

# Dietary Flavonoids Suppress Adipogenesis in 3T3-L1 Preadipocytes

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(Received: November 24, 2004; Accepted: March 1, 2005)

## ABSTRACT

This study examined how dietary flavonoids, including catechin, quercetin and kaempferol, affect the differentiation of 3T3-L1 adipocytes. Eight days following induction for differentiation with MDI-medium and simultaneously with the tested flavonoids (day 8), our study found significantly reduced ( $p < 0.05$ ) intracellular triacylglycerol accumulations of 3T3-L1 cells. This compared positively against the smaller reduction in accumulation found in the vehicle control. This suppressing effect was found to be dose-dependent, and a 54.4%, 45.7% and 29.9% decrease of triacylglycerol production was observed for catechin, quercetin and kaempferol (25-100  $\mu\text{M}$ ), respectively. Additionally, RT-PCR assays demonstrated that catechin, quercetin and kaempferol markedly reduced the mRNA expressions of C/EBP- $\alpha$ , PPAR- $\gamma$  and SREBP-1, which are key transcription-factors for 3T3-L1 adipogenesis. These results suggest that dietary flavonoids suppress 3T3-L1 differentiation by down-regulating adipogenic transcription-factors. Further study recommend to explore the potential health benefits of catechin, quercetin and kaempferol in the control of body weight.

Key words: dietary flavonoids, 3T3-L1 adipocyte, adipogenic differentiation

## INTRODUCTION

Dietary flavonoids are secondary products of plants, which humans ingest daily in fruit, vegetables, tea, wine and other foods. Research on dietary flavonoids has shown them to have a broad spectrum of biological activities, including inhibiting cell proliferation in cell cultures, inducing apoptosis, changing the activity of certain intracellular enzymes and functioning as antioxidants<sup>(1-3)</sup>. Additionally, certain studies have indicated that flavonoids may deliver a number of clinical effects including antiatherosclerotic, antiinflammatory, antitumor, antithrombogenic, antiosteoporotic and antiviral<sup>(3)</sup>.

Flavonoids also influence lipolysis and adipogenesis in adipose cells. Several flavonoids have exhibited lipolytic activity synergistically with epinephrine in primary rat adipocytes<sup>(4,5)</sup>. Quercetin, a flavone ubiquitous in fruits and vegetables, shows antilipogenic effects in inhibiting insulin-mediated lipogenesis by blocking insulin receptor tyrosine kinase from phosphorylating substrates<sup>(6)</sup>. Recently, Harmon and Harp have shown that isoflavone genistein inhibits the proliferation and terminal differentiation of both pre- and postconfluent preadipocytes<sup>(7)</sup> through increasing homologous protein expression in the CCAAT/enhancer-binding protein (C/EBP)<sup>(8)</sup> in 3T3-L1 adipocytes. Notably, the grapefruit flavanone naringenin does not

activate triacylglycerol accumulation<sup>(7)</sup> but rather inhibits insulin-stimulated glucose uptake in 3T3-L1 adipocytes<sup>(9)</sup>.

The primary adipogenic transcription factors involved in adipocyte differentiation are now well recognized as belonging to the peroxisome proliferator activator receptor (PPAR), C/EBP, and sterol regulatory element binding protein (SREBP) families. PPAR- $\gamma$  expression during differentiation is an important nuclear hormone receptor in adipocytes<sup>(10)</sup>. Moreover, C/EBPs are basic leucine zipper transcription factors and are expressed immediately following hormone induced differentiation<sup>(11)</sup>. Following the initiation of C/EBP- $\alpha$  gene transcription, continued expression is assured through transcriptional autoactivation. Additional PPAR expression is caused by C/EBP activation, which prompts the expression of other adipogenic genes. Furthermore, SREBP-1, which is also increased during the differentiation of 3T3-L1 preadipocytes and adipocytes, also regulates the transcription of various genes in enzymes and proteins involved in cholesterol and fatty acid metabolism<sup>(12,13)</sup>.

Much current understanding of adipogenesis is based on 3T3-L1 cells. As preadipocytes, 3T3-L1 cells resemble fibroblasts and replicate in culture until they form a confluent monolayer. Subsequent stimulation with 3-isobutyl-1-methylxanthine, dexamethasone, and insulin (MDI protocol) for 2 days prompts these cells to express adipocyte-specific genes, including PPAR- $\gamma$ , C/EBP- $\alpha$ , SREBP, among others<sup>(14)</sup>. Approximately five days

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following the onset of differentiation, 90% of cells exhibit the characteristic lipid-filled adipocyte phenotype.

Some herbal extracts, including pycnogenol<sup>(15)</sup> and *Ginkgo biloba*<sup>(16)</sup>, have been found to inhibit lipogenesis in adipocytes. However, it is not clear whether flavonoid compounds, such as quercetin, kaempferol, catechin (known important components of pycnogenol<sup>(17)</sup>, and *Ginkgo biloba*<sup>(18)</sup>), serve to modulate adipogenesis in adipocytes.

This study was initiated to evaluate whether dietary flavonoids, including the widely distributed flavones quercetin, kaempferol and the tea flavan-3-ol catechin, affect the differentiation of 3T3-L1 preadipocytes into adipocytes as well as to determine the mechanism through which flavonoids influence 3T3-L1 preadipocyte differentiation.

## MATERIALS AND METHODS

### I. Materials

Catechin (3,3',4',5,7-flavanpentol), quercetin (3,3',4',5,7-pentahydroxyflavone), kaempferol (3,4',5,7-tetrahydroxyflavone), insulin, dexamethasone, and 3-isobutyl-1-methylxanthine were obtained from Sigma (St. Louis, MO, USA). The tissue culture materials were from GIBCO (Grand Island, NY). Chloroform, DMSO, EDTA, TritonX-10, 37% formaldehyde were from J. T. Baker (Phillipsburg, NJ, USA).

### II. Cell Culture

Our culturing protocol was modified from the method described by Harmon and Harp<sup>(7)</sup>. 3T3-L1 preadipocytes (American Type Culture Collection, Rockville, MD, USA) were cultured in DMEM with 10% (v/v) fetal bovine serum (FBS), 1% (v/v) penicillin-streptomycin (10,000 U/mL penicillin and 10,000 µg/mL streptomycin in 0.85% saline), and 1% (v/v) 100 mM pyruvate at 37°C in 95% air -5% CO<sub>2</sub>. The differentiation process was also a modification of a Harmon and Harp method (2001). To induce differentiation, 2-day postconfluent 3T3-L1 preadipocytes (day 0) were stimulated for 72 hr by adding 0.5 mM 3-isobutyl-1-methylxanthine, 1 µM dexamethasone, 0.125 mM indomethasin, and 1 µM insulin (MDI) to the DMEM/10% FBS culture medium. Subsequently, on day three, the MDI medium was replaced with DMEM/10% FBS containing 1 µM insulin. On day 5, the MDI medium was replaced with DMEM/10% FBS and refreshed at 2 day intervals thereafter until analysis was performed on days 7~10.

Flavonoids were reconstituted using 100 mM stock solutions in DMSO, filter sterilized, and stored at -20°C. Unless otherwise stated, "vehicle" refers to 0.1 % DMSO in culture medium or MDI differentiation medium. For flavonoid and DMSO treatment, cells received flavonoids/DMSO premixed with culture medium from day 0 to day 10, a period of time which covered the entire induction and post-induction stages.

### III. Cell Viability

Cell respiration as an indicator of cell viability and proliferation was determined using a mitochondrial-dependent reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) to formazan. Preconfluent 3T3-L1 preadipocytes were seeded in 96-well plates at a density of 10,000 cells/well. Vehicle and flavonoids (25~100 µM) were subsequently added to culture medium at the time of plating. At 48 hr following plating, the cells were incubated at 37°C with 0.5 mg/mL MTT for 45 min. The medium was aspirated, and the insoluble formazan product was dissolved in DMSO (250 µL) for at least 2 hr in the dark. MTT reduction was quantified by measuring the absorbance at 550 nm.

### IV. Triacylglycerol Assay<sup>(7)</sup>

On day 8 following differentiation, 3T3-L1 cells were washed twice with PBS, scraped on ice in 100 µL of saline solution (2 M NaCl, 2 mM EDTA, 50 mM sodium phosphate, pH 7.4), sonicated to homogenize the cell suspension, and assayed for total triacylglycerol using a Merck Ecoline Triacylglycerols GPO-PAP kit (Merck, Germany) in accordance with manufacturer instructions. Results were expressed as total triacylglycerol (mg) of cellular protein (mg) (DC protein assay; Bio-Rad, Hercules, CA).

### V. Oil Red O Staining

3T3-L1 adipocytes were washed with PBS, fixed with 10% glutaraldehyde in PBS (pH 7.4), and then stained with 0.5% Oil Red O (Sigma, St. Louis, MO, USA).

### VI. mRNA Expression

RT-PCR was performed to determine the level of mRNA expression for β-actin, PPAR-γ, SREBP-1 and C/EBP-α. Briefly, on day 8 after differentiation, total RNA was extracted from cultured cells using TRIzol Reagent (Life Technologies, Rockville, MD). From each sample, 200 ng of RNA was reverse-transcribed using 200 units of Superscript II reverse transcriptase, 20 units of RNase inhibitor, 0.6 mM of dNTP, and 0.5 µg/µL of Oligo (dT12-18). PCR analyses were then performed on cDNA preparation aliquots to detect PPAR-γ, SREBP-1, C/EBP-α and β-actin (as an internal standard) mRNA expression using an ABI GeneAmp 2700 PCR System (Applied Biosystems, Foster City, CA, USA). Oligonucleotide primers for β-actin (Forward 5'-GTGGGCCGCTCTAGGCACC-3', Reverse 5'-CTCTTTGATGTACGCACGA-3'), PPAR-γ1 (Forward 5'-GTTCATGCTTGTGAAGGATGC-3', Reverse 5'-ACTCTGGGTTTCAGCTGGTCG-3'), SREBP-1c (Forward 5'-ACCCTGGTGAGTGGAGGGACCATCTTGG-3', Reverse 5'-CTTTGCTTCAGTGCCACCACCAGGTCTTT-3') and C/EBP-α (Forward 5'-AGGTGCTG-GAGTTGACCAGT-3', Reverse 5'-CAGCCTAGAGATC-

CAGCGAC-3') were obtained from Clontech (Palo Alto, CA, USA). The reactions were carried out in a volume of 50  $\mu$ L containing (final concentration) 50 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM MnCl<sub>2</sub>, 0.2 mM dNTP, 2 units of Taq DNA polymerase and 50 pmol of 5' and 3' primers. Following initial denaturation for 2 min at 95°C, 30 cycles of amplification (at 95°C for 1 min, 58°C for 1 min, and 72°C for 1.5 min) were performed, followed by 7 min of extension at 72°C. The amount of cDNA templates used for PCR was determined within the linear range.

### VII. Analysis of PCR Products

A 10  $\mu$ L aliquot from each PCR reaction was electrophoresed in a 1.5 % agarose gel containing 0.2  $\mu$ g/mL ethidium bromide. The gel was then photographed under ultraviolet transillumination. To quantify mRNA expression, the PCR bands on the photograph of the gel were scanned using a Syngene GeneGenius gel documentation system with GeneTools<sup>®</sup> software (Cambridge, UK). The levels of PPAR- $\gamma$ , SREBP-1 and C/EBP- $\alpha$  were expressed after normalization with the  $\beta$ -actin signal from the same sample.

### VIII. Statistical Analysis

Each experiment was performed in triplicate (n = 3) and repeated three times. The results were expressed as mean  $\pm$  SD. Statistical comparisons were made using the Student's T-test. Differences were considered significant when P-values were below 0.05 ( $p < 0.05$ ).

## RESULTS

### I. Effect of Flavonoids on Proliferation of Preconfluent Adipocytes

To determine whether flavonoids inhibit the cell viability or proliferation of preadipocytes, the cultured preconfluent 3T3-L1 cells were treated with or without various concentrations of catechin, quercetin and kaempferol for 48 hr. Following two days of incubation, cell viability was measured using the MTT assay. As illustrated in Figure 1, catechin, quercetin and kaempferol over a dose range from 25-100  $\mu$ M does not influence the metabolism of MTT by 3T3-L1 preadipocytes ( $P > 0.05$ ). This result indicates that none of these flavonoids in the concentration range tested exhibit cytotoxicity, and moreover they do not influence the cell viability of 3T3-L1 preadipocytes. Meanwhile, treatment using 0.1% DMSO also yielded similar results.

### II. Flavonoids Reduce Adipogenesis of 3T3-L1 Cells

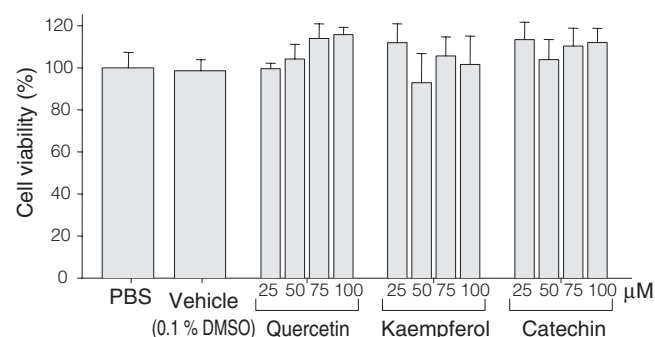
This investigation further examined the effects of flavonoids on suppressing preadipocyte differentiation. Two-day postconfluent, 3T3-L1 preadipocytes were

induced for differentiation with MDI in the presence of 100  $\mu$ M flavonoids or vehicle. Between day 3 and the induction of differentiation (on day 7 or 8), cells were maintained in culture medium with flavonoids and observed daily. As displayed by Oil Red O staining in Figure 2, most vehicle-treated cells had accumulated lipid droplets by day 10 (Figure 2A). However, adding catechin (Figure 2B), quercetin (Figure 2C) and kaempferol (Figure 2D) at 100  $\mu$ M decreased lipid accumulation and led to fewer lipids filling cells.

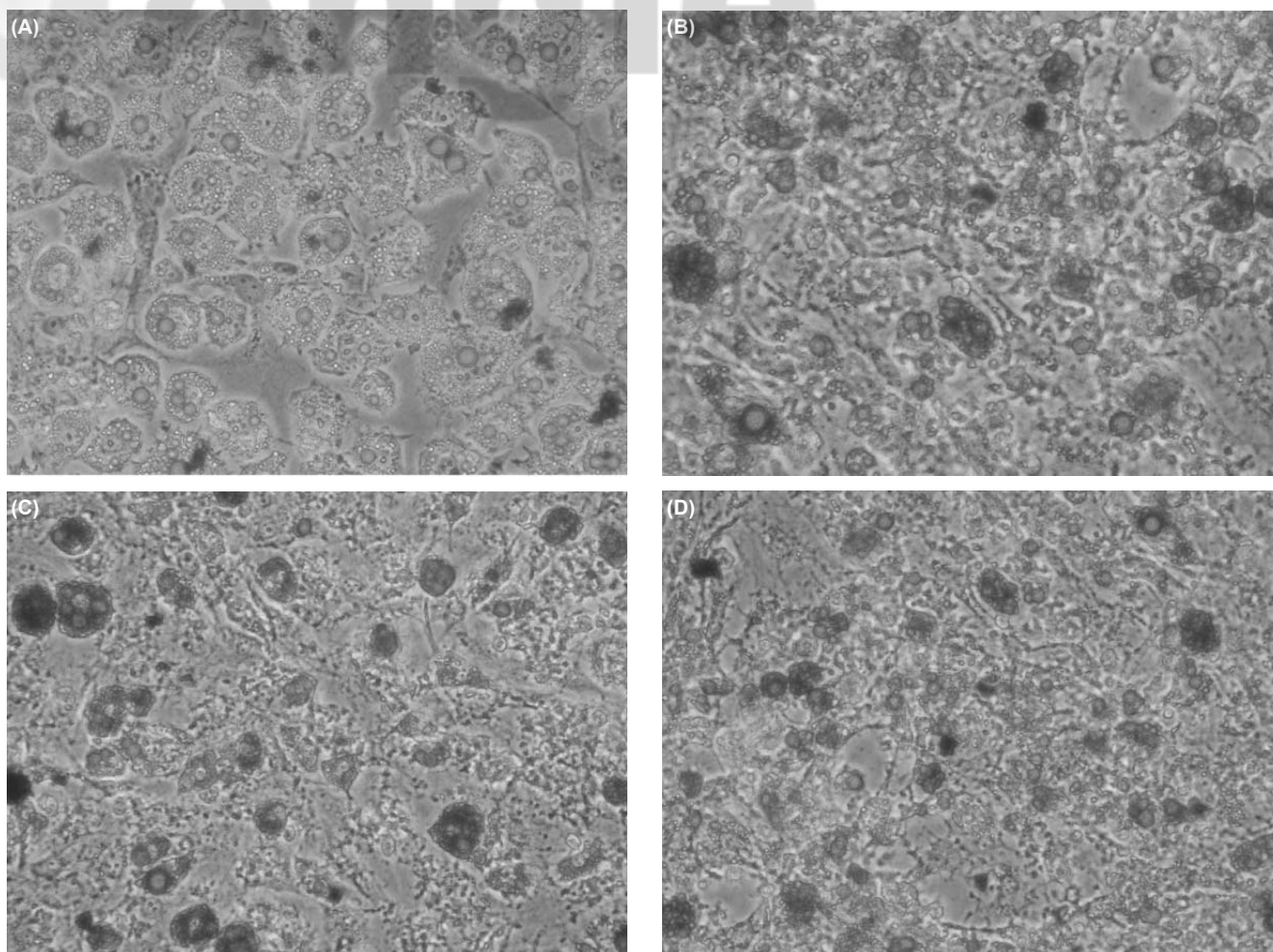
Flavonoids were also shown to reduce intracellular triacylglycerol levels in adipocytes. By day 8, intracellular triacylglycerol levels in the flavonoid-treated cells were significantly ( $p < 0.05$ ) lower than in the vehicle-treated cells (Figure 3). At a concentration of 100  $\mu$ M, catechin, quercetin and kaempferol decreased triglyceride content by 54.4%, 45.7% and 29.9%, respectively. This inhibitory effect was dose-dependent, and furthermore was consistent with observed morphology.

### III. Flavonoids Suppress mRNA Expression of PPAR- $\gamma$ , C/EBP- $\alpha$ and SREBP-1 in 3T3-L1 cells

This investigation next considered whether flavonoids changed the expression of adipogenesis-related transcription factors. The effects of flavonoids or vehicle on the expression of PPAR- $\gamma$  and C/EBP- $\alpha$  in 3T3-L1 adipocytes were further examined by RT-PCR assay. Results showed that vehicle-treated cells, which were MDI-induced and incubated without flavonoids, expressed a significant amount of PPAR- $\gamma$  mRNA on day 8. Conversely, flavonoid-treated cells expressed considerably lower PPAR- $\gamma$  mRNA levels on day 8 (Figure 4A). Intensity analysis demonstrated that catechin, quercetin and kaempferol significantly ( $P < 0.05$ ) inhibited PPAR- $\gamma$  mRNA expression in the cells (Figure 4B). Thus, the inhibitory effect was found



**Figure 1.** Influence of flavonoids on the cell viability of preconfluent 3T3-L1 preadipocytes. Preconfluent 3T3-L1 preadipocytes (10,000 cells/mL, 200  $\mu$ L per well on a 96-well plate) were treated with 0-100  $\mu$ M doses of quercetin, kaempferol, catechin or vehicle (0.1% DMSO) at the time of implantation. Cell viability was determined using an MTT assay 48 hr after implantation. Data displayed reflect the mean  $\pm$  SD (n = 3) of the three independent experiments performed. Treatment results were not statistically significant different from vehicle control results ( $P > 0.05$ ).



**Figure 2.** Photomicrographs demonstrate that flavonoids had successfully reduced lipid accumulation in differentiated 3T3-L1 adipocytes. At two days postconfluent, 3T3-L1 cells (10,000 cells/mL, 1 mL per well on a 24-well plate) were differentiated by MDI and treated with (A) vehicle, (B) catechin, (C) quercetin or (D) kaempferol at 100  $\mu$ M. Four days following differentiation, the cell culture medium was replaced with DMEM/10% FBS, which was then refreshed at 2-day intervals. Ten days following the induction of differentiation, cells were stained with Oil Rad O.

to be dose-dependent for concentrations between 25~100  $\mu$ M. At a concentration of 100  $\mu$ M, PPAR- $\gamma$  mRNA was reduced 45.1%, 47.5% and 34.0%, respectively, using catechin, quercetin and kaempferol treatments.

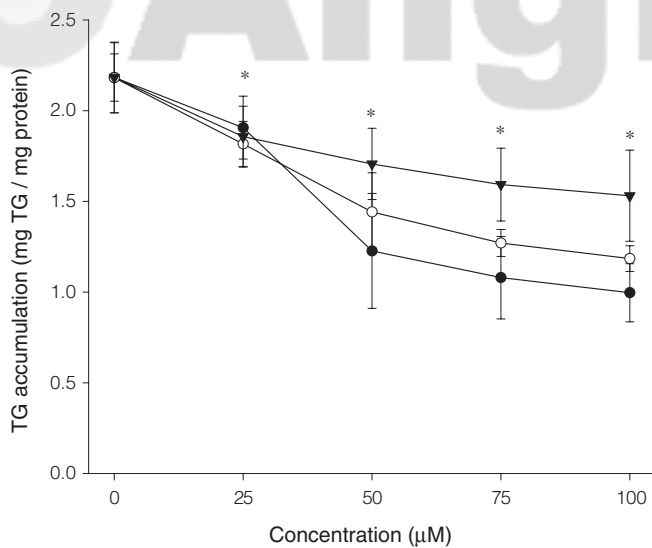
Additionally, flavonoids also influenced C/EBP- $\alpha$  expression in differentiated 3T3-L1 cells. C/EBP- $\alpha$  mRNA expression was lowered in flavonoid (25-100  $\mu$ M)-treated 3T3-L1 adipocytes to levels significantly ( $P < 0.05$ ) lower than that of vehicle-treated cells (Figure 5). At a concentration of 100  $\mu$ M, catechin, quercetin and kaempferol displayed reductions of 31.5%, 31.5% and 23.8% of C/EBP- $\alpha$  mRNA expression, respectively.

Furthermore, the effects of the flavonoids catechin, quercetin and kaempferol on SREBP-1 expression resembled those on PPAR- $\gamma$  and C/EBP- $\alpha$ . Compared with the vehicle, catechin, quercetin and kaempferol at concentrations of 100  $\mu$ M significantly decreased the level of SREBP-1 mRNA by 43.8%, 35.6% and 29.9%, respectively (Figure 6). This suppression effect was also dose-dependent at flavonoid levels of 25-100  $\mu$ M. These data

demonstrate that catechin, quercetin and kaempferol suppress the adipocytic differentiation of 3T3-L1 cells by inhibiting SREBP-1 expression and the transcriptional factors PPAR- $\gamma$  and C/EBP- $\alpha$ .

## DISCUSSION

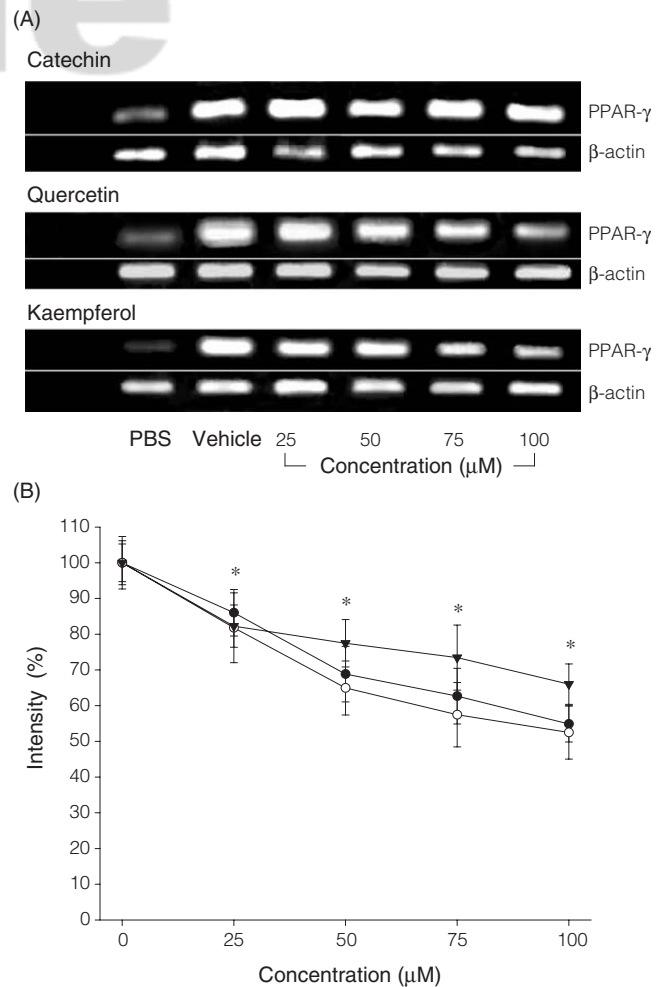
Dietary flavonoids quercetin, kaempferol and catechin were found in this study to inhibit markedly the adipogenesis of 3T3-L1 preadipocytes by suppressing adipogenesis-related transcription factor expression. These three flavonoids (25-100  $\mu$ M) did not influence cell viability and proliferation during the preadipocytic stage (Figure 1). When 3T3-L1 cells were induced using MDI-reagent for adipose differentiation in the presence of those flavonoids (days 0 – 8), the intracellular accumulation of triacylglycerol was significantly reduced (Figures 2 and 3). This inhibitory effect was demonstrated to be dosage-dependent, with catechin found to provide the most significant suppres-



**Figure 3.** Dietary flavonoids reduce triacylglycerol accumulation in differentiated 3T3-L1 cells. At two days postconfluent (day 0), 3T3-L1 cells (10,000 cells/mL, 1 mL per well on a 24-well plate) were differentiated by MDI and simultaneously treated using 0~100 µM catechin (●), quercetin (○) or kaempferol (▼). By day 4, the medium was replaced with culture medium, which was refreshed at 2-day intervals. Ten days following differentiation, cells were lysed for triacylglycerol and protein assay. The triacylglycerol level of each treatment was normalized with its protein content. Data shown reflect the mean ± SD of three independent experiments (n = 3). \*  $p < 0.05$  against the vehicle control.

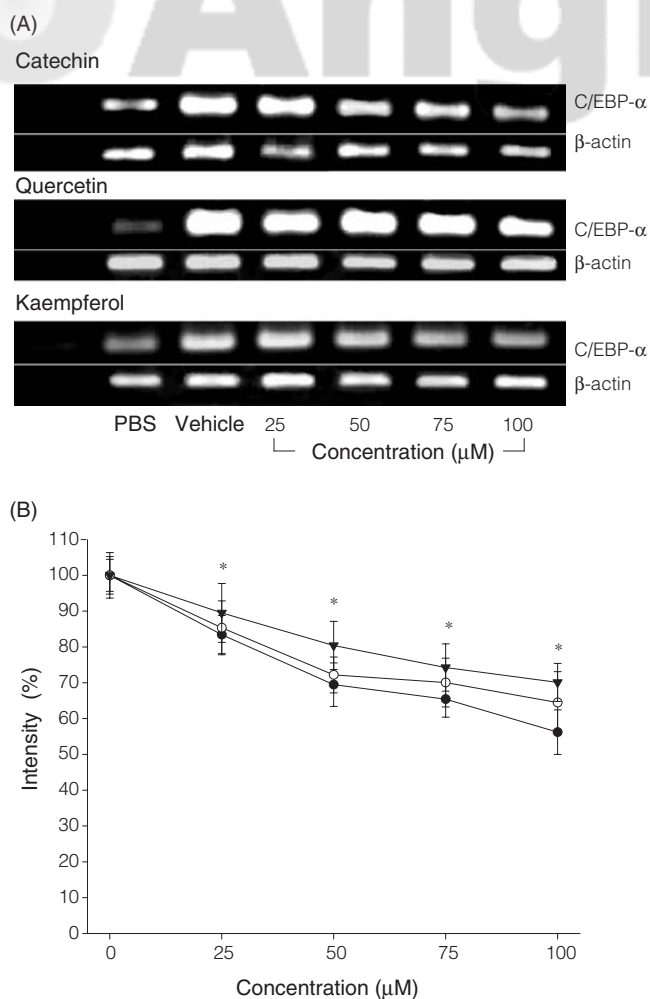
sion effect (Figure 3). However, on the third through eighth days following induction (treated on day 0) for differentiation, the posterior addition of flavonoids did not alter adipogenesis (data not shown). These results suggest that catechin, quercetin and kaempferol only alerted adipogenesis during the first three days immediately following the induction of differentiation. This finding is consistent with previous studies demonstrating irreversible differentiation of 3T3-L1 cells occurs during the initial 72-hr period<sup>(19,20)</sup>.

Adipogenesis, a complex process in which the expression of several hundred genes is altered<sup>(21)</sup>, is highly regulated by two main groups of adipogenic transcription factors – namely PPAR-γ and C/EBPs<sup>(22)</sup>. It is the cascades of these transcription factors that drive adipogenesis. When preadipocytes are induced for differentiation, C/EBP-β and C/EBP-α are rapidly expressed and synergistically promote both C/EBP-α and PPAR-γ expression<sup>(23)</sup>. PPAR-γ is considered a principal component of this cascade because adipogenesis does not occur in PPAR-γ-deficient mesenchymal stem cells<sup>(24,25)</sup>. Our study demonstrated that three flavonoids interfere with the adipogenesis process in 3T3-L1 cells by down-regulating the expression of primary adipogenic transcription factors PPAR-γ, C/EBP-α and SREBP-1 (Figures 4-6). Although this study did not examine the activities of C/EBP-β/δ, the inhibitory effect of quercetin, kaempferol and catechin, which reduced the



**Figure 4.** Dietary flavonoids inhibit PPAR-γ mRNA expression in 3T3-L1 adipocytes. At two days postconfluent (day 0), 3T3-L1 cells (10,000 cells/mL, 200 µL per well on a 96-well plate) were differentiated by MDI and treated with 25~100 µM catechin, quercetin, kaempferol or vehicle. Eight days following differentiation, the RNA was completely extracted from 3T3-L1 cells and subjected to RT-PCR, with primers specific for PPAR-γ. Expression levels were determined and quantified as described in the materials and method section. (A) 1.5% agarose gel electrophoresis of PCR-amplified cDNA obtained from PPAR-γ and β-actin stained with ethidium bromide. (B) Quantitation of mRNA levels through densitometry. PPAR-γ expression was normalized by expressing data as a ratio of PPAR-γ to β-actin. Results are expressed as mean ± SD; n = 3. \*  $p < 0.05$  against the vehicle control.

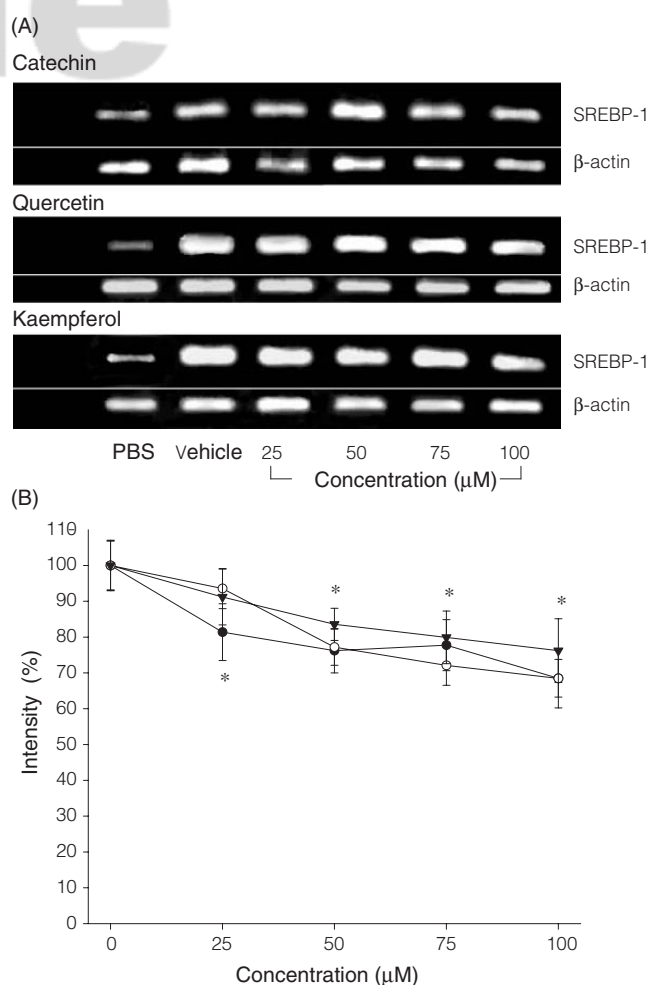
expression of PPAR-γ and C/EBP-α during the differentiation of 3T3-L1 cells, suggests that these flavonoids disrupted adipogenesis during transcription. Moreover, it has recently become known that differentiation of preadipocytes into adipocytes requires the suppression of Wnt signaling and PPAR-γ activation<sup>(26,27)</sup>. While Wnt signaling overexpression is forced in preadipocytes, adipogenesis could be blocked in part by inhibiting PPAR-γ expression<sup>(26,27)</sup>. Since quercetin, kaempferol and catechin are proven inhibitors of adipogenesis and PPAR-γ expression, further work remains to investigate the involvement of



**Figure 5.** Dietary flavonoids inhibit C/EBP- $\alpha$  mRNA expression in 3T3-L1 adipocytes. At two days postconfluent (day 0), 3T3-L1 cells (10,000 cells/mL, 200  $\mu$ L per well on a 96-well plate) were differentiated by MDI and treated with 25–100  $\mu$ M catechin, quercetin, kaempferol or vehicle. Eight days following differentiation, the RNA was completely extracted from 3T3-L1 cells and subjected to RT-PCR, with primers specific for C/EBP- $\alpha$ . Expression levels were determined and quantified as described in the materials and methods section. (A) 1.5% agarose gel electrophoresis of PCR-amplified cDNA obtained from C/EBP- $\alpha$  and  $\beta$ -actin stained with ethidium bromide. (B) Quantitation of mRNA levels through densitometry. C/EBP- $\alpha$  expression was normalized by expressing data as a ratio of C/EBP- $\alpha$  to  $\beta$ -actin. Results are expressed as mean  $\pm$  SD; n=3. \*  $p$  < 0.05 against the vehicle control.

polyphenols in the balancing of Wnt-signaling and regulation of PPAR- $\gamma$ .

The effect of polyphenols or flavonoids on adipogenesis could be related to protein-tyrosine kinase pathway and insulin signaling inhibition. Harmon, Patel and Harp reported that genistein, a known tyrosine kinase inhibitor<sup>(8)</sup>, inhibits C/EBP- $\beta$  function and C/EBP- $\alpha$ /PPAR- $\gamma$  expression by increasing the expression of C/EBP homologous proteins, which function as a dominant-negative inhibitor of C/EBP- $\alpha$ / $\beta$  DNA-binding activity. In this study, catechin, quercetin and kaempferol, which also inhibit tyrosine-



**Figure 6.** Dietary flavonoids inhibit SREBP-1 mRNA expression in 3T3-L1 adipocytes. At two days postconfluent (day 0), 3T3-L1 cells (10,000 cells/mL, 200  $\mu$ L per well on a 96-well plate) were differentiated by MDI and treated with 25–100  $\mu$ M catechin, quercetin, kaempferol or vehicle. Eight days following differentiation, the RNA was completely extracted from 3T3-L1 cells and subjected to RT-PCR, with primers specific for SREBP-1. Expression levels were determined and quantified as described in the materials and method section. (A) 1.5% agarose gel electrophoresis of PCR-amplified cDNA derived from SREBP-1 and  $\beta$ -actin stained with ethidium bromide. (B) Quantitation of mRNA levels through densitometry. SREBP-1 expression was normalized by expressing data as a ratio of SREBP-1 to  $\beta$ -actin. Results are expressed as mean  $\pm$  SD; n = 3. \*  $p$  < 0.05 against the vehicle control.

specific protein kinase<sup>(28)</sup>, were demonstrated to suppress not only adipogenesis but also the expression of key transcriptional factors, including C/EBP- $\alpha$ , PPAR- $\gamma$  and SREBP-1. These results agreed with the findings of Harmon's study<sup>(8)</sup> and implied that the anti-adipogenic effects of certain dietary flavonoids may be associated with their prominent ability to inhibit tyrosine phosphorylation. Therefore, these flavonoids may interfere with the tyrosine-phosphorylation of adipogenic transcription factors and further prevent the expression of downstream lipogenic enzymes, resulting in the inhibition of adipogenesis.

Nevertheless, catechin, quercetin and kaempferol exhibited no cytotoxicity toward 3T3-L1 preadipocytes (Figure 1).

Strobel *et al.* (2004) have recently reported that quercetin (10-100  $\mu$ M) inhibits glucose transporter GLUT4 mediated/insulin stimulated glucose uptake by adipocytes<sup>(29)</sup>. These researchers further hypothesize that flavonoids inhibit adipogenesis by interacting with GLUT4 directly, rather than through protein-tyrosine kinase related mechanisms<sup>(29)</sup>. However, the fact that our research for this report demonstrated that quercetin and kaempferol inhibited PPAR- $\gamma$  expression at the transcription level indicates that flavonoids regulate adipogenesis through direct interaction with GLUT4 as well as through other mechanisms – such as the inhibition of PPAR- $\gamma$  expression.

Catechin, one of the important tea catechins, was also tested and shown to suppress the induction of adipogenic differentiation. This result coordinates with a study by Furuyashiki *et al.*, which demonstrated tea catechins down-regulated PPAR- $\gamma$  and C/EBP- $\alpha$  expression in 3T3-L1 cells<sup>(30)</sup>. The adipogenic inhibition of tea catechins seemed to occur only in the differentiation stage and, in fully differentiated 3T3-L1 cells, catechin was unable to influence lipid metabolism and triacylglycerol accumulation<sup>(31,32)</sup>. In addition, some animal studies suggested that tea catechins might play a role in obesity prevention, as oral administration of green tea and epigallocatechin gallate (EGCG) decreased the weight of subject adipose tissue<sup>(33-35)</sup>. Moreover, EGCG, in combination with tea caffeine, was also shown to stimulate thermogenesis and fat oxidation in humans<sup>(36)</sup>. However, it was also stated that the effect of lowering plasma TG and cholesterol occurred only at high dosage levels<sup>(37)</sup>.

Inhibition of adipocyte differentiation could be related to obesity prevention. Certain herbal and plant extracts, such as pycnogenol<sup>(15)</sup>, *Ginkgo biloba*<sup>(16)</sup>, grape seed extract<sup>(38)</sup> and green tea<sup>(39)</sup>, have been reported to inhibit lipogenesis on adipocytes. Because flavonoids, including quercetin, kaempferol and catechin are important constituents of these extracts<sup>(17,18)</sup>, results point to some level of flavonoid contribution toward the anti-adipogenic effects of these foods.

In this study, quercetin, kaempferol and catechin, dietary flavonoids widely found in vegetables, fruits, green tea and herbs, were found to down-regulate the adipogenesis-related transcriptional factors PPAR- $\gamma$ , C/EBP- $\alpha$  and SREBP-1 and to inhibit adipocyte differentiation during the early stage. More studies should be performed to investigate further the related biological mechanisms. Animal experiments should also be conducted in order to confirm this adipose-inhibitory effect *in vivo* and to develop possible dietary flavonoid applications for use in the control of obesity.

#### ACKNOWLEDGEMENTS

The authors would like to thank the Kuo-Fan

Educational Foundation of the Republic of China, Taiwan (Contract No. 092E0189), and the National Science Council of the Republic of China, Taiwan (Contract No. NSC92-2313-B-264-002) for partially supporting this research.

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