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# Combined Effects of Cell Cultured Acanthopanax Senticosus Supplementation and Exercise on Lipid Profiles, Carnitine and Leptin Levels in Mice*

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**The purpose of this study was to determine the independent and the combined effects of cell cultured Acanthopanax senticosus extracts (ASE) supplementation and swimming exercise on body weight, lipid profile, carnitine and leptin levels in C57 BL/6J mice. Forty C57BL/6J mice were divided into four groups: non-supplement and non-exercise (NSNE); non-supplement and exercise (NSE); supplement and non-exercise (SNE); supplement and exercise (SE) mice. They were allowed free access to food and water. The exercised groups were forced to swim (1hr, 6 days a week) in a water bath for 12 weeks. The supplemented groups were fed Cell cultured ASE (0.5 g/kg body weight/day) for 12 weeks. In this study, we found that the combination of Cell cultured ASE supplementation and exercise significantly decreased liver triglyceride (TG) level and serum leptin level but significantly increased serum HDL-cholesterol level compare to control (NSNE) group. These improved lipid profiles and decreased serum leptin would have positive effects on obesity and cardiovascular disease.**

**Key words:** Acanthopanax senticosus, LDL-cholesterol, HDL-cholesterol, Leptin, Cardiovascular disease

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## INTRODUCTION

Acanthopanax senticosus, which is commonly known as Siberian ginseng, has been used as an adaptogenic medicine in Korea. It has been studied extensively and shown to exhibit a variety of activities such as antiviral[1)], antitumour[2)], antioxidant[3)], anti-stress[4)], exercise endurance improvement[5)], and anti-obesity.[6)] The active components in Acanthopanax senticosus have been classified as phenylpropane compounds, lignans, coumarins, polysaccharides, and other components.[7)] Unfortunately, wild Acanthopanax senticosus is expensive, and the cultivated plants require many years to develop sufficient concentration of active components. Therefore, we evaluated the efficacy of Acanthopanax senticosus by culturing isolated cells in bioreactors rather than growing plants from seeds.

Obesity leads to increased morbidity and mortality and is associated with serious medical conditions, including systematic hypertension, elevated serum cholesterol level, and insulin resistance.[8)] Caloric restriction and increased aerobic exercise are common methods of weight reduction. Aerobic exercise has been recommended as a therapeutic lifestyle change for improving lipid and lipoprotein levels in adults.[9)] Actually aerobic exercise training has been shown to significantly elevate HDL-cholesterol and reduce triglyceride concentration in normal and dyslipidemic individuals.[10)] Exercise-mediated changes in HDL-cholesterol and triglyceride are transient and have been shown to occur in the hours and days after exercise.[11)] In addition, pharmacological agents have also been introduced as an effective method of obesity treatment. Recently, we reported that Cell-cultured Acanthopanax senticosus extracts had anti-obesity effects in mice fed high fat diet.[6)] Obesity is now recognized as only one among many components of the metabolic syndromes. Most components of metabolic syndrome are independent risk factors of atherosclerosis.[12)]

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Carnitine, a nutrient, is normally synthesized from methionine and lysine in the liver and kidney. Carnitine transports long-chain fatty acid (LCFA) across the mitochondrial membrane where they undergo beta-oxidation to produce energy. Carnitine deficiency, therefore, results in LCFA accumulation in the cytoplasm, shortage of LCFA availability in mitochondria for oxidation, and decrease in ketone and energy production.[13] Other functions of carnitine include maintenance of adequate free coenzyme-A required for various metabolic pathways, protection of cells against toxic accumulation of acyl-coenzyme A compounds by shuttling acyl groups out of the mitochondria.[13] Recently, attention has been focused on the anti-oxidant effects of L-carnitine and its acyl derivatives.[14] Increased oxidative stress is associated with all cardiovascular risk factors and reactive oxygen species appear to be the principal mediators of cardiomyocite dysfunction in various cardiovascular diseases.[15] Rebuzzi and colleagues have demonstrated that infusion of carnitine significantly reduced the extent of cardiac necrosis in patients hospitalized for acute myocardial infarction.[16]

Leptin, a 167-amino acid peptide hormone produced by white adipose tissue, is primarily involved in the regulation of food intake and energy expenditure. Plasma leptin concentration is proportional to body adiposity and markedly increased in obese individuals.[12] Levels of leptin in obese individuals are increased, but the increased levels appear to fail to have no influence on energy intake or expenditure to restore fat mass to normal. It is therefore believed that obesity is a state of leptin resistance.[17] The increased leptin levels in familial combined hyperlipidemia (FCH) were found to be associated with an increased risk for cardiovascular disease, independent of gender, Body Mass Index (BMI), and insulin resistance.[18] Leptin induces the accumulation of cholesterol esters in foamcells, especially at high glucose concentrations.[19] Several clinical studies have demonstrated that high leptin level predicts acute cardiovascular events, cerebral stroke independently of traditional risk factors.[12]

In this study, we have investigated the effect of ASE supplementation with and without the combination of swimming exercise on body weight, lipid profile, carnitine and leptin levels in C57 BL/6J mice fed normal diet. We found that combination of ASE supplementation and exercise improved lipid profiles and significantly decreased serum leptin level. It would be used as basic information on a correlation between the obesity and cardiovascular disease.

## MATERIALS AND METHODS

### 1. Materials

Cultured Acanthopanax. senticocus cells with a torpedo shape were supplied by Microplants Co., Ltd. (Yusung, Korea). The Acanthopanax senticosus cells were dried and extracted with deionized water (30 times volume) for 9 hours at 80 ℃. The extracts were filtered, concentrated to the 75 brix, under vacuum at 60 ℃, and stored at 4 ℃ until used.

### 2. Animals and Diets

Male C57 BL/6J mice, aged 4 weeks, were purchased from Jackson Laboratories (Bar Harbor, ME). The animals were maintained on a chow diet (Jeil-jedang, Suwon, Korea) for 1 week, and then randomly divided into four groups: non-supplement and non-exercise (NSNE); non-supplement and exercise (NSE); supplement and non-exercise (SNE); supplement and exercise (SE) mice. The animals were randomized into groups (n=10) such that average weight in each group was comparable. The composition of experimental diet are shown in Table 1. They were allowed free access to food and water. The exercised groups were forced to swim (1 hr, 6 days a week) in a water bath for 12 weeks. The supplemented groups were fed ASE (0.5 g/kg body weight/day) for 12 weeks. Research Diets (New Brunswick, NJ) manufactured the diets. The animals were

**Table 1.** *Composition of Conditional Diet (AIN-93 modified diet for rodents)*

| | Non supplement | | supplement | |
|---|---|---|---|---|
| **Ingredient (g)** | **NSNE[1)]** | **NSE** | **SNE** | **SE** |
| Casein, lactic | 200 | 200 | 200 | 200 |
| L-cystine | 3 | 3 | 3 | 3 |
| Corn Starch | 315 | 315 | 315 | 315 |
| Maltodextrin | 35 | 35 | 35 | 35 |
| Sucrose | 350 | 350 | 350 | 350 |
| Cellulose | 50 | 50 | 50 | 50 |
| Soybean Oil | 25 | 25 | 25 | 25 |
| Lard | 20 | 20 | 20 | 20 |
| Mineral Mix | 10 | 10 | 10 | 10 |
| Dicalcium Phosphate | 13 | 13 | 13 | 13 |
| Calcium Carbonate | 5.5 | 5.5 | 5.5 | 5.5 |
| Potassium Citrate | 16.5 | 16.5 | 16.5 | 16.5 |
| Vitamin Mix | 10 | 10 | 10 | 10 |
| Choline Bitarate | 2 | 2 | 2 | 2 |
| FD&C Yellow Dye #5 | 0.05 | 0.05 | 0.05 | 0.05 |
| kcal/g | 3.8 | 3.8 | 3.8 | 3.8 |
| *Acanthopanax centicosus (ASE)* | 0.5kg/kg bw | 0.5kg/kg bw | - | - |

[1)] AIN-93 Modified deit with 4% fat (10% fat Calorie) content.
NSNE, non-supplement & non-exercise NSE, non-supplement & exercise SNE, supplement & non-exercise SE, supplement & exercise

**Table 2.** Body Weight and Food Intake in Mice

| | Non-supplement | | supplement | | ANOVA[1)] | | |
|---|---|---|---|---|---|---|---|
| | NSNE | NSE | SNE | SE | S | E | S×E |
| Initial Weight(g) | 22.03 ± 0.90 | 22.04 ± 0.96 | 22.11 ± 1.07 | 22.09 ± 0.74 | NS | NS | NS |
| Final Weight(g) | 28.00 ± 1.56$^{a}$ | 25.15 ± 1.32$^{c}$ | 27.45 ± 1.26$^{ab}$ | 26.41 ± 1.33$^{b}$ | NS | <0.0001 | NS |
| Weight gain(g/day) | 6.00 ± 1.04$^{a}$ | 3.31 ± 1.19$^{b}$ | 5.34 ± 0.89$^{a}$ | 3.92 ± 1.03$^{b}$ | NS | <0.0001 | NS |
| Food intake(g/day) | 2.33 ± 0.06$^{b}$ | 2.80 ± 0.13$^{ab}$ | 2.65 ± 0.27$^{b}$ | 3.22 ± 0.29$^{a}$ | 0.0196 | 0.0033 | NS |
| Energy intake(Kcal/day) | 8.88 ± 0.24$^{b}$ | 10.67 ± 0.51$^{ab}$ | 10.09 ± 1.03$^{b}$ | 12.26 ± 1.12$^{a}$ | 0.0201 | 0.0033 | NS |
| Feed efficiency ratio | 0.67 ± 0.06$^{a}$ | 0.32 ± 0.05$^{c}$ | 0.53 ± 0.01$^{b}$ | 0.30 ± 0.06$^{c}$ | 0.0261 | <0.0001 | NS |

All values are mean ± SD. Values with different superscripts are significantly different by ANOVA with Duncan's multiple range test at $p<0.05$.
NSNE, non-supplement & non-exercise NSE, non-supplement & exercise SNE, supplement & non-exercise SE, supplement & exercise. Feed efficiency ratio was calculated as weight gain(per day)/dietary intake(per day)
[1)] The degree of significance resulting from the two-way ANOVA is shown with effects of administration of ASE(S), exercise(E), and the interaction of administration of ASE and exercise being expressed as the numerical value or as not significant(NS) when p<0.05.

housed in a temperature-controlled environment with a 12-hour light/dark cycle. The food consumption and body weight were measured daily and weekly, respectively.

### 3. Exercise Protocol

Trained mice were exercised by swimming in a pool (Keitaro Matsumoto *et al*, 1996, Japan). After spending adaptation period for swimming for one week without a current, the animals were trained by swimming for the same time length under the same conditions (4 L/min, 34 ℃, 6 days/week, 1 hr/day) for 12 weeks.

### 4. Serum and Tissue Sample

Feed was removed 12 hours before sacrificing. Blood samples were collected from each mouse by orbital/cardiac puncture and incubated on ice water bath for 1 hour. Serum was separated from the blood by centrifugation 1,100 xg for 15 min at 4 ℃ and kept at -80 ℃ until analyzed. The abdominal fat was removed, rinsed with a phosphate- buffered saline (PBS) solution, wiped with a paper towel, weighed quickly, frozen in liquid nitrogen, and stored at -80 ℃ until assayed.

### 5. Analysis of Lipids and Leptin

The total cholesterol level and HDL-cholesterol fraction in the serum were measured by enzyme method using a commercial kit Asan Pharmaceutical Co., Seoul, Korea). The LDL-cholesterol level was calculated by the Friedwald method [20)]. The triglyceride in the blood and liver tissue were assayed with the same enzymatic commercial kit (Asan Pharmaceutical Co., Seoul, Korea), and the total amount of lipids in the blood and liver tissue was measured by the sulfo-phospho-vanillin method using a commercial kit (Kokusai Phamaceutical Co., Kobe, Japan). Liver lipids were extracted from liver tissues according to the method of Folcheta.[21)] Serum leptin levels were determined by radioimmunoassay (RIA). The serum leptin was measured using a mouse leptin RIA kit from Linco Reasearch (St. Charles, MO). Radioactivity of the samples was determined in a gamma scintillation counter.

### 6. Analysis of Carnitine

Muscle tissues (50 mg) were homogenized (20 sec) using a sonicator (Fisher Scientific Co., USA) in 99 volumes of cold distilled water and centrifuged at 1,500 xg, and then the supernatants were collected. Non-collagens of gastric tissues were extracted by adding 9 volume of 50 mmol/L KOH for 12-16 hrs, centrifuged. The level of non-collagen proteins in the supernatant was determined with a Protein Assay kit (Bio-RAD Co., USA). Nonesterified carnitine (NEC), acid-soluble acylcarnitine (ASAC), and acid-insolutble acylcarnitine (AIAC) in serum were determined by the radio immuno enzymatic procedure of Cederblad and Lindstedt,[22)] which was modified by Sachan *et al*.[23)] In this method, AIAC were precipitated with perchloric acid and centrifugation, leaving the ASAC and NEC in the supernatant. An aliquot of the supernatant was assayed to determine NEC, and another aliquot was hydrolyzed with 0.5 mol/L KOH to assay all acid-soluble carnitines (ASAC + NEC). The ASAC value was calculated as the difference between the NEC and total acid-soluble carnitines. The pellets containing the AIAC were drained, washed, and hydrolyzed in 0.5 mol/ KOH for 60 minutes in a hot water bath (60 ℃). In each case, carnitine was assayed using carnitine acetyl transferase (Sigma Chemical Co., St, Louis, MO) to esterify the carnitine to a [$^{14}$C]acetate from [1-$^{14}$C]acetyl-CoA (Amersham, Arlington Heights, IL). Radioactivity of samples was determined in a liquid scintillation counter (Beckman model LS3801, Beckman Instruments, Palo Alto, CA, USA).

**Table 3.** Lipid Concentrations in Serum and Liver

| Variable | Non-supplement | | supplement | | ANOVA[1)] | | |
|---|---|---|---|---|---|---|---|
| | NSNE | NSE | SNE | SE | S | E | S × E |
| *Serum* | | | | | | | |
| TG (mg/dl) | 65.43 ± 15.45 | 68.72 ± 14.75 | 64.50 ± 10.70 | 63.37 ± 8.78 | NS | NS | NS |
| TC (mg/dl) | 161.49 ± 19.90 | 168.70 ± 19.22 | 166.91 ± 21.53 | 162.05 ± 16.67 | NS | NS | NS |
| HDLc (mg/dl) | 24.42 ± 3.27$^{b}$ | 29.07 ± 3.06$^{ab}$ | 33.49 ± 4.84$^{a}$ | 30.35 ± 3.40$^{a}$ | 0.0033 | NS | 0.0201 |
| LDLc (mg/dl) | 139.26 ± 8.36 | 119.50 ± 12.13 | 123.96 ± 23.81 | 118.17 ± 17.86 | NS | NS | NS |
| HDLc/TC (%) | 13.34 ± 2.02$^{b}$ | 16.43 ± 0.61$^{a}$ | 18.18 ± 2.39$^{a}$ | 18.17 ± 2.40$^{a}$ | 0.0012 | NS | NS |
| LDLc/TC (%) | 77.68 ± 1.46$^{a}$ | 75.78 ± 2.51$^{ab}$ | 73.12 ± 2.99$^{b}$ | 73.80 ± 1.51$^{b}$ | 0.0189 | NS | NS |
| *Liver* | | | | | | | |
| TG (mg/g) | 93.62±4.90$^{a}$ | 74.16±8.84$^{b}$ | 78.30±9.81$^{b}$ | 76.81±4.03$^{b}$ | NS | 0.0009 | 0.0093 |
| TC (mg/g) | 9.20±4.90$^{a}$ | 6.55±1.34$^{b}$ | 7.68±1.18$^{ab}$ | 6.87±1.55$^{b}$ | NS | 0.0009 | NS |

All values are mean ± SD. Values with different superscripts are significantly different by ANOVA with Duncan's multiple range test at $p<0.05$.
NSNE, non-supplement & non-exercise; NSE, non-supplement & exercise SNE, supplement & non-exercise SE, supplement & exercise TG, triglyceride TC, total cholesterol HDLc, HDL-cholesterol; LDLc, LDL-cholesterol
[1)] The degree of significanceresulting from the two-way ANOVA is shown with effects of administration of ASE (S), exercise (E), and the interaction of administration of ASE and exercise being expressed as the numerical value or as not significant (NS) when $p<0.05$

**Table 4.** Carnitine Concentrations in Serum and Tissue

| Variable | Non-supplement | | supplement | | ANOVA[1)] | | |
|---|---|---|---|---|---|---|---|
| | NSNE | NSE | SNE | SE | S | E | S × E |
| *Serum (μmol/dl)* | | | | | | | |
| NEC | 1.18 ± 0.19$^{b}$ | 1.21 ± 0.29$^{b}$ | 1.61 ± 0.37$^{a}$ | 1.69 ± 0.57$^{a}$ | 0.0143 | NS | NS |
| ASAC | 2.15 ± 0.28 | 2.37 ± 0.12 | 1.98 ± 0.29 | 2.27 ± 0.43 | NS | NS | NS |
| AIAC | 0.21 ± 0.03 | 0.24 ± 0.06 | 0.16 ± 0.07 | 0.21 ± 0.02 | NS | NS | NS |
| TCNE | 3.72 ± 0.52 | 3.63 ± 0.54 | 3.75 ± 0.56 | 4.16 ± 0.99 | NS | NS | NS |
| Acyl/free | 1.96 ± 0.12$^{a}$ | 2.23 ± 0.23$^{a}$ | 1.37 ± 0.28$^{b}$ | 1.53 ± 0.28$^{b}$ | <0.0001 | 0.0418 | NS |
| *Liver (nmol/mg)* | | | | | | | |
| NEC | 0.545 ± 0.074$^{a}$ | 0.488 ± 0.028$^{ab}$ | 0.405 ± 0.119$^{b}$ | 0.450 ± 0.026$^{ab}$ | 0.029 | NS | NS |
| ASAC | 0.098 ± 0.064 | 0.057 ± 0.007 | 0.033 ± 0.048 | 0.083 ± 0.077 | NS | NS | NS |
| AIAC | 0.018 ± 0.005$^{b}$ | 0.028 ± 0.007$^{a}$ | 0.011 ± 0.005$^{b}$ | 0.030 ± 0.004$^{a}$ | NS | 0.0004 | NS |
| TCNE | 0.668 ± 0.125$^{a}$ | 0.569 ± 0.030$^{ab}$ | 0.431 ± 0.101$^{b}$ | 0.542 ± 0.099$^{ab}$ | 0.028 | NS | NS |
| Acyl/free | 0.178 ± 0.117 | 0.165 ± 0.045 | 0.080 ± 0.046 | 0.240 ± 0.157 | NS | NS | NS |
| *Muscle (μmol/mgNCP)* | | | | | | | |
| NEC | 8.88 ± 0.72$^{b}$ | 11.87 ± 1.78$^{a}$ | 11.53 ± 1.79$^{a}$ | 11.99 ± 1.14$^{a}$ | 0.042 | 0.015 | 0.041 |
| ASAC | 11.62 ± 0.68 | 12.13 ± 1.18 | 9.78 ± 2.52 | 9.25 ± 3.91 | NS | NS | NS |
| TCNE | 19.89 ± 1.62$^{b}$ | 23.87 ± 2.54$^{a}$ | 21.08 ± 2.21$^{ab}$ | 22.56 ± 2.29$^{ab}$ | NS | 0.014 | NS |
| Acyl/free | 1.35 ± 0.13$^{a}$ | 1.05 ± 0.33$^{ab}$ | 0.87 ± 0.16$^{b}$ | 0.79 ± 0.40$^{b}$ | 0.0109 | NS | NS |

All values are mean ± SD. Values with different superscripts are significantly different by ANOVA with Duncan's multiple range test at $p<0.05$.
NSNE, non-supplement & non-exercise; NSE, non-supplement & exercise SNE, supplement & non-exercise SE, supplement & exercise NEC, non esterfied carnitine; ASAC, acid soluable acyl carnitine; AIAC, acid insoluable acyl carnitine; Acyl/free, (ASAC+AIAC)/NEC; TCNE, total carnitine (NEC+ASAC+AIAC)
[1)] The degree of significance resulting from the two-way ANOVA is shown with effects of administration of ASE (S), exercise (E), and the interaction of administration of ASE and exercise being expressed as the numerical value or as not significant (NS) when $p<0.05$

### 7. Statistical Analysis

Data from individual experiment were expressed as the mean ± standard deviation. All statistical analysis was performed using SAS software (SAS Institute, Cary, NC, USA). The data were analyzed by two-way analysis of variance (ANOVA). Differences within the four groups were separated using Duncan's multiple range test. The accepted level of significance was $p<0.05$.

## RESULTS

### 1. Body Weight and Food Intake in Mice

Food intake and energy intake were higher in SE group than in NSNE group (Table 2). Swimming exercise significantly decreased the final body weight ($p<0.01$), body weight gain ($p<0.01$), feed efficiency ratio ($p<0.01$). However, ASE supplementation had no effect on body weight.

**Table 5.** Epididymal Fat Weight and Serum Leptin Concentration in Mice

| Variable | Non-supplement | | supplement | | ANOVA[1)] | | |
|---|---|---|---|---|---|---|---|
| | NSNE | NSE | SNE | SE | S | E | S × E |
| fat weight (g) | 0.81 ± 0.35 | 0.75 ± 0.24 | 0.97 ± 0.37 | 0.69 ± 0.17 | NS | NS | NS |
| serum leptin (ng/ml) | 5.92 ± 0.38[a] | 5.42 ± 0.80[a] | 5.99 ± 0.53[a] | 3.51 ± 0.77[b] | 0.0153 | 0.0006 | 0.0071 |

All values are mean ± SD. Values with different superscripts are significantly different by ANOVA with Duncan's multiple range test at $p<0.05$.
NSNE, non-supplement & non-exercise; NSE, non-supplement & exercise SNE, supplement & non-exercise SE, supplement & exercise
[1)] The degree of significance resulting from the two-way ANOVA is shown with effects of administration of ASE (S), exercise (E), and the interaction of administration of ASE and exercise being expressed as the numerical value or as not significant (NS) when $p<0.05$

### 2. Lipid Concentrations in Serum and Liver

The effects of ASE supplementation and exercise on lipid profiles in the serum and liver are shown in Table 3. ASE supplementation did not show any significant differences in the levels of serum triglyceride, total cholesterol, and LDL-cholesterol among groups. However, the level of serum LDL-cholesterol/TC (%) in ASE supplemented group was significantly decreased, while the levels of serum HDL-cholesterol, HDL-cholesterol/TC (%) were significantly increased compare to control (NSNE) group. Swimming exercise significantly decreased liver triglyceride, total cholesterol compare to control (NSNE) group. Combination of ASE supplementation with exercise significantly increased the level of serum HDL-cholesterol ($p<0.05$), but decreased significantly the level of liver triglyceride compare to control (NSNE) group.

### 3. Carnitine Concentration in Serum and Tissue

ASE supplementation significantly decreased the levels of serum and muscle acyl/free carnitine, liver NEC, and liver TCNE. However, levels of serum NEC and muscle NEC were significantly increased. Swimming exercise significantly increased liver AIAC and muscle TCNE (Table 4). Combination of ASE supplementation with exercise significantly increased muscle NEC level compare to control (NSNE) group ($p<0.05$).

### 4. Body Fat Weight

There were no significant differences in fat weight among groups, but swimming exercise tended to decrease fat weight (Table 5).

### 5. Serum Leptin Concentration

ASE supplementation significantly decreased the level of serum leptin ($p<0.05$), and swimming exercise also decreased significantly the level of serum leptin ($p<0.01$) (Table 5). Combination of ASE supplementation with exercise significantly decreased the level of serum leptin ($p<0.01$).

## DISCUSSION

Cardiovascular disease is the number one cause of mortality among men in Korea and the United States of America (USA). Samyah and colleagues have reported that elevated plasma LDL-cholesterol concentration was a well-established risk factor for coronary heart disease.[24)] Several epidemiological studies have demonstrated a relationship between obesity and increased risk for cardiovascular disease. However, it is not entirely clear whether this relationship is due to effects on body composition or correlated traits, such as dyslipoproteinemia and diabetes, which are themselves risk factors for cardiovascular disease.[25)] Several measures of adiposity are correlated with low concentration of HDL-cholesterol which in turn is associated with increased risk of cardiovascular disease.[26)] Our data showed that food intake and energy intake were higher in ASE supplemented group and exercise group than in control (NSNE) group. Exercise significantly decreased body weight ($p<0.01$). ASE supplementation with exercise had no synergic effects on body weight. ASE supplementation alone decreased the level of serum LDLc/TC (%) whereas the level of serum HDL-cholesterol was significantly increased compare to control (NSNE) group. We have found that swimming exercise significantly decreased the levels of liver triglyceride and total cholesterol compare to control (NSNE) group. Our results supports previous findings that aerobic exercise reduces triglyceride and total cholesterol, and increases HDL-cholesterol in men 18 years of age and older.[27)] Combination of ASE supplementation with exercise significantly increased the level of serum HDL-cholesterol, but significantly decreased liver triglyceride level compare to control (NSNE) group. The combination of ASE supplementation with exercise improved lipid profiles, suggesting that ASE supplementation with exercise has positive effects on cardiovascular disease and coronary heart disease.

Carnitine facilitates the transfer of long-chain fatty acids into the mitochondria of skeletal muscle and

cardiomyocytes, where they undergo beta oxidation. By this mechanism, carnitine profoundly influences both skeletal muscle and myocardial fatty acid oxidation and maintains low pools of fatty acid (acyl)-coenzyme A compounds, which are potentially toxic.[28] Carnitine has been shown to be effective in various pathologic conditions characterized by increased oxidative stress such as coronary heart disease as well as heart and renal failure.[13] In this study, we examined whether ASE supplementation or exercise or combination of both affects the levels of carnitine in serum, liver, and muscle. We have found that there were no significant differences in the levels of serum TCNE, ASAC, and AIAC among these groups. However, the level of serum NEC was significantly increased in the ASE supplemented group, while the level of acyl/free carnitine was significantly decreased in this group. These results suggest that ASE supplementation increases lipid oxidation in muscle by increasing serum NEC. Exercise alone significantly increased acyl/free carnitine in serum, and liver AIAC, muscle NEC and TCNE. Since the exercise can increase muscle TCNE, the process of beta-oxidation in the exercised group may be far more efficient than non-exercised groups. These results support the findings reported by Brian E. Leibovit.[29] A combination of ASE supplementation with exercise significantly increased muscle NEC level, but not the levels of TCNE in serum, liver, and muscle. However, exercise significantly increased carnitine level in serum, liver, and muscle. Our findings that exercise increases carnitine level are supported by observations reported by Reznick *et al.*[14] They reported that carnitine have effect on increased oxidative stress such as coronary heart disease, heart and renal failure.

Leptin was identified as hormone that circulates in the blood, and the level of leptin has been shown to be in proportion to whole body adipose tissue mass. Leptin-deficient and leptin receptor-deficient mice are protected from arterial thrombosis and neointimal hyperplasia in response to arterial wall injury.[12] Several *in vivo* studies strongly suggest that leptin is involved in atherogenesis.[12] Wallace *et al.*[30] have found an increased risk of approximately two-fold in cardiovascular disease in the highest two quintiles of leptin concentration compared to the lowest quintile. It has been known for a long time that *ob/ob* mice are resistant to atherosclerosis but it is usually attributed to high HDL-cholesterol level in these animals. More recent studies indicate that lack of leptin may be a key protector of these animals.[31] In the present study, combination of ASE supplementation with exercise significantly decreased serum leptin level that was almost two times less than any other groups. These findings suggest that ASE supplementation and exercise exhibit a synergic effect on serum leptin level. This decreased serum leptin level would be helpful on atherosclerosis.

In summary, the combined effects of ASE supplementation with swimming exercise on body weight, lipid profile and carnitine and leptin levels in C57BL/6J mice are as follows:

1. ASE supplementation significantly decreased the levels of serum LDLc/TC (%), serum and muscle acyl/free carnitine, liver NEC, and liver TCNE, while the levels of serum HDL-cholersterol, serum NEC, and muscle NEC were significantly increased in these groups.
2. Swimming exercise significantly decreased body weight gain, feed efficiency ratio, liver triglyceride, and total cholesterol, whereas the levels of liver AIAC and muscle TCNE were increased.
3. Combination of ASE supplementation with swimming exercise significantly increased serum HDL level, but decreased liver triglyceride and serum leptin levels.

Taken together, we propose that the combination of ASE supplementation with exercise improves lipid profiles and decreases serum leptin level and that the data can be used to control the obesity and cardiovascular disease.

## Literature Cited

1) Glatthaar-Saalmuller B, Sacher F, Esperester A. Antiviral activity of an extract derived from roots of Eleutherococcus senticosus. *Antiviral Res* 50:223-228, 2001
2) Hibasami H, Fujikawa T, Takeda H, Nishibe S, Satoh T, Fujisawa T, Nakashima K. Induction of apoptosis by Acanthopanax senticosus HARMS and its component, sesamin in human stomach cancer KATO III cells. *Oncol Rep* 7:1213-1216, 2000
3) Lin CC, Huang PC. Antioxidant and hepatoprotective effects of Acanthopanx sentocosus. *Phytother Res* 14:489-494, 2000
4) Gaffney BT, Hugel HM, Rich PA. The effects of Eleutherococcus senticosus and Panax ginseng on steroidal hormone indices of stress and lymphocyte subset numbers in endurance athletes. *Life Sci* 70:431-442, 2001
5) Szolomicki J, Samochowiec L, Wojcicki J, Drozdzik M, Szolomicki S. The influence of active components of Eleutherococcus senticosus on cellular defence and physical fitness in man. *Phytother Res* 14:30-35, 2000
6) Cha YS, Rhee SJ, Heo YR. Acanthopanax senticosus Extract

Prepared from Cultured Cells Decreases Adiposity and Obesity Indices in C57BL/6J Mice Fed a High Fat Diet. *J Med Food* 7:422-429, 2004
7) Wagner H, Norr H, Winterhoff H. Plant adaptogens. *Phytomedicine* 1:63-76, 1994
8) Chan JM, Rimm EB, Colditz GA, Stampfer MJ, Willett WC. Obesity, fat distribution, and weight gain as a risk factors for clinical diabetes in man. *Diabetes Care* 17:961-969, 1994
9) Ozcelik O, Dogan H, Kelestimur H. Effects of eight weeks of exercise training and orlistat therapy on body composition and maximal exercise capacity in obese females. *Public Health* 120:76-82, 2006
10) Durstine J, Grandjean P, Cox C. Lipids, lipoproteins, and exercise. *J Cardiopulm Rehabil* 22:385-397, 2002
11) Durstine J, Grandjean P, Davis P. Blood lipid and lipoprotein adaptations to exercise: a quantitative analysis. *Sports Med* 31:1033-1062, 2001
12) Beltowski J. Leptin and atherosclerosis. *Atherosclerosis* 18:947-60, 2006
13) Crill CM, Storm MC, Christensen ML, Hankins CT, Bruce JM, Helms RA. Carnitine supplementation in premature neonates: Effect on plasma and red blood cell total carnitine concentrations, nutrition parameters and morbidity. *Clin Nutr* (In press), 2006
14) Reznick AZ, Kagan VE, Ramsey R. Antiradical effects in L-propionyl carnitine protection of the heart against ischemia-reperfusion injury: the possible role of iron chelation. *Arch Biochem Biophys* 296:394-401, 1992
15) Calo LA, Pagnin E, Davis PA, Semplicini A, Nicolai R, Calvani M, Pessina AC. Antioxidant effect of L-carnitine and its short chain esters Relevance for the protection from oxidative stress related cardiovascular damage. *Int J Cardiol* 107:54-60, 2006
16) Rebuzzi A, Schiavoni G, Amico C. Beneficial effects of L-carnitine in the reduction of the necrotic area in acute myocardial infarction. *Drugs Exptl Clin Res* 10:219-223, 1984
17) Zhang Y, Scarpace PJ. The role of leptin in leptin resistance and obesity. *Physiol Behav* 88:249-256, 2006
18) van der Vleuten GM, Veerkamp MJ, van Tits LJ, Toenhake H, den Heijer M, Stalenhoef AF, de Graaf J. Elevated leptin levels in subjects with familial combined hyperlipidemia are associated with the increased risk for CVD. *Atherosclerosis* 183:355-360, 2005
19) O' Rourke L, Yeaman SJ, Shepherd PR. Insulin and leptin acutely regulate cholesterol ester metabolism in macrophages by novel signaling pathways. *Diabetes* 50:955-961, 2001
20) Freidwald WT, Levy RL, Fredrickso DS. Estimation of the concentration of low density lipoprotein cholesterol without use of the preparation ultracentrifuge. *Clin Chem* 18:499-502, 1972
21) Folch J, Lees M, Sloane-Stanley GHS. Simple method of the isolation of total lipids from animal tissues. *J biol Chem* 22:497-509, 1983
22) Cederblad G, Lindstedt S. A method for the determination of carnitine in the picomole range. *Clin Chim Acta* 37:235-343, 1972
23) Sachan DS, Rhew TH, Ruak RS. Ameliorating effects of carnitine and its precursors on alcohol-induced fatty liver. *Am J Clin Nutr* 123:1939-1951, 1984
24) Shadid S, LaForge R, Otvos JD, Jensen MD. Treatment of obesity with diet/exercise versus pioglitazone has distinct effects on lipoprotein particle size. *Atherosclerosis* 188(2): 370-376, 2006
25) Rainwater DL, Comuzzie AG, Vandeberg JL, Mahaney MC, Blangero J. Serum leptin levels are independently correlated with two measures of HDL. *Atherosclerosis* 132:237-243, 1997
26) Barter PJ, Rye KA. High density lipoproteins and coronary heart disease. *Atherosclerosis* 121:1-12, 1996
27) Kelley GA, Kelly KS. Aerobic exercise and lipids and lipoproteins in men: a meta-analysis of randomized controlled trials. *jmhg* 3:61-70, 2006
28) Peluso G, Barbarisi A, Savica V. Carnitine: an osmolyte that plays a metabolic role. *J cell Biochem* 80:1-10, 2001
29) Leibovitz BE. L-Carnitine the energy nutrient. In: Physical performance, pp.30-32, Kets Publishing, Los Angeles, 1998
30) Wallace AM, McMahon AD, Packard CJ, Kelly A, Shepherd J, Gaw A, Sattar N. Plasma leptin and the risk of cardiovascular disease in the west of Scotland coronary prevention study (WOSCOPS). *Circulation* 104:3052-3056, 2001
31) Schafer K, Halle M, Goeschen C, Dellas C, Pynn M, Loskutoff DJ, Konstantinides S. Leptin promotes vascular remodeling and neointimal growth in mice. *Arterioscler Thromb Vasc Bio* 24:112-117, 2004