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# Genistein Combined with Exercise Improves Lipid Profiles and Leptin Levels in C57BL/6J Mice Fed a High Fat Diet

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**Abstract** The aim of this study is to determine the anti-obesity effects of genistein and exercise, separately and in combination, in mice. Fifty male C57BL/6J mice were divided into 5 treatment groups: normal diet (ND), high fat diet (HD), high fat diet with exercise (HD+Ex), high fat diet with 0.2% genistein (HD+G), high fat diet with 0.2% genistein, and exercise (HD+G+Ex). They were allowed free access to feed and water, and exercised mice engaged in swimming on a regular basis for 12 weeks. Genistein supplemented mice gained less weight, had lower energy intake, better lipid profiles, and lower leptin than the HD mice. Furthermore, when genistein was combined with exercise (HD+G+Ex) the effects were even greater. HD, HD+Ex, and HD+G mice exhibited increased hepatic CPT-1 mRNA expression. Therefore, genistein and exercise has anti-obesity effects, as shown by changes in body weight, fat accumulation, energy intake, and leptin levels.

**Keywords:** genistein, exercise, obesity, lipid profile, leptin

## Introduction

Obesity (body mass index, BMI >30 kg/m$^2$) has been one of the most serious public health problems for most of the world in recent decades (1, 2). Obesity is a state of increased body weight, especially adipose tissue (2), and is clearly associated with increased risk for atherosclerosis, coronary artery disease, type-2 diabetes, dyslipoproteinemia, hypertension, dyslipidemia, and some cancers (3). These diseases can be caused by obesity because of its negative influence on body lipid profiles. According to Maksvytis and Stakisaitis (4), the blood concentrations of high density lipoprotein-cholesterol (HDL-C) and apo A-I of corpulent females were significantly lower compared to those of non-obese females, despite no differences in total cholesterol (TC) and triglyceride (TG). On the other hand, corpulent females had higher apo B concentrations and apo B/A-I ratios. These changes in lipid profile increase the risk of coronary heart disease. Couillard *et al.* (5) reported dissimilar effects with different types of obesity, with abdominal fat increasing the concentration of blood free fatty acids by disturbing fat metabolism, and increases in the risk of hypertriglyceridemia. In addition, elevated free fatty acids (FFAs) associated with obesity inhibit insulin-stimulated glucose uptake, glycogen synthesis, and glucose oxidation (6). It has also been reported that insulin resistance and triglyceride-rich lipoprotein remnants are significantly higher in men with abdominal obesity, resulting in decreased fat catabolism (7).

Regular exercise helps to prevent the different types of obesity, and lowers the rate of death caused by coronary heart disease and cancer (8). Maintained endurance training raises the concentrations of mitochondrial protein by activating key enzymes in the mitochondrial electron transport system, promoting lipid oxidation in trained muscle, all of which further raises the concentrations of mitochondrial proteins. Thus regular exercise encourages fat oxidation in trained muscle to enhance the utilization of energy from body lipid stores (9). For these reasons exercise activates fat oxidation in the muscle tissue of obese male, and it also reduces blood triglyceride-rich lipoproteins and the amount of lipoprotein remnants caused by high-carbohydrate diets (10). Exercise reduces the amount of body fat while increasing energy consumption, and simultaneously reducing insulin resistance which causes a decline in the ability to produce adipose cells in obese Zucker rats (11).

Recently, many researchers have reported that genistein, one of the major isoflavones, can improve body composition and lipid profiles. Naaz *et al.* (12) have reported that injecting large amounts of genistein into mice appears to have antilipogenic effects because genistein reduces the weight of fat cells and lipid droplets, and the expression of lipoprotein lipase mRNA. Genistein also affects the metabolism of adipocytes, suppressing lipogenesis, and activating lipolysis to decrease fat depots within white adipose tissue (13). Genistein improves not only lipid profiles, but also the secretion of leptin, and genistein controls the amount of pyruvate created by glucose metabolism (14). Genistein may also play a role in the anti-diabetic effects of soy isoflavones since it appears to inhibit intestinal glucose uptake and prevents glucose induced lipid peroxidation through down-regulation of the sodium dependent glucose transporter (15). These functions of genistein may be responsible for its multiple beneficial effects that include preventing obesity, cardiovascular diseases, type 2 diabetes, and cancer (16).

In this study we evaluated the effects of genistein and exercise on obesity, and whether these effects are synergistic. Accordingly, mice were given a high fat diet

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with/without genistein and were subdivided into groups with or without exercise in the form of swimming (17).

## Materials and Methods

**Animals and diets** Fifty male C57BL/6J mice, age 4 weeks, were fed a commercial pellet food (Research Diets, New Brunswick, NJ, USA) for a one week adaptation period, and were then divided by a randomized block design into 5 groups: normal diet (ND), high fat diet (HD), high fat diet with exercise (HD+Ex), high fat diet with 0.2 % genistein (HD+G), high fat diet with 0.2% genistein, and exercise (HD+G+Ex). They were fed a modified AIN-93 diet for 12 weeks (Research Diets) (Table 1) and allowed free access to feed and water. Feed consumption records were taken at 2 day intervals and weight was measured once per week. The mice were housed in stainless steel cages in a controlled environment with regard to temperature (23±1°C), humidity (53±2%), and light/dark cycle (12 hr/12 hr).

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

| Ingredient (g) | I | II | III |
|---|---|---|---|
| | Normal diet[1)] | High fat diet[2)] | High fat diet + genistein |
| Casein, lactic | 200 | 200 | 200 |
| L-Cystine | 3 | 3 | 3 |
| Corn starch | 315 | - | - |
| Maltodextrin | 35 | 125 | 125 |
| Sucrose | 350 | 68.8 | 68.8 |
| Cellulose | 50 | 50 | 50 |
| Soybean oil | 25 | 25 | 25 |
| Lard | 20 | 245 | 245 |
| Mineral mix | 10 | 10 | 10 |
| Dicalcium phosphate | 13 | 13 | 13 |
| Calcium carbonate | 5.5 | 5.5 | 5.5 |
| Potassium citrate | 16.5 | 16.5 | 16.5 |
| Vitamin mix | 10 | 10 | 10 |
| Choline bitarate | 2 | 2 | 2 |
| FD&C Yellow dye #5 | 0.05 | - | - |
| FD&C Blue dye #1 | - | 0.05 | 0.05 |
| Genistein[3)] | - | - | 1.55 |
| Total | 1,055.05 | 773.85 | 775.40 |
| kcal | 4,057 | 4,057 | 4,057 |
| Protein (kcal%) | 20 | 20 | 20 |
| Carbohydrate (kcal%) | 70 | 20 | 20 |
| Fat (kcal%) | 10 | 60 | 60 |
| kcal/g | 3.8 | 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)]AIN-93 Modified high fat diet containing 0.2% genistein.

Trained mice were exercised by swimming in a pool (17). They were adapted to swimming for one week without a current, and during the test period all of them were exercised in the same manner (1 hr/day, 6 day/week at a 4 L/min flow rate, and 34°C) for 12 weeks.

The mice were fasted for 12 hr before being sacrificed. Blood was collected and incubated on ice for 1 hr and serum was separated by centrifugation (1,000×g, 10 min), and kept at -80°C until analysis. The liver was surgically removed, rinsed in saline solution, wiped with filter paper, put into a 1.5 mL Eppendorf tube, and stored at -80°C until analysis was completed. Adipose tissues were separated from the back and around the epididymis. Epididymal and back adipose, and skeletal muscle tissues were surgically removed, weighed, quickly frozen in liquid nitrogen, and stored at -80°C until analysis.

**Analysis of lipid concentrations in serum and liver** Total cholesterol and TG in serum and liver were enzymatically measured using a commercial kit (Asan Pharm. Co., Seoul, Korea). The HDL-C was assayed with a commercial kit based on phosphotungstic acid/$MgCl_2$ precipitation (Asan Pharm. Co.).

**Analysis of carnitine concentration in serum, liver, and muscle** Muscle tissues (50 mg) were homogenized (20 sec) using a sonicator (IUL, ESLclassic; Fisher Scientific Co., New York, NY, USA) with 99 volumes of cold distilled water. Liver tissues (50 mg) were homogenized in 29 volumes of cold distilled water, centrifuged at 1,500×g and the supernatant removed.

Non-collagen protein in gastric tissue extracts was extracted by adding 1 volume to 9 volume of 50 mmol/L KOH for 12-16 hr, centrifuging, and then determining protein content with a protein assay kit (Bio-Rad Co., Hercules, CA, USA) based on the method of Bradford (18).

Carnitine was measured in blood and tissue using a modified radioisotopic method of Cederblad and Lindstedt (19, 20). Approximately 100-200 μL of samples were homogenized with 200 μL of 6% perchloric acid (PCA) at 1,500×g for 10 min and the supernatant and pellet collected. Non-esterified carnitine (NEC, free carnitine) was assayed in 150 μL of neutralized supernatant. Acid soluble acylcarnitine (ASAC, short and medium chain acylcarnitine) was measured using the supernatant after hydrolysis with 100 μL of PCA supernatant in 1 N KOH at 37°C for 30 min and neutralization. Acid insoluble acylcarnirine (AIAC, long-chain acylcarnitine) was measured using the PCA pellet washed with 6% PCA 3-4 times to remove NEC and ASAC remnants, and then hydrolyzed with 0.5 N KOH for 60 min in a hot water bath at 65°C. One hundred μL of the supernatant was then neutralized and centrifuged. The reaction mixture [1 M MOPS buffer, 0.1 M potassium ethyleneglycotetraacetate, 0.1 M sodium tetrathionate, 0.1 mM [1-$C^{14}$]acetyl CoA solution (Amersham, Little Chalfont, Buckinghamshire, England)] was added to the supernatant in each sample, and reacted with 1 unit of carnitine acetyltransferase (Sigma Chemical Co., St. Louis, MO, USA) at 37°C for 30 min. Two hundred μL of reacted supernatant was passed through a

column with ion exchange resin (AG×18, 200-400 mesh), which collected the unreacted [1-$C^{14}$] acetyl carnitine, and the $C^{14}$ isotope was measured in a Beckman LS-3801 liquid scintillation counter (Beckman Instruments, Palo Alto, CA, USA). Total carnitine was calculated as the sum of NEC, ASAC, AIAC values.

**Leptin in serum** Leptin in serum was measured using a Gamma Scintillation Counter and a $^{125}$I-labeled mouse leptin radioimmunoassay kit (Linco Research, Inc., St. Charles, MO, USA).

**CPT-1 mRNA levels in liver and muscle** Total RNA was extracted with Trizol reagent 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, Rochester, NY, USA). β-Actin was used as a control. The RT-PCR reaction was carried out using a RT-PCR kit (Primus; 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 1.0% agarose gel. For each set of reactions, 3 samples were selected randomly and run in triplicate. The relative intensity of all mRNA samples was analyzed using AlphaEaseFC software (Alpha Innotech Corporation, San Leandro, CA, USA).

**Statistical analysis** Significance of differences was determined using 2-way analysis of variance (ANOVA) using SAS software version 9 (SAS Institute, Cary, NC, USA). Significance of differences within the groups was determined by Duncan's multiple range tests. The accepted level of significance was $p<0.05$.

## Results and Discussion

**Food intake and body weight changes** The efficacy of the genistein supplementation and endurance training on weight gain is shown in Table 2. In this study, there were no significant differences in body weight initially. However at the end of the test period, both genistein and exercise significantly lowered the final weight, weight gain, and feed and energy intakes of mice fed a high fat diet.

According to the research of Kim *et al.* (21), supplementing mice on a high fat diet with genistein reduced feed intake and caused weight loss, however the genistein supplemented group was normalized for final body weight and weight gain, although there were no significant differences in feed intake.

Exercise also affects feed intake and body weight. Rats fed a high fat diet keep pace with endurance training, but have significantly lower body weight, reduced weight gain, and higher feed efficiency ratios compared to rats without exercise. The main reasons for these differences are not only increased energy expenditure due to physical activity, but also reduced body fat and increased lean body mass also due to physical exercise (22).

In this experiment feed intake and body weight were significantly decreased, with genistein supplementation being more effective than endurance exercise, and both combined being most effective. Thus we consider that there is synergistic or additive effect for taking genistein with exercise. Weight changes (g/day), were not significantly different in the genistein and exercise groups, but tended to be lower in comparison with HD mice.

**Body fat and organ weight changes** The high fat diets resulted in higher liver weights that were lowered by exercise and genistein and normalized by both in combination. However, when adjusted for body weight, liver weights were higher in the genestein supplemented animals. Both genistein and exercise groups had significantly lower epididymal and back fat, and it was even lower with both treatments combined. However, as a percentage of body weight, epididymal fat did not show synergistic effects with the combination of genistein and exercise. Statistically significant differences in back fat as a proportion of body weight was seen only with genistein (Table 3). Supplementation of genistein increased liver weight per gram body weight, but greatly decreased the weight of epididymal and back fat. This result is the same as that reported by Kim *et al.* (21), in which genistein supplementation increased the liver weight relative to body weight and decreased the amount of body fat. According to Naaz *et al.* (12), genistein injections suppressed the lipogenic enzyme LPL, and this effect decreased the size

**Table 2. Body weight and food intake in C57BL/6J mice[1)]**

| Groups | ND Control (n=10) | HD | | | | Statistical significance | | |
|---|---|---|---|---|---|---|---|---|
| | | HD | | HD+G | | | | |
| | | NEx (n=10) | Ex (n=10) | NEx (n=10) | Ex (n=10) | G | Ex | G×Ex |
| Initial weight (g) | 17.5±0.4 | 17.4±0.4 | 17.6±0.3 | 17.4±0.3 | 17.5±0.2 | | | |
| Final weight (g) | 25.2±1.6$^{c}$ | 41.1±2.4$^{a}$ | 31.7±1.8$^{b}$ | 26.4±2.5$^{c}$ | 21.5±1.0$^{d}$ | <0.0001 | <0.0001 | 0.0124 |
| Weight gain (g/day) | 0.1±0.1$^{b}$ | 0.3±0.1$^{a}$ | 0.2±0.1$^{b}$ | 0.2±0.1$^{b}$ | 0.1±0.1$^{b}$ | 0.0005 | 0.0006 | NS |
| Food intake (g/day) | 2.6±0.2$^{b}$ | 3.4±0.7$^{a}$ | 2.4±0.2$^{bc}$ | 2.6±0.6$^{b}$ | 2.3±0.2$^{c}$ | <0.0001 | <0.0001 | 0.0007 |
| Energy intake (kcal/day) | 9.9±0.6$^{d}$ | 17.4±3.6$^{a}$ | 12.5±1.3$^{bc}$ | 13.6±3.1$^{b}$ | 11.9±1.1$^{c}$ | <0.0001 | <0.0001 | 0.0007 |

[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. The degree of significance resulting from 2-way ANOVA are shown with the effects of gesnistein (G), exercise (Ex), and the interaction of genistein and exercise (G×Ex) being expressed as a numerical value or as not significant (NS) when $p>0.05$. ND, normal diet; HD, high fat diet; G, genistein; NEx, non-exercised; Ex, exercised.

**Table 3. Liver and fat weights in C57BL/6J mice[1)]**

| Groups | ND Control (n=10) | HD | | | | Statistical significance | | |
|---|---|---|---|---|---|---|---|---|
| | | HD | | HD+G | | | | |
| | | NEx (n=10) | Ex (n=10) | NEx (n=10) | Ex (n=10) | G | Ex | G×Ex |
| Liver(g) | 0.9±0.1$^{c}$ | 1.2±0.1$^{a}$ | 0.9±0.0$^{bc}$ | 1.0±0.2$^{b}$ | 0.8±0.0$^{c}$ | 0.0012 | <0.0001 | NS |
| g/body weight (%) | 3.5±0.2$^{b}$ | 3.0±0.1$^{c}$ | 2.9±0.1$^{c}$ | 3.8±0.2$^{a}$ | 3.8±0.2$^{a}$ | <0.0001 | NS | NS |
| Epididymal fat (g) | 0.4±0.1$^{cd}$ | 2.4±0.3$^{a}$ | 1.6±0.4$^{b}$ | 0.6±0.2$^{c}$ | 0.3±0.1$^{d}$ | <0.0001 | <0.0001 | 0.0404 |
| g/body weight (%) | 1.5±0.6$^{cd}$ | 5.9±0.5$^{a}$ | 5.0±1.1$^{b}$ | 2.3±0.7$^{c}$ | 1.3±0.2$^{d}$ | <0.0001 | 0.0039 | NS |
| Back fat (g) | 0.2±0.1$^{c}$ | 1.6±0.4$^{a}$ | 0.9±0.4$^{b}$ | 0.2±0.1$^{c}$ | 0.1±0.0$^{c}$ | <0.0001 | 0.0069 | 0.0419 |
| g/body weight (%) | 0.7±0.2$^{d}$ | 3.8±0.9$^{a}$ | 2.9±1.3$^{b}$ | 0.9±0.3$^{c}$ | 0.6±0.1$^{c}$ | <0.0001 | 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. The degree of significance resulting from 2-way ANOVA are shown with the effects of gesnistein (G), exercise (Ex), and the interaction of genistein and exercise (G×Ex) being expressed as a numerical value or as not significant (NS) when $p>0.05$. ND, normal diet; HD, high fat diet; G, genistein; NEx, non-exercised; Ex, exercised.

**Table 4. Lipid concentrations in serum of C57BL/6J mice[1)] (mg/dL)**

| Groups | ND Control (n=10) | HD | | | | Statistical significance | | |
|---|---|---|---|---|---|---|---|---|
| | | HD | | HD+G | | | | |
| | | NEx (n=10) | Ex (n=10) | NEx (n=10) | Ex (n=10) | G | Ex | G×Ex |
| HDL-C | 64.5±7.4 | 96.9±8.4$^{a}$ | 90.8±13.6$^{ab}$ | 78.7±9.3$^{b}$ | 78.7±8.0$^{b}$ | 0.0024 | NS | NS |
| TC | 130.8±16.9$^{c}$ | 190.8±18.9$^{a}$ | 156.7±26.3$^{b}$ | 134.8±17.1$^{bc}$ | 122.1±19.5$^{c}$ | <0.0001 | 0.0119 | NS |
| TG | 142.9±41.6$^{ab}$ | 168.1±31.7$^{a}$ | 137.9±16.8$^{ab}$ | 112.8±49.2$^{bc}$ | 89.6±22.6$^{c}$ | 0.0032 | 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. The degree of significance resulting from 2-way ANOVA are shown with the effects of gesnistein (G), exercise (Ex), and the interaction of genistein and exercise (G×Ex) being expressed as a numerical value or as not significant (NS) when $p>0.05$. ND, normal diet; HD, high fat diet; G, genistein; NEx, non-exercised; Ex, exercised; TC, total cholesterol; TG, triglyceride.

of adipocytes. Therefore, this experiment provides strong evidence that genistein suppresses the accumulation of body fat, and reduces body fat already present.

It is commonly known that low intensity exercise (40% $VO_2$ max) is more efficient at stimulating the loss of body fat since 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 (23). Therefore, the reason why epididymal and back fat decreased to levels below normal was due to the effects of exercise on lipid synthesis enzymes. Thus, both exercise and genistein reduces fat mass. It has been shown that exercise combined with isoflavones in ovariectomized mice fed high-cholesterol diets significantly decreased lipid levels (24). Therefore genistein and exercise have synergistic or additive effects on decreasing body fat accumulation.

**Blood lipid profiles** Genistein supplemented animals had lower serum lipid levels of HDL-C, TC, TG, but overall cholesterol levels were lower only in mice that were exercised (Table 4). Serum lipid concentrations were lower in the genistein-supplemented groups than in the HD group. We find similar results from other soy isoflavone studies with regard to genistein modulating blood lipid profiles. Kirk *et al.* (16) reported that total cholesterol levels were decreased by dietary isoflavones (genistein and daidzein), with decreased very low density lipoprotein (VLDL) and LDL cholesterol levels in C57BL/6 mice. Beynen *et al.* (25) demonstrated that soy protein consumption lowers plasma cholesterol concentrations in swine. According to Kim *et al.* (26), changes in lipid profiles are due to genistein induced PPARα activation, which increases the expression of PPAR target genes causing a decrease in plasma lipid levels. This tendency is also seen in type II diabetic mice injected with genistein and daidzein. Genistein and daidzein regulate plasma FFA, TG, TC, and this improves the glucose and lipid profiles of diabetic mice (27). However exercise without genistein does not improve lipid profiles in this way, except for improved TC levels. On the other hand, other researchers say that regular cardio exercise improves blood TG and cholesterol levels remarkably (28). The reason why cardio exercise did not significantly improve TG and HDL-C in this study is the same as reported in a study by Kim and Kang (29). In this report, short term cardio exercise did not change blood lipid profiles, therefore changes in blood lipid profiles are affected by differences in the duration, intensity, and frequency of exercise among experiments. Moreover, the effects of exercise performance and genistein administration were not similar, being lower than mice fed high fat diets. Kim and Kang (29) reported that cardio exercise improves blood lipid profiles, so we can assume that genistein can improve lipid profiles when combined with the beneficial effects of exercise.

**Concentration of carnitine in blood, liver, and muscle** 3-Hydroxy-4-N-trimethyl aminobutyric acid (carnitine) is an important substance for the β-oxidation of fatty acids due to its role in moving long chain fatty acids in the form of acylcartine into the mitochondrial matrix (30). AIAC transports long chain fatty acids into the mitochondrial matrix for β-oxidation to provide cellular energy. On the other hand, ASAC transports short and medium chin fatty acids into the mitochondria. Genistein supplemented mice had lower NEC and ASAC but a higher ratio of acyl/free carnitine (acyl/free). Genistein supplementation decreases NEC and ASCS, but it does not affect AIAC. As a result, we assume that supplementation of genistein increases the amount of acyl/free carnitine. Liver and kidney are known to be the main sites of carnitine synthesis. In particular, the liver transforms 4-trimethyl-aminobutyraldehyde and butyrobetaine, which are inter-mediates in the carnitine biosynthesis pathway (31). In this study, the reason for increased liver NEC with genistein administration was the increased biosynthesis of carnitine in the liver (Table 5). If energy intake decreases, the movement of fatty acids, an energy source for muscle, will decrease. If the movement of fatty acids decreases, muscle will signal for release of lipid stores, and increase lipolysis (32). In this study, supplemental genistiein decreased feeding and energy intake, and this caused a reduction in energy substrates availability to muscle. Therefore lipid metabolism in muscle was activated and increased muscle ASAC. We also observed that muscle NEC increases with exercise. This is because cardio exercise causes free carnitine to accumulate in the muscle (30). Lower AIAC levels may be due to the increased utilization of lipids, or enhanced phospholipid and cholesterol synthesis which is needed for increased membrane synthesis or the formation of eicosanoids. Also, this may be a reflection of camitine and its acylesters in preserving physiologic membrane structures from oxidative damage (44).

**Blood leptin levels** Leptin is a hormone synthesized in adipose tissue that sends information regarding the amount of body fat to the brain. Therefore the concentration of blood leptin is proportional to the amount of body fat, and when body fat is reduced the concentrations of blood leptin are also reduced (33). In this study, circulating leptin concentrations were significantly lower with genistein supplementation and exercise. Exercise combined with genestein resulted in the lowest leptin levels among the HD groups (Fig. 1). Leptin is clearly involved in the control of appetite and energy storage (34). Several studies have demonstrated that serum leptin levels are associated with body mass index (35). Jeusette *et al.* (36) showed that obese dogs have higher plasma leptin levels than lean dogs, and that weight loss decreases plasma leptin. We found that serum leptin concentrations were significantly

**Table 5. Carnitine concentrations in C57BL/6J mice[1)]**

| Groups | ND Control (n=10) | HD | | | | Statistical significance | | |
|---|---|---|---|---|---|---|---|---|
| | | HD | | HD+G | | | | |
| | | NEx (n=10) | Ex (n=10) | NEx (n=10) | Ex (n=10) | G | Ex | G×Ex |
| Serum (μmol/dL) | | | | | | | | |
| NEC | 1.0±0.2$^{b}$ | 1.6±0.2$^{a}$ | 1.5±0.2$^{a}$ | 1.1±0.3$^{b}$ | 1.1±0.2$^{b}$ | 0.0002 | NS | NS |
| ASAC | 2.6±0.3$^{a}$ | 2.5±0.2$^{ab}$ | 2.4±0.2$^{ab}$ | 1.9±0.3$^{c}$ | 2.1±0.4$^{bc}$ | 0.0113 | NS | NS |
| AIAC | 0.29±0.13 | 0.10±0.06 | 0.09±0.08 | 0.11±0.05 | 0.12±0.07 | NS | NS | NS |
| TCNE | 4.8±0.5 | 4.5±0.3 | 4.6±0.6 | 3.7±0.3 | 4.3±0.4 | NS | NS | NS |
| Acyl/free | 2.9±0.6$^{a}$ | 1.4±0.4$^{c}$ | 1.7±0.3$^{bc}$ | 2.1±0.5$^{b}$ | 2.0±0.5$^{b}$ | 0.0069 | NS | NS |
| Liver (μmol /g) | | | | | | | | |
| NEC | 0.5±0.0$^{a}$ | 0.3±0.0$^{c}$ | 0.3±0.10$^{c}$ | 0.46±0.05$^{b}$ | 0.42±0.08$^{b}$ | <0.0001 | NS | NS |
| ASAC | 0.7±0.2 | 0.4±0.1 | 0.48±0.08 | 0.37±0.09 | 0.52±0.14 | NS | NS | NS |
| AIAC | 0.06±0.02 | 0.02±0.01 | 0.02±0.01 | 0.03±0.01 | 0.02±0.02 | NS | NS | NS |
| TCNE | 1.4±0.4 | 0.8±0.1 | 0.7±0.1 | 0.8±0.1 | 0.9±0.2 | NS | NS | NS |
| Acyl/free | 1.3±0.3 | 1.6±0.4 | 1.4±0.6 | 1.0±0.4 | 1.2±0.4 | NS | NS | NS |
| Muscle (μmol/mg non collagen Protein) | | | | | | | | |
| NEC | 0.09±0.01$^{c}$ | 0.10±0.03$^{bc}$ | 0.13±0.03$^{b}$ | 0.07±0.01$^{c}$ | 0.14±0.03$^{a}$ | NS | 0.0014 | NS |
| ASAC | 0.06±0.02$^{c}$ | 0.16±0.04$^{b}$ | 0.14±0.04$^{bc}$ | 0.18±0.12$^{b}$ | 0.26±0.04$^{a}$ | 0.0248 | NS | NS |
| TCNE | 0.2±0.1 | 0.3±0.0 | 0.3±0.1 | 0.3±0.2 | 0.4±0.1 | NS | NS | NS |
| Acyl/free | 1.2±0.6 | 1.2±0.4 | 1.2±0.2 | 1.1±0.4 | 1.9±0.5 | 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. The degree of significance resulting from 2-way ANOVA are shown with the effects of gesnistein (G), exercise (Ex), and the interaction of genistein and exercise (G×Ex) being expressed as a numerical value or as not significant (NS) when $p>0.05$. ND, normal diet; HD, high fat diet; G, genistein; NEx, non-exercised; Ex, exercised; NEC, non-esterified carnitine; ASAC, acid soluble acyl carnitine; AIAC, acid insoluble acyl carnitine; TCNE. total carnitine; Acyl/free, ASAC+AIAC/NEC.

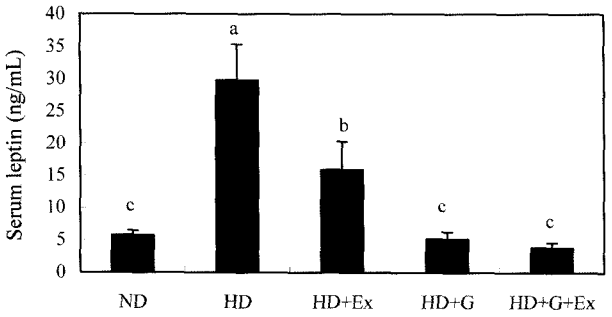


**Fig. 1. Leptin concentrations of serum in C57BL/6J mice.** Mean±SD of 10 mice per group. Values with different superscript letters within the same row are significantly different at $p<0.05$.

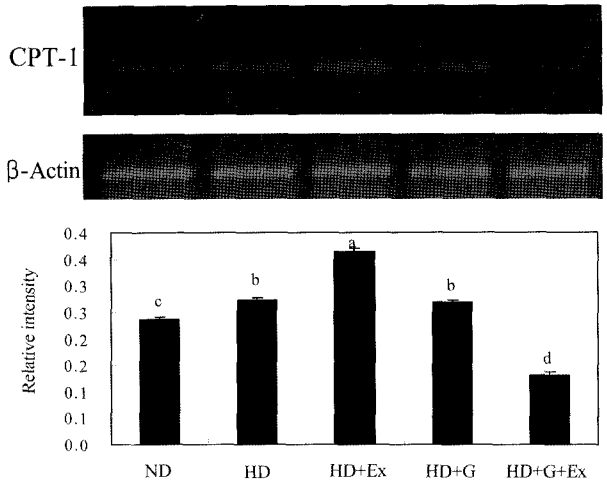


**Fig. 2. Hepatic CPT-1 mRNA levels in C57BL/6J mice.** Values with different superscript letters within the same row are significantly different at $p<0.05$.

higher in the high fat diet group than in the normal diet group. Moreover, serum leptin concentrations were lower in the genistein-supplemented groups, and leptin was lowest when exercise was combined with genistein. This is similar to the results of Miyatake *et al.* (37), in which endurance exercise in overweight males improved insulin resistance and body composition as the intensity of training increased, thus the leptin levels become significantly lower. According to Ozclik *et al.* (38), 12 weeks of continuous training by obese females notably decreased not only leptin levels, but also fat mass and leptin per kg per levels. This weight reduction was enhanced by combining exercise with Orlistat, a compound used as a remedy for obesity. Based on these results, exercise can be more efficient at changing body composition when combined with genistein, which greatly reduces leptin with continuous training.

In contrast, Phipps *et al.* (39) reported that high doses of isoflavone do not influence the blood concentration of leptin in premenopausal and postmenopausal women. However, the highest isoflavone consumption levels used were markedly lower than those used in this study, and presumably too low to change blood leptin levels.

**CPT-1 mRNA levels in liver and muscle** Carnitine palmitoyltransferase 1 (CPT-1) 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 (40). In this study, the hepatic CPT-1 mRNA levels tended to be higher with a high-fat diet, and this tendency was lowered by genistein and exercise in the HD+G+Ex group (Fig. 2). High-fat diets have been shown to increase CPT-1 and CPT-2 formation, both of which participate in β-oxidation (21). Genistein and daidzein are known to up-regulate hepatic CPT-1 enzyme activity by regulating transcription of the CPT-1 enzyme (40). The liver is the key site of adaptive regulation of β-oxidation capacity and this is modulated by exercise training (41) As shown in this experiment, the reason for the increased levels of CPT-1 in HD+Ex mice relative to the other test groups seems to be related to the characteristics of the liver. Although hepatic CPT-1 mRNA expression was higher in exercised animals, exercised animals given genistein had the lowest CPT-1 expression. This is similar to the results of Yang *et al.* (42) in which hepatic CPT-1 mRNA levels significantly decreased when exercise and growth hormone were combined. Therefore, in this study an interaction between genestein and exercise seemed to lower the effectiveness of exercise for increasing CPT-1 expression, which also presumably decreased fatty acid oxidation capacity.

Unlike in liver, however, there were no significant differences in the CPT-1 expression in muscle (Fig. 3). This result is similar to that of Cameron-Smith *et al.* (43) in which there was no specific effect on FABPpm, CPT 1, and UCP-3 formation when both high-fat and high-cholesterol diets were given to athletes. Genistein did not influence the formation of muscle CPT-1 and a reciprocal effect of exercise and genistein was not observed. Therefore, it appears that exercise combined with high-fat diet and genistein intake over a short period does not influence the formation or levels of CPT-1.

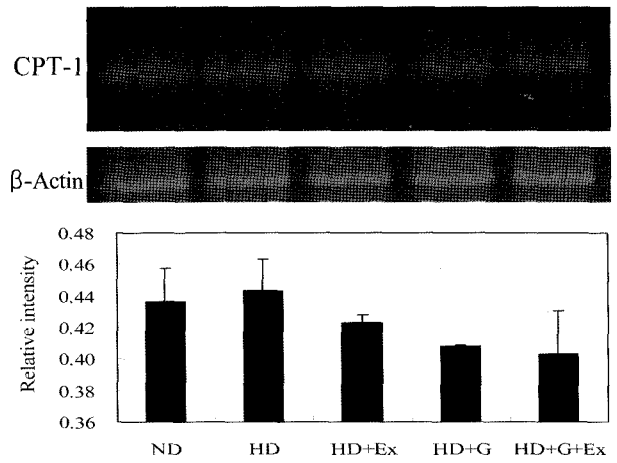


**Fig. 3. Muscle CPT-1 mRNA levels in C57BL/6J mice.** Values with different superscript letters within the same row are significantly different at $p<0.05$.

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