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Anthocyanins Inhibit Lipogenesis during Adipocyte Differentiation of 3T3-L1 Preadipocytes

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Anthocyanins have been shown to prevent obesity and to ameliorate hyperglycemia in animal studies. In this study, induction of preadipocytes in the presence of anthocyanins (1-40μg/ml), 3T3-L1 preadipocytes reduced accumulation of cytoplasmic triglycerides by dose- and time-dependent manner. This phenomenon was rapidly reversible. In addition, when applied to mature 3T3-L1 adipocytes, anthocyanins induced a moderate reduction in cellular triglyceride content. mRNA and protein expression levels of sterol regulatory element-binding protein-1c (SREBP-1c) and liver X receptor α (LXRα), which act as key transcription factors of lipogenesis, as well as their target genes such as fatty acid synthase (FAS), stearoyl-CoA desaturase-1 (SCD-1) and acetyl CoA carboxylase-α (ACC-1) were markedly suppressed by anthocyanin treatment. Anthocyanins also decreased mRNA or protein expression of peroxisome proliferators-activated receptor-γ (PPARγ) and CCAAT enhancer-binding protein-α (C/EBPα), which act as key transcription factors of adipogenesis were moderately reduced by anthocyanin treatment. Collectively, this study suggests that the inhibitory effect of anthocyanins on lipogenesis might be mediated through down-regulation of lipogenic transcription factors as well as proteins related on lipid synthesis such as SCD-1, FAS and ACC-1. These results suggest that anthocyanins might be important functional compounds in the prevention of obesity.

Key words : anthocyanins, lipogeenesis, adipogenesis, 3T3-L1, obesity

Introduction

Obesity has become a growing health problem in industrialized countries and is closely related with diabetes, cardiovascular disease, and cancer[1]. It arises from enlarged adipocytes caused by lipid accumulation and from increased number of adipocytes result from differentiation of preadipocytes to mature adipocytes. Many studies reported adipogenesis by examining the time course of expression of several key transcriptional factors known to be involved in adipocyte differentiation[2,3,4], including CCAAT enhancer-binding protein-α (C/EBPα), peroxisome proliferator-activated receptor γ (PPARγ), and sterol regulatory element-binding protein 1c (SREBP 1c). During adipogenesis, accumulation of triglyceride is increased by the activation of lipogenic pathway. Lipogenesis in adipocytes is controlled by two different transcription factor families, SREBPs and LXRs. LXRα and β are transcription factors regulating genes involved in cholesterol and fatty acid metabolism. It is demonstrated that oxysterol and LXR agonist are able to activate LXRs, leading to increased SREBP-1c expression in liver and adipose cells[4,5]. LXRα is predominantly expressed in adipose tissue, liver, kidney, and spleen, whereas LXRβ is ubiquitously expressed[6]. The activation of LXRα during adipocyte differentiation increases lipogenesis in 3T3-L1, 3T3-F442A, and primary human preadipocytes[4,7,8]. Activated LXRα induced lipogenesis involving the accumulation of lipid droplets through activation of SREBP-1c. Moreover, LXRα activation in 3T3-L1 cells increase adipocyte morphology such as lipid accumulation and expression of the lipogenic FAS and SREBP-1c genes[9]. Apart from indirect actions through SREBP-1c, LXR also directly influences transcription of FAS[10] and SCD-1[11].

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Anthocyanins are a group of naturally occurring polyphenolic compounds in the plant kingdom[12]. These compounds are widely present in the human diet due to their occurrence in fruits and vegetables, and daily anthocyanin intake has been estimated at around 180 mg/d in the United States[13]. Unlike other polyphenols, glycosides of anthocyanins are efficiently absorbed from the stomach and small intestine and excreted into bile and urine as intact glycoside as well as methylated forms and glucuronidated derivatives[14,15,16]. A wide range of biological activities of anthocyanins has been reported, such as antioxidant[17,18], anti-inflammatory[19,20], anticancer[21,22], anti-atherosclerosis[23,24], and antihyperglycemic activity in mice[25] and in streptozotocin-induced diabetic rats[26].

Recently, dietary anthocyanins significantly suppressed the development of obesity and ameliorated hyperglycemia induced by the high-fat diet feeding in mice[25]. Dietary anthocyanins suppressed the mRNA levels of the enzymes involved in the fatty acid and triacylglycerol synthesis accompanied by the reduction of the SREBP-1 mRNA level in the white adipose tissue[27]. Furthermore, anthocyanins enhanced adiponectin and leptin secretion, and up-regulated the adipocyte-specific gene expression without activation of PPARγ in isolated rat adipocytes[27]. These results suggest that anthocyanins have a potential utilization for functional foods as preventive and therapeutic use for obesity and insulin sensitivity. However, effects of anthocyanins on the lipogenesis and adipogenesis during 3T3-L1 cells and change of gene expression in cultured cells were not reported.

The purpose of the present study was to investigate the effect of anthocyanins on the lipogenesis of 3T3-L1 preadipocytes. To verify the effect of anthocyanins on the lipogenesis, we measured the accumulation of cellular triglycerides and the change of mRNAs and proteins related on lipogenesis and adipocyte differentiation.

Materials and Methods

Materials

Anthocyanins isolated from grape were provided by U. of Arkansas and their compositions were displayed in Table 1. Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum were purchased from Gibco Life Technologies (Gaithersburg, MD). Polyclonal antibodies against peroxisome proliferators-activated receptor γ (PPARγ), CCAAT enhancer-binding protein α (C/EBPα), sterol regulatory element-binding protein-1c (SREBP-1c), fatty acid synthase (FAS), acetyl-CoA carboxylase 1 (ACC-1), stearoyl-CoA desaturase-1 (SCD-1), and β-actin were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). The enhanced chemiluminescence (ECL) detection kit and secondary antibodies, horse-radish peroxidase-conjugated anti-mouse IgG and anti-goat IgG were purchased from Amersham Pharmacia Biotech (Piscataway, NJ).

Table 1. Composition of anthocyanins used in this study

Anthocyanin	Content
Delphinidin	130 mg/g
Cyanidin	30 mg/g
Petunidin	154 mg/g
Peonidin	75 mg/g
Malvidin	338 mg/g

Cell culture and treatment

Murine 3T3-L1 preadipocytes (ATCC, Rockville, MD) were cultured in DMEM with 4.5 g/liter glucose, 10% fetal bovine serum (Sigma, St Louis, MO), penicillin/streptomycin (100 U/ml penicillin and 100μg/ml streptomycin in 0.85% saline), and 100μM sodium pyruvate at 37℃ in 95% air-5% CO_2. After 2-day postconfluence, 3T3-L1 preadipocytes were induced to differentiate by the addition of 100μM 3-isobutyl-1-methylxanthine, 250 nM dexamethasone, and 170 nM insulin (MDI). On day 3, the MDI medium was replaced with DMEM containing 10% FBS and 170 nM insulin, which was changed every 2 days thereafter until analysis. When mentioned, cells were cultured in the absence (Me_2SO alone) or in the presence of anthocyanins (dissolved in Me_2SO) at concentrations and periods of time indicated under "Results". The final concentration of Me_2SO did not exceed 0.5%. During the period of anthocyanin exposure, the medium was changed every 2 days for control and treated cells. Anthocyanins were reconstituted as 50 mg/ml stock solutions in DMSO, filter sterilized, and stored at -20℃

Proliferation assay

Preconfluent 3T3-L1 preadipocytes were seeded in 96-well plates at a density of 10,000 cells/100μl/well. Vehicle or anthocyanins, in dose ranging from 0 to 40 ug/ml anthocyanins were added to culture medium with or without cells at the time of plating. At 24 and 48 h after plating, a colorimetric proliferation assay (Cell Titer 96 Aqueous nonradioactive cell proliferation assay; Promega, Madison, WI) was performed as directed by the manufacturer's manual. To calculate the absorbance values at each dose and time point, the mean absorbance of three blank wells (containing anthocyanins in culture medium without cell) was subtracted from the mean absorbance of six wells containing cells.

Triglyceride assay

Cells were washed twice with PBS and collected by scraping with a cell scraper into 50 mM sodium phosphate buffer (pH 7.4) containing 2 mM EDTA and 2 M NaCl. The harvested cells were sonicated 2 times for 5 sec at 40 watt with the microtip of a Branson model 250 Sonifier. After centrifugation at 14,000 rpm for 10 min at 4 ℃, TG content was analyzed using a commercial kit (GPO-trinder, SIGMA) according to the manufacturer's instructions.

RNA isolation and real-time RT-PCR

Total RNA was isolated from 3T3-L1 cells during differentiation using Trizol reagent (Life Technologies Inc.) and treated with DNase I (QIAGEN Inc.) according to the manufacturer's instructions. Reverse transcription and PCR were performed with QuantiTect SYBR Green RT-PCR Kit according to the manufacturer's instructions (QIAGEN Inc.) on an ABI Prism 7900 Sequence Detection System (Applied Biosystems). Total RNA was diluted in the range of 20-100 ng and used to establish standard curves for each individual gene. Post-PCR melting curves confirmed the specificity of single-target amplification. The quantitation of each mRNA was normalized by the expression levels of β-actin, and all experiments were repeated for 5 - 7 times. Primers used in this study were as follows: PPARγ, 5'-CCCTGGCAAAGCATTTGTAT-3' (forward) and 5'-GAAACTGGCACCCTTGAA AA-3' (reverse); SREBP-1c, 5'-AGCTCAAAGACCTGGTGGTG-3' (forward) and 5'-TCATGCCCTCCATAGACACA-3' (reverse); C/EBPα 5'-TTACAACAGGCCA GGTT TCC-3' (forward) and 5'-AACTCCAGTCCCTCTGGGAT-3' (reverse); LXRα, 5'-TCTGCAATTGAGGTCATGCT (forward) and 5'-TGCAGAGAAGATGCTGATGG-3 (reverse); SCD-1, 5'-GAACTTACAAGGC TCGGCTG-3' (forward) and 5'-AGACATGTCCAGTTTTCCGC-3' (reverse); GLUT 4, 5'-ATCTTGATGACCGTGGC TCT-3' (forward) and 5'-TAGCATCC GCAACATACTGG-3' (reverse); ACC-1, 5'-TCTGATTTGGGGATCTCTGG-3' (forward) and 5'-CAAATTCTGCTGGAGAAGCC-3' (reverse); FAS 5'-GCTG TGCTTGCAGCTTACTG-3' (forward) and 5'-TGTCCTCAGAGTTGTGGCAG-3' (reverse) and β-actin, 5'-CTGACTGACTACCTCA TGAAGATCCT-3' (forward) and 5'-CTTAATGTCACGC ACGATTTCC-3' (reverse).

Western immunoblot analysis

3T3-L1 cells were washed twice with ice-cold PBS, layered with lysis buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 % Nonidet-40, 1 % Tween-20, 0.1% SDS, 1 mM Na_3VO_4, 10μg/ml leupeptin, 50 mM NaF, 1 mM PMSF), and stood on ice for 1 hr to lysis. After centrifuge at 18,000 g for 10 min, protein content of supernatant was measured, and aliquots (40μg) of protein were separated by SDS-PAGE and transferred onto a nitrocellulose membrane. The membrane was blocked with 5 % non-fat dried milk in TBST buffer (10 mM Tris-HCl, pH 8.0, 150 mM NaCl,, and 0.06% Tween-20) for 1 hr, and incubated for 1 hr with primary antibody in TBST containing 5 % non-fat dried milk. The blots were treated with horseradish peroxidase-conjugated secondary antibody in TBST containing 5 % non-fat dried milk for 30 min, and immune complex was detected using ECL detection kit (Amersham Pharmacia).

Statistical analysis

Data are expressed as the mean ± SEM. Data were analyzed using one-way ANOVA, followed by each pair of Student's t-tests for multiple comparisons. Differences were considered significant if $P < 0.05$. All analyses were performed using SPSS for Windows, version 10.07 (SPSS, Chicago, IL).

Results

Cytotoxicity of anthocyanins on 3T3-L1

Because few studies had reported that anthocyanin caused cytotoxicity, we characterized whether the anthocyanins induce toxicity on 3T3-L1. Anthocyanins used in this study were not shown cytotoxicity up to 40 μg/ml, and anthocyanin-induced suppression of TG accumulation was not relevant to the cell cytotoxicity (data not shown).

Effects of anthocyanins on TG accumulation during adipocyte differentiation

To evaluate the effects of anthocyanins on the suppression of triglyceride accumulation, 3T3-L1 preadipocytes were exposed from confluence with various concentration of anthocyanins for 7 days. Triglyceride content decreased significantly from 2.5 μg/ml of anthocyanins and continued remarkable reduction with increasing anthocyanins (Fig. 1). The reduction of triglyceride was maximal at 40 μg/ml of anthocyanin treatment, with a 75% reduction in triglyceride content as compared with control cells. The half maximal effect was obtained around 10 μg/ml of anthocyanin treatment.

To determine the time dependence of anthocyanin effect on triglyceride accumulation, 3T3-L1 cells were differentiated on day 0 in the absence or presence of 2.5 and 20 μg/ml of anthocyanins and continued anthocyanin treatment to harvesting day. Until 3 days of treatment following induction, cellular triglyceride content was not changed. However, triglyceride accumulation was rapidly increased after 5 day induction (Fig. 2). Triglyceride accumulation by 20 μg/ml anthocyanin treatment was not significantly changed until 11 day culture.

To confirm the influence of anthocyanins on the expression of adipogenic transcription factors, 3T3-L1 cells were treated from induction with various concentrations of anthocyanins for 7 days and mRNA expression level of adipogenic transcription factors such C/EBPα and PPARγ were analyzed by real-time RT-PCR. During adipogenesis, the expression of C/EBPα mRNA was suppressed with dose dependent pattern from 1 μg/ml anthocyanin treatment and showed 20% of control group at 40 μg/ml anthocyanins. However, suppression of PPARγ mRNA was moderately suppressed by exposure to low anthocyanin

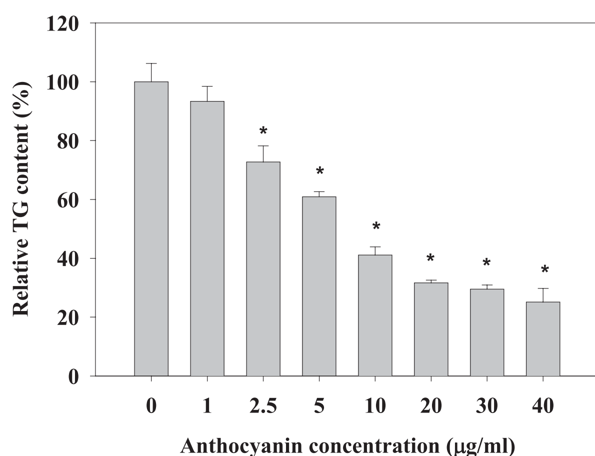

Fig. 1. Effect of anthocyanins on triglyceride accumulation during adipogenesis of 3T3-L1. 2-Day post-confluent 3T3-L1 preadipocytes (day 0) were stimulated for 3 day with MDI medium containing indicated concentration of anthocyanins. On day 3, the MDI medium was replaced with DMEM/10 % FBS culture medium containing 10 μg/ml insulin and indicated concentrations of anthocyanins. Medium was changed at day 3 and 5, and cells were harvested at day 7 for triglyceride analysis. Results represent the mean ± S.E of 3 separate experiments. *, p<0.01; **, p<0.001, anthocyanin treated versus control cells.

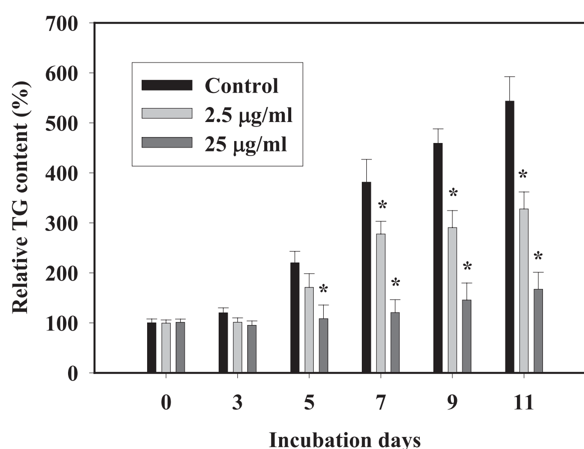

Fig. 2. Effect of culture time on triglyceride accumulation during adipogenesis of 3T3-L1 with or without anthocyanins. 2-Day postconfluent 3T3-L1 preadipocytes (day 0) were stimulated for 3 day with MDI medium containing 2.5 and 20 μg/ml anthocyanins. On day 3, the MDI medium was replaced with DMEM/10 % FBS culture medium containing 10ug/ml insulin and 2.5 and 20 μg/ml anthocyanins. Medium containing indicated concentration of anthocyanins was changed at day 3, day 5, day 7 and day 9. Cells were harvested at day 0, 3, 5, 7, 9 and 11 after induction. Control was contained same volume of DMSO.

concentrations, with a more pronounced effect at 40 μg/ml anthocyanins (Fig. 3).

In addition to regulation of adipogenesis by LXRα [41], activation of LXRα stimulates lipogenic genes including FAS, ACC-1, and SCD-1 through transcriptional activation of SREBP-1c[28]. In order to evaluate anthocyanins on the lipogenesis in 3T3-L1 cells, we determined mRNA expression levels of LXRα, SREBP-1c, FAS, ACC-1, and SCD-1 in anthocyanin-induced 3T3-L1 cells. As shown in Fig. 3, anthocyanins caused dramatic down-regulation of LXRα and SREBP-1c mRNA level, which is significantly decreased at low concentrations. Suppression of LXRα and SREBP-1c mRNA was a maximal approximately 65% and 80%, respectively, at 40 μg/ml anthocyanins and a half maximal effect was approximately 15 ug/ml anthocyanins. However, expression of PPARγ was not much suppressed by anthocyanins, with a maximal 50% inhibition at 40 ug/ml anthocyanins.

To ensure the functional consequences of LXRα, C/EBPα, SREBP-1c, and PPARγ down-regulation by anthocyanins, we analyzed the effect of anthocyanins on the expression of their target genes, such as those coding for fatty acid synthase (FAS) and stearyl-CoA desaturase-1 (SCD-1). As shown in Fig. 4, a dose-dependent decrease in mRNA levels of FAS and SCD-1 was observed. Down-regulation of these genes was detected over 10 μg/ml of anthocyanin concentation and mRNA levels of FAS and SCD-1 treated with 40 μg/ml anthocyanins represented 56 and 33%, respectively, of the control value. Therefore, the effect of anthocyanins on SREBP-1c target proteins such as FAS and SCD-1 closely related expression of transcription factors.

To clarify whether anthocyanins influence only mRNA expression level or affect both mRNA and protein expression in 3T3-L1 cells, Western blot analysis was conducted. Transcription factors related with lipogenesis such as SREBP-1c, and its downstream proteins such as SCD-1, FAS and ACC-1 were analyzed with Western blot. Fig. 5 shows that anthocyanin treatment induced a reduced expression of SREBP-1c, which is important transcription factor for lipogenesis. The inhibition of SREBP-1c expression was accompanied by a sharp reduction in the protein expression of SREBP-1c target genes such as FAS and SCD-1, which is a good agreement with gene express-

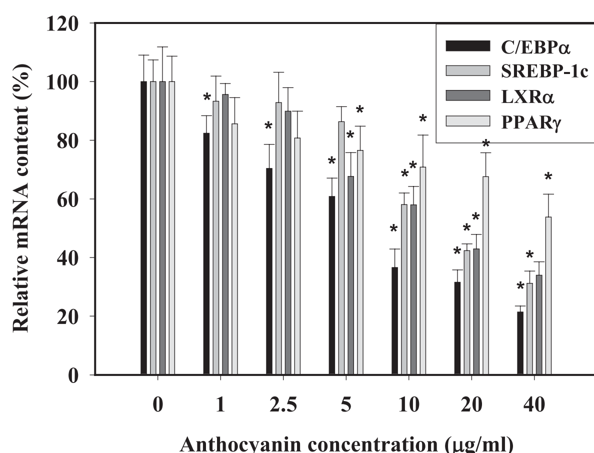

Fig. 3. Dose-dependent effect of anthocyanins on LXRα, C/EBPα, SREBP-1c, and PPARγ mRNA levels in differentiating 3T3-L1 cells. 2-day postconfluent 3T3-L1 preadipocytes (day 0) were stimulated for 3 day with MDI medium containing indicated concentrations of anthocyanins. On differentiation day 3, the MDI medium was replaced with DMEM/10 % FBS culture medium containing 10 μg/ml insulin and indicated concentrations of anthocyanins. Medium was changed at differentiation day 3 and 5, and cells were harvested at differentiation day 7 for RT-PCR. Expression levels of mRNA were compensated with that of β-actin.

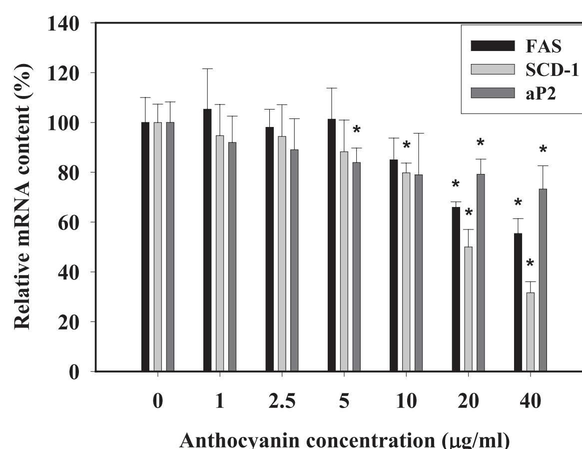

Fig. 4. Dose-dependent effect of anthocyanins on AP2, FAS and SCD-1 mRNA levels in differentiating 3T3-L1 cells. 2-day postconfluent 3T3-L1 preadipocytes (day 0) were stimulated for 3 day with MDI medium containing indicated concentrations of anthocyanins. On differentiation day 3, the MDI medium was replaced with DMEM/10 % FBS culture medium containing 10 μg/ml insulin and indicated concentrations of anthocyanins. Medium was changed at differentiation day 3 and 5, and cells were harvested at differentiation day 7 for RT-PCR. Expression levels of mRNA were compensated with that of β-actin.

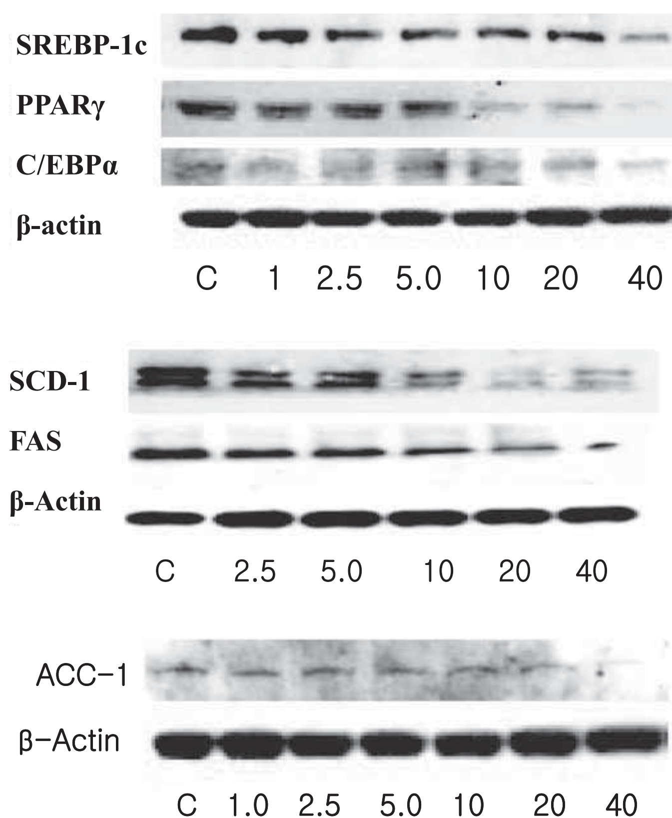

Fig. 5. Dose-dependent effect of anthocyanins on SREBP-1c, PPARγ, C/EBPα,, FAS, and SCD-1 protein expression. 2-day postconfluent 3T3-L1 preadipocytes (day 0) were stimulated for 3 day with MDI medium containing indicated concentrations of anthocyanins (μg/ml). On differentiation day 3, the MDI medium was replaced with DMEM/10% FBS culture medium containing 10μg/ml insulin and indicated concentrations of anthocyanins. Medium was changed at differentiation day 3, 5 and 7 day, and the cells were harvested at differentiation day 9 for Western blotting analysis.

ion result (Fig. 4). However, marked down-regulation of ACC-1 was only clearly detectable at the highest concentration of anthocyanins at 40μg/ml. In addition, anthocyanins induced suppression of adipogenic transcription factors such as C/EBPα and PPARγ with high concentration of anthocyanins (10-40μg/ml). The result confirmed that anthocyanins have more influence on lipogenesis than adipogenesis.

Discussion

Anthocyanins are water-soluble pigments of the red to blue range of the visible spectrum and a group of naturally occurring polyphenolic compounds in the plant kingdom. The few existing animal studies that have evaluated the effects of anthocyanin treatment on lipid metabolism or body weight support our findings in 3T3-L1 cells. Tsuda et al.[25,27] examined the effect of anthocyanins from purple corn on the lipid metabolism of high-fat diet rats and found that dietary anthocyanins significantly decreases liver total lipid and liver triglyceride. They demonstrated that decreased lipid levels were result from suppressed mRNA expression of FAS, acetyl-CoA synthase 1 (ACS 1), glycerol-3-phosphate acyltransferase (GPAT), and SREBP-1 in liver and white adipose tissue. Furthermore, the suppression effect of anthocyanins on obesity was demonstrated by increase of adipocytokine (adiponectin and leptin) secretion and by up-regulation of adipocyte specific genes (LPL, aP2 and UCP2) without activation of PPARγ[27] in isolated rat adipocytes. In the present study, semi-purified anthocyanins (72.8% anthocyanins) from grape were treated during adipogenesis of 3T3-L1 cells and intracellular lipid store was significantly decreased. This result was probably related to a strong reduction in the expression of the lipogenic transcription factor SREBP-1c and its upstream nuclear factor LXRα.

Anthocyanins clearly prevented the cells from accumulating lipid when incubated with preadipocytes during the dif-

ferentiation process. We demonstrated that the anthocyanins used with low concentrations prevented lipid storage and cell differentiation during adipose conversion of 3T3-L1 preadipocytes. These two effects displayed distinct sensitivities of anthocyanins to inhibition of triglyceride accumulation occurring at lower concentrations of anthocyanins. Dietary sesamin decreased lipogenic enzyme gene expression through the reduction of the SREBP-1 mRNA level and protein content of the precursor and its mature forms[28]. Also dietary purple corn anthocyanins could remarkably reduce FAS mRNA level through a reduction in the SREBP-1 mRNA level[25]. Anthocyanins from grape also reduce lipid accumulation in mature adipocytes. This phenomenon is probably related to a strong reduction in the expression of the lipogenic transcription factor SREBP-1c (Fig. 3). Anthocyanins decreased SREBP-1c mRNA levels and the expression of the related mature 68 kDa protein. Because of the mature form of SREBP-1c is known to promote lipogenic gene expression[29], decrease in the levels of the 68 kDa SREBP-1c protein contribute to suppressed lipogenesis in anthocyanin-treated cells in adipogenesis as well as in mature cells. In agreement with anthocyanin-induced down-regulation of SREBP-1c at transcriptional and post-translational level, we observed a reduction in adipocyte lipogenic activity and decrease in the expression in SREBP-1c target genes, such as FAS, ACC-1, and SCD-1, important enzymes for lipogenesis.

Recent studies have also shown that administration of anthocyanins to C57BL/6 mice caused a dramatic reduction of lipid accumulation in the liver, which suggest that anthocyanins might regulate lipid metabolism by affecting hepatic lipid oxidation and lipogenesis[30]. Cyanidin aglycone and its glycoside up-regulated the adipocyte-specific gene expression[27] and genes involved in lipid metabolism[31]. Suppression of LXRα by anthocyanin treatment induced down-regulation of lipogenic transcription factor, SREBP-1c, with similar dose-dependent pattern in this study (Fig. 3). SREBP-1c plays a critical role in the transcriptional regulation of a number of genes in the lipogenic pathway, including FAS, SCD-1, and ACC-1[32]. Decreased expression of SREBP-1c in the anthocyanin-treated 3T3-L1 caused suppressed expression of lipogenic genes, FAS, ACC-1, and SCD-1 displayed in Fig. 4. This result suggests that suppression of lipogenesis by anthocyanin treatment in 3T3-L1 cells was mainly caused by down-expression of lipogenic genes (FAS, ACC-1, and SCD-1) through suppression of LXRα and SREBP-1c.

With treatment of anthocyanins during differentiation of 3T3-L1 preadipocytes, expression level of C/EBPα was remarkably reduced, however, that of PPARγ and its target gene, aP2, was moderately suppressed by exposure to high concentration of anthocyanins (Fig. 3). However, the mRNA level of the PPARγ and its target genes such as LPL, aP2, and UCP in isolated rat adipocytes treated with cyanidin were significantly increased by the treatment of cyanidin compared to the control[27], which is a discrepancy with present result. They suggested that up-regulation of adipocyte-specific genes by cyanidin was not due to the stimulation of the PPARγ ligand activity, but possibly due to the mechanism of AMPK activations. Since C/EBPα and PPARγ are key genes in differentiated adipocytes, it is likely that the suppression of C/EBPα, not PPARγ, at low concentration of anthocyanins partially influenced on the adipocyte differentiation. This result suggests that low concentration of anthocyanins exert much influence on the lipogenic process than adipogenic process in 3T3-L1 cells.

At high concentration (40 μg/ml), anthocyanins remarkably changed adipocyte differentiation. This result can be brought together with the observation that C/EBPα and PPARγ gene and protein expression with decreased level at this high concentration of anthocyanins. Supression of PPARγ at high concentration of anthocyanins may influence on the adipogenesis of 3T3-L1 as shown in Fig. 3. The pattern of aP2 mRNA expression, which corresponds to a PPARγ and C/EBPα [3] target gene but not to a SREBP-1c target gene, is quite similar to that of PPARγ transcript. Thus, a high concentration of anthocyanins may only influence on the suppression of adipose conversion through PPARγ and aP2 expression.

The concentrations of anthocyanins required to elicit its effects on lipogenesis are within the range of those plasma concentration[33]. Thus, it is possible that the effect of anthocyanins on the 3T3-L1 cell lines observed in vitro may also occur in vivo. Interestingly, a recent work has reported that administration of low dose grape powder (150 μg of total polyphenol per day) exerted a reduction of

cholesterol accumulation and attenuate atherosclerosis development in apolipoprotein D deficient mice[23]. In this study, anthocyanins primarily affect the lipogenic pathway in differentiating and mature fat cells. It is generally recognized that whereas the lipogenic activity has a central role in energy storage in cultured preadipose cell lines and in adipose tissue from rodent species[34], this pathway has been reported to exert an accessory function in human adipose tissue[35]. In humans, the liver is the central organ for de novo lipogenesis. As suggested in our experimental results, the antilipogenic properties of anthocyanins in adipocyte may help to ameliorate obesity in addition to liver function.

In summary, we provide evidence that anthocyanins decrease lipid accumulation in differentiating preadipocytes and mature adipose cells through suppression of lipogenic and adipogenic genes. Our results suggest that consumption of grape anthocyanins has the potential to prevent obesity. The present study indicated that possible mechanism for the suppression of lipid accumulation by anthocyanins is relevant to the down-regulation of LXRa, SREBP-1c and their target genes. Further experimental investigations will be helpful to understand which anthocyanin compound has key role in the suppression of lipogenesis or adipogenesis in 3T3-L1 adipose cells.

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