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Effect of FC-GT Supplement on Body Fat and Lipid Metabolism in Rats

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The objective of this study was to investigate the effects of supplementation of an antiobese functional formula (FC-GT) on body weight and lipid metabolism in rats fed a high-fat diet. Three groups of male Sprague-Dawley rats were fed different diets for 6 weeks: normal control (NC), high-fat (HF), and high-fat supplemented with powdered antiobese functional formula (FC-GT) (5%, wt/wt) groups. Although body weight was not significantly different among the groups, relative weights of epididymal and perirenal white adipose tissues were significantly lower in the FC-GT group than in the HF group. FC-GT supplementation significantly lowered the plasma total cholesterol and triglyceride concentrations, whereas it elevated the ratio of HDL-C/total-C and improved the atherogenic index. Hepatic cholesterol and triglyceride concentrations were significantly lowered in the FC-GT group compared to the HF group. The accumulation of hepatic lipid droplets and the epididymal white adipocyte size of the FC-GT group were diminished compared to the HF group. Hepatic HMG-CoA reductase activity was significantly lower in the FC-GT group than in the HF group. Plasma GPT activity was significantly lowered in the FC-GT group compared to the HF group. Additionally, fecal weight was significantly increased in the FC-GT group than in the HF group. In addition, contents of fecal triglyceride and cholesterol were significantly higher in the FC-GT group compared to the other groups. The antioxidant activities of hepatic SOD, CAT, and GR were significantly increased in the FC-GT group compared to the HF group. Hepatic TBARS and plasma TBARS levels were significantly lowered in the FC-GT group compared to the NC group. Accordingly, we conclude that supplementation of FC-GT improves plasma and hepatic lipid levels in high-fat fed rats.

Key words: Antiobese functional formula (FC-GT), Body weight, Lipid metabolism, Adipose tissue, Atherogenic index

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INTRODUCTION

The prevalence of obesity has increased dramatically worldwide in recent years. Modern lifestyles, with abundant nutrient supply and reduced physical activity, has resulted in dramatic increases in obesity-associated diseases, metabolic syndromes including obesity, dislipidemia, diabetes, and cardiovascular disease.[1)] Dietary fat is one of the most important environmental factors associated with the obesity that results in the accumulation of excess body fat.[2)] White adipose tissue (WAT) is a major site of energy storage and is important for energy homeostasis; it stores energy in the form of triglycerides during calorie abundance and releases it as FFAs during deprivation.[3)] Although WAT provides a survival advantage in times of starvation, excess WAT is now linked to obesity-related health problems in the current environment.

Epidemiological data, in conjunction with *in vitro* studies, strongly indicate that foods containing phytochemicals with antioxidant potential have strong protective effects against major disease risks including cancer, diabetes and cardiovascular diseases.[4)] Consumption of fruits, vegetables, and various teas has been strongly linked to reduced risk of those diseases.[5)] Plant polyphenols exert cardiovascular benefits by altering concentrations of blood lipid components. A high intake of

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polyphenols (flavonoids) can significantly reduce the risk of mortality from cardiovascular diseases.[6] Therefore, using the functional foods used with natural plants is an important part of the prevention and improvement of chronic diseases.

In Asia, green tea is a widely consumed beverage that for centuries has been regarded as possessing significant health promoting effects. The health-promoting effects of green tea are mainly attributed to its polyphenol content. Green tea is a rich source of polyphenols, especially of flavanols and flavonols, which represent approximately 30% of dry weight of the fresh leaf.[7] Many of the aforementioned beneficial effects of green tea are attributable to its most abundant catechin, epigallocatechin gallate (EGCG).[8] In recent years, research has mainly focused on effects of green tea related to the prevention of cancer[9] and cardiovascular disease and on its antioxidative properties.[10] In chicory, inulin is stored as a reserve carbohydrate in the fleshly tap root which constitutes about 70-80% of the chicory root in dry weight. Inulin and oligofructose are functional food ingredients since they affect physiological and biochemical processes in rats and human beings, resulting in better health and reduction in the risk of many diseases.[11] Stevioside is a natural sweetener extracted from leaves of *Stevia rebaudiana* Bertoni. In Brazil, Korea, and Japan, *Stevia* leaves, stevioside, and highly refined extracts are widely used as low-calorie sweeteners.[12] Taurine (2-amino ethane sulphonic acid) has various biological and physiological functions and has been extensively reviewed with focus on involvement in diabetes and its complications. Taurine supplementation has been found to decrease fat storage in abdominal cavity and to affect the cholesterol metabolism in the livers of broiler chicks.[13] Buckwheat leaves are known to contain 3-8% and are potential source for industrial extraction of this compound.[14] The main components of these extracts or plant parts are phenolics such as rutine. Ascorbic acid functions as an ubiquitous antioxidant in both animals and plants by scavenging reactive oxygen species via enzymatic and non-enzymatic reactions. Hydroxycitric acid (HCA) is an active ingredient that is extracted from the rind of the fruit *Garcinia cambogia*, an anative species native to India, and is promoted as a weight loss agent. HCA is an inhibitor of ATP-citrate-lyase, a cytosolic (extramitochondrial) enzyme that catalyses the cleavage of citrate to oxaloacetate and acetyl-CoA.[15] L-carnitine (β -hydroxy- γ -trimethylaminobutyrate; (LC) is an endogenous compound that plays an important physiological role in the transfer of long-chain fatty acids across the inner matrix membrane of mitochondria for their β -oxidation with energy production. In humans, LC homeostasis is maintained by a modest biosynthesis within the body, absorption from dietary sources, and by efficient renal tubular reabsorption from glomerular filtrate.[16]

A high fat intake itself can contribute to the development of obesity and hyperlipidemia in human and rodents by altering cholesterol and triglyceride levels in plasma and tissues. Accordingly, the objective of this study was to investigate the supplementary effects of an antiobese functional formula we prepared, a mixture of plant-origin functional components including green tea extract, chicory fiber, and stevioside, on the regulation of body weight gain and lipid lowering in rats fed a high-fat diet.

MATERIALS AND METHODS

1. Preparation of Powdered Antiobese Functional Formula and Analysis of General Composition

Antiobese functional formula, prepared from Bionutrigen Inc. (Daejeon, Korea), was composed of a mixture of powdered chicory fiber (77.875%, wt/wt), stevioside (0.125%, wt/wt), powdered green tea extract (8.75%, wt/wt), powdered persimmon leaf extract (4.375%, wt/wt), powdered buckwheat leaf extract (4.3750%, wt/wt), taurine (3.75%, wt/wt), ascorbic acid (0.25%, wt/wt), L-carnitine (0.25%, wt/wt), and hydroxycitric acid (HCA, 0.25%. wt/wt).

Chicory, persimmon leaf, and green tea were purchased from a local market in Daejeon, Korea and dried in the shade for a week. Buckwheat leaf was obtained from Gangwon province in Korea. Chicory, green tea, and buckwheat leaf extracts were prepared in boiling water for 4-8 hours at the temperature of 96-100 ℃ with constant stirring. The extracts were strained using a strainer and spray-dried or freeze-dried. These dried powders were passed through 60-100 mesh sieves. Taurine, ascorbic acid, L-carnitine and hydroxycitrate were purchased from Sigma Chemical Co., St. Louis, MO, USA. Stevioside was purchased from Wako Pure Chemical Industries (Osaka, Japan). The crude protein and the crude fat contents in FC-GT powder were determined by the Kjedahl method and Soxhelt extraction method, respectively. Its carbohydrate and the total fiber contents were analyzed by AOAC[17] method and the Prosky-AOAC[18] method, respectively.

2. Animals and Diets

Thirty male Sprague-Dawley rats aged 3 weeks (40~50 g) were purchased from Orients, Inc. (Seoul, Korea). The animals were housed individually in stainless steel cages with a 12:12-h light-dark cycle and at ambient temperature of 24 °C. All rats were fed a pelletized commercial chow diet for 1 week after arrival. They were then randomly divided into 3 groups and fed a normal control diet (NC, n=10) and two high-fat diets (HF, n=10, FC-GT, n=10) for 6 weeks, respectively. The high-fat group consisted of two groups, without (HF) or with 5% (wt/wt) powdered FC-GT. The composition of the experimental diet (Table 1) was based on the AIN-76 semisynthetic diet.[19] The energies of the high-fat diets in the HF and the FC-GT groups were 435.0 kcal/100 g and 433.6 kcal/100 g whereas that of NC group was 385.0 kcal/100 g. The animals were given food and distilled water *ad libitum* during the experimental period. Food consumption and weight gain were measured daily and weekly, respectively.

At the end of the experimental period, the rats were sacrificed following a 14-h fast by removing the food from the cages at 7 P.M. of the previous day and collecting the blood samples at 9 A.M. of the experiment day. Animals were anesthetized with ketamine and blood samples were taken from the inferior vena cava for the determination of plasma lipid profiles. The livers were removed under anesthesia and rinsed with physiological saline. The adipose tissues (epididymal white adipose tissue, perirenal white adipose tissue, interscapular white adipose tissue, and interscapular brown adipose tissue) were immediately weighed. All samples were stored at -70 °C until analyzed. The current study protocol was approved by the Ethics Committee at Kyungpook National University for Animal Studies.

Table 1. Compositions of the experimental diets (%)

Component	NC[1]	HF[2]	FC-GT[3]
Casein	20	20	20
D,L-methionine	0.3	0.3	0.3
Corn starch	15	5	-
Sucrose	50	50	50
Cellulose	5	5	5
Mineral mixture[4]	3.5	3.5	3.5
Vitamin mixture[5]	1	1	1
Choline bitartrate	0.2	0.2	0.2
Corn oil	5	5	5
Lard	-	10	10
Antiobese functional formula[6]	-	-	5
Total (%)	100	100	100
kcal/100g diet	385.0	435.0	433.6
Calorie from fat (%)	11.7	31.0	31.1
Calorie from carbohydrate (%)	67.5	50.6	46.8
Calorie from protein (%)	20.8	18.4	18.5
Dietary fiber(%)	5	5	6.31

[1] Normal control group
[2] High-fat fed group
[3] High-fat with powdered antiobese functional formula (Fatclean-Green Tea, FC-GT) group
[4] AIN-76 mineral mixture contained (in g/kg of mixture): calcium phosphate, dibasic 500.0; sodium chloride, 74.0; potassium citrate, monohydrate, 220.0; potassium sulfate, 52.0; magnesium oxide, 24.0; manganous carbonate, 3.5; ferric citrate, 6.0; zinc carbonate, 1.6; cupric carbonate, 0.3; potassium iodate, 0.01; sodium selenite, 0.01; chromium potassium sulfate, 0.55; sucrose, finely powdered, 118.03
[5] AIN-76A vitamin mixture contained (in g/kg of mixture): thiamine HCl, 0.6; riboflavin, 0.6; pyridoxine HCl, 0.7: niacin, 3.0; d-calcium pantothenate, 1.6; folic acid, 0.2: d-biotin, 0.02; cyanocobalamin (vitamin B_{12}), 1.0; dry vitamin A palmitate (500,000 U/g), 0.8; dry vitamin E acetate (500 U/g), 10.0; vitamin D_3 trituration (400,000 U/g), 0.25; menadione sodium bisulfite complex, 0.15; sucrose, fine powder, 981.08
[6] Antiobese functional formula (Fatclean-Green Tea, FC-GT) ; powdered chicory fiber 77.875%, stevinoside 0.125%, powdered green tea extract 8.75%, powdered persimmon leaf extract 4.375%, powdered buckwheat leaf extract 4.375%, taurine 3.7500%, ascorbic acid 0.25%, L-Carnitine 0.25%, HCA (hydroxycitrate) 0.25%.

3. Plasma, Hepatic and Fecal Lipids

Plasma total cholesterol (Total-C) and high-density lipoprotein (HDL)-cholesterol concentrations were determined using a commercial kit (Sigma) based on the modification of the cholesterol oxidase method of Allain *et al.*.[20] The HDL-fractions were separated using a kit (Sigma) based on the heparin-manganese precipitation procedure.[21] The plasma triglyceride concentrations were measured enzymatically using a kit (Sigma), a modification of the lipase-glycerol phosphate oxidase method.[22] The hepatic cholesterol and triglycerides were extracted using the procedure developed by Folch *et al.*[23] Triton X-100 and a sodium cholate solution were added to 200 μL of the dissolved lipid solution in 1 mL of ethanol to yield final concentrations of 5 g/L and 3 mmol/L, respectively. The cholesterol and triglyceride concentrations were analyzed with the same enzymatic kit as used in the plasma analysis.

4. Hepatic and Adipose Tissue Morphology

Livers and epididymal white adipose tissue were removed and fixed in a buffer solution of 10% formalin. Fixed tissues were processed routinely for paraffin embedding, and 4-μm sections were prepared and dyed with hematoxylin-eosin. Stained areas were viewed using an optical microscope with magnifying power of × 200.

5. 3-Hydroxy-3-methylglutaryl CoA (HMG-CoA) Reductase and Acyl-CoA:Cholesterol Acyltransferase (ACAT) Activities

The microsomes were prepared according to the method developed by Hulcher and Oleson[24] with a slight modification. One gram of liver tissue was homogenized in 4 mL

of an ice-cold buffer (pH 7.0) containing 0.1 mol/L triethanolamine, 0.02 mol/L EDTA, and 2 mmol/L dithiothreitol. The homogenates were centrifuged twice at 10,000 g for 15 min at 4 °C. Then, the supernatants were ultracentrifuged twice at 100,000 g for 60 min at 4 °C. The resulting microsomal pellets were then redissolved in 1 mL of a homogenation buffer for protein determination[25] and finally analyzed for their HMG-CoA reductase and ACAT activities. The HMG-CoA reductase activities were determined as described by Shapiro *et al.*[26] with a slight modification using freshly prepared hepatic microsomes. The incubation mixture (60 μL) containing the microsomes (100~150 μg of protein) and 500 nmol of NADPH (dissolved in a reaction buffer containing 0.1 mol/L triethanolamine and 10 mmol/L EDTA) was preincubated at 37 °C for 5 min. Then, 10 μL of 50 nmol [^{14}C]HMG-CoA (specific activity, 2.1083 GBq/mmol; NEN™ Life Science Products, Boston, MA) was added, and the incubation was continued for 15 min at 37 °C. The reaction was terminated by the addition of 15 μL of 10 mol/L HCl, and the resultant reaction mixture was incubated at 37 °C for an additional 15 min to convert the mevalonate into mevalonolactone. The incubation mixture was centrifuged at 10,000 g for 5 min, and the supernatant was spotted on a Silica Gel 60 F_{254} thin-layer chromatography plate using mevalonolactone as the standard. The plate was developed in benzene-acetone (1:1, vol/vol) and air-dried. Finally, the ratio of fronts (R_f) 0.3-0.6 region was removed by scraping with a clean razor blade, and its ^{14}C radioactivity was determined using a liquid scintillation counter (Tricarb 1600 TR, Packard Instrument, Meriden, CT). The results were expressed as picomoles of mevalonate synthesized per min per mg of protein. The ACAT activities were determined in freshly prepared hepatic microsomes, by the method of Erickson *et al.*[27] as modified by Gillies *et al.*.[28] To prepare the cholesterol substrate, 6 mg of cholesterol and 600 mg of Tyloxapol (Triton WR-1339, Sigma) were each dissolved in 6 mL of acetone, mixed well, and completely dried in N_2 gas. The dried substrate was then redissolved in 20 mL of distilled water to a final concentration of 300 μg of cholesterol/mL. Then, reaction mixtures containing 20 μL of the cholesterol solution (6 μg of cholesterol), 20 μL of a 1 mol/L potassium phosphate buffer (pH 7.4), 10 μL of 0.6 mmol/L bovine serum albumin, 10 μg of the microsomal fraction, and distilled water (up to 180 μL) were preincubated at 37 °C for 30 min. The reaction was then initiated by adding 20 μL of 5.62 nmol [^{14}C]oleoyl-CoA (specific activity, 1.9795 GBq/mmol; NEN Life Science Products) to a final volume of 200 μL; the reaction time was 30 min at 37 °C. The reaction was terminated by the addition of 500 μL of isopropanol-heptane (4:1, vol/vol), 300 μL of heptane, and 200 μL of 0.1 mol/L potassium phosphate (pH 7.4), and the reaction mixture was allowed to stand at room temperature for 2 min. Finally, an aliquot (200 μL) of the supernatant was subjected to scintillation counting. ACAT activities were expressed as picomoles of cholesteryl oleate synthesized per min per mg of protein.

6. Adipocyte LPL Activities

Five hundred milligrams of the epididymal white adipose tissue were homogenized using a glass Teflon homogenizer in 4.5 mL of a 25 mM ammonium-HCl buffer (pH 8.2) containing 5 mM EDTA, 10 mg/mL Triton X-100, 1 mg/mL sodium dodecyl sulfate, 5 IU/mL heparin, 10 μg/mL leupeptin, 1 μg/mL pepstatin A, and 3.5 μg/mL aprotinin. The homogenates were centrifuged at 20,000 × g for 20 min at 4 °C. The LPL activity was then analyzed based on a modification of the method by Nilsson-Ehle and Schotz.[29] A stock emulsion containing [^{3}H]triolein (2.5 × 10^9 dpm), 600 mg of triolein, 36 mg of phosphatidylcholine (egg yolk; Sigma), and 10 mL of glycerol was briefly sonicated. Before assay, 1 volume of the stock emulsion, 1 volume of heat-inactivated fasted rat serum (heated at 56 °C for 30 min), and 4 volumes of a 0.3 M Tris-HCl (pH 8.5) buffer containing 12% (w/v) bovine serum albumin, 0.02% (w/v) heparin, and 0.2 M NaCl were mixed and incubated at 37 °C for 5 min. For the assay, 120 μL of the activated substrate mixture was added to 80 μL of the diluted tissue extract and incubated at 37 °C for 60 min. The fatty acids produced during the incubation was isolated using a modification of the liquid-liquid partition system described by Belfrage and Vaughan.[30] The reaction was stopped by adding 3.25 mL of chloroform: methanol: *n*-heptane (1.25:1.41:1, v/v/v), followed by 1.05 mL of a 0.1 M sodium carbonate buffer (pH 10). After vigorous mixing, the tubes were centrifuged for 15 min at 1500 × g and a 0.4 mL aliquot of the methanol-water upper phase was counted using a Packard Tricarb 1600 TR. The LPL activity was expressed as the number of nanomoles free fatty acids released/hour/grams tissue.

7. Antioxidant Enzyme Activities

The SOD activity was spectrophotometrically measured using a modified version of the method developed by Marklund and Marklund.[31] Briefly, SOD was detected on the basis of its ability to inhibit superoxide-mediated

reduction. One unit was determined as the amount of enzyme that inhibited the oxidation of pyrogallol by 50%. The activity was expressed as unit/mg protein. The CAT activity was measured using Aebi's[32] method with a slight modification, in which the disappearance of hydrogen peroxide was monitored spectrophotometrically at 240 nm for 5 min. A molar extinction coefficient of 0.041 $mM^{-1}cm^{-1}$ was used to determine the CAT activity. The activity was defined as the decrease in H_2O_2 μmol/min/mg protein.

8. Glutathione and Related Enzyme Activities

GSH-Px activity was measured using Paglia and Valentine's[33] method with a slight modification. The reaction mixture contained 1 mM glutathione, 0.2 mM NADPH, and 0.24 units of glutathione reductase in a 0.1 M Tris-HCl (pH 7.2) buffer. Reaction was initiated by adding 0.25 mM H_2O_2 and the absorbance was measured at 340 nm for 5 min. A molar extinction coefficient of 6.22 $mM^{-1}cm^{-1}$ was used to determine the activity, which was expressed as the oxidized NADPH nmol/min/mg protein. The GR activity was determined using the method of Pinto and Bartley[34] by monitoring the oxidation of NADPH at 340 nm. The reaction mixture contained 1 mM EDTA and 1 mM GSSG in a 0.1 M potassium phosphate buffer (pH 7.4). The activity was expressed as the oxidized NADPH nmol/min/mg protein. The G6 PD activity was determined using the method of Pitkanen et al.[35] The reaction mixture contained 55 mM Tris-HCl (pH 7.8), a 3.3 mM $MgCl_2$ buffer, and 6 mM G6P. The activity was expressed as the reduced NADPH nmol/min/mg protein.

9. Plasma and Hepatic Lipid Peroxidation (TBARS assay)

The TBARS (thiobarbituric acid-reactive substances) were monitored according to the procedure previously described.[36] Briefly, 500 μL of plasma was well mixed with 3 mL of 5% trichloroacetic acid and 1 mL of freshly prepared 60 mmol/L thiobarbituric acid (TBA). After incubation at 80 °C for 90 min, the samples were cooled at room temperature, centrifuged at 1,000 × g for 15 min at 4 °C, and the supernatant absorbance was read at 535 nm.

The levels of hepatic lipid peroxide were determined using the method of Ohkawa et al.[37] with a slight modification. Tissue homogenates were prepared based on a ratio of 1 g of wet tissue to 9 mL of a 1.15% KCl solution using a glass or Teflon Potter-Elvehjem homogenizer. A reaction mixture containing a 0.2 mL aliquot of the homogenates, 0.2 mL of 8.1% sodium dodecyl sulfate (SDS), and 0.6 mL of distilled water was allowed to sit at room temperature for 5 min, then mixed with 1.5 mL of 20% acetic acid (pH 3.5) and 1.5 mL of a 0.8% aqueous solution of TBA, and finally heated at 95 °C for 60 min. After cooling with tap water, 1 mL of distilled water and 5.0 mL of a mixture of n-butanol and pyridine (15:1, v/v) were added and the mixture was vigorously vortexed. Then, after centrifugation at 4,000 rpm for 10 min, the absorbance of the upper layer was measured at 535 nm. A malondialdehyde (MDA) solution freshly made by the hydrolysis of 1,1,3,3-tetramethoxypropane (TMP) was used as the standard. The results were expressed as the nmol MDA/mL plasma and nmol MDA/g liver.

10. GOT and GPT Activities

The GOT (aspartate aminotransferase) and GPT (alanine aminotransferase) activities were measured using a commercially available kit (Sigma).

11. Statistical Analysis

All data were presented as the mean ± standard error of the mean. The data were evaluated by one-way analysis of variance using SPSS program, and the differences between the means was assessed using Duncan's multiple-range test. Statistical significance was defined as $p<0.05$.

RESULTS

1. The General Composition and the Total Phenolic Content of Powdered Antiobese Functional Formula (FC-GT)

The general composition of powdered antiobese functional formula (FC-GT) is shown in Table 2. In 100 g of FC-GT, the following components were included: 61.1 ± 0.01 g carbohydrate, 5.6 ± 0.10 g crude protein, 0 g

Table 2. The general composition of powdered Fatclean-Green Tea formula[1)]

Component	Antiobese functional formula (Fatclean-Green Tea)
Energy (kcal/100 g)	372.0 ± 2.2
Carbohydrate (g/100 g)	61.1 ± 0.0
Crude protein (g/100 g)	5.6 ± 0.1
Crude fat (g/100 g)	0
Fiber (g/100 g)	26.2 ± 1.0
Ash (g/100g)	2.8 ± 0.1
Moisture (g/100g)	4.3 ± 0.2

[1)] Mean ± S.E.M (n=3)

Table 3. Effects of supplementation of FC-GT on food intake, body weight gain and food efficiency ratio in rats fed high-fat diet[1)]

Dietary group	NC[2)]	HF[3)]	FC-GT[4)]
Food Intake (g/day)	23.61 ± 0.7	23.72 ± 0.5	23.16 ± 0.5
Initial body weight (g)	100.9 ± 1.5	101.0 ± 1.4	101.0 ± 1.4
Final body weight (g)	403.2 ± 12.9[a]	450.6 ± 6.3[b]	433.3 ± 8.1[b]
Body Weight Gain (g/day)	7.20 ± 0.3[a]	8.32 ± 0.1[b]	7.92 ± 0.2[b]
FER[5)]	0.30 ± 0.01[a]	0.34 ± 0.01[b]	0.34 ± 0.01[b]
Energy Intake (kcal/day)	90.9 ± 2.6[a]	103.1 ± 2.4[b]	100.4 ± 2.1[b]

1) Means ± S.E.M (n=10)
2) Normal control group
3) High-fat fed group
4) High-fat with powdered antiobese functional formula (FC-GT)group
5) Food efficiency ratio = body weight gain / food intake
a,b Means in the same row not sharing a common superscript are significantly different (p<0.05) between groups

Table 4. Effects of FC-GT supplementation on organs and adipose tissues weights in rats fed high-fat diet[1)]

	NC[2)]	HF[3)]	FC-GT[4)]
Organs(mg/g B.W.)			
Liver	36.50 ± 1.3	36.49 ± 1.3	33.90 ± 0.9
Heart	3.29 ± 0.1[a]	3.32 ± 0.1[a]	4.01 ± 0.2[b]
Kidney	6.94 ± 0.7	7.68 ± 0.2	7.97 ± 0.3
Adipose tissue(mg/g B.W.)			
Epididymal WAT[5)]	15.52 ± 1.2[a]	22.73 ± 1.7[b]	17.16 ± 0.6[a]
Perirenal WAT	24.33 ± 2.4[a]	31.21 ± 2.6[b]	21.83 ± 1.6[a]
Interscapular WAT	3.45 ± 0.2[a]	4.83 ± 0.5[b]	4.89 ± 0.1[b]
Interscapular BAT[6)]	1.93 ± 0.1[a]	2.32 ± 0.1[a]	3.08 ± 0.2[b]
Total WAT	43.30 ± 3.6[a]	58.77 ± 3.9[b]	43.88 ± 2.1[a]

1) Means ± S.E.M (n=10)
2) Normal control group
3) High-fat fed group
4) High-fat with powdered antiobese functional formula (FC-GT) fed group
5) WAT; white adipose tissue
6) BAT; brown adipose tissue
a,b Means in the same row not sharing a common superscript are significantly different (p<0.05) between groups

crude fat, 4.3±0.10 g moisture, 2.8±0.10 g ash and 26.2± 1.01 g fiber. Energy content was 372 kcal per 100 g diet.

2. Food Intakes, Weight Gains and Food Efficiency Ratio

Energy density of the two high-fat diets used was higher than that of normal control diet (435.0 kcal/100 g and 433.6 kcal/100 g vs. 385.0 kcal/100 g) as shown in Table 1. Food intake was not significantly different among the groups (Table 3).

Initial and final body weights of three groups were not significantly different between the groups (Table 3). However, the average weight gain, food efficiency ratio (FER), and energy intake were significantly higher in the HF and FC-GT groups than in the NC group.

Table 5. Effects of FC-GT supplementation on plasma and hepatic lipid concentration in rats fed high-fat diet[1)]

	NC[2)]	HF[3)]	FC-GT[4)]
Plasma			
Triglyceride(mgl/dL)	146.35 ± 24.9[ab]	169.64 ± 18.3[b]	94.61 ± 8.0[a]
Total cholesterol(mg/dL)	90.48 ± 4.9[ab]	106.62 ± 9.4[b]	84.17 ± 2.3[a]
HDL-cholesterol(mg/dL)	58.05 ± 4.7	53.39 ± 3.1	56.34 ± 3.0
HDL-cholesterol/Total-cholesterol(%)	60.57 ± 4.0[a]	59.51 ± 3.1[a]	70.71 ± 3.1[b]
AI[5)]	0.56 ± 0.1[a]	1.00 ± 0.1[b]	0.49 ± 0.1[a]
Liver			
Triglyceride(mg/g)	125.31 ± 9.7[b]	162.70 ± 26.3[b]	61.05 ± 9.6[a]
Cholesterol(mg/g)	70.03 ± 8.8[ab]	78.22 ± 5.7[b]	48.12 ± 8.1[a]

1) Means ± S.E.M (n=10)
2) Normal control group
3) High-fat fed group
4) High-fat with powdered antiobese functional formula (FC-GT) fed group
5) Atherogenic index : (Total cholesterol - HDL-cholesterol) / HDL-cholesterol
a,b Means in the same row not sharing a common superscript are significantly different (p<0.05) between groups

3. Organ Weights

Organ weights were expressed as their relative weight per body weight. The relative weights of the liver and the kidney were not significantly different between the groups. However, the heart weight was significantly higher in the FC-GT group than in the HF group (Table 4). Epididymal WAT, perirenal WAT, and total adipose tissue weights were significantly lower in the NC or FC-GT group than in the HF group. Interscapularl WAT and BAT weights were significantly higher in the FC-GT group compared to the NC group. Overall, FC-GT supplementation significantly lowered the weight of white adipose tissues compared to the HF group (Table 4).

4. Plasma and Hepatic Lipids

Concentrations of plasma and hepatic lipids are shown in Table 5. FC-GT supplementation significantly lowered the plasma total cholesterol concentration by 21% and the triglyceride concentration by 44% compared to the HF group, respectively (p<0.05). Plasma total cholesterol and triglyceride concentration of NC group were between those of HF and FC-GT group. Although HDL-cholesterol concentration was not significantly different among the groups, the ratio of HDL-C/Total-C exhibited higher values in the FC-GT group than in the HF group mainly due to the differences in plasma total cholesterol concentrations. For this reason, the atherogenic index was significantly higher in the HF group than in the NC or FC-GT group. Hepatic cholesterol and triglyceride concentrations were also significantly lower in the FC-GT group than in the HF group by 38% and 62%, respectively (p<0.05).

Table 6. Effects of FC-GT supplementation on sizes of epididymal adipocyte in rats fed high-fat diet[1)]

(μm)

	NC[2)]	HF[3)]	FC-GT[4)]
Epididymal Adipocyte	291.30 ± 15.9[b]	461.11 ± 34.0[c]	221.2 ± 9.9[a]

[1)] Means ± S.E.M (n=10)
[2)] Normal control group
[3)] High-fat fed group
[4)] High-fat with powdered antiobese functional formula (FC-GT) fed group
[a,b,c] Means in the same row not sharing a common superscript are significantly different ($p<0.05$) between groups

Table 7. Effects of FC-GT supplementation on activities of hepatic ACAT and HMG-CoA reductase and adipocyte LPL in rats fed high-fat diet[1)]

	NC[2)]	HF[3)]	FC-GT[4)]
ACAT (pmol/min/mg protein)	28.86 ± 1.5	32.58 ± 3.0	24.09 ± 3.1
HMG-CoA reductase (pmol/min/mg protein)	222.58 ± 19.8[ab]	240.30 ± 19.8[b]	163.88 ± 27.8[a]
Lipoprotein lipase (nmol/hour/g)	94.21 ± 4.9	93.45 ± 1.2	100.38 ± 1.2

[1)] Means ± S.E.M (n=10)
[2)] Normal control group
[3)] High-fat fed group
[4)] High-fat with powdered antiobese functional formula (FC-GT) fed group
HMG-CoA: 3-hydroxy-3-methylglutaryl CoA reductase, ACAT: cholesterol acyltransferrase
[a,b] Means in the same row not sharing a common superscript are significantly different ($p<0.05$) between groups

Table 8. Effects of FC-GT supplementation on feces weight and fecal lipid contents in rats fed high-fat diet[1)]

	NC[2)]	HF[3)]	FC-GT[4)]
Fecal weight (g/day)	4.30 ± 0.3[a]	4.24 ± 0.1[a]	6.23 ± 0.4[b]
Fecal triglyceride (mg/day)	72.72 ± 4.7[a]	154.57 ± 17.7[b]	198.83 ± 18.8[b]
Fecal cholesterol (mg/day)	113.36 ± 8.6[a]	118.16 ± 6.7[a]	174.75 ± 13.5[b]

[1)] Means ± S.E.M (n=10)
[2)] Normal control group
[3)] High-fat fed group
[4)] High-fat with powdered antiobese functional formula (FC-GT) fed group
[a,b] Means in the same row not sharing a common superscript are significantly different ($p<0.05$) between groups

5. Morphological Comparisons in Epididymal Adipocytes and Hepatocytes

Fig. 1 shows the histological appearance and size of epididymal adipose tissue. The sizes of adipocytes in the NC and FC-GT groups were smaller than those of the HF group when compared and measured under light microscopy (Table 6). Accumulation of hepatic lipid droplets was appeared the highest in HF group, but they were relatively lower in the NC group and FC-GT groups (Fig. 2) that corresponded to the hepatic triglyceride and cholesterol profile in Table 5.

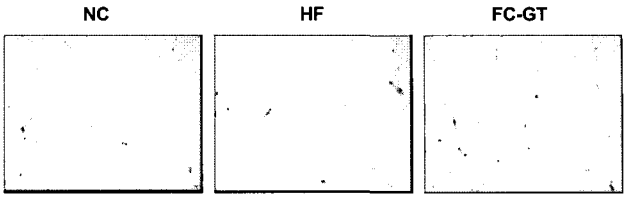

Fig. 1. Light micrography of epididymal adipocytes in rats fed high-fat diet with or without an antiobese functional formula (FC-GT) (×200).

Representative pictures of hematoxylin and eosin- stained sections of epididymal adipocytes from rats fed a normal control diet (NC) or high-fat diet supplemented with antiobese functional formula (FC-GT) show smaller sizes of adipocyte than in rats fed a high-fat diet (HF)

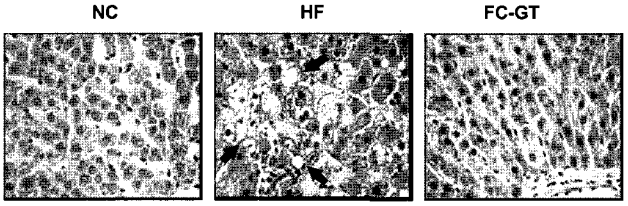

Fig. 2. Effects of FC-GT supplementation on hepatic tissue morphology in rats fed high-fat diet (×400).

Fat accumulation, indicated by the arrowheads, in the form of large fat droplet is present in liver of rats fed a high-fat diet (HF). Representative pictures of hematoxylin and eosin-stained sections of liver tissue from rats fed a normal control diet (NC) or high-fat diet supplemented with antiobese functional formula (FC-GT) show few fat droplets.

6. Activities of Hepatic HMG-CoA Reductase and ACAT and Adipocyte LPL

Hepatic HMG-CoA reductase activity was significantly lower in the FC-GT group than in the HF group (Table 7). However, activities of hepatic ACAT and adipocyte LPL activity were not significantly different between the groups.

7. Fecal Lipid Levels

Dried fecal weight was significantly higher in the FC-GT group than in the HF group. FC-GT supplementation significantly elevated the excretion of fecal cholesterol and triglyceride compared to the HF or NC group (Table 8).

8. Antioxidant Enzyme Activities and Peroxidation (TBARS)

Activities of hepatic antioxidant enzymes and hepatic and plasma TBARS levels are shown in Table 9. The hepatic SOD, CAT, and GR activities were significantly increased in the FC-GT group than in the HF group; however, GSH-Px and G6PD activities were not significantly different between the groups. The levels of

Table 9. Effects of an FC-GT supplementation on hepatic antioxidant enzyme activities and hepatic and plasma TBARS levels in rats fed high-fat diet[1)]

	NC[2)]	HF[3)]	FC-GT[4)]
SOD (units/mg protein)	16.21 ± 2.2[ab]	14.66 ± 1.2[a]	20.97 ± 1.9[b]
CAT (umol/min/mg protein)	0.82 ± 0.1[b]	0.58 ± 0.0[a]	0.93 ± 0.1[b]
GSH-Px (nmol/min/mg protein)	3.00 ± 0.1	2.89 ± 0.2	2.64 ± 0.2
GR (nmol/min/mg protein)	78.90 ± 3.4[b]	65.17 ± 3.2[a]	76.16 ± 4.4[b]
G6PD (nmol/min/mg protein)	73.12 ± 6.1[b]	39.79 ± 4.2[a]	49.78 ± 4.7[a]
Hepatic TBARS (nnmol/g)	18.53 ± 1.1[b]	19.43 ± 1.0[b]	14.39 ± 1.2[a]
Plasma TBARS (nmol/dL)	3.67 ± 0.9[b]	4.34 ± 0.6[b]	2.30 ± 0.36[a]

1) Means ± S.E.M (n=10)
2) Normal control group
3) High-fat fed group
4) High-fat with powdered antiobese functional formula (FC-GT) fed group
SOD: superoxide dismutase, CAT: catalase, GSH-Px: glutathione peroxidase, GR: glutathione reductase, G6PD: glucose-6-phosphate dehydrogenase, TBARS: thiobabituric acid reactive substance.
a,b Means in the same row not sharing a common superscript are significantly different ($p<0.05$) between groups.

Table 10. Effects of FC-GT supplementation on activities of plasma GOT and GPT in rats fed high-fat diet[1)]

(karman/mL)

	NC[2)]	HF[3)]	FC-GT[4)]
GOT	62.64 ± 2.7	67.55 ± 3.6	64.49 ± 5.4
GPT	9.42 ± 0.9[b]	10.56 ± 0.8[b]	6.27 ± 0.9[a]

1) Means ± S.E.M (n=10)
2) Normal control group
3) High-fat fed group
4) High-fat with powdered antiobese functional formula (FC-GT) fed group
a,b Means in the same row not sharing a common superscript are significantly different ($p<0.05$) between groups.

hepatic and plasma TBARS were significantly lowered in the FC-GT group compared to the HF group.

9. Plasma GOT and GPT Activities

Plasma GPT activity was significantly lower in the FC-GT group than in the other two groups; however, there were no differences in GOT activities between the groups (Table 10).

DISCUSSION

Two high-fat diets, HF and FC-GT, were almost isonitrogenous and isocaloric although fiber content were higher in the FC-GT diet (6.31%) than in the HF diet (5%) that is mainly due to high fiber content of FC-GT. The food intake was approximately the same for all groups, although the final body weight, body weight gain, food efficiency ratio and energy intake were significantly higher in the HF and FC-GT groups than in the NC group. As shown in Table 3, the high-fat diet (approximately 31% energy as fat) for 6 weeks significantly increased body weight by 47 g based on the NC group (450.6 ± 6.3 g in the HF group vs. 403.2 ± 12.9 g in the NC group, $p<0.05$) (Figure 1). In general, a high-fat diet itself results in reducing the food intake when provided as about 50% of energy in the diet[38)]; however, in the present study, this was not true when dietary fat provided approximately 31% of the total energy. There was no abnormality in the growth performance; however, the relative weights of the hearts were significantly higher in the FC-GT group compared to the NC and HF groups. Although body weight was not significantly different between the HF and FC-GT groups, the relative weights of epididymal and perirenal white adipose tissues were dramatically lower in the FC-GT group than in the HF group. However, that of interscapular BAT was opposite. Surprisingly, relative weight of total white adipose tissue in the FC-GT group was similar to the NC group. These seemed to have caused a decrease in the epididymal adipocyte size of the FC-GT group.

An elevated plasma total cholesterol concentration or LDL-cholesterol is known to increase the risk of coronary heart disease (CHD).[39)] The clinical complications of atherosclerosis can be diminished when plasma lipids are lowered by hypocholesterolemic agents.[40)] The effect of fiber and phytochemicals or flavonoids on serum and hepatic lipids are very relevant to cardiovascular diseases and some cancers.[41)] In the current study, FC-GT supplement improved plasma lipid profile significantly by lowering plasma total cholesterol concentration and triglyceride concentration compared to the HF group. Regular ingestion of green tea catechins decreased plasma total cholesterol and blood triglyceride levels in human,[42)] and other animal studies showed reduction in plasma triglycerides by regular ingestion of both green and black tea.[43)] The plasma HDL-cholesterol concentration was not different between the groups; however, the ratio of HLD-C/Total-C was increased by about 18.82% compared with the HF or NC group. Our results indicate that the supplementation with FC-GT improves high-fat diet induced atherogenic index. Generally, high-fat diet significantly increases the total cholesterol levels in serum and liver compared to normal control diet in rats. In the present study, FC-GT significantly lowered hepatic cholesterol and triglyceride levels by 38.48% and 62.48% compared to the HF group and diminished the accumulation of hepatic droplets. In

particular, plasma and hepatic lipid levels were the lowest in the FC-GT group among the three groups. The activity of plasma GPT was significantly lower in the FC-GT group than in the HF group. Gorinstein et al.[44] reported that two diets, fortified with 7% whole dry persimmon and 7% phenolic compound-free dry persimmon, given to cholesterol-fed rats improved plasma lipid levels. The action of hydroxycitrate reduces the acetyl-CoA and subsequently the malonyl-CoA pool, limiting the availability of two-carbon groups required for the synthesis of fats and cholesterol. It has been found that mice lacking malonyl-CoA show decreased fat accumulation in adipose tissue and liver.[45] In the leaf of persimmon *diospyros kaki*, flavonoid oligomers, tannins, phenols, organic acids, chlorophyll, vitamin C, and caffeine are present and the leaf is commonly used as a tea in Asia.[46] Tannin or tannin with gallate group have various physiological functions such as antilipidemic actions in rats with hypercholesterolemia.[47] Taurine has various biological and physiological functions including the prevention of some cancers and lowering the cholesterol level in the plasma.[48] In green tea *(Camellia sinensis L)*, fiber, chlorophyll, catechin, kaempferol, quercetin, myricetin, phenols, vitamin C, and caffeine are present. Flavonoid aglycones such as catechin, kaempferol, and quercetin reportedly possess strong antioxidative activities acting as oxygen radical scavengers, metal chelators and lipid peroxidation inhibitors and effective inhibitor on cholesterol and triglyceride concentration in plasma.[49] In addition, a high-fat diet supplemented with FC-GT significantly lowered both the HMG-CoA reductase activities compared to the HF group. Plasma cholesterol lowering action of the FC-GT supplementation is possibly mediated by the inhibition of hepatic HMG-CoA reductase. Similar results were observed in our previous study with high cholesterol fed rats supplemented with a citrus flavonoid that lowered plasma and hepatic cholesterol level.[50]

Inclusion of chicory roots (mainly inulin) in the diet of saturated fat fed rats significantly reduced the high triglyceride content of blood and liver.[51] Kaur et al.[52] reported the cholesterol lowering effect of an inulin rich diet in caffeine-fed rats. Daily fecal weight was significantly higher in the FC-GT group than in the HF group, which seemed to be due to additional fiber content in FC-GT diet. The increase in fecal weight might vary widely with the type and quantity of dietary fiber being consumed. Chau et al.[53] reported that fecal dry weight was significantly higher when hamsters were supplemented with the water-insoluble fiber-rich fraction isolated from the peel of *Citru sinensis* L. cv. Liucheng and cellulose diets, 55~56% increase relative to the fiber-free diet, and suggested that the consumption of insoluble fiber could significantly increase the fecal weight, as well as fecal bulk. We have previously reported that some plant powders, their extracts or their respective phytochemicals exhibited lipid-lowering and body fat-lowering properties in experimental animal models.

In the current study, the activities of antioxidant enzymes in hepatocyte were not consistently affected by the supplementation of FC-GT. However, hepatic SOD, CAT, and GR activities were significantly higher in the FC-GT group than in the HF group, whereas the concentration of plasma and hepatic TBARS was significantly lower in the FC-GT group. Antioxidants are of great interest because they may help to protect the body against damage by reactive oxygen species (ROS). In general, there is convincing epidemiological evidence that the consumption of fruits and vegetables is beneficial to health due to their antioxidant phytonutrients.[54] Among numerous properties, many polyphenolics exhibit antioxidative properties, especially oxygen species scavenging, and reduce atherosclerosis in high-fat diet fed rabbit.[55] The combined effect of high fiber and high phenolic content in persimmon leaf itself enhanced the fecal excretion of neutral and acidic sterols.[38] In general, many effects of dietary fibers, such as decreased transit time, higher bile acid adsorption, and decreased cholesterol absorption, could lead to a greater excretion of fecal sterols, and subsequently to a decrease in the serum cholesterol level.[56] Functional components in FC-GT that resulted to regulate cholesterol and fat metabolism may include a variety of phenolic compounds, fiber, and other possible bioactive compounds. The bioavailability of phytochemicals can be influenced by intrinsic factors in food and/or in human, in general, the substances are little adsorbed, largely metabolized and rapidly eliminated.[57]

In conclusion, the supplementation with FC-GT that is rich in fiber and phenolic compounds or phytochemicals remarkably lowered plasma and hepatic lipid concentrations as well as cholesterol regulating enzyme activities and TBARS levels in rats fed a high-fat diet. FC-GT would be a beneficial functional formula for lowering body fat and for the regulation of lipid metabolism in this animal model.

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