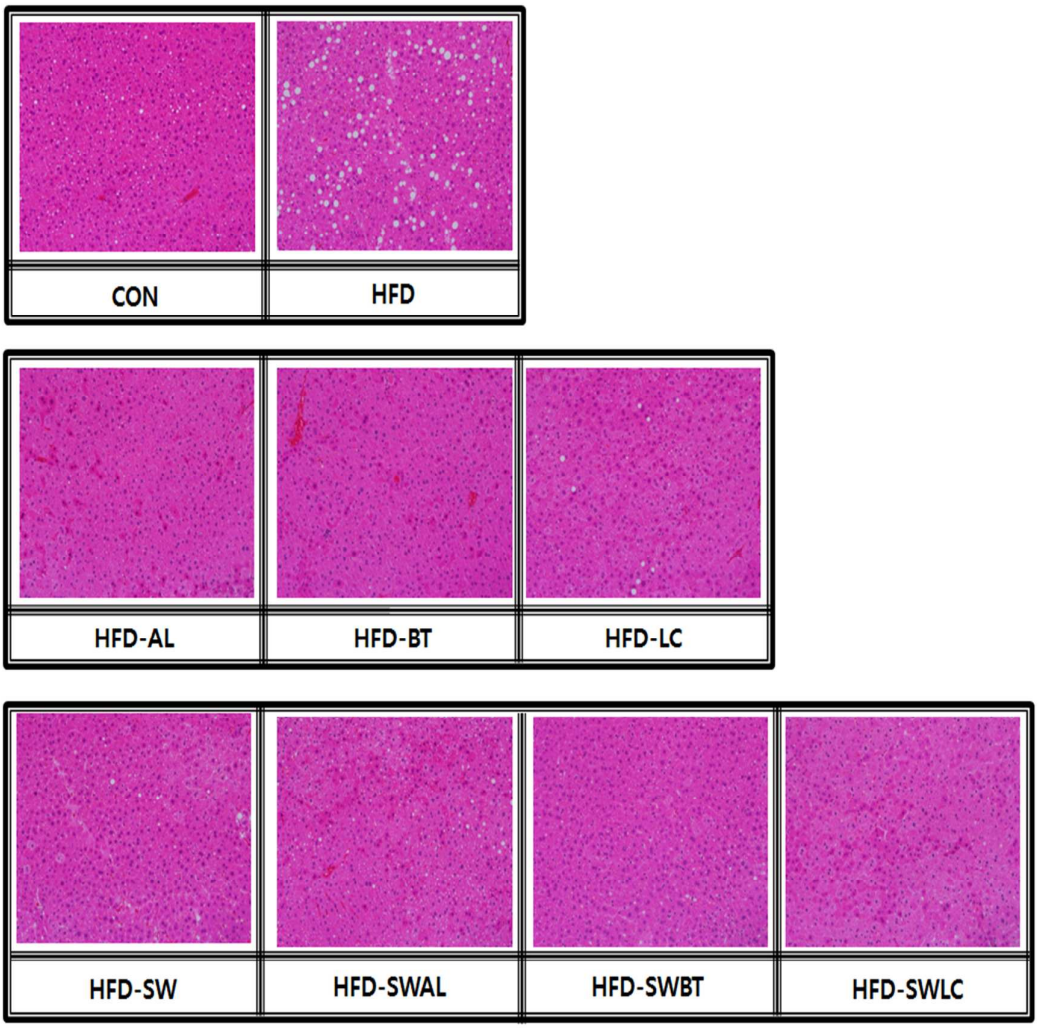

455

456 **Fig. 2.**

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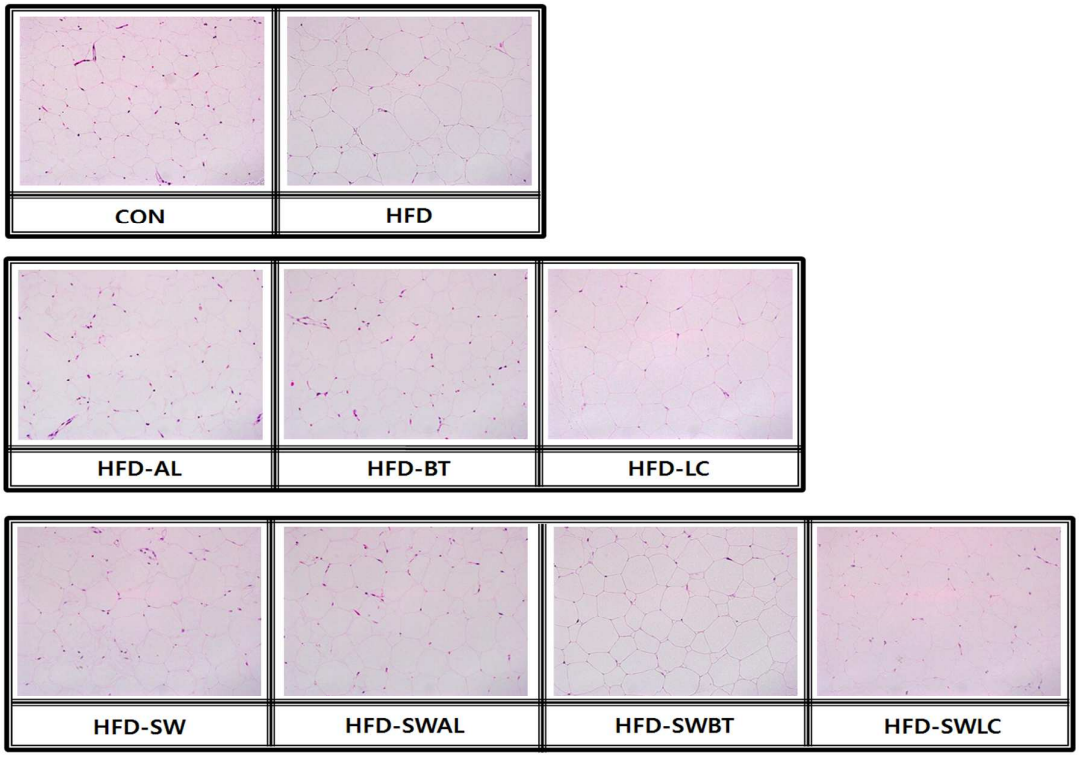

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Treatments	Epididymal adipose tissue (μm^2)
Con	74.97 ± 0.829^{d}
HFD	106.87 ± 1.481^{a}
HFD-AL	90.11 ± 0.771^{bc}
HFD-BT	87.87 ± 1.793^{bc}
HFD-LC	90.86 ± 1.249^{b}
HFD-SW	88.90 ± 0.790^{bc}
HFD-SWAL	86.99 ± 1.178^{c}
HFD-SWBT	88.60 ± 0.942^{b}
HFD-SWLC	71.40 ± 0.453^{e}

460

461 **Fig. 3.**

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463

464 **Table 1.** Experimental design of treatments

No	Groups (n=10)	Treatments
1	Con	Basal diets (10% fat)
2	HFD	High fat diets (60% fat)
3	HFD-AL	α-lipoic acid (60% fat)
4	HFD-BT	Betaine (60% fat)
5	HFD-LC	L-carnitine (60% fat)
6	HFD-SW	High fat diets (60% fat) with swimming
7	HFD-SWAL	α-lipoic acid (60% fat) with swimming
8	HFD-SWBT	Betaine (60% fat) with swimming
9	HFD-SWLC	L-carnitine (60% fat) with swimming

465

466

467 **Table 2.** Effect of exercise and antioxidants (AL, BT, and LC) supplementation on weight
468 gain and feed efficiency ratio of C57BL/6 mice fed high fat diet

Treatments	Body weight (g)		Weight gain (g/day)	Feed efficiency ratio
	Initial	Final		
Con	18.04±0.112	27.14±0.759^{d}	0.11±0.009^{cd}	0.05±0.004^{d}
HFD	18.04±0.154	34.60±0.944^{a}	0.21±0.013^{a}	0.10±0.006^{a}
HFD-AL	18.06±0.317	30.42±0.558^{bc}	0.17±0.007^{b}	0.08±0.003^{b}
HFD-BT	18.06±0.246	29.04±0.556^{cd}	0.15±0.008^{bc}	0.07±0.004^{bc}
HFD-LC	18.04±0.374	32.28±0.874^{b}	0.18±0.020^{b}	0.08±0.009^{b}
HFD-SW	18.04±0.144	27.48±0.325^{d}	0.12±0.005^{cd}	0.05±0.002^{d}
HFD-SWAL	18.04±0.129	27.38±1.044^{d}	0.11±0.017^{d}	0.05±0.007^{d}
HFD-SWBT	18.04±0.291	28.30±0.553^{cd}	0.12±0.005^{cd}	0.06±0.002^{cd}
HFD-SWLC	18.04±0.163	26.84±0.779^{d}	0.11±0.009^{cd}	0.05±0.004^{d}

469 Con, fed basal diet containing 10% fat; HFD, fed high fat diet containing 60 % fat; HFD-AL, fed high fat diet containing 60 %
470 fat and α-lipoic acid; HFD-BT, fed high fat diet containing 60 % fat and betaine; HFD-LC, fed high fat diet containing 60 %
471 fat and L-carnitine; HFD-SW, fed high fat diet containing 60 % fat and swimming; HFD-SWAL, fed high fat diet containing
472 60 % fat and α-lipoic acid and swimming; HFD-SWBT, fed high fat diet containing 60 % fat and betaine and swimming;
473 HFD-SWLC, fed high fat diet containing 60 % fat and L-carnitine and swimming

474 $^{a-d}$ Means±SE with different superscript in the same column differ significantly at $p<0.05$.

475

476 **Table 3.** Effect of exercise and antioxidants (AL, BT, and LC) supplementation on relative
477 organ and tissue weight to body weight (%) of C57BL/6 mice fed high fat diet

Treatments	Liver	Kidneys	Spleen	Testis	Retroperitoneal adipose	Epididymal adipose
Con	3.22±0.063^{a}	0.98±0.034^{abc}	0.19±0.003	0.70±0.013^{a}	1.07±0.046^{c}	3.03±0.160^{d}
HFD	2.78±0.055^{c}	0.92±0.023^{c}	0.17±0.012	0.59±0.044^{b}	2.62±0.089^{a}	6.21±0.249^{a}
HFD-AL	2.92±0.058^{bc}	1.01±0.009^{ab}	0.18±0.005	0.67±0.022^{ab}	1.86±0.067^{b}	4.87±0.123^{b}
HFD-BT	3.03±0.065^{abc}	1.01±0.021^{a}	0.20±0.018	0.70±0.008^{a}	1.34±0.070^{c}	3.54±0.150^{cd}
HFD-LC	3.07±0.124^{ab}	0.93±0.037^{bc}	0.18±0.006	0.67±0.020^{ab}	1.85±0.199^{b}	5.35±0.452^{b}
HFD-SW	3.04±0.064^{ab}	1.05±0.019^{a}	0.18±0.011	0.72±0.043^{a}	1.22±0.074^{c}	4.02±0.293^{c}
HFD-SWAL	2.94±0.104^{bc}	1.00±0.025^{ab}	0.17±0.016	0.71±0.050^{a}	1.29±0.127^{c}	4.11±0.200^{c}
HFD-SWBT	3.02±0.089^{abc}	0.98±0.011^{abc}	0.18±0.005	0.70±0.010^{a}	1.24±0.016^{c}	3.89±0.173^{c}
HFD-SWLC	3.02±0.061^{abc}	0.99±0.014^{abc}	0.19±0.008	0.70±0.023^{a}	1.15±0.049^{c}	3.51±0.152^{cd}

478 Con, fed basal diet containing 10% fat; HFD, fed high fat diet containing 60 % fat; HFD-AL, fed high fat diet containing 60 %
479 fat and α-lipoic acid; HFD-BT, fed high fat diet containing 60 % fat and betaine; HFD-LC, fed high fat diet containing 60 %
480 fat and L-carnitine; HFD-SW, fed high fat diet containing 60 % fat and swimming; HFD-SWAL, fed high fat diet containing
481 60 % fat and α-lipoic acid and swimming; HFD-SWBT, fed high fat diet containing 60 % fat and betaine and swimming;
482 HFD-SWLC, fed high fat diet containing 60 % fat and L-carnitine and swimming

483 $^{a-d}$ Means±SE with different superscript in the same column differ significantly at $p<0.05$.

484

485

Table 4. Effect of exercise and antioxidants (AL, BT, and LC) supplementation on serum glucose, BUN, insulin, and leptin contents of C57BL/6 mice fed high fat diet

Treatments	Glucose (mg/dL)	BUN (mg/dL)	Insulin (uIU/ml)	Leptin (ng/ml)
Con	203.33±1.333^{cd}	17.23±0.726	5.67±0.521	7.14±0.304^{h}
HFD	263.00±15.948^{a}	17.10±0.529	6.93±0.133	27.07±0.402^{a}
HFD-AL	218.67±2.333b^{c}	17.50±1.750	5.93±0.696	12.56±0.451^{c}
HFD-BT	182.33±4,485^{d}	15.10±1.229	5.27±0.371	8.96±0.341^{fg}
HFD-LC	239.00±5.686^{ab}	14.33±1.298	6.53±0.742	17.58±0.503^{b}
HFD-SW	218.33±3.180^{bc}	16.57±0.953	6.47±0.636	10.30±0.309^{ef}
HFD-SWAL	182.67±19.802^{d}	14.83±0.940	7.60±1.301	10.67±0.477^{de}
HFD-SWBT	184.00±14.189^{d}	15.57±1.660	6.87±0.521	11.89±0.250^{cd}
HFD-SWLC	208.00±7.506^{bcd}	14.40±0.400	5.87±0.867	7.56±1.003^{gh}

Con, fed basal diet containing 10% fat; HFD, fed high fat diet containing 60 % fat; HFD-AL, fed high fat diet containing 60 % fat and α-lipoic acid; HFD-BT, fed high fat diet containing 60 % fat and betaine; HFD-LC, fed high fat diet containing 60 % fat and L-carnitine; HFD-SW, fed high fat diet containing 60 % fat and swimming; HFD-SWAL, fed high fat diet containing 60 % fat and α-lipoic acid and swimming; HFD-SWBT, fed high fat diet containing 60 % fat and betaine and swimming; HFD-SWLC, fed high fat diet containing 60 % fat and L-carnitine and swimming

$^{a-h}$ Means±SE with different superscript in the same column differ significantly at $p<0.05$.

Table 5. Effect of exercise and antioxidants (AL, BT, and LC) supplementation on serum lipid profile of C57BL/6 mice fed high fat diet

Treatments	TG (mg/dL)	TC (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	AI (mg/dL)
Con	32.63±1.228^{b}	139.00±3.606^{e}	87.33±7.333	45.14±5.668^{e}	0.59±0.042^{c}
HFD	57.67±4.702^{a}	172.00±0.577^{a}	91.67±5.840	68.80±5.948^{a}	0.88±0.006^{a}
HFD-AL	49.33±5.239^{a}	153.67±6.839^{bcd}	87.00±4.359	56.80±3.863^{bcd}	0.77±0.078^{ab}
HFD-BT	24.67±4.807^{b}	141.00±5.568^{de}	85.67±3.712	50.40±0.902^{cde}	0.65±0.064^{bc}
HFD-LC	24.33±0.333^{b}	152.67±1.202^{bcd}	92.33±1.453	55.47±0.371^{bcde}	0.66±0.012^{bc}
HFD-SW	29.67±4.807^{b}	156.67±2.667^{bc}	92.33±1.764	58.40±1.442^{abc}	0.70±0.027^{bc}
HFD-SWAL	23.67±8.172^{b}	154.00±4.163^{bcd}	87.67±1.453	61.60±1.973^{ab}	0.76±0.047^{ab}
HFD-SWBT	20.60±2.572^{b}	159.00±3.606^{b}	95.67±0.333	59.21±2.863^{abc}	0.66±0.036^{bc}
HFD-SWLC	23.00±2.309^{b}	144.33±4.256^{cde}	92.67±3.480	47.07±1.671^{de}	0.56±0.046^{c}

TG, triglyceride; TC, total cholesterol; HDL-C, HDL-cholesterol; LDL-C, LDL-cholesterol; AI, atherosclerosis index

Con, fed basal diet containing 10% fat; HFD, fed high fat diet containing 60 % fat; HFD-AL, fed high fat diet containing 60 % fat and α-lipoic acid; HFD-BT, fed high fat diet containing 60 % fat and betaine; HFD-LC, fed high fat diet containing 60 % fat and L-carnitine; HFD-SW, fed high fat diet containing 60 % fat and swimming; HFD-SWAL, fed high fat diet containing 60 % fat and α-lipoic acid and swimming; HFD-SWBT, fed high fat diet containing 60 % fat and betaine and swimming; HFD-SWLC, fed high fat diet containing 60 % fat and L-carnitine and swimming

$^{a\text{-}e}$ Means±SE with different superscript in the same column differ significantly at $p<0.05$.

Table 6. Effect of exercise and antioxidants (AL, BT, and LC) supplementation on the hepatic triglyceride and cholesterol of C57BL/6 mice fed high fat diet

Treatments	TG (mg/dL)	TC (mg/dL)	HDL-C (mg/dL)
Con	450.82±28.160^{f}	124.62±3.201^{de}	2.92±0.326^{a}
HFD	1704.31±53.459^{a}	162.27±1.578^{a}	1.48±0.365^{c}
HFD-AL	1431.02±146.162^{b}	137.29±2.896^{bed}	1.90±0.267^{bc}
HFD-BT	1232.55±117.029^{b}	131.29±1.233^{bcde}	1.50±0.332^{b}
HFD-LC	1170.63±131.934^{bc}	128.97±2.191^{bcde}	1.68±0.270^{bc}
HFD-SW	956.83±77.126^{cd}	126.34±5.405^{cde}	1.96±0.339^{bc}
HFD-SWAL	885.68±34.356^{d}	142.08±11.047^{b}	1.38±0.270^{c}
HFD-SWBT	758.36±50.635^{de}	140.227±0.674^{bc}	2.50±0.111^{ab}
HFD-SWLC	595.59±36.843^{ef}	117.01±1.967^{e}	2.86±0.244^{a}

TG, triglyceride; TC, total cholesterol; HDL-C, HDL-cholesterol; LDL-C, LDL-cholesterol

Con, fed basal diet containing 10% fat; HFD, fed high fat diet containing 60 % fat; HFD-AL, fed high fat diet containing 60 % fat and α-lipoic acid; HFD-BT, fed high fat diet containing 60 % fat and betaine; HFD-LC, fed high fat diet containing 60 % fat and L-carnitine; HFD-SW, fed high fat diet containing 60 % fat and swimming; HFD-SWAL, fed high fat diet containing 60 % fat and α-lipoic acid and swimming; HFD-SWBT, fed high fat diet containing 60 % fat and betaine and swimming; HFD-SWLC, fed high fat diet containing 60 % fat and L-carnitine and swimming

$^{a-f}$ Means±SE with different superscript in the same column differ significantly at $p<0.05$.

518 **Table 7.** Effect of exercise and antioxidants (AL, BT, and LC) supplementation on the fecal
519 triglyceride and cholesterol of C57BL/6 mice fed high fat diet

Treatments	TG (mg/dL)	TC (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)
Con	17.45±2.139^{c}	30.76±3.410^{d}	6.15±1.311^{b}	21.12±2.045^{bc}
HFD	40.06±4.144^{b}	42.21±3.423^{c}	13.17±0.813^{a}	21.04±2.050^{bc}
HFD-AL	45.08±2.932^{ab}	37.83±0.910^{c}	11.10±1.937^{ab}	17.71±1.621^{c}
HFD-BT	41.85±4.023^{b}	50.17±1.411^{ab}	8.84±2.033^{ab}	32.96±2.021^{a}
HFD-LC	42.62±4.300^{ab}	50.03±0.695^{ab}	8.94±2.572^{ab}	32.56±2.651^{a}
HFD-SW	42.62±4.406^{ab}	43.92±1.315^{bc}	9.97±1.523^{ab}	25.43±0.938^{b}
HFD-SWAL	45.79±2.379^{ab}	42.98±2.643^{c}	9.04±1.907^{ab}	24.79±0.741^{b}
HFD-SWBT	51.37±2.588^{ab}	51.60±0.580^{a}	6.97±1.405^{b}	34.35±1.350^{a}
HFD-SWLC	53.97±3.726^{a}	51.61±1.187^{a}	6.67±1.566^{b}	34.14±1.273^{a}

520 TG, triglyceride; TC, total cholesterol; HDL-C, HDL-cholesterol; LDL-C, LDL-cholesterol
521 Con, fed basal diet containing 10% fat; HFD, fed high fat diet containing 60 % fat; HFD-AL, fed high fat diet containing 60 %
522 fat and α-lipoic acid; HFD-BT, fed high fat diet containing 60 % fat and betaine; HFD-LC, fed high fat diet containing 60 %
523 fat and L-carnitine; HFD-SW, fed high fat diet containing 60 % fat and swimming; HFD-SWAL, fed high fat diet containing
524 60 % fat and α-lipoic acid and swimming; HFD-SWBT, fed high fat diet containing 60 % fat and betaine and swimming;
525 HFD-SWLC, fed high fat diet containing 60 % fat and L-carnitine and swimming
526 $^{a-d}$ Means±SE with different superscript in the same column differ significantly at $p<0.05$.
527