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Effects of Cell Cultured *Acanthopanax senticosus* Extract Supplementation and Swimming Exercise on Lipid and Carnitine Profiles in C57BL/6J Mice Fed a High Fat Diet

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Abstract This study investigated the effects of cell cultured *Acanthopanax senticosus* extract (ASE) supplementation and swimming exercise on lipid profiles and carnitine concentrations in C57BL/6J mice fed high fat diets. Male C57BL/6J mice (n=50), aged 4 weeks, were divided into 5 groups based on exercise and/or ASE supplementation (0.5 g/kg of body weight): normal diet (N-C), high fat diet (H-C), high fat diet non-supplement & exercise (H-NSE), high fat diet supplement & no exercise (H-SNE), high fat diet supplement & exercis (H-SE). Liver nonesterified carnitine (NEC) was significantly higher in the H-SNE group than in the H-C group, and liver total carnitine (TCNE) levels were significantly higher in the H-SNE group than in the H-NSE and H-SE groups. Liver and muscle carnitine palmitoyltransferase-I (CPT-I) mRNA levels tended to be higher with ASE supplementation and/or exercise. These results suggest that supplementation with ASE and/or exercise might have a role in improving lipid oxidation.

Keywords: *Acanthopanax senticosus*, lipid profile, carnitine, carnitine palmitoyltransferase-I (CPT-I)

Introduction

Obesity is a serious public health threat, thus there have been intensified efforts to control weight and such efforts are especially widespread in developed countries (1, 2). The degree of obesity is dependent on the amount of body fat and is regarded as a complex syndrome associated with various factors including genetic influences, nutrition, and environmental and social conditions (3). Complications associated with obesity are responsible for most obesity related morbidity and mortality. Obesity increases circulating cholesterol and triglyceride levels, which in turn increase the risk of developing hypertension, cardiovascular diseases, and stroke (4). Furthermore, increased triglycerides induce peripheral tissue insulin resistance, which is deemed one of the risk factors for age-related diseases such as Type 2 diabetes (5). Given the health consequences of obesity, there have been numerous studies of weight loss and management. Some studies have focused on the prevention of obesity in people with inactive lifestyles and the relationship between exercise and lipid concentrations in blood (6). Exercise-based treatments for the overweight and obese aim to lower body fat by improving metabolic rates and utilizing fatty acids from adipose tissue as a source of energy (7).

Acanthopanax senticosus (AS) is a member of the *Acanthopanax* family of plants classified as Araliaceae. When the perennially deciduous shrub is dried, and compared to other *Acanthopanax* species AS has such a high density of thorns that it has the appearance of hair covering the entire plant (8). AS is a high altitude plant that originated in the Korean peninsular region and spread to Siberia and China. Thorny *Acanthopanax* is known to have acanthosides E, D, chiisanoside, eleutherosides A, B, C, D, E, I, K, M, saponins, and sesamin (9), making it a popular ingredient in Chinese medicine. It has strong medicinal properties, similar to ginseng, because of its eleutheroside B and E contents, which are physiologically active and make up about 80% of its bioactive components (10). The level of physiological activity is usually quoted in product information for goods containing this herb (11). It is also known that acanthoside D and chisanoside enhance endurance capacity (12).

Earlier studies of AS centered around its efficacy for modulating the immune system, hypertension, antivirus, anti-cancer, anti-stress, and exercise capacity (13). In animal-based studies, its efficacy for excitation/relaxation, increase in sleeping hours, antioxidant activity and lipid improvement were also tested (8, 9, 14). Lately, most research involving this herb is regarding disease or exercise performance. Studies that examined effects on exercise performance, immune function, antioxidation, and lipid improvement of ginseng or red ginseng are well known (15, 16). These previous studies addressed much about herbal medicine extracts and exercise performance, but studies that closely examine lipid and blood sugar adjustments with the combination of herbal medicine extracts and aerobic exercise are limited.

AS is known to improve aerobic exercise performance ability, therefore combining AS and exercise may have a synergistic effect. However, wild AS is expensive, and the cultivated plants require many years to develop sufficient concentrations of the active components. As a result there have been many attempts to develop methods for cultivating fully developed plants more quickly (17, 18). One potential method for developing large amounts of active herb is to culture isolated cells in bioreactors rather than growing plants from seeds. If successful, this technique could make possible the production of functional botanicals in days

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rather than years (19). For this study, cell cultured AS was grown by a novel, proprietary method, involving the culture of isolated cells in a bioreactor. However, few studies have investigated the efficacy of cell cultured AS. This study investigates the effect of the cell cultured ASE supplementation and exercise on lipid and carnitine profiles in C57BL/6J mice fed high fat diets.

Materials and Methods

Materials Cell cultured AS with a torpedo shape was supplied by Microplants Co., Ltd. (Microplants Co., Daejeon, Korea). The cell cultured AS cells were dried, ground to a fine powder, and extracted with deionized water (30 times volume) for 9 hr at 80°C. The resulting extracts were filtered, concentrated under vacuum at 60°C, and stored at 4 until used.

Animals and diets Male C57BL/6J mice, aged 4 weeks, were purchased from Charles River Laboratories (Charles River Laboratories, Tokyo, Japan). The animals were maintained on a pellet diet (Research Diets, New Brunswick, NJ, USA) for 1 week, and then randomly divided into 5 groups: normal diet (N-C), high fat diet (H-C), high fat diet non-supplement & exercise (H-NSE), high fat diet with supplement & no exercise (H-SNE), high fat diet supplement & exercise (H-SE). The compositions of the experimental diets are shown in Table 1. The animals were randomly assigned to each group (n=10) such that the average weight in each group was comparable. The H-SNE and H-SE groups were orally administered cell cultured ASE (0.5 g/kg BW) suspended in distilled water once a day for 12 weeks; distilled water only was administered to the N-C, H-C, and H-NSE groups. Research Diets manufactured the experimental diets. The animals were housed in a temperature-controlled environment with a 12-hr light/dark cycle. Food consumption and body weight were measured daily and weekly, respectively. The experimental protocol was approved by the Animal and Use Committee of Chonbuk National University.

Exercise protocol Trained mice were exercised by swimming in a pool (20) under the same conditions (34°C, 6/week, 1 hr/day) without a current for 12 weeks.

Collection of serum and tissue samples Feed was removed 12 hr before sacrificing. Blood samples were collected from each mouse by orbital/cardiac puncture and incubated on ice water for 1 hr. Serum was separated from

Table 1. Composition of experimental diets

Ingredient	Normal diet[1]	High fat diet[2]			
		Non-supplement		Supplement[3]	
		No exercise	Exercise	No exercise	Exercise
	N-C	H-C	H-NSE	H-SNE	H-SE
Casein	200	200	200	200	200
L-Cystine	3	3	3	3	3
Corn starch	315	-	-	-	-
Maltodextrin	35	125	125	125	125
Sucrose	350	68.8	68.8	68.8	68.8
Cellulose	50	50	50	50	50
Soybean Oil	25	25	25	25	25
Lard	20	245	245	245	245
Mineral mix	10	10	10	10	10
Dicalcium phosphate	13	13	13	13	13
Calcium carbonate	5.5	5.5	5.5	5.5	5.5
Potassium citrate	16.5	16.5	16.5	16.5	16.5
Vitamin mix	10	10	10	10	10
Choline bitarate	2	2	2	2	2
FD&C Yellow dye #5	0.05	-	-	-	-
FD&C Blue dye #1	-	0.05	0.05	0.05	0.05
Total	1,055.05	773.85	773.85	773.85	773.85
kcal	4,057	4,057	4,057	4,057	4,057
Protein (kcal%)	20	20	20	20	20
Carbohydrate (kcal%)	70	20	20	20	20
Fat (kcal%)	10	60	60	60	60
kcal/g	3.8	5.2	5.2	5.2	5.2

[1]AIN-93 Modified diet with 4% fat (10% fat calories) content.
[2]AIN-93 Modified high fat diet with 35% fat (60% fat calories) content.
[3]High fat diet+SNE, SE groups, *Acanthopanax senticosus* extract (0.5 g/kg BW) was administered as described in Material and Methods.

blood by centrifugation at 1,100×g for 15 min at 4°C and kept at -80°C until analyzed. The epididymal fat, liver, kidney, heart, brain, and muscle were removed, rinsed with a phosphate-buffered saline solution, wiped with a paper towel, weighed quickly, frozen in liquid nitrogen, and stored at -80°C until assayed.

Analysis of lipids and leptin Total cholesterol in the serum was measured enzymatically using a commercial kit (Asan Pharmaceutical Co., Seoul, Korea), and the high density lipoprotein-cholesterol (HDL-c) fraction was determined by the dextran sulfate-Mg^{++} method. Liver lipids were extracted from liver tissues according to the method of Folch *et al.* (21). Triglycerides in serum and liver tissue were measured enzymatically with a commercial kit (Assn Phamaceutical Co.), and total lipids in serum and liver tissue were measured by the sulfo-phospho-vanillin method using a commercial kit (Kokusai Phamaceutical Co., Kobe, Japan).

Serum leptin levels were determined by radioimmunoassay (RIA) using a mouse leptin RIA kit from Linco Reasearch (St. Charles, MO, USA). Radioactivity was measured in a gamma scintillation counter.

Analysis of carnitine Muscle tissues (50 mg) were homogenized (20 sec) using a sonicator (Fisher Scientific Co., Toronto, ONT, USA) with 99 volumes of cold distilled water. Liver tissues were homogenized by adding 50 mg to 29 volumes of cold distilled water, centrifuging at 1,500×g and collecting the supernatant. Non-collagen protein in gastric tissue extracts was extracted by adding 1 to 9 volumes of 50 mmol/L KOH for 12-16 hr, centrifuging at 1,500×g, and then quantitatively analyzing the supernatant with a protein assay kit (Bio-Rad Lab., Hercules, CA, USA) based on the method of Bradford (22).

Nonesterified carnitine (NEC), acid-soluble acylcarnitine (ASAC), and acid-insoluble acylcarnitine (AIAC) in serum were determined by the radioimmunoenzymatic procedure of Cederbad and Lindstedt (23) as modified by Sachan *et al.* (24). In this method, AIAC was precipitated with perchloric acid and centrifuged, leaving the ASAC and NEC in the supernatant. An aliquot of the supernatant was assayed to determine the amount of NEC, and another aliquot was hydrolyzed with 0.5 mol/L KOH to assay all acid-soluble carnitine (ASAC+NEC). The ASAC value was calculated as the difference between the NEC and total acid-soluble carnitine. The pellets containing AIAC were drained, washed, and hydrolyzed in 0.5 mol/ KOH for 60 min in a hot water bath 60°C. In each case, carnitine was assayed using carnitine acetyltransferase (Sigma Chemical Co., St. Louis, MO, USA) to esterify the carnitine with [^{14}C]acetate from [1-^{14}C]acetyl-CoA (Amersham, Arlington Heights, IL, USA). Radioactivity was measured in a Beckman model LS3801 liquid scintillation counter (Beckman Instruments, Palo Alto, CA, USA).

CPT-I mRNA levels of liver and muscle Total RNA was extracted with Trizol reagent (Ambion, Austin, TX, USA) and the concentration measured spectrophotometrically. Reverse transcription polymerase chain reaction (RT-PCR) was used for cDNA synthesis using a one-step RT-PCR kit (ABgene, New York, NY, USA). β-Actin was used as a control. The RT-PCR reaction was carried out using an RT-PCR kit (MWG-Biotech, High Point, NC, USA). At the end of the RT-PCR reaction, the results were confirmed by verifying the cDNA product by electrophoresis on a 1.5% agarose gel.

Statistical analysis Data from individual experiments are expressed as the mean ± standard deviation. All statistical analyses were performed using SAS software (SAS Institute, Cary, NC, USA). The data were analyzed by 2-way analysis of variance (ANOVA) (ASE supplementation × swimming exercise). When significant differences were indicated, mean values were compared by Tukey's test. Statistical significance is defined as $p<0.05$.

Results and Discussion

Weight gain and feed efficiency The H-C group gained more weight and at a faster rate than the H-NSE, H-SNE, and H-SE groups (Table 2). The feed efficiency ratio was significantly higher in the H-C group compared to the H-NSE, H-SNE, and H-SE groups (Table 2). The feed efficiency ratios were significantly decreased in the ASE supplementation or exercise groups compared to the H-C group. This finding supports the hypothesis that cell cultured ASE supplementation leads to weight loss in mice fed a high

Table 2. Weight gain and feed efficiency ratio[1)]

Group	ND	HD				Statistical significance[2)]		
	N-C	Non-supplement		Supplement				
		No exercise	Exercise	No exercise	Exercise			
		H-C	H-NSE	H-SNE	H-SE	S	E	SE
Initial weight (g)	22.03±0.90	22.68±0.58	22.65±0.91	22.77±1.00	12.33±1.10	NS	NS	NS
Weight gain (g/day)	5.82±0.06^{c}	16.69±4.27^{a}	13.21±2.01^{b}	12.49±3.45^{b}	12.33±1.36^{b}	0.01	0.01	NS
Food intake (g/day)	2.33±0.06	2.37±0.17	2.35±0.09	2.31±0.07	2.33±0.08	NS	NS	NS
Energy intake (kcal/day)	8.88±0.24	12.31±0.89	12.25±0.72	11.99±0.37	11.89±0.6	NS	NS	NS
Feed efficiency ratio	0.067±0.006^{b}	0.086±0.002^{a}	0.061±0.002^{b}	0.065±0.001^{b}	00.60±0.003^{b}	0.023	0.02	NS

[1)]Mean±SD of 10 mice per group. Values with different superscript letters within the same row are significantly different at $p<0.05$ by ANOVA and Duncan's multiple range tests. N-C, normal diet; H-C, high fat diet; H-NSE, high fat diet, non-supplement & exercise; H-SNE, high fat diet, supplement & no exercise; H-SE, high fat diet, supplement & exercise.
[2)]The degrees of significance resulting from 2-way ANOVA are shown with the effects of supplementation (S), exercise (E), and the interaction of supplementation and exercise (S×E) being expressed as the numerical value or as not significant (NS) when $p<0.05$.

fat diet (19). Chung *et al.* (25) found that exercise prevents diet-induced weight gain and lowers the feed efficiency ratio in all mice fed diets high in fat compared with non-exercised mice. The authors suggested that physical exercise boosts fat breakdown by increasing energy expenditure while suppressing hyperplasia synthesis of fatty cells.

Epididymal fat pad weight Epididymal fat pad weights were significantly lower in the H-NSE, H-SNE, and H-SE groups than in the H-C group, and the H-NSE, H-SE groups were lower than the H-SNE group demonstrating that exercise decreases epididymal fat pad weights (Fig. 1). Regular exercise helps reduce body fat by increasing energy consumption and suppressing insulin resistance and the formation of adipose cells in obese Zucker rats (26). It is commonly known that low intensity exercise (40% VO_2 max) is more efficient for losing body fat because the body uses lipid stores for energy during submaximal exercise. Low intensity exercise increases total fat oxidation, and in animal experiments, cardio exercise down-regulates FAS activity which decreases lipid synthesis and accumulation thereby reducing body fat (27). In this study, epididymal fat pad weights were also significantly decreased with exercise or ASE supplementation combined with exercise, which was more effective than ASE supplementation only. Therefore, we can assume that ASE decreases epididymal fat pad weights when combined with the beneficial effects of exercise.

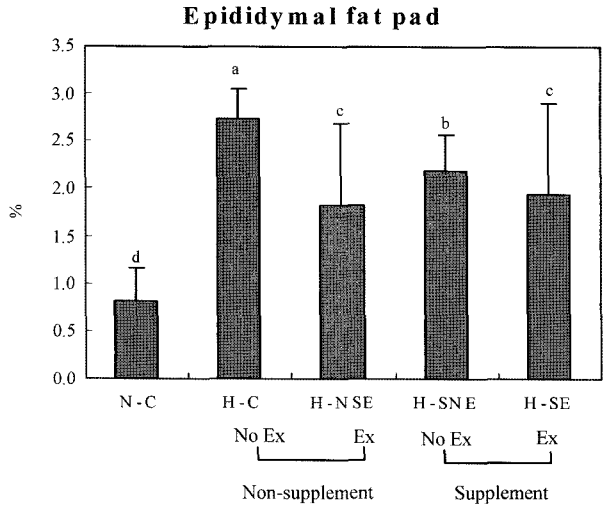

Fig. 1. Epididymal fat pad weight. N-C, normal diet; H-C, high fat diet; H-NSE, high fat diet non-supplement & exercise; H-SNE, high fat diet supplement & no exercise; H-SE, high fat diet supplement & exercise; Ex, exercise. Mean±SD of 10 mice per group. Bars with different superscript letters are significantly different at $p<0.05$ by ANOVA and Duncan's multiple range tests.

Serum and liver lipid levels The effects of ASE supplementation and exercise on lipid profiles in serum and liver are shown in Table 3. Serum triglyceride (TG) concentrations were significantly lower in the H-SE group compared to the H-C group. Serum TG levels were significantly lower with ASE supplementation combined with exercise, which was most effective. Thus we consider there to be a synergistic or additive effect when ASE supplementation is combined with exercise. Liver TG levels were significantly lower in the H-NSE, H-SNE, and H-SE groups than in the H-C group. The hepatic TG concentrations were significantly lower with exercise training and ASE supplementation. The level of dietary fat has an impact on the amount of body fat, serum cholesterol levels, and formation of fatty acids (28). However, cell cultured ASE supplementation significantly reduced serum and liver TG concentrations in mice fed the high fat diet, as was previously demonstrated (19). Nishibe *et al.* (29) also reported a decrease in body lipid levels in rats injected with water extracts of ASE when exercised in a pool. TC concentrations in serum were significantly higher in the H-C group than in the H-SNE and H-SE groups. Similar results were obtained in another AS study. Cha *et al.* (30) reported that total cholesterol levels were decreased by ASE supplementation, with decreased LDL

Table 3. Lipid concentrations in mice[1)]

Group	ND	HD				Statistical significance[2)]		
		Non-supplement		Supplement				
	N-C	No exercise	Exercise	No exercise	Exercise			
		H-C	H-NSE	H-SNE	H-SE	S	E	SE
				Serum (mg/dL)				
TG	67.02±16.28^{ab}	95.06±25.84^{a}	73.46±15.10^{ab}	70.06±5.65^{ab}	48.97±19.61^{b}	0.029	NS	NS
TC	159.09±20.66^{b}	203.35±33.72^{a}	175.09±14.78^{ab}	155.02±19.36^{b}	154.35±19.55^{b}	0.002	NS	NS
HDL-c	23.72±2.64	40.59±24.19	50.49±17.95	30.45±15.79	35.64±8.74	NS	NS	NS
HDL-c/TC	0.12±0.02^{c}	0.23±0.01^{b}	0.31±0.03^{a}	0.19±0.03^{b}	0.23±3.68^{b}	0.0002	<0.0001	NS
				Liver (mg/g)				
TG	80.08±34.94^{c}	377.01±75.08^{a}	197.31±38.46^{b}	191.19±32.89^{b}	162.54±17.27^{b}	0.0002	0.0002	0.0008
TC	8.10±3.23	24.82±5.51	23.45±6.58	23.13±3.68	20.18±2.19	NS	NS	NS

[1)]Mean±SD of 10 mice per group. Values with different superscript letters within the same row are significantly different at $p<0.05$ by ANOVA and Duncan's multiple range tests. N-C, normal diet; H-C, high fat diet; H-NSE, high fat diet, non-supplement & exercise; H-SNE, high fat diet, supplement & no exercise; H-SE, high fat diet, supplement & exercise; TC, total cholesterol; TG, triglyceride; HDL-c, HDL-cholesterol.
[2)]The degrees of significance resulting from 2-way ANOVA are shown with the effects of supplementation (S), exercise (E), and the interaction of supplementation and exercise (S×E) being expressed as the numerical value or as not significant (NS) when $p<0.05$.

cholesterol levels in C57BL/6J mice. In this study, exercise without ASE supplementation did not improve lipid profiles more than ASE supplementation. Kim *et al.* (31) found that exercise could not significantly improve TG and HDL-c levels. They reported that short term cardio exercise did not change blood lipid profiles, therefore changes in blood lipid profiles are affected by differences in duration, intensity, and frequency of exercise among experiments. Thus, we can assume that ASE extract can improve lipid profiles when combined with the beneficial effects of exercise. There were no significant differences in serum HDL-c concentrations among the no exercise groups or exercise groups with ASE supplementation. However, the HDL-c/TC ratios were significantly higher in the H-NSE group than in the H-C, H-SNE, and H-SE groups.

Although the serum HDL-c levels did not change much, the HDL-c/TC ratio was significantly higher in the non-supplemented exercise group. Combination measures such as the HDL-c/TC index have been shown to have a stronger association with coronary heart disease than individual cholesterol components (32). To control serum cholesterol levels, regular aerobic exercise is needed because exercise reduces body fat and serum cholesterol levels, and the risk of developing coronary artery disease is reduced as a result (33).

Serum, liver, and muscle carnitine levels Carnitine (3-hydroxy-4-N-trimethyl-ammonium butyrate) transports fatty acids into the mitochondria where fatty acids undergo β-oxidation, playing a vital role in activating the oxidation of fatty acids in tissues (34). This means that a lack of carnitine will slow the oxidation of fatty acids and fuel an increase in serum lipid levels. Serum NEC, ASAC, AIAC, and TCNE levels were not significantly different among the exercise and ASE supplemented groups. However, the acyl/free carnitine levels were significantly higher in the H-NSE, H-SNE, and H-SE groups than in the H-C group. These findings are likely due to the fact that mice fed high fat diets had higher levels of NEC, on the other hand, ASAC and AIAC concentrations in plasma were lower in high fat diet groups compared to the normal diet group. The higher serum acyl/free carnitine ratios in the ASE supplemented and exercised groups is likely attributable to the transportation of acyl-carnitine from muscle to the bloodstream during exercise (35). Further investigation of the mechanism of how ASE supplementation and exercise affect carnitine levels is needed.

The liver ASAC, AIAC, and acyl/free carnitine ratio levels were not significantly different among exercised and ASE supplemented groups. However, the TCNE levels were significantly higher in the H-SNE group than in the H-NSE and H-SE groups. NEC levels were significantly higher in the H-SNE group than in the H-C group. NEC levels in muscle were significantly higher in the ASE supplemented groups than in the H-C group (Table 4). In this study, NEC and TCNE concentrations were elevated in the liver after ASE supplementation, which was likely

Table 4. Carnitine concentrations[1)]

Group	ND	HD				Statistical significance[2)]		
		Non-supplement		Supplement				
	N-C	No exercise	Exercise	No exercise	Exercise			
		H-C	H-NSE	H-SNE	H-SE	S	E	SE
Serum (μmol/dL)								
NEC	1.18±0.19	1.98±0.49	2.07±0.24^{a}	1.92±0.34	2.12±0.19	NS	NS	NS
ASAC	2.15±0.28	1.15±0.31	1.63±0.32	1.78±0.30	1.66±0.40	NS	NS	NS
AIAC	0.21±0.03	0.12±0.05	0.11±0.05	0.11±0.04	0.11±0.01	NS	NS	NS
TCNE	3.72±0.52	3.79±0.36	3.18±0.43	3.82±0.56	3.89±0.50	NS	NS	NS
Acyl/free	1.96±0.52^{a}	0.66±0.08^{c}	0.94±0.08^{b}	1.05±0.10^{b}	0.92±0.09^{b}	NS	NS	0.029
Liver (mol/g)								
NEC	0.56±0.08^{a}	0.37±0.05^{c}	0.41±0.04^{bc}	0.47±0.05^{b}	0.40±0.06^{bc}	0.035	NS	0.015
ASAC	0.12±0.007	0.03±0.007	0.03±0.009	0.03±0.007	0.02±0.008	NS	NS	NS
AIAC	0.19±0.02	0.05±0.02	0.05±0.02	0.10±0.02	0.05±0.02	NS	NS	NS
TCNE	0.71±0.01^{a}	0.52±0.06^{bc}	0.47±0.05^{c}	0.57±0.07^{b}	0.48±0.05^{c}	NS	0.03	NS
Acyl/free	0.84±0.54	0.20±0.90	0.17±0.29	0.28±0.54	0.18±0.47	NS	NS	NS
Muscle (nmol/mg non collagen protein)								
NEC	8.60±0.87^{b}	8.92±1.60^{b}	11.12±2.44^{ab}	11.50±1.91^{a}	11.99±1.14^{a}	0.01	NS	NS
ASAC	11.05±2.50	10.41±2.87	13.07±1.13	13.01±1.69	12.43±1.97	NS	NS	NS
TCNE	19.98±1.97	19.98±1.97	23.53±3.35	21.55±3.95	22.07±3.38	NS	NS	NS
Acyl/free	1.29±0.38	1.13±0.34	1.51±0.21	1.22±0.18	1.41±0.11	NS	NS	NS

[1)]Mean±SD of 10 mice per group. Values with different superscript letters within the same row are significantly different at $p<0.05$ by ANOVA and Duncan's multiple range test. N-C, normal diet; H-C, high fat diet; H-NSE, high fat diet non-supplement & exercise; H-SNE, high fat diet supplement & no exercise; H-SE, high fat diet supplement & exercise; NEC, non-esterified carnitine; ASAC, acid soluble acyl carnitine; AIAC, acid insoluble acyl carnitine; TCNE, total carnitine; Acyl/free, ASAC + AIAC/TCNE.

[2)]The degrees of significance resulting from 2-way ANOVA are shown with the effects of supplementation (S), exercise (E), and the interaction of supplementation and exercise (S×E) being expressed as the numerical value or as not significant (NS) when $p<0.05$.

due to increased carnitine biosynthesis in the liver. ASE supplementation also triggers an increase in fatty acid oxidation and muscle NEC. Thus cell cultured ASE supplementation, combined with exercise, may have reduced body lipid levels by accelerating carnitine biosynthesis and the oxidation of fatty acids.

Serum leptin levels Leptin is a protein hormone encoded by the ob gene and secreted by adipose cells. Leptin was found to exert effects on the hypothalamic area involved in the regulation of food and water. By combining with its receptors in the hypothalamic area, it inhibits the production and secretion of neuropeptide Y and eventually reduces food intake and body weight (36). Several investigators claim that a high fat diet increases leptin production and its level in the blood (37). Leptin reduces weight in normal weight animals as it signals fullness and cues them to stop eating, but its activation is blunted in high fat diet-induced obese animals as they develop resistance to leptin (38). Serum leptin levels were significantly higher in the no exercise groups. The supplementation of ASE had no effect on serum leptin concentrations in the any exercise groups, however serum leptin concentrations were significantly lower in both NSE and SE groups (Fig. 2). The obesity-related increase in leptin levels can be improved through a variety of exercises such as aerobic and resistance exercise (39). This result suggests that swimming decreases the level of serum leptin, which helps regulate insulin sensitivity and the size of fatty tissues (40). In this study, ASE supplementation did not significantly improve leptin production, however exercise did improve leptin levels. Therefore, we can assume that ASE extract can improve leptin production when combined with the beneficial effects of exercise.

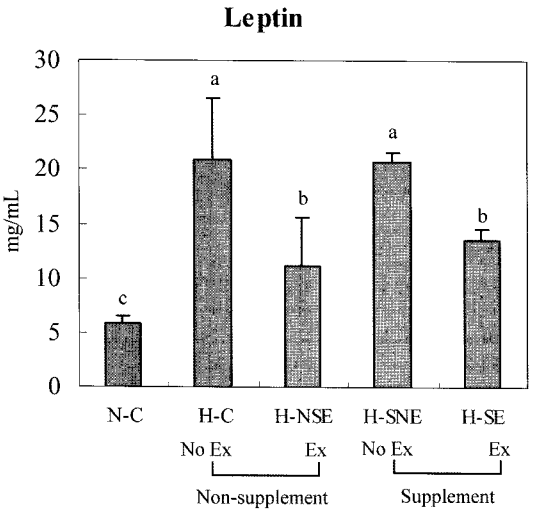

Fig. 2. Leptin concentrations in serum. N-C, normal diet; H-C, high fat diet; H-NSE, high fat diet non-supplement & exercise; H-SNE, high fat diet supplement & no exercise; H-SE, high fat diet supplement & exercise. Mean±SD of 10 mice per group. Bars with different superscript letters are significantly different at $p<0.05$ by ANOVA and Duncan's multiple range tests.

Hepatic and muscle CPT-I mRNA levels Carnitine palmitoyltransferase-I (CPT-I) is located on the outer mitochondrial membrane and facilitates the movement of long chain fatty acids (LCFA) into the mitochondrial matrix. Therefore, it functions as a rate-limiting enzyme of β-oxidation in the mitochondria by having a key role in the transport of LCFA (41). The effects of ASE supplementation and exercise on CPT-I expression in serum and

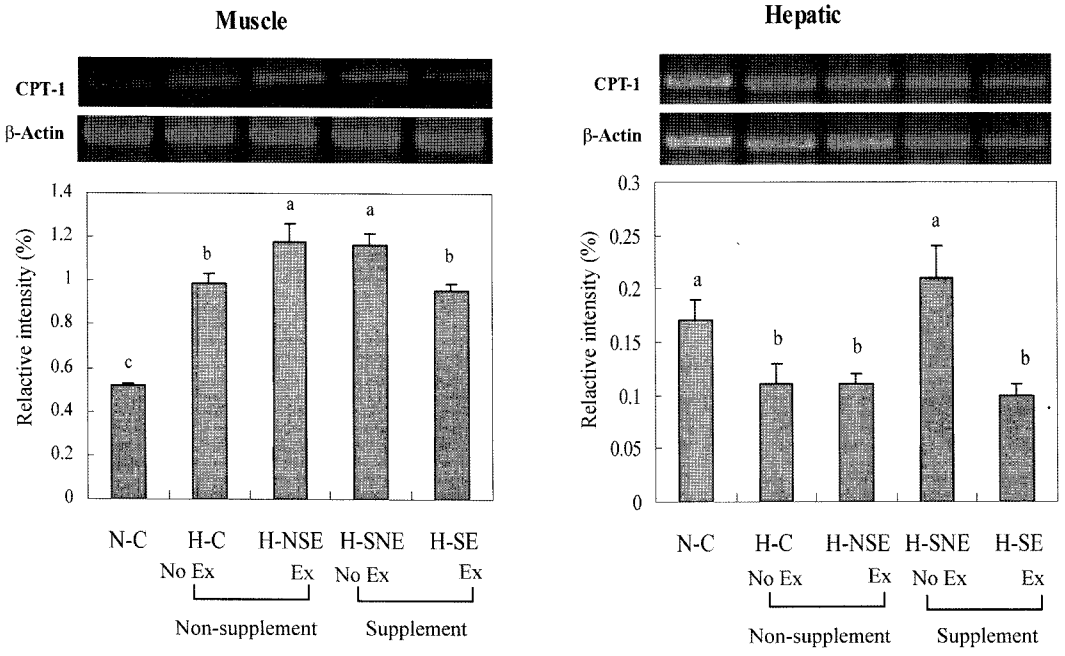

Fig. 3. Hepatic and muscle CPT-I mRNA levels. N-C, normal diet; H-C, high fat diet; H-NSE, high fat diet non-supplement & exercise; H-SNE, high fat diet supplement & no exercise; H-SE, high fat diet supplement & exercise. Mean±SD of 10 mice per group. Bars with different superscript letters are significantly different at $p<0.05$ by ANOVA and Duncan's multiple range tests.

liver are shown in Fig. 3.

Hepatic CPT-I mRNA levels, which are associated with fatty acid β-oxidation, were higher in the H-NSE and H-SNE groups than the intermediate levels observed in the H-C and H-SE groups. Earlier studies reported that hepatic CPT-I mRNA expression is increased by exercise and high fat diet, suggesting that the control of CPT-I mRNA expression is a key feature in the regulation of fatty acid oxidation during exercise (42, 43). Another study claimed that CPT-I enzyme activity is increased by the up-regulation of CPT-I transcription mediated by genisetin, soy isoflavone, and daidzein (44). The present study supports the results of these earlier studies by demonstrating increased hepatic CPT-I mRNA expression in the ASE supplemented groups. Given the vital role of CPT-I in regulating fatty acid oxidation, a large number of studies have been carried out to explore the effects of exercise on this enzyme and suggest an increase in CPT-I and fatty acid oxidation after regular exercise (45). It was found in this study, however, that hepatic CPT-I mRNA expression is lower in the group supplemented with ASE and exercised. This finding is similar to that of Yang *et al.* (46) in which hepatic CPT-I mRNA expression was reduced when the injection of growth hormone was combined with exercise. These findings suggest that the efficacy of cell cultured ASE is undermined by counteractive effects of exercise, resulting in a decreased capacity for fatty acid oxidation. CPT-I mRNA levels in muscle were higher in the H-SNE group than in the H-C, H-NSE, and H-SE groups. This finding can be explained by the observation that with long-term physical training, CPT-I activity is adequate to support fatty acid oxidation (47). In this study, ASE influenced the formation of muscle and hepatic CPT-I, and the reciprocal effect of ASE and exercise was not observed. Therefore, it appears that exercise combined with high-fat diet and ASE extract intake in a short period of time does not influence the formation or levels of CPT-I.

Based on these results, ASE and exercise can each reduce body fat stores, and we conclude that the combination of ASE with exercise is even more effective at improving lipid profiles.

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