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Microarray profiling of gene expression in human adipocytes in response to anthocyanins

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ACOX1, acylCoA oxidase1
CCAAT/enhancer binding protein, C/EBP
C3G, cyanidin 3-O-β-D-glucoside
Cy, cyanidin
DMEM, Dulbecco's modified Eagle's medium
gp130, glycoprotein 130
IL, interleukin
OCM, oncostatin M
PAI-1, plasminogen activator inhibitor-1
PLN, perilipin

ABSTRACT

Adipocyte dysfunction is strongly associated with the development of obesity and insulin resistance. It is accepted that the regulation of adipocytokine secretion or the adipocyte specific gene expression is one of the most important targets for the prevention of obesity and amelioration of insulin sensitivity. Recently, we demonstrated that anthocyanins, which are pigments widespread in the plant kingdom, have the potency of anti-obesity in mice and the enhancement adipocytokine secretion and its gene expression in adipocytes. In this study, we have shown the gene expression profile in human adipocytes treated with anthocyanins (cyanidin 3-glucoside; C3G or cyanidin; Cy). The human adipocytes were treated with 100 μM C3G, Cy or vehicle for 24 h. The total RNA from the adipocytes was isolated and carried out GeneChip microarray analysis. Based on the gene expression profile, we demonstrated the significant changes of adipocytokine expression (up-regulation of adiponectin and down-regulation of plasminogen activator inhibitor-1 and interleukin-6). Some of lipid metabolism related genes (uncoupling protein2, acylCoA oxidase1 and perilipin) also significantly induced in both common the C3G or Cy treatment groups. These studies have provided an overview of the gene expression profiles in human adipocytes treated with anthocyanins and demonstrated that anthocyanins can regulate adipocytokine gene expression to ameliorate adipocyte function related with obesity and diabetes that merit further investigation.

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PPAR, peroxisome proliferator-activated receptor
TZD, thiazolidinediones
UCP2, uncoupling protein 2

Adipocyte is the primary site of energy storage and accumulates triacylglycerol during nutritional excess. In recent years, obesity is one of the central causal components in metabolic syndrome and it is well known that adipocyte dysfunction plays an important role in the development of this syndrome. Adipocyte synthesizes and secretes biologically active molecules called adipocytokines [1]. For example, leptin is the product of the ob gene and is secreted from adipocytes, and reduces food intake and increases energy expenditure [2]. Adiponectin is one of the most important adipocytokines, and is specifically and highly expressed in adipocytes [3]. The plasma adiponectin concentration and mRNA expression level are decreased in the obese and insulin resistant state [4,5]. The administration of adiponectin improves insulin action in humans [6,7]. Dysregulation of adipocytokines production is strongly associated with metabolic syndrome and amelioration of adipocyte dysfunction including adipocytokines expression is one of the important targets for prevention and therapies of metabolic syndrome [8].

There are some drugs, which are the target for regulation of the adipocyte function, that improve insulin sensitivity or glucose homeostasis [9,10]. Recently, much attention has been focused on some food factors that may be beneficial for the prevention of body fat accumulation and possibly reduce the risk of diabetes and heart disease. Although some drugs are used for the therapy of obese-related metabolic diseases or possibility discussed as preventing body fat accumulation, there has been little evidence that food factors themselves are directly beneficial for the improvement of the dysfunction of the adipocyte responsible for adipocytokine expression and lipid metabolism.

Anthocyanins are the largest group of water-soluble pigments in the plant kingdom. They are widely distributed in the human diet through crops, beans, fruits, vegetables and red wine [11], suggesting that we ingest significant amounts of anthocyanins from plant-based daily diets. In general, anthocyanin pigments are stable under acidic conditions, but are unstable and rapidly broken down under neutral conditions [12]. Therefore, anthocyanins have not been recognized as a physiological functional food factor [12]. However, we demonstrated that cyanidin 3-*O*-β-D-glucoside (C3G) (Fig. 1), which is a typical anthocyanin, had antioxidative and anti-inflammatory activities based on in vitro and in vivo studies [13–16]. These findings suggest that C3G has more beneficial effects beyond its antioxidant activity.

Recently, we demonstrated that dietary anthocyanins significantly suppressed the development of obesity, normalized hypertrophy of the adipocytes in the epididymal white adipose tissues and ameliorated hyperglycemia induced by the high-fat diet feeding of C57BL/6J mice [17]. These results suggest that anthocyanins can regulate obesity and insulin sensitivity associated with adiponectin and leptin secretion and peroxisome proliferator-activated receptor (PPAR) γ activation in adipocytes. Moreover, we have clearly demonstrated for the first time that anthocyanins enhance adipocytokine (adiponectin and leptin) secretion, the expression of PPARγ and adipocyte specific genes in isolated rat adipocytes without the stimulation of the PPARγ ligand activity [18]. It will promote an increased understanding of how anthocyanins influence gene expression including adipocytokine and regulate those responsible for the prevention of obesity and the amelioration of insulin sensitivity. However, the molecular action and mechanism of the anthocyanins responsible for the amelioration of insulin sensitivity and prevention of obesity through regulation of the adipocyte function is not fully understood and there has been also little evidence on whether or not anthocyanins affect expression including adipocytokines, lipid metabolism and diabetes-related genes in human adipocytes. Based on these background, the present study was designed to examine the gene expression profiling in human adipocytes treated with anthocyanins (C3G or cyanidin (Cy), which is an aglycon of C3G) (Fig. 1) using a DNA microarray. Also, based on the DNA microarray analysis, we found that anthocyanins affect the expression of the adipocytokines and lipid or energy metabolism-related genes in human adipocytes.

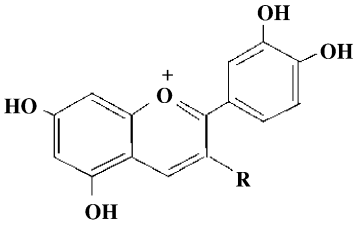
1. Materials and methods

1.1. Chemicals

C3G and Cy were obtained from Extrasynthèse (Genay, France), and their purities were greater than 99%. Human subcutaneous preadipocytes isolated from adipose tissue of healthy, non-diabetic woman (age 33 years, body mass index, 25.77) were obtained from Zen Bio, Inc. (Research Triangle Park, NC).

1.2. Cell culture of human adipocyte

The human preadipocytes were cultured using PM-1 medium (Zen-Bio Inc.) containing Dulbecco's modified Eagle's medium (DMEM)/Ham's F-10 medium (1:1, v/v), HEPES 15 mM (pH 7.4), 10% (v/v) of fetal bovine serum, penicillin (100 U/ml) streptomycin (100 μg/ml) and amphotericin B (0.25 μg/ml) in a humidified atmosphere (5% CO_2/95% air). After 3–4 days, the confluent cells were placed in differentiation medium (DM-2/10, Zen-Bio Inc.) containing DMEM/Ham's F-10 medium (1:1, v/v), HEPES 15 mM (pH 7.4), 3% (v/v) of fetal bovine serum, biotin (33 μM), pantothenate (17 μM), human insulin (100 nM), dexamethasone (1 μM), penicillin (100 U/ml), streptomycin (100 μg/ml), amphotericin B (0.25 μg/ml), isobutylmethylxanthine (0.20 mM) and PPARγ agonist (10 μM) for 3 days. The medium was then changed to AM-1 medium (Zen-Bio Inc.) containing DMEM/Ham's F-10 medium (1:1, v/v), HEPES 15 mM



R = -*o*-β-D-glucose; cyanidin 3-glucoside (C3G)
R = OH; cyanidin (Cy)

Fig. 1 – Chemical structures of cyanidin 3-O-β-D-glucoside (C3G) and cyanidin (Cy).

(pH 7.4), 3% (v/v) of fetal bovine serum, biotin (33 μM), pantothenate (17 μM), human insulin (100 nM), dexamethasone (1 μM), penicillin (100 U/ml), streptomycin (100 μg/ml) and amphotericin B (0.25 μg/ml). Medium was changed every 2–3 days and cells were cultured for additional 10 days. At 13 days after differentiation of preadipocytes into adipocytes, the adipocytes were treated with 100 μM C3G, Cy or vehicle (0.1% DMSO) for 24 h at 37 °C in a humidified atmosphere (5% CO_2/95% air).

1.3. *Isolation of total RNA and GeneChip microarray assay*

All experiments were performed in triplicate. The total RNA from the adipocytes was isolated using the RNeasy Lipid Tissue Mini Kit (QIAGEN, Tokyo, Japan) according to the manufacturer's directions. Subsequent RNA processing procedures followed protocols in the GeneChip Expression Analysis Technical Manual (Affymetrix, Santa Clara, CA). Briefly, the first and second cDNAs were synthesized from the total RNA (5.0 μg) using the Superscript Choice System (Life Technologies, Rockville, MD) with a T7-dT24 primer. Biotinylated cRNA was synthesized using the BioArray High Yield RNA Transcriptional Labeling Kit (Enzo Diagnostics, Farmingdale, NY). After purification of the cRNA using the RNeasy Mini Kit (Qiagen, Tokyo, Japan), 20 μg of the cRNA was fragmented by heating at 94 °C for 35 min, then an aliquot of the obtained fragmented cRNA (12.5 μg) was hybridized to the Human Genome Focus Array (Affymetrix, Santa Clara, CA) for 16 h at 45 °C. After hybridization, the arrays were washed and stained using streptavidin phycoerythrin (Molecular Probe, Eugene, OR), and the fluorescent signals were measured on the arrays using a GeneArray Scanner (Hewlett-Packard, Palo Alto, CA).

1.4. *Microarray data analysis and statistical treatment*

A DNA microarray data analysis was performed using GeneSpring Ver. 7.2 software (Silicongenetics, Redwood City, CA). Briefly, the array measurements for all samples were normalized using the control samples (treated with 0.1% dimethyl sulfoxide). After the normalization, the detectable expressed genes were defined using P-, M- or A-calls (according to Affymetrix algorithm) and the intensity for the genes. We found as the detectable expressed genes by filtering that both required (1) a minimum of four P- or M-calls out of nine measurements, and (2) all of sample signals was >35.0 out of nine measurements. After the filtering step, 4538 genes were selected as the detectable expressed genes from all genes. Treatment effects were analyzed using one-way ANOVA based on the filtered 4538 genes. If the ANOVA was significant ($P < 0.05$), post hoc Student–Newman–Keuls test was applied. Differences were considered significant at $P < 0.05$. Fold changes (>1.5- or <1.5-fold) compared to the controls were performed based on the significant different genes after the post hoc test.

1.5. *Quantification of gene expression level using real-time PCR*

Total RNA (1.0 μg) was reverse transcribed to cDNA in a reaction mixture in a final 20 μl using Takara RNA PCR kit (AMV) Ver. 3.0 (Takara Bio Inc., Shiga, Japan) according to the manufacture's directions. Quantification of gene expression in the human adipocytes treated with anthocyanins was measured using the real-time PCR system (ABI PRISM 7300 Sequence Detection System, Applied Biosystems, Tokyo, Japan). Amplification was performed in a final 25 μl containing 50 ng of cDNA, optimized specific primers and probes (TaqMan Gene Expression Assays, Applied Biosystems) and TaqMan Universal PCR Master Mix reagents (Applied Biosystems) according to the manufacture's directions. The assay ID No. of the TaqMan Gene Expression Assays were as follows; adiponectin: Hs00605917_m1, plasminogen activator inhibitor-1 (PAI-1): Hs00167155_m1, interleukin-6 (IL-6): Hs00174131_m1, uncoupling protein2 (UCP2): Hs00163349_m1, acylCoA oxidase1 (ACOX1): Hs00244515_m1, perilipin (PLN): Hs00160173_m1, β-actin: Hs99999903_m1. Results were expressed as fold increase relative to the controls after normalization using β-actin gene expression level.

1.6. *Statistical analysis for real-time PCR data*

The differences among the means were analyzed by Fisher's protected least significant difference test after one-way ANOVA. Differences in the $P < 0.05$ were considered significant. All the statistical analyses were performed using the StatView version 5.0 software for Macintosh (SAS Institute Inc., Cary, NC, USA).

2. Results

2.1. *Anthocyanins alter many genes expression in human adipocytes*

Table 1 shows the number of the significant up-regulated (>1.5-fold) or down-regulated (<1.5-fold) genes treated human adipocytes with C3G or Cy compared to the controls using the Human Genome Focus Array. Although the data were not shown, treatment of adipocytes with 100 μM of C3G or Cy for 24 h produced no cytotoxic effects (cell viability; more than 95%) using 3-(4,5-dimethyl-thiazol-2yl)-2,5-diphenyl tetrazolium bromide assays. The treated adipocytes with anthocyanins caused significant changes in the gene expression. The number of up-regulated genes in the Cy-treated group was greater than those of the C3G-treated group. A total of 33 genes were up-regulated (>1.5-fold) and 29 genes were down-

Table 1 – Number of the significant up- or down-regulated genes in human adipocytes treated with C3G or Cy

	Up-regulated (>1.5-fold)	Down-regulated (<1.5-fold)
C3G	49	36
Cy	93	111
Cy and C3G*	33	29

Determination of the significant up- or down-regulated genes were described in Section 1.
* Significant up- or down-regulated genes in both the common C3G and Cy-treated groups.

regulated (<1.5-fold) in both the common C3G and Cy-treated groups, respectively. However, the other genes were also up-regulated for only the C3G (16 genes) or Cy (60 genes) treatment and down-regulated for only the C3G (7 genes) or Cy (82 genes) treatment.

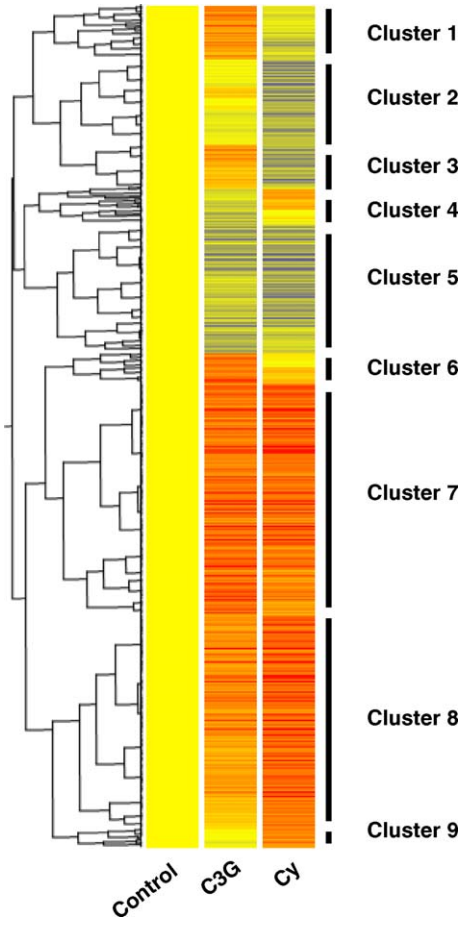


Fig. 2 – Hierarchical clustering display of data in adipocytes treated with C3G or Cy based on the significant 845 genes. The clustering display was performed using GeneSpring data analysis software. The details were described in Section 1. Each gene is represented by a single row of colored bars. The red color indicates up-regulation of the gene expressions and the blue color denotes down-regulation of the gene expressions compared to the controls.

Fig. 2 shows the hierarchical clustering display of data for adipocytes treated with C3G or Cy based on the significant 845 genes. Each gene is represented by a single row of colored bars. The genes were grouped into nine clusters each containing 19–234 genes. Fig. 3 shows the profiles of the clustering (up-regulated, down-regulated and not changed compared to the control group). The profiles of clusters 1 and 6 (83 genes in total) were affected (up-regulated) by only the C3G treatment. The cluster 2 (85 genes) summarized the down-regulation by only the Cy treatment. The cluster 3 (45 genes) summarized up-regulation by the C3G treatment and the down-regulation by the Cy treatment and compared to the control. The cluster 4 (37 genes) summarized the down-regulation by the C3G treatment and up-regulation by the Cy treatment. No significant difference was observed in the profiles of cluster 5 (128 genes), cluster 7 (234 genes) and cluster 8 (214 genes) between the C3G and Cy treatment. The cluster 9 (19 genes) summarized the up-regulation by only the Cy treatment.

Tables 2–5 show the list of the significant up-regulated genes (>1.5-fold) (Tables 2 and 3) and the down-regulated genes (<1.5-fold) (Tables 4 and 5) in human adipocytes treated with C3G or Cy. Treatment with C3G or Cy resulted significant influence of adipocytokine, cell cycle, enzyme including lipid metabolism or carbohydrate metabolism, signal transduction, stress response, transcription factor, receptor and transport genes. The number of up- or down-regulated genes by the treatment with Cy were more than that by the treatment of C3G.

2.2. *Up-regulation of adipocytokines and lipid metabolism related genes*

Table 6 shows the up-regulated (>1.5-fold) adipocytokine and lipid metabolism related genes list in the Array by the treatment with C3G or Cy. The treatment with C3G enhanced the energy expenditure related gene (UCP2; 2.26-fold, ACOX1; 1.58-fold, adiponectin; 1.57-fold) and PLN (1.54-fold) which were related with triacylglycerol degradation. UCP2, ACOX1, adiponectin and PLN were also up-regulated by the treatment of Cy (UCP2; 2.24-fold, ACOX1; 1.68-fold, adiponectin; 1.82-fold, PLN; 1.80-fold). CCAAT/enhancer binding protein (C/EBP) α, D component of complement (adipsin) and cathepsin D were specifically up-regulated by the treatment of Cy.

2.3. *Down-regulation of adipocytokines and lipid metabolism related genes*

Table 7 shows the down-regulated (<1.5-fold) adipocytokines and lipid metabolism related genes list in the Array by the treatment with C3G or Cy. Significant down-regulation was observed in PAI-1 (C3G treatment; 0.49-fold, Cy treatment; 0.39-fold) and IL-6 (C3G treatment; 0.58-fold, Cy treatment; 0.56-fold) in both the common C3G and Cy treated groups, respectively.

2.4. *Quantification of gene expression level using real-time PCR system*

The array data resulted in the fact that adipocytokine (adiponectin, PAI-1 and IL-6) were significantly altered (up-regulated; adiponectin, down-regulated; PAI-1 and IL-6) in both the common C3G and Cy treated groups. UCP2, ACOX1

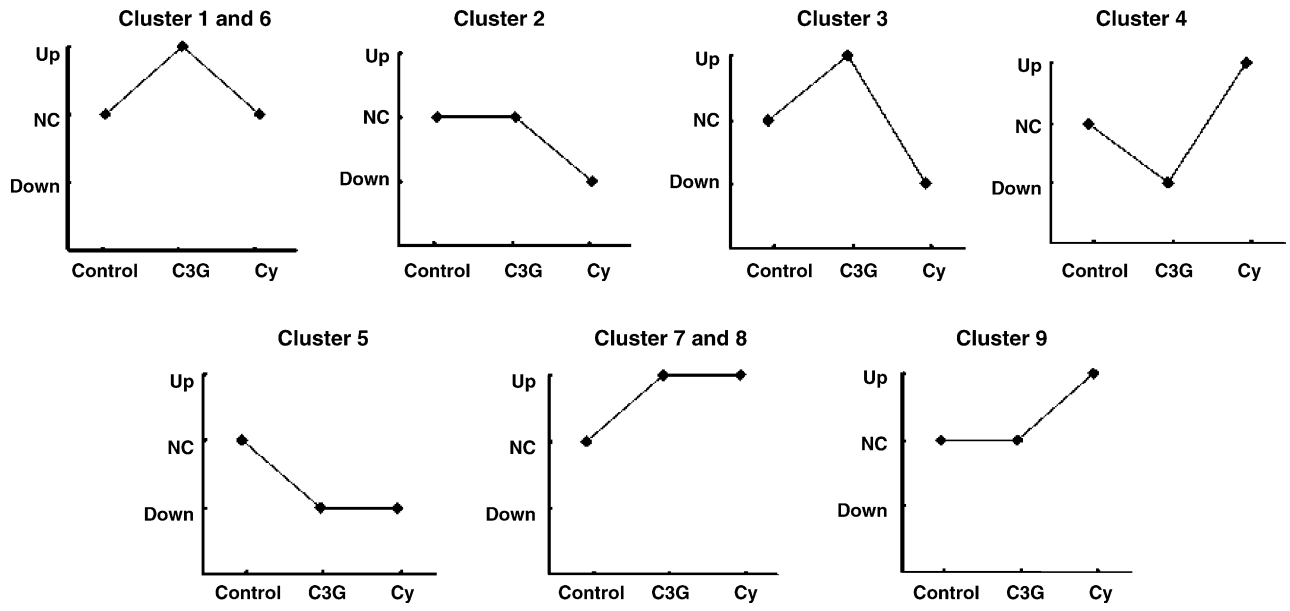


Fig. 3 – Profiles of each cluster (clusters 1–9) summarized in a hierarchical clustering display of data in adipocytes treated with C3G or Cy based on the significant 845 genes. These profiles indicate whether genes in a given cluster are significantly up-regulated (up), down-regulated (down), or not changed (NC) by the treatment with C3G or Cy.

and PLN related with lipid and energy metabolism were also up-regulated in both the common C3G and Cy treated groups. Based on these results, we confirmed the gene expression level of these genes using the quantitative real-time PCR system and compared with the array data. Treatment of the human adipocytes with C3G or Cy in 24 h caused a significant increase in the adiponectin mRNA level (C3G treatment; 1.73-fold, Cy treatment; 2.03-fold compared to the control) (Fig. 4A) and decrease in the PAI-1 (Fig. 4B) (C3G treatment; 0.58-fold, Cy treatment; 0.45-fold compared to the control) and IL-6 (Fig. 4C) (C3G treatment; 0.45-fold, Cy treatment; 0.43-fold compared to the control). UCP2 (Fig. 4D), ACOX1 (Fig. 4E) and PLN (Fig. 4F) were also significantly increased in both the common C3G and Cy treated groups compared to the control group in the 24 h incubation as well as these of the array data (UCP2; C3G treatment, 1.81-fold, Cy treatment, 1.95-fold, ACOX1; C3G treatment, 1.91-fold, Cy treatment, 2.04-fold, PLN; C3G treatment, 1.93-fold, Cy treatment, 1.99-fold compared to the control). The expression level of these genes was consistent with that of the array data. Although the data was not shown, preliminary experiments for dose response suggest that 100 μM dose of anthocyanins was the most effective on these gene expression without toxicity.

3. Discussion

Adipocyte dysfunction plays an important role in the development of obesity and insulin resistance. Some drugs are used for the therapy of obese-related metabolic diseases or in the discussion about the possibility of preventing body fat accumulation. In this study, we examined the gene expression profile of human adipocytes treated with anthocyanins and the potency of a unique pharmacological function of anthocyanins in human adipocytes through the DNA microarray analysis.

The human adipocytes treated with anthocyanins caused many changes in the gene expression. A hierarchical clustering profile display of data based on the significant 845 genes also suggest that 32% of the genes did not the same response in human adipocytes between the C3G and Cy treatment. These results raised a question as to why the structure of the anthocyanins affected the gene expression profile in the different ways. Cy is an aglycon of C3G, more hydrophilic than C3G. The affinity of the compounds for membrane may affect the response. The structure–activity relationship for the anthocyanins responsive genes should be clarified.

Adiponectin is one of the most important adipocytokines, and is specifically and highly expressed in adipocytes. We previously demonstrated that feeding C3G-rich diet significantly up-regulated adiponectin gene expression level in epididymal white adipose tissue in mice [18]. In this study, the gene expression level of adiponectin was significantly up-regulated in both the common C3G and Cy treated groups. The results of quantitative real-time PCR analyze were also consistent with the array data. These results suggest that anthocyanins have a potency regulating the gene expression level of adiponectin and have a therapeutic advantage involved in the regulation of the adipocyte function to improve insulin sensitivity.

Iwaki et al. showed that adiponectin gene expression was regulated via PPARγ and liver receptor homolog-1 [19]. PPAR γ and its target genes such as lipoprotein lipase or fatty acid binding protein 4 did not significantly changed in our microarray analyze. Also, our previous study demonstrated that anthocyanins did not stimulate the PPRE-dependent luciferase activities, indicating that up-regulation of the adipocyte specific genes by anthocyanins is not due to

Table 2 – The up-regulated (>1.5-fold) genes in human adipocytes treated with C3G

Accession no.	Fold change	Description	Function	Cluster
NM_005897	1.53	Intracisternal A particle-promoted polypeptide	Actin cytoskeleton	7
NM_004797	1.57	Adipose most abundant gene transcript 1 (adiponectin)	Adipocytokine	7
AW024335	1.64	Homo sapiens cDNA FLJ38630 fis, clone HHDPC2000070, mRNA sequence	Apoptosis	2
NM_000095	1.61	Cartilage oligomeric matrix protein (pseudoachondroplasia, epiphyseal dysplasia 1, multiple)	Cell adhesion	7
NM_005675	1.76	DiGeorge syndrome critical region gene 6	Cell adhesion	7
N33167	1.80	Cyclin-dependent kinase inhibitor 1C (p57, Kip2)	Cell cycle	7
AI922519	1.57	rab6 GTPase activating protein (GAP and centrosome-associated)	Cell cycle	7
NM_014454	1.60	p53 regulated PA26 nuclear protein	Cell proliferation	7
NM_001321	1.77	Cysteine and glycine-rich protein 2	Cell proliferation, differentiation	7
BC005264	1.60	Replication protein A3, 14 kDa	DNA replication	7
NM_000104	2.16	Cytochrome P450, subfamily I (dioxin-inducible), polypeptide 1 (glaucoma 3, primary infantile)	Electron transport	7
NM_000690	1.52	Aldehyde dehydrogenase 2 family (mitochondrial)	Enzyme	7
NM_001823	1.62	Creatine kinase, brain	Enzyme	7
NM_004480	1.66	fucosyltransferase 8 (alpha (1,6) fucosyltransferase)	Enzyme	2
NM_000637	1.66	Glutathione reductase	Enzyme	7
AL527430	1.68	Glutathione S-transferase M3 (brain)	Enzyme	7
NM_014214	1.55	Inositol(myo)-1(or 4)-monophosphatase 2	Enzyme	7
NM_000240	1.73	Monoamine oxidase A	Enzyme	7
NM_000285	1.59	Peptidase D	Enzyme	7
NM_004753	1.66	Short-chain dehydrogenase/reductase 1	Enzyme	8
NM_018973	1.81	Dolichyl-phosphate mannosyltransferase polypeptide 3	Enzyme/carbohydrate metabolism	7
NM_020379	1.64	Mannosidase, α, class 1C, member 1	Enzyme/carbohydrate metabolism	6
NM_016931	1.87	NADPH oxidase 4	Enzyme/electron transport	8
S69189	1.58	Acyl-Coenzyme A oxidase 1 (ACOX1), palmitoyl	Enzyme/lipid metabolism	7
BE895437	1.54	Thymidine kinase 2, mitochondrial	Enzyme/nucleic acid metabolism	7
NM_003748	1.61	Aldehyde dehydrogenase 4 family, member A1	Enzyme/proline metabolism	7
AI130969	1.54	Collagen, type V, α1	Extracellular matrix structural constituent	7
NM_004669	1.64	Chloride intracellular channel 3	Ion transport	7
NM_002666	1.80	Perilipin (PLN)	Lipid metabolism	7
NM_006400	1.54	Dynactin 2 (p50)	Microtubule-based process, mitosis	6
NM_005584	1.54	mab-21-like 1 (*C. elegans*)	Morphogenesis	7
NM_005258	1.64	GTP cyclohydrolase I feedback regulatory protein	Neurotransmitter metabolism	7
U79718	1.57	nth endonuclease III-like 1 (*E. coli*)	Nucleic acid metabolism	7
AL157437	1.62	GPAA1P anchor attachment protein 1 homolog (yeast)	Protein modification	7
NM_007038	1.91	A disintegrin-like and metalloprotease (reprolysin type) with thrombospondin type 1 motif, 5 (aggrecanase-2)	Proteolysis	
U94592	2.26	Uncoupling protein 2 (UCP2)	Proton transport	7
NM_004750	1.56	Cytokine receptor-like factor 1	Receptor	7
L37882	1.71	Frizzled homolog 2 (Drosophila)	Receptor	8
U91903	1.54	Frizzled-related protein	Receptor	7
NM_016235	1.51	G protein-coupled receptor, family C, group 5, member B	Receptor	7
NM_005619	1.55	Reticulon 2	Receptor	7
NM_001954	1.79	Discoidin domain receptor family, member 1	Receptor/cell adhesion	7
NM_001145	1.75	Angiogenin, ribonuclease, RNase A family, 5	RNA catabolism	7
NM_014424	1.72	Heat shock 27 kDa protein family, member 7 (cardiovascular)	Stress response	7
NM_014757	1.53	Mastermind-like 1 (Drosophila)	Transcription coactivator	8
NM_012082	1.56	Zinc finger protein, multitype 2	Transcription corepressor	7
NM_000151	1.76	Glucose-6-phosphatase, transport (glucose-6-phosphate) protein 1	Transport	7
NM_002555	1.89	Solute carrier family 22 (organic cation transporter), member 1-like	Transport	7
NM_012382	1.51	Osmosis responsive factor	Unknown	7

The altered genes and fold changes were determined as described in Section 1.

Table 3 – The up-regulated (>1.5-fold) genes in human adipocytes treated with Cy

Accession no.	Fold change	Description	Function	Cluster
NM_004797	1.82	Adipose most abundant gene transcript 1 (adiponectin)	Adipocytokine	7
NM_001928	1.67	D component of complement (adipsin)	Adipocytokine	8
NM_001909	1.52	Cathepsin D (lysosomal aspartyl protease)	Adipocytokine	7
X56841	1.66	Major histocompatibility complex, class I, E	Antigen presentation	8
NM_022094	1.51	Cell death activator CIDE-3	Apoptosis	7
NM_005675	1.78	DiGeorge syndrome critical region gene 6	Cell adhesion	7
N33167	2.10	Cyclin-dependent kinase inhibitor 1C (p57, Kip2)	Cell cycle	7
NM_002430	1.61	Meningioma (disrupted in balanced translocation) 1	Cell cycle	8
NM_002579	1.74	Paralemmin	Cell motility	8
NM_001981	1.54	Epidermal growth factor receptor pathway substrate 15	Cell proliferation	8
NM_001321	2.08	Cysteine and glycine-rich protein 2	Cell proliferation, differentiation	7
NM_006709	2.02	HLA-B associated transcript 8	Chromatin modification	8
BC005264	1.61	Replication protein A3, 14 kDa	DNA replication	7
NM_000104	4.35	Cytochrome P450, subfamily I (dioxin-inducible), polypeptide 1 (glaucoma 3, primary infantile)	Electron transport	8
NM_004753	2.09	Short-chain dehydrogenase/reductase 1	Enzyme	8
NM_006319	1.86	CDP-diacylglycerol–inositol 3-phosphatidyltransferase (phosphatidylinositol synthase)	Enzyme	8
NM_000713	1.70	Biliverdin reductase B (flavin reductase (NADPH))	Enzyme	8
NM_021198	1.70	Nuclear LIM interactor-interacting factor	Enzyme	8
NM_015974	1.66	Crystallin, lambda 1	Enzyme	7
NM_001823	1.64	Creatine kinase, brain	Enzyme	7
NM_001387	1.62	Dihydropyrimidinase-like 3	Enzyme	7
NM_003102	1.62	Superoxide dismutase 3, extracellular	Enzyme	7
NM_000637	1.61	Glutathione reductase	Enzyme	7
AL527430	1.59	Glutathione S-transferase M3 (brain)	Enzyme	7
NM_000285	1.57	Peptidase D	Enzyme	7
NM_000848	1.53	Glutathione S-transferase M2 (muscle)	Enzyme	7
BC002515	1.53	Aldehyde dehydrogenase 7 family, member A1	Enzyme	7
NM_002133	1.51	Heme oxygenase (decycling) 1	Enzyme	8
NM_012088	1.62	6-Phosphogluconolactonase	Enzyme/carbohydorate metabolism	8
NM_018973	1.90	Dolichyl-phosphate mannosyltransferase polypeptide 3	Enzyme/carbohydrate metabolism	7
NM_020379	1.87	Mannosidase, α, class 1C, member 1	Enzyme/carbohydrate metabolism	7
NM_000263	1.66	N-Acetylglucosaminidase, α-(Sanfilippo disease IIIB)	Enzyme/carbohydrate metabolism	7
NM_000690	1.50	Aldehyde dehydrogenase 2 family (mitochondrial)	Enzyme/carbohydrate metabolism	7
NM_016931	1.61	NADPH oxidase 4	Enzyme/electron transport	7
D13119	1.53	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit c (subunit 9), isoform 2	Enzyme/electron transport	8
BC001669	1.52	Oxidase (cytochrome c) assembly 1-like	Enzyme/electron transport	7
S69189	1.68	Acyl-Coenzyme A oxidase 1(ACOX1), palmitoyl	Enzyme/lipid metabolism	7
BE895437	1.72	Thymidine kinase 2, mitochondrial	Enzyme/nucleic acid metabolism	7
NM_002937	1.54	Ribonuclease, RNase A family, 4	Enzyme/nucleic acid metabolism	7
NM_003748	1.90	Aldehyde dehydrogenase 4 family, member A1	Enzyme/proline metabolism	7
AI130969	1.52	Collagen, type V, α1	Extracellular matrix structural constituent	7
NM_015937	1.74	Phosphatidyl inositol glycan class T	GPI anchor biosynthesis	8
NM_021070	1.61	Latent transforming growth factor β binding protein 3	Growth factor binding	8
NM_005532	1.90	Interferon, α-inducible protein 27	Immune response	8
NM_005031	1.78	FXYD domain containing ion transport regulator 1 (phospholemman)	Ion transport	8
X62078	1.83	GM2 ganglioside activator protein	Lipid metabolism	8
NM_002666	1.80	Perilipin (PLN)	Lipid metabolism	7
NM_005584	1.61	mab-21-like 1 (C. elegans)	Morphogenesis	7
NM_007165	1.76	Splicing factor 3a, subunit 2, 66 kDa	mRNA splicing	8
AF196468	1.52	Chromosome 6 open reading frame 28	mRNA splicing	7
NM_022719	1.76	DiGeorge syndrome critical region gene 14	Neurogenesis	8
NM_005258	1.97	GTP cyclohydrolase I feedback regulatory protein	Neurotransmitter metabolism	7
U79718	2.09	nth endonuclease III-like 1 (*E. coli*)	Nucleic acid metabolism	7
NM_006026	2.17	H1 histone family, member X	Nucleosome assembly	8
AI004246	1.54	Eukaryotic translation elongation factor 2	Protein biosynthesis	8
AL157437	1.70	GPAA1P anchor attachment protein 1 homolog (yeast)	Protein modification	7
NM_007038	2.10	A disintegrin-like and metalloprotease (reprolysin type) with thrombospondin type 1 motif, 5 (aggrecanase-2)	Proteolysis	7
NM_005040	1.51	Prolylcarboxypeptidase (angiotensinase C)	Proteolysis	8

Table 3 (*Continued*)

Accession no.	Fold change	Description	Function	Cluster
U94592	2.24	Uncoupling protein 2 (UCP2)	Proton transport	7
NM_016235	1.92	G protein-coupled receptor, family C, group 5, member B	Receptor	8
L37882	2.44	Frizzled homolog 2 (Drosophila)	Receptor	8
NM_005619	2.01	Reticulon 2	Receptor	8
NM_004750	1.84	Cytokine receptor-like factor 1	Receptor	7
NM_001954	1.84	Discoidin domain receptor family, member 1	Receptor/cell adhesion	7
NM_001280	1.57	Cold inducible RNA binding protein	Response to cold	7
NM_001145	1.90	Angiogenin, ribonuclease, RNase A family, 5	RNA catabolism	7
NM_012294	1.87	Guanine nucleotide exchange factor for Rap1; M-Ras-regulated GEF	Signal transduction	8
NM_003881	1.78	WNT1 inducible signaling pathway protein 2	Signal transduction	8
NM_001745	1.65	Calcium modulating ligand	Signal transduction	8
AK000095	1.64	Homo sapiens cDNA FLJ20088 fis, clone COL03869, mRNA sequence	Signal transduction	8
NM_021818	1.56	WW45 protein	Signal transduction	7
NM_000906	1.54	Natriuretic peptide receptor A/guanylate cyclase A (atrionatriuretic peptide receptor A)	Signal transduction	8
NM_003278	1.90	Tetranectin (plasminogen binding protein)	Skeletal development	8
NM_014424	2.87	Heat shock 27 kDa protein family, member 7 (cardiovascular)	Stress response	8
NM_005346	1.88	Heat shock 70 kDa protein 1B	Stress response	8
NM_005345	1.69	Heat shock 70 kDa protein 1A	Stress response	7
AF279899	1.54	Proline rich 2	Transcription coactivator	8
NM_014757	1.53	Mastermind-like 1 (Drosophila)	Transcription coactivator	7
NM_003662	1.66	Pirin	Transcription cofactor	8
NM_004364	1.75	CCAAT/enhancer binding protein (C/EBP), α	Transcription factor	8
NM_005461	1.71	v-maf musculoaponeurotic fibrosarcoma oncogene homolog B (avian)	Transcription factor	8
BF058726	1.59	TAF6-like RNA polymerase II, p300/CBP-associated factor (PCAF)-associated factor, 65 kDa	Transcription factor	8
NM_016202	1.52	LDL induced EC protein	Transcription factor	7
NM_002555	1.92	Solute carrier family 22 (organic cation transporter), member 1-like	Transport	7
AL138717	1.70	Rag D protein	Transport	8
NM_006744	1.54	Retinol binding protein 4, plasma	Transport	7
AL046054	1.67	Prostate tumor over expressed gene 1	tRNA aminoacylation	7
NM_007071	1.65	HERV-H LTR-associating 3	Unknown	8
H93077	1.61	Chromosome 5 open reading frame 4	Unknown	8
NM_006848	1.61	Hepatitis delta antigen-interacting protein A	Unknown	8
NM_013265	1.59	Chromosome 11 open reading frame2	Unknown	8
NM_015392	1.54	Neural proliferation, differentiation and control, 1	Unknown	7
AA451996	1.52	H2A histone family, member O	Unknown	8

The altered genes and fold changes were determined as described in Section 1.

stimulation of the PPARγ ligand activity, but is due to a PPARγ independent mechanism [18].

PAI-1 is known as one of the adipocytokines. It is expressed in adipose tissue and secreted into the plasma. PAI-1 is the primary inhibitor of plasminogen activation and increased plasma PAI-1 promotes thrombosis. The elevation of plasma PAI-1 level are closely related with the development of cardiovascular disorders [20,21]. It is known that the elevation of adipose PAI-1 expression is considered to be an important contributor to elevated plasma PAI-1 associated with both obesity and type 2 diabetes, suggesting that the regulation of PAI-1 expression is one of the important therapeutic targets for metabolic syndrome including cardiovascular disease based on obesity or hyperinsulinaemia [22–24]. In this study, treatment of human adipocytes with C3G or Cy clearly down-regulated PAI-1 gene expression level. These anthocyanins can be clinically useful as food factors to control PAI-1 level associated with metabolic syndrome.

The regulatory mechanism for the gene expression of PAI-1 in adipocytes is complex. Recent report showed intracellular reactive oxygen species production activates transcription factors such as nuclear factor-κB and induced PAI-1 synthesis [25]. Thiazolidinediones (TZD) attenuates PAI-1 expression in human adipose tissue and adipocytes [26,27]. Recent studies demonstrated that obesity is associated with macrophages infiltration into adipose tissue and the activation of inflammatory pathway caused with the development of insulin resistance [28,29]. Anti-inflammation function of TZD may contribute to improve insulin sensitivity as well as normalization of PAI-1 expression. Based on these observations, as the one of the possible mechanisms, the antioxidant and/or anti-inflammatory action of anthocyanins in adipocytes may contribute to the reduction of PAI-1 gene expression.

IL-6 is one of the cytokines and an important regulator of acute phase response. It is secreted by different cell types

Table 4 – The down-regulated (<1.5-fold) genes in human adipocytes treated with C3G

Accession no.	Fold change	Description	Function	Cluster
AL574210	0.49	Serine (or cysteine) proteinase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1 (PAI-1)	Adipocytokine	5
NM_000600	0.58	Interleukin-6 (IL-6)	Adippocytokine	5
NM_016109	0.56	Angiopoietin-like 4	Angiogenesis	5
AF001294	0.53	Tumor suppressing subtransferable candidate 3	Apoptosis	5
NM_000930	0.58	Plasminogen activator, tissue	Blood coagulation	5
BC004188	0.55	Tubulinβ, 2	Cytoskeleton	5
AF141347	0.56	Tubulinα, 3	Cytoskeleton	5
NM_006086	0.62	Tubulinβ, 4	Cytoskeleton	5
NM_000161	0.65	GTP cyclohydrolase 1 (dopa-responsive dystonia)	Enzyme	4
BC003143	0.57	Dual specificity phosphatase 6	Enzyme/cell cycle	5
BC001886	0.66	Ribonucleotide reductase M2 polypeptide	Enzyme/nucleic acid metabolism	5
U16996	0.51	Dual specificity phosphatase 5	Enzyme/signal transduction	5
AA780381	0.63	Mitogen-activated protein kinase kinase 3	Enzyme/signal transduction	5
L14561	0.54	ATPase, Ca^{++} transporting, plasma membrane 1	Enzyme/transport	5
U65585	0.54	Major histocompatibility complex, class II, DR β1	Immune response	5
BG252490	0.63	DnaJ (Hsp40) homolog, subfamily B, member 4	Protein folding	5
NM_016639	0.41	Tumor necrosis factor receptor superfamily, member 12A	Receptor	5
NM_001964	0.45	Early growth response 1	Receptor	5
NM_003483	0.61	High mobility group AT-hook 2	Regulation of transcription	4
NM_013285	0.63	Nucleolar GTPase	Ribosome biogenesis	5
NM_003090	0.63	Small nuclear ribonucleoprotein polypeptide A′	RNA splicing	5
NM_004244	0.66	CD163 antigen	Scavenger receptor	5
NM_007107	0.60	Signal sequence receptor γ (translocon-associated protein γ)	Signal sequence binding	5
NM_030775	0.41	Wingless-type MMTV integration site family, member 5B	Signal transduction	5
NM_005842	0.57	Sprouty homolog 2 (Drosophila)	Signal transduction	5
BG251266	0.49	FOS-like antigen 1	Transcription factor	5
NM_002467	0.57	v-myc myelocytomatosis viral oncogene homolog (avian)	Transcription factor	5
X63381	0.63	MADS box transcription enhancer factor 2, polypeptide A (myocyte enhancer factor 2A)	Transcription factor	5
NM_016270	0.65	Kruppel-like factor 2 (lung)	Transcription factor	5
AI951185	0.63	Homo sapiens full length insert cDNA clone, YW26E10, mRNA sequence	Transcription factor	5
NM_005415	0.43	Solute carrier family 20 (phosphate transporter), member 1	Transport	5
BC005032	0.45	Sec23 homolog B (*S. cerevisiae*)	Transport	5
NM_006931	0.55	Solute carrier family 2 (facilitated glucose transporter), member 3	Transport	5
D87920	0.66	Solute carrier family 5 (sodium iodide symporter), member 5	Transport	5
AY014180	0.61	E3 ubiquitin ligase SMURF2	Ubiquitin cycle	4
NM_004907	0.59	Immediate early protein	Unknown	5

The altered genes and fold changes were determined as described in Section 1.

including adipocytes. The plasma IL-6 is elevated in obese subjects and type 2 diabetes patients, particularly in subjects also having features of the metabolic syndrome [30–32]. Also, Tsigos et al. reported that the administration of IL-6 to humans increase in fasting plasma glucose level [33]. Interestingly, Rega et al. showed that IL-6 induces PAI-1 in human adipose tissue [34]. They also demonstrated that oncostatin M (OSM), which is a member of the glycoprotein 130 (gp130) ligand family as well as IL-6, up-regulated PAI-1 expression in human adipocytes. These results indicate that the expression of IL-6 and OCM link with PAI-1 expression and contribute to the increased cardiovascular risk of patients with obesity and diabetes. It is noteworthy that treatment of Cy down-regulated IL-6 signal inducer (gp130, OCM receptor) (Table 5), although treatment of C3G did not affect the gene expression. The down-regulation of IL-6 and OCM receptor by anthocyanin treatment can contribute to the down-regulation of PAI-1. Inflammatory state in adipose tissue including up-regulation of pro-inflammatory cytokines such as IL-6 and tumor necrosis factor-α resulted in up-regulation of PAI-1 and down-regulation of adiponectin. Hou et al. showed that anthocyanidins (Cy or delphinidin) blocked 12-O-tetradecanoylphorbol-13-acetate-induced inflammation though inhibition of mitogen-activated protein kinase signaling pathway and activator protein-1 transcription activity in mouse epidermal cell line [35]. They also showed that lipopolysaccaride induced cyclooxygenase-2 expression was inhibited by anthocyanidins in macrophage RAW264 cells [36]. These reports imply anthocyanins would contribute to normalize these adipocytokines expression due to the anti-inflammatory action.

In this study, lipid and energy metabolism related genes (UCP2, ACOX1 and PLN) were significantly up-regulated in both the common C3G and Cy treated groups. The up-regulation of the genes may be performed in adipocytes to prevent excess lipid accumulation in them. The gene expression level of fatty

Table 5 – The down-regulated (<1.5-fold) genes in human adipocytes treated with Cy

Accession no.	Fold change	Description	Function	Cluster
AL574210	0.39	Serine (or cysteine) proteinase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1 (PAI-1)	Adipocytokine	5
NM_000600	0.56	Interleukin-6 (IL-6)	Adipocytokine	5
BC005254	0.62	C-type (calcium dependent, Carbohydrate-recognition domain) lectin, superfamily member 2 (activation-induced)	Antimicrobial	2
NM_007051	0.40	Fas (TNFRSF6) associated factor 1	Apoptosis	2
AF001294	0.54	Tumor suppressing subtransferable candidate 3	Apoptosis	5
NM_000930	0.55	Plasminogen activator, tissue	Blood coagulation	5
M63310	0.67	Annexin A3	Calcium ion binding	5
NM_001627	0.58	Activated leukocyte cell adhesion molecule	Cell adhesion	2
NM_020307	0.42	Cyclin L ania-6a	Cell cycle	2
AL136877	0.56	SMC4 structural maintenance of chromosomes 4-like 1 (yeast)	Cell cycle	2
D80000	0.59	SMC1 structural maintenance of chromosomes 1-like 1 (yeast)	Cell cycle	5
NM_003882	0.63	WNT1 inducible signaling pathway protein 1	Cell growth, cell adhesion	5
NM_003035	0.56	TAL1 (SCL) interrupting locus	Cell proliferation	2
NM_005807	0.59	Proteoglycan 4	Cell proliferation	2
AF112345	0.59	Integrin, α10	Cell-matrix adhesion	5
NM_001270	0.65	Chromodomain helicase DNA binding protein 1	Chromatin assembly	2
NM_006729	0.59	Diaphanous homolog 2 (Drosophila)	Cytokinesis	3
J05021	0.61	Villin 2 (ezrin)	Cytoskeletal anchoring	5
NM_004342	0.47	Caldesmon 1	Cytoskeleton	3
NM_004010	0.50	Dystrophin (muscular dystrophy, Duchenne and Becker types)	Cytoskeleton	2
NM_004415	0.52	Desmoplakin (DPI, DPII)	Cytoskeleton	2
BC004188	0.55	Tubulinβ, 2	Cytoskeleton	5
NM_006086	0.62	Tubulinβ, 4	Cytoskeleton	5
NM_016357	0.62	Epithelial protein lost in neoplasm β	Cytoskeleton	2
NM_012334	0.63	Myosin X	Cytoskeleton	3
BE877796	0.65	Collagen, type VIII, α1	Cytoskeleton	3
AI688418	0.54	Plexin A2	Development	5
NM_021154	0.63	Phosphoserine aminotransferase	Enzyme/amino acid biosynthesis	5
NM_000254	0.63	5-Methyltetrahydrofolate-homocysteine methyltransferase	Enzyme/amino acid biosynthesis	2
BC001886	0.57	Ribonucleotide reductase M2 polypeptide	Enzyme/DNA replication	5
NM_001379	0.50	DNA (cytosine-5-)-methyltransferase 1	Enzyme/nucleic acid modification	2
U16996	0.38	Dual specificity phosphatase 5	Enzyme/signal transduction	5
N22548	0.46	Rho-associated, coiled-coil containing protein kinase 1	Enzyme/signal transduction	2
NM_004760	0.50	Serine/threonine kinase 17a (apoptosis-inducing)	Enzyme/signal transduction	5
BC003143	0.57	Dual specificity phosphatase 6	Enzyme/signal transduction	5
NM_002841	0.58	Protein tyrosine phosphatase, receptor type, G	Enzyme/signal transduction	2
AA780381	0.58	Mitogen-activated protein kinase kinase 3	Enzyme/signal transduction	5
AF100763	0.61	Protein kinase, AMP-activated, α1 catalytic subunit	Enzyme/signal transduction	2
AL049383	0.63	Rho-associated, coiled-coil containing protein kinase 2	Enzyme/signal transduction	3
BC002755	0.64	MAP kinase-interacting serine/threonine kinase 1	Enzyme/signal transduction	2
NM_004834	0.64	Mitogen-activated protein kinase kinase kinase kinase 4	Enzyme/signal transduction	2
NM_013233	0.66	Serine threonine kinase 39 (STE20/SPS1 homolog, yeast)	Enzyme/signal transduction	2
L14561	0.35	ATPase, Ca^{++} transporting, plasma membrane 1	Enzyme/transport	5
NM_006447	0.65	Ubiquitin specific protease 16	Enzyme/ubiquitin-dependent protein catabolism	3
NM_018844	0.57	B-cell receptor-associated protein BAP29	Intracellular protein transport	5
NM_023010	0.61	Similar to yeast Upf3, variant B	mRNA catabolism	1
N36997	0.59	KIAA1966 protein	Nuclear mRNA splicing	3
BG254869	0.63	Splicing factor, arginine/serine-rich 2	Nuclear mRNA splicing	5
NM_004768	0.65	Splicing factor, arginine/serine-rich 11	Nuclear mRNA splicing	3
BG532929	0.54	Sjogren syndrome antigen B (autoantigen La)	Nucleic acid metabolism	3
NM_001380	0.62	Dedicator of cyto-kinesis 1	Phagocytosis	5
AI375486	0.64	Adenomatosis polyposis coli	Protein complex assembly	3
NM_004244	0.41	CD163 antigen	Receptor	5
NM_016639	0.42	Tumor necrosis factor receptor superfamily, member 12A	Receptor	5
NM_002184	0.52	IL-6 signal transducer (gp130, oncostatin M receptor)	Receptor	2
NM_003856	0.54	IL-1 receptor-like 1	Receptor	5
NM_000118	0.55	Endoglin (Osler-Rendu-Weber syndrome 1)	Receptor	5
NM_000916	0.63	Oxytocin receptor	Receptor	3
NM_002224	0.66	Inositol 1,4,5-triphosphate receptor, type 3	Receptor	2
NM_003082	0.57	Small nuclear RNA activating complex, polypeptide 1, 43 kDa	Regulation of transcription	5

Table 5 (*Continued*)

Accession no.	Fold change	Description	Function	Cluster
NM_012081	0.62	ELL-related RNA polymerase II, elongation factor	Regulation of transcription	2
D13889	0.64	Inhibitor of DNA binding 1, dominant negative helix-loop-helix protein	Regulation of transcription	2
NM_006824	0.60	EBNA1 binding protein 2	Ribosome biogenesis	5
NM_012341	0.63	G protein-binding protein CRFG	Ribosome biogenesis	5
NM_013285	0.64	Nucleolar GTPase	Ribosome biogenesis	5
NM_004728	0.51	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 21	RNA processing	5
AW089673	0.41	Cisplatin resistance-associated overexpressed protein	RNA splicing	2
NM_004698	0.57	U4/U6-associated RNA splicing factor	RNA splicing	5
NM_003090	0.60	Small nuclear ribonucleoprotein polypeptide A′	RNA splicing	5
NM_004741	0.66	Nucleolar and coiled-body phosphoprotein 1	rRNA processing	3
NM_030775	0.37	Wingless-type MMTV integration site family, member 5B	Signal transduction	5
AB003476	0.43	A kinase (PRKA) anchor protein (gravin) 12	Signal transduction	2
AA308853	0.45	Nuclear pore complex interacting protein	Signal transduction	2
NM_005842	0.47	Sprouty homolog 2 (Drosophila)	Signal transduction	5
AB019691	0.59	A kinase (PRKA) anchor protein (yotiao) 9	Signal transduction	1
NM_005475	0.61	Lymphocyte adaptor protein	Signal transduction	5
NM_016141	0.65	Dynein, cytoplasmic, light intermediate polypeptide 1	Signal transduction	5
AL042733	0.67	BRCA1 associated protein	Signal transduction	2
NM_006107	0.50	Acid-inducible phosphoprotein	Stress response	3
BG252490	0.50	DnaJ (Hsp40) homolog, subfamily B, member 4	Stress response	5
AF007217	0.41	Thyroid hormone receptor interactor 11	Transcription co-activator	2
X63381	0.55	MADS box transcription enhancer factor 2, polypeptide A (myocyte enhancer factor 2A)	Transcription co-activator	5
AF113514	0.38	Monocytic leukemia zinc finger protein-related factor	Transcription factor	3
NM_001964	0.40	Early growth response 1	Transcription factor	5
BG251266	0.43	FOS-like antigen 1	Transcription factor	5
NM_002467	0.45	v-myc myelocytomatosis viral oncogene homolog (avian)	Transcription factor	5
NM_004735	0.46	Leucine rich repeat (in FLII) interacting protein 1	Transcription factor	5
NM_016270	0.56	Kruppel-like factor 2 (lung)	Transcription factor	5
AI951185	0.61	Homo sapiens full length insert cDNA clone, YW26E10, mRNA sequence	Transcription factor	5
NM_003750	0.44	Eukaryotic translation initiation factor 3, subunit 10θ, 150/170 kDa	Translational initiation	2
AI768122	0.49	Eukaryotic translation initiation factor 4γ, 3	Translational initiation	2
NM_005415	0.29	Solute carrier family 20 (phosphate transporter), member 1	Transport	5
NM_002078	0.38	Golgi autoantigen, golgin subfamily a, 4	Transport	3
Z22551	0.39	Kinectin 1 (kinesin receptor)	Transport	2
NM_002956	0.45	Restin	Transport	2
BF110993	0.50	Translocated promoter region (to activated MET oncogene)	Transport	2
NM_003566	0.51	Early endosome antigen 1, 162 kDa	Transport	3
BC005032	0.52	Sec23 homolog B (S. cerevisiae)	Transport	5
D87920	0.64	Solute carrier family 5 (sodium iodide symporter), member 5	Transport	5
NM_006931	0.64	Solute carrier family 2 (facilitated glucose transporter), member 3	Transport	5
AV699347	0.47	Human XIST, coding sequence ‘a’ mRNA (locus DXS399E), mRNA sequence	Unknown	3
NM_015577	0.48	Retinoic acid induced 14	Unknown	2
NM_004907	0.50	Immediate early protein	Unknown	5
NM_016374	0.52	RBP1-like protein	Unknown	5
NM_003611	0.52	Oral-facial-digital syndrome 1	Unknown	2
NM_023012	0.58	Hypothetical protein FLJ11021similar to splicing factor, arginine/serine-rich 4	Unknown	2
NM_016644	0.60	Mesenchymal stem cell protein DSC54	Unknown	5
NM_006851	0.64	GLI pathogenesis-related 1 (glioma)	Unknown	5
NM_014890	0.65	Down-regulated in ovarian cancer 1	Unknown	2
AK023637	0.65	Alport syndrome, mental retardation, midface hypoplasia and elliptocytosis chromosomal region, gene 1	Unknown	5
NM_007124	0.66	Utrophin (homologous to dystrophin)	Unknown	3

The altered genes and fold changes were determined as described in Section 1.

Table 6 – The up-regulated (>1.5-fold) adipocytokines and lipid metabolism related genes in human adipocytes treated with C3G or Cy

Accession no.	Fold change	Description
C3G treatment		
U94592	2.26	Uncoupling protein 2 (UCP2)
S69189	1.58	AcylCoA oxidase 1 (ACOX1), palmitoyl
NM_004797	1.57	Adipose most abundant gene transcript 1 (adiponectin)
NM_002666	1.54	Perilipin (PLN)
Cy treatment		
U94592	2.24	Uncoupling protein 2 (UCP2)
X62078	1.83	GM2 ganglioside activator protein
NM_004797	1.82	Adipose most abundant gene transcript 1 (adiponectin)
NM_002666	1.80	Perilipin (PLN)
NM_004364	1.75	CCAAT/enhancer binding protein (C/EBP), α
S69189	1.68	AcylCoA oxidase 1(ACOX1), palmitoyl
NM_001928	1.67	D component of complement (adipsin)
NM_001909	1.52	Cathepsin D (lysosomal aspartyl protease)

The altered genes and fold changes were determined as described in Section 1.

Table 7 – The down-regulated (<1.5-fold) adipocytokines and lipid metabolism related genes in human adipocytes treated with C3G or Cy

Accession no.	Fold change	Description
C3G treatment		
AL574210	0.49	Serine (or cysteine) proteinase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1 (PAI-1)
NM_000600	0.58	Interleukin-6 (IL-6) (interferon, β2)
Cy treatment		
AL574210	0.39	Serine (or cysteine) proteinase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1 (PAI-1)
NM_000600	0.56	Interleukin-6 (IL-6) (interferon, β2)

The altered genes and fold changes were determined as described in Section 1.

acid binding protein 4, lipoprotein lipase, hormone sensitive lipase, leptin and PPARγ was not significantly up-regulated by the administration of anthocynains in human adipocytes, however, these gene expression level was significantly up-regulated in rat adipocytes study [18]. It is speculated that it may be due to the difference in response to abdominal (obtained from rat epididymal adipose tissue) or subcutaneous (human) adipocytes rather than species.

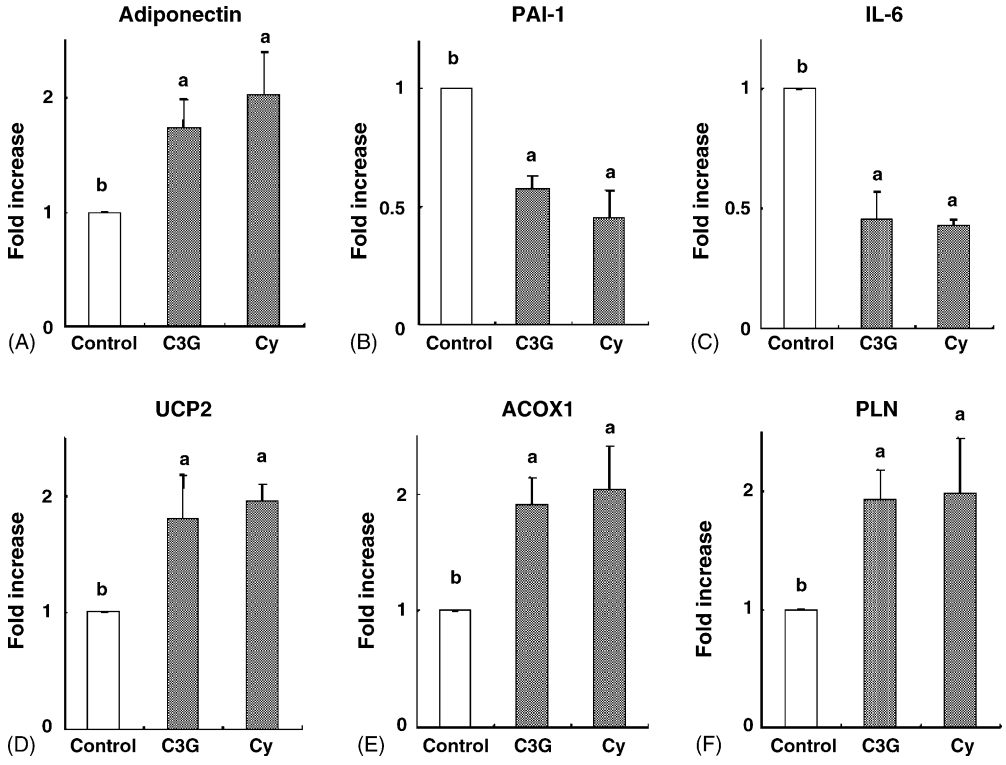


Fig. 4 – Gene expression level of adiponectin (A), PAI-1 (B), IL-6 (C), UCP2 (D), ACOX1 (E) and PLN (F) in human adipoytces treated with C3G or Cy determined by real-time PCR analyses. The gene expression level was expressed as fold increase relative to the control group after normalization using the β-actin gene expression level. Values are means ± S.E.M., $n = 3$. Means without a common letter differ, $P < 0.05$.

In conclusion, this study demonstrates that anthocyanins modulate the gene expression of the adipocytokine in human adipocytes, suggesting that anthocyanins have a unique therapeutic advantage responsible for the regulation of the adipocyte function. Our findings provide a biochemical basis for the use of anthocyanins, which can also have important implications for preventing obesity and diabetes.

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REFERENCES

[1] Matsuzawa Y. Adipocytokines and metabolic syndrome. Semin Vasc Med 2005;5:34–9.
[2] Friedman JM, Halaas JL. Leptin and the regulation of body weight in mammals. Nature 1998;395:763–70.
[3] Scherer PE, Williams S, Fogliano M, Baldini G, Lodish HF. A novel serum protein similar to C1q, produced exclusively in adipocytes. J Biol Chem 1995;270:26746–9.
[4] Maeda K, Okubo K, Shimomura I, Funahashi T, Matsuzawa Y, Matsubara K. cDNA cloning and expression of a novel adipose specific collagen-like factor, apM1 (adipose most abundant gene transcript 1). Biochem Biophys Res Commun 1996;221:16286–9.
[5] Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, et al. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. Biochem Biophys Res Commun 1999;257:79–83.
[6] Bajaj M, Suraamornkul S, Piper P, Hardies LJ, Glass L, Cersosimo E, et al. Decreased plasma adiponectin concentrations are closely related to hepatic fat content and hepatic insulin resistance in pioglitazone-treated type 2 diabetic patients. J Clin Endocrinol Metab 2004;89:200–6.
[7] Diez JJ, Iglesias P. The role of the novel adipocyte-derived hormone adiponectin in human disease. Eur J Endocrinol 2003;148:293–300.
[8] Matsuzawa Y, Funahashi T, Nakamura T. Molecular mechanism of metabolic syndrome X: contribution of adipocytokines adpocyte-derived bioactive substances. Ann NY Acad Sci 1999;892:146–54.
[9] Lee CH, Olson P, Evans RM. Minireview: lipid metabolism, metabolic diseases, and peroxisome proliferator-activated receptors. Endocrinology 2003;144:2201–7.
[10] Zhou G, Myers R, Li Y, Chen Y, Shen X, Fenyk-Melody J, et al. Role of AMP-activated protein kinase in mechanism of metformin action. J Clin Invest 2001;108:1167–74.
[11] Harborne JB, Grayer RJ. The anthocyanins. In: Harborne JB, editor. The flavonoids. London: Chapman & Hall; 1988. p. 1–20.
[12] Brouillard R. Flavonoids and flower color. In: Harborne JB, editor. The flavonoids. London: Chapman & Hall; 1988. p. 525–38.
[13] Tsuda T, Horio F, Osawa T. Dietary cyanidin 3-O-β-D-glucoside increases ex vivo oxidation resistance of serum in rats. Lipids 1998;33:583–8.
[14] Tsuda T, Horio F, Kitoh J, Osawa T. Protective effects of dietary cyanidin 3-O-β-D-glucoside on ischemia-reperfusion injury in rats. Arch Biochem Biophys 1999;368:361–6.
[15] Tsuda T, Horio F, Kato Y, Osawa T. Cyanidin 3-O-β-D-glucoside attenuates the hepatic ischemia-reperfusion injury through a decrease in the neutrophil chemoattractant production in rats. J Nutr Sci Vitaminol 2002;48:134–41.
[16] Tsuda T, Horio F, Osawa T. Cyanidin 3-O-β-D-glucoside suppresses nitric oxide production during a zymosan treatment in rats. J Nutr Sci Vitaminol 2002;48:305–10.
[17] Tsuda T, Horio F, Uchida K, Aoki H, Osawa T. Dietary cyanidin 3-O-β-D-glucoside-rich purple corn color prevents obesity and ameliorates hyperglycemia in mice. J Nutr 2003;133:2125–30.
[18] Tsuda T, Ueno Y, Aoki H, Koda T, Horio F, Takahashi N, et al. Anthocyanin enhances adipocytokine secretion and adipocyte-specific gene expression in isolated rat adipocytes. Biochem Biophys Res Commun 2004;316:149–57.
[19] Iwaki M, Matsuda M, Maeda N, Funahashi T, Matsuzawa Y, Makishima M, et al. Induction of adiponectin, a fat-derived antidiabetic and antiatherogenic factor, by nuclear receptors. Diabetes 2003;52:1655–63.
[20] Juhan-Vague I, Alessi MC. Plasminogen activator inhibitor 1 and athero-thrombosis. Thromb Haemost 1993;70:138–43.
[21] Eren M, Painter CA, Atkinson JB, Declerck PJ, Vaughan DE. Age-dependent spontaneous coronary arterial thrombosis in transgenic mice that express a stable form of human plasminogen activator inhibitor-1. Circulation 2002;106:491–6.
[22] Juhan-Vague I, Alessi MC, Mavri A, Morange PE. Plasminogen activator inhibitor-1, inflammation, obesity, insulin resistance and vascular risk. J Thromb Haemost 2003;1:1575–9.
[23] Alessi MC, Bastelica D, Morange P, Berthet B, Leduc I, Verdier M, et al. Plasminogen activator inhibitor 1, transforming growth factor-beta1, and BMI are closely associated in human adipose tissue during morbid obesity. Diabetes 2000;49:1374–80.
[24] Eriksson P, Reynisdottir S, Lonnqvist F, Stemme V, Hamsten A, Arner P. Adipose tissue secretion of plasminogen activator inhibitor-1 in non-obese and obese individuals. Diabetologia 1998;41:65–71.
[25] Uchida Y, Ohba K, Yoshioka T, Irie K, Muraki T, Maru Y. Cellular carbonyl stress enhances the expression of plasminogen activator inhibitor-1 in rat white adipocytes via reactive oxygen species-dependent pathway. J Biol Chem 2004;279:4075–83.
[26] Zirlik A, Leugers A, Lohrmann J, Ernst S, Sobel BE, Bode C, et al. Direct attenuation of plasminogen activator inhibitor type-1 expression in human adipose tissue by thiazolidinediones. Thromb Haemost 2004;70:138–43.
[27] Harte AL, McTernan PG, McTernan CL, Smith SA, Barnett AH, Kumar S. Rosiglitazone inhibits the insulin-mediated increase in PAI-1 secretion in human abdominal subcutaneous adipocytes. Diab Obesity Metab 2003;5:302–10.
[28] Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante Jr AW. Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest 2003;112:1796–808.
[29] Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, et al. Chronic inflammation in fat plays a crucial role in the development of obesity related insulin resistance. J Clin Invest 2003;112:1821–30.
[30] Fried SK, Burkin DA, Greenberg AS. Omental and subcutaneous adipose tissues of obese subjects release

interleukin-6: depot difference and regulation by glucocorticoid. J Clin Endocrinol Metab 1998;83:847–50.
[31] Pickup JC, Chusney GD, Thomas SM, Burt D. Plasma interleukin-6, tumor necrosis factor alpha and blood cytokine production in type 2 diabetes. Life Sci 2000;67:291–300.
[32] Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. J Am Med Assoc 2001;286:327–34.
[33] Tsigos C, Papanicolaou DA, Kyrou I, Raptis SA, Chrousos GP. Dose-dependent effects of recombinant human interleukin-6 on the pituitary-testicular axis. J Interferon Cytokine Res 1999;19:1271–6.
[34] Rega G, Kaun GC, Weiss TW, Demyanets S, Zorn G, Kastl SP, et al. Inflammatory cytokines interleukin-6 and oncostatin M induce plasminogen activator inhibitor-1 in human adipose tissue. Circulation 2005;111:1938–45.
[35] Hou DX, Kai K, Li JJ, Lin S, Terahara N, Wakamatsu M, et al. Anthocyanidins inhibit activator protein 1 activity and cell transformation: structure–activity relationship and molecular mechanisms. Carcinogenesis 2004;25:29–36.
[36] Hou DX, Yanagita T, Uto T, Masuzaki S, Fujii M. Anthocyanidins inhibit cyclooxygenase-2 expression in LPS-evoked macrophages: structure–activity relationship and molecular mechanisms involved. Biochem Pharmacol 2005;70:417–25.