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# *Garcinia cambogia* Extract Ameliorates Visceral Adiposity in C57BL/6J Mice Fed on a High-Fat Diet

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**The aim of present study is to evaluate the effects of *Garcinia cambogia* on the mRNA levels of the various genes involved in adipogenesis, as well as on body weight gain, visceral fat accumulation, and other biochemical markers of obesity in obesity-prone C57BL/6J mice. Consumption of the *Garcinia cambogia* extract effectively lowered the body weight gain, visceral fat accumulation, blood and hepatic lipid concentrations, and plasma insulin and leptin levels in a high-fat diet (HFD)-induced obesity mouse model. The *Garcinia cambogia* extract reversed the HFD-induced changes in the expression pattern of such epididymal adipose tissue genes as adipocyte protein aP2 (aP2), sterol regulatory element-binding factor 1c (SREBP1c), peroxisome proliferator-activated receptor $\gamma$2 (PPAR$\gamma$2), and CCAT/enhancer-binding protein $\alpha$ (C/EBP$\alpha$). These findings suggest that the *Garcinia cambogia* extract ameliorated HFD-induced obesity, probably by modulating multiple genes associated with adipogenesis, such as aP2, SREBP1c, PPAR$\gamma$2, and C/EBP$\alpha$ in the visceral fat tissue of mice.**

**Key words:** adipogenesis; *Garcinia cambogia*; gene expression; high-fat diet; mice

Obesity, particularly that caused by visceral fat accumulation, is a serious risk factor for so-called lifestyle-related diseases such as diabetes, coronary heart diseases, hyperlipidemia, hypertension, and cancer.[1] Insulin resistance is considered the most common underlying abnormality in human obesity and is influenced by genetic and environmental factors, and particularly by changes in diet and physical activity.[2] High-fat-fed rodents appear to be the best model for the visceral obesity syndrome[3] because of the similar pathogenesis of obesity to that found in humans.[4] The recent and rapid increase in obesity in developed countries points to the important interaction between the series of genes that predispose to obesity and an environment that facilitates the expression of the obese phenotype, another trait shared with high-fat-diet (HFD)-induced rodent obesity models.[5] Preventive or therapeutic strategies to control most of human obesity should target these abnormalities.[6]

Due to the adverse side effects associated with many of the anti-obesity drugs, more recent drug trials have focused on screening the herbal medicines that have been reported to treat obesity and that generally have minimal side effects.[7] Anti-obesity foods and food ingredients may avert the condition, possibly leading to the prevention of lifestyle-related diseases, if they are effective in reducing the visceral fat mass.[8] Traditional herbal medicines such as a *Panax ginseng* berry extract,[9] *Zingiber officinale Roscoe*,[10] and *Platycodi radix*[11] have been reported to be effective in managing obesity in HFD-fed mice and rats, while such natural products as *Ephedra sinica*, *Taraxacum officinale* (dandelion), and *Rhamnus purshiana* (cascara) have been specifically studied for weight loss in humans.[12]

*Garcinia cambogia*, an edible fruit native to Southeastern Asia, exhibits a distinctive sweet and sour taste, and has been used for centuries in Asian countries for preparing culinary dishes.[13,14] A *Garcinia cambogia* extract has been available as an anti-obesity herbal supplement around the world for decades, although some mild adverse events such as headache, and upper respiratory tract and gastrointestinal symptoms have been reported in overweight subjects.[15] Hydroxy citric acid (HCA), the principal acid in the fruits of *Garcinia cambogia*, is a competitive inhibitor of ATP-citrate lyase (EC 4.1.3.8), the enzyme responsible for fatty acid, cholesterol, and triglyceride biosynthesis.[16,17] The inhibitory action of HCA reduces the acetyl-CoA pool, thus limiting the availability of the two carbon units required for the initial steps of fatty acid and cholesterol biosynthesis.[13,16] Although the weight loss effects of *Garcinia cambogia* have been documented,[14,15,18] a comprehensive study examining its ability to combat

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*Abbreviations*: aP2, adipocyte P2; C/EBP$\alpha$, CCAAT/enhancer-binding protein $\alpha$; GLUT4, glucose transporter 4; HCA, hydroxyl citric acid; HFD, high-fat diet; HFD+G, *Garcinia cambogia* extract-supplemented diet; ND, normal diet; PPAR$\gamma$2, peroxisome proliferators-activated receptor $\gamma$2; SREBP1c, sterol regulatory element-binding protein 1c; TNF$\alpha$, tumor necrosis factor $\alpha$

visceral adiposity in an HFD-induced obesity model is lacking. Especially, the interaction between obesity-related genes and consumption of the *Garcinia cambogia* extract needs to be confirmed in animal models of diet-induced obesity.

The present work was therefore undertaken to study the effects of *Garcinia cambogia* on the expression of multiple genes associated with adipogenesis as well as other visceral obesity-related biomarkers in an HFD-induced obesity mouse model. The focus of this study is on the molecular effects of *Garcinia cambogia* on the mRNA expression of adipocyte protein aP2 (aP2), sterol regulatory element-binding factor 1c (SREBP1c), peroxisome proliferator-activated receptor $\gamma 2$ (PPAR$\gamma 2$), and CCAT/enhancer-binding protein $\alpha$ (C/EBP$\alpha$) in the epididymal adipose tissue of a diet-induced obesity mouse model.

## Materials and Methods

*Animals and diets.* Thirty-six 7-wk-old male C57BL/6J mice (Jung-Ang Lab Animal, Seoul, Korea) were individually housed in standard cages and placed in a room where the temperature was kept at $21 \pm 2.0\,^{\circ}C$, the relative humidity at $50 \pm 5\%$, and the light on a 12-h light/dark cycle. All the mice consumed a commercial diet and tap water *ad libitum* for 1 wk prior to their division into three weight-matched groups: the normal diet (ND) group, HFD group, and *Garcinia cambogia* extract-supplemented diet (HFD+G) group. ND was a purified diet based on the AIN-76 rodent diet composition. HFD was identical to ND except that 200 g of fat/kg (170 g of lard plus 30 g of corn oil) and 1% (w/w) cholesterol were added. HFD was formulated to provide 39% of the total energy generated by the diet from fat by replacing carbohydrate energy with lard and corn oil, and had the same amount of vitamins and minerals per kilojoule as ND did. HFD+G was identical to HFD and contained 1a % *Garcinia cambogia* extract composed of the natural and highly water-soluble potassium and calcium salt of 60% hydroxycitric acid (Super Citrimax®, HCA-600-SXS, Lot no. 0503006 supplied by InterHealth Nutraceuticals, Benicia, CA, USA.) (Table 1). The diets were given in the form of pellets for 12 wk.

The mice's food intake was recorded daily, and their body weights were monitored every three days, from 10:00 a.m. to 11:00 a.m., during the feeding period. At the end of the experimental period, the animals were anesthetized with ether, following a 12-h period of fasting. Blood was drawn from the inferior vena cava into a heparin-coated tube, and the plasma was obtained by centrifuging the blood at $1{,}000 \times g$ for 15 min at $4\,^{\circ}C$. The livers and visceral fat pads were removed, rinsed with phosphate-buffered saline, and then weighed. The plasma, liver and visceral fat pad samples were stored at $-70\,^{\circ}C$ until they were analyzed. This study adhered to the *Guide for the Care and Use of Laboratory Animals* developed by the Institute of Laboratory Animal Resources of the National Research Council, and approved by the Institutional Animal Care and Use Committee of Yonsei University in Seoul, South Korea.

**Table 1.** Composition of the Experimental Diets

| Ingredient | ND | HFD | HFD+G |
|---|---|---|---|
| | | (g/kg diet) | |
| Casein | 200 | 200 | 200 |
| DL-Methionine | 3 | 3 | 3 |
| Corn starch | 150 | 111 | 101 |
| Sucrose | 500 | 370 | 370 |
| Cellulose | 50 | 50 | 50 |
| Corn oil | 50 | 30 | 30 |
| Lard | — | 170 | 170 |
| Vitamin mix[1)] | 10 | 12 | 12 |
| Mineral mix[2)] | 35 | 42 | 42 |
| Choline bitartrate | 2 | 2 | 2 |
| Cholesterol | — | 10 | 10 |
| tert-Butylhydroquinone[3)] | 0.01 | 0.04 | 0.04 |
| *Garcinia cambogia* extract[4)] | — | — | 10 |
| Total (g) | 1,000 | 1,000 | 1,000 |
| Fat (% Calorie) | 11.5 | 39.0 | 39.0 |
| Total energy, kJ/kg of diet | 16,439 | 19,315 | 19,315 |

[1)]AIN-76 vitamin mixture (g/kg of mix): thiamin·HCl, 0.6; riboflavin, 0.6; nicotinamide, 25; pyridoxine·HCl, 0.7; nicotinic acid, 3; D-calcium pantothenate, 1.6; folic acid, 0.2; D-biotin, 0.02; cyanocobalamin (vitamin $B_{12}$), 0.001; retinyl palmitate (250,000 IU/gm), 1.6; DL-$\alpha$-tocopherol acetate (250 IU/gm), 20; cholecalciferol (Vitamin $D_3$), 0.25; menaquinone (Vitamin $K_2$), 0.05; finely powdered sucrose, 972.9.
[2)]AIN-76 mineral mixture (g/kg of mix): $CaHPO_4$, 500; NaCl, 74; $K_2H_6O_7H_2O$, 220; $K_2SO_4$, 52; MgO, 24; $MnCO_3$, 3.57; Fe $(C_6H_5O_7)\cdot 6H_2O$, 6; $ZnCO_3$, 1.6; $CuCO_3$, 0.3; $KIO_3$, 0.01; $Na_2SeO_3\cdot 5H_2O$, 0.01; CrK $(SO_4)_2$, 0.55; finely powdered sucrose, 118.
[3)]Antioxidative agent, 0.01 g/ 50 g of lipids.
[4)]*Garcinia cambogia* extract contained 60% hydroxyl citric acid.

*Histology and mean adipocyte surface area.* Tissue samples of the epididymal fat pads were fixed with 4% buffered formalin and embedded in paraffin. Standard sections of 5 μm were cut and stained with hematoxylin and eosin, viewed with an optical microscope (Vanox-S, Olympus Optical, Tokyo, Japan), and photographed at a final magnification of 200×. The average size of adipocytes was measured by using image analysis software (Image-Pro Plus 4.5, Media Cybernetics, Silver Spring, MD, USA).

*Biochemical analysis.* The plasma concentrations of total cholesterol and triglyceride were determined enzymatically by using commercial kits (BioClinical System, Gyeonggi-do, Korea). Hepatic lipids were extracted as described,[19)] and the dried lipid residues were dissolved in 1 ml of ethanol. The concentrations of cholesterol and triglyceride in the hepatic lipid extracts were determined by using an automatic analyzer (Express Plus, Chiron Diagnostics, East Walpole, MA, USA) with reagents (Bayer, Tarrytown, NY, USA). The plasma insulin, leptin, and adiponectin levels were measured by a radioimmunoassay (Linco Research,

**Table 2.** Primer Sequences and PCR Conditions

| Accession No. | Gene description | Primer | Sequence (5′→3′) | PCR product (bp) | Annealing temperature (°C) |
|---|---|---|---|---|---|
| NM024406 | Adipocyte protein aP2 (aP2) | F<br>R | CACCATCCGGTCAGAGAGTA<br>TGATGCTCTTCACCTTCCTG | 104 | 60 |
| NM011480 | Sterol regulatory element-binding factor 1 (SREBP1c) | F<br>R | TTGTGGAGCTCAAAGACCTG<br>TGCAAGAAGCGGATGTAGTC | 94 | 60 |
| NM011146 | Peroxisome proliferator-activated receptor gamma (PPAR$\gamma$2) | F<br>R | ACCACAGTTGATTTCTCCAG<br>TGTTGTAGAGCTGGGTCTTT | 178 | 60 |
| NM007678 | CCAAT/enhancer-binding protein, alpha (C/EBP$\alpha$) | F<br>R | GAGGGACTGGAGTTATGACA<br>GTGAAGAGTCTCAGTTTGGC | 194 | 60 |
| NM013693 | Tumor necrosis factor alpha (TNF$\alpha$) | F<br>R | TGTCTCAGCCTCTTCTCATT<br>AGATGATCTGAGTGTGAGGG | 156 | 60 |

Charles, MO, USA). The plasma glucose concentration was measured enzymatically by using a commercial kit (BioClinical System).

*Real-time RT-PCR analysis.* Total RNA was isolated from the epididymal fat tissues of each mouse by using Trizol (Invitrogen, Carlsbad, CA, USA) and was reverse-transcribed using the Superscript II kit (Invitrogen) according to the manufacturer's recommendations. The GenBank accession numbers of the relevant templates and the forward (F) and reverse (R) primer sequences are shown in Table 2. Primers were also designed to amplify the 530-bp cDNA fragment encoding $\beta$-actin (sense: 5′-ACCTTCAACACCCCAGCCATGTACG-3′; anti-sense: 5′-CTGATCCACATCTGCTGGAAGGTGG-3′) as an internal control. Real-time PCR reactions were then carried out in a 20-μl reaction mixture (2 μl of cDNA, 16 μl of SYBR green PCR master mix, which included 2 μl of 1×LightCycler, 2.4 μl of 1.5 mmol/l $MgCl_2$ and 11.6 μl of $H_2O$, and 1 μl of 0.5 μmol/l of a specific gene primer pair) in a LightCycler instrument (Roche Diagnostics, Indianapolis, IN, USA).

The PCR program was initiated by 10 min at 95 °C, before 40 thermal cycles, each of 10 s at 95 °C, 5 s at 55 °C, and 30 s at 70 °C. The data obtained were analyzed by using the comparative cycle threshold method, and were normalized by the $\beta$-actin expression value. Melting curves for each PCR reaction were generated to ensure the purity of the amplification product.

*Statistical analysis.* All the results obtained are expressed as the mean ± SEM of 12 mice in each group. Statistical evaluation was done by using one-way ANOVA and followed by Duncan's multiple-range test. The level of significance was set at $p < 0.05$ for all statistical tests.

## Results

*Body weight and visceral fat-pad weights*

The body weights in the ND, HFD, and HFD+G groups began to diverge from d 24 and stayed different for the remainder of the experimental period ($p < 0.05$) (Fig. 1). The final body weight and 12-wk body weight gain of mice in the HFD group were 43% and 202% greater, respectively, than the equivalent values for the ND mice. Dietary supplementation with the *Garcinia cambogia* extract significantly reduced the final body weight (22% lower) and body weight gain (46% lower) of the mice consuming HFD. The food intake and food efficiency ratio (FER) of mice in the HFD+G group were significantly lower than the values for the HFD group ($p < 0.05$) (Table 3).

The relative weights of the total visceral fat depots were significantly higher by feeding HFD than the value for the ND mice (137% greater, $p < 0.05$) and were significantly lower by supplementing HFD with the *Garcinia cambogia* extract (39% lower, $p < 0.05$). The epididymal, perirenal, retroperitoneal, and mesenteric fat-pad weights were reduced by 37%, 48%, 36%, and 42%, respectively, in the mice fed HFD+G than in the HFD mice ($p < 0.05$) (Fig. 2). The adipocytes from the epididymal fat-pad of the mice fed HFD+G were 34% smaller than those of the animals fed HFD (Fig. 3).

*Plasma and hepatic biochemistry*

The HFD-induced hypercholesterolemia was significantly improved by dietary supplementation with the *Garcinia cambogia* extract. The plasma total cholesterol and triglyceride concentrations were 24% and 25%

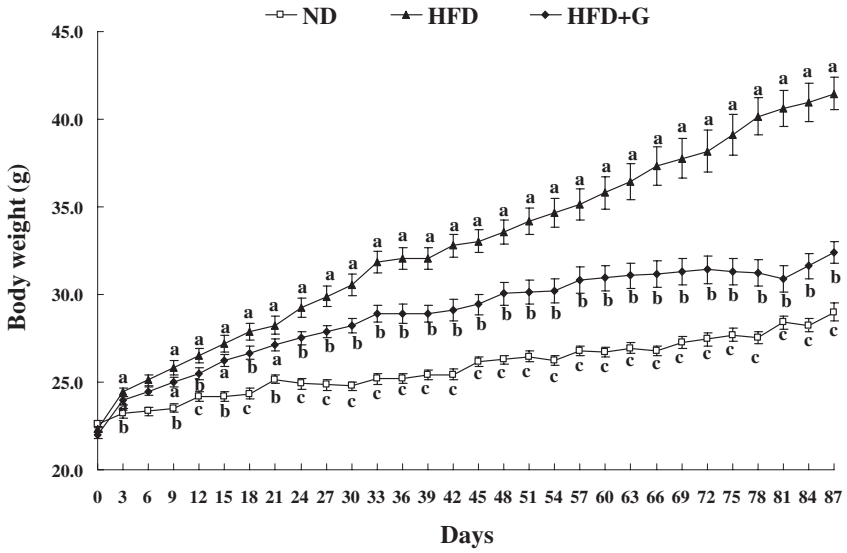


**Fig. 1.** Body Weight of Mice Fed on the Experimental Diets for 12 Weeks.
Each value is the mean ± SEM (n = 12). [a,b,c]Means not sharing a common superscript are significantly different ($p < 0.05$).

**Table 3.** Body Weight Gain, and Plasma and Hepatic Biochemisty of Mice Fed on the Experimental Diets for 12 Weeks

| Group | ND | HFD | HFD+G |
|---|---|---|---|
| Initial body weight (g) | $22.6 \pm 0.21$ | $22.3 \pm 0.17$ | $22.0 \pm 0.20$ |
| Final body weight (g) | $29.0 \pm 0.51^{c}$ | $41.5 \pm 0.91^{a}$ | $32.4 \pm 0.61^{b}$ |
| Body weight gain (g/12 weeks) | $6.35 \pm 0.50^{c}$ | $19.2 \pm 1.55^{a}$ | $10.4 \pm 0.77^{b}$ |
| Food intake (g/d) | $2.29 \pm 0.01^{c}$ | $2.58 \pm 0.01^{a}$ | $2.37 \pm 0.03^{b}$ |
| FER[1)] | $0.03 \pm 0.002^{c}$ | $0.09 \pm 0.004^{a}$ | $0.05 \pm 0.003^{b}$ |
| ***Plasma*** | | | |
| Total cholesterol (mmol/l) | $2.83 \pm 0.11^{c}$ | $4.96 \pm 0.17^{a}$ | $3.76 \pm 0.18^{b}$ |
| Triglyceride (mmol/l) | $0.50 \pm 0.01^{a}$ | $0.56 \pm 0.02^{a}$ | $0.42 \pm 0.03^{b}$ |
| Glucose (mmol/l) | $9.94 \pm 0.62$ | $11.8 \pm 0.53$ | $10.4 \pm 0.65$ |
| Insulin (pmol/l) | $35.3 \pm 6.4^{b}$ | $68.3 \pm 10.2^{a}$ | $39.0 \pm 7.1^{b}$ |
| Leptin (ng/ml) | $6.58 \pm 0.86^{b}$ | $22.2 \pm 1.15^{a}$ | $10.7 \pm 0.59^{b}$ |
| Adiponectin (μg/ml) | $7.15 \pm 0.78$ | $6.54 \pm 0.70$ | $6.50 \pm 1.41$ |
| ***Liver*** | | | |
| Liver weight (mg/g of body weight) | $34.5 \pm 0.8^{b}$ | $61.9 \pm 2.5^{a}$ | $50.7 \pm 2.3^{b}$ |
| Triglyceride (μmol/g of liver) | $28.6 \pm 3.30^{c}$ | $43.5 \pm 2.49^{a}$ | $37.1 \pm 1.51^{b}$ |
| Cholesterol (μmol/g of liver) | $10.8 \pm 0.64^{c}$ | $23.9 \pm 1.06^{a}$ | $20.5 \pm 1.10^{b}$ |

Each value is the mean ± SEM (n = 12).
[a,b,c]Means in a row with superscripts without a common letter differ, $p < 0.05$.
[1)]Food efficiency ratio (FER) = $\frac{\text{Body weight gain during experimental period (g/d)}}{\text{Food intake during experimental period (g/d)}}$

lower, respectively, in the mice fed with HFD+G than those for the HFD-fed mice ($p < 0.05$). The enlargement of the liver and the accumulations of triglyceride and cholesterol in the liver of the mice fed HFD were ameliorated by dietary supplementation with the *Garcinia cambogia* extract (a 18% reduction in the liver weight, $p < 0.05$; 14–15% reductions in the hepatic triglyceride and cholesterol levels, $p < 0.05$). The elevated levels of plasma insulin and leptin which had been caused by feeding HFD were reduced by 43% and 52%, respectively, by dietary supplementation with the *Garcinia cambogia* extract ($p < 0.05$). The plasma glucose and adiponectin levels were not affected by the experimental diets (Table 3).

*Expression levels of adipogenesis-related genes*

Results from the real-time RT-PCR analyses of total RNA prepared from the epididymal adipose tissue of mice indicate that HFD significantly down-regulated the expression of the SREBP1c, C/EBP$\alpha$, and PPAR$\gamma$2 genes, whereas it significantly up-regulated the TNF$\alpha$ gene. The aP2 gene expression tended to be decreased in the mice fed with HFD than in the ND animals, but without showing statistical significance. These HFD-induced changes in the expression pattern of the edipidymal adipose tissue genes implicated in adipogenesis were reversed by feeding the *Garcinia cambogia* extract. Significant up-regulation of the aP2 (120% increase, $p < 0.05$), SREBP1c (12% increase, $p < 0.05$),

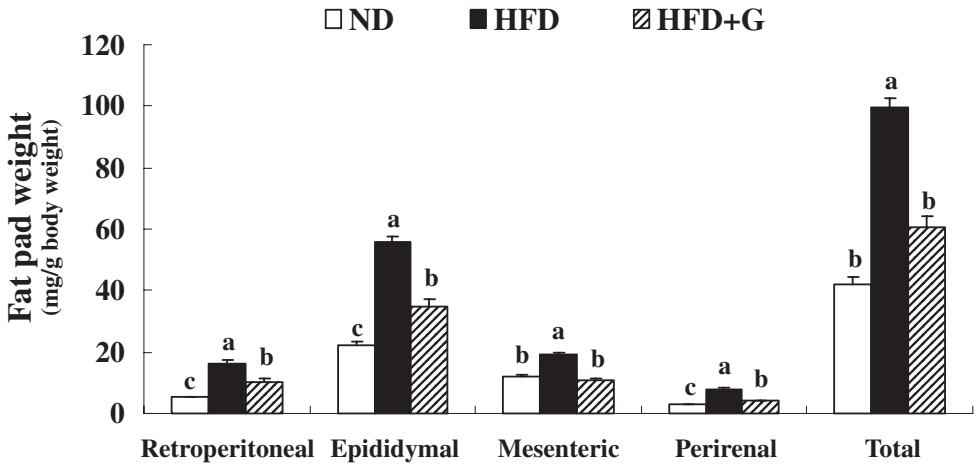


**Fig. 2.** Visceral Fat Pad Weights of Mice Fed on the Experimental Diets for 12 Weeks.
Each value is the mean ± SEM (n = 12). [a,b,c]Means not sharing a common superscript are significantly different ($p < 0.05$).

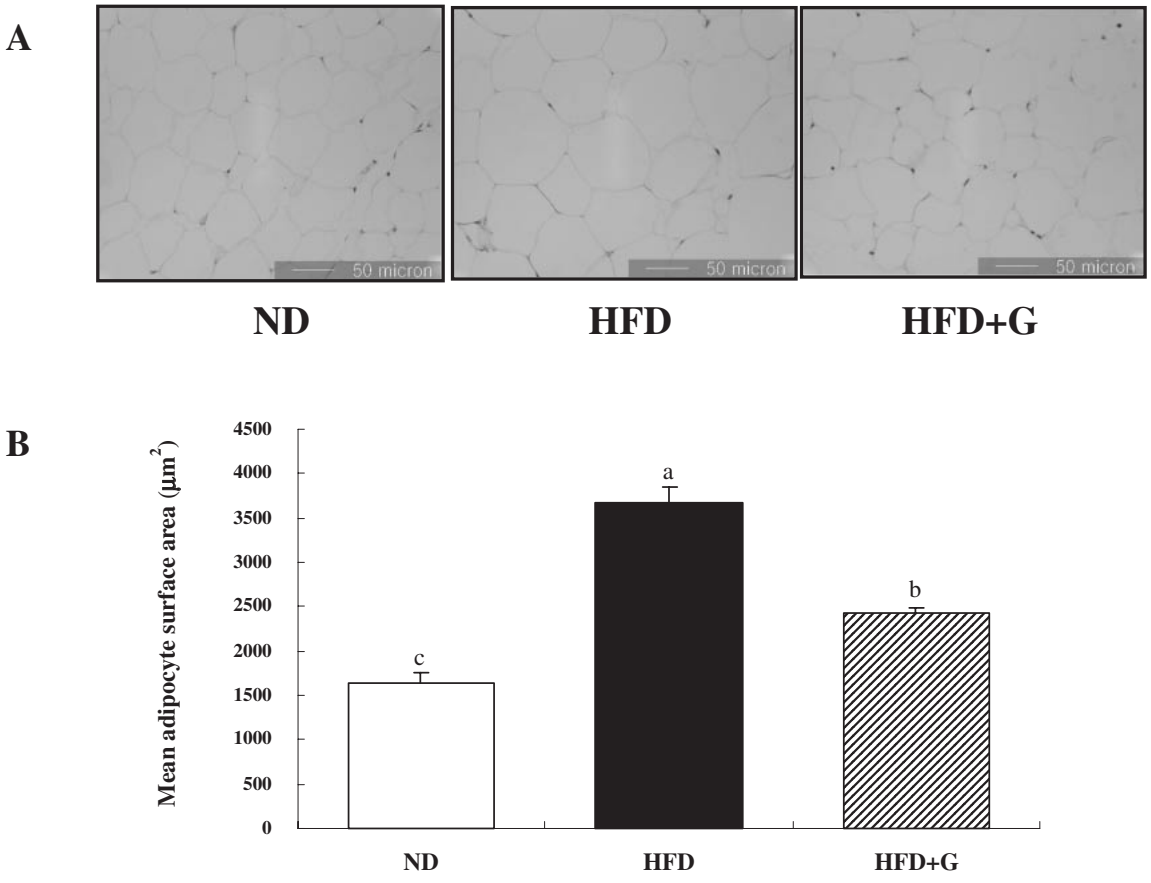


**Fig. 3.** Histology of Epididymal Adipose Tissue and Mean Adipocyte Surface Area of Mice Fed on the Experimental Diets for 12 Weeks.
A, Representative photomicrographs of the epididymal adipose tissue. All sections were stained with hematoxylin and eosin; magnification, ×200. Magnification bar = 50 μm. B, Epididymal mean adipocyte surface area. Each value is the mean ± SEM (n = 12). [a,b,c]Means not sharing a common superscript are significantly different ($p < 0.05$).

C/EBPα (174% increase, $p < 0.05$), and PPARγ2 (70% increase, $p < 0.05$) genes, in conjunction with significant down-regulation of the TNFα gene (20% reduction, $p < 0.05$) were observed in the epididymal adipose tissue of mice fed with HFD+G compared to those observed in the HFD mice ($p < 0.05$) (Fig. 4).

## Discussion

HCA, an active ingredient of the *Garcinia cambogia* extract, reduces food consumption in humans and in rodent models of obesity by possibly diverting carbohydrates and fatty acids that would have become fat in the

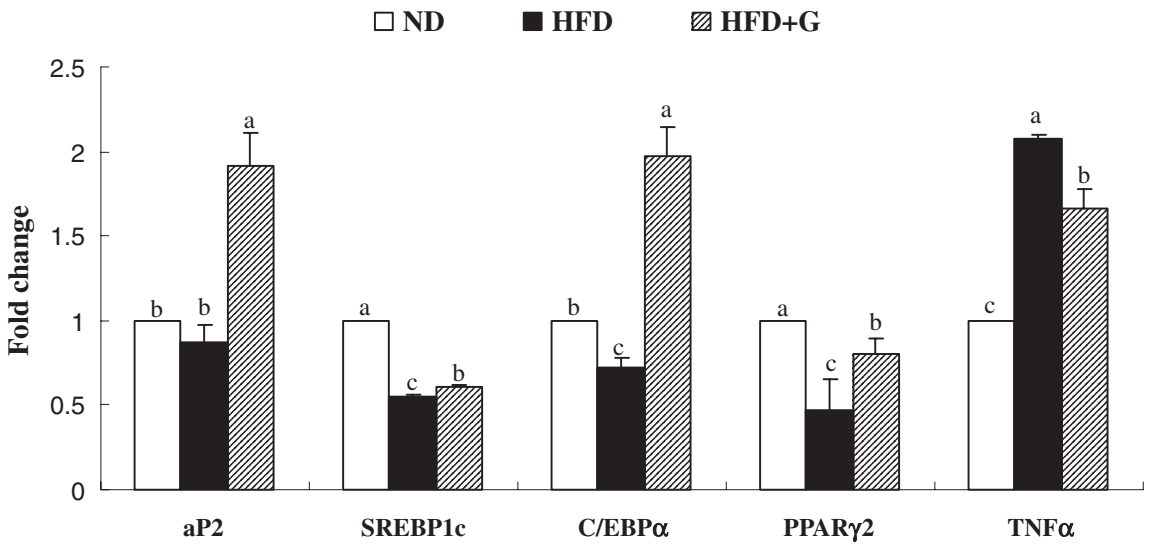


**Fig. 4.** Fold Changes in Gene Expression as Determined by the Real-Time PCR Analyses in the Epididymal Fat Tissues of Mice Fed on the Experimental Diets for 12 Weeks.

Each value is shown as the mean ± SEM of triplicate analyses of RNA samples pooled from 12 mice. Results were normalized to $\beta$-actin mRNA expression. The mRNA levels of mice fed with HFD or HFD+G are expressed as the fold changes compared with the ND mice. [a,b,c]Means not sharing a common superscript are significantly different ($p < 0.05$).

liver into hepatic glycogen.[13,14,17,18] This metabolic change may send a signal to the brain that results in a reduced appetite.[16] In the present study, a suppressed food intake does not appear to be the only cause of weight loss in the mice fed with the *Garcinia cambogia* extract, since, in addition to the food intake, FER was also significantly lower in this group than in the HFD mice, which implies that the mice administered the *Garcinia cambogia* extract were less efficient in transforming the nutrients fed into their own biomass. HCA resulted in a significant increase in the serum serotonin level, concomitant with a reduced appetite, weight loss, a favorable lipid profile, and a reduction in the plasma leptin level in human clinical trials.[20] The brain serotonin level has also been increased by HCA in obese Zucker rats.[21] It therefore appears that the ability of the *Garcinia cambogia* extract to reduce body weight gain could have been due to its combined effects on the metabolic and serotonin pathways.[21]

The plasma and hepatic concentrations of cholesterol and triglyceride were significantly lower in animals that were given the *Garcinia cambogia* extract than in the HFD control mice. These results were expected because HCA is known to inhibit the lipogenesis catalyzed by ATP citrate-lyase in the liver and peripheral tissues, as reported previously.[22] It is well known that HCA prevents the production of acetyl-CoA and, subsequently, malonyl-CoA.[23] The results of the present study clearly show that the *Garcinia cambogia* extract ameliorated HFD-induced hyperinsulinemia. In addition, *Garcinia cambogia*, in a previous report by other investigators, restored the impaired glucose tolerance in obese Zucker rats.[24] Taken together, these results suggest that the *Garcinia cambogia* extract might have a beneficial role in improving the insulin resistance induced by HFD, although the mechanism for this needs to be clarified.

Leptin is a fat-derived key regulator of appetite and energy expenditure, and is exclusively secreted by adipocytes in proportion to their triglyceride stores.[25] Thereby, the circulating leptin level is correlated with the extent of obesity.[26] In the present study, the *Garcinia cambogia* extract decreased not only the leptin concentration but also the sizes of adipocytes, indicating that it decreased lipid accumulation in the visceral adipocytes of rats rendered obese by HFD. Adiponectin is also secreted by fat cells and has anti-atherosclerotic and insulin-sensitizing properties that suppress the hepatic glucose production and enhance the glucose uptake into the skeletal muscles.[27] In the current study, feeding HFD to mice did not lead to any significant decrease in the plasma adiponectin level. The inverse association of body weight and the serum adiponectin level has not been observed consistently in rodent models,[27] although Barnea, M., *et al.*[28] have recently reported that the circulating adiponectin level of mice fed HFD remained relatively constant and was highly regulated by a mechanisms that has yet to be investigated.

To maintain lipid homeostasis, adipocytes carry out two reciprocal biochemical processes: lipogenesis and lipolysis. These two processes are tightly controlled by several hormones, lipid metabolites, and nutritional conditions such as feeding and fasting. With these signals, lipogenic transcription factors, including SREBP1c, liver X receptors, PPAR$\gamma$, and C/EBP$\alpha$, actively participate in the lipid metabolism of adipocytes.[29] These transcription factors are highly expressed in the adipose tissue and play a key role in adipocyte

differentiation by coordinating lipogenic and adipocyte-specific gene expression.[30] PPAR$\gamma$, one of the first identified nuclear receptors which bind many fatty acids as ligands, interacts with several other transcription factors.[31] C/EBP$\alpha$ and PPAR$\gamma$ are initially expressed at low levels, and are then able to induce each other's expression *via* a positive feedback loop in the differentiated adipocytes.[32] Another transcription factor that PPAR$\gamma$ interacts with is SREBP1. Co-expression of this transcription factor with PPAR$\gamma$ increases the transcriptional activity of PPAR$\gamma$. Since SREBP1 is able to increase the expression of several genes involved in fatty acid metabolism, it has been suggested that SREBP1 induces the production of an endogenous ligand that enhances the transcriptional activity of PPAR$\gamma$.[33] Several genes specializing in lipid metabolism and storage are induced in the adipogenic differentiation process, many of which contain functional PPAR-response elements. aP2, which is a marker of terminal adipocyte differentiation and involved in free fatty acid transportation and shunting within the cell, is one such gene,[34] together with a cluster of other adipocyte-specific genes such as adiponectin, insulin receptor, leptin, glucose transporter 4 (GLUT4) and glycerol phosphate dehydrogenase.[30]

In the present study, the aP2, SREBP1c, C/EBP$\alpha$, and PPAR$\gamma$2 mRNA levels were not increased, but rather decreased in the visceral adipose tissue of HFD-induced obese mice. This is in agreement with the previous reports, showing that the fatty acid synthase, acetyl-CoA carboxylase 1, and SREBP1c mRNA concentrations were decreased in the adipose tissue of obese human subjects[35] and of the obese animal models.[36] The HFD-induced down-regulation of the PPAR$\gamma$2 and C/EBP$\alpha$ genes supports the response seen after the treatment of 3T3-L1 adipose cells with TNF$\alpha$.[37] These could be explained as an adaptive response of the visceral adipose tissue aimed at limiting a further expansion of fat storage in the adipose tissue. From a proteomic approach, Schmid, G. M., *et al.*[38] have observed that the proteins involved in oxidative phosphorylation were over-expressed in the brown adipose tissue of rats by feeding HFD. Therefore, the adaptive response of animals to HFD does not appear to be limited to the visceral fats, but expanded into the brown adipose tissue for the purpose of increasing energy expenditure to compensate for the weight gain.

TNF$\alpha$ is involved in pro-inflammation, apoptosis, lipid metabolism, and insulin resistance.[39] Both the mRNA and protein expression of TNF$\alpha$ are increased in the adipose tissue from obese rodents and humans,[40] and a high level of TNF$\alpha$ suppresses such transcription factors as PPAR$\gamma$ and C/EBP$\alpha$ which, in turn, activates the GLUT4 gene.[41,42] The addition of TNF$\alpha$ to such adipocytes as 3T3-L1 and TA1 causes down-regulation of enzymes involved in lipid metabolism and of such adipocyte specific genes as aP2 and fat-specific protein 27.[43,44] Xing H., *et al.*[42] have observed that TNF$\alpha$ prevented the mRNA expression of PPAR$\gamma$ and corresponding proteins from increasing in the adipogenic cells (3T3-L1 cells).

The expression levels of PPAR$\gamma$2 and C/EBP$\alpha$ in the epididymal adipose tissue showed significantly more up-regulation (70–174% increases) in the animals fed with HFD supplemented with the *Garcinia cambogia* extract than in the mice fed with HFD alone in the present study. These remarkable changes in the mRNA expression of PPAR$\gamma$2 and C/EBP$\alpha$ induced by the *Garcinia cambogia* extract supplemented to HFD were also associated with noticeable regulation of the aP2 gene (a 120% increase), one of the target genes of PPAR$\gamma$. These results are in agreement with the previous observation by other investigators that the inhibitory effect of the *Garcinia cambogia* extract against the insulin-induced differentiation of 3T3-L1 cells targeted the adipogenic transcription factor, C/EBP$\alpha$.[45] Furthermore, the result that the expression of the TNF$\alpha$ gene, which was up-regulated by HFD, was down-regulated again by the *Garcinia cambogia* extract supplemented to HFD corresponds well with the diet-induced changes in the expression of such other genes as PPAR$\gamma$2, C/EBP$\alpha$, and SREBP1c. Therefore, the *Garcinia cambogia* extract appears to have modulated the expression of a critical nuclear transcription factor that can trigger the entire process of adipocyte differentiation. It could be speculated from the gene expression data, together with the relevant biochemical results for mice, that the blood levels of insulin and leptin, and the visceral fat-pad weights might have inverse relationships with the expression levels of PPAR$\gamma$2, C/EBP$\alpha$, SREBP1c, and aP2, and that they might be positively related with the TNF$\alpha$ expression level in their visceral adipose tissues.

In conclusion, the *Garcinia cambogia* extract was effective in reducing the body weight gain, and in improving fatty liver, dyslipidemia, hyperinsulinemia, and hyperleptinemia in mice rendered obese by HFD. We report here for the first time that these anti-obesity effects of the *Garcinia cambogia* extract were associated with modulation of the multiple genes associated with visceral adipogenesis such as PPAR$\gamma$2, SREBP1c, C/EBP$\alpha$, and aP2 in the visceral fat tissue of mice.

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## References

1) Nakamura, T., Tokunaga, K., Shimomura, I., Nishida, M., Yoshida, S., Kotani, K., Islam, A. H. M. W., Keno, Y., Kobatake, T., Nagai, Y., Fujioka, S., Tarui, S., and

Matuzawa, Y., Contribution of visceral fat accumulation to the development of coronary artery disease in non-obese men. *Atherosclerosis*, **107**, 239–246 (1994).
2) Uauy, R., and Dias, E., Consequences of food energy excess and positive energy balance. *Public Health Nutr.*, **8**, 1077–1099 (2005).
3) Hansen, P. A., Han, D. H., Nolte, L. A., Chen, M., and Holloszy, J. O., DHEA protects against visceral obesity and muscle insulin resistance in rats fed a high-fat diet. *Am. J. Physiol.*, **273**, 1704–1708 (1997).
4) Katagiri, K., Arakawa, S., Kurahashi, R., and Hatano, Y., Impaired contact hypersensitivity in diet-induced obese mice. *Dermatol. Sci.*, **46**, 117–126 (2007).
5) Levin, B. E., Triscari, J., and Sullivan, A. C., Metabolic features of diet-induced obesity without hyperphagia in young rats. *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, **251**, 433–440 (1986).
6) Velasquez, M. T., and Bhathena, S. J., Role of dietary soy protein in obesity. *Int. J. Med. Sci.*, **26**, 72–82 (2007).
7) Kishino, E., Ito, T., Fujita, K., and Kiuchi, Y., A mixture of the *Salacia reticulata* (Kotala himbutu) aqueous extract and cyclodextrin reduces the accumulation of visceral fat mass in mice and rats with high-fat diet-induced obesity. *J. Nutr.*, **136**, 433–439 (2006).
8) Saito, M., Ueno, M., Ogino, S., Kubo, K., Nagata, J., and Takeuchi, M., High dose of *Garcinia cambogia* is effective in suppressing fat accumulation in developing male Zucker obese rats, but highly toxic to the testis. *Food Chem. Toxicol.*, **43**, 411–419 (2005).
9) Attele, A. S., Zhou, Y. P., Xie, J. T., Wu, J. A., Zhang, L., Dey, L., Pugh, W., Rue, P. A., Polonsky, K. S., and Yuan, C. S., Anti-diabetic effects of *Panax ginseng* berry extract and the identification of an effective component. *Diabetes*, **51**, 1851–1858 (2002).
10) Han, L. K., Gong, X. J., Kawano, S., Saito, M., Kimura, Y., and Okuda, H., Antiobesity actions of *Zingiber officinale* Roscoe. *Yakugaku Zasshi*, **125**, 213–217 (2005).
11) Han, L. K., Xu, B. J., Kimura, Y., Zheng, Y., and Okuda, H., *Platycodi radix* affects lipid metabolism in mice with high-fat diet-induced obesity. *J. Nutr.*, **130**, 2760–2764 (2000).
12) Bank, M. S., "Physicians' Desk Reference for Herbal Medicines," Medical Economics Company, New Jersey (2000).
13) Sullivan, A. C., and Triscari, J., Metabolic regulation as a control for lipid disorders. I. Influence of (−)-hydroxycitrate on experimentally induced obesity in the rodent. *Am. J. Clin. Nutr.*, **30**, 767–776 (1977).
14) Ohia, S. E., Opere, C. A., LeDay, A. M., Bagchi, M., Bagchi, D., and Stohs, S. J., Safety and mechanism of appetite suppression by a novel hydroxycitric acid extract (HCA-SX). *Mol. Cell. Biochem.*, **238**, 89–103 (2002).
15) Heymsfield, S. B., Allison, D. B., Vasselli, J. R., Pietrobelli, A., Greenfield, D., and Nunez, C., *Garcinia cambogia* (hydroxycitric acid) as a potential antiobesity agent: a randomized controlled trial. *JAMA*, **280**, 1596–1600 (1998).
16) Sullivan, A. C., Triscari, J., Hamilton, J. G., Miller, O. N., and Wheatley, V. R., Effect of (−)-hydroxycitrate upon the accumulation of lipid in the rat. I. Lipogenesis. *Lipids*, **9**, 121–128 (1974).
17) Sullivan, A. C., Triscari, J., Hamilton, J. G., and Miller, O. N., Effect of (−)-hydroxycitrate upon the accumulation of lipid in the rat. II. Appetite. *Lipids*, **9**, 129–134 (1973).
18) Leonhardt, M., and Langhans, W., Hydroxycitrate has long-term effects on feeding behavior, body weight regain and metabolism after body weight loss in male rats. *J. Nutr.*, **132**, 1977–1982 (2002).
19) Folch, J., Lees, M., and Sloane-Stanley, G. H., A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.*, **226**, 497–509 (1957).
20) Preuss, H. G., Bagchi, D., Bagchi, M., Rao, C. V. S., Dey, D. K., and Satayanarayana, S., Effects of natural extract of (−)-hydroxycitric acid (HCA-SX) and a combination of HCA-SX plus niacine-bound chromium and Gymnema sylvestre extract in weight loss. *Diabetes Obes. Metab.*, **24**, 45–58 (2004).
21) Asghar, M., Zeyssig, R., Monjok, E., Kouamou, G., Ohia, S. E., Lokhandwala, M. F., and Bagchi, D., Hydroxycitric acid (HCA-SX) decreases oxidative stress and insulin resistance and increases brain serotonin levels in obese Zucker rats. *Exp. Biol. Meet.*, **20**, 655 (2006).
22) Sullivan, A. C., Singh, M., Srere, P. A., and Glusker, J. P., Reactivity and inhibitor potential of hydroxycitrate isomers with citrate synthase, citrate lyase, and ATP citrate lyase. *J. Biol. Chem.*, **252**, 7583–7590 (1977).
23) Saha, A. K., Vavvas, D., Kurowshi, T. G., Apanidis, A., Witters, L. A., Shafrir, E., and Ruderman, N. B., Malonyl-CoA regulation in skeletal muscles, its link to cell citrate and the glucose-fatty acid cycle. *Am. J. Physiol.*, **272**, 641–648 (1997).
24) Asghar, M., Monjok, E., Kouamou, G., Ohia, S. E., Bagchi, D., and Lokhandwala, M. F., Super CitriMax (HCA-SX) attenuates increases in oxidative stress, inflammation, insulin resistance, and body weight in developing obese Zucker rats. *Mol. Cell. Biochem.*, **304**, 93–99 (2007).
25) Staiger, H., Tschritter, O., Machann, J., Thamer, C., Fritsche, A., Maerker, E., Schick, F., Haring, H. U., and Stumvoll, M., Relationship of serum adiponectin and leptin concentrations with body fat distribution in humans. *Obes. Res.*, **11**, 368–372 (2003).
26) Maffei, M., Halaas, J., Ravussin, E., Pratley, R. E., Lee, G. H., Zhang, Y., Fei, H., Kim, S., Lallone, R., and Ranganathan, S., Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. *Nat. Med.*, **1**, 1155–1161 (1995).
27) Naderali, E. K., Estadella, D., and Rocha, M., A fat-enriched, glucose-enriched diet markedly attenuates adiponectin mRNA levels in rat epididymal adipose tissue. *Clin. Sci.* (Lond), **105**, 403–408 (2003).
28) Barnea, M., Shamay, A., Stark, A. H., and Madar, Z., A high-fat diet has a tissue-specific effect on adiponectin and related enzyme expression. *Obesity*, **14**, 2145–2153 (2006).
29) Park, J., Rho, H. K., Kim, K. H., Choe, S. S., Lee, Y. S., and Kim, J. B., Overexpression of glucose-6-phosphate dehydrogenase is associated with lipid dysregulation and insulin resistance in obesity. *Mol. Cell. Biol.*, **25**, 5146–

5157 (2005).
30) Rosen, E. D., Walkey, C. J., Puigserver, P., and Spiegelman, B. M., Transcriptional regulation of adipogenesis. *Genes Dev.*, **14**, 1293–1307 (2000).
31) Xu, H. E., Lambert, M. H., Montana, V. G., Parks, D. J., Blanchard, S. G., Brown, P. J., Sternbach, D. D., Lehmann, J. M., Wisely, G. B., Willson, T. M., Kliewer, S. A., and Milburn, M. V., Molecular recognition of fatty acids by peroxisome proliferator-activated receptors. *Mol. Cell*, **3**, 397–403 (1999).
32) Rosen, E. D., Hsu, C. H., Wang, X., Sakai, S., Freeman, M. W., Gonzalez, F. J., and Spiegelman, B. M., C/EBP$\alpha$ induces adipogenesis through PPAR$\gamma$: a unified pathway. *Genes Dev.*, **16**, 22–26 (2002).
33) Kim, J. B., Wright, H. M., Wright, M., and Spiegelman, B. M., ADD1/SREBP1 activates PPAR$\gamma$ through the production of endogenous ligand. *Proc. Natl. Acad. Sci. USA*, **95**, 4333–4337 (1998).
34) Tontonoz, P., Hu, E., Graves, R. A., Budavari, A. I., and Spiegelman, B. M., mPPAR gamma 2: tissue-specific regulator of an adipocyte enhancer. *Genes Dev.*, **8**, 1224–1234 (1994).
35) Diraison, F., Dusserre, E., Vidal, H., Sothier, M., and Beylot, M., Increased hepatic lipogenesis but decreased expression of lipogenic gene in adipose tissue in human obesity. *Am. J. Physiol.*, **282**, 46–51 (2002).
36) Nadler, S., Stoehr, J., Schueler, K., Tanimoto, G., Yandell, B., and Attie, A., The expression of adipogenic genes is decreased in obesity and diabetes mellitus. *Proc. Natl. Acad. Sci. USA*, **97**, 11371–11376 (2000).
37) Ron, D., Brasier, A. R., McGehee, R. E. Jr., and Habener, J. F., Tumor necrosis factor-induced reversal of adipocytic phenotype of 3T3-L1 cells is preceded by a loss of nuclear CCAAT/enhancer binding protein (C/EBP). *J. Clin. Invest.*, **89**, 223–233 (1992).
38) Schmid, G. M., Converset, V., and Walter, N., Effect of high-fat diet on the expression of proteins in muscle, adipose tissues, and liver of C57BL/6 mice. *Proteomics*, **4**, 2270–2282 (2004).
39) Hammarstedt, A., Andersson, C. X., Rotter-Sopasakis, V., and Smith, U., The effect of PPAR$\gamma$ ligands on the adipose tissue in insulin resistance. *Prostaglandins Leukot. Essent. Fatty Acids*, **73**, 65–75 (2005).
40) Hotamisligil, G. S., Shargill, N. S., and Spiegelman, B. M., Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Science*, **259**, 87–91 (1993).
41) Stephens, J. M., and Pekala, P. H., Transcriptional repression of the C/EBP$\alpha$ and GLUT4 genes in 3T3-L1 adipocytes by tumor necrosis factor-alpha. Regulations is coordinate and independent of protein synthesis. *J. Biol. Chem.*, **267**, 13580–13584 (1992).
42) Xing, H., Northrop, J. P., Grove, J. R., Kilpatrick, K. E., Su, J. L., and Ringold, G. M., TNF$\alpha$-mediated inhibition and reversal of adipocyte differentiation is accompanied by suppressed expression of PPAR$\gamma$ without effects on Pref-1 expression. *Endocrinology*, **138**, 2776–2783 (1997).
43) Chapman, A. B., Knight, D. M., Dieckman, B. S., and Ringold, G. M., Analysis of gene expression during differentiation of adipogenic cells in culture and hormonal control of the developmental program. *J. Biol. Chem.*, **259**, 15548–15555 (1984).
44) Torti, F. M., Torti, S. V., Larrick, J. W., and Ringold, G. M., Modulation of adipocyte differentiation by tumor necrosis factor and transforming growth factor beta. *J. Cell Biol.*, **108**, 1105–1113 (1989).
45) Kim, M. S., Kim, J. K., Kwon, D. Y., and Park, R., Anti-adipogenic effects of *Garcinia* extract on the lipid droplet accumulation and the expression of transcription factor. *BioFactors*, **22**, 193–196 (2004).